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Effect of Phytic Acid Administration on the Zinc concentration, Uric Acid Biosynthesis, and Serum Lipid Components in Rats

Ziwen Jin, Ryota Hosomi, Kenji Fukunaga, Munehiro Yoshida*

Laboratory of Food and Nutritional Sciences, Faculty of Chemistry, Materials and Bioengineering, Kansai University

Summary

The effects of phytic acid on the absorption of several minerals including zinc, iron, copper, calcium, magnesium, manganese and molybdenum, the biosynthesis of uric acid, and the serum lipid components were examined. Weaning male Wistar rats were fed a basal AIN93G diet or the basal diet supplemented with 0.5% or 1.0% sodium phytate for 4 weeks. Phytic acid administration reduced the serum and femur zinc levels in a dose-dependent manner but did not affect the liver and kidney zinc levels. In addition, significant reductions with phytic acid administration were observed in the liver iron, serum and liver copper, liver and kidney calcium, kidney magnesium, and liver and kidney manganese concentrations. In phytic-acid-administered rats, the molybdenum concentration and xanthine oxidase activity in the liver and the serum uric acid decreased in a dose-dependent manner. In addition, phytic acid administration also reduced the serum lipid components including triacylglycerol and total cholesterol. Since phytic acid is hardly absorbed, these results indicate that phytic acid inhibits the absorption of several dietary components including minerals, and that the decrease in the serum uric acid concentration that occurs when phytic acid is ingested is due to the decrease in molybdenum absorption. When utilizing the functionality of phytic acid for health promotion, it is necessary to pay sufficient attention to the intake of minerals.

Key words: phytic acid, zinc, uric acid, molybdenum, xanthine oxidase, serum lipid components

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*Correspondence:
Yamate 3-3-35, Suita, Osaka 564-8680, Japan
Tel: 81-6-6368-0970 (cell phone number: +81-90-9990-1853)
E-mail: hanmyou4@kansai-u.ac.jp

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Introduction

Phytic acid (inositol hexaphosphate) is a major phosphorus storage form present in plant tissues, such as seeds, and is often present as phytin, which is a mixed salt of calcium and magnesium [1]. Therefore, a high intake of beans and unrefined grains leads to a high intake of phytic acid. Phytic acid strongly chelates to many metal ions, which may inhibit their intestinal absorption [2]. In particular, it was believed that a large intake of phytic acid causes zinc deficiency, as Egyptian boys who had growth inhibition due to zinc deficiency ate whole grain breads

high in phytic acid on a daily basis [3]. However, zinc deficiency caused by phytic acid occurs only in the case of insufficient zinc intake, and it is not necessary to worry about zinc deficiency associated with phytic acid when the zinc intake is sufficient [4].

In recent years, there have been increasing reports that phytic acid intake has a positive effect on maintaining good health [5]. Epidemiological studies and animal experiments have shown that phytic acid is effective in preventing neurodegenerative diseases [6], renal stones [7], several types of cancer [8-10], and fatty liver [11]. Furthermore, it has been reported that phytic acid lowers the serum uric acid concentration [12, 13].

Thus, phytic acid is now expected to be a functional ingredient that has a positive effect on health, rather than an antitrophic factor that inhibits mineral absorption. However, many studies examining the effects of phytic acid intake are one-sided, and few examine both positive and unfavorable effects at the same time. In this study, in order to re-evaluate the health effects of phytic acid, phytic acid was administered to rats, and the effects on the concentrations of several minerals including zinc, as well as the serum uric acid and lipid components were examined at the same time. In addition, the effects of phytic acid administration on the liver xanthine oxidase (XOX) activity involved in the production of uric acid [14] and the concentration of molybdenum, which is an essential component of XOX [15], were also investigated.

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 was reviewed and approved by the Animal Ethics Committee of Kansai University (Approval No. 2005).

