Effect of Dietary Magnesium Level on Nephrocalcinosis and Growth in Rats

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Summary We studied the extent of kidney calcification by varying dietary levels of Mg, based on pathological examinations and calcium (Ca) and magnesium (Mg) balance tests. AIN-76 diets containing varying levels of Mg—0.3 (–M), 1.3 (1/20M), 2.4 (1/10M), 9.2 (1/5M), 19 (control), 38 (2M), 102 (5M), and 187 (10M) mmol/kg diet—were fed to 3-week-old male Fischer-344 rats for 14 d. Although the magnitude of abnormality was highest in kidney of rats fed the –M diet, the damage was normalized as the dietary level of Mg increased, with increasing serum Mg concentration and urinary excretion of Mg. We found almost no deposition of Ca in rats fed the 10M diet. The mechanism by which the high dietary Mg induces these effects most likely involves a competition between Mg and Ca for reabsorption in proximal and/or distal tubules, since these diets increased the urinary excretion of Ca. However, these high Mg diets decreased food intake and body weight gain compared with the control diet, although these indices were not decreased in rats fed the 2M diet. The results suggest that a dietary magnesium level approximately twice the normal level effectively reduces kidney calcification while maintaining normal growth in rats.

Key Words calcium deposition, magnesium, nephrocalcinosis, pathological examination, rats

For 16 years, the American Institute of Nutrition's Rodent Diet, AIN-76 (1), has been used extensively worldwide. A major problem with this diet, however, is its propensity to produce calcium deposits in rat kidney (2, 3), which many studies

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have suggested is the result of a low molar ratio of calcium (Ca) to phosphorus (P) (4–6).

Factors besides the Ca:P molar ratio are also suspected of contributing to the calcium deposits, including low dietary levels of magnesium (Mg) (7). The mechanism of calcification in the kidney is unclear. One study found that the main cause was a decreasing urinary excretion of Ca (8), and several others reported that high dietary intakes of Mg reduced kidney calcification in rats (9, 10).

The aim of the present study was to investigate the proliferation of kidney deposits accompanying changes in dietary Mg levels, based on pathological examinations and Ca and Mg balance tests.

MATERIALS AND METHODS

General treatment of animals. The study was approved by the National Institute of Health and Nutrition Animal Use Committee, and all rats were maintained in accordance with guidelines for the care and use of laboratory animals, National Institute of Health and Nutrition. Three-week-old male Fischer-344 rats were purchased from Charles River Japan (Kanagawa, Japan). They were housed in individual cages with screen bottoms of stainless steel in a room maintained at $23 \pm 1^\circ\text{C}$ and lighted from 0800–2000 h. Body weight and food intake were recorded daily in the morning before the diet was replenished.

Experimental diet. For the first 7 d, rats were fed the control (C) diet (shown in Table 1), an AIN-76 diet (I) slightly modified. Nephrocalcinogenic sucrose was

Table 1. Composition of the experimental diets.

	–M	1/20M	1/10M	1/5M	C	2M	5M	10M
Ingredients (g/kg diet)								
Constant components ¹	350.000							
Sucrose	632.300	632.258	632.217	631.885	631.470	630.640	628.150	624.000
CaHPO ₄	17.700							
MgO	0	0.042	0.083	0.415	0.830	1.660	4.150	8.300
Chemical analysis (mmol/kg)								
Magnesium (Mg)	0.3	1.3	2.4	9.2	19.0	38.6	102.4	187.4
Calcium (Ca)	124.6	125.4	123.5	120.8	124.1	124.4	125.0	123.8
Phosphorus (P)	166.2	166.7	161.6	142.8	133.7	150.7	141.8	156.8
Ca/Mg	415.0	96.4	51.4	13.1	6.5	3.2	1.2	0.6

