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Calcium Metabolism in Rats Fed Diets Containing Various Concentrations of Magnesium

H.E. Mohamed, A. Alhaidary and A.C. Beynen
Department of Animal Production, College of Food and Agricultural Sciences,
King Saud University, Riyadh, Kingdom of Saudi Arabia

Abstract: It has been shown earlier in rats that an increase in the dietary magnesium concentration causes higher rates of calcium excretion with urine. The hypothesis tested in this study was that the magnesium-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 purified diets containing magnesium concentrations of 0.02, 0.04, 0.06, 0.12 or 0.24% for a period of 6 weeks. There was no effect of dietary treatment on growth and feed intake. Dietary magnesium concentrations higher than 0.02% produced a significant increase in urinary calcium excretion. Apparent calcium absorption and tibia calcium concentrations were not affected by magnesium intake. The efficiency of apparent calcium absorption fell markedly with increasing age. It remains unknown how calcium homeostasis is attained in rats fed magnesium-rich diets.

Key words: Rats, magnesium, calcium, metabolism, excretion, tibia

INTRODUCTION

In rats, it has been shown frequently that high intakes of calcium depress magnesium absorption (Hoek *et al*., 1988; Brink *et al*., 1992a) which is explained by an increased formation of insoluble calcium-magnesium-phosphate complexes in the small intestinal content.

When magnesium is present in the solid phase of the digesta rather than the soluble phase, it is not available for absorption (Brink *et al*., 1992a). A decreased absorption of magnesium is associated with a decreased excretion with the urine in order to maintain magnesium homeostasis in the body.

Based on the earlier described calcium-magnesium interaction it would be predicted that high intakes of magnesium will inhibit calcium absorption and thus decrease urinary calcium excretion. In contrast to the prediction, we have shown earlier in rats that an increase in dietary magnesium concentration caused higher rates of calcium excretion with urine (Mars *et al*., 1988).

It could be suggested that magnesium can replace calcium in the calcium-magnesium-phosphate complexes, leading to more soluble calcium in the intestine and thus, enhanced intestinal absorption and more urinary excretion of calcium. However, apparent calcium absorption in young growing rats fed extra magnesium was found to be unaffected (Bergstra *et al*., 1993; Mars *et al*., 1988; Sterck *et al*., 1992). Possibly, there was a delayed adaptation of calcium absorption which was not seen in those studies because the duration was only 4 weeks. Alternatively, the increased urinary calcium excretion in growing rats fed extra magnesium may have been compensated for by less deposition of calcium in the skeleton.

The present study with young rats was carried out to verify the possible responses of calcium metabolism to high magnesium intakes. The feeding trial lasted 42 days instead of 28 days (Bergstra *et al*., 1993; Mars *et al*., 1988; Sterck *et al*., 1992). The questions addressed were whether magnesium loading inhibits calcium deposition in tibia and/or stimulates apparent calcium absorption in order to compensate for an increased loss of calcium with urine.

MATERIALS AND METHODS

Rats and treatments: In this study, 3 weeks-old, female Wistar rats (CPB:WU) were used. During the pre-experimental period of 7 days, all rats were fed on the diet containing 0.04% magnesium (Table 1). The rats had free access to food and demineralized water. After the pre-experimental period (day 0), the rats were divided into five groups of six rats each so that group mean body weights and distributions were similar. Each group was randomly

Corresponding Author: H.E. Mohamed, Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia

Table 1: Ingredient and analyzed composition of the experimental diets

	Diet code (magnesium[1]%)				
Items	0.02	0.04[2]	0.06	0.12	0.24
Ingredients (g/1000 g)					
Glucose	710.1	709.4	708.0	705.2	699.6
$MgCO_3$	0.700	1.400	2.800	5.600	11.20
Constant components[3]	289.2	289.2	289.2	289.2	289.2
Total	1000	1000	1000	1000	1000
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

[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)

assigned to one of the five experimental diets. One group remained on the diet with 0.04% magnesium and the other 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 and magnesium.

The diets were in powdered form and were stored at 4°C until feeding. Experimental feeds and demineralized water were supplemented *ad libitum* for a period of 6 weeks. Feed consumption and body weights were measured. 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: Feces and urine of each rat were collected quantitatively during days 17-21 and 39-42. Urine was collected in containers to which 50 μL of 0.2% (w/v) NaN_3 had been added as a preservative. After each balance period, total urine volume was recorded.

