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Original Article

Thyroparathyroidectomy Suppresses Urinary Albumin Excretion and Urinary N-Acetyl- β -D-Glucosaminidase Activity in Rats Fed a High-Phosphorus Diet

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Abstract

This study examined the effects of thyroparathyroidectomy (TPTX) on kidney function in rats fed a high-phosphorus (P) diet. Female Wistar rats received either a sham operation (sham) or TPTX. Each surgical group was fed diets containing P concentrations of either 0.3% (control diet) or 1.2% (high-P diet) for 21 days. Calcium (Ca) deposits were observed in the sham rats fed the high-P diet. In contrast, there was no evidence of Ca deposits in the TPTX rats fed the high-P diet. With respect to kidney function, creatinine clearance was significantly decreased in the TPTX rats compared to sham rats. However, dietary treatments had no significant effect on creatinine clearance in sham and TPTX rats. Urinary albumin excretion was significantly increased in sham rats fed the high-P diet, while no significant differences were observed in TPTX rats fed the control and high-P diets. N-acetyl- β -D-glucosaminidase (NAG) activity in the urine was significantly increased in sham rats fed the high-P diet. In TPTX rats, no significant difference in urinary NAG activity was observed between the control and high-P diet groups. This study suggests that TPTX suppresses diminished kidney function under high-P diet administration.

Key words : Thyroparathyroidectomy, Kidney Function, High-phosphorus Diet, Rats

I. INTRODUCTION

One of the characteristic phenomena caused by high-phosphorus (P) diet feeding is the induction of nephrocalcinosis, a disorder involving calcium (Ca) deposits in the kidney. Experimental animals fed a high-P diet have been shown to display increased kidney Ca and P concentrations and an increased incidence of Ca deposits, as demonstrated by histological examination[1-3]. On the other hand, it is speculated that a high-P diet may induce diminished kidney function as well as the development of nephrocalcinosis. Therefore, previous studies examined the effects of high-P diet on the biochemical indicators of kidney function. Ritskes-Hoitinga et al.[4] and Van Camp et al.[5] reported that urinary albumin excretion, which is positively correlated with kidney Ca concentration, is elevated in rats fed a high-P diet. Our previous studies showed that urinary N-acetyl- β -D-glucosaminidase (NAG) activity and β 2-microglobulin excretion were increased in rats fed a high-P diet[6,7]. Thus, results in previous studies suggest that a high-P diet not only induces nephrocalcinosis, but also diminishes kidney function.

Other previous studies have reported that a high-P diet does not induce nephrocalcinosis in thyropara-thyroidectomized (TPTX) rats or parathyroidectomized (PTX) rats[8-10]. It is important to note the results of these previous studies that TPTX or PTX prevents nephrocalcinosis in rats fed a high-P diet. Based on these observations, we speculate that because the changes in kidney function induced by a high-P diet are suggested to result from the development of nephrocalcinosis, TPTX may have a preventive effect on diminished kidney function due to high-P diet, as well as on the development of nephrocalcinosis. However, no study has yet assessed whether TPTX has preventive effect on diminished kidney function due to a high-P diet. Accordingly, to determine the effects of TPTX, the present study examined the biochemical indicators of kidney function in TPTX rats fed a high-P diet.

II. MATERIALS AND METHODS

1. Animals and diets

Six-week-old female Wistar rats were divided into two surgical groups; the groups underwent either TPTX or a sham operation (sham). TPTX and sham rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and individually housed in stainless-steel wire-mesh cages. During the experiment, cages were located in a room with controlled lighting under a 12-h light:dark cycle (light, 08:00-20:00), at a temperature of 22 ± 1° C and relative humidity of 60-65%. In this study, animals subjected to TPTX had serum Ca levels ≤ 7.0 mg/dL, in accordance with levels reported in previous study[10]. Serum Ca levels of TPTX rats were between 5.0 and 6.95 mg/dl, while those of sham rats were between 9.71 and 10.69 mg/dL. Experimental diets were based on AIN-93G diet[11]. Two experimental diets containing the two different P concentrations (control diet, 0.3%; high-P diet, 1.2%). All the experimental diets had the same Ca (0.5%) and Mg (0.05%) concentration. There was a 4-d acclimatization period prior to the beginning of the study during which all rats were given free access to control diet and deionized water. After the acclimatization period, each surgical group was randomly divided into two groups, each of which was assigned to one of the experimental diets. Rats were given free access to the experimental diet and deionized water for 21 d. On the last day of the experiment, urine was collected over a 24 h period from each rat using stainless-steel metabolic cages, and the urine was analyzed to determine the levels of various indicators of kidney function. At the end of the experiment, rats were sacrificed under diethyl ether. Blood was collected in tubes, and centrifuged to obtain the serum. The right kidney was collected for histological examination. The present study was approved by the Animal Use Committee at the Ibaraki Christian University, and all animals were maintained in accordance with the university's guidelines for the care and use of laboratory animals.

