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Research Note

Early Changes in Bone Formation and Resorption of Magnesium-deficient Rats

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Abstract

Magnesium (Mg) deficiency is a risk factor for osteoporosis. It is important to clarify the effects of Mg deficiency on bone formation and resorption to prevent bone impairment by Mg deficiency. This study examined the effects of a Mg-deficient diet on bone formation and resorption in rats, with a particular focus on early changes in bone formation and resorption. Male Wistar rats were fed a control diet or a Mg-deficient diet. Serum Mg level was significantly decreased in the Mg-deficient group at all feeding periods. Femoral Mg content significantly decreased in the Mg-deficient group at 2, 4, and 7 d. Serum osteocalcin levels significantly decreased in the Mg-deficient group at 4 and 7 d. Excretion of urinary C-terminal telopeptides of type Ⅰ collagen significantly increased in the Mg-deficient group at 2 and 4 d. These results suggest that Mg-deficient diet rapidly induces decreased bone formation and increased bone resorption.

Key words: Bone Formation, Bone Resorption, Magnesium-deficient Diet, Rats

Ⅰ. INTRODUCTION

Magnesium (Mg) plays an important role in the maintenance of bone growth, and Mg deficiency is a risk factor for osteoporosis. Several studies have reported that Mg intake is correlated with bone mineral content and/or bone mineral density (BMD) in humans [1-3]. In animal studies, Mg deficiency has been shown to cause a decrease in bone Mg content and BMD [4,5]. In terms of bone formation, serum osteocalcin, a biochemical marker of bone formation, was decreased in Mg-deficient rats [6,7]. Our previous study [8] using bone histomorphometry revealed that mineralizing bone surface, mineral apposition rate, and surface referent bone formation rate were decreased in Mg-deficient rats. Rude et al. [9,10] also reported a decrease in osteoblasts and an increase in osteoclasts in Mg-deficient rats. Results of previous studies [6-10] indicate that Mg deficiency leads to a decrease in bone formation and an increase in bone resorption.

Rates of bone formation and resorption determine bone growth. Therefore, it is important to clarify the effects of Mg deficiency on bone formation and resorption to prevent bone impairment by Mg deficiency. Although many studies have been conducted, the effect of Mg deficiency on bone formation and resorption has until now been superficial. Furthermore, no studies have investigated early changes in bone turnover due to Mg deficiency. Accordingly, this study examined the effects of a Mg-deficient diet on bone turnover in rats, with particular attention focused on early changes in bone formation and resorption.

Ⅱ. MATERIALS AND METHODS

1. Animals and Diets

Four-week-old male Wistar rats (Clea Japan, Tokyo, Japan) were housed in individual stainless-steel wire-mesh cages. During the experiment, cages were located in a room with controlled lighting under a 12-h light:dark cycle (light, 0800-2000 h), a temperature of 24 ± 1° C, and a relative humidity of 55 ± 5%.

The composition of the experimental diets is shown in Table 1. Experimental diets were based on an AIN-93G

diet [11]. The two experimental diets contained different Mg concentrations (control diet, 0.05% and Mg-deficient diet, Mg-free). All the experimental diets had the same calcium (Ca) and phosphorus (P) concentrations. The Mg, Ca and P concentrations, as measured from an analysis of the experimental diets, are shown in Table 1.

2. Experimental design

Before the study began, there was a 5 d acclimatization period during which all rats were given free access to the control diet and demineralized water. After the acclimatization period, rats were divided into six groups of 5 rats, with each group having a similar mean body weight. Three groups were switched to the Mg-deficient diet, and the other three groups continued to receive the control diet. Rats were given free access to the experimental diet and demineralized water throughout the experimental period. Before dissection, the rats were housed individually in metabolic cages, and urine was collected from each rat for 24 h. Rats were sacrificed at 2, 4, and 7 d of the experimental period. Blood was collected in tubes, and was centrifuged to obtain serum. The left femur was removed and cleaned of muscles and connective tissue. Animals were treated in accordance with the guidelines of the National Research Council for the Care and Use of Laboratory Animals (1985).

3. Chemical analysis

Samples of the experimental diet and femur were ashed at 550°C for 48 h in a muffle furnace, and minerals were extracted in 1 mol/L of HCl for analysis. Mg and Ca in the experimental diet and femur were determined by atomic absorption spectrometry (Hitachi A-2000)[12]. P in the experimental diet and femur was determined using the method of Gomori [13]. Osteocalcin in the serum was measured with an osteocalcin rat ELISA system (Amersham Biosciences K.K., Tokyo, Japan). C-terminal telopeptide of type Ⅰ collagen (CTx) in urine were measured with a RatLaps ELISA (Nordic Bioscience Diagnostics A/S, Denmark). Creatinine in urine was measured with a Creatinine-Test Wako (Wako Pure Chemical Industries, Osaka, Japan). Mg in serum was measured with Magnesium B (Wako Pure Chemical Industries, Osaka, Japan).

