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Influence of Long-term Lactose Feeding on Intestinal Alkaline Phosphatase in Rats

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Lactose is known to enhance passive calcium absorption independent of vitamin D endocrine system. However, the effect of lactose on other metabolism is not fully understood. The purpose of this study was to examine the effects of long-term lactose feeding on intestinal alkaline phosphatase (ALP) activity. A total of 30 female Sprague-Dawley rats (6 weeks old) were divided into three groups, and fed a control diet, a 3% lactose or a 10% lactose diet, respectively. After 56 days, intestinal ALP activity in the ileum from the 3% or 10% lactose group was significantly higher than the control group ($p<0.01$, respectively). These data suggest that the long-term lactose feeding influences on intestinal ALP activity.

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INTRODUCTION

Lactose is a disaccharide contained in milk, and several experiments have demonstrated that lactose enhances calcium homeostasis and may be beneficial for bone mineralization.[1)-6)] However, the mechanisms of these actions are still unclear. Moreover, the effect of lactose on other metabolism such as phosphate is not fully understood.

Alkaline phosphatase (ALP, EC 3.1. 3.1) hydrolyzes a variety of monophosphate esters into inorganic phosphoric acid and alcohol at a high pH optimum (pH 8-10). In rats, ALP is classified into two types: tissue non-specific (liver/bone/kidney: TNSALP) and intestinal ALP.[7)] The level of ALP activity in the intestine is higher compared with other tissues, and it is located at the brush border of intestinal epithelial cells, suggesting the participation of this enzyme in the transport of nutrients, such as inorganic phosphate across the membrane, but little is known about the physiological function of intestinal ALP.

Previously, we reported that intestinal ALP activity in the rat intestine was markedly enhanced after 14 days of being fed a 10% lactose diet, and that lactose is one of the factors responsible for intestinal ALP activity.[8)] In the present study, we examined the influence of different amount of lactose feeding on intestinal ALP activity in longer period than previous study.

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. Six-week-old female Sprague Dawley rats ($n=30$) were allowed to acclimate for nine days prior to any study procedure. Then, rats were separated into three groups to be fed an AIN-93 M diet[9)] (the control group), a 3% lactose diet, or a 10% lactose diet (the 3% and 10% lactose groups, respectively). The lactose diets were modified from AIN-93 M and contained 30 g or 100 g lactose/kg as a substitute for sucrose. The Ca, P, protein, and lipid contents were identical in the three diets. The animals were housed individually in wire cages with free access to ion-exchanged distilled water. Twelve hour light/dark cycles, 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. After 56 days from starting the experimental diet, 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: jejunum and ileum. Ten centimeters of proximal jejunum and ileum were used for the assay. 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 at 10,000×g for 5 min was used for the enzyme assay. ALP activity was determined with 10 mM p-nitro-phenylphosphate 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.[10] 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).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Polyacrylamide gel (7.5%) electrophoresis in the presence of sodium dodecyl sulfate (SDS) was carried out according to the method of Weber and Osborn.[11] After electrophoresis, ALP isozymes in the gels were stained by the method of coupling beta-naphthyl-phosphoric acid monosodium salt with Fast Violet B salt.[12]

Statistical analysis

Values are shown as means±S.E. Dunnet's multiple comparison tests was used after ANOVA to compare the statistical significance of the differences between control group and each lactose group respectively by using SPSS 13.0J (SPSS Inc., IL, USA).

RESULTS

Body weight gain and food intake

The initial body weights among the control group, the 3% lactose group, and the 10% lactose group were not significantly different. As shown in Table 1, there was also no significant difference among the three groups in body weight gain (g/day), food intake (g/day), and food efficiency [body weight gain (g/day)/food intake (g/day)]. Diarrhea was not observed during the feeding of the experimental diet in any group.

ALP activities in the intestine

As shown in Figs. 1 and 2, the levels of ALP activity

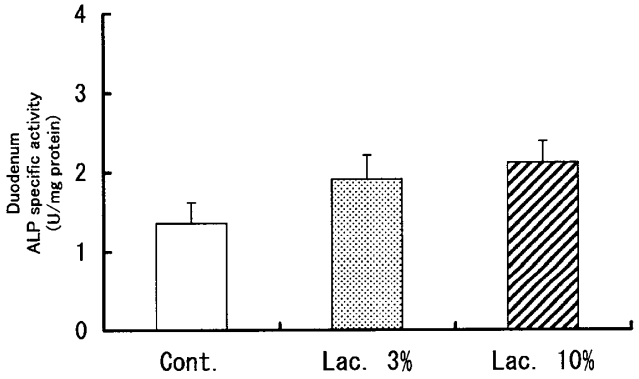

Fig. 1. ALP specific activity in duodenum
Results are mean±S.E.

