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Calcium Metabolism in Rats Fed Diets Containing Supplemental Chloride

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Abstract: It has been observed earlier that an increase in the dietary chloride concentration causes higher rates of calcium excretion with urine in rats. The hypothesis tested in this study was that the chloride-induced rise in urinary calcium excretion is associated by an increase in intestinal calcium absorption and/or a lowering of calcium deposition in tibia. Female rats aged 4 weeks were fed a purified control diet or a diet containing either 1.61% ammonium chloride or 1.67% calcium chloride. The three diets had identical calcium concentrations and were fed for a period of 6 weeks. There was no effect of dietary treatment on growth and feed intake. Chloride loading produced a significant increase in urinary calcium excretion, the source of chloride not having a differential effect. Apparent calcium absorption and tibia calcium concentrations were not affected by high chloride intake. It remains unknown how calcium homeostasis is attained in rats fed chloride-rich diets.

Key words: Rats, chloride, calcium, metabolism, excretion, tibia, Saudi Arabia

INTRODUCTION

In various species, the ingestion of supplemental chloride, in the form of either ammonium or calcium chloride, acidifies the urine and raises urinary excretion of calcium (Lemann *et al*., 1967; Whiting and Cole, 1986; Ching *et al*., 1989; Kootstra *et al*., 1991; Schonewille *et al*., 1994). In rats, it was found that the urinary acid excretion correlated with calcium excretion (Jacob *et al*., 1983). It would be expected that the chloride-induced increase in urinary calcium excretion is associated with enhanced intestinal calcium absorption in order to maintain calcium homeostasis. At identical calcium intake and without compensation for the increased excretion of calcium with urine there will be demineralization of the bones in adult animals and diminished deposition of calcium in the skeleton of young growing animals.

An increase in calcium absorption has indeed been shown in dairy cows fed supplemental chloride (Schonewille *et al*., 1994) but such an effect was not seen in rats (Greger *et al*., 1991). Some researchers have noted that chloride loading caused loss of bone minerals (Petito and Evans, 1984; Kunkel *et al*., 1986; Kaup and Greger, 1990; Greger *et al*., 1991) but researchers observed no effect (Newell and Beauchene, 1975). Thus, it remains unknown if calcium homeostasis is achieved after the feeding of diets enriched with chloride.

It is likely that there is a form of adaptation after prolonged high-chloride intake in order to ensure maintenance or growth of bone. In the present study, the researchers measured the possible responses of calcium metabolism to the feeding of high-chloride diets. The questions addressed were whether the feeding of diets containing either ammonium chloride or calcium chloride would raise intestinal calcium absorption and/or reduce calcium deposition in tibia in an attempt to compensate for the increased excretion of calcium with urine.

MATERIALS AND METHODS

Rats and treatments: Female Wistar rats (CPB:WU), aged about 3 weeks were used. During the pre-experimental period of 1 week, all rats were fed on the control diet containing 0.5 calcium, 0.4 phosphorus and 0.04% magnesium (Table 1). At the end of the pre-experimental period (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 control diet and the other groups were fed either the diet supplemented with 0.3 mol ammonium chloride or 0.15 mol calcium chloride kg^{-1}. Ammonium chloride was added to the control diet at the expense of an equimolar amount of ammonium carbonate. Calcium chloride was subsituted for calcium carbonate (Table 1).

The control diet was formulated to have a positive cation-anion balance so that the effect of acid loading would be more pronounced. The diets were in powdered form and were stored at 4°C until feeding. Food and demineralized water were supplemented *ad libitum*

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Table 1: Ingredient and analyzed composition of the experimental diets

	Diet code		
Diets	Control	NH_4Cl	$CaCl_2$
Ingredient (g/1000 g)			
$CaCO_3$	15.00	15.00	-
$(NH_4)_2CO_3$	14.40	-	14.40
NH_4Cl	-	16.10	-
$CaCl_2$	-	-	16.70
Glucose	692.40	690.70	690.70
Constant components[1]	278.20	278.20	278.20
Chemical analysis (g 100 g^{-1})			
Calcium	0.59	0.58	0.58[1]

The constant components consisted of (g/1000 g diet): casein, 151; corn oil, 25; coconut fat, 25; cellulose, 30; $NaH_2PO_4.2H_2O$, 15.1; $MgCO_3$, 1.4; KCl, 1.0; $KHCO_3$, 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)

for a period of 6 weeks. Feed consumption and initial and final body weights were recorded. The rats were housed individually in metabolic cages placed in a room with controlled temperature (20-22°C), relative humidity (40-60%) and controlled lighting (light: 06.00-18.00 h).

Collection of samples: During days 24-27 and 38-41, feces and urine of each rat were collected quantitatively. Urine was collected in tubes containing two drops of a 0.2% (w/v) sodium azide solution. Urinary volumes and pH were measured.

On day 42, blood samples were taken by orbital puncture in heparinized tubes while the rats were under diethyl ether anesthesia. Plasma was collected by low speed centrifugation and then stored at -20°C until analyses. The rats were killed by decapitation. Tibias were removed, weighed and stored at -20°C.

Chemical procedures: Samples of feed, plasma, feces and urine were processed and analyzed exactly as has been described elsewhere (Mohamed *et al*., 2010a).

Statistics: Results are shown as means±SD for 10 rats per dietary group. The data were statistically analyzed using a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL 1998). Differences between treatments were evaluated with the use of Duncan's multiple range test. The level of statistical significance was pre-set at $p<0.05$.

RESULTS

Supplemental ammonium chloride or calcium chloride did not significantly influence final body weight and feed intake (Table 2). Weight gain of the 3 diet groups was similar.

