Diminished Kidney Function and Nephrocalcinosis in Rats Fed a Magnesium-Deficient Diet

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Summary The effect of a magnesium-deficient diet on kidney function was studied in young male rats. The rats were fed a purified diet with a magnesium content of either 20.5 (control diet) or 2.6 mmol/kg (magnesium-deficient diet) for 21 d. In rats fed the magnesium-deficient diet, kidney wet and dry weights were significantly increased, and calcium and phosphorus concentrations in the kidney were significantly higher than in rats fed the control diet. Upon histological examination, an increase in the mesangial matrix of the glomeruli and injury to the brush border of the proximal tubules were observed in rats fed the magnesium-deficient diet. Also, a deposition of calcium was observed in the tubules of the corticomedullary junction and medulla of these rats. Total protein and albumin concentrations in serum were significantly decreased in rats fed the magnesium-deficient diet. Urinary albumin excretion was significantly higher, and N-acetyl-β-D-glucosaminidase activity in the urine was significantly increased in rats fed the magnesium-deficient diet. These findings indicate diminished glomerular and proximal tubular functions. We suggest that a magnesium-deficient diet not only induces nephrocalcinosis, but it also diminishes kidney function.

Key Words magnesium-deficient diet, kidney function, nephrocalcinosis, rats

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Nephrocalcinosis refers to the deposition of calcium phosphate mainly in the entire corticomedullary junction of the kidney in rats (1, 2). The composition of the diet is an important factor in the etiology of nephrocalcinosis (2–6). Especially, a magnesium-deficient diet has been repeatedly reported to induce an increase in kidney calcium concentration and nephrocalcinosis as demonstrated in several studies (7–10). Numerous attempts have been made by researchers to demonstrate the effect of a magnesium-deficient diet on nephrocalcinosis.

Furthermore, rats fed a magnesium-deficient diet may display diminished kidney function, because nephrocalcinosis results from rats being fed the magnesium-deficient diet (7–10). With regard to kidney function in rats fed this diet, Van Camp et al (11) reported that urinary albumin excretion and plasma urea concentration were increased in rats fed a low-magnesium diet. The results of this report (11) indicate that a magnesium-deficient diet may induce diminished kidney function. However, the details of kidney function in rats fed the magnesium-deficient diet is not revealed in this report (11). Although urinary albumin excretion and plasma urea concentration serve as biochemical indicators of kidney function, the details of kidney function cannot be elucidated by these two indicators alone. Moreover, only a few attempts have so far been made to assess kidney function in rats fed a magnesium-deficient diet by using biochemical indicators and histological examinations. In other words, the study of the effect of magnesium-deficient diet on kidney function has until now been superficial.

Accordingly, the present study examined the effect of a magnesium-deficient diet on kidney function and the occurrence of nephrocalcinosis by means of biochemical indicators and histological examinations. The main purpose of this study was to reveal the kidney function in rats fed a magnesium-deficient diet.

MATERIALS AND METHODS

Animals. Four-week-old male Wistar-strain rats weighting approximately 90–100 g (Clea Japan, Tokyo, Japan) were housed in individual stainless steel wire-mesh cages. During the experiment, the rat cages were in a room with controlled lighting (12-h light: dark cycle; light, 0800–2000 h), temperature (22 ± 1°C) and humidity (60–65%).

Experimental diets. The composition of each of the experimental diets is shown in Table 1. The experimental diets were prepared according to the AIN-76 guidelines (12, 13). AIN-76 mineral mixture was used, excluding magnesium oxide. The dietary magnesium content was adjusted to control and deficient levels by using magnesium oxide (Wako Pure Chemical Industries, Osaka, Japan). The magnesium content in the control and magnesium-deficient diets was 20.5 and 2.6 mmol/kg, respectively. All experimental diets were stored at 4°C until use.

Experimental design. The study was approved by the Tokyo University of Agriculture Animal Use Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Tokyo University.
Table 1. Composition of the experimental diets.

| Ingredient            | Control diet | Magnesium-deficient diet |
|-----------------------|--------------|--------------------------|
| Casein, milk          | 200.00       | 200.00                   |
| Starch, corn          | 150.00       | 150.00                   |
| Cellulose powder      | 50.00        | 50.00                    |
| Oil, corn             | 50.00        | 50.00                    |
| Mineral mixture\(^1\) | 34.16        | 34.16                    |
| Vitamin mixture\(^2\) | 10.00        | 10.00                    |
| DL-Methionine         | 3.00         | 3.00                     |
| Choline bitartrate    | 2.00         | 2.00                     |
| MgO                   | 0.84         | 0.08                     |
| Sucrose               | 500.00       | 500.76                   |
| Chemical analysis     |              |                          |
| Magnesium content     | 20.50        | 2.60                     |

\(^1\) This mineral mixture is a modification of AIN-76 mineral mixture without magnesium source.
\(^2\) AIN-76A vitamin mixture.

of Agriculture. All rats were given free access to the control diet and demineralized water for a 7-d acclimation period before initiation of the study. After the acclimation period, the rats were divided into two groups of six rats each, all having a similar mean body weight. One experimental diet was assigned to each group. During the period of the experiment, rats fed the control diet were given an amount equivalent to that consumed by rats fed the magnesium-deficient diet. Rats were given free access to demineralized water during the period of the experiment, which was set at 21 d. Food intake and body weight were recorded daily.