¹ The constant components consisted of the following (g): milk casein, 200; corn oil, 50; cellulose, 50; vitamin mixture based on AIN-76, 10; L-methionine, 3; choline bitartrate, 2; and a mineral mixture based on AIN-76 without magnesium oxide and calcium phosphate, dibasic, 35.

substituted as a carbohydrate source for corn starch (11). After 7 d, the rats were divided into 8 groups ($n=6$), based on body weight, and fed the experimental diets for 14 d. The AIN-76 diet was modified to contain varying levels of Mg with 0.3, 1.3, 2.4, 9.2, 19, 38, 102, and 187 mmol/kg diet, coded -M, 1/20M, 1/10M, 1/5M, C, 2M, 5M, and 10M, respectively.

Sample collection and analysis. From day 7 to day 10, urine was collected in beakers containing 10 mL of 5 mol/L HCl, as were feces for mineral determination. However, urine and feces in rats fed the 10M diet were not used for mineral determination because separation could not be achieved. After 14 d, rats were decapitated, and blood, right kidneys, and femur were collected for the measurement of Mg, Ca, and P levels. The blood was centrifuged, and the serum was kept at -20°C until analysis. The left kidney was fixed with 10% formalin for pathological examination. The right kidney was digested by a method combining acid hydrolysis and dry heat. Briefly, the kidney was digested and dried with analytical grade nitric acid ($80\text{--}120^{\circ}\text{C}$), and the residue was then heated to $270\text{--}300^{\circ}\text{C}$. After cooling to room temperature, the residue was digested and dried with hydrogen peroxide ($80\text{--}120^{\circ}\text{C}$), then dissolved in 0.5 mol/L HCl for mineral determination. Femur mineral concentration was determined by the method of Calvo et al (12). Mg and Ca were analyzed by atomic absorption spectrometry (model Spectre AA-40, Varian, Victoria, Australia) with a 2.5 mg/L final concentration of strontium in the sample. P concentration was determined by the Gomori method (13). Bovine liver 1577a from the National Institute of Standard and Technology (Maryland, USA) was digested and analyzed along with samples to confirm accuracy.

Pathological examination. Paraffin slices of the formalin-fixed left kidney were stained with hematoxylin-eosin and Von Kossa's stain. The degree of kidney damage and nephrocalcinosis was scored on a scale from - (not detected) to + + + (severe).

Statistical analysis. Statistical differences were determined by ANOVA and then by Duncan's multiple range test (14). The significance of relationships between data was established by linear regression analysis (15). A probability value of $p < 0.05$ was considered significant.

RESULTS

Body weight and food intake

Final body weight, body weight gain, food intake, and food efficiency were significantly lower in rats fed the -M, 1/20M, 1/10M, and 10M diets than in those fed the control diet (Table 2). Only food intake was significantly lower in rats fed the 5M diet relative to the control diet. There were no significant differences in any variables among rats fed the 1/5M, C, and 2M diets.

Mineral balance

With a concomitant increase in Mg intake, the amount of Mg absorbed and fecal and urinary excretions of Mg also increased (Table 3). Higher intakes of Mg

Table 2. Final body weight, body weight gain, and food intake in rats fed the respective diets.¹

Diet	Initial body weight (g)	Final body weight (g)	Body weight gain (g/14 d)	Food intake (g/14 d)	Food efficiency (g/g diet)
—M	61.7 ± 1.6	80.9 ± 1.7 ^a	19.2 ± 0.3 ^a	94 ± 2 ^a	0.21 ± 0.003 ^a
1/20M	61.7 ± 1.4	86.0 ± 2.8 ^{ab}	24.3 ± 1.5 ^b	99 ± 3 ^{ab}	0.25 ± 0.009 ^b
1/10M	61.8 ± 1.2	90.4 ± 1.2 ^b	28.5 ± 0.9 ^{bc}	108 ± 2 ^b	0.27 ± 0.008 ^{bc}
1/5M	61.7 ± 1.1	107.9 ± 4.0 ^c	46.2 ± 2.9 ^d	139 ± 5 ^{cd}	0.33 ± 0.012 ^d
C	61.6 ± 1.0	109.8 ± 1.7 ^c	48.1 ± 1.5 ^d	144 ± 3 ^d	0.34 ± 0.008 ^d
2M	61.7 ± 1.0	110.5 ± 1.5 ^c	48.8 ± 0.8 ^d	143 ± 2 ^{cd}	0.34 ± 0.005 ^d
5M	61.7 ± 0.9	107.6 ± 2.3 ^c	45.8 ± 1.5 ^d	134 ± 4 ^c	0.34 ± 0.005 ^d
10M	61.8 ± 0.9	91.3 ± 1.1 ^b	29.4 ± 1.7 ^c	108 ± 2 ^b	0.27 ± 0.015 ^c

