The frequency of magnesium consumption directly influences its serum concentration and the amount of elutable bone magnesium in rats

Yumi Nakaya1, Mariko Uehara1, Shin-ichi Katsumata1, Kazuharu Suzuki1, Kensuke Sakai2, Ryuko Ohnishi3, Satoko Akiyama1, Atsutane Ohta2

1 Department of Nutritional Science, Tokyo University of Agriculture, Setagaya-ku, Tokyo; 2 Faculty of Clinical Pharmaceutical Science, Division of Pharmaceutical Science, Josai International University, Togane-shi, Chiba; 3 School of Food and Nutritional Science, The University of Shizuoka, Shizuoka-shi, Shizuoka, Japan

Correspondence: A. Ohta, Josai International University, 1 Gumyo, Togane, Chiba, 283-8555 Japan
<aohta@jiu.ac.jp>

Abstract. We investigated the influence of Mg feeding frequency on the variation in serum Mg concentration and tissue Mg levels in Mg-deficient rats. Sprague-Dawley rats, which had been fed a Mg-deficient diet for 14 d, were divided into 3 groups that were kept on 3 diets differing in their Mg content. The rats were fed 0.5-fold (Mg250 group), 1-fold (Mg500 group), or 1.5-fold (Mg750 group) the amounts of recommended Mg in their standard AIN-93G diet (Mg: 478 mg/kg diet) during the recovery period (12 d). The Mg500 and Mg750 groups were intermittently fed (Mg500, every 2 d; Mg750, every 3 d) so that their total intake of Mg during the recovery period could equal the Mg intake of the Mg250 group. The serum Mg concentrations increased in the 3 groups after feeding with a Mg-containing diet. However, serum Mg levels were only maintained within the normal range in the Mg250 group. After feeding on the Mg-deficient diet, in the intermittently fed groups, serum Mg concentrations decreased. Urinary Mg excretion was higher and Mg retention was lower in the Mg500 and Mg750 groups than in the Mg250 group. Moreover, bone Mg, especially elutable bone Mg, was lower in the Mg500 and Mg750 groups than in the Mg250 group. The elutable fraction of bone Mg correlated to the coefficient of variation of serum Mg concentration. In conclusion, for the maintenance of serum Mg concentration, it is important to increase the amount of elutable bone Mg by frequent Mg consumption.

Key words: magnesium recovery, continuous Mg intake, physiological Mg pool, rats

Mg, a physiologically essential element, is involved in ATP production [1], regulation of intracellular sodium, potassium, and calcium concentration [2], and changes in glucose metabolism and insulin secretion [3]. Mg intake is important because its deficiency disturbs the homeostasis of these reactions, which in turn leads to several diseases. Meta-analysis studies have suggested that Mg intake is inversely related to the incidence of metabolic syndrome [4], type 2 diabetes [5], and hypertension [6]. The National Health and Nutrition Survey conducted in Japan revealed that the average Mg intake in men (263 mg/d) and women (237 mg/d) was below the recommended dietary allowance (RDA); more than 50% of the adult population did not meet the RDA for Mg [7]. These results indicate that many people need to increase their dietary intake of Mg.

Generally, serum concentrations of numerous nutrients are usually good biomarkers for the evaluation of nutritional sufficiency. The levels of serum
and bone Mg are known to increase with the dietary Mg intake in rats [8, 9]. However, serum Mg concentration does not reflect nutritional Mg sufficiency because serum Mg is usually maintained within a narrow range by supplies from other tissues [10, 11]. At least, potential Mg deficiency may not be detected by measuring the serum Mg concentration, although it is possible to evaluate Mg status by evaluation of Mg pools within the body by using stable isotopes of Mg [12, 13]. Several investigations have been conducted to develop clinical methods for diagnosing potential Mg deficiency [14-16]. However, a brief and suitable clinical test for diagnosing Mg deficiency has not yet been developed. More detailed information is needed to use serum Mg concentration for the diagnosis of Mg deficiency.

