Comparison of Various Phosphate Salts as the Dietary Phosphorus Source on Nephrocalcinosis and Kidney Function in Rats

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Summary The effects of various phosphate salts as the dietary phosphorus sources on the development of nephrocalcinosis and kidney function were examined in rats fed diets containing monophosphate salts (sodium dihydrogenphosphate, NaH₂PO₄, or potassium dihydrogenphosphate, KH₂PO₄) or polyphosphate salts (sodium tripolyphosphate, Na₅P₃O₁₀, or potassium tripolyphosphate, K₅P₃O₁₀), at levels representing normal phosphorus (normal phosphorus diet) or high phosphorus (high phosphorus diet) contents for 21 d. High phosphorus diet-feeding increased the kidney calcium and phosphorus concentrations. Kidney calcium and phosphorus concentrations were higher in rats fed the high phosphorus diet containing Na₅P₃O₁₀ or K₅P₃O₁₀ than in rats fed the high phosphorus diet containing NaH₂PO₄ or KH₂PO₄. Nephrocalcinosis was observed in all rats fed a high phosphorus diet, and the degree of nephrocalcinosis was more severe in rats fed Na₅P₃O₁₀ or K₅P₃O₁₀ than in rats fed NaH₂PO₄ or KH₂PO₄. In rats fed the high phosphorus diet, creatinine clearance was higher in rats fed Na₅P₃O₁₀ or K₅P₃O₁₀ than in rats fed NaH₂PO₄ or KH₂PO₄. In rats fed Na₅P₃O₁₀ or K₅P₃O₁₀, urinary albumin excretion and N-acetyl-β-D-glucosaminidase (NAG) activity in the urine were increased in rats fed the high phosphorus diet. These were higher in rats fed the high phosphorus diet containing Na₅P₃O₁₀ than in rats fed the high phosphorus diet containing NaH₂PO₄ or KH₂PO₄. This study observed that the development of nephrocalcinosis and kidney function in rats fed the high phosphorus diet was influenced by the difference in monophosphate or polyphosphate salts provided as

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the dietary phosphorus source, while the effects of sodium and potassium salts were not evident. We suggest that the development of nephrocalcinosis and kidney function in rats fed a high phosphorus diet was altered depending on the form of phosphate salts provided as the dietary source of phosphorus. Additionally, the development of nephrocalcinosis and diminished kidney function in rats fed the high phosphorus diet was more severe for polyphosphate salts as compared to monophosphate salts.

Key Words phosphate salts, nephrocalcinosis, kidney function, rats

Mineral concentration in the diet is an important factor in the development of nephrocalcinosis. A magnesium-deficient diet (1, 2) and a diet with a calcium:phosphorus ratio below one (3, 4) produce nephrocalcinosis, and high concentrations of magnesium (5, 6) and fluoride (7, 8) in the diet prevent nephrocalcinosis. Especially, it is known that dietary concentrations of phosphorus play a major role in the development of this disease (9–11). Experimental animals fed a high phosphorus diet have often been shown to display an increase in kidney calcium concentration and nephrocalcinosis as demonstrated by histological examination, whereupon deposition of calcium is found primarily in the corticomedullary junction of the kidney, and in severe cases, this extends towards the cortex or medulla (9–17).

A high phosphorus diet induces not only nephrocalcinosis but also diminished kidney function. Schaafsma et al (18) showed that a high phosphorus diet induces an increase in plasma urea concentration. Ritskes-Hoitinga et al (16) and Van Camp et al (19) reported that urinary albumin excretion was increased in rats fed a high phosphorus diet, and urinary albumin excretion was positively correlated with kidney calcium concentration. In previous studies, we showed that rats fed a high phosphorus diet display an increase in N-acetyl-β-D-glucosaminidase (NAG) activity in the urine, an increase in urinary β2-microglobulin excretion, and ultrastructural changes in the proximal tubules, indicating that a high phosphorus diet depresses proximal tubular function (13, 14).

Thus, there has been a large number of studies examining the effects of a high phosphorus diet on the development of nephrocalcinosis and kidney function (9–14, 16, 18). We have noted that the phosphate salts employed as the source of dietary phosphorus by researchers conducting these studies have not been consistent. This difference in phosphate salts may have influenced the development of nephrocalcinosis and kidney function.