¹ Data are expressed as the mean ± SE. Within a column, values not sharing a superscript letter are significantly different from one another ($p < 0.05$). Among the experimental groups, the letter “a” indicates the smallest value with ascending order of “b,” “c,” and “d.”

were associated with a significant increase in urinary Ca excretion (Table 4). Although Ca intake was increased by increasing the food intake, the Ca absorption and retention ratio were similar at all dietary Mg levels tested. However, urinary excretion of Ca (Y) was significantly increased at higher levels of Mg intake and urinary excretion of Mg (X) ($Y = 11.878 + 0.153X$, $R = 0.632$, $p < 0.0001$). Although P intake was increased by increasing the food intake, the amount of P absorption was similar at all dietary Mg levels tested (Table 5). Urinary excretion of P was significantly lowered by increasing Mg intake in rats fed the 2M, 5M, and 10M diets, compared with the control diet.

Serum mineral concentrations

A rise in the dietary level of Mg was accompanied by an increase in the serum Mg concentration and a decrease in the ratio of Ca to Mg, although there were no significant differences in serum Mg concentrations among rats fed the —M, 1/20M, and 1/10M diets (Table 6). Nor were there any significant differences in serum Ca and P concentrations among rats fed the C, 2M, 5M, and 10M diets, although serum Ca concentration was significantly higher in rats fed the —M, 1/20M, and 1/10M diets, and serum P concentration was significantly lower in rats fed the 1/20M and 1/10M diets, compared with the control diet.

Femur weight and femur mineral concentrations

Femur fat-free dry weights did not differ among rats fed the 1/5M, C, 2M, 5M, and 10M diets (Table 7). A rise in the dietary level of Mg was accompanied by an increase in the femur Mg concentration and a decrease in the femur Ca

Table 3. Magnesium balance in rats fed the respective diets.¹

Diet	Intake ($\mu\text{mol/d}$)	Fecal output ($\mu\text{mol/d}$)	Absorption ($\mu\text{mol/d}$)	Absorption (%)	Urinary output ($\mu\text{mol/d}$)	Retention ($\mu\text{mol/d}$)	Retention (%)
-M	2 $\pm$ 0.09 ^a	4.5 $\pm$ 0.3 ^a	—	—	1.54 $\pm$ 0.58 ^a	—	—
1/20M	9 $\pm$ 0.33 ^a	5.8 $\pm$ 0.6 ^a	3.5 $\pm$ 0.3 ^a	37.9 $\pm$ 6.0 ^a	1.15 $\pm$ 0.08 ^a	2.4 $\pm$ 0.6 ^a	25.6 $\pm$ 6.4
1/10M	18 $\pm$ 0.59 ^a	10.8 $\pm$ 0.9 ^a	8.1 $\pm$ 0.5 ^a	42.7 $\pm$ 4.5 ^a	1.64 $\pm$ 0.13 ^a	6.4 $\pm$ 0.8 ^a	34.1 $\pm$ 4.3
1/5M	99 $\pm$ 3.76 ^b	43.2 $\pm$ 3.2 ^b	56.2 $\pm$ 0.9 ^b	56.5 $\pm$ 2.8 ^b	21.05 $\pm$ 1.76 ^a	35.1 $\pm$ 2.0 ^{ab}	35.4 $\pm$ 1.8
C	219 $\pm$ 3.52 ^c	94.1 $\pm$ 7.2 ^c	125.4 $\pm$ 3.6 ^c	57.1 $\pm$ 3.1 ^b	60.45 $\pm$ 3.56 ^b	64.9 $\pm$ 6.8 ^b	29.6 $\pm$ 3.1
2M	426 $\pm$ 11.20 ^d	190.7 $\pm$ 9.1 ^d	235.6 $\pm$ 6.7 ^d	55.2 $\pm$ 1.9 ^b	103.13 $\pm$ 7.77 ^c	132.4 $\pm$ 7.9 ^c	31.1 $\pm$ 1.7
5M	1,034 $\pm$ 37.62 ^e	449.7 $\pm$ 17.5 ^e	584.5 $\pm$ 11.03 ^e	56.4 $\pm$ 1.4 ^b	221.09 $\pm$ 11.38 ^d	363.4 $\pm$ 31.8 ^d	34.9 $\pm$ 2.1
10M	1,760 $\pm$ 47.51 ^f						