Table 2: Growth performance of the rats fed the experimental diets

	Diet code		
Body weight (g)	Control	NH_4Cl	$CaCl_2$
Initial, day 0	82.3±3.70	82.9±3.70	83.1 ± 4.0
Final, day 42	205.3±17.1	206.9±16.5	201.4±21.5
Feed intake, g day^{-1}	14.1±1.80	14.7±1.90	13.7±1.50
Growth, g day^{-1}	2.9±0.40	3.0±0.30	2.8±0.40

Table 3: Urinary pH, urinary calcium excretion and intestinal calcium absorption in rats fed the experimental diets

	Diet code		
Parameters	Control	NH_4Cl	$CaCl_2$
Urinary pH			
Days 24-27	8.9±0.30	5.6±0.90	5.4±0.50
Days 38-41	9.5±0.10	5.4±0.30	5.6±0.40
Urinary calcium excretion (μ mol day^{-1})			
Days 24-27	14.3±10.8[a]	102.3±42.2[b]	80.8±27.9[b]
Days 38-41	15.8±9.2[a]	108.9±29.7[b]	69.1±27.0[b]
Apparent calcium absorption (intake %)			
Days 24-27	57.0±3.80	57.4±10.4	55.4±8.00
Days 38-41	48.1±11.0	45.4±9.30	46.4±15.5

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

Table 4: Plasma calcium concentrations and calcium deposition in tibia of rats fed the experimental diets

	Diet code		
Results	Control	NH_4Cl	$CaCl_2$
Plasma calcium (mmol L^{-1})	2.69±0.08	2.65±0.08	2.62±0.05
Tibia calcium (dry weight %)	23.7±2.30	24.5±1.20	23.8±1.00

The addition of either ammonium chloride or calcium chloride to the diet significantly lowered urinary pH (Table 3). Urinary calcium excretion was markedly raised by feeding the high-chloride diets, the increase being similar for ammonium chloride and calcium chloride. Apparent calcium absorption was lower during days 38-41 than during days 24-27 but there was no significant effect of high chloride intake.

Plasma concentrations of calcium were similar for the three dietary groups (Table 4). The addition of choride to the diet did not significantly influence calcium concentration in tibia.

DISCUSSION

As would be expected, the addition of either ammonium chloride or calcium chloride to the diet lowered urinary pH and produced an increase in urinary calcium excretion. The feeding of a chloride-rich diet has been shown to reduce the blood concentration of bicarbonate (Gevaert *et al*., 1991; Schonewille *et al*., 1999) and the excretion of bicarbonate with urine (Schonewille *et al*., 1999). In dogs, tubular reabsorption of calcium was negatively correlated with the excretion of bicarbonate in urine (Peraino and Suki, 1980). It may be suggested that acid loading enhances urinary calcium excretion through diminshed tubular reabsorption of calcium.

Supplemental chloride in the diet did neither influence calcium deposition in tibia nor apparent calcium absorption. The rats fed the high-chloride diets were able to maintain normal plasma calcium concentrations and were apparently healthy as indicated by the unaffected growth performance. At least for the duration of the experiment the loss of calcium with urine had no negative impact on the rats. However, continuation of the extra urinary calcium loss without enhanced calcium absorption efficiency must lead to lower calcium concentrations in bone. The daily extra calcium loss as induced by chloride feeding was in the order of 7% of the daily apparent calcium absorption and thus will have an impact sooner or later.

The high-chloride diets did not influence the efficiency of calcium absorption. The literature data on chloride feeding and calcium absorption do not provide a clear picture. In dairy cows fed rations enriched with chloride there was an increase in calcium absorption (Schonewille *et al*., 1994). In cats, chloride feeding in the form of calcium chloride has been shown to raise calcium absorption (Pastoor *et al*., 1994a, b) but in another study with cats fed on a diet with supplemental ammonium chloride, a decrease in intestinal calcium absorption was found (Ching *et al*., 1989). In a study with rats given drinking water containing 1% ammonium chloride, a decrease in calcium absorption was observed (Petito and Evans, 1984). As explained earlier, it is most likely that the feeding of supplemental chloride raises urinary calcium excretion which in turn causes stimulation of calcium absorption to compensate for the extra calcium loss.

It has been suggested that the extra urinary calcium in cows fed a chloride-rich ration originates from an increase in bone resorption (Block, 1984) or from a decrease in bone accretion (Van Mosel *et al*., 1994). These suggestions would imply demineralization of the skeleton or depressed deposition of minerals. However, such situations cannot be everlasting and the amount of calcium in the skeleton has to reach a steady state at some point. This steady state can only be reached when the increase in urinary calcium is compensated for by an increase in calcium absorption. However, before or after the steady state is reached, the calcium content of bone may be lowered. Kunkel *et al*. (1986) shown that the feeding of a diet containing ammonium chloride lowered calcium concentrations in the femur of rats. In this study, there was no influence of chloride feeding on the calcium concentration in tibia. Other researchers (Newell and Beauchene, 1975; Greger *et al*., 1991) also showed that chloride feeding to rats did not affect calcium concentrations in bone.

CONCLUSION

This study shows that high-chloride intakes by rats raised urinary calcium excretion but did not influence intestinal calcium absorption and calcium deposition in tibia. Thus, it remains unknown how calcium homeostasis is attained in rats fed chloride-rich diets. The same holds for other dietary variables that raise urinary calcium excretion such a high magnesium intakes (Mohamed *et al*., 2010a) and the feeding of a diet containing galacturonic acid (Mohamed *et al*., 2010b).

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