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ORIGINAL ARTICLE

Effect of Sodium Phytate and Phytin on the Absorption and Organ Concentration of Several Minerals in Rats

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Summary

The effects of sodium phytate or phytin administration on several mineral utilizations in rats were examined. Eighteen male 4-week-old Wistar/ST rats were divided into 3 groups. One group (control group) was fed a basal AIN93G diet, the other two groups (SP group and phytin group) were fed the basal diet containing sodium phytate (1.0wt%) or rice bran-derived phytin (1.0wt%), respectively, for 4 weeks. Because the phytin used contained magnesium, zinc, and manganese, the phytin group consumed more of these minerals than the other two groups. The SP group excreted more magnesium in their feces and had a lower apparent absorption than the control group, which had a similar magnesium intake. In addition, the SP group had lower zinc concentrations in the serum and several organs than the control group. There was also a trend toward lower serum and organ zinc concentrations in the phytin group compared to the control group, but the only significant difference was in the femoral zinc concentration. The phytin group had lower apparent iron absorption and lower serum and organ iron concentrations than the other two groups. The serum phosphorus concentrations and urinary phosphorus excretion were increased in the SP and phytin groups. These results indicated that 1) phytic acid inhibits the absorption of magnesium and zinc, 2) minerals bound to phytin, possibly manganese, inhibit iron absorption, 3) phytic acid is partially hydrolyzed in the digestive tract, phosphoric acid and the minerals bound to phytic acid are released and utilized.

Introduction

Phytic acid is a hexaphosphate ester of myoinositol, which is abundant in beans and cereals and is commonly found as an insoluble mineral-mixed salt also called phytin[1]. The Southeast Asian diet, based on plant-based products, tends to have a high intake of phytic acid[2]. For decades, phytic acid has been considered an anti-nutritional factor because it forms insoluble salts with divalent metal cations and affects the absorption of minerals in the small intestine[3]. It is believed that phytic acid intake particularly affects zinc absorption, as severe growth suppression due to zinc deficiency was commonly observed in an Iranian village people that consumed whole grain bread with high concentrations of phytic acid and no animal protein at all[4]. Furthermore, calcium and iron deficiencies also have been reported to be induced by the intake of phytic acid[5,6].

On the other hand, there has been growing interest in exploring the health advantages of phytic acid administration in recent years. Several studies have reported that phytic acid intake has powerful prevention and treatment on different symptoms, including Alzheimer's disease[7], enterocolitis[8], hyperlipidemia[9], and hyperuricemia[10] in human and animal models. As a result, phytic acid is expected to have great potential for improving human health and preventing diseases.

However, there are two types of studies on phytic acid, one using water-soluble sodium phytate and the other using insoluble phytin. Accordingly, the adverse or beneficial health effects of phytic acid is difficult to be interpreted uniformly at present. Therefore, in order to evaluate phytic acid nutritionally, it is necessary to conduct experiments using different forms of phytic acid simultaneously.

In this study, we performed an experiment in which rats were administered sodium phytate as a soluble phytate or phytin as an insoluble phytate and the organ accumulation and balance of several minerals were measured.

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Materials and methods

Animal feeding

The experimental protocol followed the Guide for the Care and Use of Experimental Animals issued by the Prime Minister's Office of Japan and approved by the Animal Ethics Committee of Kansai University (Approval No. 2113).

Eighteen 4-week-old male Wistar/ST rats (SHIMIZU Laboratory Supplies Co., Kyoto) were divided into 3 groups. One group (control group) was fed a basal diet prepared according to the AIN93G formulation[11], and the other two groups (SP group and phytin group) were fed the basal diet containing 1.0 wt% sodium phytate (Sigma-Aldrich, St. Louis) or 1.0 wt% phytin (Tokyo Chemical Industry, Tokyo), respectively. The animals were allowed to access tap water and the experimental diet ad libitum.

