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Nutritional Availability of Zinc Contained in Phytin in Rats with Adequate and Low Zinc Status

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Abstract

In cereals and legumes, phytic acid is present as phytin bound with magnesium and zinc. In this study, the nutritional availability of zinc-containing phytin as a zinc source was compared between rats with an adequate zinc status and those with a low zinc status. Twenty-four male 4-week-old Wistar rats were divided into four groups (AZ, AZP, LZ, and LZP): the AZ group was fed a basal AIN93G diet containing adequate amounts of zinc (zinc concentration, 35.6 mg/kg), the AZP group was fed a phytin-supplemented basal diet (40.7 mg/kg), the LZ group was fed a low zinc diet (5.0 mg/kg), and the LZP group was fed a phytin-supplemented low zinc diet (10.2 mg/kg) for 4 weeks. The LZ group showed significantly lower feed intake, body weight, organ weights, organ and serum zinc concentrations, and serum alkaline phosphatase activity than the AZ group, indicating that they were moderately zinc-deficient. In the LZP group fed the phytin-supplemented low zinc diet, the apparent absorption rate of zinc was lower than in the LZ group, but the amount of apparent absorption was higher than in the LZ group, and feed intake, body weight, and organ weights recovered to the same levels as in the AZ group. Organ and serum zinc levels in the LZP group were also significantly higher than in the LZ group. On the other hand, although the AZP group consumed more zinc than the AZ group, they excreted markedly more zinc in their feces, and the apparent amount of zinc absorption was significantly lower than that of the AZ group. In addition, serum and femoral zinc concentrations and serum alkaline phosphatase activity were significantly lower in the AZP group than in the AZ group. These results indicate that zinc-containing phytin is utilized as a zinc source in the presence of a low zinc status, but inhibits zinc utilization when the zinc status is adequate.

Keywords: phytin, phytate, zinc, nutritional availability, balance study, absorption

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Introduction

Zinc is the second most abundant essential trace element in the human body after iron, with approximately 90% of zinc stored in skeletal muscle and bone [1]. Zinc has been found to be necessary to maintain the function of over 300 enzymic reactions. Adult zinc intake shown in the 2019 Japanese National Health and Nutrition Survey [2] exceeds the estimated average requirement shown in the Dietary Reference Intakes for Japanese (2020) [3], with the exception of men over 75 years of age.

Phytic acid (myo-inositol hexaphosphate), which is abundant

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in grains and legumes, is a storage form of phosphorus in plants and is known to inhibit the absorption of minerals, including zinc, in the digestive tract in non-ruminant animals [4]. It was suggested that the stunting caused by zinc deficiency in Egyptian boys, in addition to low zinc intake, was related to the consumption of whole-grain bread, which contains high levels of phytic acid [5]. However, it is also considered that as long as the diet contains sufficient minerals, the effect of phytic acid intake from grains and legumes on mineral absorption is minor and negligible [6]. In recent years, there have been increasing reports that phytic acid intake has a positive effect on maintaining good health [7]. Epidemiological studies and animal experiments have shown that phytic acid is effective in preventing several chronic diseases such as cancer [8] and neurodegenerative diseases [9]. Furthermore, it has been reported that phytic acid lowers the serum uric acid concentration [10].

Phytic acid is present in plant seeds in a multiple metal-bound form called phytin. Our previous studies showed that phytin from rice bran contains magnesium, zinc, and manganese [11]. This indicates that a significant portion of the zinc in grains and legumes is bound to phytic acid. Since zinc intake from grains and legumes accounts for about one-third of the total zinc intake in Japanese [2], it is important to investigate the nutritional availability of zinc in phytin. However, it is unclear whether the zinc bound to phytic acid has nutritional significance as a source of zinc. Although many animal studies have reported that phytate adversely affects the absorption of trace elements in the diet [12-14], no studies focusing on zinc bound to phytic acid have been reported to our knowledge. Here, we administered zinc-containing phytin to rats with an adequate or low zinc nutritional state to determine its effect on growth and the tissue zinc concentration, and conducted a one-week metabolic balance study.

