Effect of dietary magnesium level on urinary and faecal excretion of calcium, magnesium and phosphorus in adult, ovariectomized cats

BY F. J. H. PASTOOR[1], A. TH. VAN 'T KLOOSTER[2], R. OPITZ[1] AND A. C. BEYNEN[1,2]

[1] *Department of Laboratory Animal Science and* [2] *Department of Large Animal Medicine and Nutrition, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

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Dietary Mg restriction is generally considered to contribute to the prevention of struvite urolithiasis in cats, but its effects on faecal and urinary excretion of Ca and P have not been systematically investigated. The present study seeks to fill the gap. In a 4 × 4-week crossover study, ovariectomized cats were fed on purified diets containing either 0·40, 0·79, 1·59 or 3·17 mmol Mg/MJ (0·19, 0·38, 0·76 and 1·52 g Mg/kg diet). Increasing the dietary Mg level from 0·40 to 3·17 mmol Mg/MJ by the addition of extra $MgCO_3$ raised urinary and faecal excretion of Mg from 0·14 to 0·68 mmol/MJ and from 0·28 to 1·66 mmol/MJ respectively. The 8-fold increase in Mg intake significantly raised urinary excretion of Ca from 0·06 to 0·09 mmol/MJ. Apparent absorption and urinary excretion of P were depressed by 13 and 25% respectively when the dietary Mg level was raised from 0·40 to 3·17 mmol/MJ. A dietary level of 0·40 mmol Mg/MJ (0·19 g Mg/kg diet) was found to be sufficient to maintain Mg balance in the adult ovariectomized cats fed on the purified diet.

Magnesium: Mineral excretion: Urolithiasis: Cat

Urethral obstruction due to uroliths composed of struvite (magnesium ammonium phosphate hexahydrate) is a common disorder in cats (Hesse & Sanders, 1985; Lawler *et al.* 1985). In controlled experiments, struvite urolithiasis and urethral obstruction have been produced by the feeding of diets with Mg contents ranging from 7·5 to 10 g/kg dry matter (Lewis *et al.* 1978; Kallfelz *et al.* 1980; Finco *et al.* 1985). The levels of Mg in the urolithiagenic diets used are high when compared with those in commercial cat diets, which generally range between 0·5 and 3·0 g/kg dry feed (Feldmann *et al.* 1977; Graser *et al.* 1981; Sauer *et al.* 1985). Nevertheless, low-Mg diets are generally considered advantageous in the prevention of urolithiasis.

In young growing rats an increase in dietary Mg concentration above the recommended requirement raises urinary Ca excretion without affecting apparent Ca absorption (Mars *et al.* 1988; Sterck *et al.* 1992; Bergstra *et al.* 1993). These results point either to a delayed adaptation of Ca absorption not seen in those studies, to Ca mobilization from the bones, or to diminished Ca deposition in the skeleton. The influence of dietary Mg restriction on the urinary excretion of Ca in cats has not been systematically described. Thus in the present experiment the effects on urinary and faecal Ca excretion of four, practically realistic dietary levels of Mg (0·19, 0·38, 0·76 and 1·52 g/kg diet) were studied in adult, ovariectomized cats. The effects on urinary and faecal excretion of Mg and P were determined as well.

MATERIALS AND METHODS

The protocol of the experiment was approved by the animal experiments committee of the Department of Laboratory Animal Science, Utrecht University.

Animals and housing

Nine SPF-derived cats (*n* 7, Ico:FecEur(Tif), Iffa Credo, L'Arbresle, France; *n* 2, Hsd/Cpb:CaDs, Harlan Cpb, Zeist, The Netherlands) were housed as a group under conventional conditions in a room (2·2 × 4·5 × 3·0 m) with nine open stainless steel cages (1·16 × 0·56 × 0·67 m). A controlled light cycle (light: 07.00–19.00 hours), temperature (20–23°) and humidity (50–65%) were maintained in the room. At the start of the experiment the cats were aged about 2·5 years. They had been ovariectomized at the age of either 8–9 months (*n* 7) or 2 years (*n* 2). We decided to ovariectomize the cats for two reasons. When intact female cats are on heat they often refuse to eat, which would interfere with the execution of a dietary balance study. Furthermore, female cats kept as pets are often neutered. Thus by using ovariectomized cats the outcome of the study may have greater practical value.