On day 42, the rats were anesthetized with diethyl ether and blood samples were taken by orbital puncture into heparinized tubes. Plasma was collected by centrifugation at 3000 rpm for 15 min and stored at -20°C until analysis. The anesthetized rats were immediately killed by decapitation and tibias were removed, weighed and stored at -20°C.

Chemical procedures: Feces and feed samples were freeze-dried, homogenized and a sample (about 150 mg) was ashed (500°C, 17 h), followed by dissolving it in 3 mL of 6 moL L^{-1} HCl. After appropriate dilution with distilled water, magnesium in feed and calcium in feed and feces were analyzed by atomic absorption spectroscopy in the presence of 5% (w/v) $LaCl_3$ (Varian Atomic Absorption Spectrophotometer type AA-475, Springvale, Australia).

Urine samples were acidified to pH 1-2 with 6 mol L^{-1} HCl and centrifuged (1200×g) for 10 min and the supernatant was frozen at -20°C. Calcium in urine was analyzed as described for feces. For the analysis of plasma calcium, the same method was used. Tibias were cleaned of adhering tissue and dried for 16 h at 106°C. Then, they were weighed, ashed (500°C, 17 h) and dissolved in 6 mol L^{-1} HCl followed by calcium analysis as described for feces.

Statistics: Results are presented as means±SD for six rats. Statistical analysis of the data was done using a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL 1998). Significant differences between treatments were identified using Duncan's multiple range test. The level of statistical significance was pre-set at $p<0.05$.

RESULTS AND DISCUSSION

The amount of magnesium in the diet did not influence final body weight and growth rate (Table 2). Feed intake was similar for the five dietary treatments. When the dietary magnesium concentration was increased above 0.02% there was a rise in urinary calcium excretion (Table 3).

For all four groups, the rise was statistically significant for days 17-21 but for days 39-42 it reached significance for the diet with 0.12% magnesium only. The four diets with dietary magnesium levels higher than 0.02% produced similar rates of calcium output with urine.

During days 17-21 and 39-42, apparent calcium absorption was not affected by the level of magnesium intake (Table 3). The efficiency of calcium absorption was much greater during days 17-21 than during days 39-42 (Table 3).

Plasma concentrations of calcium did not differ between the dietary groups (Table 4). The magnesium content of the diet had no effect on the calcium concentration in tibia.

In agreement with earlier research (Mars *et al*., 1988), extra magnesium in the diet significantly raised calcium excretion with urine. In order to compensate for the increased urinary calcium loss, it would be expected that calcium absorption would be increased.

This expectation is based on other dietary variables that stimulate urinary calcium excretion. In ruminants (Schonewille *et al*., 1994), it has been shown that the feeding of a ration with a negative instead of positive cation-anion balance raises both urinary calcium excretion and apparent calcium absorption.

When rats were fed a diet containing lactulose, there was an increase in urinary calcium excretion that was associated with an increase in apparent calcium absorption (Heijnen *et al*., 1993). The feeding to rats of

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

	Diet code (magnesium%)				
Groth performance	0.02	0.04	0.06	0.12	0.24
Body weight (g)					
Initial (day 0)	74.6±4.60	77.8±5.5	76.2±5.30	76.3 ±5.9	76.0±6.60
Final (day 42)	212.3±37.6	196.1±9.7	207.3±24.2	209.0±22.0	199.6±17.0
Feed intake (g day^{-1})	14.1±1.40	13.5±0.7	14.4±1.10	13.6±0.80	13.2±0.90
Growth (g day^{-1})	3.2±0.80	2.8±0.3	3.1±0.50	3.2±0.40	3.0±0.30

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

	Diet code (magnesium%)				
Days	0.02	0.04	0.06	0.12	0.24
Urinary calcium excretion (μmoL day^{-1})					
Days 17-21	9.2±3.0^{a}	19.8±10.6^{b}	22.5±8.70^{b}	20.1±12.2^{b}	20.8±9.0^{b}
Days 39-42	10.9±1.2^{a}	16.1±6.7^{a,b}	21.3±11.9^{a,b}	24.3±4.5^{b}	18.5±6.1^{a,b}
Apparent calcium absorption (intake %)					
Days 17-21	66.6±17.3	67.0±10.6	60.6±14.9	66.5±7.90	65.7±9.2
Days 39-42	39.8±12.9	45.9±26.0	39.4±10.6	40.3±10.2	40.4±8.7