Collection of samples. On days 20 to 21, the rats were housed individually in metabolism cages, and urine was collected every 24 h from each rat. Immediately after collection, the 24-h urine volume was measured. At the end of the experiment, the rats were sacrificed by decapitation. Blood was collected and centrifuged to separate the serum. Both kidneys were removed and weighed after the capsule was discarded. The right kidney was used for chemical analysis and the left kidney for histological examination. The urine, serum, and right kidney were stored at \(-40^\circ\text{C}\) until analysis.

Chemical analysis. The kidney was dried overnight at 100°C, and the dry weight was measured. The kidney was ashed at 550°C for 48 h and the minerals were extracted in 1 mol/L HCl solution for analysis. Calcium and magnesium in the kidney and magnesium in the diet were analyzed by atomic absorption spectrophotometry (Shimadzu AA-640-13) \((/4)\). Phosphorus in the kidney was
analyzed according to the method of Gomori (15). Creatinine in the serum and urine was measured by using the commercial assay kit Creatinine-TEST Wako (Wako Pure Chemical Industries). Urea nitrogen, uric acid, and total protein and albumin in the serum were measured by using the commercial assay kits Urea nitrogen-TEST Wako, Uric acid C-TEST Wako, and A/G B-TEST Wako (Wako Pure Chemical Industries), respectively. Albumin in the urine was measured by using the commercial assay kit PANATEST Rat Albumin (Panapharm Laboratories, Kumamoto, Japan). The N-acetyl-β-D-glucosaminidase (NAG) activity in the urine was measured by using the commercial assay kit NAG TEST Shionogi (Shionogi, Osaka, Japan).

Histological examination of the kidney. Immediately after collection, half of the left kidney was fixed in 10% neutral formalin phosphate buffer. The tissue samples were embedded in paraffin wax, cut into sections 5 μm thick, and the sections were stained with hematoxylin-eosin, periodic acid methenamine silver (PAM) and Von Kossa’s.

Statistical analysis. All data are expressed as means ± SE. Statistical analysis was performed by one-way ANOVA (16). Differences were considered significant when the p value was <0.05 between groups, comparing rats fed the control diet and those fed the magnesium-deficient diet.

RESULTS

Growth performance

Body weight and weight gain are presented in Table 2. Final body weight and weight gain were not significantly different between the two groups. Rats were given the diets by an equalized feeding method; therefore this was a necessary result.

Kidney analysis

Kidney weight, water content, and calcium, magnesium, and phosphorus concentrations in the kidney are presented in Table 3. Wet and dry weights of the kidney were significantly increased in rats fed the magnesium-deficient diet. Water content and calcium and phosphorus concentrations in the kidney were significantly

| Table 2. Growth performance in rats fed the control and magnesium-deficient diets. 1 |
|---------------------------------|-----------------|----------------------|
|                                 | Control diet    | Magnesium-deficient diet |
| Body weight (g)                |                 |                      |
| Initial                        | 170 ± 3         | 169 ± 3              |
| Final                          | 311 ± 7         | 299 ± 10             |
| Weight gain (g/d)              | 6.8 ± 0.4       | 6.2 ± 0.4            |

1 Values are means ± SE for six rats.
higher in rats fed the magnesium-deficient diet. Magnesium concentration in the kidney was not significantly different between the two groups.

**Histological examination of the kidney**

The results of histological examination of the kidney are presented in Fig. 1. Dilatation of the distal tubules was observed in rats fed the magnesium-deficient diet. A positive reaction upon treatment with PAM stain was detected in the glomerular basement membrane and mesangial matrix of the glomeruli of rats fed the magnesium-deficient diet, and from this result an increase in the mesangial matrix of the glomeruli was evident. The positive reaction upon treatment with PAM stain was weak in the brush border of the proximal tubules of rats fed the magnesium-deficient diet. A positive reaction upon treatment with eosin and PAM stain was detected in the tubules of the corticomedullary junction of rats fed the magnesium-deficient diet. Deposition of calcium was observed in the tubules of the corticomedullary junction in these rats. Furthermore, a slight deposition of calcium was observed in the tubules of the medulla of rats fed the magnesium-deficient diet (photomicrograph not shown).