4. Statistical analysis

Results are expressed as means ± SD. Data were analyzed by two-way ANOVA to determine the effect of dietary Mg concentration, the effect of the feeding period, and their interaction. Fisher's PLSD was used to determine the significant differences of multiple comparisons among groups.

Table 1 Composition of the experimental diets

	Control diet	Mg-deficient diet
Ingredient	g/kg	
Corn starch	529.486	529.486
Casein	200	200
Sucrose	100	100
Soybean oil	70	70
Cellulose powder	50	50
Mineral mix	35.0 [1]	35.0 [2]
Vitamin mix [3]	10	10
L-Cystine	3	3
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.014	0.014
Chemical analysis	%	
Mg	0.048	0.004
Ca	0.518	0.502
P	0.315	0.315

[1] AIN-93G mineral mix.
[2] AIN-93G mineral mix without Mg source.
[3] AIN-93 vitamin mix.

Differences were considered significant at $p<0.05$.

Ⅲ. RESULTS

1. Body Weight, Food Intake and Serum Mg Level

Dietary Mg concentration had no significant influence on final body weight (Table 2). Final body weight was significantly increased with feeding period, irrespective of the dietary Mg concentration. Dietary Mg concentration, feeding period and their interaction had no significant influence on food intake. Serum Mg level was significantly decreased in the Mg-deficient group at all feeding periods. In the Mg-deficient group, serum Mg level was significantly decreased with feeding period.

2. Femoral Mineral Contents and Biochemical Markers of Bone Turnover

Femoral Mg content was significantly decreased in the Mg-deficient group at 2, 4, and 7 d (Table 3). Femoral Mg level in the Mg-deficient group was significantly decreased with feeding period. Dietary Mg concentration, feeding period and their interaction had no significant influence on femoral Ca and P contents. Serum osteocalcin level was significantly decreased in the Mg-deficient group at 4 and 7 d. Urinary CTx excretion was significantly increased in the Mg-deficient group at 2 and 4 d. Urinary CTx excretion in the Mg-deficient

Table 2 Body weight, food intake, and serum Mg level in rats fed a control or Mg-deficient diet[1]

	2 days		4 days		7 days		Two-way ANOVA[2]
	Control diet	Mg-deficient diet	Control diet	Mg-deficient diet	Control diet	Mg-deficientdiet	
Initial body weight(g)	100.6 ± 4.3	101.1 ± 4.0	101.3 ± 3.7	100.9 ± 4.1	100.3 ± 2.0	99.8 ± 3.8	
Final body weight(g)	113.2 ± 5.5[a]	113.4 ± 4.7[a]	128.8 ± 6.6[b]	128.5 ± 5.9[b]	150.9 ± 4.3[c]	146.7 ± 9.1[c]	F
Food intake (g/day)	13.0 ± 1.0	13.3 ± 0.8	13.3 ± 0.9	13.0 ± 1.5	14.2 ± 0.5	13.1 ± 0.8	
Serum Mg (mg/dl)	1.98 ± 0.20	1.22 ± 0.11[*,a]	1.94 ± 0.04	0.98 ± 0.11[*,b]	1.97 ± 0.10	0.60 ± 0.03[*,c]	D, F, D × F

[1] Values are means ± SD (n=5).
[2] Significant effect ($p<0.05$): D=effect of dietary Mg concentration; F=effect of feeding period; D × F=effect of interaction.
[*] Significantly different from control diet with the same feeding period ($p<0.05$).
[a,b,c] Values with different superscript letters in the same diet are significantly different ($p<0.05$).

Table 3 Femoral mineral contents and biochemical markers of bone turnover in rats fed a control or Mg-deficient diet[1]