¹ Data are expressed as the mean $\pm$ SE. Within a column, values not sharing a superscript letter are significantly different from one another ($p < 0.05$). Among the experimental groups, the letter "a" indicates the smallest value with ascending order of "b," "c," "d," "e," and "f."

Table 4. Calcium balance in rats fed the respective diets.¹

Diet	Intake (mmol/d)	Fecal output (mmol/d)	Absorption (mmol/d)	Absorption (%)	Urinary output ($\mu\text{mol/d}$)	Retention (mmol/d)	Retention (%)
-M	0.80 $\pm$ 0.03 ^a	0.214 $\pm$ 0.025 ^a	0.587 $\pm$ 0.024 ^a	73.4 $\pm$ 2.4 ^b	5.28 $\pm$ 1.50 ^{ab}	0.582 $\pm$ 0.025 ^a	72.7 $\pm$ 2.3 ^c
1/20M	0.90 $\pm$ 0.03 ^{ab}	0.290 $\pm$ 0.029 ^{ab}	0.611 $\pm$ 0.018 ^a	68.0 $\pm$ 2.2 ^{ab}	3.99 $\pm$ 0.23 ^a	0.607 $\pm$ 0.018 ^a	67.6 $\pm$ 2.2 ^{bc}
1/10M	0.96 $\pm$ 0.03 ^b	0.366 $\pm$ 0.032 ^{bc}	0.601 $\pm$ 0.012 ^a	62.4 $\pm$ 2.1 ^a	4.28 $\pm$ 0.40 ^a	0.597 $\pm$ 0.012 ^a	61.9 $\pm$ 2.1 ^{ab}
1/5M	1.29 $\pm$ 0.04 ^{de}	0.463 $\pm$ 0.037 ^d	0.832 $\pm$ 0.035 ^{bc}	64.3 $\pm$ 2.0 ^a	13.55 $\pm$ 0.66 ^b	0.819 $\pm$ 0.035 ^b	63.2 $\pm$ 2.0 ^{ab}
C	1.43 $\pm$ 0.02 ^f	0.528 $\pm$ 0.050 ^d	0.906 $\pm$ 0.040 ^c	63.3 $\pm$ 3.1 ^a	40.41 $\pm$ 3.39 ^d	0.865 $\pm$ 0.041 ^b	60.4 $\pm$ 3.2 ^a
2M	1.37 $\pm$ 0.03 ^{ef}	0.479 $\pm$ 0.025 ^d	0.892 $\pm$ 0.030 ^{bc}	65.0 $\pm$ 1.4 ^a	48.65 $\pm$ 5.39 ^d	0.843 $\pm$ 0.027 ^b	61.5 $\pm$ 1.5 ^{ab}
5M	1.26 $\pm$ 0.04 ^{cd}	0.455 $\pm$ 0.017 ^{cd}	0.807 $\pm$ 0.034 ^b	63.8 $\pm$ 0.8 ^a	29.77 $\pm$ 3.89 ^e	0.777 $\pm$ 0.032 ^b	61.5 $\pm$ 0.8 ^{ab}
10M	1.16 $\pm$ 0.03 ^c						