Eighteen 4-week-old male Wistar rats (SHIMIZU Laboratory Supplies Co., Kyoto) were divided into 3 groups. One group (control group) was fed a basal AIN93G diet [16], and the other two groups (0.5PA group and 1.0PA group) were fed the basal diet containing 0.5 or 1.0% sodium phytate (Sigma-Aldrich, St. Louis), respectively. Sodium chloride was added to the diets of the control group and the 0.5PA group in order to equalize the sodium intake of each group. During the feeding period, the animals ingested the diets and water (tap water) *ad libitum*. After feeding for 4 weeks, the liver, kidney, femur, and blood were collected under isoflurane (Fujifilm Wako Pure Chemical Co., Tokyo) anesthesia. The blood was centrifuged at 1500 x g for 15 minutes to obtain the serum. The livers, kidneys and femurs were frozen in liquid nitrogen and stored at −30°C until analysis.

Analysis

Analysis of metals. Approximately 1 g of liver, kidney, and femur were heated with 5 mL of nitric acid until there were no solids. The obtained solution was filtered through a 0.45 μm filter, and the concentrations of zinc, iron, copper, manganese, molybdenum, calcium, and magnesium were determined using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto) or an inductively coupled plasma mass spectrometer (ICPMS-2030, Shimadzu, Kyoto). In the analysis with ICPMS, ^{45}scandium was used as an internal standard. The serum zinc and copper levels were measured using a commercial kit (Metalloassay LS, Metallogenics Co., Chiba).

Assay of hepatic xanthine oxidase (XOX) activity. About 1 g of liver was homogenized with 9 mL of saline. The homogenate was centrifuged at 8000 x g for 20 minutes and the XOX activity in the supernatant was measured as follows [17]. To 200 μL of the homogenate, 800 μL of 0.5 mM xanthine solution and 3 mL of 0.1 M Tris-HCl buffer (pH 7.4) were added, and the mixture was incubated for 20 minutes at 37°C. After the incubation, 1 mL of 30% perchloric acid was added and centrifuged, and the uric acid contained in the supernatant was determined by high performance liquid chromatography (HPLC). The condition of HPLC was as follows: equipment, LC-20Ai (Shimadzu, Kyoto); column, Develosil ODS-HG (4.6 mm φ x 250 mm, Nomura Kagaku, Seto); mobile phase, 20 mM sodium phosphate buffer (pH 3.0)/acetonitrile=99/1 (v/v); column oven, 30°C; flow rate: 1.0 mL/minute; detection, absorbance at 292 nm (SPD-20A, Shimadzu, Kyoto). The activity of XOX that produces 1 μmol of uric acid per minute was defined as 1 unit. Protein in the supernatant of the liver homogenate was determined by Lowry's method [18].

Determination of serum components. The serum calcium, magnesium, iron, uric acid, triacyl glycerol (TAG), total lipid (TL), total cholesterol (TCHOL) and HDL-cholesterol (HDL-CHOL) concentrations were measured with an automatic biochemistry

analyzer (Olympus AU5431; Olympus Co., Tokyo) by Japan Medical Laboratory Co. (Kaizuka).

Statistics

For each measurement, the differences among the groups were tested by one-way ANOVA followed by Tukey-Kramer's multiple comparison test. GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego) was used as the statistical analysis application.

Results

There was no difference in growth among the experimental groups, and the body weight (mean ± SEM) after the end of the feeding period was 287 ± 7 in the control group, 253 ± 18 in the 0.5PA group, and 282 ± 13 g in the 1.0PA group.

Table 1 shows the zinc concentrations in the serum and organs of each experimental group. There was no difference in the zinc concentrations in the liver and kidney among the experimental groups, but the zinc concentrations in the serum and femur were significantly lower in the two groups administered phytic acid than in the control group. Increased phytic acid doses tended to lower the serum and femoral zinc levels.

Table 2 summarizes the concentrations of several metals in the serum, liver, and kidney. As with zinc, the concentration of some metals was reduced by phytic acid administration. Significant reductions with phytic acid administration were observed in the liver iron, serum and liver copper, liver and kidney calcium, kidney magnesium, and liver and kidney manganese concentrations.

Figure 1 shows the serum uric acid concentration, liver XOX activity, and molybdenum concentration. All of these three parameters were significantly lower in a dose-dependent manner after the administration of phytic acid.