2. Chemical analysis and histological examination

Samples were ashed at 550° C for 48 h in a muffle furnace, and minerals were extracted in 1 mol/L of HCl for analysis. P was analyzed using the method of Gomori[12]. Ca and magnesium (Mg) were determined by atomic absorption spectrophotometry (ANA-182; Tokyo Photo Electric, Tokyo, Japan)[13]. Urea nitrogen in serum was determined with a Urea N B (Wako Pure Chemical Industries, Osaka, Japan). Creatinine in serum and urine was determined with a LabAssay™ Creatinine (Wako Pure Chemical Industries, Osaka, Japan). Urinary NAG activity was determined with an NAG Test Shionogi (Shionogi, Osaka, Japan). Albumin in urine was determined with a Panatest Rat Albumin (Mitsubishi Chemical Medience, Kumamoto, Japan). The right kidney was fixed in 4% paraformaldehyde for subsequent processing immediately after being removed and paraffin embedded following routine methods for histopathology. Sections (3 μm) were cut and stained with von Kossa's solution. The degree of Ca deposits was graded on a scale from 0 (not detected) to 4 (severe) (Fig. 1).

3. Statistical analysis

Results are expressed as means ± SE. Data were analyzed by two-way ANOVA to determine the effect of surgical operation and effect of dietary P concentration. Tukey's 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 and intake of food and minerals

Final body weight and intake of food, Ca and Mg were significantly decreased in TPTX rats compared to the sham rats (Table 1). P intake was significantly decreased in TPTX rats fed the high-P diet compared to the sham rats fed the high-P diet, but was significantly increased in the high-P diet group, irrespective of the surgical operation.

2. Biochemical indicators of kidney function

In control diet group, creatinine clearance was significantly decreased in TPTX rats compared to the sham rats, but no significant differences in the creatinine clearance of high-P diet group was observed between the sham and TPTX rats (Table 2). Urinary albumin excretion was significantly increased in sham rats fed the high-P diet compared to the sham rats fed the control diet. In TPTX rats, no significant difference in urinary albumin excretion was observed between the control and high-P diet groups. Urinary NAG activity of high-P diet group was significantly decreased in TPTX rats. Urinary NAG activity was also significantly increased in sham rats fed the high-P diet compared with those fed the control diet, while no significant differences were observed in TPTX rats fed the control and high-P diets. Urinary P excretion of high-P diet group was significantly decreased in the TPTX rats compared to the sham rats. Furthermore, urinary P excretion was significantly increased in rats fed the high-P diet, irrespective of the surgical operation. In TPTX rats, urinary Ca excretion was significantly decreased in the high-P diet group compared to