Materials and Methods

Reagents

Phytin derived from rice bran was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Analysis showed that the phytin used contained 19.2% phosphorus, 13.6% magnesium, 1.04% calcium, and 513 μg/g zinc. Ingredients of animal feed were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Animals and diets

Twenty-four 4-week-old male Wistar rats (mean ± SD of body weight, 81 ± 4 g) purchased from Shimizu Laboratory Supply Co. (Kyoto) were divided into four groups of equal body weight. Four kinds of diets (adequate zinc without phytin, adequate zinc with phytin, low zinc without phytin, and low zinc with phytin) based on the AIN93G formula [15] were prepared and fed to each group; the four dietary groups were named the adequate zinc group (AZ group), the adequate zinc-phytin-added group (AZP group), the low zinc group (LZ group), and the low zinc-phytin-added group (LZP group). **Table 1** shows the composition of the experimental diets. The total zinc concentrations in the diets were measured as 35.6 ± 2.5 mg/kg (AZ group), 40.7 ± 1.3 mg/kg (AZP group), 4.97 ± 0.90 mg/kg (LZ group), and 10.2 ± 0.6 mg/kg (LZP group). The diets of the AZP and LZP groups contained more phosphorus and magnesium than those of the AZ and LZ groups because of the addition of phytin-derived phosphorus and magnesium.

In order to administer the low zinc diet as early as possible, each group of rats was immediately fed each experimental diet without an acclimatization period. The rats were housed in a room at a room temperature of 22 ± 1°C with a 12-hour light/dark cycle (8:00-20:00). Body weight and food intake were recorded every two days. The feeding period lasted 28 days and rats were allowed access to tap water and experimental diets ad libitum. On day 23, each rat was transferred to an individual metabolism cage, and fecal samples were collected every two days for zinc balance studies. When the rearing period ended, blood was collected from the abdominal vena cava under deep anesthesia with isoflurane (MSD Animal Health, Tokyo, Japan) under non-fasting conditions, and the blood samples were centrifuged at 2,000 × g for 15 minutes to obtain serum. Organs were collected and weighed, frozen in liquid nitrogen, and stored at −30°C until analysis.

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.: 2203).

Measurements

Portions of the liver, kidney, testis, spleen, femur, and serum collected as well as the experimental diets were digested using concentrated nitric acid. The digests were quantitatively diluted with ultrapure water and filtered through a 0.45-μm filter. The zinc concentration of the sample solution was determined using a flame atomic absorption spectrophotometer (AA-7000,

Table 1. | Composition of experimental diets

Ingredients (g/kg)	AZ	AZP	LZ	LZP
Corn starch	397.486	387.486	397.486	387.486
Casein	200	200	200	200
Gelatinized corn starch	132	132	132	132
Sucrose	100	100	100	100
Soybean oil	70	70	70	70
Cellulose	50	50	50	50
AIN93G mineral mixture	35	35	–	–
Zn-free AIN93G mineral mixture	–	–	35	35
AIN93 vitamin mixture	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
L-Cystine	3	3	3	3
tert-Butylhydroquinone	0.014	0.014	0.014	0.014
Zinc-containing phytin	–	10	–	10
Zinc content (mg/kg) *	35.6 ± 2.5	40.7 ± 1.3	5.0 ± 0.9	10.2 ± 0.6
Phosphorus content (mg/kg)*	3145 ± 32	4998 ± 82	3186 ± 115	5224 ± 188
Magnesium (mg/kg)*	579 ± 32	1877 ± 11	530 ± 41	1867 ± 5

*, Actual measured values (means ± SD for 3 determinants).