**Biochemical indicators of kidney function**

The indicators of kidney function are presented in Table 4. There was no significant difference in urinary volume, creatinine clearance, serum urea nitrogen, or serum uric acid concentrations between the two groups. Total protein and albumin concentrations in serum were significantly decreased in rats fed the magnesium-deficient diet. Urinary albumin excretion was significantly increased and NAG activity in the urine was significantly higher in rats fed the magnesium-deficient diet in comparison with those fed the control diet.
Fig. 1.
Table 4. Biochemical indicators of kidney function in rats fed the control and magnesium-deficient diet.1

|                          | Control diet | Magnesium-deficient diet |
|--------------------------|--------------|--------------------------|
| Urinary volume (mL/d)    | 10.4 ± 1.6   | 12.2 ± 1.3               |
| Creatinine clearance (mL/min/100 g body weight) | 0.291 ± 0.012 | 0.325 ± 0.009 |
| Urea nitrogen in the serum (mmol/L) | 6.90 ± 0.78    | 7.23 ± 0.34             |
| Uric acid in the serum (μmol/L)      | 76.1 ± 6.2    | 74.2 ± 4.2              |
| Total protein in the serum (g/dL)     | 6.90 ± 0.39   | 5.47 ± 0.20*           |
| Albumin in the serum (g/dL)           | 4.13 ± 0.08   | 3.39 ± 0.07*           |
| Albumin in the urine (mg/d)           | 0.09 ± 0.01   | 0.33 ± 0.08*           |
| NAG activity in the urine (U/d)       | 0.19 ± 0.01   | 0.26 ± 0.01*           |

1 Values are means ± SE for six rats.

* Significantly different from the control diet (p < 0.05).

DISCUSSION

Animals fed a magnesium-deficient diet have been reported to display an increase in calcium concentration in the kidney and nephrocalcinosis as determined by histological observation (7–10). In the present study, the calcium concentration in the kidney was increased in rats fed the magnesium-deficient diet, and a deposition of calcium in the dilated tubules of the corticomedullary junction and medulla was also evident upon histological examination of the kidney. Furthermore, a positive reaction upon treatment with eosin and PAM stain at the sites of calcium deposition was detected in rats fed the magnesium-deficient diet, which indicates a presence of glycoprotein. The mechanism of magnesium-deficient diet-induced nephrocalcinosis remains unclear. Our findings suggest that some type of glycoprotein is present at sites of calcium deposition in rats fed a magnesium-deficient diet.

Fig. 1. Kidney section from a rat fed the magnesium-deficient diet examined by light microscopy. A: Dilatation of the distal tubules was observed. Hematoxylin-eosin-stained section, magnification ×100. B: A positive reaction upon treatment with periodic acid methenamine silver (PAM) stain was observed in the glomerular basement membrane and mesangial matrix of the glomerulus. A weak positive reaction was observed in the brush border of the proximal tubules (arrows). PAM-stained section, magnification ×100. C: A positive reaction upon treatment with eosin stain was detected in the tubules of the corticomedullary junction (arrows). Hematoxylin-eosin-stained section, magnification ×100. D: A positive reaction upon treatment with PAM stain was detected in the tubules of the corticomedullary junction (arrows). PAM-stained section, magnification ×100. E: A deposition of calcium was observed in the tubules of the corticomedullary junction (arrow). Von Kossa’s-stained section, magnification ×100.
Wet and dry kidney weights and water content and calcium and phosphorus concentrations in the kidney were increased in rats fed the magnesium-deficient diet. It seems likely that the increase in water content and the calcium and phosphorus concentrations in the kidney were mainly responsible for the increase in kidney weight in these rats.

In the present study, total protein and albumin concentrations in serum were lower in rats fed the magnesium-deficient diet. Furthermore, an increase in urinary albumin excretion was evident in rats fed the magnesium-deficient diet. This finding is similar to results reported by other investigators (11). In rats fed the magnesium-deficient diet, the decrease in total protein and albumin concentrations in serum were ascribed to an increase in urinary albumin excretion. It is known that an increase in urinary albumin excretion occurs as the result of a defect in the glomerular function (17, 18). In this study, an increase in the mesangial matrix was observed in the glomeruli of rats fed the magnesium-deficient diet, and this may be indicative of diminished glomerular function. Thus the increase in urinary excretion of albumin in rats fed the magnesium-deficient diet may be due to diminished glomerular function. Moreover, the increase in urinary albumin excretion is caused by a defect in the proximal tubular function, since albumin reabsorption occurs mainly in the proximal tubules (19). In the present study, proximal tubular function was examined by using NAG activity in the urine as an indicator and by histological observation. NAG activity in the urine was elevated in rats fed the magnesium-deficient diet. Furthermore, the PAM-positive reaction upon histological examination was weak in the brush border of the proximal tubules of rats fed the magnesium-deficient diet. It was evident that injury to the brush border of the proximal tubules occurred in rats fed the magnesium-deficient diet. The results concerning NAG activity in the urine and histological findings indicate that a magnesium-deficient diet induces diminished proximal tubular function, and this may lead to an obstruction of albumin reabsorption in the proximal tubules. We suggest that an increase in urinary albumin excretion in rats fed the magnesium-deficient diet may be due to a defect in the glomerular and proximal tubular functions.

In conclusion, nephrocalcinosis was induced in rats fed the magnesium-deficient diet, and histological changes in the glomeruli and proximal tubules were observed. The biochemical indicators of kidney function, urinary albumin excretion, and NAG activity in the urine were elevated in rats fed the magnesium-deficient diet, suggesting that such a diet induces not only nephrocalcinosis, but also diminished kidney function. Furthermore, the diminished kidney function induced by the magnesium-deficient diet refers to diminished glomerular and proximal tubular functions.

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