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Effects of Phytate and Calcium on the Reabsorption of Endogenous Zinc in Zinc-Depleted Rats

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ABSTRACT

Endogenous zinc is important for maintaining zinc homeostasis because the size of endogenous zinc pool is almost 3-4 times bigger than that of dietary zinc. The purpose of this study was to examine the phytate effect on the reabsorption of endogenous zinc and the additional Ca effect on the phytate effect. Rats were fed a casein-based diet with added sodium phytate containing either high(1.6%) or low(0.8%) Ca concentrations for 4 weeks to reduce the body zinc pool. After the depletion period, ^{65}Zn was given by intraperitoneal injection to label the endogenous zinc pool. Rats were then assigned into phytate or non-phytate group within the same Ca group. Feces were collected for 2 weeks of the initial collection period and 1 week after dietary crossover. The ratios of excreted fecal ^{65}Zn radioactivity of phytate group : non-phytate group were determined as a measure of the phytate effect on the endogenous zinc. Mean fecal ^{65}Zn radioactivity was higher in the phytate group than in the non-phytate group during the entire 3 weeks of the collection period in the low Ca group, and during the initial collection period in the high Ca group($p<0.0001$). This study showed an adverse phytate effect on endogenous zinc at both high and low dietary Ca levels. Elevated dietary Ca levels showed a synergistic effect on the phytate effect on endogenous zinc($p<0.05$). These results imply greater phytate effect on zinc homeostasis rather than on zinc bioavailability through complexing with the endogenous zinc which is larger portion than the dietary zinc on zinc homeostasis. (*Korean J Nutrition* 30(4) : 394~405, 1997)

KEY WORDS : phytate · endogenous zinc · calcium · zinc hemeostasis · rats.

Introduction

Phytate(myo-inositol-1, 2, 3, 5/4, 6-hexakis dihydrogen phosphate) is a naturally occurring compound formed during the maturation of seeds and cereal grains. It is present in plant seeds, many roots, tubers, and some fruits. It is found in an especially high concentration in legumes, the bran and germ of cereal grains, where it represents 70 to 90% of the organically-bound phosphorus storage compound. Phytate was first isolated from the aleurone of the plant seeds by Pfeffer in 1872[1-2]. Phytate is a chelator that has been considered as the primary factor responsible for the poorer absorbability of various cationic metals. O'Dell and Savage(1960)[3] first demonstrated that phytate complexed with zinc in chick gastrointestinal tract and precipitated it out. Phytate-related zinc deficiency in man was first reported in some male adolescents in the Middle East in the early 1960s[4]. They showed poor appetities, marked growth retardation, and hypogonadism with calcium deficiency. The main factor for this zinc deficiency was the high phytate content in their

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meals. Since then, others also found that dietary zinc was being affected by dietary phytate.

For a long time, excessive dietary intake of phytate was thought to inhibit the bioavailability of zinc through the formation of insoluble phytate-metal complexes in the gastrointestinal tract of monogastric animals [5-9], including humans[10-13]. There are numerous research reports that the bioavailability of dietary zinc is affected by decreased absorption in the presence of phytate. These studies have measured only apparent zinc absorption. However, out of the total amount of zinc in the duodenal gastrointestinal tract, a large amount of endogenous zinc is secreted through pancreatic/biliary secretion.

Therefore, it is essential, considering the large amounts of zinc secreted, that a major portion of the zinc must be reabsorbed. The reabsorption of endogenous zinc is an important component of overall zinc homeostasis because it is almost 4 times that of daily dietary zinc absorption[14-17]. The present concept of the phytate effect on zinc bioavailability is that dietary phytate decreases the absorption of dietary zinc. However, with the indication that the endogenous zinc pool is bigger in size than the dietary zinc pool, it should be considered that the effect of phytate on endogenous zinc may be more important than the effect on dietary zinc in understanding whole zinc ho-meostasis. With the phytate effect influencing dietary zinc absorption, only a small portion of the potential effect of phytate might be observed. The phytate effect as an important entity in whole zinc homeostasis may be ignored. This problem should be considered as to whether dietary phytate inhibits the reabsorption of endogenous zinc. The need exists to study the relative vulnerability of zinc secreted into the gastroin-testinal tract to bind by phytate and to test the effect of phytate on endogenous zinc reasborption for zinc homeostasis.

Calcium is also known as an anti-nutrient factor for zinc absorption along with phytate. Zinc uptake progressively decreased as the ratio of calcium to phytate was increased. Calcium potentiates the negative effect of phytate on dietary zinc bioavailability by enhancing phytate-zinc chemical interaction[7)11)18-20]. On examination of the effect of phytate on endogenously secreted zinc, this study was also validated to test calcium effects by the phytate : zinc and phytate × calcium : zinc molar ratio relationships.

The purpose of the present study was to examine the effect of phytate added to a casein-based diet on endogenously secreted zinc and to examine the synergistic effect of calcium on the phytate effect on endogenously secreted zinc in zinc-depleted rats.

Materials and Method

1. Experimental Design, Diet and Animals

The experimental design was a randomized complete block design with a 2×2 factorial arrangement of treatments. The two variables in this study were the two levels of phytate(0 and 4.7g/kg) achieved by adding sodium phytate to the supplemented diets and two levels of calcium(0.8% and 1.6%) adjusted with calcium carbonate to elucidate the effect of [phytate] : [Zn] or [phytate]×[Ca] : [Zn] molar ratio relationships. The amount, a 4.7g/kg diet, of sodium phytate (Phytic Acid, Inositol Hexaphosphoric Acid from corn, Sodium Salt, Sigma Chemical Co., St. Louis) for the phytate groups was calculated to be a molar ratio, [phytate] : [Zn] of 30 : 1.

