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Influence of High-Phosphorus or High-Salt Feeding on Intestinal Alkaline Phosphatase Activity in Rats

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Alkaline phosphatase (ALP) hydrolyzes a variety of monophosphate esters into inorganic phosphoric acid and alcohol, but little is known about the physiological function of intestinal ALP. We investigated the influence of a high-phosphorus or high-salt dietary intake on intestinal alkaline phosphatase activity in rats. A total of 33 female Sprague-Dawley rats (6-weeks-old) were divided into four groups: control, 1.0% phosphorus (P 1.0%) group, 1.5% phosphorus (P 1.5%) group, and 1.0% sodium chloride (High Salt) groups. At 56 days after the beginning of the experiment, intestinal segments from the duodenum, jejunum, and ileum were obtained and used for enzyme assays. There was no significant difference in the levels of intestinal ALP activity between the high-phosphorus groups (P 1.0% and P 1.5% groups) and the control group. Interestingly, the levels of intestinal ALP activity in the duodenum and jejunum from the High Salt group were significantly lower than those from the control group ($p<0.05$ and $p<0.01$, respectively). These findings suggest that a high-salt dietary intake is one of the factors that decrease intestinal ALP activity. Further studies on the mechanism of the regulation of intestinal ALP activity would provide useful data on the physiological function of intestinal ALP.

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Keywords: alkaline phosphatase (ALP), phosphorus, high salt, intestine, rats.

INTRODUCTION

Alkaline phosphatase (ALP, EC 3.1.3.1) hydrolyzes a variety of monophosphate esters into inorganic phosphoric acid and alcohol at a high optimal pH (pH 8-10). In humans, studies on the genes of this enzyme have revealed that there are at least four ALP isozymes: tissue non-specific (TNSALP), intestinal, placental, and germ cell types.[1)-4)] Based on studies of hypophosphatasia, which is a systemic skeletal disorder resulting from a TNSALP deficiency,[5)-10)] TNSALP was suggested to be indispensable for bone mineralization.

In rats, ALP is classified into two types: TNSALP and intestinal ALP. The strong activity of intestinal ALP, which is located at the brush border of intestinal epithelial cells, suggests the involvement of this enzyme in the transport of nutrients such as inorganic phosphate (Pi) across the membrane, but little is known about the physiological function of intestinal ALP. Intestinal ALP appears to have an important role in active metabolism by hydrolyzing phosphocompounds to supply free inorganic phosphate.

It is well known that intestinal ALP is affected by several kinds of nutrients. Previously, we reported that intestinal ALP activity was increased by fat or lactose feeding.[11)-13)] We also demonstrated that the intestinal ALP activities in both the duodenum and jejunum from rats in a powdered green tea group were significantly lower that those from control rats.[14)]

Several studies on the effects of a high-phosphorus or high-salt intake have been investigated.[15)-18)] However, there is little data concerning the influence of a high-phosphorus or high-salt intake on intestinal ALP activity.

In this study, we attempted to examine the influence of a high-phosphorus or high-salt dietary intake on the activity of intestinal ALP. Our aim was to clarify the physiological function of intestinal ALP using the relationship between intestinal ALP and various nutritional factors.

MATERIALS AND METHODS

Experimental animals

The care and use of the rats in the present study followed the guidelines of governmental legislation in

Japan on the proper use of laboratory animals. Thirty-three six-week-old female Sprague Dawley rats were acclimated for nine days prior to any study procedure. Then, rats were separated into four groups: a control group fed an AIN-93M diet containing 0.3% P and 0.26% sodium chloride (Cont.),[19] a 1.0% phosphorus diet (P 1.0%) group, a 1.5% phosphorus diet (P 1.5%) group, and a 1.0% sodium chloride diet (High Salt) group. The experimental diets were modified from AIN-93M, and both the control and High Salt diets contained 0.3% phosphorus. The Ca, protein, and lipid contents were identical in the four diets. The animals were housed individually in wire cages with free access to ion-exchanged distilled water. Twelve hour light/dark cycles, a constant temperature (23±1℃), and constant humidity (50±5%) were maintained. All rats were observed each day. Their food intake was monitored and body weights were obtained every second day. At 56 days after the beginning of the experiment, the animals were fasted overnight and sacrificed by bleeding from the abdominal aorta under anesthesia.