Table 3 summarizes the concentrations of the serum lipid components (TAG, TL, TCHOL, HDL-CHOL) in each group. Phytic acid administration significantly reduced the values for all the measured components. However, unlike the serum and femoral zinc or serum uric acid, no further decrease was observed when the dose of phytic acid was increased.

Discussion

Since phytic acid is abundant in unrefined grains and legumes, its intake level varies depending on the content of the dish. In a recent review, phytic acid intake is roughly divided into three types according to eating habits [1]: (i) general Western-style diets low in phytate rich plant foods results in a low intake level of 200 to 350 mg/d, (ii) Western-style diets with enhanced portions of whole grain products or other phytate rich foods results in a higher intake level of 500 to 800 mg/d, and (iii) diets dominated by legumes and unrefined grains such as vegetarian diets or some diets in developing countries in Asia, Africa, and Latin America result in a high intake level of >1000 mg/d. The diets eaten by Egyptian boys with zinc deficiency [3] would fall into this category (iii). In some developing countries corresponding to category (iii), phytic acid intakes in excess of 2000 mg/d have been reported [19-22]. Regarding East Asia, where the Westernization of eating habits is progressing, the daily phytate intake for adult males (21–70 years) was 839 ± 400 mg and for females 752 ± 407 mg in the Republic of Korea [23]. However, phytic-acid-enriched foods and supplements are designed for phytic acid intakes of more than 500 mg/d [12]. Therefore, even in East Asia including Japan, where Westernization is progressing, it can be estimated that there are cases where the phytic acid intake falls into category (iii) due to the intake of phytic-acid-enriched foods. Since the amount of human diet is 400 to 500 g/d by dry weight, the phytic acid dose in this experiment corresponds to 2000 to 5000 mg/d; that is, it corresponds to the phytic acid intake of category (iii).

In this experiment, the serum and femur zinc levels were significantly reduced in rats fed diets supplemented with phytic acid (**Table 1**). This strongly suggests that phytic acid inhibited the absorption of zinc. However, the zinc concentration in the liver and kidney did not change even after the administration of phytic acid, and a decrease in food intake and growth inhibition associated with zinc deficiency did not occur. That is, phytic acid did inhibit zinc absorption, but did not cause a serious deficiency. This is probably because the AIN93G used as the basal diet contains 30 μg/g of zinc, which is sufficient for growth.

The concentrations of some minerals other than zinc in serum and organs also decreased (**Table 2**), and it can be inferred that phytic acid also inhibited the absorption of minerals other than zinc. Similarly to zinc, reduced absorption of these minerals did not lead to the manifestation of a severe deficiency. For example, in the case of iron, administration of phytic acid decreased the iron concentration in the liver, but did not decrease the hemoglobin concentration or the transferrin saturation rate of serum (data not shown). That is, because the iron concentration in the basal AIN93G diet was sufficiently high, the decrease of iron absorption by phytic acid did not lead to the development of iron deficiency.

Table 1. | Effect of phytic acid administration on the zinc concentration in experimental animals

	Zinc concentration		
	Control	**0.5PA**	**1.0PA**
Serum (μg/mL)	2.94 ± 0.20^{b}	2.38 ± 0.10^{a}	2.20 ± 0.05^{a}
Liver (μg/g)	22.9 ± 0.7^{a}	23.7 ± 1.0^{a}	24.8 ± 0.5^{a}
Kidney (μg/g)	24.7 ± 0.6^{a}	24.6 ± 0.4^{a}	23.0 ± 0.6^{a}
Femur (μg/g)	109.2 ± 6.1^{b}	82.8 ± 7.6^{ab}	60.5 ± 8.1^{a}

Values are means ± SEM (n=6).
[a,b)] Means in the same row not sharing a common superscript differ significantly ($p < 0.05$)

Table 2. | Effect of phytic acid administration on several metal concentrations in the serum, liver, and kidney