The experimental plan and design is shown in Fig. 1. Twenty four young Sprague-Dawley male rats (Harlan Sprague Dawley Inc., Indianapolis) were used for each low or high Ca group. The initial mean body weight was 105±2g in the low Ca group and was 97±1g in the high Ca group. Rats were housed individually in suspended stainless steel cages in a temperature and light-controlled room(22℃, 12-h light/dark cycle). All forty-eight rats were fed a low Ca, non-phytate diet for the first 3 weeks of the growth period to increase body weight. Following the growth period was a 4-week zinc depletion period. The rats of the low Ca group were fed the casein-based low Ca, phytate-containing diet, and the rats of the high Ca group were fed the casein-based high Ca, phytate-containing diet for 4 weeks to deplete body zinc. At the end of the depletion period, just before being assigned and then placed in metabolism cages, each of the 48 rats was injected intraperitoneally with 0.1mL of radioactive ^{65}Zn(chemical form, $ZnCl_2$, DuPont, Boston, MA) in 0.001mol/L HCl diluted with 0.9% saline. This provided 3.7×10^5 Bq to form an equilibrium with the endogenous metabolic zinc pool. Im-

mediately after the injection of radioactive ^{65}Zn, the rats were placed in metabolism cages for fecal collection. For the first 2 weeks of the initial collection period, the rats were fed a phytate or a non-phytate diet within the same Ca group. After the initial collection period, test rats given a low-Ca, non-phytate diet were switched to a low-ca diet containing phytate. The rats in the low Ca, phytate diet group were changed to a phytate-free diet. This was done to make a crossover design to examine the phytate effect on endogenous zinc during the following 1 week of the crossover collection period without any additional ^{65}Zn injection(Fig. 1). Rats in the high Ca dietary group were fed in the same manner.

Four experimental treatment diets were prepared from ingredients that were purchased from Dyets, Inc. (Bethlehem, PA). The composition of the four experimental diets is shown in Table 1. The diets were supplied in aluminum feed cups, and the deionized and distilled water was supplied from polyethylene water bottles with butyl rubber(neoprene) stoppers to prevent zinc contamination. The animals were given free access to food and water.

2. Fecal Sample Collection and Radioactive ^{65}Zn Measurement in the Feces

The total rat feces were collected at the nearly the same time, about 2 : 00 p.m. to 4 : 00 p.m. daily during the combined 3 weeks of the collection periods ; 2 weeks of the initial collection period and 1 week of the crossover collection period. Feces from each animal for each day was placed in a counting tube.

The collected total feces in the individual tube were counted for ^{65}Zn radioactivity by an γ-scintillation counter(Packard, Cobra Ⅱ Auto-Gamma Counting System. Meriden, CT) set at the photopeak for radioactive ^{65}Zn within 24 hours. The ratio of fecal ^{65}Zn ra-

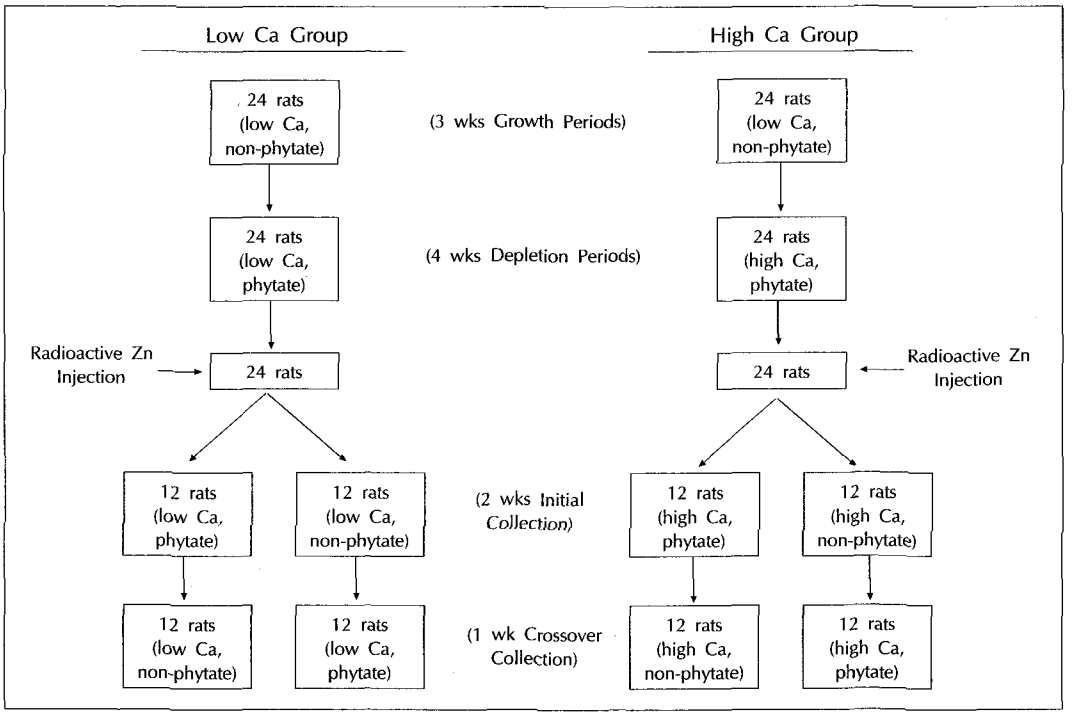

Fig. 1. Experimental design and diet plan. Twenty-four young Sprague-Dawley strain male rats were fed with the low Ca, non-phytate diet for the first 3 weeks of the growth period to increase body weight. Following the growth period was 4 weeks depletion period ; the rats of the low Ca group were fed with the casein-based low Ca, phytate containing diet, while the rats of the high Ca group were fed the casein-based high Ca, phytate containing diet to deplete body zinc. After 7 weeks of the growth and depletion period, radioactive ^{65}Zn was given by intraperitoneal injection to form an equilibrium with the endogenous metabolic zinc pool. After ^{65}Zn injections, rats were reassigned to each phytate or non-phytate group. After the initial 2 weeks of the collection period, each diet was switched over to feed the rats in crossover design to examine the phytate effect within the same calcium dietary group.