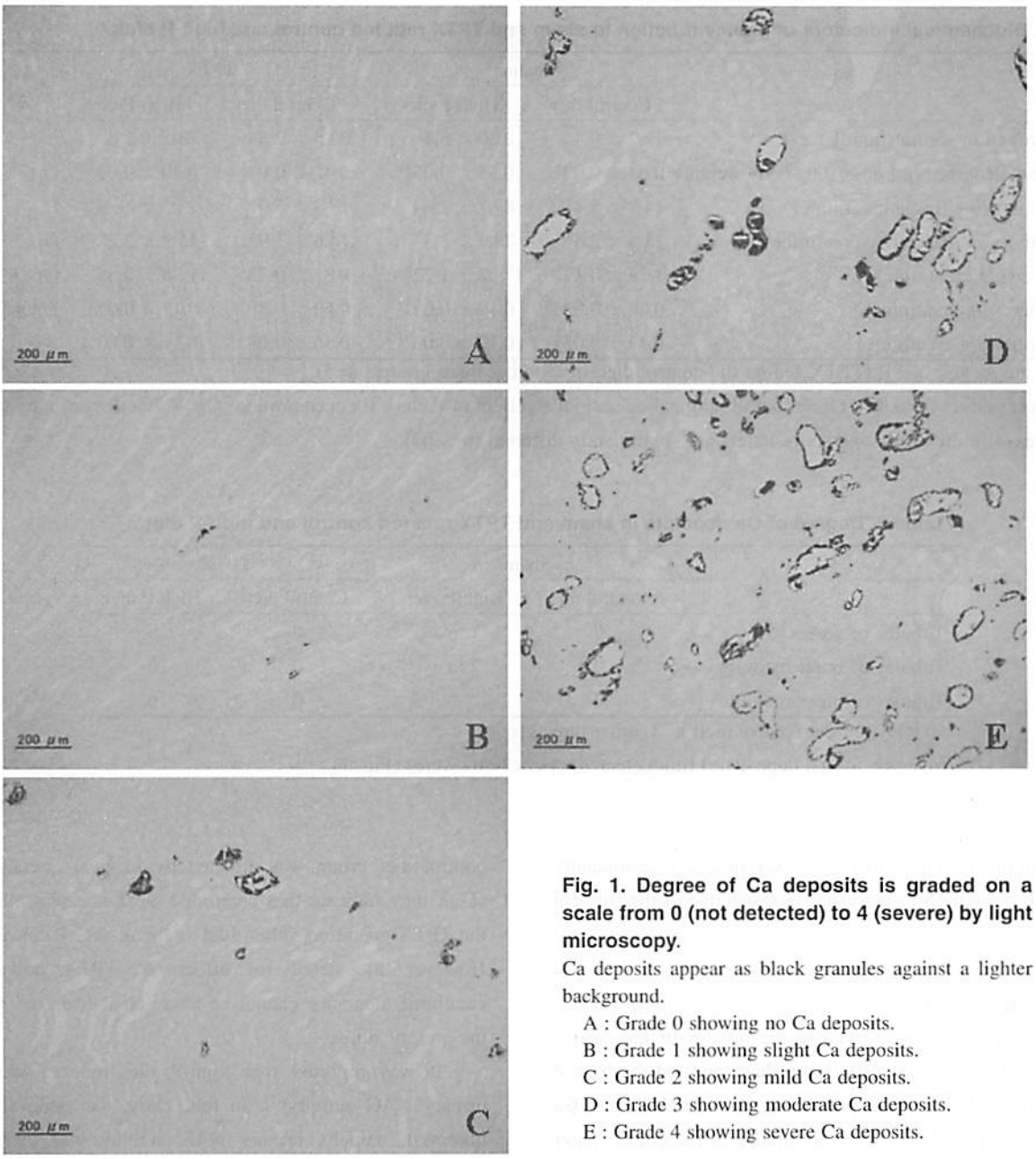

Fig. 1. Degree of Ca deposits is graded on a scale from 0 (not detected) to 4 (severe) by light microscopy.

Ca deposits appear as black granules against a lighter background.

A : Grade 0 showing no Ca deposits.
B : Grade 1 showing slight Ca deposits.
C : Grade 2 showing mild Ca deposits.
D : Grade 3 showing moderate Ca deposits.
E : Grade 4 showing severe Ca deposits.

Table 1. Body weight and intake of food and minerals in sham and TPTX rats fed control and high-P diets[1].

	Sham		TPTX		Two-way ANOVA[2]
	Control diet	High-P diet	Control die t	High-P diet	
Initial body weight (g)	178.7 ± 3.5	180.0 ± 2.8	168.2 ± 5.6	170.7 ± 4.0	Op
Final body weight (g)	251.0 ± 5.1[a]	239.3 ± 6.3[a]	188.4 ± 9.4[b]	167.8 ± 5.1[b]	Op, P
Food intake (g/d)	16.3 ± 0.6[a]	16.3 ± 0.6[a]	10.9 ± 0.8[b]	9.3 ± 0.8[b]	Op
P intake (mg/d)	50.0 ± 2.0[a]	197.4 ± 7.1[b]	33.6 ± 2.4[a]	112.5 ± 9.3[c]	Op, P, Op × P
Ca intake (mg/d)	84.4 ± 3.3[a]	83.2 ± 3.0[a]	56.7 ± 4.0[b]	47.4 ± 3.9[b]	Op
Mg intake (mg/d)	7.58 ± 0.30[a]	7.82 ± 0.28[a]	5.09 ± 0.36[b]	4.46 ± 0.37[b]	Op