Mechanisms by which Mg is supplied from tissues to the serum have been investigated, but the details remain unclear. It is known that Mg homeostasis is maintained by its intestinal absorption and renal excretion; however, little has been reported on the direct hormonal regulation of Mg homeostasis [17, 18]. A few studies have reported that the parathyroid hormone (PTH) stimulates the reabsorption of Mg in the kidney, its absorption from the intestine, and its release from the bone; these results suggest that PTH plays a role in maintaining Mg homeostasis [19]. In our previous study, we did not observe regulation of intestinal Mg absorption when the serum Mg concentration was high. Indeed, increased serum Mg with excessive doses of Mg did not decrease intestinal Mg absorption [20]. Therefore, we supposed that a negative feedback mechanism on intestinal Mg absorption by the administration of excessive Mg in rats does not exist. The soft tissues and bones, which act as Mg pools of the body, release Mg in response to a decrease in the serum Mg concentration in order to ensure that the serum Mg concentration is maintained within the normal range [11]. However, a decrease in the amount of serum and tissue Mg caused by the intake of a chronically Mg-Insufficient diet depletes the Mg pool. In these cases, Mg intake may directly affect the serum Mg concentration. Therefore, under such conditions, variations in serum Mg concentration after feeding on a Mg-deficient diet could be used as an indicator for potential Mg deficiency.

It could be debated that the nutritional effect of Mg supplementation is affected not only by the quantity but also by the source of Mg and possibly the frequency of Mg intake. Therefore, in the present study, we fed groups of rats with a Mg-containing diet at different intervals. Some rats were fed everyday with a diet containing only half the amount of Mg recommended in the normal diet, while others were fed a diet containing the recommended Mg amounts every 2 d or 1.5 times the amount of Mg recommended in the normal diet every 3 d. On other days, the rats were fed a Mg-deficient diet so that the total intake of Mg during the 12-d feeding period would be the same in all the rats. The purpose of this study was to clarify the mechanism that underlies the regulation of Mg homeostasis by investigating the correlation between serum Mg concentration and the Mg stored in bone and soft tissues and by examining the influence of Mg feeding frequency in Mg-deficient rats.

Materials and methods

Animals and diets

This study was approved by the Animal Management Committee of the Josai International University, and rats were maintained in accordance with the Committee guidelines for the care and use of laboratory animals. We housed 6-week-old male Sprague-Dawley rats (n = 37) (Clea Japan, Tokyo, Japan) in individual stainless-steel metabolic cages (with wire-mesh bottoms) placed in a temperature-and humidity-controlled room (temperature, 25°C; relative humidity, 55%) with a 12-h light/dark cycle.

Four experimental diets were used during this study. These diets were based on the AIN-93G formulation [21], and the ingredients used were obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan). The mineral mixture was a modification of the AIN-93G mineral mixture without magnesium oxide. The Mg level in Mg-containing diets, i.e., Mg250, Mg500, and Mg750, were adjusted to each level by using magnesium oxide, while a Mg-deficient diet was made with Mg-free additives only. The amount of Mg in the Mg250, Mg500, and Mg750 diets was 0.5, 1, and 1.5 folds the amount of Mg in the normal diet, based on the AIN-93G formula. The Mg-deficient, Mg250, Mg500, and Mg750 diet contained 36, 274, 478, and 761 mg/kg of Mg, respectively. The rats were allowed to freely consume the experimental diets and deionized water.

Experimental design

The rats (n = 30) were fed a Mg-deficient diet for 14 d (Mg-deficient period). Of these, some rats (n = 8) were classified into the Mg-deficient group. The control group (n = 7) comprised rats that were...
fed on the Mg500 diet for 14 d. After the Mg-deficient period, the rats from the control and Mg-deficient groups were anesthetized with diethyl ether. After laparotomy, whole blood was collected by abdominal vein puncture, and the rats were sacrificed. The remaining rats (n = 22) were weighed, and a blood sample was collected by the tail vein puncture; these samples were subsequently used to measure the serum Mg concentration. These rats were divided into 3 recovery groups, with 7–8 rats in each group, such that the body weight and serum Mg concentration would remain similar in all rats. The first group (n = 7) was fed the Mg250 diet everyday during the recovery period (12 d). The second group (n = 7) was alternatively fed the Mg500 and Mg-deficient diet during the recovery period. The third group (n = 8) was fed the Mg750 diet every 3 d and was fed the Mg-deficient diet on other days. The food intake and body weight were recorded everyday. Blood samples were collected everyday from all the rats and were used to determine the serum Mg concentration.

