

## Nephrocalcinosis and Urinary Mineral Concentrations in Rats Fed Diets Containing Supplemental Chloride

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**Abstract:** It has been shown that the feeding of an anion-rich diet with supplemental ammonium chloride inhibits the development of nephrocalcinosis in female rats. The present study was carried out to obtain further insight into the inhibitory effect of dietary chloride on nephrocalcinosis. Female rats were fed high-chloride diets with the same chloride contents but containing either ammonium chloride or calcium chloride. The diets were formulated so that chloride was the only variable versus the control diet. Chloride feeding markedly lowered kidney calcium concentrations and nephrocalcinosis scores. The high-chloride diets reduced urinary pH values and raised urinary concentrations of calcium and magnesium but did not influence those of phosphorus. It is concluded that high intakes of ammonium chloride or calcium chloride lower the degree of nephrocalcinosis in female rats through a decrease in urinary pH and an increase in urinary magnesium concentrations.

**Key words:** Rats, nephrocalcinosis, diet, chloride, urine, pH

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### INTRODUCTION

In female rats, acidification of urine by the feeding of diets containing either ammonium chloride or ammonium sulphate inhibits the intratubular deposition of calcium phosphates in the corticomedullary junction of the kidney (Kootstra *et al.*, 1991). The development of kidney calcification (nephrocalcinosis) depends on the pH and mineral composition of urine. High pH values, high calcium and high phosphate concentrations promote the precipitation of calcium phosphates (Greenwald, 1945) whereas the addition of magnesium counteracts precipitation (Boulet *et al.*, 1962).

The observed reduction of nephrocalcinosis after ingestion of ammonium chloride can be explained by urine acidification (Kootstra *et al.*, 1991) which causes dissolution of existing calcium phosphates in the urine and prevents precipitation (Greenwald, 1945). The feeding of high-chloride diets to rats raises urinary calcium excretion (Kootstra *et al.*, 1991; Alhaidary *et al.*, 2010). High urinary calcium concentrations are expected to stimulate nephrocalcinogenesis (Greenwald, 1945; Hoek *et al.*, 1988). Thus, it appears that the chloride-induced lowering of urinary pH has a greater impact on nephrocalcinogenesis than the elevation of urinary calcium concentrations. However, it was not known whether chloride-mediated changes in urinary magnesium

and phosphorus concentrations, if any play a role in the reduction of nephrocalcinosis. This study with female rats was carried out to describe the impact of chloride feeding on nephrocalcinosis in combination with the simultaneous effects on urinary pH and the concentrations of calcium, magnesium and phosphorus in urine.

The rats were fed diets containing supplemental chloride in the form of either ammonium chloride or calcium chloride. It was anticipated that this study contributes to further understanding of the inhibitory effect of dietary chloride on nephrocalcinosis.

### MATERIALS AND METHODS

**Rats and treatments:** The rats and diets were also used in another study described elsewhere (Alhaidary *et al.*, 2010). Female Wistar rats (CPB:WU), aged about 3 weeks were fed on the pre-experimental, purified diet (Table 1) for 1 week. Subsequently (day 0), the rats were divided into three groups of 10 rats each so that group mean body weights and distributions were similar.

One group remained on the pre-experimental control diet and the other groups were fed the diets rich in chloride. The high-chloride diets contained either 0.3 mol ammonium chloride or 0.15 mol calcium chloride per kg. The ammonium chloride was substituted for an equimolar amount of ammonium carbonate and calcium

Table 1: Ingredient and analyzed composition of the experimental diets

Ingredients	Diet code		
	Control	NH <sub>4</sub> Cl	CaCl <sub>2</sub>
<b>Ingredient (g/1000 g)</b>			
CaCO <sub>3</sub>	15.00	15.00	-
(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	14.40	-	14.40
NH <sub>4</sub> Cl	-	16.10	-
CaCl <sub>2</sub>	-	-	16.70
Glucose	692.40	690.70	690.70
Constant components <sup>1</sup>	278.20	278.20	278.20
Total	1000.00	1000.00	1000.00
<b>Chemical analysis (g/100 g)</b>			
Calcium	0.59	0.58	0.58
Magnesium	0.04	0.04	0.04
Phosphorus	0.40	0.38	0.39