Table 1 summarizes the actual mineral content of the diets administered to each group. The phytin used in this study was sold as calcium phytate derived from rice bran, but the analysis showed that it contained no calcium, but 20.0% phosphorus, 11.7% magnesium, 0.1% zinc, and 0.2% manganese. Thus, the diet fed to the phytin group contained higher concentrations of magnesium, zinc, and manganese than the other diets. In addition, the SP and phytin groups also received higher doses of phosphorus than the control group due to the phytate-derived phosphorus.

After 20 days of feeding, each rat was housed separately in a metabolic cage (Natsume Seisakusho Co., Ltd., Osaka), and all feces and urine were collected every 48 hours. One mL of 1M HCl was added to the flasks which were used to collect the urine. The food and water consumption as well as body weight were recorded every 2 days.

On day 28, the rats were weighed and sacrificed under isoflurane (Fujifilm Wako Pure Chemical Co., Tokyo) anesthesia. The liver, kidney, spleen, and femur were removed, then weighed, rinsed with cold saline, and frozen in liquid nitrogen. Blood was collected from the abdominal aorta, and serum was obtained by centrifugation at 1,500 × g for 15 min. All the specimens were stored at -30℃ until analysis.

Analysis

Approximately 1 g of liver, kidney, spleen, femur, experimental diets, and 1 mL of serum were heated with 5 mL of nitric acid until there were no solids. The obtained solution was diluted with pure water and filtrated through a 0.45 μm filter. The feces samples were freeze-dried overnight and then ground into a mill. The feces powder was partially weighed in crucibles and then heated in an electric furnace at 500℃ for 16 hours. The ashes obtained were dissolved in 0.1M nitric acid and filtrated through a 0.45 μm filter. The urine samples were centrifuged at 20,000 x g for 5 min and the supernatant obtained was used for analysis.

The contents of calcium, magnesium, iron, zinc, manganese, and copper in the solutions were determined using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto) or an inductively coupled plasma mass spectrometer (ICPMS-2030, Shimadzu, Kyoto). For the determination of calcium and magnesium, lanthanum chloride solution was added to a final concentration of 3000 ppm to eliminate the interference from coexisting phosphorus. In the analysis by ICPMS, ^{115}indium was used as an internal standard. The inorganic phosphorus determination was performed using the vanadomolybdate method[12].

It is difficult to avoid contamination of a very small amount of the experimental diets when collecting the urine. Therefore, only the concentrations of calcium, phosphorus, and magnesium were measured because urine is the main excretion route and the effect of dietary contamination is negligible.

Table 1 Measured mineral concentrations (μg/g) of the experimental diets administered to each group

Minerals	Control group	SP group	Phytin group
Calcium	4980 ± 172	4894 ± 48	5048 ± 37
Magnesium	525 ± 30	512 ± 11	1694 ± 28
Phosphorus	3158 ± 107	5114 ± 33	5156 ± 121
Iron	37.1 ± 0.1	41.9 ± 0.2	38.9 ± 1.2
Zinc	41.3 ± 1.0	43.8 ± 1.5	50.9 ± 1.7
Copper	6.0 ± 0.2	6.3 ± 0.5	6.3 ± 0.8
Manganese	8.9 ± 2.5	9.4 ± 0.6	30.5 ± 3.5

Values are means ± SD of triplicate measurements.

Statistical analysis

The statistical differences among the groups were evaluated by Tukey's honestly significant difference test after one-way analysis of variance (ANOVA). SPSS Statistics 27 for windows (IBM Japan, Ltd., Tokyo) was used as the statistical analysis application.

Results

Table 2 shows the body weight, feed intake and water consumption in each group. After 4 weeks of feeding, the weight gain of the rats in each group was almost equal, and the addition of sodium phytate or phytin to the diet did not affect the growth of the rats.

Table 3 summarizes the balance calculated from fecal and urinary excretion for several minerals. For calcium, there were no differences among the three groups for all parameters.

For magnesium, the apparent absorption rate was lower in the phytin group, which had a very high intake, than in the other two groups. On the other hand, when the SP group was compared to the control group with equal magnesium intake, the SP group had higher fecal excretion and lower amounts of apparent absorption. Thus, although there was a difference in apparent absorption among the groups, there was no difference in the final retention amounts among the three groups because urinary excretion was higher in the phytin group, the control group, and the SP group, in that order.