To assess the degree of Mg absorption and retention, feces and urine samples were collected throughout the recovery period. On the last day of the recovery period, all the rats were anesthetized with diethyl ether. After laparotomy, whole blood was stored on ice until centrifugation at 2,500 rpm for 10 min at 4°C to obtain the serum. The heart, liver, kidney, duodenum, soleus muscle, and femur were immediately sampled.

**Mg balance**

The feces were dried, weighed, and milled. Powdered fecal samples and urine samples were used for Mg analysis. The absorption and retention of Mg were calculated by the following formulae.

Absorption (mg/d) = intake – fecal excretion

Absorptivity (%) = absorption/intake × 100

Retention (mg/d) = absorption – urinary excretion

Retentivity (%) = retention/intake × 100

**Bone preparation for determination of the elutable bone Mg**

The femur was powdered according to the method described by Alfrey and Miller [22]. A portion of the powdered bone was used for elution studies. A second portion was weighed, dried for 16 h at 130°C, re-weighed, ashed for 16 h at 550°C, and weighed again. All data were calculated on the basis of the ashed weight. The amount of residual bone Mg was obtained by subtracting the amount of elutable bone Mg from the amount of total bone Mg.

**Analytical procedures**

The Mg concentration in the diets, fecal samples, urine samples, soft tissues, and bones were determined using an inductively coupled plasma emission spectrometer (ICPS-7000; Shimadzu, Kyoto, Japan). Approximately 5 g of each diet, 100 mg of feces, and 1 ml of urine were ashed by heating at 550°C for 24 h. Approximately 300 mg of the heart, liver, kidney, and spleen and approximately 150 mg of the duodenum and the whole right soleus muscle were dried and ashed by heating at 550°C for 24 h. The ashed samples were dissolved in 3 ml HCl solution (1 mol/L) and diluted further with HCl solution (0.1 mol/L) for atomization. The working standard solution was prepared by diluting MgCl₂ (Wako Pure Chemical Industries, Osaka, Japan). The detection limit for Mg was 0.1 mg/L.

The serum Mg was colorimetrically analyzed with the Mg B-test Wako assay kit (Wako Pure Chemical Industries).

**Calculation of coefficient of variation of serum Mg concentration**

In the Mg250, Mg500, and Mg750 groups, the means and standard deviations (SDs) were calculated on the basis of the daily serum Mg concentrations determined during the recovery period. In order to evaluate the change in serum Mg concentration during this period, a coefficient of variation was calculated by the following formula.

Coefficient of variation (%) = standard deviation/mean value × 100

**Statistical analysis**

Data are expressed as the means along with their SDs. These were analyzed by one-way analysis of variance (ANOVA), and significant differences among the groups were determined by the Tukey-Kramer test (SPSS ver. 12.0J; SPSS, Chicago, IL, USA). Differences were considered significant at a P value of < 0.05.

**Results**

**Initial and final body weight, body weight gain, food intake, and food efficiency**

During the Mg-deficient period, the final body weight, weight gain, food intake, and food efficiency were significantly lower in rats from the Mg-deficient
group than in those of the control group (table 1). During the recovery period, the final body weight, weight gain, food intake, and food efficiency did not vary among the 3 groups.