<sup>1</sup>The constant components consisted of (g/1000 g diet): casein, 151; corn oil, 25; coconut fat, 25; cellulose, 30; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 15.1; MgCO<sub>3</sub>, 1.4; KCl, 1.0; KHCO<sub>3</sub>, 7.7; mineral premix, 10.0; vitamin premix, 12.0. The composition of the mineral and vitamin premix has been published elsewhere (Mars *et al.*, 1988)

chloride was substituted for calcium carbonate (Table 1). The diets which were in powdered form were stored at 4°C until used for feeding. The rats had free access to feed and demineralized water for a period of 6 weeks.

Body weights and feed consumption were measured. The rats were housed individually in metabolic cages. The animal room had controlled temperature (20-22°C), relative humidity (40-60%) and controlled lighting (light: 06.00-18.00 h).

**Methods:** The methods of collection of samples, chemical analyses and histological examination have been described elsewhere.

**Statistics:** The results are presented as means±SD for 10 rats per dietary treatment. Statistical analysis was performed as described with  $p < 0.05$  as criterion of statistical significance.

## RESULTS

Average initial and final body weights were 82.8 and 204.5 g. The diets high in chloride did not influence final body weight and growth rate. Feed intake was unaffected by dietary treatment; it was on average 14.2 g day<sup>-1</sup>.

The degree of nephrocalcinosis was evaluated by mineral analyses and histological assessment. Table 2 shows that the diets containing either ammonium chloride or calcium chloride markedly lowered kidney calcium concentrations and histological scores. Kidney phosphorus concentrations were also decreased after the feeding of the high-chloride diets but magnesium contents were not affected. The diets with ammonium or calcium chloride significantly reduced urinary pH values

Table 2: Mineral concentrations in kidney and nephrocalcinosis in rats fed the experimental diets

Mineral concentration	Diet code		
	Control	NH <sub>4</sub> Cl	CaCl <sub>2</sub>
<b>Kidney measures</b>			
Dry weight (mg)	178±25 <sup>a</sup>	171±15 <sup>a</sup>	164±13 <sup>a</sup>
Calcium (%)	4.04±2.59 <sup>a</sup>	0.41±0.24 <sup>b</sup>	0.47±0.27 <sup>b</sup>
Magnesium (%)	0.19±0.02 <sup>a</sup>	0.18±0.02 <sup>a</sup>	0.17±0.01 <sup>a</sup>
Phosphorus (%)	3.42±0.59 <sup>a</sup>	1.26±0.11 <sup>a</sup>	1.27±0.11 <sup>a</sup>
<b>Nephrocalcinosis</b>			
Incidence	10/10	0/10	0/10
Severity	2.4 <sup>a</sup>	0.2 <sup>b</sup>	0.2 <sup>b</sup>

Means in the same row not sharing the same superscript are significantly different

Table 3: Urinary pH and urinary mineral concentrations in rats fed the experimental diets

