High P Diet Induces Acute Secretion of Parathyroid Hormone without Alteration of Serum Calcium Levels in Rats

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To find whether a high phosphorus (P) diet stimulate the secretion of PTH, a high-P diet was fed to rats and an increase in serum P levels has occurred. All rats were fed a control diet (0.5% calcium (Ca), 0.5% P) for 7 days, while they were being adapted, for 1 hour at 8:00 AM and again at 8:00 PM. Four groups were switched to the high-P diet (0.5% Ca, 1.5% P) at the time of their morning meal for 1 hour. The other 4 groups continued to receive the control diet. Blood samples were collected from the rats in the remaining group, which served as a pre-feeding control. Every 30 minutes after the start of feeding (30, 60, 90, 120 min), blood samples were collected from the rats in the groups fed the control and high-P diets. Serum P concentrations increased upon intake of the high P diet, within 30 minutes after the start of feeding. Serum PTH levels also increased upon intake of the high P diet, within 30 minutes after the start of feeding, and the levels were significantly higher in the high-P group than in the control group. However, no significant difference was observed in serum Ca levels between the two groups. From these results, our findings suggest that an increase in serum P concentration might be a trigger of PTH secretion without any changes of serum calcium levels.

Key words: calcium; phosphorus; parathyroid hormone

Parathyroid hormone (PTH) serves to maintain serum calcium (Ca) concentrations within a limited range by inducing an increase in Ca reabsorption in the kidney and by promoting Ca absorption in the intestine through a mechanism involving the synthesis of 1,25(OH)_2D_3. Conversely, in phosphorus (P) homeostasis, PTH decreases serum P concentrations by inducing an increase in urinary P excretion. In studies of humans and animals, it has been demonstrated that PTH secretion is induced by a low-Ca diet, a high-P diet or a diet lacking an adequate balance in terms of the Ca:P ratio. The factors capable of triggering an increase in PTH secretion are still not fully understood. It is generally recognized that PTH secretion is induced by a decrease in serum Ca concentrations in vivo. In a previous in vitro study, it was found that a high extracellular concentration of phosphorus has a direct stimulatory effect on PTH secretion. Therefore, it seems likely that administered phosphorus may serve as a stimulatory factor capable of triggering an increase in PTH secretion. In an in vivo study, it was found that a high P diet directory stimulated PTH secretion. The changes in serum PTH levels that occur in response to increasing serum P levels caused by a high P load have been demonstrated in studies using rats, and the serum levels of both PTH and P increased at the same time. The changes in PTH occurring as a result of stimulation by dietary P, including hyperphosphatemia, were quite immediate and could be seen within 2 h. The matter of how fast the PTH secretion response occurs upon stimulation by dietary P, is still controversial. In this study, to be certain of an increase in the serum P concentration, a high-P diet was fed to rats. After confirming that an increase in serum P levels had occurred, we immediately assessed whether the high-P diet influenced the levels of PTH.

Materials and Methods

Experimental procedures. Weanling 4-week-old male Wistar rats (n = 45) were obtained from Clea Japan (Tokyo, Japan) and housed individually in stainless-steel cages in a room maintained at 22°C with a 12-h light-dark cycle. The Tokyo University of Agriculture Animal Use Committee approved this study and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Tokyo University of Agriculture. All rats were fed a control diet (0.5% Ca, 0.5% P; Table 1) for 7 days, while they were being adapted for 1 hour at 8:00 AM and again at 8:00 PM. During the 7 days of this pre-experimental period, all rats grew normal-
Dietary Phosphorus and Calcium Metabolism

ly and equally (initial weight = 77.2 ± 2.7, final weight = 109.3 ± 5.3 values are means of all rats ± SD), and then, were randomly divided into 9 groups of 5 each. Consequently, no differences of body weight among the 9 groups were observed. Four groups were switched to the high-P diet (0.5% Ca, 1.5% P; Table 1) at the time of their morning meal for 1 hour. The other 4 groups continued to receive the control diet. Blood samples were collected before the morning meal from the rats in the remaining group, which served as a pre-feeding control. Every 30 minutes after the start of feeding (30, 60, 90, 120 min), blood samples were collected from the rats in the groups fed the control and high-P diets.

