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Original Article

Effect of High Dietary Phosphorus Concentration and Different Dietary Phosphorus Forms on Bone Strength and Bone Turnover in Rats

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Abstract

The purpose of this study was to examine the effect of high dietary phosphorus (P) concentration and different dietary P forms on bone strength and bone turnover in rats. The P concentration of the experimental diets was adjusted to either 0.3% (normal-P diet) or 1.5% (high-P diet) using potassium dihydrogenphosphate or potassium tripolyphosphate as dietary P sources. Rats were divided into four groups and fed one of four experimental diets for 21 d. Bone mineral density (BMD) and breaking energy were significantly lower in rats fed the high-P diet than in rats fed the normal-P diet. Serum osteocalcin level and urinary C-terminal telopeptide of type Ⅰ collagen excretion as a biochemical marker of bone turnover were significantly higher in rats fed the high-P diet than in rats fed the normal-P diet. Different dietary P forms had no significant influence on the BMD, breaking energy, and biochemical markers of bone turnover. These results suggest that a high dietary P concentration leads to a high bone turnover, which induces decreased bone strength. Moreover, our results indicate that bone strength and bone turnover are not influenced by different dietary P forms.

Key words： Bone Strength, Bone Turnover, Dietary Phosphorus Concentration, Dietary Phosphorus Form, Rats

Ⅰ. INTRODUCTION

Phosphorus (P) is present in most foods and many phosphate salts are used as additives in processed foods to maintain quality. In recent decades, processed foods have become an increasing proportion of our diet. As a result, P is consumed in amounts that are too high for optimal bone health [1, 2].

Epidemiologic studies have linked the dietary calcium (Ca):P ratio to bone mineral density (BMD)[3] and bone fractures [4]. In healthy young females, high P intake induced a decrease in serum bone-specific alkaline phosphatase activity and an increase in urinary N-terminal telopeptide of collagen type Ⅰ excretion[5]. Urinary excretion of hydroxyproline[6], deoxypyridinoline[7], and C-terminal telopeptide of type Ⅰ collagen (CTx)[8], biochemical markers of bone resorption, was increased in rats fed a high-P diet. Results of previous studies suggest that although adequate P intake is a necessary dietary factor for maintenance of bone growth, high P intake is considered to be one of the risk factors for osteoporosis. Thus, although many studies have been conducted, the details of the effect of high P intake on bone metabolism remain unclear.

On the other hand, processed foods are supplemented with various kinds of phosphate salts as food additives, and as a result, various forms of dietary P have been introduced into our bodies. It is speculated that different dietary P forms may affect bone metabolism, because P plays an important role in bone metabolism. However, the effect of different dietary P forms on bone metabolism has not yet been examined. Accordingly, this study examined the effect of high dietary P concentration and different dietary P forms on bone strength and bone turnover.

Ⅱ. MATERIALS AND METHODS

1. Animals and Diets

Four-week-old male Wistar rats (Clea Japan, Tokyo, Japan) were housed in individual stainless-steel wire-mesh cages. During the experiment, the cages were located in a room with controlled lighting under a 12-h light:dark cycle (light, 0800-2000 h), a temperature of 24 ± 1°C, and relative humidity of 55 ± 5%. Experimental diets were based on an AIN-93G diet[9] (Table 1), but the mineral mix was a modification of the AIN-93G mineral mix without potassium dihydrogenphosphate (KH_2PO_4). We used KH_2PO_4 and potassium tripolyphosphate ($K_5P_3O_{10}$) as dietary P sources. The P concentration of the experimental diets was adjusted to either 0.3% (normal-P diet) or 1.5% (high-P diet). All of the experimental diets had the same Ca concentration. The P and Ca concentrations, as measured from an analysis of the experimental diets, are shown in Table 1. All rats were treated in accordance with the guidelines of the National Research Council for the Care and Use of Laboratory Animals (1985). Before the study period began, there was a 5-d acclimatization period during which all rats were given free access to normal-P diet containing KH_2PO_4 and deionized water. After the acclimatization period, the rats were divided into four groups (n = 6 per group), with each group having a similar mean body weight, and fed one of four experimental diets differing in dietary P concentration and dietary P form for 21 d. Rats fed the other experimental diets were fed the mean weight of food consumed by the rats fed the high-P diet containing $K_5P_3O_{10}$ on the previous day. Rats were given free access to deionized water. Body weight and food intake were recorded daily. On the last day of the experimental periods, the rats were housed individually in stainless-steel metabolic cages and each rat's urine was collected for 24 h and analyzed to determine the bone resorption marker. At the end of the experimental period, rats were killed by exsanguination under nembutal anesthesia, and blood and femur samples were collected for analysis.