Experimental diet	Diet code		
	Control	NH <sub>4</sub> Cl	CaCl <sub>2</sub>
<b>Urine production (mL day<sup>-1</sup>)</b>			
Days 24-27	6.2±3.7 <sup>a</sup>	8.7±3.7 <sup>b</sup>	8.9±4.0 <sup>b</sup>
Days 38-41	8.3±1.6 <sup>a</sup>	10.6±4.3 <sup>b</sup>	9.0±3.0 <sup>b</sup>
<b>Urinary pH</b>			
Days 24-27	8.9±0.3 <sup>a</sup>	5.6±0.9 <sup>b</sup>	5.4±0.5 <sup>b</sup>
Days 38-41	9.5±0.1 <sup>a</sup>	5.4±0.3 <sup>b</sup>	5.6±0.4 <sup>b</sup>
<b>Urinary calcium (mg mL<sup>-1</sup>)</b>			
Days 24-27	0.09±0.07 <sup>a</sup>	0.47±0.19 <sup>b</sup>	0.36±0.12 <sup>b</sup>
Days 38-41	0.08±0.05 <sup>a</sup>	0.41±0.03 <sup>b</sup>	0.31±0.12 <sup>b</sup>
<b>Urinary magnesium (mg mL<sup>-1</sup>)</b>			
Days 24-27	0.13±0.06 <sup>a</sup>	0.18±0.08 <sup>a</sup>	0.15±0.03 <sup>a</sup>
Days 38-41	0.09±0.03 <sup>a</sup>	0.14±0.03 <sup>b</sup>	0.13±0.04 <sup>b</sup>
<b>Urinary phosphorus (mg mL<sup>-1</sup>)</b>			
Days 24-27	1.75±1.12 <sup>a</sup>	1.33±0.68 <sup>a</sup>	1.43±0.19 <sup>a</sup>
Days 38-41	1.59±0.35 <sup>a</sup>	1.42±0.49 <sup>a</sup>	1.45±0.04 <sup>a</sup>

Means in the same row not sharing the same superscript are significantly different

and increased urinary volume (Table 3). Chloride feeding significantly raised the urinary concentrations of calcium. Group mean urinary magnesium concentrations were increased after feeding the high-chloride diets. The concentrations of phosphorus in urine were not significantly influenced by the diets rich in chloride.

## DISCUSSION

In this study, a nephrocalcinogenic diet was used. The diet consisted of semipurified ingredients, contained 0.6% calcium, 0.4% phosphorus and 0.04% magnesium and induced an alkaline urinary pH. Rats fed the control diet had a mean kidney calcium concentration of 4.0%. This amount of kidney calcium can be considered very high when compared to levels seen in rats fed on commercial diets that are based on natural ingredients (Ritskes-Hoitinga *et al.*, 1991). As would be expected (Kootstra *et al.*, 1991), the addition of ammonium chloride to the diet drastically diminished the severity of nephrocalcinosis. There was no difference in the nephrocalcinosis-lowering activity of dietary ammonium

chloride and calcium chloride. The control and high-chloride diets were formulated so that the dietary concentration of chloride was the only variable. The diets with either ammonium chloride or calcium chloride contained identical amounts of chloride. Thus, the nephrocalcinosis-lowering activity of the high-chloride diets was caused by the supplemental chloride only.

The two high-chloride diets not only induced the same degree of nephrocalcinosis but they also had similar effects on urinary pH and urinary concentrations of calcium and magnesium whereas urinary phosphorus concentrations were not affected. The development of nephrocalcinosis is inhibited by lowering of urinary pH values, calcium and phosphorus concentrations and elevation of urinary magnesium concentrations (Greenwald, 1945; Boulet *et al.*, 1962).

The high-chloride diets did not influence urinary phosphorus. The chloride induced increase in urinary magnesium concentrations seems to be small but the changes may have been sufficient to decrease nephrocalcinosis. This notion is based on the study describing the dose-response relationship between magnesium intake and the severity of nephrocalcinosis.

The high-chloride diets produced a marked decrease in urinary pH. The increase in urinary calcium concentrations by itself would stimulate nephrocalcinosis but this effect was masked by the nephrocalcinosis-lowering action of low urinary pH and increased urinary magnesium concentrations.

The increase in urinary calcium concentrations seen after feeding the diets with either ammonium chloride or calcium chloride may be explained by diminished tubular reabsorption of calcium. It has been shown that a chloride-rich diet reduced the blood concentration of bicarbonate (Gevaert *et al.*, 1991; Schonewille *et al.*, 1999) and the excretion of bicarbonate with urine (Schonewille *et al.*, 1999) which in turn may inhibit the tubular reabsorption of calcium (Peraino and Suki, 1980). Possibly, a similar mechanism holds for magnesium.

## CONCLUSION

It is clear from this study that high intakes of ammonium chloride or calcium chloride lower the degree

of nephrocalcinosis in female rats. This effect can be explained by a decrease in urinary pH and an increase in urinary magnesium concentrations.

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