¹ Data are expressed as the mean $\pm$ SE. Within a column, values not sharing a superscript letter are significantly different from one another ($p < 0.05$). Among the experimental groups, the letter "a" indicates the smallest value with ascending order of "b," "c," "d," "e," and "f."

Table 5. Phosphorus balance in rats fed the respective diets.¹

Diet	Intake (mmol/d)	Fecal output (mmol/d)	Absorption (mmol/d)	Absorption (%)	Urinary output (mmol/d)	Retention (mmol/d)	Retention (%)
-M	1.06±0.04 ^a	0.130±0.015 ^a	0.939±0.032 ^a	87.9±1.2 ^d	0.755±0.033 ^b	0.183±0.022 ^a	17.2±2.2 ^{ab}
1/20M	1.19±0.04 ^b	0.161±0.010 ^a	1.037±0.038 ^a	86.5±0.6 ^d	0.769±0.023 ^b	0.267±0.032 ^a	22.1±2.0 ^b
1/10M	1.26±0.04 ^b	0.219±0.018 ^b	1.046±0.039 ^a	82.6±1.4 ^c	0.810±0.016 ^b	0.235±0.032 ^a	18.3±2.2 ^b
1/5M	1.53±0.05 ^{cd}	0.284±0.020 ^c	1.248±0.042 ^b	81.5±0.7 ^c	1.019±0.061 ^c	0.228±0.025 ^a	15.2±2.1 ^{ab}
C	1.54±0.02 ^{cd}	0.319±0.024 ^c	1.226±0.022 ^b	79.3±1.3 ^{bc}	1.061±0.026 ^c	0.164±0.038 ^a	10.6±2.5 ^a
2M	1.66±0.04 ^d	0.374±0.025 ^d	1.288±0.049 ^b	77.4±1.5 ^b	0.799±0.051 ^b	0.488±0.040 ^b	29.5±2.4 ^c
5M	1.43±0.05 ^c	0.442±0.011 ^e	0.988±0.054 ^a	68.8±1.4 ^a	0.566±0.034 ^a	0.422±0.049 ^b	29.2±2.7 ^c
10M	1.47±0.04 ^c						

¹ Data are expressed as the mean±SE. Within a column, values not sharing a superscript letter are significantly different from one another (*p*<0.05). Among the experimental groups, the letter “a” indicates the smallest value with ascending order of “b,” “c,” “d,” and “e.”

Table 6. Serum magnesium, calcium, and phosphorus concentrations in rats fed the respective diets.¹

Diet	Magnesium (mmol/L)	Calcium (mmol/L)	Phosphorus (mmol/L)	Ca/Mg (mol/mol)
—M	0.23 ± 0.01 ^a	2.91 ± 0.07 ^c	—	12.53 ± 0.28 ^d
1/20M	0.23 ± 0.01 ^a	2.91 ± 0.03 ^c	2.45 ± 0.03 ^a	12.94 ± 0.63 ^d
1/10M	0.22 ± 0.01 ^a	2.89 ± 0.04 ^c	2.40 ± 0.06 ^a	13.21 ± 0.45 ^d
1/5M	0.52 ± 0.01 ^b	2.73 ± 0.03 ^b	2.89 ± 0.03 ^b	5.39 ± 0.15 ^c
C	0.69 ± 0.01 ^c	2.61 ± 0.04 ^{ab}	3.03 ± 0.05 ^{bc}	3.77 ± 0.06 ^b
2M	0.81 ± 0.004 ^d	2.54 ± 0.04 ^a	2.97 ± 0.05 ^b	3.15 ± 0.06 ^{ab}
5M	0.98 ± 0.02 ^e	2.49 ± 0.08 ^a	3.14 ± 0.03 ^c	2.55 ± 0.11 ^a
10M	1.06 ± 0.02 ^f	2.56 ± 0.02 ^a	2.99 ± 0.06 ^b	2.42 ± 0.06 ^a