	Control	**0.5PA**	**1.0PA**
Iron			
Serum (μg/mL)	2.93 ± 0.21^{a}	2.74 ± 0.16^{a}	2.51 ± 0.13^{a}
Liver (μg/g)	76.1 ± 4.5^{b}	58.4 ± 5.2^{a}	60.5 ± 3.5^{ab}
Kidney (μg/g)	46.9 ± 1.3^{a}	45.2 ± 1.2^{a}	42.5 ± 1.1^{a}
Copper			
Serum (μg/mL)	2.34 ± 0.26^{b}	1.56 ± 0.10^{a}	1.87 ± 0.05^{a}
Liver (μg/g)	3.35 ± 0.11^{ab}	3.52 ± 0.13^{b}	3.08 ± 0.08^{a}
Kidney (μg/g)	8.62 ± 0.67^{a}	7.66 ± 0.21^{a}	7.93 ± 0.97^{a}
Calcium			
Serum (μg/mL)	107 ± 2^{a}	106 ± 2^{a}	109 ± 2^{a}
Liver (μg/g)	53.4 ± 2.5^{b}	46.1 ± 2.1^{ab}	38.8 ± 1.2^{a}
Kidney (μg/g)	61.1 ± 0.3^{b}	59.6 ± 2.5^{ab}	52.3 ± 2.8^{a}
Magnesium			
Serum (μg/mL)	18.9 ± 0.5^{a}	18.3 ± 0.2^{a}	18.3 ± 0.4^{a}
Liver (μg/g)	267 ± 7^{a}	247 ± 7^{a}	244 ± 7^{a}
Kidney (μg/g)	201 ± 2^{b}	199 ± 2^{b}	166 ± 2^{a}
Manganese			
Liver (μg/g)	2.36 ± 0.08^{b}	1.97 ± 0.06^{a}	1.74 ± 0.08^{a}
Kidney (μg/g)	0.76 ± 0.01^{b}	0.84 ± 0.02^{b}	0.59 ± 0.01^{a}

Values are means ± SEM (n=6).
[a,b)] Means in the same row not sharing a common superscript differ significantly ($p < 0.05$)

Table 3. | Effect of phytic acid administration on serum lipid components

Components	**Control**	**0.5PA**	**1.0PA**
TAG (mg/dL)	71 ± 12^{b}	42 ± 6^{a}	54 ± 2^{ab}
TL (mg/dL)	305 ± 19^{b}	226 ± 8^{a}	252 ± 7^{a}
TCHOL (mg/dL)	86 ± 6^{b}	71 ± 4^{a}	74 ± 5^{a}
HDL-CHOL (mg/dL)	56 ± 3^{b}	47 ± 1^{a}	45 ± 2^{a}

Values are means ± SEM (n=6).
[a,b)] Means in the same row not sharing a common superscript differ significantly ($p < 0.05$)