Furthermore, in recent years, our eating habits show a tendency to a remarkably high rate of processed foods. The processed foods are supplemented with various phosphate salts for the purpose of preventing spoiling, and as a result, various phosphate salts are gaining acceptance in our eating habits. Additionally, the intake of various phosphate salts may influence physiological conditions. We must draw attention to the physiological effects of the various phosphate salts provided as dietary phosphorus.

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Thus, it seems very important to compare the physiological effects of the various phosphate salts used in these investigations, as no such comparative study has been reported previously. In this study, we examined whether various phosphate salts as dietary phosphorus sources could differentially effect the development of nephrocalcinosis and kidney function in rats fed diets with normal or high phosphorus contents.

MATERIALS AND METHODS

Animals and diets. Four-week-old male Wistar rats (Clea Japan, Tokyo, Japan) were housed in individual stainless-steel wire-mesh cages in a room with controlled lighting on a 12-h light:dark cycle (light, 0800–2000 h), temperature (22 ± 1°C) and relative humidity (60–65%). For a 7-d acclimation period before initiation of the studies, all rats were given free access to a diet containing 147 mmol phosphorus/kg diet, with sodium dihydrogenphosphate (NaH$_2$PO$_4$) as the phosphorus source, and demineralized water. After the acclimation period, the rats were divided into eight groups of 5 rats each, having a similar mean body weight. Each group was assigned one of the experimental diets. Rats were given free access to the assigned diet and demineralized water throughout the experimental period. At the end of the experiment, all rats were killed by exsanguination from the carotid artery. The experimental period was set at 21 d.

The compositions of the experimental diets are shown in Table 1. The experimental diets were prepared according to the AIN-76 guidelines (20, 21), but the mineral mixture was a modification of AIN-76 mineral mixture without calcium and phosphorus sources. The phosphorus content of the experimental diets was adjusted to either a normal phosphorus content (normal phosphorus diet) or a high phosphorus content (high phosphorus diet). The phosphate salts used as the dietary phosphorus source used were monophosphate salts (NaH$_2$PO$_4$ and potassium dihydrogenphosphate, KH$_2$PO$_4$) and polyphosphate salts (sodium triphosphate, Na$_5$P$_3$O$_10$, and potassium triphosphate, K$_5$P$_3$O$_10$). The analyzed values of phosphorus and calcium contents in the experimental diets and calculated values of sodium and potassium contents in the experimental diets are shown in Table 1. All experimental diets were in powdered form and were stored at 4°C until use.

The study was approved by the Tokyo University of Agriculture Animal Use Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Tokyo University of Agriculture.

Collections of samples. On days 20 to 21, the rats were housed individually in stainless-steel metabolism cages and urine was collected for 24 h from each rat. Blood was collected into tubes from the carotid artery at the time of sacrifice, and centrifuged to obtain the serum. Both kidneys were removed, and the right kidney was used for chemical analysis. The left kidney was used for histological examination. The urine, serum and right kidney were stored at −40°C until chemical analysis.

Chemical analysis. The right kidney was removed and weighed after the renal
Table 1. Compositions of the experimental diets.

