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Nephrocalcinosis and Urinary Mineral Concentrations in Rats Fed Diets Containing Various Concentrations of Magnesium

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Abstract: High magnesium intakes are known to inhibit the development of nephrocalcinosis in female rats but there was no information on the dose-response relationship and the underlying mechanism. In an attempt to collect the lacking information, female rats were fed diets containing 0.02-0.24% magnesium as the only variable. Increasing dietary magnesium concentrations were found to reduce nephrocalcinosis in a dose-dependent fashion. The lowest dietary magnesium level produced a kidney calcium concentration of 10.6% in the dry matter whereas the highest magnesium intake reduced kidney calcium to 0.2%. Increasing dietary magnesium concentrations produced increasing urinary magnesium concentrations in combination with decreasing phosphorus concentrations. It is suggested that the magnesium-induced inhibition of nephrocalcinosis is caused by a decrease in urinary phosphorus and increase in urinary magnesium.

Key words: Rats, nephrocalcinosis, diet, urine, magnesium, phosphorus, concentrations

INTRODUCTION

In young female rats fed purified diets, nephrocalcinosis is a common condition (Hoek *et al*., 1988; Mars *et al*., 1988) that involves the intratubular deposition of calcium phosphates in the corticomedullary junction of the kidney (Nguyen and Woodard, 1980). The mineral composition of the diet is an important determinant of the development of nephrocalcinosis. Diets that are low in magnesium (Bunce *et al*., 1965), high in phosphorus (Mars *et al*., 1988) or high in calcium (Hoek *et al*., 1988) promote the renal deposition of calcium phosphates. It is likely that such diets change the mineral composition of urine leading to the formation of calcium phosphate precipitates in the tubular fluid, thereby inducing nephrocalcinosis. This notion is supported by test tube experiments showing that high calcium and high phosphate concentrations promote the precipitation of calcium phosphates (Greenwald, 1945) whereas, the addition of magnesium has an inhibitory effect (Boulet *et al*., 1962). The protecting effect of high magnesium intake against the development of nephrocalcinosis in female rats has been described by various researchers (Bunce *et al*., 1965; Bergstra *et al*., 1992; Sterck *et al*., 1992). However, the dose-response relationship for dietary magnesium and nephrocalcinosis, if any had not been described. In this study, female rats were fed diets containing different concentrations of magnesium as the only variable and the degree of nephrocalcinosis was assessed. An attempt was made to disclose to what extent the inhibitory effect of dietary magnesium on nephrocalcinosis is mediated through changes in the urinary concentrations of calcium, phosphorus and magnesium.

MATERIALS AND METHODS

Rats and treatments: The rats and diets were also used in researchers study described elsewehere (Mohamed *et al*., 2010). About 3 weeks old, female Wistar rats (CPB:WU) were fed on a purified diet containing 0.5% calcium, 0.04% magnesium and 0.4% phosphorus (Table 1) for a period of 7 days. Then, the rats were divided into five groups with similar group mean body weights. Each group was allocated to one of the five experimental diets containing different amounts of magnesium. There were six rats per dietary treatment. One group continued on the diet with 0.04% magnesium. The other four groups received diets containing either 0.02, 0.06, 0.12 or 0.24% magnesium. Table 1 shows the ingredient composition of the diets and the analysed concentrations of calcium, magnesium and phosphorus. The diets were in powdered form and were stored at 4°C until feeding. The rats had free access to feed and demineralized water for a period of 6 weeks. Feed consumption and body weights were recorded. The rats were housed individually in metabolic cages.

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Table 1: Ingredient and analyzed composition of the experimental diets

	Diet code, magnesium[1] (%)				
Parameters	0.02	0.04[2]	0.06	0.129	0.24
Ingredients (g/1000 g)					
Glucose	710.10	709.40	708.00	705.2	699.60
$MgCO_3$	0.70	1.40	2.80	5.6	11.20
Constant components[3]	289.20	289.20	289.20	289.2	289.20
Total	1000.00	1000.00	1000.00	1000.0	1000.00
Chemical analysis (g/100 g)					
Magnesium	0.02	0.04	0.06	0.12	0.24
Calcium	0.47	0.48	0.49	0.49	0.49
Phosphorus	0.38	0.38	0.38	0.36	0.36

[1]Calculated magnesium concentrations; [2]Pre-experimental diet, [3]The constant components consisted of (g/1000 g diet): casein, 151; corn oil, 25; coconut fat, 25; cellulose, 30; $CaCO_3$, 12.4; $NaH_2PO_4.2H_2O$, 15.1; 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)

The animal room had controlled temperature (20-22°C), relative humidity (40-60%) and controlled lighting (light: 06.00-8.00 h).