Regarding phosphorus, due to the phytic-acid-derived phosphorus, the SP and phytin groups, which had a higher intake than the control group, had a higher apparent absorption, but these two groups also had higher urinary excretion, and there was no difference among the three groups in the final amount of phosphorus retained.

For iron, fecal excretion was higher in the phytin group, and the amount and rate of apparent absorption was lower than in the other two groups. Although the intake of zinc was higher in the SP and phytin groups than in the control group, the apparent absorption amounts of zinc was not different among the three groups because the fecal excretion was also higher in these groups. For copper, no difference in apparent absorption was observed among the three groups. For manganese, there was no difference in the apparent absorption among the three groups because the phytin group, which had a higher intake, also excreted more manganese in their feces.

Table 4 shows the mineral concentrations in the serum and organs of each group. For calcium, the SP group showed higher levels in the kidneys. For magnesium, there was no difference in the serum and organ concentrations among the three groups. For phosphorus, the serum concentrations in the SP and phytin groups were significantly higher than in the control group. In addition, the liver and spleen concentrations were higher in the phytin group than in the other two groups.

For iron, the phytin group had significantly lower concentrations in the serum, liver, and spleen than the other two groups. The zinc concentration in the SP group was significantly lower than that in the control group in the serum, kidney, spleen, and femur. The phytin group also tended to show lower values than the control group, and there was a significant difference in the concentration in the femur. For copper, the serum levels in the SP and phytin groups were significantly lower than in the control group. For manganese, there was no difference in the serum and organ concentrations among the three groups.

Discussion

As mentioned in the materials and methods section, the rice-bran-derived phytin used in this study contained magnesium, zinc, and manganese, not calcium, even though it was clearly labeled calcium phytate. To separate phytin from rice bran, phytic acid has been extracted from rice bran with acid, and ethanol, magnesium oxide, calcium chloride, etc. are added to the resulting acid extract solution to recover phytin as a precipitate[13]. When calcium is added, phytin is recovered as calcium phytate, but calcium phytate is not formed in the other methods. The weight ratio of phosphorus, magnesium, zinc, and manganese in the phytin used in this study (200:117:1:2) is close to the weight ratio of these minerals in rice bran (200:85:0.6:1.5)[14]. In other words, the phytin used in this

Table 2 Body weights, feed intake and water consumption of each group

	Control group	SP group	Phytin group
Body weight (g)	281.5 ± 4.5^{a}	299.1 ± 5.0^{b}	294.1 ± 4.5^{ab}
Feed intake (g/d)	16.7 ± 0.3	17.3 ± 0.2	17.8 ± 0.6
Water consumption (mL/d)	18.1 ± 1.2	18.9 ± 0.85	17.2 ± 1.0

Values are means ± SEM (n=6). Means in the same row not sharing a common superscript differ significantly ($p < 0.05$). No significant differences were observed among groups for the items without a superscript in the mean.