Shimadzu Co., Kyoto, Japan) or inductively coupled plasma mass spectrometer (ICPMS-2030, Shimadzu Co., Kyoto, Japan). Phosphorus and magnesium in the experimental diets were measured by vanadomolybdate absorption spectrophotometry [16] and flame atomic absorption spectrophotometry, respectively. In ICPMS analysis, ^{103}rhodium was used as an internal standard.

For a portion of the serum and whole-blood samples, biochemical testing was contracted to a clinical laboratory providing testing services (Japan Medical Laboratory Co., Kaizuka, Japan).

Statistical analysis

The groups in the animal study were divided by two factors: zinc concentration in the diet and presence or absence of phytin addition. However, because phytin contains zinc, the addition of phytin increases the zinc concentration in the diet. In other words, the two factors are not independent. Therefore, one-way analysis of variance (ANOVA) was used for statistical analysis, rather than two-way ANOVA. For some results, data were transformed into ranks and Kruskal-Wallis tests were performed. If the results of one-way ANOVA or Kruskal-Wallis tests were significant ($p < 0.05$), Tukey's multiple comparisons were performed to test for differences between the groups. Statistical software IBM SPSS Statistics 27 (IBM Japan, Ltd., Tokyo, Japan) was used for these analyses.

Results

Table 2 summarizes body and organ weights at the end of the rearing period, as well as total feed intake and feed efficiency during the rearing period. The LZ group, which received the lowest zinc diet, had significantly lower feed intake and lower body and organ weights, while the LZP group, which received zinc-containing phytin in addition to the low zinc diet, showed increased feed intake and similar body and organ weights as the AZ and AZP groups. Feed efficiency tended to be higher in the LZ group, which had the lowest feed intake.

Zinc concentrations in serum and organs are summarized in **Table 3**. The LZ group showed the lowest zinc concentrations in

Table 2. | Body weight, feed intake, and organ weights of rats fed experimental diets

	AZ	AZP	LZ	LZP
Final body weight (g)	295 ± 7^{b}	292 ± 5^{b}	252 ± 4^{a}	282 ± 6^{b}
Body weight gain (g/28 d)	215 ± 6^{b}	211 ± 5^{b}	172 ± 4^{a}	200 ± 5^{b}
Feed intake (g/28 d)	432 ± 6^{b}	428 ± 12^{b}	327 ± 6^{a}	424 ± 11^{b}
Feed efficiency*	0.498 ± 0.002^{b}	0.492 ± 0.004^{b}	0.524 ± 0.001^{c}	0.472 ± 0.001^{a}
Liver weight (g)	13.59 ± 0.83^{b}	11.25 ± 0.37^{a}	10.92 ± 0.45^{a}	12.05 ± 0.32^{ab}
Kidney weight (g)	2.04 ± 0.03^{b}	2.02 ± 0.04^{ab}	1.85 ± 0.03^{a}	2.05 ± 0.07^{b}
Spleen weight (g)	0.60 ± 0.03^{a}	0.57 ± 0.02^{a}	0.51 ± 0.03^{a}	0.55 ± 0.01^{a}
Testis weight (g)	2.75 ± 0.22^{b}	2.74 ± 0.05^{b}	2.15 ± 0.06^{a}	2.83 ± 0.05^{b}

*, Feed efficiency calculated as (body weight gain)/(feed intake).

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

Table 3. | Zinc concentration in organs of rats

Organs	AZ	AZP	LZ	LZP
Liver (μg/g)	22.6 ± 0.6^{b}	25.5 ± 0.4^{c}	19.9 ± 0.7^{a}	22.2 ± 0.7^{ab}
Kidney (μg/g)	26.1 ± 0.2^{c}	26.0 ± 0.2^{c}	16.9 ± 0.2^{a}	19.1 ± 0.2^{b}
Spleen (μg/g)	18.9 ± 0.4^{b}	18.3 ± 0.1^{b}	15.9 ± 0.5^{a}	18.0 ± 0.3^{b}
Testis (μg/g)	22.1 ± 0.3^{b}	21.7 ± 0.2^{b}	18.7 ± 0.4^{a}	21.3 ± 0.1^{b}
Femur (μg/g)	121.8 ± 2.4^{d}	114.4 ± 1.5^{c}	31.5 ± 1.1^{a}	50.5 ± 1.9^{b}
Serum (μg/dL)	124.2 ± 4.9^{d}	104.3 ± 3.2^{c}	17.8 ± 2.9^{a}	38.2 ± 4.5^{b}