Experimental design

During the first month of the experiment the cats were trained to eat a pelleted (diameter 4 mm), purified diet (0·79 mmol Mg/MJ (0·38 g Mg/kg diet), Table 1) during a restricted period of 2 h/d (09.00–11.00 hours). During the 2 h feeding period each cat had free access to food while locked up in its own cage. Apart from the feeding interval the cats could move freely within the room. Demineralized water was always freely available.

After the training period the cats were fed on the purified diets (Table 1) containing either 0·40, 0·79, 1·59 or 3·17 mmol Mg/MJ (0·19, 0·38, 0·76, 1·52 g Mg/kg diet) according to a 4 × 4 crossover design. Each cat was given each diet in such a sequence that diets followed each other a comparable number of times to take into account possible carry-over effects. The diets were provided for 2 h/d on an *ad lib.* basis. Except for the content of Mg the diets were formulated according to the nutrient requirements of cats (National Research Council, 1986). The diets were in pelleted form (diameter 4 mm). The ingredients and analysed composition of the diets are given in Table 1.

The entire experiment consisted of four dietary periods, each lasting 28 d. Body weight was recorded weekly. For the last 7 d of each period the cats were confined individually in their own cages. Feed intake was recorded and urine and faeces were collected. At the end of each period heparinized fasting blood samples were taken from the jugular vein while the cats were anaesthetized (20 mg ketamine/kg and 0·5 mg atropine, administered intramuscularly and subcutaneously respectively).

The method used to collect excreta of the cats has been published elsewhere (Pastoor *et al.* 1990).

Preparation of samples

Faeces, urine and plasma samples were prepared for analysis as described previously (Pastoor *et al.* 1994).

Chemical analyses

Ca, Mg and P in feed, faeces, urine and blood, plasma activity of alkaline phosphatase (*EC* 3.1.3.1), creatinine and urea in plasma and urine, and urinary pH were determined as described previously (Pastoor *et al.* 1994). For all chemical analyses, accuracy was verified to be within 5% deviation from the targets with reference samples (reference serum, Roche N, Roche Diagnostica, Basel, Switzerland and in-house reference pools of feed, faeces and urine).

Table 1. *Composition of the experimental diets**

Mg (mmol/MJ)...	0·40	0·79	1·59	3·17
Mg (g/kg diet)...	0·19	0·38	0·76	1·52
Ingredient (g/kg)				
$MgCO_3$	0·263	0·923	2·242	4·881
Dextrin	337·727	337·067	335·748	333·109
Constant components†	662·01	662·01	662·01	662·01
Chemical analysis (mmol/kg)				
Mg	9·5	15·3	27·6	52·7
Ca	173	173	168	169
P	164	166	166	166

* Calculated dietary Mg concentrations are indicated; the calculated, metabolizable energy density of the diets was 19·7 MJ/kg.

† The constant components consisted of the following (g): egg-white 186·5, herring meal 56·2, beef tallow 197·2, maize oil 8·5, glucose 56·2, cooked maize starch 56·2, cellulose 11·2, $NaH_2PO_4.2H_2O$ 21·99, $CaCO_3$ 16·36, Na_2CO_3 19·28, taurine 0·38, vitamin premix 12, mineral premix 20. The diets were formulated taking into account analysed Mg, Ca and P concentrations in the egg-white and herring-meal preparations. These concentrations were as follows (mmol/kg product): herring meal; Mg 74·04, Ca 426·65, P 645·79; egg-white; Mg 2·88, Ca 2·00, P 24·22. The vitamin premix consisted of (mg/12 g): retinyl acetate and retinyl palmitate (150 μg/mg) 6·3, cholecalciferol (12·5 μg/mg) 0·94, DL-α-tocopheryl acetate (0·5 mg/mg) 56·6, menadione 0·094, thiamin 4·7, riboflavin 3·78, pyridoxine 3·78, nicotinamide 37·8, DL-calcium pantothenate (0·45 mg/mg) 10·48, pteroylmonoglutamic acid 0·755, biotin 3·0, cyanocobalamin (0·1 μg/mg) 18·9, choline chloride (0·5 mg/mg) 5228·46, myo-inositol 200, cooked maize starch 6424·411. The mineral premix consisted of (mg/20 g): KCl 7191, $FeSO_4.7H_2O$ 375·6, $CuSO_4.5H_2O$ 18·5, MnO_2 7·4, $ZnCl_2$ 98·3, KI 0·45, $Na_2SeO_3.5H_2O$ 0·31, cooked maize starch 12308·44.