Preparation and measurement of intestinal enzymes

We removed the small intestines from the pylorus side to the beginning of the cecum and rinsed them with ice-cold saline. From the pylorus, we took the first 3 cm as the duodenum, and then separated the remaining part into two: 10 cm from the pylorus side of the upper half for the jejunum and the lower half for the ileum. The segments were slit open longitudinally, and the mucosa was scraped with a piece of slide glass after being rinsed and stored at −30℃ prior to use. The sample was homogenized with 10 mM Tris-buffered saline containing 1% TritonX-100 (pH 7.3) using a Polytron homogenizer (Kinematica, Switzerland). The supernatant obtained via centrifugation after 10,000×g for 5 min was used for the enzyme assay.

ALP activity was determined with 10 mM p-nitrophenylphosphate as a substrate in 100 mM 2-amino-2-methyl-1,3-propanediole HCl buffer containing 5 mM $MgCl_2$, pH 10.0, at 37℃, as previously reported.[13] The enzyme activity was determined by the rate of hydrolysis of p-nitrophenyl phosphate and expressed in units (U= μmol p-nitrophenol formed/min). Protein concentrations were determined using BCA protein assay reagent (Pierce, Rockford, IL, USA).

Balance study of phosphorus

On days 28 and 54 of the dietary intervention, urine and feces were collected using stainless steel metabolic cages and a collection apparatus designed to keep the urine and feces separate. The feces were ashed and diluted with HNO_3. Phosphorus was determined by the method of p-methylaminophenol reduction.[20] The apparent intestinal absorption and accumulation of phosphorus were determined. The absorption of phosphorus was calculated as the difference between the amount of oral intake and excretion in feces, and the retention of phosphorus was calculated as the difference between absorption and excretion in urine.

Biochemical analysis of serum

Sera were separated by centrifugation and stored at −30℃ until being thawed for analyses. Phosphorus contents were measured as described above. Ca contents in the serum were measured by the o-cresolphthalein complexion color development method,[21] and the total amount of protein was measured by the biuret method.[22]

Statistical analysis

Values are shown as means±SE, and significance was determined by Scheffé's test after one-way analysis of variance (ANOVA) (SPSS 13.0 J, SPSS Inc., IL, USA).

Table 1. Body weight gain, food intake, and food efficiency

Groups	n	Body weight gain (g/day)	Food intake (g/day)	Food efficiency
Control	9	2.02±0.07	13.48±0.27	0.15±0.00
P 1.0%	8	1.97±0.08	13.92±0.27	0.14±0.00
P 1.5%	8	1.50±0.09***	13.27±0.30	0.11±0.01***
High Salt	8	2.05±0.05	14.15±0.20	0.15±0.00

Food efficiency=body weight gain/food intake. Each value represents the mean±SE. ***: Significant difference between the control and P 1.5% groups ($p<0.001$).

RESULTS

Body weight gain and food intake

The body weight gain, food intake, and food efficiency during the experiments are shown in Table 1. There were no significant differences in weight gain, food intake, and food efficiency [body weight gain (g/day)/food intake (g/day)] among the control, P 1.0%, and High Salt groups, although the body weight gain, food intake (g/day), and food efficiency in the P 1.5% group were significantly lower than those in the control group ($p<0.001$).

Biochemical assays of serum

The levels of total protein, calcium, phosphorus, and ALP activity in the serum are shown in Table 2. There were no significant differences in the levels of calcium, phosphorus, ALP activity, and total protein among the four groups.

ALP activities in the intestine

As shown in Fig. 1, the ALP-specific activity (U/mg protein) in the duodenum in the High Salt group was significantly lower than that in the control group ($p<0.05$), while the P 1.0% and P 1.5% groups were lower, but this was not significant. In the jejunum, the ALP-specific activity in the High Salt group was significantly lower than in the control group ($p<0.01$), but phosphate loading did not affect ALP activity in the jejunum (Fig. 2). As shown in Fig. 3, there were no significant differences in ALP-specific activity among the four groups in the ileum.

Balance study of phosphorus

As shown in Fig. 4A and 4B, the apparent absorption or the accumulation of phosphorus in the P 1.0% and P 1.5% groups were significantly higher than in the control group ($p<0.01$, respectively). On the other hand, the High-Salt diet did not affect the phosphorus balance.