Table 3 Balance of several minerals in rats fed the experimental diets

	Control group	SP group	Phytin group
Diet intake (g/d)	21.8 ± 0.4	23.4 ± 0.4	23.2 ± 1.0
Calcium			
Intake (mg/d)	108.7 ± 2.0	114.5 ± 2.1	116.9 ± 5.2
Fecal excretion (mg/d)	48.6 ± 2.2	52.7 ± 1.2	56.2 ± 4.4
Apparent absorption (mg/d)	60.1 ± 1.8	61.8 ± 1.4	60.7 ± 3.4
Apparent absorption (%)	55.4 ± 1.7	54.0 ± 0.8	52.1 ± 2.6
Urinary excretion (mg/d)	0.85 ± 0.11	0.60 ± 0.03	0.87 ± 0.12
Retention (mg/d)	59.3 ± 1.8	61.2 ± 1.5	59.8 ± 3.4
Retention (%)	54.6 ± 1.6	53.4 ± 0.8	51.4 ± 2.6
Magnesium			
Intake (mg/d)	11.46 ± 0.20^{a}	11.98 ± 0.19^{a}	39.2 ± 1.6^{b}
Fecal excretion (mg/d)	3.40 ± 0.15^{a}	5.30 ± 0.14^{b}	23.0 ± 1.6^{c}
Apparent absorption (mg/d)	8.06 ± 0.17^{b}	6.68 ± 0.17^{a}	16.3 ± 1.3^{c}
Apparent absorption (%)	70.4 ± 1.1^{c}	55.7 ± 1.0^{b}	41.5 ± 3.1^{a}
Urinary excretion (mg/d)	5.46 ± 0.29^{b}	4.43 ± 0.12^{a}	11.5 ± 0.8^{c}
Retention (mg/d)	2.60 ± 0.27	2.24 ± 0.14	4.72 ± 1.93
Retention (%)	22.8 ± 2.5	18.7 ± 1.1	11.6 ± 4.7
Phosphorus			
Intake (mg/d)	68.9 ± 1.1^{a}	119.7 ± 2.2^{b}	119.4 ± 4.2^{b}
Fecal excretion (mg/d)	18.0 ± 1.0^{a}	38.5 ± 0.5^{b}	45.9 ± 3.3^{b}
Apparent absorption (mg/d)	50.9 ± 1.0^{a}	81.2 ± 1.8^{b}	73.5 ± 3.8^{b}
Apparent absorption (%)	70.4 ± 1.1^{c}	67.8 ± 0.6^{b}	61.6 ± 2.0^{a}
Urinary excretion (mg/d)	15.1 ± 1.0^{a}	41.8 ± 1.1^{c}	29.9 ± 1.4^{b}
Retention (mg/d)	35.8 ± 1.4	39.4 ± 1.1	43.6 ± 3.6
Retention (%)	52.0 ± 2.0^{b}	32.9 ± 0.6^{a}	38.5 ± 2.5^{a}
Iron			
Intake (μg/d)	810 ± 15^{a}	980 ± 15^{b}	901 ± 38^{b}
Fecal excretion (μg/d)	610 ± 31^{a}	731 ± 11^{ab}	782 ± 49^{b}
Apparent absorption (μg/d)	200 ± 17^{b}	249 ± 11^{b}	119 ± 38^{a}
Apparent absorption (%)	24.8 ± 2.3^{b}	25.4 ± 0.8^{b}	13.4 ± 4.2^{a}
Zinc			
Intake (μg/d)	901 ± 16^{a}	1025 ± 16^{b}	1179 ± 49^{c}
Fecal excretion (μg/d)	615 ± 32^{a}	717 ± 10^{ab}	894 ± 60^{b}
Apparent absorption (μg/d)	286 ± 23	318 ± 11	285 ± 49
Apparent absorption (%)	31.8 ± 2.7	31.0 ± 0.9	24.2 ± 3.8
Copper			
Intake (μg/d)	131 ± 2^{a}	147 ± 2^{b}	146 ± 6^{b}
Fecal excretion (μg/d)	117 ± 4	123 ± 3	125 ± 8
Apparent absorption (μg/d)	14 ± 4	24 ± 2	21 ± 7
Apparent absorption (%)	10.9 ± 3.1	16.3 ± 1.3	14.4 ± 4.4
Manganese			
Intake (μg/d)	194 ± 4^{a}	220 ± 3^{a}	706 ± 30^{b}
Fecal excretion (μg/d)	172 ± 10^{a}	191 ± 10^{a}	624 ± 41^{b}
Apparent absorption (μg/d)	22 ± 6	29 ± 2	82 ± 33
Apparent absorption (%)	11.6 ± 3.2	13.3 ± 0.8	11.5 ± 4.3

Values are means ± SEM (n=6). Means in the same row not sharing a common superscript differ significantly ($p < 0.05$). No significant differences were observed among groups for the items without a superscript in the mean.