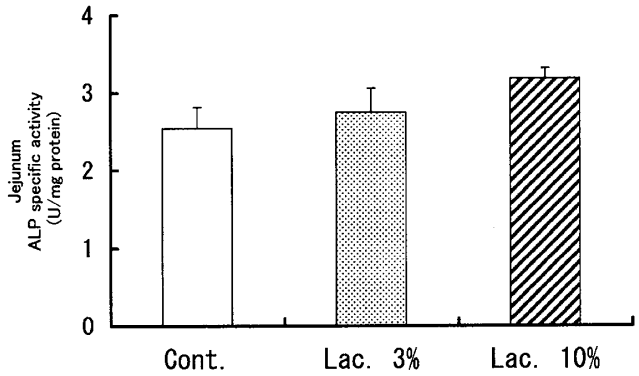

Fig. 2. ALP specific activity in jejunum
Results are mean±S.E.

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

Groups	Body weight gain (g/day)	Food intake (g/day)	Food efficiency
Control	1.16±0.06	13.05±0.29	0.089±0.003
Lactose 3%	1.21±0.09	13.93±0.35	0.087±0.005
Lactose 10%	1.20±0.05	13.50±0.14	0.089±0.007

Each value represents mean±S.E. Food efficiency=Body weight gain/Food intake.

in the duodenum and jejunum tend to be higher in the 3% and 10% lactose groups than the control group. The level of ALP activity in the ileum was significantly higher in the 3% and 10% lactose groups than the control group ($p<0.01$, $p<0.01$, respectively) (Fig. 3).

SDS-PAGE

Further identification of intestinal ALP isozymes by SDS-PAGE was carried out. In the each three group, there were two main bands of 110-kDa and 90-kDa in the duodenum (Fig. 4, lanes 1-3) and the jejunum (Fig. 4, lanes 4-6). In duodenum, 110-kDa band was stronger than 90-kDa band, while 110-kDa band was weaker than 90-kDa band in the jejunum. It was determined that the main ALP isozyme in the ileum was 90-kDa band and long-term feeding of both 3% and 10% lactose enhanced this isozyme (Fig. 4, lanes 8-9).

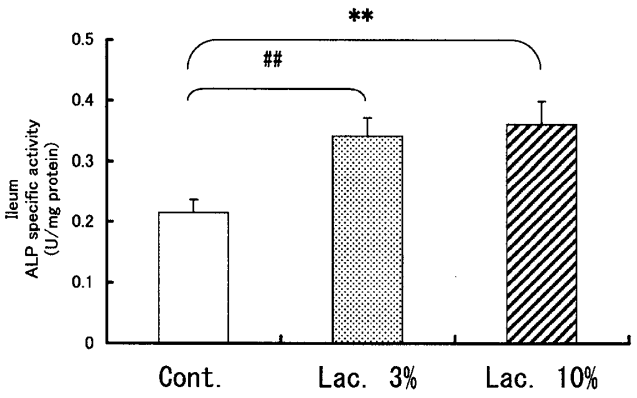

Fig. 3. ALP specific activity in ileum

Results are mean±S.E. #Significant difference between the 3% lactose group and control group (##: $p<0.01$). *Significant difference between the 10% lactose group and control group (**: $p<0.01$).

DISCUSSION

Previously, we reported that intestinal ALP activity in the rat intestine was markedly enhanced after less than 14 days of 10% lactose feeding.[8]

In the present study, we clarified that both 3% and 10% lactose feeding increased intestinal ALP activity in 56 days feeding. We also examined the effective lactose amount and we confirmed that even if the 3% lactose addition have the same effect on the increase of intestinal ALP activity.

By SDS-PAGE analysis, we showed that there are two ALP isozymes in the intestine mainly. As shown in Fig. 4, 110 kDa band was major isozyme in the region of intestine of pylorus side. In the region near to the cecum side, 110 kDa band was weaker and 90 kDa band was detected. The main ALP isozyme was the 90-kDa band in ileum, (Fig. 4, lanes 7-9), and lactose feeding apparently increased ALP of 90-kDa isozyme.