Table 1. Experimental diets[1-5]

Ingredient	Low Ca phytate	Low Ca non-phytate	High Ca phytate	High Ca non-phytate
	(g/kg diet)			
Glucose	665.3	670	645.3	650
Vegetable oil	40	40	40	40
Fat-soluble vit premix	10	10	10	10
Cellulose	30	30	30	30
Casein	200	200	200	200
Mineral premix	30	30	30	30
Calcium carbonate	0	0	20	20
Trace element premix	10	10	10	10
B-complex premix	10	10	10	10
Na phytate	4.7	0	4.7	0

1) Any substitutions were made at the expense of glucose.
2) Fat-soluble vitamin premix(g/kg diet) : vitamin A acetate, 0.00069 ; vitamin D, 0.0000075 ; Menadione, 1 ; Alpha tocopherol acetate, 3 ; Butylate hydroxy anisole(antioxidant), 10 ; Vegetable oil, 985.3.
3) Mineral premix(g/kg diet) : Calcium carbonate, 210 ; Calcium phosphate(2 waters), 330 ; Magnesium oxide, 15 ; Potassium chloride, 10 ; Sodium chloride, 30 ; Ferrous sulfate(7 waters), 4 ; Potassium acid phosphate, 120 ; Manganese carbonate, 2 ; Cupric sulfate(5 waters), 0.8 ; Potassium iodate, 0.1 ; Sodium fluoride, 0.4 ; Glucose, 277.7.
4) Trace element premix(g/kg diet) : Sodium molybdate(2 waters), 0.00126 ; Chromium Ⅲ acetate(1 water), 2.4 ; Sodium selenite(5 waters), 0.0167 ; Glucose, 997.5.
5) B-complex vitamin premix(g/kg diet) : Thiamine HCl, 1.6 ; Riboflavin, 1.6 ; Nicotinic acid, 17.32 ; Phridoxine HCl, 1.6 ; Calcium pantothenate, 4 ; Biotin, 0.02 ; Folic acid, 0.5 ; Cyanocobalamin(0.1% in manitol), 5.0 ; DL-Methionine, 200 ; Choline bitartrate, 300 ; Glucose, 468.36.

dioactivity of the phytate group to that of the non-phytate group was calculated to test the effect of phytate on the excretion of endogenous zinc.

3. Zn and Ca Analysis in the Diet

Zn and Ca concentrations in the diet were measured by a modified method of Anderson et al.(1985)[21]. All glassware used in the extraction were cleaned by soaking for 24 hrs in a 10% nitric acid(HNO_3) solution, followed by rinsing in a large volume of deionized and distilled water. All the samples were dry-ashed for the determination of Zn and Ca. For analysis, diet and feces samples were dried for 24 hours in a convection oven at 105℃. They were then ashed in a muffle furnace with no exposed metal heating elements. The furnace was initially set at 100℃ and the temperature was raised 50℃ every hour to 300℃. The temperature was then raised to 450℃ and the samples were ashed for 24 hours. The ashed samples were cooled in a desiccator. To each cooled tube was added 50μL each of 50% hydrogen peroxide and concentrated nitric acid. Concentrated nitric acid and hydrogen peroxide were to destroy any organic matter left in the sample. The tubes were dried in a dry bath at 100℃ for 3 hours and samples were then ashed at 400℃ for an additional 12 hours. The drying and ashing procedure was repeated until white ash remained. After ashing, the samples were diluted with 10% nitric acid and analyzed using a Perkin Elmer Model 5000 atomic absorption spectrophotometer at 213.8nm wavelength(slit 0.7H, 15mA) with Zeeman backgrou-nd correction.

The accuracy of the atomic absorption analysis and the completeness of digestion was verified by using the National Institute of Standards and Technology(NIST) peach leaves standard(standard reference material 1547). The zinc value found for the standard peach leaves(17.9μg Zn/g sample) with 98% recovery, compared with the zinc value of the standard peach leaves(18.3μg Zn/g sample) reported by NIST.

4. Phytate Analysis in the Diet

For analysis of phytate in the diets, a modification of the AOAC Official Method was used[22]. Samples of the four diets(1g) were extracted for for more than 3 hrs at room temperature with 20mL of 2.4% HCl. Before extraction, the diet samples were defatted by ether extraction. The extracts were then vacuum-filtered. To avoid hydrolysis from mold or bacterial enzymes, the filtrate was refrigerated. Glass barrel columns(0.7× 15cm) equipped with a 3-way Luer fit stopcock were clamped vertically and packed with about 0.5g of anion exchange resin(Bio-Rad laboratories, Richmond, CA), 100-200 mesh AG1-X4 chloride form. Before use, the columns were washed first with 10mL of 0.7 M NaCl to assure chloride saturation of the resin, and then with distilled water until the eluate was salt-free. 1mL of the diet extracts and 1mL of NaEDTA-NaOH mixture were combined into a beaker and diluted to 15mL with distilled water. This mixture was quantitatively poured onto the column and the the