Table 4 Mineral concentrations in the serum and organs of rats fed the experimental diets

	Control group	SP group	Phytin group
Calcium			
Serum (mg/dL)	10.3 ± 0.1	10.5 ± 0.1	10.6 ± 0.1
Liver (μg/g)	44.1 ± 0.3	44.7 ± 1.5	44.4 ± 1.0
Kidney (μg/g)	79.8 ± 0.8^{ab}	84.2 ± 3.9^{b}	74.2 ± 1.1^{a}
Spleen (μg/g)	45.6 ± 1.2	49.2 ± 1.5	49.7 ± 1.2
Magnesium			
Serum (mg/dL)	1.83 ± 0.02	1.78 ± 0.02	1.83 ± 0.02
Liver (μg/g)	194 ± 1	199 ± 5	203 ± 1
Kidney (μg/g)	194 ± 2	191 ± 2	195 ± 2
Spleen (μg/g)	204 ± 2	201 ± 1	207 ± 2
Phosphorus			
Serum (mg/dL)	7.5 ± 0.2^{a}	8.6 ± 0.1^{b}	8.5 ± 0.2^{b}
Liver (mg/g)	1.02 ± 0.07^{a}	1.08 ± 0.08^{a}	1.75 ± 0.18^{b}
Kidney (mg/g)	1.47 ± 0.04	1.50 ± 0.04	1.57 ± 0.11
Spleen (mg/g)	0.52 ± 0.02^{a}	0.44 ± 0.03^{a}	0.68 ± 0.02^{b}
Femur (mg/g)	27.1 ± 1.4	28.9 ± 1.3	31.4 ± 2.7
Iron			
Serum (μg/dL)	190 ± 11^{ab}	215 ± 11^{b}	168 ± 15^{a}
Liver (μg/g)	62.7 ± 4.6^{b}	63.9 ± 5.9^{b}	39.4 ± 5.9^{a}
Kidney (μg/g)	43.1 ± 1.7	41.7 ± 0.6	40.6 ± 2.2
Spleen (μg/g)	147 ± 7^{b}	170 ± 8^{b}	113 ± 5^{a}
Femur (μg/g)	35.1 ± 1.2	31.7 ± 1.3	29.0 ± 2.3
Zinc			
Serum (μg/dL)	100 ± 5^{b}	64 ± 2^{a}	85 ± 7^{b}
Liver (μg/g)	21.7 ± 0.8	22.1 ± 1.0	24.8 ± 0.6
Kidney (μg/g)	26.4 ± 0.7^{b}	22.8 ± 0.5^{a}	24.2 ± 0.8^{ab}
Spleen (μg/g)	18.5 ± 0.2^{b}	17.1 ± 0.3^{a}	17.6 ± 0.5^{ab}
Femur (μg/g)	124 ± 2^{b}	97 ± 3^{a}	109 ± 5^{a}
Copper			
Serum (μg/dL)	95 ± 4^{b}	80 ± 3^{a}	77 ± 1^{a}
Liver (μg/g)	3.60 ± 0.10	3.39 ± 0.20	3.66 ± 0.22
Kidney (μg/g)	10.7 ± 1.2	11.5 ± 1.2	10.1 ± 1.9
Spleen (μg/g)	0.65 ± 0.04	0.54 ± 0.04	0.62 ± 0.03
Manganese			
Serum (μg/dL)	0.89 ± 0.03	0.78 ± 0.03	0.87 ± 0.04
Liver (μg/g)	2.27 ± 0.11	2.10 ± 0.08	2.37 ± 0.06
Kidney (μg/g)	0.97 ± 0.02	0.95 ± 0.01	1.00 ± 0.01
Spleen (μg/g)	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.01

Values are means ± SEM (n=6). Means in the same row not sharing a common superscript differ significantly ($p < 0.05$). No significant differences were observed among groups for the items without a superscript in the mean.

study is considered to reflect the mineral composition of the phytin in rice bran, although the details of the isolating process are unknown.

Thus, in this experiment, the magnesium, zinc, and manganese doses were higher in the phytin group than in the other two groups. In addition, the phosphorus doses were higher in the SP and phytin groups due to phosphorus derived from phytic acid. Since the absorption rate of minerals generally decreases with increasing dosage, in this experiment, it was difficult to determine the effect of phytic acid on mineral absorption based on the apparent absorption rate alone, so the amount excreted in urine and the amount accumulated in the serum and organs were also used to determine the effect.