Statistical analyses

All statistical analyses were carried out using a SPSS/PC+ computer program (SPSS Inc., 1988*a*, *b*). The two-sided level of statistical significance was pre-set at $P < 0·05$. All data within dietary groups were normally distributed as based on the Kolmogorov–Smirnov one-sample test. Multivariate analyses of variance (MANOVA) with cat, diet and time as main effects were carried out. Homogeneity of variances was checked (Barlett's test). In the case of unequal variances the data were subjected to logarithmic transformation. For the analysis of selected data, feed intake was used as a covariate. Data were checked for interaction between covariate and main effects. Significant effects of diet were identified by multiple comparisons (Tukey test) in a one-way analysis of variance, using the residual sum of squares of the MANOVA. Significant diet effects were also tested for linearity. In case of inhomogeneity of variances after logarithmic transformation the data were subjected to non-parametrical testing by means of Friedman's test. Multiple comparisons were performed with paired Student's *t* tests with adjusted levels of significance according to Bonferroni's adaptation. Significant diet effects in non-parametric tests were not tested for linearity.

RESULTS

Body weight and feed intake

Body weight of the cats at the beginning of the experiment was 2·9 (SE 0·1) kg (*n* 9). There was no change in body weight during the balance periods (results not shown). Feed intake was not affected by dietary Mg concentration (Table 2).

Table 2. *Feed intake, urinary volume and balance of magnesium, calcium, and phosphorus in ovariectomized cats fed on diets containing various amounts of magnesium**

(Mean values with their standard errors for nine cats)

	Dietary Mg (mmol/MJ)								
	0·40		0·79		1·59		3·17		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Significant†
Feed intake (g/d)	39·8	3·2	42·2	2·4	38·8	2·9	40·1	3·2	
Urinary volume (ml/d)	60·9	4·9	62·5	4·3	62·8	5·0	69·1	6·5	Mg, F, L
Mg balance (mmol/MJ)									
Intake	0·48	0·00	0·78	0·00	1·40	0·00	2·67	0·00	
(mmol/d)	0·38	0·03	0·65	0·04	1·07	0·08	2·11	0·17	
Urinary output	0·14[d]	0·01	0·24[c]	0·02	0·41[b]	0·02	0·68[a]	0·06	Mg, F, L
Faecal output	0·28[d]	0·02	0·43[c]	0·03	0·87[b]	0·07	1·66[a]	0·16	Mg, L
Absorption	0·20[d]	0·02	0·35[c]	0·03	0·53[b]	0·07	1·01[a]	0·16	Mg, L
Retention	0·06	0·03	0·10	0·04	0·12	0·06	0·33	0·12	Mg
Ca balance (mmol/MJ)									
Intake	8·8	0·0	8·8	0·0	8·5	0·0	8·6	0·0	
(mmol/d)	6·9	0·6	7·3	0·4	6·5	0·5	6·8	0·5	
Urinary output	0·06[b]	0·01	0·07[b]	0·01	0·07[a,b]	0·01	0·09[a]	0·01	Mg, L
Faecal output	7·6	0·3	7·5	0·4	7·8	0·5	7·6	0·6	
Absorption	1·1	0·3	1·3	0·4	0·7	0·5	1·0	0·6	
Retention	1·1	0·3	1·2	0·4	0·6	0·5	0·9	0·6	
P balance (mmol/MJ)									
Intake	8·3	0·0	8·4	0·0	8·4	0·0	8·4	0·0	
(mmol/d)	6·5	0·5	7·0	0·4	6·5	0·5	6·7	0·5	
Urinary output	6·2[a]	0·4	5·6[a,b]	0·4	5·5[a,b]	0·2	4·7[b]	0·2	Mg, L
Faecal output	2·2[b]	0·2	2·2[b]	0·3	2·6[a,b]	0·3	3·1[a]	0·4	Mg, F, L
Absorption	6·1[a]	0·2	6·2[a]	0·3	5·8[a,b]	0·3	5·3[b]	0·4	Mg, F, L
Retention	−0·1	0·5	0·6	0·2	0·3	0·2	0·6	0·3	