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

serum and all organs. The decrease in zinc concentration in the femur and serum was particularly marked. On the other hand, the LZP group, which ingested zinc-containing phytin, showed higher zinc concentrations in serum and all organs than the LZ group, especially in the liver, spleen, and testis, which were similar to those of the AZ group. The AZP group, which consumed more zinc than the AZ group due to the addition of phytin to the diet, had higher zinc concentrations in the liver than the AZ group, but zinc concentrations in the kidney, spleen, and testis were similar to those of the AZ group, and lower than those of the AZ group in the femur and serum.

Table 4 shows the results of the zinc balance study. When comparing AZ and AZP groups, which have sufficient zinc in their diets, the zinc intake of rats in the AZP group was higher than in the AZ group due to the addition of phytin to the diet, as there was no difference in feed intake in the two groups. However, the AZP group excreted more zinc in the feces and had the lowest apparent absorption rate of the four groups. Furthermore, although the AZP group consumed more zinc than the AZ group, the amount of apparent absorption was lower in the AZP group. On the other hand, the LZ group, which consumed the lowest amount of zinc, had the highest zinc absorption rate. The LZP group, which consumed more zinc than the LZ group due to the addition of phytin to the diet, had a lower apparent absorption rate than the LZ group, but a higher amount of apparent absorption.

Table 5 summarizes the results of blood biochemical tests. Significant differences between groups were observed in several items. There were several patterns in the appearance of differences. The first pattern was that the LZ group differed from the other three groups, with the LZ group having lower values for total protein and higher values for iron, transferrin saturation, and aspartate aminotransferase than the other three groups. The second pattern was that the AZ group had higher values than the other three groups for triacylglycerols, total cholesterol, and total lipids. The third pattern was that the AZP and LZP groups

Table 4. | Zinc balance in rats fed experimental diets

	AZ	AZP	LZ	LZP
Intake (μg/d)	773.1 ± 25.4^{c}	871.5 ± 21.5^{d}	72.9 ± 2.7^{a}	212.0 ± 5.2^{b}
Fecal excretion (μg/d)	565.3 ± 28.3^{c}	722.9 ± 5.0^{d}	16.6 ± 0.5^{a}	119.9 ± 4.9^{b}
Apparent absorption (μg/d)*	207.8 ± 11.6^{d}	148.6 ± 22^{c}	56.2 ± 2.7^{a}	92.1 ± 2.6^{b}
Apparent absorption (%)	27.1 ± 2.0^{b}	16.8 ± 2.2^{a}	76.3 ± 1.5^{d}	43.4 ± 1.2^{c}

Values are means ± SEM (n = 6). Means in the same row not sharing a common superscript differ significantly ($p < 0.05$).
*, Data were converted to ranks and then statistical tests were performed.