eluate was discarded. The column was eluted with 15mL of 0.1M NaCl to elute the inorganic phosphate. The phytate was then eluted from the column with 10mL of 0.7M NaCl and collected in a micro-Kjeldahl digestion flask. To the digestion vessel was added 0.5mL of concentrated H_2SO_4 and a few boiling beads. Digestion was continued until only the H_2SO_4 remained(25-30min). The end point had been reached when a white cloud of vapors separated from and remained suspended above the H_2SO_4 in the digestion vessel for 5 min. One mL H_2O_2 was added for digestion until solution was clear. Digestion timing was critical because incompletely digested products do not react with color reagents and over-digestion causes sublimation of the phosphorus. About 10-15mL of distilled water was added to the digestion vessels and the contents were mixed thoroughly and heated near boiling for 10min. 2mL of NH_4MoO_4 in 5N H_2SO_4 and 1mL sulfonic acid reagent were added and read at 640nm.

5. Statistical Analysis

The statistical analytical system program(SAS/STAT Version 6, SAS Institute Inc., Cary, NC) was used for statistical analysis. Analysis of variance(Anova) was performed as a 2×2 factorial treatment which is the main effects of phytate and of calcium and the interaction between phytate and calcium. Student's t-test was used for the comparison of ^{65}Zn radioactivity between phytate group and non-phytate group within the same Ca group at each collection period, and for ratios of phytate group : non-phytate group between low and high calcium dietary group. A $p<0.05$ or less was considered to be significanty different.

Results

1. Analysis of the Diet Composition

The analyzed calcium level was 7.9g/kg diet for the low Ca group and 15.7g/kg diet for the high Ca group. Dietary zinc was 6.8μg/kg for the low Ca, phytate group, 6.2μg/kg for the low Ca, non-phytate group, 6.6μg/kg for the high Ca, phytate group, and 6.8μg/kg for the high Ca, non-phytate diet group, respectively. The phytate content of the low Ca, phytate group was 2.2g/kg diet which gives a [phytate] : [Zn] molar ratio of 32.3. Phytate content of the high Ca, phytate group was 1.8g/kg diet which gives a [phytate] : [Zn] molar ratio of 27.0. The molar ratio of [phytate]×[Ca] : [Zn] was 6.3 for the low Ca, phytate diet, and 12.3 for the high Ca, phytate diet. The moles and molar ratios of phytate, calcium, and zinc are in Table 2. The analyzed Ca concentration was 7.836g/kg diet(low Ca, phytate), 7.890g/kg diet (low Ca, non-phytate), 18.240g/kg diet(high Ca, phytate), and 13.104g/kg diet(high Ca, non-phytate). This yielded the molar concentration(weight/molecular weight ; molecular weight of Ca, 40.08) of 0.1955M(low Ca, phytate), 0.1969M(low Ca, non-phytate), 0.4551M(high Ca, phytate), and 0.3269M(high Ca, non-phytate), respectively. The concentration of the phytate in each low Ca or high Ca phytate diet was corrected for a non-phytate phosphate-containing fraction that appeared in the non-phytate casein-based diets.

2. ^{65}Zn Radioactivity in the Feces

The statistical data summary showed a large effect of phytate levels on endogenous zinc. In the low Ca dietary groups, mean fecal ^{65}Zn radioactivity per day during the initial collection period was higher in the phytate group(4582±345cpm/d) than in the non-phytate group(3990±215cpm/d)($p<0.0001$), and was higher in the phytate group(3077±146cpm/d) than in the non-phytate group(2376±96cpm/d) during the crossover collection period($p<0.0001$)(Fig. 2).

For the high Ca dietary groups, mean fecal ^{65}Zn radioactivity during the initial collection periods was

Table 2. The moles and molar ratios of phytate, calcium and zinc in the diets[1]

Diet	phytate[1](mole)	Ca(mole)	Zn(mole)	phytate : Zn(molar)	phytate×Ca : Zn(molar)
Low Ca, phytate	3.357×10^{-3}	0.1955	1.039×10^{-4}	32.3	6.3
Low Ca, Non-phytate	–	0.1969	0.948×10^{-4}	–	–
High Ca, phytate	2.725×10^{-3}	0.4551	1.009×10^{-4}	27.0	12.3
High Ca, Non-phytate	–	0.3269	1.039×10^{-4}	–	–

1) The concentration of the phytate in each low Ca, phytate or high Ca, phytate group was corrected by the concentration of the hexaphosphate equivalent in each low Ca, non-phytate or high Ca, non-phytate group.

higher in the phytate group(4250±310cpm/d) than in the non-phytate group(2790±140cpm/d)($p<0.0001$), while no significant difference was shown between the two groups during the crossover collection periods($p>0.05$)(Fig. 2).

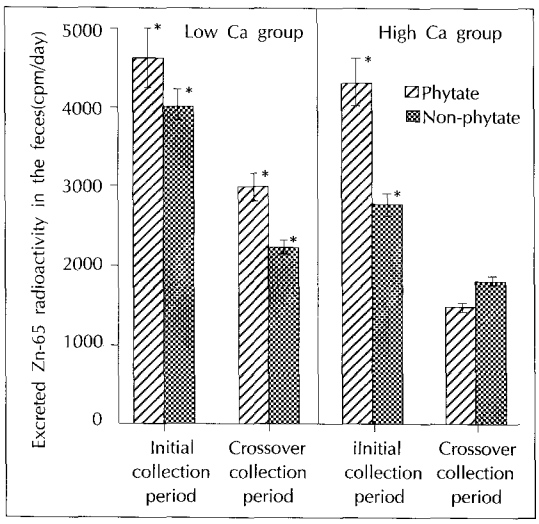

Fig. 2. Fecal excretion of ^{65}Zn radioactivity in low and high calcium group. Values are mean±SEM of total measured ^{65}Zn radioactivity during each collection period : 14 days of the initial collection period and 7 days of the crossover collection period(n=12 rats per group). Bars that have asterisks(*) are significantly different at $p<0.0001$ between phytate group and non-phytate group. Each feces sample was collected daily during the whole collection period. ^{65}Zn radioactivity of each sample was measured with a γ-scintillation counter daily. For the comparison of fecal Zn-65 radioactivity of phytate groups to that of non-phytate groups in each collection period, paired t-test was used.