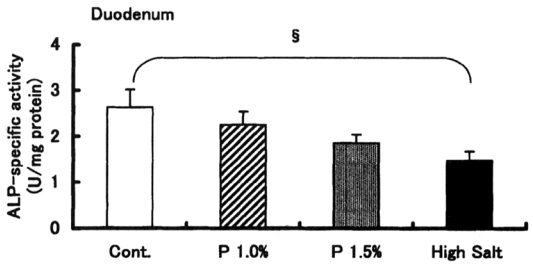

Fig. 1. Specific ALP activity in the duodenum

Each result is the mean±SE. §: Significant difference between the control and High Salt groups ($p<0.05$).

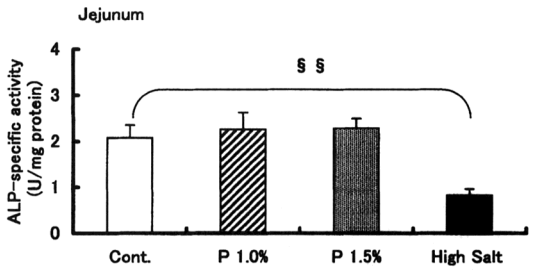

Fig. 2. Specific ALP activity in the jejunum

Each result is the mean±SE. §§: Significant difference between the control and High Salt groups ($p<0.01$).

DISCUSSION

In this study, we examined the influence of a high-phosphorus or high-salt dietary intake on intestinal ALP activity. Interestingly, the ALP activities of the

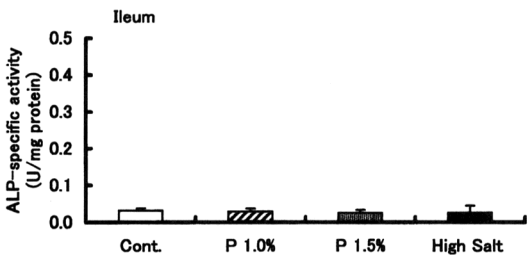

Fig. 3. Specific ALP activity in the ileum

Each result is the mean±SE.

Table 2. The levels of serum total protein, Ca, P, and alkaline phosphatase

Groups	Total protein (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (U/l)
Control	6.1±0.3	10.6±0.3	8.1±0.7	28.5±1.0
P 1.0%	5.7±0.2	10.0±0.2	6.9±0.3	33.3±2.4
P 1.5%	5.4±0.2	9.8±0.1	7.2±0.6	31.9±2.1
High Salt	6.2±0.3	10.1±0.3	8.3±0.6	28.0±1.8

Each value represents the mean±SE.

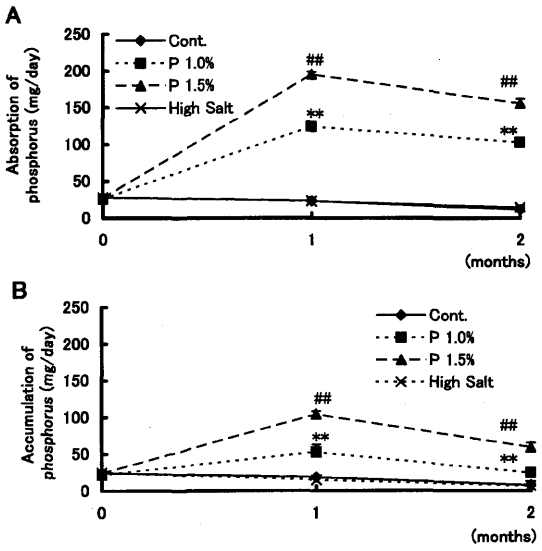

Fig. 4. Balance study of phosphorus

A: Absorption, B: Accumulation. **: Significant difference between the control and P 1.0% groups ($p<0.01$). ##: Significant difference between the control and P 1.5% groups ($p<0.01$).

duodenum and jejunum in the High Salt group were significantly lower than in the control group (Figs. 1 and 2).

A high-salt diet is known to induce or aggravate hypertension in both animal models of hypertension and humans. Several studies have reported the effect of a high-NaCl diet (4.0%) on hypertension using a rat model.[16)-18)] In our study, the high-salt diet contained 1% NaCl, and this NaCl amount was four-fold higher than that of the control diet (0.26%).[19)] Therefore, we considered the 1.0% NaCl diet as a moderate high-salt diet. As shown in Table 1, there were no significant differences in weight gain, food intake, and food efficiency between the High Salt and the control groups.

In vitro, the effect of salt concentration on ALP activity depends on the pH of the reaction mixture, the type of ions involved, and the source of the enzyme.[23)] Halford *et al.* suggested that conformational changes were strongly influenced by the concentration of sodium chloride during the action of ALP on substrates.[24)] However, *in vivo*, the mechanisms underlying the kinetic effects of sodium chloride are not entirely clear. Further study is necessary on the regulation of the high-salt feeding effect on intestinal ALP activity.