Blood biochemistry. The blood samples were centrifuged at 3000×g for 20 min and the supernatants were used as serum samples. Each 300 μl of serum samples were filtered with ultrafiltration membranes (Microcon-10, Amicon, Inc. MA) at 25°C 14000×g. The filtrated samples were separated from large molecules, and were collected with unbound and a small amount of low-bound calcium. It was regarded as an ultrafiltered Ca in this study. The Ca concentrations in serum were measured using an atomic absorption spectrophotometer (Shimazu AA 640-13) by the method of Gimblet et al. P concentrations were determined colorimetrically by the method of Gomori. Serum PTH levels were assayed by means of a radioimmunoassay kit (Nichols Inc., San Clemente, CA). This method is a two-site immunoradiometric assay (IRMA) using two different goat antibodies to measure both intact ratPTH and its N-terminal fragments.

Statistical analysis. The data are presented as the mean ± SE for a group of 5 rats. One-way analysis of variance (ANOVA) was used to analyze the data, and a P value of less than 0.05 between the control and the high phosphorus group was considered significant.

Results

Feeding analysis

During the 1-hour feeding period, food intake in the control group was significantly higher than in the high-P group (Table 2). The Ca intake, which was dependent on the total food intake, was higher in the control group than in the high-P group. No significant difference in P intake was observed between the two groups in spite of the finding that the total food intake was significantly lower in the high-P group than in the control group, considering that the P concentration in the high-P diet was three-fold greater than that in the control diet.

Serum analysis

Serum P concentrations increased upon intake of the high P diet, within 30 minutes after the start of feeding. Until 2 hours after the start of feeding, significantly high serum P levels were still observed in the high-P group, compared with the control group. Serum PTH levels also increased upon intake of the high P diet, within 30 minutes after the start of feeding, and the levels were significantly higher in the high-P group than in the control group. The levels of PTH in the high-P group remained significantly high for 2 hours after the start of feeding. Serum Ca levels

### Table 1. Diet Compositions

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (%)</th>
<th>High-P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mil casein</td>
<td>200</td>
<td>200</td>
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<tr>
<td>DL-methionine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>K₃PO₄</td>
<td>19</td>
<td>57</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Sucrose</td>
<td>469</td>
<td>431</td>
</tr>
</tbody>
</table>

1) Calcium and phosphorus-free mineral mixture which had the AIN-76 mineral mixture without Ca and P from.

2) AIN-76 vitamin mixture 1), 2) J. Nutr. 1977; 107, 1340-1348.

### Table 2. Food Intake

<table>
<thead>
<tr>
<th>Food Intake (g)</th>
<th>Control</th>
<th>High-P</th>
<th>Control</th>
<th>High-P</th>
<th>Control</th>
<th>High-P</th>
<th>Control</th>
<th>High-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>5.5 ± 0.1</td>
<td>1.5 ± 0.3 **</td>
<td>6.2 ± 0.4</td>
<td>2.1 ± 0.2 **</td>
<td>5.4 ± 0.4</td>
<td>1.9 ± 0.2 **</td>
<td>5.0 ± 0.1</td>
<td>2.3 ± 0.2 **</td>
</tr>
<tr>
<td>60 min</td>
<td>30.7 ± 0.5</td>
<td>8.2 ± 1.6 **</td>
<td>34.1 ± 2.2</td>
<td>11.5 ± 1.1 **</td>
<td>29.7 ± 2.2</td>
<td>10.4 ± 1.1 **</td>
<td>27.5 ± 0.5</td>
<td>12.6 ± 1.1 **</td>
</tr>
<tr>
<td>90 min</td>
<td>27.8 ± 0.7</td>
<td>23.4 ± 2.1</td>
<td>31.4 ± 2.3</td>
<td>32.8 ± 1.9</td>
<td>27.0 ± 2.3</td>
<td>29.4 ± 1.9</td>
<td>25.3 ± 0.8</td>
<td>34.3 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5).

** Significant difference from control group (p<0.05, 0.01).
changed slightly in response to different dietary P levels, however, no significant difference was observed between the two groups (Fig. 1).