3. Ratios of Phytate Group : Non-Phytate Group on Fecal ^{65}Zn Excretion

The ratios of phytate group : non-phytate group calculated from endogenous ^{65}Zn excretion in total feces per day during initial collection period and crossover collection period are shown in Table 3. This data is also graphed in Fig. 3 for low Ca group and in Fig. 5 for high Ca group. In comparison of diets with and without phytate within the same dietary calcium group on fecal ^{65}Zn excretion per day, if a ratio of endogenous ^{65}Zn excretion of phytate group : non-phytate group in the total feces is greater 1, it indicates that phytate was complexed with endogenous zinc through the gastrointestinal tract, and excreted into the feces.

Since the mean ratio of ^{65}Zn excretion in the total feces between phytate group : non-phytate group was higher in the high Ca dietary groups(1.48±0.08) than that of low Ca dietary groups(1.09±0.07) during the initial collection period($p<0.001$), dietary Ca synergized the phytate complexation with zinc. During the crossover collection period, the mean ratio of phytate group : non-phytate group in fecal ^{65}Zn excretion was 1.34±0.14 in low Ca dietary groups and 0.84±0.04 in high Ca dietary groups($p<0.05$). Ratios of phytate group : non-phytate group on fecal ^{65}Zn excretion range from 0.66-1.77 for the low Ca diet and 0.64-1.99 for the high Ca diet.

4. Pattern of Ratios of Phytate Group : Non-phytate Group on Fecal ^{65}Zn Excretion

Low Ca group : The pattern of ratios of phytate

Table 3. Ratio of phytate group : non-phytate group on the ^{65}Zn excretion in the total feces from endogenously labelled rats fed casein-based diet[1-3]

Dietary	Days(initial collection period)														
treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	mean±SD
Low Ca	0.7	1.3	1.3	1.2	1.5	1.1	1.4	1.3	1.1	1.0	0.9	0.8	1.1	0.7	1.09±0.07*
High Ca	1.9	2.0	1.8	1.6	1.3	1.2	1.2	1.0	1.1	1.6	1.4	1.5	1.5	1.7	1.48±0.08*
Dietary	Days(crossover collection period)														
treatment	15	16	17	18	19	20	21	mean±SD							
Low Ca	0.9	1.8	1.6	1.7	1.1	1.4	0.9	1.34±0.14**							
High Ca	0.6	0.9	0.9	0.9	0.8	0.9	0.8	0.84±0.04**							

1) Ratios were based on the ^{65}Zn excretion in the total feces. Fecal samples were collected daily during the whole 3 weeks of the collection period : 2 weeks of the initial collection period and 1 week of the crossover collection period. ^{65}Zn radioactivity was measured at the γ-sintillation counter daily. Ratio of phytate : non-phytate was calculated with the average of the measured radioactivity each day. Ratios greater than 1 indicate that the excreted ^{65}Zn radioactivity was greater in the phytate group than in the non-phytate group.

2) Ratio were rounded to the second decimal point.

3) *$p<0.001$, low Ca group vs. high ca group during the initial collection period. **$p<0.001$, low Ca group vs. high Ca group during the crossover collection period.

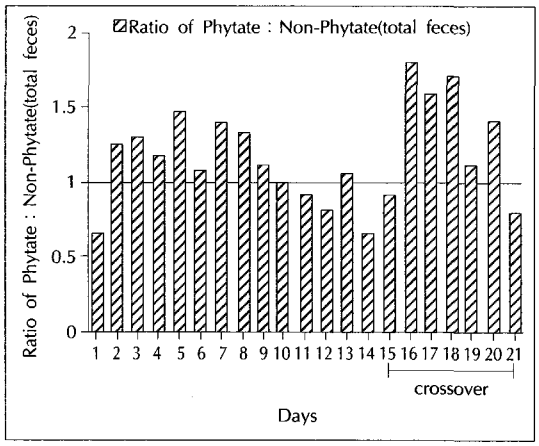

Fig. 3. Ratio(phytate group : non-phytate group) of ^{65}Zn radioactivity daily excreted in the total feces in low calcium group during the whole 3 weeks of the collection period. Each rat feces(12 rats per group) was collected during the collection period in each counting tube at nearly same time daily. ^{65}Zn radioactivity of each sample was measured at γ-scintillation counter. Ratio of phytate group : non-phytate group was graphed with the average of fecal excretion of ^{65}Zn radioactivity for each day. Ratio greater than 1 indicates that endogenous zinc is complexed with the phytate and excreted into the feces.

group : non-phytate group on fecal ^{65}Zn excretion in the total feces per day is shown in Fig. 3. The daily ratio of ^{65}Zn excretion in total feces between phytate group : non-phytate group of was always greater than 1.0 during the initial 14day collection period except day 1(0.7), day 11(0.9), day 12(0.8) and day 14(0.7). During the crossover collection period, the ratio of phytate group : non-phytate group total fecal ^{65}Zn excretion was still greater than 1.0 except on the first(0.9) and last days(0.9). In Fig. 3, the endogenous zinc excreted by phytate group is shown high on the second day and stayed high until the tenth day of the initial collection period. The ratio of radioactive ^{65}Zn excretion was also very high from early in day 2 to late in day 6 during the crossever collection period, in which the phytate and non-phytate diet was switched over within the same low Ca dietary group. Since the ratio of phytate group : non-phytate group endogenous ^{65}Zn excretion in the total feces is greater than 1, these results indicate a definite phytate effect on the endogenous zinc during both the initial and crossover collection period in low Ca dietary groups.