| Ingredient       | NaH$_2$PO$_4$ | KH$_2$PO$_4$ | Na$_3$P$_3$O$_10$ | K$_3$P$_3$O$_10$ | g/kg diet | NaH$_2$PO$_4$ | KH$_2$PO$_4$ | Na$_3$P$_3$O$_10$ | K$_3$P$_3$O$_10$ |
|------------------|---------------|--------------|-------------------|------------------|-----------|---------------|--------------|-------------------|------------------|
| Casein           | 200.0         | 200.0        | 200.0             | 200.0            |           | 200.0         | 200.0        | 200.0             | 200.0            |
| Corn starch      | 150.0         | 150.0        | 150.0             | 150.0            |           | 150.0         | 150.0        | 150.0             | 150.0            |
| Cellulose powder | 50.0          | 50.0         | 50.0              | 50.0             |           | 50.0          | 50.0         | 50.0              | 50.0             |
| Corn oil         | 50.0          | 50.0         | 50.0              | 50.0             |           | 50.0          | 50.0         | 50.0              | 50.0             |
| Mineral mixture$^1$ | 35.0        | 35.0         | 35.0              | 35.0             |           | 35.0          | 35.0         | 35.0              | 35.0             |
| Vitamin mixture$^2$ | 10.0       | 10.0         | 10.0              | 10.0             |           | 10.0          | 10.0         | 10.0              | 10.0             |
| DL-Methionine    | 3.0           | 3.0          | 3.0               | 3.0             |           | 3.0           | 3.0          | 3.0               | 3.0              |
| Choline bitartrate | 2.0          | 2.0          | 2.0               | 2.0             |           | 2.0           | 2.0          | 2.0               | 2.0              |
| CaCO$_3$         | 12.5          | 12.5         | 12.5              | 12.5             |           | 12.5          | 12.5         | 12.5              | 12.5             |
| Na$_3$H$_2$PO$_4$ | 11.6         | —            | —                 | —               |           | 54.2          | —            | —                 | —               |
| K$_3$H$_2$PO$_4$ | —             | 13.2         | —                 | —               |           | —             | 61.5         | —                 | —               |
| Na$_3$P$_3$O$_10$ | —             | —            | 11.9              | —               |           | —             | —            | 55.4              | —               |
| K$_3$P$_3$O$_10$ | —             | —            | —                 | 14.5            |           | —             | —            | —                 | 67.5             |
| Sucrose          | 475.9         | 474.3        | 475.6             | 473.0            |           | 433.3         | 426.0        | 432.1             | 420.0            |

| Analyzed values | mmol/kg diet |
|-----------------|--------------|
| Phosphorus content | 149          | 148          | 150              | 145          | 484          | 464          | 452              | 478             |
| Calcium content  | 123          | 124          | 125              | 128          | 123          | 119          | 121              | 116             |
| Sodium content   | 141          | 44           | 206              | 44           | 496          | 44           | 797              | 44              |
| Potassium content| 92           | 189          | 92               | 253          | 92           | 543          | 92               | 844             |

$^1$ The mineral mixture is a modification of AIN-76 mineral mixture without calcium and phosphorus sources.

$^2$ AIN-76A vitamin mixture.
capsule was discarded. The kidney was dried overnight at 100°C, and the dry weight was measured. The kidney and experimental diets were ashed at 550°C for 48 h in a muffle furnace, and the minerals were extracted in a 1-mol/L HCl solution for analysis. Phosphorus in the kidney and experimental diets was analyzed by the method of Gomori (22). Calcium in the kidney and experimental diets was analyzed by atomic absorption spectrophotometry (Shimadzu AA-640-13) (23).

Immediately after completion of urine collection, 24-h urine volume was measured. Creatinine in the serum and urine was determined using the Creatinine-TEST Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Urea nitrogen in the serum was determined using Urea Nitrogen-TEST Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Albumin in the urine was determined using the PANATEST Rat Albumin (Panapharm Laboratories, Co., Ltd., Kumamoto, Japan). The activity of NAG in the urine was determined using NAG TEST Shionogi (Shionogi Co., Ltd., Osaka, Japan).

Urinary excretion of albumin and NAG activity in the urine were expressed in relation to urinary excretion of creatinine.

**Histological examination of the kidney.** Immediately after the left kidney was removed, half of the left kidney was fixed in 10% neutral formalin phosphate buffer. The tissue samples were embedded in paraffin wax, cut into sections 5 μm thick, and the sections were stained with hematoxylin-eosin and Von Kossa’s. The degree of nephrocalcinosis was graded on a scale from 0 (nephrocalcinosis not detected) to 4 (severe nephrocalcinosis) according to a previous report (13).

**Statistical analysis.** Data are expressed as means ± SE. Data were analyzed by two-way ANOVA. Two-way ANOVA was used to determine the effect of dietary phosphorus content and form of phosphate salts. Tukey’s test was used to determine the significant differences of multiple comparisons among groups. Differences were considered significant at p < 0.05. All statistical analyses were performed using the SPSS package program Ver. 6.1J.

**RESULTS**

Body weight is shown in Table 2. Final body weight was lower in rats fed the high phosphorus diet. In rats fed the high phosphorus diet, final body weight was lower in rats fed Na₅P₃O₁₀ or K₅P₃O₁₀ than in rats fed NaH₂PO₄ or KH₂PO₄.