¹ Data are expressed as the mean ± SE. Within a column, values not sharing a superscript letter are significantly different from one another ($p < 0.05$). Among the experimental groups, the letter "a" indicates the smallest value with ascending order of "b," "c," "d," "e," and "f."

Table 7. Femur weight and femur magnesium, calcium, and phosphorus concentrations in rats fed the respective diets.¹

Diet	Dry weight (mg/100 g BW)	Fat-free dry weight (mg/100 g BW)	Magnesium (μmol/g fat free femur)	Calcium (mmol/g fat free femur)	Phosphorus (mmol/g fat free femur)
—M	153 ± 1.9 ^c	149 ± 1.2 ^c	56.6 ± 1.3 ^a	5.38 ± 0.07 ^d	2.95 ± 0.03 ^e
1/20M	150 ± 2.4 ^c	146 ± 2.5 ^c	57.7 ± 1.3 ^a	5.13 ± 0.05 ^{bc}	2.24 ± 0.02 ^c
1/10M	151 ± 2.2 ^c	148 ± 2.4 ^c	66.7 ± 2.0 ^b	5.29 ± 0.10 ^{cd}	2.32 ± 0.02 ^{cd}
1/5M	137 ± 1.9 ^b	132 ± 1.5 ^b	123.0 ± 2.3 ^c	5.18 ± 0.04 ^{bc}	2.37 ± 0.01 ^d
C	130 ± 2.3 ^a	127 ± 2.3 ^{ab}	155.4 ± 0.9 ^d	5.16 ± 0.03 ^{bc}	1.96 ± 0.06 ^b
2M	131 ± 1.9 ^{ab}	128 ± 2.5 ^{ab}	170.4 ± 1.1 ^e	5.04 ± 0.03 ^{ab}	2.41 ± 0.01 ^d
5M	130 ± 2.4 ^a	127 ± 2.5 ^{ab}	189.7 ± 0.8 ^f	4.93 ± 0.02 ^a	2.39 ± 0.01 ^d
10M	128 ± 2.0 ^a	124 ± 1.5 ^a	209.8 ± 2.4 ^g	5.02 ± 0.05 ^{ab}	1.59 ± 0.03 ^a

¹ Data are expressed as the mean ± SE. Within a column, values not sharing a superscript letter are significantly different from one another ($p < 0.05$). Among the experimental groups, the letter "a" indicates the smallest value with ascending order of "b," "c," "d," "e," "f," and "g."

concentration, but change was not significant compared with the control diet.

Kidney weight and kidney mineral concentrations

Kidney weight, kidney Ca and P concentrations, and the ratio of Ca to Mg decreased as the level of Mg in the diets increased (Table 8). There was a negative

Table 8. Kidney weight and kidney magnesium, calcium, and phosphorus concentrations in rats fed the respective diets.¹