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

Table 4: Calcium concentrations in plasma and tibia of rats fed the experimental diets

	Diet code (magnesium%)				
Factors	0.02	0.04	0.06	0.12	0.24
Plasma calcium (mmol L^{-1})	2.74±0.12	2.82±0.40	2.69±0.08	2.73±0.11	2.73±0.08
Tibia calcium (dry weight %)	24.7±1.100	24.3±2.600	24.4±1.200	24.5±1.40	24.5±0.80

cow's milk versus soybean beverage caused an increase in the excretion of calcium with urine in combination with enhanced apparent calcium absorption (Brink *et al*., 1992b).

However, in this study the magnesium-induced increase in urinary calcium excretion did not go hand in hand with an increase in calcium absorption.

It was found that apparent calcium absorption fell with increasing age. When the rats were aged 10 weeks, the efficiency of calcium absorption was about 40% lower than when they were 7 weeks old. The age-dependent efficiency of calcium absorption is a well-known phenomenon (Pastoor *et al*., 1985) and is explained by a decreasing calcium requirement as the skeleton matures. Thus, the rats used in this study displayed adequate regulation of calcium absorption. This substantiates the conclusion that high magnesium intake did not affect calcium absorption.

In adult animals at maintenance with constant body weight and body composition there is calcium balance, implying that the calcium intake equals calcium losses with feces and urine. When calcium intake remains unchanged, an increased excretion of calcium with urine should be compensated for so that a negative calcium balance will not persist.

A long-term, negative calcium balance will cause demineralization of the skeleton, leading eventually to fractures. In young growing animals, extra calcium loss from the body leads to less deposition in the developing skeleton. As described earlier, an increased urinary excretion of calcium normally will be negated by more efficient calcium absorption. In this study with young growing rats, the magnesium-induced increase in calcium excretion was not associated with enhanced calcium absorption. Thus, it would be expected that calcium deposition in bone was depressed by high magnesium intake.

However, an increase in the concentration of magnesium in the diet did not affect calcium deposition in tibia. Parker (1985) found that the ash content of tibia was lowered in rats fed high-magnesium diets but this could relate to minerals other than calcium. It should be stressed that a magnesium-induced lowering of calcium deposition in bones other than tibia cannot be excluded.

CONCLUSION

This study shows that high magnesium intake 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 magnesium-rich diets.

The same holds for rats fed a diet enriched with galacturonic acid. In a recent study, we found that the feeding of galacturonic acid raised urinary calcium excretion but left unchanged apparent calcium absorption (Mohamed *et al*., 2010).

REFERENCES

Bergstra, A.E., A.G. Lemmens and A.C. Beynen, 1993. Dietary fructose versus glucose stimulates nephrocalcinogenesis in female rats. J. Nutr., 123: 1320-1327.

Brink, E.J., P.R. Dekker, E.C.H. Van Beresteijnand and A.C. Beynen, 1992a. Bioavailability of magnesium and calcium from cow s milk and soybean beverage in rats. Br. J. Nutr., 68: 571-582.

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

Heijnen, A.M., E.J. Brink, A.G. Lemmensand and A.C. Beynen, 1993. Ileal pH and apparent absorption of magnesium in rats fed on diets containing either lactose or lactulose. Br. J. Nutr., 70: 747-756.

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.

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. Nephrocalcinosis in female rats fed diets containing either pectin, galacturonic acid or glucose. Am. J. Vet. Anim. Sci., 5: 110-113.

Parker, H.E., 1985. Magnesium and phosphorus inter-relationships at deficient to normal dietary levels. Nutr. Rep. Int., 32: 983-997.

Pastoor, F.J.H., R. Opitz, A.T. Van t Klooster and A.C. Beynen, 1985. Dietary phosphorus restriction to half the minimum required amount slightly reduces weight gain and length of tibia, but sustains femur mineralization and prevents nephrocalcinosis in female kittens. Br. J. Nutr., 74: 85-100.

Schonewille, J.Th., A.Th. Van t Klooster, A. Dirkzwager and A.C. Beynen, 1994. Stimulatory effect of an anion (chloride) rich ration on apparent calcium absorption in dairy cows. Livestock Production Sci., 40: 233-240.

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.