The ratio pattern of phytate group : non-phytate group fecal ^{65}Zn excretion on the basis of gram feces per day in the low Ca groups was graphed in Fig. 4. On the basis of gram feces per day, the adverse phytate effect on the zinc bioavailability was more prominent. Most collection days, except the 13th day, showed a ratio higher than 1. The general pattern of ^{65}Zn excretion on the basis of gram feces per day was similar to the pattern of the ratio in the total feces per day(Fig. 3). During both the initial and crossover collection periods, ^{65}Zn excretion increased from the early days to the middle of the collection period, and then decreased to the end of the collection period.

High Ca group : In the high Ca groups, the pattern of phytate group : non-phytate group of ^{65}Zn excretion in the total feces was graphed in Fig. 5. The daily ratio of phytate group : non-phytate group ^{65}Zn excretion in the total feces was also always greater than 1.0 through out the 2-week initial collection period(1.0-2.0). In the high Ca dietary group, the pattern for 2 weeks of the initial collection period showed that much of the radioactive ^{65}Zn was excreted very early(day 1-day 7) in the initial collection period. There were 6 days out of the 14 days of the initial collection period in which the ratio exceeded 1.5. This was the highest ratio in the low Ca group during the same initial collection period. The ratios in days(day 1, 2, 3, 4, 6, 10, 11, 12, 13, 14) were higher than those

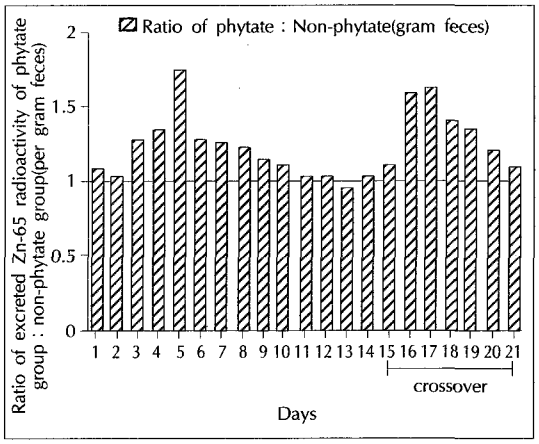

Fig. 4. Ratio(phytate group : non-phytate group) of ^{65}Zn radioactivity excreted daily on a gram basis of feces in low calcium group during the entire 3 weeks of the collection period. The conditions for sample collection and measuremnt are same as on Fig. 4. Ratio greater than 1 indicates that endogenous zinc is complexed with the phytate and excreted into the feces.

in the present study ranged from 6.2-6.8μg/kg. These levels are quite a bit below the normal zinc intake required and that is sufficient to deplete the body zinc pool in rats. [Phytete] : [Zn] molar ratios of 8 or less were equivalent to the dietary zinc source for the normal growth of rats[24]. Therefore, the [phytate] : [Zn] molar ratio of 32.3 in the low Ca, phytate group and of 27.0 in the high Ca, phytate group in this present study, was enough to deplete the body zinc in rats.

In the present study, excretion of endogenous zinc from the second day to the tenth day of the initial collection period in the low Ca dietary group has bean shown to be greater than 1(Fig. 3). Radioactive ^{65}Zn excretion was also very high during the early part of the crossover collection period, from the second day to the sixth day, in the low Ca dietary group during the crossover collection period(Fig. 3). In the high Ca dietary group, an extremely high amount of radioactive ^{65}Zn was secreted from the first day to the seventh day during the initial collection period. After the seventh day, the pancreas may decrease secretion of zinc when homeostatic equilibrium has been achieved, but only until the ninth day. On this day a large amount of endogenous zinc would be excreted until the end of the initial collection period(Fig. 5). During days that show a ratio higher than 1, the pancreas may serve an important function in the maintenance of zinc homeostasis by excreting excess zinc. This secreted endogenous zinc in both Ca dietary groups, appears to be as vulnerable to phytate binding as endogenous zinc and dietary zinc in the gastrointestinal tract, where the pH is about 6 and the phytate-zinc complex is the most insoluble[8]. Some amount of endogenous zinc secreted from the pancreas is to be excreted for the maintenance of zinc homeostasis. This excreted endogenous zinc complexes with the phytate. At this time, the phytate effect might be considered as a homeostatic adjustment rather than zinc bioavailability itself through the phytate-endogenous zinc complex, which is insoluble and thus excreted into the feces[25-26].