[a,b,c,d] Group means within a row not sharing the same superscript were significantly different ($P < 0·05$ Tukey test).

* For details of diets and procedures, see Table 1 and pp. 78–79.

† Multivariate ANOVA ($P < 0·05$) or Friedman's test ($P < 0·05$); Mg, magnesium effect; F, feed intake as significant covariate; L, linear effect of dietary magnesium level ($P < 0·05$).

Mineral balance

Mineral balances are expressed as mmol/MJ (Table 2). A rise in the dietary level of Mg was accompanied by increased urinary and faecal excretion of Mg and an increase in the amount of Mg absorbed. Although Mg retention did not differ significantly from zero throughout, the group means were raised by increased Mg intake. Higher intakes of Mg were associated with a slight, but significant, rise in urinary Ca excretion. Faecal excretion of Ca was similar at the four dietary Mg levels tested and so were Ca absorption and retention. Urinary excretion of P was significantly lowered by increasing Mg intakes and faecal P output was raised. Thus, extra Mg lowered P absorption, but P retention was not affected.

Relation between magnesium intake and output

Fig. 1 shows the relation between dietary Mg concentration and Mg excretion in urine plus faeces expressed as mmol/MJ metabolizable dietary energy. Output and intake were essentially equal at the four intakes studied.

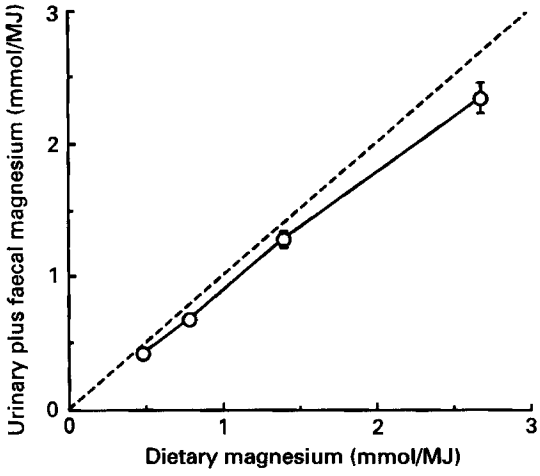

Fig. 1. Relation between dietary magnesium concentration and magnesium excretion in urine plus faeces by ovariectomized cats fed on diets containing four different magnesium concentrations. Dietary magnesium concentrations were based on chemical analyses. Results are expressed as means with their standard errors for nine cats; the line of equality is shown. The linear regression equation for the four mean values is: $Y = 0{\cdot}01 + 0{\cdot}877X$ (r 0·999).

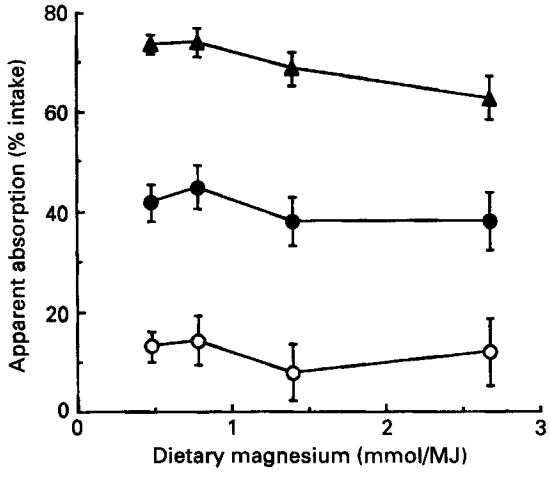

Fig. 2. Relation between dietary magnesium concentration and percentage apparent absorption of magnesium, calcium and phosphorus in cats fed on diets containing four different magnesium concentrations. Dietary magnesium concentrations were based on chemical analyses. Mineral absorption was calculated as mineral intake minus faecal excretion, and expressed as a percentage of intake. Values are means for nine cats, with their standard errors indicated by vertical bars. (○–○), Calcium; (●–●), magnesium; (▲–▲), phosphorus. Multivariate ANOVA revealed a significant effect ($P < 0{\cdot}05$) of dietary magnesium level on the absorption of phosphorus.