Moreover, we investigated the influence of high-phosphorus supplementation on intestinal ALP activity. As shown in Tables 1 and 2, there were significant differences in weight gain, and food efficiency between the P 1.5% and control groups. These results suggested that the P 1.5% diet may have an inhibitory effect on the weight gain of the experimental animals. However, there were no significant differences in those parameters between the control and P 1.0% groups (Tables 1 and 2).

Matsuzaki *et al.* reported the kidney mineral concentrations in rats given feed containing various phosphorus levels: 0.6% P, 0.9% P, 1.2% P, and 1.5% P diet groups.[15)] As their results, the P concentrations in the kidney were higher in rats given 0.6% P, 0.9% P, 1.2% P, and 1.5% P diets than in those given 0.3% P feed, while the final body weight and food intake were lower in the rats given the 1.5% P feed, but not in those given 0.6% P, 0.9% P, and 1.2% P feed.[15)]

In our study, we demonstrated that the absorption and accumulation of phosphorus in the P 1.0% and P 1.5% groups were significantly higher than the control group ($p<0.01$). Although there were no significant differences in intestinal ALP activity between the control and P 1.0% groups, elucidation of the correlation between intestinal ALP and a high-phosphorus dietary intake is helpful for elucidating the role of this enzyme in phosphate metabolism.

The physiological role of ALP is still unclear; however, strong evidence for its role is provided by the rare genetic disease hypophosphatasia (HOPS). HOPS is an inherited disorder characterized by a defect in skeletal mineralization due to TNSALP deficiency.[5)-10)] Thus, TNSALP was thought to be indispensable for bone mineralization. Quite recently, we revealed a significant association between the 787 T>C (Tyr 246 His) TNSALP gene and bone mineral density among 501 postmenopausal women.[25)]

As a result of studies on cDNA encoding ALP isozymes, it is known that the primary structure in the catalytic region is well conserved in the ALPs of humans, animals, and *Escherichia coli*,[1)] suggesting that both intestinal ALP and TNSALP play important roles in active metabolism by hydrolyzing phosphocompounds.

Recently, Narisawa *et al.* suggested that fat absorption was accelerated in intestinal ALP knockout mice, and suggested that intestinal ALP participates in a rate-limiting step regulating fat absorption.[26)]

In conclusion, the present study indicated that a high-salt intake is one of the factors that decrease intestinal ALP activity. Further studies on the

mechanism of intestinal ALP would provide useful data on its physiological function.

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高リン食摂取，または高食塩食摂取が小腸アルカリホスファターゼ活性に及ぼす影響について

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アルカリホスファターゼ（ALP）は，アルカリ性（pH 8-10）に至適pHを持つ亜鉛含有酵素で，リン酸エステルを無機リン酸とアルコールに加水分解する反応を触媒する．小腸アルカリホスファターゼ（ALP）は，小腸刷子縁膜に高濃度に存在し，小腸内の無機リン酸の取り込みに深く関与していると考えられているが，未だ不明な点が多い．近年の食生活において，リンや食塩の過剰摂取が問題となってきている．そこで，本研究では，高リン食摂取，または高食塩食摂取が小腸ALP活性に及ぼす影響について検討を行った．6週齢SD系雌ラット（33匹）を，AIN-93Mを与えるコントロール群，飼料重量の1.0%および1.5%のリンを含む飼料を与える高リン食群（P 1.0%およびP 1.5%），さらに1.0%の塩化ナトリウムを含む高食塩食群の4群に分けた．実験食投与開始から56日後に十二指腸，空腸，回腸に分けて採取し，それぞれの部位でALP比活性を比較した．高リン食群（P 1.0%およびP 1.5%）では，小腸ALP比活性において，コントロール群と比べ有意な差は認められなかった．一方，興味深いことに，高食塩食群ではコントロール群に比べ，十二指腸および空腸で，それぞれ有意な低値を示した（$p<0.05$, $p<0.01$）．今回の結果から，高食塩食摂取が小腸ALPの活性を低下させる作用があることが示唆された．今後，さらに小腸ALP活性調節メカニズムについて検討を重ねることによって，小腸ALPの生理的機能の解明に役立つ証拠が得られるであろう．

キーワード：アルカリホスファターゼ，リン，食塩，小腸，ラット．