Diet	Kidney weight (mg/100 g BW)	Magnesium (μ mol/g kidney)	Calcium (μ mol/g kidney)	Phosphorus (μ mol/g kidney)	Ca/Mg (mol/mol)
– M	967 $\pm$ 13 ^d	7.2 $\pm$ 0.1 ^a	213.5 $\pm$ 11.2 ^e	197 $\pm$ 6 ^e	29.44 $\pm$ 1.32 ^e
1/20M	993 $\pm$ 14 ^d	7.5 $\pm$ 0.1 ^a	234.5 $\pm$ 12.7 ^e	208 $\pm$ 8 ^e	31.30 $\pm$ 1.31 ^e
1/10M	1,038 $\pm$ 27 ^e	7.4 $\pm$ 0.2 ^a	187.5 $\pm$ 12.5 ^d	179 $\pm$ 7 ^d	25.34 $\pm$ 0.95 ^d
1/5M	658 $\pm$ 16 ^c	10.1 $\pm$ 0.2 ^c	118.1 $\pm$ 6.6 ^c	154 $\pm$ 4 ^c	11.72 $\pm$ 0.52 ^c
C	542 $\pm$ 18 ^b	10.7 $\pm$ 0.4 ^d	89.4 $\pm$ 5.9 ^b	137 $\pm$ 4 ^b	8.30 $\pm$ 0.35 ^b
2M	510 $\pm$ 4 ^a	8.4 $\pm$ 0.1 ^b	17.2 $\pm$ 2.4 ^a	99 $\pm$ 2 ^a	2.03 $\pm$ 0.27 ^a
5M	492 $\pm$ 7 ^a	8.2 $\pm$ 0.1 ^b	1.9 $\pm$ 0.1 ^a	90 $\pm$ 1 ^a	0.24 $\pm$ 0.01 ^a
10M	528 $\pm$ 7 ^a	8.4 $\pm$ 0.04 ^b	1.9 $\pm$ 0.1 ^a	90 $\pm$ 1 ^a	0.23 $\pm$ 0.01 ^a

¹ Data are expressed as the mean $\pm$ SE. Within a column, values not sharing a superscript letter are significantly different from one another ($p < 0.05$). Among the experimental groups, the letter “a” indicates the smallest value with ascending order of “b,” “c,” “d,” and “e.”

Fig. 1. A logarithmic curve between renal Ca concentration (mean $\pm$ SE) and dietary Mg level.

correlation between kidney Ca (Y) and serum Mg (X) concentration ($Y = 267 - 269X$, $R = 0.953$, $p < 0.0001$), although there were no significant correlations between kidney Ca and Mg concentrations. Kidney Mg was lower in rats fed the –M, 1/20M, 1/10M, 2M, 5M, and 10M diets than in rats fed the control diet. Figure 1 shows a logarithmic curve between renal Ca concentration and dietary Mg level.

Table 9. Pathological findings of kidney in rats fed the respective diets.¹

Diet	Deposit of calcium	Glo-merulus	Cortex	Medulla			Cell Infiltration	Fibrosis
				Outer stripe	Inner stripe	Inner zone		
–M	1,200–1,700 (1,401) ²	–	+	+++	++	+	+	±
1/20M	1,150–1,700 (1,409)	–	+	+++	++	+	+	±
1/10M	950–1,500 (1,228)	–	+	+++	+	+	+	±
1/5M	550–750 (607)	–	–	++	+	±	±	–
C	200–350 (303)	–	–	++	±	±	±	–
2M	60–120 (91)	–	–	+	±	±	±	–
5M	3–10 (5)	–	–	±	–	–	–	–
10M	0–2 (1)	–	–	–	–	–	–	–

¹ –: normal. ±, +, ++, and +++: magnitude of abnormality (± < + < ++ < +++).

² Within parentheses, values are expressed as means.

Pathological examination of kidney calcification

A rise in the dietary level of Mg was accompanied by a decrease in Ca deposition and a normalization of pathological indices (Table 9). Moreover, we found almost no deposition of Ca and no abnormality by pathological examination in rats fed the 10M diet. Abnormalities in the cortex were found in rats fed the –M, 1/20M, and 1/10M diets and were associated with increasing kidney weights and kidney and serum Ca levels. Furthermore, abnormalities in the medulla were found in these three groups. Most abnormalities occurred in the outer stripe of medulla, in which proximal and distal tubules are located. Fibrosis was found in rats fed the –M, 1/20M, and 1/10M diets. Fewer abnormalities of the medulla were present in rats fed the 2M, 5M, and 10M diets than those fed the control diet. Representative sections of kidneys from rats fed the –M, control, and 10M diets are shown in Fig. 2.