Percentage mineral absorption

Dietary Mg concentration did not affect apparent absorption of Mg, when expressed as a percentage of intake (Fig. 2). Apparent percentage absorption of Ca also remained unchanged when dietary Mg level was raised, but the percentage P absorption decreased.

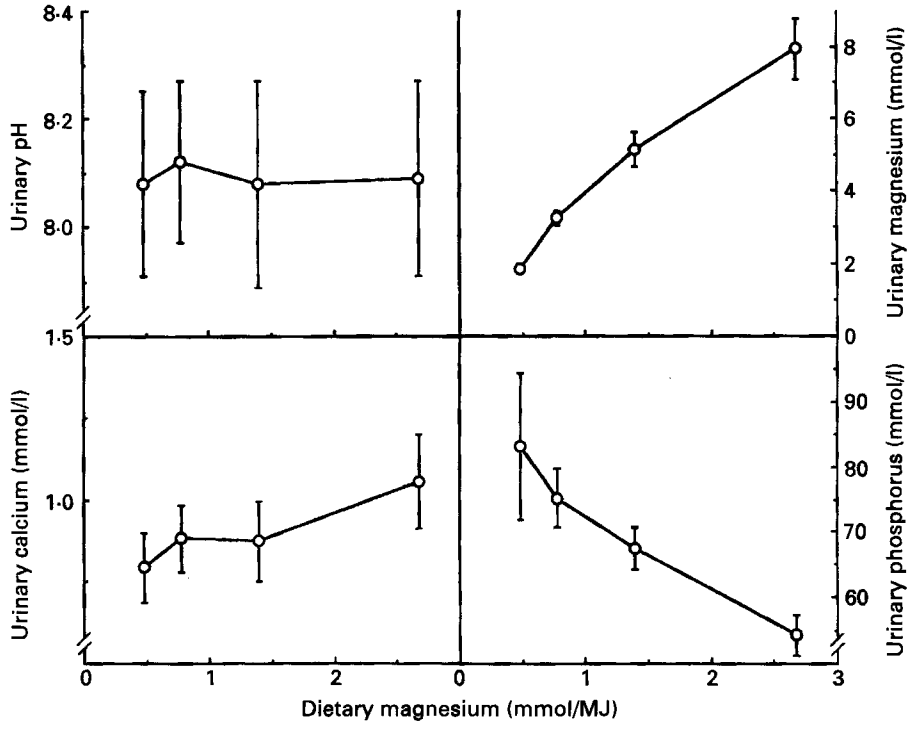

Fig. 3. Relation between dietary magnesium concentration and urinary pH and urinary concentrations of magnesium, calcium and phosphorus in cats fed on diets containing four different magnesium concentrations. Dietary magnesium levels were based on chemical analyses. Values are means for nine cats, with their standard errors indicated by vertical bars. Multivariate ANOVA showed significant effects ($P < 0{\cdot}05$) of dietary magnesium levels on urinary concentrations of magnesium, calcium and phosphorus.

Urinary composition

Increasing levels of dietary Mg were associated with increases in urinary volume (Table 2), but left urinary pH unchanged (Fig. 3). Urinary concentrations of Mg and Ca were systematically raised by increasing intakes of Mg, whereas urinary concentrations of P dropped.

Plasma mineral concentrations and activity of alkaline phosphatase

Plasma concentrations of Mg were raised at the highest dietary Mg level studied; values were 0·85 (SE 0·02), 0·88 (SE 0·02), 0·89 (SE 0·03) and 0·96 (SE 0·02) mmol/l (*n* 9) for cats fed on diets containing 0·40, 0·79, 1·59 and 3·17 mmol Mg/MJ respectively. Plasma levels of Ca and P and alkaline phosphatase activity were not affected by dietary Mg content (results not shown here but reported elsewhere; Pastoor, 1993).