Table 1 Composition of the experimental diets

	Normal-P diet		High-P diet	
	KH_2PO_4	$K_5P_3O_{10}$	KH_2PO_4	$K_5P_3O_{10}$
Ingredient	g/kg			
Corn starch	522.632	521.957	469.909	464.042
Casein	200.0	200.0	200.0	200.0
Sucrose	100.0	100.0	100.0	100.0
Soybean oil	70.0	70.0	70.0	70.0
Cellulose powder	50.0	50.0	50.0	50.0
Mineral mix[1]	35.0	35.0	35.0	35.0
Vitamin mix[2]	10.0	10.0	10.0	10.0
L-Cystine	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5
Tert-butylhydroquinone	0.014	0.014	0.014	0.014
KH_2PO_4	6.854	—	59.577	—
$K_5P_3O_{10}$	—	7.529	—	65.444
Chemical analysis	%			
P	0.33	0.32	1.57	1.52
Ca	0.51	0.50	0.50	0.51

[1] The mineral mix is a modification of the AIN-93G mineral mix without KH_2PO_4.
[2] AIN-93 vitamin mix.

2. Chemical analysis

Samples of the experimental diet were ashed at 550°C for 48 h in a muffle furnace and minerals were extracted for analysis with 1 M HCl. The Ca content in the experimental diet was determined by atomic absorption spectrophotometry (Hitachi A-2000; Hitachi, Tokyo, Japan) according to the method of Grimblet et al.[10]. The P content in the experimental diet was determined colorimetrically according to the method of Gomori [11]. Blood was collected and centrifuged to separate the serum. Serum Ca was measured with a commercial calcium C kit (Wako Pure Chemical Industries, Osaka, Japan). Serum parathyroid hormone (PTH) was measured with a rat intact PTH ELISA kit (Immutopics Inc., San Clemente, CA, USA). Serum osteocalcin was measured with an osteocalcin rat ELISA system (Amersham Biosciences K.K., Tokyo, Japan). Urinary C-terminal telopeptide of type Ⅰ collagen (CTx) was measured with a RatLaps ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). Urinary creatinine was measured with a commercial creatinine test (Wako Pure Chemical Industries, Osaka, Japan). Urinary CTx was expressed relative to the urinary creatinine.

3. Measurement of bone mineral density, breaking force, and breaking energy

Femora were excised from rats and the muscles and connective tissues were removed. BMD of the right femur was measured by using a DSC-600EX-Ⅲ R bone densitometer (Aloka, Tokyo, Japan). The breaking force and breaking

Table 2 Changes in body weight

	Normal-P diet		High-P diet		Two-way ANOVA (P-values)[1]		
	KH_2PO_4	$K_5P_3O_{10}$	KH_2PO_4	$K_5P_3O_{10}$	C	F	C × F
Initial body weight (g)	98.6 ± 3.3	99.6 ± 2.0	99.2 ± 3.6	99.2 ± 2.3	NS	NS	NS
Final body weight (g)	169.0 ± 3.8	170.9 ± 3.7	165.0 ± 4.6	166.6 ± 13.5	NS	NS	NS

Values are means ± SD (n = 6).

[1] C: effect of dietary P concentration; F: effect of dietary P form; C × F: effect of interaction; NS: not significant ($p>0.05$).

Table 3 Bone mineral density, breaking force, breaking energy and biochemical markers of bone turnover

	Normal-P diet		High-P diet		Two-way ANOVA (P-values)[1]		
	KH_2PO_4	$K_5P_3O_{10}$	KH_2PO_4	$K_5P_3O_{10}$	C	F	C × F
BMD (mg/cm^2)	75.1 ± 2.1	76.7 ± 1.2	72.5 ± 1.9[a]	72.9 ± 1.9[a]	0.0003	NS	NS
Breaking force (N)	55.1 ± 1.9	56.2 ± 2.2	52.8 ± 2.1	53.7 ± 2.0	0.01	NS	NS
Breaking energy (N.mm)	563.3 ± 62.5	542.3 ± 86.7	456.5 ± 50.6[a]	465.2 ± 49.3[a]	0.003	NS	NS
Serum osteocalcin (ng/ml)	150.0 ± 19.2	132.0 ± 16.7	230.8 ± 26.6[a]	222.9 ± 35.8[a]	<0.0001	NS	NS
Urinary CTx (μg/mmol creatinine)	30.6 ± 9.8	27.8 ± 10.5	326.7 ± 68.7[a]	253.4 ± 95.8[a]	<0.0001	NS	NS

Values are means ± SD (n = 6).

[1] C: effect of dietary P concentration; F: effect of dietary P form; C × F: effect of interaction; NS: not significant ($p>0.05$).

[a] Significant differences from normal-P diet with the same dietary P form ($p<0.05$).

energy of the left femur were determined by the three-point bending procedure using a materials testing system (MZ-500S; Maruto, Tokyo, Japan).

4. Statistical analysis

Data are expressed as mean values with SD. Data were analyzed by two-way analysis of variance to determine the effect of dietary P concentration, the effect of dietary P form, and their interaction. Fisher's protected least significant difference test was used to determine the significant differences of multiple comparisons among groups. Differences were considered significant at $p<0.05$.

Ⅲ. RESULTS

1. Body Weight

Dietary treatments had no significant influence on the final body weight (Table 2).