From previous reports, two kinds of cDNA clones for rat intestinal ALP, rIAP-1 and rIAP-2, were isolate,[13][14] respectively. Their cDNA sequences have 79% homology at the amino acid level. The sizes of rIAP-1 mRNA is 2.7 kb and rIAP-2 mRNA is 3.0 kb.[15] The larger mRNA transcript, rIAP-2, exists in the upper part of the intestine,[16] while rIAP-1 isozyme expressed mainly in whole area of intestine.[15][17]

It was reported that rIAP-1 carboxyl-terminal peptide is smaller than that of rIAP-2 protein by Western blotting.[18] We considered that the 90 kDa band in the ileum might correspond to the product of rIAP-1. However, the ALP molecule contains about 20% (w/w) carbohydrates,[19] it could not be excluded the possibility that the isozymes may be glucosylated differently depending on the location in the intestine. It has been also reported that sugar chains play a role in

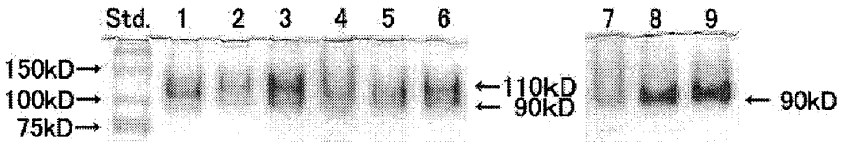

Fig. 4. Rat intestine ALP isozymes separated by polyacrylamide gel electrophoresis after two months

S: molecular-size standard (Precision Plus Protein Standards, Bio-Rad Lab.). Lane 1: duodenum from the control group; lane 2: duodenum from the 3% lactose group; lane 3: duodenum from the 10% lactose group; lane 4: jejunum from the control group; lane 5: jejunum from the 3% lactose group; lane 6: jejunum from the 10% lactose group; lane 7: ileum from the control group; lane 8: ileum from the 3% lactose group; lane 9: ileum from the 10% lactose group.

regulating ALP activity.[20]

As a result of the studies on cDNA encoding intestinal ALP and TNSALP, it is known that the primary structure in the catalytic region is well conserved in the ALPs of humans, animals and *E. coli*,[21] suggesting that both intestinal ALP and TNSALP may play important roles in active metabolism by hydrolyzing phospho-compounds to supply free inorganic phosphate (Pi). Although the physiological role of ALP is unknown, strong evidence for this 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.[22)-28)] Thus, TNSALP was thought to be indispensable for bone mineralization. Moreover, we revealed recently that a significant association between the 787T>C (Tyr246His) TNSALP gene and bone mineral density among 501 postmenopausal women.[29]

The role of intestinal ALP *in vivo* is more obscure. The previous studies indicated that intestinal ALP was affected by nutritional factors. Intestinal ALP is known to increasing by fat- feeding,[10)30)] and decreasing by green tea powder intake,[31] or high-salt feeding.[32] On fat-feeding, the mRNA for rIAP-2 enhanced markedly but that for rIAP-1 did not increase as much.[10] These data suggest that rIAP-1 and rIAP-2 may be regulated in a different way at the transcriptional level.

Since phosphate is an essential nutrient in nucleic acids, proteins, lipids, and sugars in all organisms, we formed the hypothesis that mammalian intestinal ALP may hydrolyze phospho-compounds and supply free Pi to the intestinal Pi transport system just before the use of the intestinal bacterium. However, little is known about how the hydrolysis of phospho-compounds works to increase phosphate absorption.

We suppose that the lactose feeding may elevate ALP activity at the transcriptional or translational level, and lead to apply the Pi for mineral metabolism.

Further studies on the mechanism of increased intestinal ALP activity induced by lactose is still unknown, would provide useful data on the clarification of the physiological function of intestinal ALP.

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ラットにおけるラクトース長期投与による小腸アルカリホスファターゼ活性への影響

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アルカリホスファターゼ（ALP）は，アルカリ性に至適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．ラクトース（乳糖）は，小腸でCaの受動輸送による吸収を促進していると考えられているが，生体内の他の代謝に及ぼす影響についてほとんど研究されていない．本研究では，ラクトース長期摂取が小腸ALPに及ぼす作用について検討を行った．6週齢SD系雌ラット30匹を3群に分け，AIN-93 Mを与えるコントロール群，飼料重量の3%，10%をラクトースに置き換えたラクトース3%群，ラクトース10%群とした．56日間飼育した後，十二指腸，空腸，回腸のALPの比活性を測定した．回腸のALP活性は，ラクトース3%群および10%群がコントロール群より高値を示した（それぞれ$p<0.01$）．今回の結果から，ラクトース長期投与が小腸ALP活性に影響を及ぼしていることが明らかになった．

キーワード：ラクトース，ラット，アルカリホスファターゼ，小腸．