Plasma and urinary urea and creatinine

Plasma urea and creatinine concentrations were not affected by Mg intake. Urinary urea excretion was not affected by dietary Mg, but an effect of feed intake was found. Urinary creatinine excretion and creatinine clearance were almost identical for the four dietary Mg levels (results not shown here but reported elsewhere; Pastoor, 1993).

DISCUSSION

The Mg requirement of cats has been set at 0·79 mmol/MJ dietary metabolizable energy (0·38 g Mg/kg diet; 19·7 MJ/kg diet) (National Research Council, 1986) but actually refers to the minimum requirement for growing kittens fed on a purified diet. At the four dietary levels of Mg studied, retention of Mg did not differ significantly from zero. Thus, for adult ovariectomized cats fed on a purified diet, a Mg level as low as 0·40 mmol/MJ (0·19 g magnesium/kg diet) is sufficient to maintain Mg balance. Increasing rates of Mg absorption were followed by rises in urinary excretion. Only when the dietary level of Mg was raised to 3·17 mmol/MJ (1·52 g Mg/kg diet) were plasma levels of Mg in the cats significantly elevated.

The intake of extra Mg produced an augmented urinary excretion of Ca which agrees with our earlier work in rats (Mars *et al.* 1988; Sterck *et al.* 1992; Bergstra *et al.* 1993). It can be explained by a competition between Mg and Ca for tubular reabsorption (Cruikshank *et al.* 1981; Rude & Singer, 1981). The increased amounts of renally filtered Mg may depress reabsorption of Ca. There was no effect of increased Mg intake on Ca absorption in the cats, but an increase matching the extra loss of Ca with urine would not have been detected because of its smallness. It is not known whether the Mg-induced increase in urinary Ca excretion is transient. In any event, in the long run whole-body Ca balance during consumption of a high-Mg diet can only be maintained by a rise in Ca absorption and/or a rebound urinary Ca excretion.

An increase in Mg intake lowered apparent absorption of P in the cats. As a result, extra Mg in the diet lowered urinary P excretion. These results agree with previous work in cats (Lewis *et al.* 1978) and rats (Clark, 1968, 1969). The drop in apparent P absorption and rise in faecal P excretion as caused by high Mg intakes in the cats may be explained by the formation of an insoluble Mg–phosphate complex in the lumen of the intestine, which lowers the availability of P for absorption (Brink *et al.* 1992).

There appears to be no clear correlation between urinary composition and urethral obstruction (Carbone, 1965). However, the formation of struvite must depend on the urinary concentrations of its constituent ions (Buffington *et al.* 1989). Increasing intakes of Mg were associated with increases in urinary volume. An increase in urinary Mg excretion may lower tubular reabsorption of Na (Carney *et al.* 1980), leading to osmotic diuresis (Chesley & Tepper, 1958). Greater urinary volumes are favourable in the prevention of urolithiasis, because urinary mineral concentrations will be lowered. In the present study, increasing intakes of Mg elevated urinary concentrations of Mg. When urinary pH is relatively high (pH > 6·5), increased urinary Mg concentrations may provoke urolithiasis (Buffington *et al.* 1985). Increasing Mg intakes lowered urinary concentrations of P in the cats, which may have diminished precipitation of struvite crystals since phosphate is a component of struvite. Urinary pH was not affected by dietary Mg, which was unexpected because it has been reported that high intakes of Mg in the form of $MgCO_3$ or MgO raise urinary pH (Lewis *et al.* 1978; Buffington *et al.* 1985). Probably, in the present study baseline urinary pH values, which were already relatively high, did not rise further because the amount of added $MgCO_3$ was relatively small.

In summary, increasing levels of dietary Mg, in the form of $MgCO_3$, caused increases in urinary excretion of Mg and Ca. Apparent absorption and urinary excretion of P in the cats fell when Mg intake was raised. A Mg level of 0·40 mmol/MJ (0·19 g Mg/kg diet) was found to be sufficient to maintain Mg balance in adult, ovariectomized cats fed on a purified diet.

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