[1] Values are means ± SE (TPTX fed on the control diet: n =3, other three groups: n=5).
[2] Significant effect ($p<0.05$): Op=effect of surgical operation; P=effect of dietary P concentration; Op × P=effect of interaction.
[a,b] Values with different superscript letters are significantly different ($p<0.05$).

Table 2. Biochemical indicators of kidney function in sham and TPTX rats fed control and high-P diets[1].

	Sham		TPTX		Two-way ANOVA[2]
	Control diet	High-P diet	Control diet	High-P diet	
Urea nitrogen in serum (mg/dL)	17.2 ± 0.4	21.0 ± 3.4	22.5 ± 2.8	23.5 ± 1.6	
Creatinine clearance (mL/min/100g body weight)	0.63 ± 0.03^{a}	0.53 ± 0.05^{a,c}	0.42 ± 0.03^{b,c}	0.40 ± 0.03^{b,c}	Op
Albumin in urine (mg/g creatinine)	14.7 ± 5.4^{a}	87.7 ± 25.4^{b}	18.3 ± 5.9^{a,b}	23.5 ± 10.9^{a}	P
NAG activity in urine (U/g creatinine)	18.5 ± 2.3^{a}	28.9 ± 2.3^{b}	14.6 ± 3.9^{a}	15.9 ± 2.2^{a}	Op, P
P in urine (g/g creatinine)	1.79 ± 0.17^{a}	17.02 ± 1.22^{b}	0.81 ± 0.23^{a}	11.38 ± 0.59^{c}	Op, P, Op × P
Ca in urine (g/g creatinine)	0.27 ± 0.05^{a,b}	0.19 ± 0.03^{a}	0.60 ± 0.20^{b}	0.17 ± 0.05^{a}	P, Op × P
Mg in urine (g/g creatinine)	0.49 ± 0.05^{a}	0.19 ± 0.03^{b,c}	0.36 ± 0.08^{a,b}	0.14 ± 0.03^{c}	P

[1] Values are means ± SE (TPTX fed on the control diet: n =3, other three groups: n=5).

[2] Significant effect ($p<0.05$): Op=effect of surgical operation; P=effect of dietary P concentration; Op × P=effect of interaction.

^{a,b,c} Values with different superscript letters are significantly different ($p<0.05$).

Table 3. Degree of Ca deposits in sham and TPTX rats fed control and high-P diets[1,2].

	Sham		TPTX	
	Control diet	High-P diet	Control diet	High-P diet
Tubules of cortex	0	1 to 3	0	0
Tubules of outer medulla	0	2 to 4	0	0
Tubules of inner medulla	0	2	0	0

[1] TPTX fed the control diet: n=3, other three groups: n=5.

[2] Degree for Ca deposits: 0 (not detected)<1<2<3<4(severe) (Fig. 1)

the control diet group. Urinary Mg excretion was significantly decreased in the high-P diet group, irrespective of the surgical operation.

3. Ca deposits in kidney

Ca deposits were observed in the tubules of the cortex, outer medulla and inner medulla of sham rats fed the high-P diet (Table 3). Notably, despite being fed a high-P diet, Ca deposits were not observed in the tubules of the cortex, outer medulla or inner medulla of TPTX rats. On the other hand, no evidence of Ca deposits was observed in the control diet group, irrespective of the sham operation and TPTX.

IV. DISCUSSION

Previous studies have reported the effects of TPTX or PTX on the development of nephrocalcinosis[8-10], however, the effects of TPTX or PTX on the kidney function under high-P diet administration have not been reported. Accordingly, this study examined the effects of TPTX on kidney function in rats fed a high-P diet.