2. Bone Mineral Density, Breaking Force, Breaking Energy, and Biochemical Markers of Bone Turnover

BMD was significantly lower in rats fed the high-P diet than in rats fed the normal-P diet, irrespective of the dietary P form (Table 3). Breaking energy was significantly lower in rats fed the high-P diet than in rats fed the normal-P diet. Dietary P form had no significant influence on the BMD, breaking force, and breaking energy. Serum osteocalcin level and urinary CTx excretion were significantly higher in rats fed the high-P diet than in rats fed the normal-P diet. However, the different dietary P forms had no significant influence on the serum osteocalcin level and urinary CTx excretion.

3. Serum Calcium and Parathyroid Hormone Levels

Dietary treatments had no significant influence on serum Ca level (Table 4). Serum PTH level was significantly higher in rats fed the high-P diet than in rats fed the normal-P diet. The different forms of dietary P had no significant influence on the serum PTH level.

Ⅳ. DISCUSSION

Previous studies reported that a high-P diet induced decreases in femur BMD [12-14] and lumbar BMD [7, 12] in rats. Similarly, the femur BMD in this study was decreased in rats fed the high-P diet. In addition, this study showed that breaking energy was lower in rats fed the high-P diet than in rats fed the normal-P diet. These results suggest that high-P diet induced bone loss and decreased bone strength, which is related to the bone turnover, because BMD and bone strength reflect the end result of bone modeling and remodeling

Table 4 Serum calcium and parathyroid hormone levels

	Normal-P diet		High-P diet		Two-way ANOVA (P-values)[1]		
	KH2PO4	$K_5P_3O_{10}$	KH_2PO_4	$K_5P_3O_{10}$	C	F	C × F
Ca (mg/dl)	9.28 ± 0.18	9.18 ± 0.35	8.88 ± 0.59	9.23 ± 0.39	NS	NS	NS
PTH (pg/ml)	562.0 ± 242.5	609.1 ± 132.0	1715.0 ± 1000.2[a]	1699.8 ± 827.5[a]	0.0005	NS	NS

Values are means ± SD (n = 6).

[1] C: effect of dietary P concentration; F: effect of dietary P form; C × F: effect of interaction; NS: not significant ($p>0.05$).

[a] Significant differences from normal-P diet with the same dietary P form ($p<0.05$).

activities.

Bone metabolism is characterized by the formation of new bone by osteoblasts and the resorption of old bone by osteoclasts, and is an important factor in the determination of bone quality. Therefore, we assessed bone formation and resorption using serum osteocalcin level and urinary CTx excretion as biochemical markers of bone turnover. Results in this study showed that serum osteocalcin level and urinary CTx excretion were increased in rats fed the high-P diet. This result indicates a high turnover bone metabolism in rats fed the high-P diet. It is speculated that high bone turnover has an adverse effect on BMD and bone strength. High bone turnover is a significant etiological factor for bone loss [15]. In other words, the reduction of BMD and bone strength induced by high-P diet intake could be explained by an increase in bone turnover.

It is known that bone resorption is regulated by PTH. With regard to the relationship between PTH and bone resorption in rats fed a high-P diet, Katsumata et al.[12] reported that the elevated serum PTH level caused by a high-P diet stimulated the receptor activator of nuclear factor-kappa B ligand (RANKL) mRNA expression. RANKL mediates osteoclast differentiation and activation [16-18]. Several bone histomorphometry studies showed that osteoclast number was increased by a high dietary P concentration [12, 14]. In other words, the increased serum PTH level induced by a high-P diet enhances osteoclast number via increased RANKL expression, thus accelerating bone resorption in this study. Also, PTH is an important factor in bone formation, as this hormone affects osteoblast activity. This study observed that serum PTH level was remarkably increased in rats fed the high-P diet. Thus, we suggest that the high bone turnover induced by a high-P diet is due to an increased serum PTH level.

No studies have ever tried to evaluate the effect of different dietary P forms on bone metabolism. Therefore, this study investigated the effect of different dietary P forms on bone strength and bone turnover. Results in this study observed that BMD, breaking force, and breaking energy were not influenced by dietary P form. In addition, we observed that dietary P form had no influence on the serum osteocalcin level and urinary CTx excretion as biochemical markers of bone turnover, indicating that bone turnover is not influenced by a difference in dietary P form. This result supports unchanged BMD, breaking force, and breaking energy in this study. In other words, the different dietary P form does not affect bone turnover; consequently, bone strength is unchanged. We suggest that bone strength and bone turnover are strongly influenced by the high dietary P concentration rather than by the different forms of dietary P. This study also showed that although serum PTH level was increased by a high-P diet, the different forms of dietary P had no influence on the serum PTH level. We suggest that no change of serum PTH level accounts for the any change of bone turnover in different forms of dietary P.

In conclusion, we examined the effect of high dietary P concentration and different dietary P forms on bone strength and bone turnover in rats. These results suggest that high dietary P concentration leads to a high bone turnover, which induces decreased bone strength. Moreover, our results indicate that bone strength and bone turnover are not influenced by different dietary P forms.

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