Nevertheless, in the case of magnesium, it is clear that phytic acid inhibited magnesium absorption, as the appar-

ent absorption in the SP group was significantly lower than in the control group with approximately equal intake (Table 3). Thus, phytic acid inhibited magnesium absorption, but there were no differences in the magnesium retention or organ concentrations among the three groups (Table 4). This means that when magnesium is supplied in sufficient amounts, as in the case of the AIN93G diet, magnesium homeostasis in the body is sufficiently maintained even when magnesium absorption is suppressed by phytic acid.

In the SP and phytin groups, femur zinc concentrations were significantly lower than in the control group (Table 4). We have observed that among serum and organs, bone zinc concentration is the most sensitive to change in dependence on decreased zinc absorption[15]. Although no clear difference was observed in the balance study (Table 3), these decreased femur concentrations suggest that phytic acid inhibited zinc absorption. In the balance study, no clear difference could be detected because the SP group with decreased zinc status is thought to have decreased endogenous fecal excretion, and the difference between the apparent and true absorption is larger than in the control group. The milder decrease in the zinc status in the phytin group may be due to some utilization of the zinc that was bound to phytin.

Furthermore, the serum copper concentrations were significantly lower in the SP and phytin groups than in the control group. This suggests that phytic acid may inhibit the absorption of copper in addition to magnesium and zinc.

For iron, the apparent absorption and serum and organ concentrations were lower only in the phytin group than in the control group (Tables 3 and 4). Since such a decrease was not observed in the SP group, the decreased iron utilization in the phytin group was most likely due to the effects of magnesium, zinc, and manganese bound to phytin. In this connection, it is known that the divalent metal transporter 1 (DMT1) acts on divalent iron ions in addition to transporting manganese ions for uptake into the small intestine mucosal cells[16]. It could be supposed that the high amount of manganese contained in the ingestion of phytin may exacerbate the antagonistic effect of iron and manganese when bound to DMT1, leading to a decrease in iron absorption. A similar result, leading to reduced iron accumulation in organs when high manganese diets are administered to growing rats, has also been reported[17].

In the phosphorus analysis, we observed an increase in the serum inorganic phosphorus levels in the SP and phytin groups (Table 4) and a significant increase in the release of phosphorus from urine (Table 2). It indicates that phytic acid was partially hydrolyzed and releases inorganic phosphorus in the intestinal tract. In addition, magnesium and zinc bound to phytin were also presumed to be partially absorbed. These results indicate the chelating ability of metal ions is much lower after phytic acid hydrolysis[5], which facilitates the utilization of minerals in the intestine. However, the utilization of phytate in rats has not been well-understood. One study reported that phytin phosphorus utilization was similar to inorganic phosphorus when the vitamin D intake was adequate[18]. Another study has noted that phytic acid is hydrolyzed in the cecum and colon of rats by the gut flora[19]. Furthermore, a study on phytase in the rat small intestine indicated that the phytase activity was active in the duodenum yet still insufficiently hydrolyzed phytic acid[20]. This suggests that the hydrolysis of phytic acid in the gastrointestinal tract needs to be discussed further in future studies. The hepatic and splenic phosphorus levels were significantly increased only after rats were administrated phytin. The exact reason for this is unknown and it might be since the excessive magnesium intake affects the Na^+-K^+ dynamics in the blood, which in turn affects the amount of Na-dependent phosphate transporters located in the liver, resulting in increased phosphorus accumulation in the organs.

In the present experiment, phytic acid clearly inhibited the absorption of magnesium and zinc, and may also have affected the absorption of copper. However, there was no effect on calcium absorption, which has been pointed out in the past[21]. In other words, phytic acid may affect the absorption of magnesium, but not calcium. The effect on iron was observed only when phytin, which is phytic acid bound to magnesium, zinc, and manganese, was administered. The diversity in the results of studies examining the relationship between phytic acid and mineral absorption may be due to the diversity in the mineral composition of the phytic acid used.

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