Dietary P concentration had no significant influence on the creatinine clearance, which is consistent with the results of other study[4]. On the other hand, creatinine clearance of control diet group was affected by surgical operation. This result may indicate that creatinine clearance was affected by the TPTX operation rather than by the dietary P concentration. However, the details of effects of TPTX operation on creatinine clearance cannot be made clear from the results of the present study.

It was reported that high-P diet induces increase in urinary NAG activity[6,7]. In this study, the same result was observed, namely, urinary NAG activity was increased in sham rats fed the high-P diet. Because renal tubular lesions enhance urinary NAG activity, the increase in urinary NAG activity due to a high-P diet may be explained by occurrence of Ca deposits in the renal tubules. This study observed that Ca deposits were induced in the renal tubules of sham rats fed the high-P diet. Therefore, increased NAG activity in the urine due to a high-P diet is thought to be the result of lesions with Ca deposits in renal tubules. Moreover, this study showed that in spite of high-P diet feeding, NAG activity in the urine was not increased in TPTX rats. This finding is especially noteworthy in that TPTX inhibited urinary NAG activity under a high-P diet. The inhibitory effect of TPTX on urinary NAG activity observed in this study is related to alterations in renal tubule Ca deposits. As described above, although Ca deposits appeared in the renal tubules of sham rats fed the high-P diet,

no evidence of Ca deposits was observed in the renal tubules of TPTX rats fed the high-P diet. Thus, we suggest that TPTX does not induce Ca deposits in the renal tubules and as a result, inhibits increased urinary NAG activity due to high-P diet administration.

A high-P diet has been reported to induce an increase in urinary albumin excretion[4,5], and an increase in urinary albumin excretion is known to be caused by a defect in the permeability of the renal glomerular basement membrane[14]. Albumin resorption occurs mainly in the proximal tubules[15]. With regard to increased urinary albumin excretion under high-P diet administration, our previous studies[6,7] showed that a high phosphorus diet induced an increase in indicators of proximal tubular function and injury of proximal tubules, but did not cause injury of the renal glomerular basement membrane, and suggested that the increase in urinary albumin excretion due to high-P diet may be due to the obstruction of proximal tubular albumin resorption. In other words, renal tubular function insufficiency has been linked to an increase in urinary albumin excretion in rats fed a high-P diet. In the present study, Ca deposits in the renal tubules and an increase in urinary NAG activity were evident in sham rats fed the high-P diet, which is indicative of renal tubular function insufficiency. Therefore, we suggest that increased urinary albumin excretion in sham rats fed the high-P diet may be due to renal tubular function insufficiency. Furthermore, we observed that increased urinary albumin excretion was not induced in TPTX rats fed the high-P diet. This result indicates that TPTX has an inhibitory effect on urinary albumin excretion under high-P diet administration. Moreover, results in our study suggest that inhibition of Ca deposits in the renal tubules by TPTX, leading to the prevention of renal tubular function insufficiency, was responsible for the inhibition in urinary albumin excretion under high-P diet administration.

In this study, urinary P excretion of high-P diet group was decreased in the TPTX rats compared to the sham rats, whereas no differences in urinary Ca and Mg excretion were found between sham and TPTX rats. Results in this study indicate that TPTX had no effect on the urinary excretion of Ca and Mg. On the other hand, other studies reported that PTX rats showed decreased urinary excretion of Ca, P and Mg[16,17] and increased urinary Ca excretion[18]. Clark and Rivera-Cordero[19] reported that no significant differences in urinary excretion of Ca, phosphate and Mg were found between intact and PTX rats. Thus, the changes in urinary excretion of Ca, P and Mg observed in these studies were not congruent. One reason for these differences may be variances in dietary condition, age and sex in used rats and feeding period. However, the changes in urinary excretion of minerals may, at least in part, account for the preventive effect of TPTX or PTX on nephrocalcinosis development[20].

In conclusion, we examined the effects of TPTX on kidney function in rats fed a high-P diet. Ca deposits in the renal tubules and increased urinary albumin excretion and NAG activity were observed in sham rats fed the high-P diet. However, no evidence of Ca deposits was observed in the TPTX rats fed the high-P diet. Furthermore, TPTX suppressed increases in urinary albumin excretion and urinary NAG activity under high-P diet. Accordingly, our results suggest that TPTX suppresses diminished kidney function under high-P diet administration.

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