Table 5. | Blood biochemical examination of rats fed experimental diets

	AZ	AZP	LZ	LZP
Serum				
Total protein (mg/dL)	5.2 ± 0.1^{b}	5.1 ± 0.1^{ab}	4.9 ± 0.1^{a}	5.1 ± 0.1^{ab}
Triacylglycerol (mg/dL)	144 ± 17^{b}	83 ± 12^{a}	89 ± 13^{a}	91 ± 7^{a}
Total cholesterol (mg/dL)	93 ± 7^{b}	84 ± 3^{ab}	68 ± 6^{a}	78 ± 6^{ab}
Total lipids (mg/dL)	423 ± 29^{b}	315 ± 13^{a}	286 ± 16^{a}	312 ± 12^{a}
Aspartate aminotransferase (IU/L)	65 ± 1^{a}	64 ± 1^{a}	74 ± 3^{b}	67 ± 3^{ab}
Alanine aminotransferase (IU/L)	29 ± 2^{a}	28 ± 1^{a}	29 ± 1^{a}	26 ± 2^{a}
Alkaline phosphatase (U/L)	99.5 ± 3.6^{c}	83.4 ± 4.7^{b}	40.1 ± 4.0^{a}	48.5 ± 0.7^{a}
Inorganic phosphorus (mg/dL)	8.1 ± 0.1^{a}	9.3 ± 0.2^{b}	7.5 ± 0.1^{a}	8.9 ± 0.2^{b}
Magnesium (mg/dL)	1.9 ± 0.03^{a}	2.1 ± 0.03^{b}	1.9 ± 0.04^{a}	2.1 ± 0.02^{b}
Iron (μg/dL)	235 ± 26^{ab}	193 ± 9^{a}	294 ± 18^{b}	197 ± 11^{a}
Transferrin saturation (%)	47.3 ± 4.8^{ab}	42.6 ± 1.5^{a}	64.7 ± 4.7^{b}	40.5 ± 1.6^{a}
Whole blood				
Hemoglobin (g/dL)	13.0 ± 0.1^{a}	13.4 ± 0.1^{a}	13.0 ± 0.1^{a}	13.1 ± 0.1^{a}
Hematocrit (%)	41.0 ± 0.3^{a}	42.1 ± 0.3^{a}	41.0 ± 0.2^{a}	41.2 ± 0.4^{a}

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

had higher values than AZ and LZ groups for phosphorus and magnesium. The zinc-containing enzyme alkaline phosphatase (ALP) showed specific changes, with the AZ group having the highest values, followed by the AZP group, while the LZ and LZP groups had much lower values. This pattern was similar to the pattern of intergroup variation in serum and femoral zinc.

Discussion

When casein or egg white is a primary protein source, the US National Research Council (US-NRC) recommends a diet containing approximately 12 mg/kg of zinc for weaning rats [17]. The LZ group in this study received a low zinc diet of about 5 mg/kg, which depended solely on the zinc contained in the casein used as a protein source. In the LP group, the rats showed reduced feed intake and growth retardation from the first week of feeding, and low organ weights and reduced organ and serum zinc concentrations were observed after the 4-week feeding period. In our previous study, feeding diets with a zinc concentration of 5.5 mg/kg, using egg white as the main protein source, caused a decrease in appetite and growth retardation [18]. In the present experiment, relative testicular weight in the LZ group was not significantly lower than in the AZ group (data not shown). In other words, the LZ diet caused growth retardation, but did not lead to the reproductive dysfunction observed in severe zinc

deficiency [19]. Furthermore, although food intake was reduced, the cyclic increase or decrease in food intake observed with severe zinc deficiency [20] was also not observed. Accordingly, it can be concluded that rats in the LZ group, which were fed diets with a zinc concentration of approximately 5 mg/kg, were indeed suffering from moderate zinc deficiency. However, since the feed efficiency of this group was higher than that of the other three groups, the growth retardation was not due to inadequate protein utilization in the body, but rather to an undernutritional state caused by a low feed intake, and the decreased concentrations of serum total protein and lipid-related components observed in the LZ group also reflect the state of undernutrition. Furthermore, the elevation in serum iron markers and aspartate aminotransferase activity observed in the LZ group should also be interpreted as a secondary effect of undernutrition.

The diet of the LZP group increased the zinc concentration to approximately 10 mg/kg with the addition of zinc-containing phytin. As a result, in the LZP group, the low levels of feed intake, body and organ weights, and serum parameters indicative of the nutritional status observed in the LZ group were restored to values comparable with those of AZ and AZP groups. In the balance study (**Table 4**), the amounts of apparent absorption of zinc in the LZP group was significantly higher than in the LZ group, clearly indicating that the zinc contained in the phytin was absorbed. These results show that a significant portion of the zinc contained in the phytin was effectively utilized in the LZP group.