Discussion

This study was done to examine whether PTH secretion is influenced directly by an increase in serum P concentrations in vivo, and to find how much time is required for stimulation of PTH secretion upon intake of dietary P at a high level. Feeding of a high P diet resulted in the development of secondary hyperparathyroidism by decreasing calcitriol synthesis and serum Ca levels. Previously, it has been demonstrated that dietary P has an influence on Ca metabolism and PTH stimulation, however, hyperphosphatemia was not observed despite chronic high P loading. Hypocalcemia has also been observed following intake of a high P diet in cases of hyperparathyroidism. Consequently it was difficult to observe an independent effect in vivo of P on parathyroid function. Therefore, to separate the effects of hyperphosphatemia from hypocalcemia, the rats were given a high P diet acutely and the feeding period until blood collection was arranged to be short, compared with a previous report. Within 30 min after the start of feeding, before the serum Ca levels decreased, an immediate increase in serum levels of P and PTH, as a result of high-level P supplementation, was observed. Test feeding was done after meal feeding for 1 week at separate times, and food intake decreased along with the increase in dietary P levels. Even though there was lower intake of the high-P diet, there was no difference of P-intake between the two groups, because the dietary P concentration was 3 times higher in the high P diet than in the control diet. Under such conditions, there was not a long lag-time before the increase in serum P occurred as a result of high-level dietary P loading. It was decided based on the results of a previous study that the time of blood collection would be set at 1 h after start of the feeding. In that study a decrease in serum Ca concentration influenced by high P status was observed 1 h after the start of feeding a high-P diet. On the other hand, Hernandes et al. have shown that a high P diet containing 1.2% P tended to induce hyperparathyroidism directly within 2 hrs after the start of feeding, regardless of the finding that there was no change in serum levels of calcium or calcitriol. The serum P levels were increased as a result of intake of the high-P diet, and this increase occurred earlier than expected in this study. The differences in the levels of dietary P may be responsible for this difference, affecting the speed of response to the diet. In this study, the high-P diet contained 1.5% P, a 3 times higher P content than that of the control diet. After ingestion of this high P diet, P is readily absorbed in the intestine and the serum P concentration increases immediately. Approximately half of the total intestinal P uptake had been reported in this diffusion pathway. In this study, high-P diet caused an increase in serum P despite P intake between two groups being the same quantity. It was speculated that serum P levels were easy to increase according to P contents per amount of the diet. The Ca intake was found to be lower in the high-P group compared with the control group. Initially, we speculate that the serum Ca level was lower in the high-P group than in the control group as a result of the difference in Ca intake, and this might have been a factor involved in stimulation of PTH secretion. In practice, regardless of low Ca intake, the serum Ca level was almost the same as that in the control group just after feeding the test diet, which was observed within the normal range reported previously. Hypocalcemia has been observed in rats chronically feeding on very high levels of P and in the animal models of renal failure while hyperparathyroidism appeared. Therefore feeding a high-P diet has been used for the induction of secondary hyperparathyroidism in renal failure. We previously reported the indexes of renal function of the rats chronically fed a high-P diet and the result showed the high-P diet favored renal deterioration. In these cases, an expression of renal PTH receptor was down-regulated by the stimulated PTH, then, the production of 1,25(OH)2D3 might be consequently inhibited. However, even if the levels of dietary P was very high, the first change of serum P and PTH had no influence on the apparent serum Ca homeostasis, such like a result of the chronic feeding of some high levels of P. P loading inhibited calcitriol synthesis examined in healthy human subjects and animal, and the calcitriol synthesis deficit caused by P was counterbalanced with the
concurrent increase in PTH levels to maintain normal calcitriol levels, keeping normal Ca homeostasis. We hypothesized that the result of serum Ca in this study was a normal reflection of the high-P diet, because the feeding period was too short to cause renal insufficiency so calcitriol synthesis might be counter-balanced by a high level of PTH.

In this study, 30 min after high-P intake an abrupt change rather than a gradual time-dependent change was observed. For this reason, the response the serum PTH rising after the feeding of the high-P diet was rapid beyond expectation, so the trial to isolate the single effects of serum P on PTH stimulation was unresolved. It is just speculated, however, that the high-P intake may result in an increase in serum P concentration initially, followed by PTH stimulation. Further consideration about a detailed design of blood collection times with high-P loading is needed. In conclusion, our findings suggest that an increase in serum P concentration might be a trigger of PTH secretion without any changes of serum calcium levels.

References