Food and mineral intake are shown in Table 3. High phosphorus diet-feeding decreased the food and calcium intake. Phosphorus intake was increased in rats fed the high phosphorus diet. Food, phosphorus and calcium intake were lower in rats fed the high phosphorus diet containing Na₅P₃O₁₀ or K₅P₃O₁₀ than in rats fed the high phosphorus diet containing NaH₂PO₄ or KH₂PO₄. Irrespective of the dietary phosphorus contents, sodium intake was decreased in rats fed KH₂PO₄ or K₅P₃O₁₀, and potassium intake was increased in rats fed KH₂PO₄ or K₅P₃O₁₀ as compared to rats fed NaH₂PO₄ or Na₅P₃O₁₀.

Kidney weight and water content and mineral concentrations in the kidney
Table 2. Body weight in rats fed the experimental diets.

|                      | Normal phosphorus diet | High phosphorus diet |
|----------------------|------------------------|----------------------|
|                      | NaH$_2$PO$_4$  | KH$_2$PO$_4$ | Na$_2$P$_3$O$_{10}$ | K$_3$P$_3$O$_{10}$ | NaH$_2$PO$_4$  | KH$_2$PO$_4$ | Na$_2$P$_3$O$_{10}$ | K$_3$P$_3$O$_{10}$ | Two-way ANOVA |
| Initial weight (g)   | 130 ± 3            | 128 ± 2          | 129 ± 2             | 130 ± 2            | 127 ± 3        | 128 ± 1          | 129 ± 1             | 128 ± 2            | L, S, L × S   |
| Final weight (g)     | 284 ± 5            | 276 ± 8          | 283 ± 7             | 286 ± 3            | 219 ± 6*       | 218 ± 10*        | 184 ± 5b*           | 163 ± 5b*           |               |

Values are means ± SE (n = 5).

Significant effects (p < 0.05): L, effect of dietary phosphorus content; S, effect of form of phosphate salts; L × S, effect of interaction.

* Significantly different from normal phosphorus diet matched with the form of phosphate salt (p < 0.05).

Values with different superscript letters in the same row are significantly different (p < 0.05).

Table 3. Food and mineral intake in rats fed the experimental diets.

|                      | Normal phosphorus diet | High phosphorus diet |
|----------------------|------------------------|----------------------|
|                      | NaH$_2$PO$_4$  | KH$_2$PO$_4$ | Na$_2$P$_3$O$_{10}$ | K$_3$P$_3$O$_{10}$ | NaH$_2$PO$_4$  | KH$_2$PO$_4$ | Na$_2$P$_3$O$_{10}$ | K$_3$P$_3$O$_{10}$ | Two-way ANOVA |
| Food intake (g/d)    | 17.8 ± 0.2           | 17.7 ± 0.7        | 18.3 ± 0.5          | 18.9 ± 0.2         | 13.1 ± 0.5ab* | 14.3 ± 0.7*       | 10.8 ± 0.4bc*       | 10.1 ± 0.5cd*       | L, S, L × S |
| Phosphorus intake (mmol/d) | 2.98 ± 0.05   | 2.93 ± 0.14      | 2.95 ± 0.15         | 2.92 ± 0.02       | 7.20 ± 0.35bc*| 7.25 ± 0.17a*    | 4.59 ± 0.26b*        | 4.83 ± 0.28b*        | L, S, L × S |
| Calcium intake (mmol/d) | 2.46 ± 0.04    | 2.45 ± 0.11      | 2.44 ± 0.13         | 2.61 ± 0.02       | 1.83 ± 0.09bc*| 1.85 ± 0.04b*    | 1.23 ± 0.07b*        | 1.17 ± 0.06b*        | L, S, L × S |
| Sodium intake (mmol/d) | 2.81 ± 0.05ab | 0.875 ± 0.042b  | 4.04 ± 0.21a        | 0.899 ± 0.006a    | 7.38 ± 0.36bc*| 0.693 ± 0.016b  | 8.10 ± 0.46a**       | 0.447 ± 0.026a       | L, S, L × S |
| Potassium intake (mmol/d) | 1.83 ± 0.03a | 3.73 ± 0.18ab | 1.81 ± 0.09a        | 5.14 ± 0.04a      | 1.37 ± 0.07a  | 8.50 ± 0.20b**  | 0.936 ± 0.053a       | 8.51 ± 0.49ab**       | L, S, L × S |

Values are means ± SE (n = 5).