DISCUSSION

Nephrocalcinosis, induced in rats fed an Mg-deficient diet, is a pathological condition of the kidney in which intratubular calcified deposits occur along the

Fig. 2. Typical histopathological appearance of kidney tissue of rats fed the $-M$, control, and 10M diets. Paraffin slices of the formalin-fixed left kidney were stained with hematoxylin-eosin ($\times 100$). Photograph A is from rats fed the $-M$ diet, B from rats fed the control diet, and C from rats fed the 10M diet. Paraffin slices of the formalin-fixed left kidney were stained with Von Kossa's ($\times 100$). Photograph a is from rats fed the $-M$ diet, b from rats fed the control diet, and c from rats fed the 10M diet.

entire corticomedullary junction (16). In the present study, by examining dietary levels of Mg, we found that abnormalities proliferated from the outer stripe in the medulla to the cortex and inner zone in the medulla of kidney as the dietary level of Mg decreased. Furthermore, we found that urinary excretion of Ca decreased as the dietary level of Mg decreased, while the serum Ca level slightly increased and Ca absorption slightly decreased, compared with the normal level of Mg. We suggest that increased Ca reabsorption promotes Ca deposition in the kidney of rats fed a low Mg diet.

High dietary intakes of Mg were associated with reduced kidney calcification

in rats (9, 10). Woodard and Jee (17) reported that renal Ca was linearly decreased in rats fed the Mg 82 and 144 mmol/kg diets, although they did not prepare an Mg-deficient diet in the study. They suggested singly that renal Ca deposition was reduced in rats fed the high Mg diet. In the present study, we prepared different-dose Mg diets, from 0.3 to 187.4 mmol/kg diet, and obtained a logarithmic curve between renal Ca concentration and dietary Mg level. In particular, renal Ca concentration was decreased much more in rats fed a dietary Mg level 2 times higher than the control. We suggest that the 2M diet effectively reduced Ca deposition of kidney. Cruikshank et al (18) and Rude and Singer (19) reported that Mg competed with Ca for tubular reabsorption. Pastoor et al (8) speculated that increased amounts of renal-filtered Mg depress reabsorption of Ca and increase the urinary excretion of Ca. In the present study, a rise in the dietary level of Mg was accompanied by an increase in the serum Mg concentration. We suggest that nephrocalcinosis is reduced at high dietary Mg levels because of an effect on the reabsorption of Mg and Ca in the proximal and/or distal tubules.

Ritskes-Hoitinga and Beynen (16) reported that a low Mg diet reduces food intake in rats. However, it is not to be emphasized that food intake is also changed in rats fed a high Mg diet. In the present study, food intake was reduced in rats fed the low Mg diet, and also the 10M and 5M diets, but the 2M diet did not reduce food intake. Siu et al (20) focused on feedback regulation of P intake and reported that food intake was mediated by the mineral content in diet, and that the mechanism most likely involved plasma Ca homeostasis. In the present study, serum Ca levels were regulated in a narrow range. We speculate that a feedback mechanism exists by which rats regulate their Ca intake.

We consider that an extremely high Mg diet is not suitable for elucidating the mechanism of Ca deposition in normal rats because food intake is reduced, although a diet high in Mg clearly reduces Ca deposition. However, the 2M diet did not reduce food intake, body weight gain, or femur Ca concentrations, although Ca deposition in the kidney of rats fed the 2M diet was a third that of rats fed the control diet. Furthermore, abnormalities in the medulla of kidney were normalized in rats fed the 2M diet.

We suggest that a dietary Mg level 2 times higher than the currently recommended level would effectively reduce kidney calcification in rats while maintaining normal growth.

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