Collection of samples: Urine of each rat was collected quantitatively from days 17-21 and 39-42. Urine was collected in containers containing 50 μL of 0.2% (w/v) NaN_3 as a preservative. On day 42, the rats were killed by decapitation and the kidneys were removed. After removal of capsules, kidneys were weighed. The left kidneys were fixed in formalin for histological analysis and the right kidneys were frozen at -20°C until analyses.

Chemical procedures: Homogenized feed samples and freeze-dried kidneys were ashed (500°C, 17 h) and dissolved in a solution of 6 mol L^{-1} HCl. After appropriate dilution with distilled water, magnesium and calcium were analysed by atomic absorption spectroscopy in the presence of 5% (w/v) $LaCl_3$ (Varian Atomic Absorption Spectrophotometer type AA-475, Springvale, Australia). Total phosphorus was analysed with the use of a commercial test combination (Phosphate, MA-KIT 10 ROCHE, Roche Diagnostics, Basel, Switserland) and a Cobas-Bio autoanalyser (Hoffman-La Roche BV, Mijdrecht, The Netherlands). For complete recovery of phosphate, analysis was performed at least 5 days after dissolving the sample in Hcl. Urine samples were acidified to pH 1-2 with 6 mol L^{-1} HCl and centrifuged; the supernatant was frozen at -20°C. Calcium, magnesium and phosphorus in urine were analysed as described above.

Histological methods: The left kidneys were embedded in paraffine wax after being sectioned longitudinally. Sections of 5 μm thickness were stained by Von Kossa's method. The severity of nephrocalcinosis was graded on a scale from 0 (absence of calcium deposits) to 3 (severe calcinosis). To aid in scoring, four reference slides were used. The kidneys were scored in random order and blind by two researchers. The score of each rat was the average score of the assessors.

Statistics: Results are presented as means±SD for six rats per dietary treatment. Statistical analysis was done with the use of a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL 1998). Differences between group means were evaluated with the use of Duncan's multiple range test. Mann-Whitney U-test was used to compare histological scores of different diet groups. The level of statistical significance was pre-set at $p<0.05$.

RESULTS AND DISCUSSION

Initial and final body weights on average were 76.2 and 204.9 g. The amount of magnesium in the diet did not influence final body weight and growth rate. Feed intake which was on average 13.8 g day^{-1} was similar for the five dietary treatments. Both mineral analyses and histological evaluation were used to assess the degree of nephrocalcinosis. Table 2 shows that increasing dietary magnesium concentrations lowered kidney calcium and phosphorus concentrations and also the histological scores in a dose-dependent fashion. The dry weight of kidney was reduced by higher intakes of magnesium. The magnesium concentration in whole kidney was raised by increasing dietary magnesium levels, the effect being dose dependent. The amount of magnesium in the diet influenced the urinary concentrations of calcium, magnesium and phosphorus. During days 17-21, urinary calcium concentration was increased when the magnesium level of the diet was higher than 0.02% but the four higher levels produced similar urinary calcium concentrations. During days 39-42, group mean urinary calcium concentrations were elevated when the diet contained more than 0.02% magnesium but the increase reached statistical significance for the diet with 0.12% magnesium only. Higher intakes of magnesium generally induced higher group mean urinary concentrations of magnesium. Urinary phosphorus concentrations fell with increasing magnesium intakes.