Significant effects (p < 0.05): L, effect of dietary phosphorus content; S, effect of form of phosphate salts; L × S, effect of interaction.

* Significantly different from normal phosphorus diet matched with the form of phosphate salt (p < 0.05).

Values with different superscript letters in the same row are significantly different (p < 0.05).
Table 4. Kidney weight and water content and mineral concentrations in the kidney in rats fed the experimental diets.

|                     | Normal phosphorus diet | High phosphorus diet | Two-way ANOVA |
|---------------------|------------------------|----------------------|---------------|
|                     | NaH₂PO₄ | KH₂PO₄ | Na₃P₂O₁₀ | K₃P₂O₁₀ | NaH₂PO₄ | KH₂PO₄ | Na₃P₂O₁₀ | K₃P₂O₁₀ | L, S, L × S |
| Wet weight (g/100 g body weight) | 0.372 ± 0.006 | 0.371 ± 0.005 | 0.391 ± 0.006 | 0.375 ± 0.007 | 0.934 ± 0.031** | 0.730 ± 0.026b** | 1.06 ± 0.08** | 0.752 ± 0.039b** | L, S, L × S |
| Dry weight (g/100 g body weight) | 0.086 ± 0.001 | 0.086 ± 0.001 | 0.096 ± 0.002 | 0.092 ± 0.002 | 0.167 ± 0.003** | 0.130 ± 0.003** | 0.227 ± 0.017** | 0.169 ± 0.010** | L, S, L × S |
| Water (g) | 0.813 ± 0.023 | 0.786 ± 0.029 | 0.836 ± 0.019 | 0.811 ± 0.019 | 1.68 ± 0.08** | 1.30 ± 0.04** | 1.54 ± 0.11b, c** | 0.949 ± 0.045c | L, S, L × S |
| Calcium (mmol/100 g dry weight) | 0.926 ± 0.021 | 0.918 ± 0.040 | 0.902 ± 0.051 | 0.912 ± 0.031 | 108 ± 13** | 70.2 ± 12.2a | 302 ± 22b** | 255 ± 33** | L, S, L × S |
| Phosphorus (mmol/100 g dry weight) | 41.5 ± 0.4 | 42.3 ± 0.2 | 39.2 ± 0.2 | 39.0 ± 0.2 | 98.8 ± 6.7** | 66.4 ± 4.2** | 189 ± 11b** | 166 ± 16** | L, S, L × S |

Values are means ± SE (n = 5).
Significant effects (p < 0.05): L, effect of dietary phosphorus content; S, effect of form of phosphate salts; L × S, effect of interaction.
* Significantly different from normal phosphorus diet matched with the form of phosphate salt (p < 0.05).
a,b,c Values with different superscript letters in the same row are significantly different (p < 0.05).
Table 5. Incidence and degree of nephrocalcinosis in rats fed the high phosphorus diet.

|          | NaH₂PO₄ | KH₂PO₄ | Na₅P₃O₁₀ | K₅P₃O₁₀ |
|----------|----------|--------|----------|----------|
| Incidence| 5/5      | 5/5    | 5/5      | 5/5      |
| Degree score¹ |          |        |          |          |
| 0        | 0        | 0      | 0        | 0        |
| 1        | 2        | 4      | 0        | 0        |
| 2        | 3        | 1      | 0        | 1        |
| 3        | 0        | 0      | 1        | 2        |
| 4        | 0        | 0      | 4        | 2        |
| Mean score² | 1.6      | 1.2    | 3.8      | 3.2      |

¹ Degree score of nephrocalcinosis: 0 (nephrocalcinosis not detected)<1<2<3<4 (severe nephrocalcinosis). Degree score of nephrocalcinosis was graded according to the previous report (13).