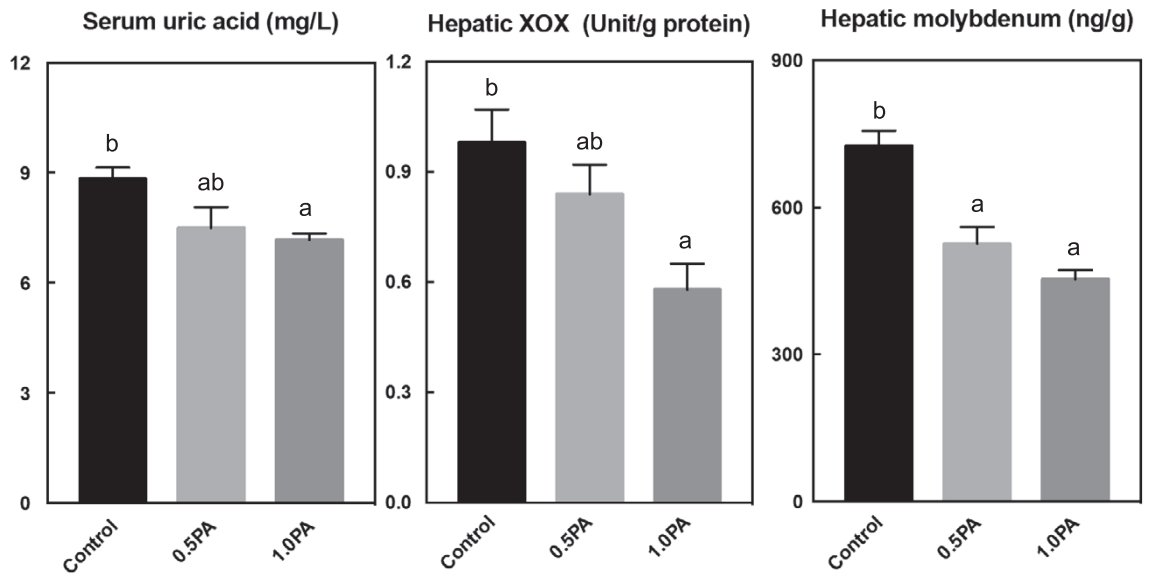

Figure 1. Effect of phytic acid administration on the serum uric acid concentration, hepatic XOX activity, and hepatic molybdenum concentration

Heights of the bars and vertical lines indicate means and SEM (n=6), respectively.
[a,b)] Heights of bars in the same frame not sharing a common superscript differ significantly ($p < 0.05$)

It has been reported that the intake of phytic acid reduces serum uric acid in humans [12, 13]. In the present study as well, it was confirmed that the serum uric acid concentration was decreased in the rats to which phytic acid was administered (**Fig. 1**). For the decrease in serum uric acid caused by phytic acid, a mechanism has been proposed in which the intestinal absorption of a purine base that is the source of the serum uric acid is reduced because phytic acid inhibits the conversion of nucleic acids in the diet to purine bases [24]. However, in the present study, rats were fed a nucleic-acid-free diet. Therefore, the decrease in the serum uric acid concentration cannot be explained by the decrease in the amount of purine base absorbed. It has been reported that phytic acid itself inhibits the XOX activity involved in uric acid production [25], but since phytic acid is hardly absorbed, it is unlikely that it will reach a concentration that inhibits the XOX activity in the liver.

We measured the liver XOX activity and molybdenum concentration, and found that both decreased depending on the dose of phytic acid (**Fig. 1**). Since molybdenum has a strong affinity for phosphate [26], it is quite possible that the phosphate group of phytic acid binds to molybdenum in the intestinal tract in phytic-acid-administered rats, and the absorption of molybdenum is reduced. It is considered that as a result of phytic acid inhibiting molybdenum absorption, the biosynthesis of XOX, which is a molybdenum-containing enzyme, decreased, and the production of uric acid decreased. In this regard, we have observed that a decrease in the liver molybdenum concentration leads to a decrease in the XOX activity in rats administered tungsten, which antagonizes molybdenum *in vivo* [27].

Administration of phytic acid reduced the serum lipid components in addition to serum uric acid (**Table 3**). The mechanism by which phytic acid lowers the serum lipid content is unknown. In the small intestine of monogastric animals, phytic acid forms an insoluble complex with divalent or trivalent cations, and its phosphate ester is not hydrolyzed, so that neither the inositol moiety nor the phosphate is absorbed [28, 29]. In fact, in this experiment, no change was observed in the serum inorganic phosphate concentration of the rats to which phytic acid was administered (data not shown), and it can be estimated that there was almost no release of phosphate from phytic acid. Thus, since phytic acid is hardly absorbed, it is most likely that phytic acid interfered with the absorption of several dietary components including lipids and carbohydrates in the gastrointestinal tract. If so, phytic acid also inhibits the absorption of dietary components other than minerals.

If many of the functions of phytic acid depend on the inhibition of absorption of dietary components in the gastrointestinal tract, phytic acid has a positive effect on people who are overeating. However, on the contrary, it can be said that it has an unfavorable effect on people who have an insufficient intake of nutritional components such as minerals. When considering the function of phytic acid, it is important to fully understand the dietary intake status of each individual.

Soluble sodium phytate was used in this experiment. It is necessary to investigate whether the same result can be obtained by using calcium phytate, which is insoluble in water.

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