	2 days		4 days		7 days		Two-way ANOVA[2]
	Control diet	Mg-deficient diet	Control diet	Mg-deficient diet	Control diet	Mg-deficient diet	
Mg (mg/g dry weight)	3.41 ± 0.17	2.95 ± 0.18[*,a]	3.17 ± 0.21	2.48 ± 0.28[*,b]	3.24 ± 0.20	2.05 ± 0.11[*,c]	D, F, D × F
Ca (mg/g dry weight)	173.4 ± 9.6	171.5 ± 10.2	182.5 ± 10.9	175.1 ± 17.3	180.3 ± 7.7	172.5 ± 5.1	
P (mg/g dry weight)	89.5 ± 2.6	88.8 ± 4.9	90.3 ± 4.2	87.0 ± 8.3	92.4 ± 4.6	91.9 ± 3.0	
Osteocalcin in serum (ng/ml)	122.7 ± 27.4	114.5 ± 12.6[a]	141.5 ± 7.5	99.8 ± 8.3[*,a,b]	137.2 ± 16.6	88.1 ± 24.0[*,b]	D, D × F
CTx in urine (μg/mmol creatinine)	59.8 ± 7.6	98.7 ± 39.3[*]	48.5 ± 9.7	83.8 ± 12.2[*]	49.8 ± 10.5	72.8 ± 20.5	D

[1] Values are means ± SD (n=5).
[2] Significant effect ($p<0.05$): D=effect of dietary Mg concentration; F=effect of feeding period; D × F=effect of interaction.
[*] Significantly different from control diet with the same feeding period ($p<0.05$).
[a,b,c] Values with different superscript letters in the same diet are significantly different ($p<0.05$).

group also showed a tendency to increase at 7 d ($p=0.08$).

Ⅳ. DISCUSSION

This study showed that femoral Mg content was decreased in the Mg-deficient group. This result is similar to results of previous studies [4,5]. We also observed that femoral Mg content was decreased as early as 2 d after the start of the Mg-deficient diet. This result suggests that a Mg-deficient diet rapidly induces a decrease in femoral Mg content.

Our previous study [8] reported results of mineralizing bone surface, mineral apposition rate, and surface referent bone formation rate and showed that bone formation was suppressed by a Mg-deficient diet. Other researchers have shown a decrease in osteoblasts in Mg-deficient rats [9]. This study assessed bone formation using serum osteocalcin levels as a biochemical marker of bone formation. Results showed that serum osteocalcin levels were decreased in Mg-deficient group. This study and previous studies [8,9] indicate that bone formation is reduced in Mg-deficient rats. We also observed early changes in bone formation in Mg-deficient rats. After the start of the Mg-deficient diet, serum osteocalcin levels decreased at 4 d. This result suggests that reduction in bone formation is rapidly induced by Mg deficiency. With regard to the mechanism responsible for the reduction of bone formation induced by Mg-deficient diet, parathyroid hormone (PTH), 1,25$(OH)_2$-vitamin D and insulin-like growth factor Ⅰ (IGF-Ⅰ) are important factors in bone formation. Previous studies reported that serum PTH and 1,25$(OH)_2$-vitamin D levels [9,10] and serum IGF-Ⅰ levels [14,15] were decreased in rats fed a Mg-deficient diet. Decreases in serum PTH, 1,25$(OH)_2$-vitamin D, and IGF-Ⅰ levels may adversely influence bone formation in Mg-deficient rats. Because of the decreases in bone formation caused early on by a Mg-deficient diet in this

study, we speculate that an Mg-deficient diet may rapidly induce a decrease in serum PTH, 1,25$(OH)_2$-vitamin D, and IGF-Ⅰ levels. Additional studies are needed to verify this hypothesis.

This study showed that excretion of urinary CTx, a biochemical marker of bone resorption, was increased in the Mg-deficient group. This result indicates that a Mg-deficient diet induces an increase in bone resorption. Bone resorption is mediated by osteoclasts. Receptor activator of nuclear factor-kappa B ligand (RANKL) mediates osteoclast differentiation and activation [16-18]. Our previous study [15] observed that serum soluble RANKL level was increased in rats fed a Mg-restricted diet. Therefore, we suggest that the increases in bone resorption in this study may be due to an increase in osteoclast differentiation and activation. In other words, the Mg-deficient diet may enhance RANKL expression, thus elevating osteoclast differentiation and activation. Subsequently, increases in bone resorption are seen in Mg-deficient rats. This study observed that Mg-deficient diet rapidly induced a increase in urinary CTx excretion. This result suggests that increases in bone resorption causes early on by a Mg-deficient diet. Therefore, we speculate that RANKL levels may be sensitive to Mg deficiency as well as PTH, 1,25$(OH)_2$-vitamin D, and IGF-Ⅰ levels.

As described above, Mg-deficient diet rapidly induces changes in bone formation and resorption. At present, the details of bone formation and resorption in Mg deficiency remain unclear. However, results in this study indicate that 1) bone turnover is sensitive to Mg deficiency; and 2) Mg plays an important role in the maintenance of bone turnover. Therefore, it may provide valuable information into understanding the mechanisms responsible for bone impairment in Mg-deficiency.

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