² Values are means (n=5).

are shown in Table 4. Kidney wet and dry weights increased in rats fed the high phosphorus diet, and these were lower in rats fed the high phosphorus diet containing KH₂PO₄ or K₅P₃O₁₀ than in rats fed the high phosphorus diet containing NaH₂PO₄ or Na₅P₃O₁₀. The high phosphorus diet resulted in a higher kidney water content. In rats fed the high phosphorus diet, kidney water content decreased in rats fed KH₂PO₄ or K₅P₃O₁₀. Kidney calcium and phosphorus concentrations were higher in rats fed the high phosphorus diet. In rats fed the high phosphorus diet, Na₅P₃O₁₀ or K₅P₃O₁₀-feeding increased kidney calcium and phosphorus concentrations.

The results of histological examination of the kidney are shown in Table 5. Nephrocalcinosis was observed in all of the rats fed the high phosphorus diet. The degree of nephrocalcinosis was more severe in rats fed Na₅P₃O₁₀ or K₅P₃O₁₀ than in rats fed NaH₂PO₄ or KH₂PO₄. Nephrocalcinosis was not observed in rats fed the normal phosphorus diet, irrespective of the form of phosphate salts provided as the dietary phosphorus source (data not shown).

Urinary volume, creatinine clearance, serum urea nitrogen concentration, urinary albumin excretion and NAG activity in the urine are shown in Table 6. In rats fed the high phosphorus diet, creatinine clearance increased more in rats fed Na₅P₃O₁₀ or K₅P₃O₁₀ as compared to rats fed NaH₂PO₄ or KH₂PO₄. Serum urea nitrogen concentration increased in rats fed the high phosphorus diet, and there was no change dependent on the form of phosphate salts provided as the dietary phosphorus source. In rats fed Na₅P₃O₁₀ or K₅P₃O₁₀, high phosphorus diet-feeding increased the urinary albumin excretion and NAG activity in the urine. Urinary albumin excretion and NAG activity in the urine were higher in rats fed the high phosphorus diet containing Na₅P₃O₁₀ than in rats fed the high phosphorus diet containing NaH₂PO₄ or KH₂PO₄.
### Table 6. Indicators of kidney function in rats fed the experimental diets.

|                              | Normal phosphorus diet | High phosphorus diet | Two-way ANOVA |
|------------------------------|------------------------|----------------------|---------------|
|                              | NaH₂PO₄ | KH₂PO₄ | Na₅P₃O₁₀ | K₃P₃O₁₀ | NaH₂PO₄ | KH₂PO₄ | Na₅P₃O₁₀ | K₃P₃O₁₀ |              |
| Urinary volume (mL/d)        | 11.9 ± 2.4 | 8.48 ± 1.53 | 7.48 ± 0.92 | 11.5 ± 2.5 | 14.1 ± 1.4 | 14.4 ± 1.9 | 22.6 ± 1.5* | 18.8 ± 2.2 | L, S            |
| Creatinine clearance (mL/min/100 g body weight) | 0.279 ± 0.010 | 0.255 ± 0.005 | 0.295 ± 0.016 | 0.309 ± 0.014 | 0.250 ± 0.009* | 0.280 ± 0.023* | 0.345 ± 0.034* | 0.490 ± 0.021* | L, S, L × S |
| Urea nitrogen in serum (mmol/L) | 7.38 ± 0.39 | 7.39 ± 0.55 | 7.79 ± 0.11 | 7.93 ± 0.40 | 12.7 ± 1.0* | 13.8 ± 1.1* | 10.8 ± 0.9 | 11.9 ± 0.8* | L |
| Albumin in urine (g/mol creatinine) | 1.46 ± 0.12 | 1.83 ± 0.28 | 1.71 ± 0.49 | 1.71 ± 0.32 | 6.76 ± 1.03* | 7.04 ± 1.54* | 19.9 ± 4.8* | 11.3 ± 1.6* | L, S, L × S |
| NAG activity in urine (U/mmol creatinine) | 2.52 ± 0.54 | 2.62 ± 0.55 | 2.36 ± 0.24 | 2.26 ± 0.36 | 4.06 ± 0.54* | 3.62 ± 0.21* | 6.02 ± 0.65* | 4.53 ± 0.45* | L, L × S |

Values are means ± SE (n = 5).
Significant effects (p < 0.05): L, effect of dietary phosphorus content; S, effect of form of phosphate salts; L × S, effect of interaction.
* Significantly different from normal phosphorus diet matched with the form of phosphate salt (p < 0.05).