On the other hand, although zinc concentrations in the organs of the LZP group were elevated compared with the LZ group, zinc concentrations in the serum and femur as well as serum ALP activity were markedly lower than in the AZ and AZP groups, which had adequate zinc intake. Although the LZP group was not zinc-deficient to the point of loss of appetite, the zinc status may still be considered to be low. In an experimental study on induced zinc deficiency in rats, it was noted that when zinc was added stepwisely to a zinc-deficient diet (zinc content <1 mg/kg), recovery of body weight gain saturated at a dietary zinc concentration of 9 to 12 mg/kg [19]. Another study on zinc requirements in growing rats noted that less than 3 mg/kg of dietary zinc induces severe deficiency, from 3 to 6 mg/kg moderate deficiency, from 6 to 9 mg/kg mild deficiency, and from 9 to 15 mg/kg marginal deficiency [21]. These results are similar to the present experimental results.

The results of administering zinc-containing phytin in the presence of the zinc-sufficient condition differed significantly from the results of administering zinc-containing phytin under the zinc-deficient condition. That is, although the AZP group had a higher zinc intake than the AZ group, there was little increase in organ zinc concentrations, and the femoral and serum zinc concentrations, as well as serum ALP, were lower than in the AZ group (**Tables 3 and 5**). In the zinc balance study, the AZP group excreted markedly more zinc in the feces than the AZ group, and absorbed less zinc (**Table 4**). These results indicate that when zinc-containing phytin is ingested when the zinc nutritional status is adequate, the inhibition of zinc absorption by phytic acid is marked. It is known that the expression of ZIP4, which is involved in zinc absorption in the small intestine, is markedly increased in the zinc-deficient state [22]. The different effects of zinc-containing phytin depending on the zinc nutritional status may mean that the inhibitory effect of phytate is less when expression of the transporter involved in zinc absorption is high, and the inhibitory effect of phytate is greater when the expression is low.

The concentration of serum lipid components was decreased in the AZP compared with AZ group (**Table 5**). This result is similar to our previous study in which sodium phytate was administered to rats with adequate zinc intake [14]. Although the details of the mechanism are not clear, it is considered to be an effect of phytic acid.

The World Health Organization states that zinc is less available when the molar ratio of phytate to zinc exceeds 15 [23]. In fact, one study of dietary monitoring of young men showed that when the phytate to zinc molar ratio exceeds 15, zinc absorption is markedly inhibited and the zinc balance may even become negative [24]. Similar studies have been conducted in rats and chicks, with both reaching similar conclusions [25, 26]. However, in these experiments, the phytic acid concentration in the diet was high and the zinc concentration in the diet was also high, so it is likely that the inhibition of zinc absorption by phytic acid occurred more strongly than in reality. Furthermore, the phytic acid given was purified sodium phytate. However, sodium phytate does not occur naturally, and the phytin present in grains and legumes contains magnesium and zinc [11]. In other words, it would be unrealistic to discuss the effects of phytic acid in grains and legumes based on experiments using sodium phytate.

The molar ratio of phytic acid to zinc in the present study was 17 in the AZP group and 70 in the LZP group. If previous reports [23-26] are to be followed, inhibition of zinc absorption by phytic acid should be more pronounced in the LZP group. Indeed, the apparent zinc absorption rate of the LZP group was about 40%, which is lower than that of the LZ group (**Table 4**). However, this decrease in absorption was not sufficient to counteract the ameliorative effect of zinc-containing phytin on zinc-