For the urine collection period of days 17-21, the dose dependency was more evident than for the period of days 39-42 (Table 3). The purified diets used in this study contained 0.5% calcium and 0.4% phosphorus. This combination of calcium and phosphorus level may be considered nephrocalcinogenic in female rats (Hoek *et al*., 1988; Mars *et al*., 1988). The present study confirms that an increase in magnesium intake lowers the severity of nephrocalcinosis in female rats. The new finding is that the inhibitory effect of dietary magnesium on the

Table 2: Mineral concentrations in kidney and degree of nephrocalcinosis

	Diet code (magnesium %)				
Parameters	0.02	0.04	0.06	0.12	0.24
Kidney measures					
Dry weight (mg)	243±55^{a}	198±23^{ab}	181±2.5^{b}	172±21^{b}	165±5^{b}
[1]Calcium (%)	10.6±4.1^{a}	6.5±3.8^{ab}	2.7±2.5^{bc}	1.9±1.8^{bc}	0.2±0.2^{c}
[1]Magnesium (%)	0.15±0.01^{a}	0.17±0.01^{ab}	0.18±0.03^{ab}	0.21±0.06^{ab}	0.23±0.05^{b}
[1]Phosphorus (%)	6.0±2.0^{a}	4.4±1.6^{ab}	2.8±0.03^{bc}	2.8±0.7^{bc}	2.1±0.1^{c}
Nephrocalcinosis					
Incidence	6/6	6/6	5/6	4/6	0/6
Severity (0-3)	3.8^{a}	2.6^{ab}	1.8^{b}	1.03^{b}	0^{c}

[1]On a dry weight basis Means in the same row not sharing the same superscript are significantly different

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

Urinary	Diet code (magnesium %)				
concentrations	0.02	0.04	0.06	0.12	0.24
Urinary calcium (mg mL^{-1})					
Days 17-21	0.05±0.02^{a}	0.10±0.05^{b}	0.11±0.04^{b}	0.10±0.06^{b}	0.10±0.04^{b}
Days 39-42	0.05±0.01^{a}	0.08±0.04^{a,b}	0.11±0.06^{a,b}	0.12±0.02^{b}	0.09±0.03^{a,b}
Urinary magnesium (mg mL^{-1})					
Days 17-21	0.06±0.05^{a}	0.11±0.03^{b}	0.19±0.05^{b}	0.27±0.21^{b}	0.79±0.10^{c}
Days 39-42	0.05±0.02^{a}	0.11±0.02^{b}	0.18±0.07^{b}	0.46±0.21^{c}	0.42±0.17^{c}
Urinary phosphorus (mg mL^{-1})					
Days 17-21	1.69±0.31^{a}	1.65±0.32^{a}	1.35±0.13^{a}	0.64±0.36^{b}	0.29±0.13^{c}
Days39-42	2.33±0.30^{a}	2.04±0.09^{a}	1.99±0.33^{a}	1.61±0.37^{a}	0.62±0.17^{b}

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

development of nephrocalcinosis is dose dependent. At the highest dietary magnesium concentration of 0.24%, the histological mehod did not detect calcium phosphate in the kidney anymore. The absence of histological evidence for nephrocalcinosis was accompanied with a kidney calcium concentration of 0.2%. In female rats fed diets based on natural ingredients rather than purified ingredients, kidney calcium concentratins as low as 0.05% may be detected (Ritskes-Hoitinga *et al*., 1991). The lowest dietary magnesium concentration of 0.02% had induced a kidney calcium level as high as 10.6%.

It can be suggested that the concentrations in urine of magnesium, calcium and phosphorus reflect those in the tubular fluid. There is evidence that high concentrations of calcium and phosphorus will stimulate the development of nephrocalcinosis whereas high magnesium concentrations have an inhibitory action (Greenwald, 1945; Boulet *et al*., 1962). Increasing intakes of magnesium were found to raise urinary magnesium concentrations and to lower those of phosphorus. These effects would explain the anti-neprocalcinogenic activity of high magnesium intake.

The diets with higher magnesium concentrations raised urinary calcium concentration which in itself would stimulate nephrocalcinogenesis. Clearly, the inhibitory effect on nephrocalcinogenesis of increased urinary magnesium concentrations and decreased phosphorus concentrations was stronger than the stimulatory effect of increased urinary calcium concentrations. Acidification of urine by the feeding of diets containing either ammonium chloride (Kootstra *et al*., 1991) or calcium chloride (Alhaidary *et al*., 2010) inhibitis nephrocalcinogenesis. This effect can be explained by dissolution of existing calcium phosphates or by prevention of precipitation. Unfortunately, urinary pH was not measured in this study. Based on the ingredient composition of the diets, the urinary pH probably was in the order of 7. It is likely that the inhibitory effect of high magnesium intake on nephrocalcinosis is greater when the urinary pH is alkaline when compared to acidic.