It has been suggested that there were two endogenous pools, one in which the zinc complexes formed in the pancreas are quite stable and not affected by phytate. In this stable pool, the zinc is tightly bound, probably to carboxypeptidase A or carboxypeptidase B and other zinc-dependent enzymes. The second pool is labile and thus is affected by phytate. In the labile pool, zinc is loosely bound and subject to binding by phytate. The labile zinc pool is depletable, while the stable pool continues to secrete zinc after the labile pool is exhausted[27]. Once zinc homeostasis is established, small amounts of zinc continue to be secreted from the pancreas into the gastrointestinal tract. Some of the endogenous zinc is vulnerable to complexing with phytate. As the molar ratio of [phytate] : [Zn] in the diet increases, more zinc is depleted from the endogenous pool. In this case, the complexed zinc is primarily from the labile endogenous pancreatic pool. The pattern of ^{65}Zn excretion in the high calcium dietary group during the crossover collection period, which shows ratios below 1 between phytate group : non-phytate group, may be explained with the presence of this labile zinc pool. With a synergistic calcium effect on the phytate-zinc complex, the labile zinc pool was almost depleted until the initial collection period. The exact size of the two zinc pools has not been determined. However, the labile pool is somewhat larger, than the stable pool. Once the labile zinc pool is depleted, the phytate effect on endogenous zinc might not be apparent. In this case, during the crossover collection period in the high Ca dietary group, the ratios of phytate group : non-phytate group fecal ^{65}Zn excretion might not reflect the effect of phytate as acutely as in the initial collection period. That shows the ratios of phytate group : non-phytate group fecal ^{65}Zn excretion below 1.

Shinoda and Yoshida(1989)[8] reported that 1% sodium phytate added to a casein-based diet, which is under the same diet condition of the present study, insolubilized zinc in the rat's digestive tract in which both dietary and endogenous zinc were present. Flanagan(1984)[27] reported that phytate reduced the reabsorption of endogenous zinc secreted into the intestinal tract. Sodium phytate, added to a tube-fed diet to rats fed a deficient diet(1.0 ± 0.4 ppm) and then supplemented with zinc(48.5 ± 4.2 ppm), significantly increased the zinc content in luminal washings and fecal zinc excretion. This zinc increase in luminal washings and fecal zinc excretion resulted from interfering with endogenous zinc reabsorption. Dietary phytate increased the excretion of endogenous

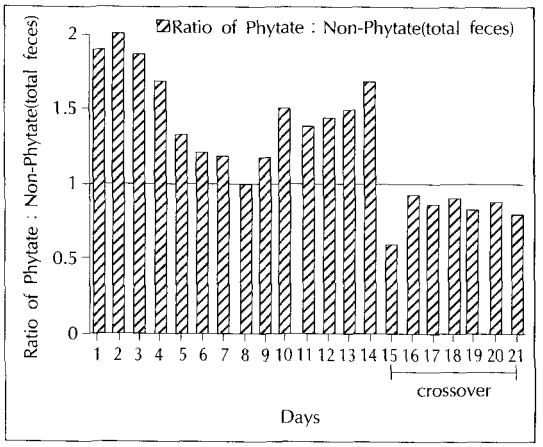

Fig. 5. Ratio(phytate group : non-phytate group) of ^{65}Zn radioactivity excreted daily in the total feces in high calcium group during the entire 3 weeks of the collection period. Each rat feces(12 rats per group) was collected during the collection period in counting tube at nearly same time daily. ^{65}Zn radioactivity of each sample was measured by γ-scintillation counter. Ratio of phytate group : non-phytate group was graphed with the average of fecal excretion of ^{65}Zn radioactivity for each day. Ratios greater than 1 indicate that endogenous zinc is complexed with phytate and excreted into feces.

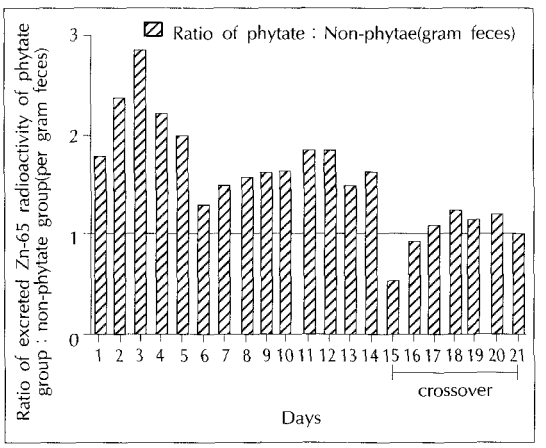

Fig. 6. Ratio(phytate group : non-phytate group) of ^{65}Zn radioactivity excreted daily on a gram basis of feces in high calcium group during the entire 3 weeks of the collection period. The conditions for sample collection and measurement are same as for Fig. 5. Ratios greater than 1 indicate that endogenous zinc is complexed with phytate and excreted into the feces.

of the low Ca dietary treatment during the initial collection period(Table 3). Compared to the pattern of low Ca dietary groups, these results also strongly support the fact that calcium accentuates the phytate effect on endogenous zinc as the higher calcium does on dietary zinc. This binding of phytate to zinc increases the endogenous zinc excretion. However, in the high Ca group, the ratios of phytate group : non-phytate group were below 1(0.6-0.9) through out the 7 days of the crossover collection period, which implies the presence of a labile endogenous zinc pool and calcium's synergistic effect on that pool. This result will be discussed for the presence of the labile endogenous zinc pool in discussion.

The ratio pattern of phytate group : non-phytate group fecal ^{65}Zn excretion on the basis of gram feces per day in the high Ca groups is graphed in Fig. 6. The general pattern of ^{65}Zn excretion on the basis of gram feces per day is similar to the pattern of the ratio in the total feces per day(Fig. 5). The whole initial collection period showed a ratio higher than 1. During the crossover collection period, three days(day 15, 16, 21) showed a ratio less than 1 for fecal ^{65}Zn excretion between phytate group : non-phytate group. In Comparison to the pattern of ^{65}Zn excretion in the total feces(Fig. 5), in which the whole crossover collection periold showed a ratio less than 1, even fewer days showed a ratio below 1. However, about half of the collection days still showed a ratio below 1.