a,b,c Values with different superscript letters in the same row are significantly different (p < 0.05).
DISCUSSION

Previous studies (9–17) have reported the effects of dietary phosphorus content on the development of nephrocalcinosis and kidney function, however, the effects of various phosphate salts provided as the dietary phosphorus source on the development of nephrocalcinosis and kidney function have not been reported. Accordingly, in this study, we examined two issues: 1) the effects of various phosphate salts as the dietary phosphorus source on the development of nephrocalcinosis in rats fed a diet with normal or high phosphorus contents; and 2) the effects of various phosphate salts as dietary phosphorus source on kidney function in rats fed a diet with normal or high phosphorus contents.

In previous studies, we reported that rats fed a high phosphorus diet with K₅P₃O₁₀ as the dietary phosphorus source displayed increased kidney mineral (calcium and phosphorus) concentrations, as determined by chemical analysis, and nephrocalcinosis, as demonstrated by histological examination (12–15). Additionally, in this study, we observed increased kidney mineral concentrations and nephrocalcinosis in all of the rats fed the high phosphorus diet containing either NaH₂PO₄, KH₂PO₄, Na₅P₃O₁₀ or K₅P₃O₁₀ as the dietary source of phosphorus. However, kidney mineral concentrations in rats fed polyphosphate salts were higher than those in rats fed monophosphate salts, and the degree of nephrocalcinosis was more severe in rats fed polyphosphate salts. Thus, our findings in this study demonstrate that kidney mineral concentrations and the degree of nephrocalcinosis in rats fed a high phosphorus diet differ depending on the form of phosphate salts provided as the phosphorus source in the diet. These results suggest that, in rats fed a high phosphorus diet, various phosphate salts in the diet can vary the effect of nephrocalcinosis development, although high phosphorus diet-feeding induces nephrocalcinosis irrespective of the form of phosphate salts provided as the dietary phosphorus source.

This study observed that phosphorus intake in rats fed the high phosphorus diet decreased in rats fed polyphosphate salts as compared to rats fed monophosphate salts provided as the dietary phosphorus source. Additionally, the degree of nephrocalcinosis was more severe in rats fed polyphosphate salts than that in rats fed monophosphate salts. The results concerning phosphorus intake and degree of nephrocalcinosis was seen to introduce the following contradiction: polyphosphate salts-feeding induced severe nephrocalcinosis, although phosphorus intake was lower in rats fed polyphosphate salts as compared to rats fed monophosphate salts. With regard to the mechanism responsible for the difference in development of nephrocalcinosis in animals fed different phosphate salts, this cannot be ascertained from the results of the present study. However, we speculate that changes in calcium and phosphorus bioavailability (due to changes in absorption, retention and/or excretion) may, at least in part, account for the difference in development of nephrocalcinosis observed. Thus, it is of interest to further examine the effects of various phosphate salts provided as the dietary
phosphorus source on calcium and phosphorus bioavailability. Furthermore, we observed that sodium or potassium intake were differed among the groups of rats fed various phosphate salts provided as the dietary phosphorus source. However, kidney mineral concentrations and the development of nephrocalcinosis were not different between the groups of rats fed sodium salts or potassium salts provided as the phosphorus source in the diet. These findings indicate that the development of nephrocalcinosis is not influenced by sodium or potassium intake. Additionally, we suggest that the development of nephrocalcinosis is influenced by the difference of monophosphate salts or polyphosphate salts rather than the difference of sodium salts or potassium salts provided as the dietary phosphorus source.

A high phosphorus diet has been shown to induce an increase in kidney water content, and edema is observed upon histological examination (13). In this study, increased kidney water content was evident in the case of rats fed a high phosphorus diet, and this result implies that a high phosphorus diet induces renal edema. Additionally, in rats fed a high phosphorus diet, kidney water content was higher in rats fed sodium salts than in rats fed potassium salts. This finding indicates that, in rats fed a high phosphorus diet, more severe renal edema is induced by sodium salts than by potassium salts provided as the phosphorus source in the diet.