deficient rats. We hypothesize that the administration of zinc-containing phytin to zinc-deficient rats may cause the following series of changes in the gastrointestinal tract. First, when the zinc-containing phytin, or zinc phytate, enters the stomach, the low pH environment causes some of the chelated zinc to be released and the phytate itself becomes soluble. Next, when the zinc enters the duodenum and jejunum, the zinc in the digested material is efficiently absorbed from the small intestinal mucosa because the expression of ZIP4 is increased due to the zinc-deficient state [22]. Remaining unabsorbed zinc and endogenous zinc excreted from the gastrointestinal tract are bound to phytic acid in the ileum or large intestine and are not absorbed and excreted in feces. This results in a higher amount of zinc excretion in the feces in the LZP group and a lower apparent absorption rate. That is, although inhibition of zinc absorption by phytic acid also occurs with low zinc nutrition, this inhibition occurs after most of the zinc has been absorbed in the duodenum or jejunum, so the symptoms associated with zinc deficiency may have been ameliorated in the LZP group.

Phytic acid has been considered one of the factors causing zinc deficiency since cases of zinc deficiency were reported by Prasad in Egypt [5]. In this Egyptian study, those who developed the deficiency were dependent on a diet consisting mainly of whole wheat bread and had little access to animal products. With such a diet, the source of zinc would have been wheat, and the zinc would likely have been zinc phytate. According to the results of the present experiment, in Egyptian boys with zinc deficiency, the phytin (zinc phytate) in whole grain bread did not inhibit zinc absorption, but rather should supplement zinc intake. Nevertheless, zinc deficiency could have occurred for the following reasons. Their routine diet was extremely unbalanced [5] and the intake of major nutrients was marginal to the requirements. Under such low nutritional conditions, the various defense mechanisms, involving increased protein synthesis that occurs in response to loading, often fail to operate. In other words, in the Egyptian case, low nutrition may have resulted in inhibition of zinc absorption by phytic acid as in the case of adequate zinc intake, without the increased expression of ZIP4 that should occur under low zinc conditions.

The purpose of this experiment was to qualitatively examine the potential of zinc-containing phytin to be a source of zinc, separately for low zinc status and zinc adequate status. The results, as summarized in **Table 4**, show that when the zinc status is low, the zinc in zinc-containing phytin functions to some extent as a source of zinc and improves the zinc status, but when the zinc status is adequate, the phytic acid has a strong effect and acts in the direction of inhibiting zinc absorption. It has been believed that non-refined grains, even though they contain more zinc than refined grains, have a negative effect on the zinc status because phytic acid inhibits zinc absorption [5]. However, the present results suggest that intake of non-refined grains in low zinc status can improve the zinc status, even if the phytic acid intake is higher, because the zinc bound to phytic acid is available to some extent. On the other hand, intake of non-refined grains when the zinc status is adequate, even if zinc intake is increased, will result in less zinc absorption due to the greater effect of absorption inhibition by phytic acid. However, the decrease in zinc absorption due to phytic acid when the zinc status is adequate is small and does not cause a serious effect on zinc status. Furthermore, we are also planning to conduct an experiment to quantitatively examine the utilization rate of zinc-containing phytin with uniform dietary zinc concentrations.

In the present study, inorganic phosphorus and magnesium concentrations in serum were significantly increased in the AZP and LZP groups treated with phytin (**Table 5**). This suggests that a portion of phytic acid was hydrolyzed in the gastrointestinal tract, resulting in increased absorption of phosphorus and magnesium. Recently, it was reported that phytase secreted by the intestinal microflora improves zinc absorption in the large intestine [4]. On the other hand, it remains unclear whether phytase is secreted in the intestines of non-ruminant animals. The nutritional significance of phosphorus derived from phytic acid will need to be examined in the future.

The relationship between phytic acid and micronutrients is very complex, and further studies on the effects of phytic acid on minerals are needed. A fundamental understanding of the concentrations and molecular species of nutrients and other chemicals in the diet will lead to a better understanding of the interaction between micronutrients and phytic acid.

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