Discussion

The effect of phytate(molecular weight of the phytate ion, 666) on zinc(atomic weight, 65.4) bioavailability is best defined by the phytate : zinc molar ratios in the food or diet. Even though the phytate has potentially twelve dissociable hydrogens and could theoretically combine with six divalent cations or has a phytate : cation molar ratio of 1 : 6, only eight of the protons are actually titratable. Therefore, phytate can complex a maximum of four divalent cations per molecule[2]. Thus, there is considerably more complexing capacity in dietary phytate than is necessary to produce zinc deficiency. The inhibitory effect of phytate in the diet may function through the formation of zinc-ligand complexes, which are either insoluble[5-6)9)] or which reduce the binding of zinc to zinc's mucosal receptor[23)]. About 30μg Zn/kg diet(AIN : American Institute of Nutrition), or at least 12μg Zn/kg diet[24)], are considered as necessary for optimal growth in rats. Zinc concentration in the four dietary groups

in the present study ranged from 6.2-6.8μg/kg. These levels are quite a bit below the normal zinc intake required and that is sufficient to deplete the body zinc pool in rats. [Phytete] : [Zn] molar ratios of 8 or less were equivalent to the dietary zinc source for the normal growth of rats[24]. Therefore, the [phytate] : [Zn] molar ratio of 32.3 in the low Ca, phytate group and of 27.0 in the high Ca, phytate group in this present study, was enough to deplete the body zinc in rats.

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zinc and decreased the reabsorption of endogenous zinc, contributing to maintenance of zinc homeostasis. In the present study, the excretion of radioactive fecal endogenous zinc was higher with phytate group than without phytate in both Ca test groups, except during the crossover collection period in the high Ca group. This result clearly shows that the phytate has an adverse effect on endogenous zinc as wall as on exogenous zinc.

However, House et al.(1982)[29] reported that endogenous zinc was not affected by phytate in a four-day fecal collection experiment with rats. Mills(1985)[30] also reported that no differences were observed in effects of added phytate and calcium on endogenous or exogenous zinc. House et al.(1982)[29] reported that whole-body retention of ^{65}Zn was higher, and endogenous fecal zinc excretion was lower in rats fed phytate than in those fed the basal diet. They explained that these responses to phytate might reflect a homeostatic adjustment of decreased absorption of zinc. The [phytate] : [Zn] molar ratio of their diet was 60 : 1. However, since equilibrium with endogenous zinc did not occur until the 10th day in the low Ca group(Fig. 3) and the 8th day in the high Ca group(Fig. 5) after ^{65}Zn injection in the present study, a four-day fecal collection might be too short a period to examine the phytate effect on endogenous zinc.

Since the detrimental effect of phytate can be accentuated by elevated levels of dietary calcium, and the [phytate] : [Zn] molar ratio is a simple mathematical representation of equilibrium between phytate and Zn, the net replacement value of the Zn for homeostasis is represented by [phytate] : [Zn] or [phytate]×[Ca] : [Zn] molar ratios. If 10 or more molecules of phytate are consumed in the diet, one or more atoms of zinc from the dietary and endogenous zinc pool are restrained from being absorbed or reabsorbed[4,31-33]. The ratio does not, however, take into account the significant synergistic influence of the dietary intake of calcium on phytate complexing action with zinc. Even [phytate] : [Zn] molar ratios greater than 5 from bran-and cereal-based meals showed low zinc absorption in man[13]. On the other hand, Morris and Ellis(1980)[33] emphasized much more the [phytate]×[Ca] : [Zn] molar ratio to determine zinc bioavailability. Their explanation was that an increasing dietary zinc concentration sufficiently overcame the growth depressing effects of phytate. They reported that the maximum [phytate] : [Zn] molar ratio that did not depress the growth of young rats was greatly influenced by dietary calcium levels and somewhat influenced by total dietary zinc concentrations. Since calcium potentiates the negative effect of phytate on zinc bioavailability, Fordyce et al.(1987)[14] and Davies et al.(1975)[34] also have suggested zinc availability can be more accurately predicted by using the molar ratio of [phytate]×[Ca] : [Zn] rather than [phytate] : [Zn]. The molar ratios of [phytate]×[Ca] : [Zn], 6.3 for the low Ca, phytate diet, and 12.3 for the high Ca, phytate diet, show higher ratios for the adverse zinc nutrition in the present study. The amount of zinc precipitated by phytate at pH 6.0 was much greater if calcium was present. By increasing the dietary calcium content at a fixed [phytate] : [Zn] molar ratio, a profound decrease in growth rate in rats has been reported[8,35],

In conclusion, since mean fecal ^{65}Zn radiocativity was higher in the phytate group than in the non-phytate group($p < 0.0001$) and ratios of phytate group : non-phytate group of the ^{65}Zn excretion in the total feces were above 1 for both low calcium and high calcium diets, phytate decreases the reabsorption of endogenous zinc. Since the ratio of phytate group : non-phytate group ^{65}Zn radioactivity excretion are higher in high Ca groups than in low Ca groups($p<0.0001$), dietary calcium synergized the phytate precipitation of endogenous zinc. Since reabsorption of endogenously secreted zinc is essential for the maintenance of zinc homeostasis, the results of this study suggest that the phytate effect on endogenous zinc is more important for the zinc homeostasis and bioavailability, because the endogenous zinc pool is bigger than the dietary zinc pool for one day.

However, the exact site or sites of secretion and the vulnerability of secreted zinc to precipitation by phytate or other complexing agents has not been fully studied. Those questions would be further research topics for a full understanding of zinc homeostasis.

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