The magnesium-induced increase in urinary magnesium concentrations relates to an increase in magnesium absorption and subsequent excretion of the surplus with the urine (Bergstra *et al*., 1992). The observed magnesium-induced increase in urinary calcium concentrations cannot be easily explained because there is no evidence for stimulation of calcium absorption after high magnesium intakes (Mohamed *et al*., 2010).

The lowering of urinary phosphorus concentrations after the feeding of diets with increasing magnesium concentrations may be explained by a decreased intestinal absorption of phosphorus. The small intestinal contents contain insoluble calcium-magnesium-phosphate complexes (Brink *et al*., 1992). It is possible that the feeding of extra magnesium stimulates the formation of more insoluble complex so that additional phosphate is bound which is thereby withdrawn from the process of absorption.

CONCLUSION

In conclusion, this study with female rats shows that increasing dietary magnesium concentrations within the range of 0.02-0.24% reduce nephrocalcinosis in a dose-dependent fashion. The magnesium-induced reduction of nephrocalcinosis is explained by increased magnesium concentrations in urine combined with decreased phosphorus concentrations.

REFERENCES

Alhaidary, A., H.E. Mohamed and A.C. Beynen, 2010. Nephrocalcinosis and urinary mineral concentrations in rats fed diets containing supplemental chloride. J. Ani. Vet. Adv. (in Press).

Bergstra, A.E., A.G. Lemmens and A.C. Beynen, 1992. Dietary fructose versus glucose stimulates nephrocalcinogenesis in female rats. J. Nutr., 123: 1320-1327.

Boulet, M., J.R. Marier and D. Rose, 1962. Effect of magnesium on formation of calcium phosphate precipitates. Arch. Biochem. Biophys., 96: 629-636.

Brink, E.J., A.C. Beynen, P.R. Dekker, E.C. van Beresteijn and R. van der Meer, 1992. Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats. J. Nutr., 122: 580-586.

Bunce, G.E., H.E. Sauberlich, P.G. Reeves and T.S. Oba, 1965.. Dietary phosphorus and magnesium deficiency in the rat. J. Nutr., 86: 406-414.

Greenwald, I., 1945. The effect of phosphate on solubility of calcium carbonate and of bicarbonate on the solubility of calcium and magnesium phosphates. J. Biol. Chem., 161: 697-704.

Hoek, A.C., A.G. Lemmens, J.W.M.A. Mullink and A.C. Beynen, 1988. Influence of dietary calcium: Phosphorus ratio on mineral excretion and nephrocalcinosis female in rats. J. Nutr., 118: 1210-1216.

Kootstra, Y., J. Ritskes-Hoitinga, A.G. Lemmens and A.C. Beynen, 1991. Diet-induced calciuria and nephrocalcinosis in female rats. Int. J. Vitam. Nutr. Res., 61: 100-101.

Mars, Y.W.H.M., A.G. Lemmens and A.C. Beynen, 1988. Dietary phosphorus and nephrocalcinosis in female rats. Nutr. Rep. Int., 38: 249-258.

Mohamed, H.E., A. Alhaidary and A.C. Beynen, 2010. Calcium metabolism in rats fed diets containing various concentrations of magnesium. Res. J. Biol. Sci. (In Press).

Nguyen, H.T. and J.C. Woodard, 1980. Intranephronic calculosis in rats an ultrastructural study. Am. J. Pathol., 100: 39-56.

Ritskes-Hoitinga, J., J.N.J.J. Mathot, L.H.J.C. Danse and A.C. Beynen, 1991. Commercial rodent diets and nephrocalcinosis in weanling female rats. Lab. Ani., 25: 126-132.

Sterck, J.G., J. Ritskes-Hoitinga and A.C. Beynen, 1992. Inhibitory effect of high protein intake on nephrocalcinogenesis in female rats. Br. J. Nutr., 67: 223-233.