Previously, we reported that creatinine clearance was increased in rats fed a high phosphorus diet (12-14), whereas Schaafsma et al (18) reported that creatinine clearance was decreased and Ritskes-Hoitinga et al (16) reported that there was no change. These differences may be explained by the results of this study. In this study, creatinine clearance in rats fed the high phosphorus diet was increased in rats fed polyphosphate salts as compared to rats fed monophosphate salts. Furthermore, in rats fed polyphosphate salts, the high phosphorus diet increased creatinine clearance, but the effects of the high phosphorus diet on creatinine clearance were not evident in rats fed monophosphate salts. These findings suggest that creatinine clearance is influenced by the form of phosphate salts provided as the dietary phosphorus source rather than the phosphorus content of the diet. We conclude that the differences in results obtained in previous studies (12-14, 16, 18) were due to differences in the phosphorus source used. K₅P₃O₁₀ was used as the phosphorus source in our previous studies (12-14), whereas Ritskes-Hoitinga et al (16) used NaH₂PO₄·2H₂O.

Urinary albumin excretion was increased in the rats fed a high phosphorus diet, consistent with the results of other studies (16, 19). In rats fed the high phosphorus diet, urinary albumin excretion was higher in rats fed polyphosphate salts than in rats fed monophosphate salts provided as the phosphorus source in the diet. This difference in urinary albumin excretion, depending on the form of phosphate salt in the diet, may be a reflection of the degree of diminished proximal tubular function. Our previous studies (13, 14) showed that a high phosphorus diet induced diminished proximal tubular function but did not cause injury to the renal glomerular basement membrane, and suggested that the increase in urinary albumin excretion in rats fed a high phosphorus diet may be due to the obstruction of
proximal tubular albumin reabsorption. In this study, NAG activity in the urine was increased in rats fed the high phosphorus diet. This result indicates that diminished proximal tubular function is induced in rats fed a high phosphorus diet, consistent with previous reports (13, 14). In addition, in rats fed the high phosphorus diet, NAG activity in the urine was higher in rats fed polyphosphate salts than in rats fed monophosphate salts. These findings indicate that the degree of diminished proximal tubular function is more severe in rats fed the high phosphorus diet containing polyphosphate salts than in rats fed the high phosphorus diet containing monophosphate salts. In other words, the obstruction of albumin reabsorption in the proximal tubules is more severe and thus more clearly evident in rats fed polyphosphate salts than in rats fed monophosphate salts. We suggest that, in rats fed a high phosphorus diet, different phosphate salts affect urinary albumin excretion to a different degree and this is responsible for the difference in the degree of diminished proximal tubular function. Additionally, the difference in the degree of diminished proximal tubular function in rats fed various phosphate salts can be explained by the degree of nephrocalcinosis. This study shows that, in rats fed the high phosphorus diet, nephrocalcinosis was more severe in rats fed polyphosphate salts than in rats fed monophosphate salts. This result suggests that rats fed polyphosphate salts would have severe calcium deposition and injury in the proximal tubules as well; consequently, proximal tubular function is more severely depressed in rats fed polyphosphate salts than in rats fed monophosphate salts. Additionally, we suggest that the difference in the degree of diminished proximal tubular function observed with various phosphate salts as the dietary phosphorus source is due to the difference in the development of nephrocalcinosis. Furthermore, the effects of sodium salts and potassium salts on urinary albumin excretion and NAG activity in the urine were not evident. Therefore, we suggest that urinary albumin excretion and NAG activity in the urine are influenced by the difference of monophosphate salts or polyphosphate salts rather than the difference of sodium salts or potassium salts.

In conclusion, the results of this study indicate that the development of nephrocalcinosis and kidney function in rats fed the high phosphorus diet were influenced by the form of phosphate salts provided as the dietary phosphorus source. Such differences in the physiological effects of various phosphate salts provided as the dietary phosphorus source were not evident in rats fed the diet with normal phosphorus content. Additionally, this study observed that the development of nephrocalcinosis and kidney function in rats fed the high phosphorus diet was strongly influenced by the difference of monophosphate salts or polyphosphate salts rather than the difference of sodium salts or potassium salts. The results in this study suggest that we should consider not only the amount of phosphate salts but also the form of phosphate salts used as food additives for the prevention of nephrocalcinosis and diminished kidney function.
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