Concentration and daily change of serum Mg
At the end of the Mg-deficient period, the serum Mg concentration in rats that were fed a Mg-deficient diet (0.50 ± 0.21 mg/dL) was significantly reduced when compared with rats that were fed the control diet (2.22 ± 0.18 mg/dL) (p < 0.05). In rats that were fed Mg-containing diets, i.e., the Mg250, Mg500, and Mg750 diets, the serum Mg levels were significantly higher at the end of the recovery period than in the beginning of the recovery period (figure 1). At the start of the recovery period, the serum Mg concentration in rats of all groups increased within a day of consuming a Mg-containing diet (figure 1). In the Mg250 group, the serum Mg concentrations reached normal levels on the 4th day of the recovery period, and this level was maintained through the experimental period. During feeding of the Mg-deficient diet, the serum Mg concentrations decreased within a day in the Mg500 and Mg750 groups. Also, the serum Mg concentrations in these rats increased within a day after they were fed the Mg-containing diet.

Urinary excretion, absorption, and retention of Mg
The intake and fecal excretion of Mg did not vary among the 3 groups (table 2). Urinary Mg excretion was significantly lower in the Mg250 group than in the Mg500 and Mg750 groups. Mg absorption and Mg excretion (mg/12 d) were significantly lower in the Mg500 and Mg750 groups than in the Mg250 group.

Table 1. Initial and final body weight, body weight gain, food intake, and food efficiency of rats in Mg-deficient period and Mg-recovery period.

<table>
<thead>
<tr>
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<th>Mg-deficiency period</th>
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<th>Mg-recovery period</th>
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<tbody>
<tr>
<td></td>
<td>C (n = 7)</td>
<td>MgD (n = 8)</td>
<td>Mg250 (n = 7)</td>
</tr>
<tr>
<td>Initial weight, g</td>
<td>130 ± 4.2</td>
<td>129 ± 5.2a</td>
<td>204 ± 8.2a</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>249 ± 11.7</td>
<td>203 ± 8.7b</td>
<td>292 ± 19.5a</td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>119 ± 9.7</td>
<td>74 ± 5.6c</td>
<td>87 ± 13.6</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>285 ± 18.4</td>
<td>240 ± 15.3e</td>
<td>240 ± 18.9</td>
</tr>
<tr>
<td>Food efficiency</td>
<td>0.42 ± 0.03</td>
<td>0.31 ± 0.02e</td>
<td>0.36 ± 0.04a</td>
</tr>
</tbody>
</table>

Values are means ± SDs.
* p < 0.05 significant difference as compared to the control group.
Values with different superscript letters in the same row among all groups, excluding the control group are significantly different (p < 0.05), when analyzed by Tukey-Kramer test.

Mg concentration in the bone and soft tissues
The total, residual, and elutable bone Mg concentrations were significantly lower in rats of the Mg-deficient group than in those of the control group (table 3). The amounts of total and residual bone Mg were significantly higher in the recovery groups than in the Mg-deficient group; further, these values were significantly lower in the Mg750 group than in the Mg250 and Mg500 groups. There were significant differences between the 3 recovery groups with respect to the amount of elutable bone Mg.

Mg concentrations in soft tissues did not vary between the control and Mg-deficient group, and among the 3 recovery groups (table 3).

Relationship between the coefficient of variation of serum Mg and elutable bone Mg
Figure 2 shows a negative correlation between the coefficient of variation of serum Mg concentration and the amount of elutable bone Mg. The correlation coefficient was –0.652 (p = 0.001).

Discussion
In this study, Mg deficiency (MgD) in rats was obtained by feeding them a Mg-deficient diet for 14 d. Serum Mg concentration in the MgD rats was markedly lower (0.5 mg/dL) than that in the control rats (C) (2.2 mg/dL). We observed that the serum Mg concentration increased in rats from all the recovery groups within 1 d after they were fed a Mg-containing diet. In the Mg250 group, on the 4th day of the recovery period, the serum Mg concentrations reached normal levels and were maintained...
Figure 1. Changes in the food intake, body weight gain, and serum Mg concentration of the Mg250, Mg500 and Mg750 groups. Each value is mean ± SD. Significant differences analyzed by Tukey-Kramer test (p < 0.05) among the three groups at each day point are represented by adding superscript letters to each symbol of parameters i.e. the food intake (●) with a or b, the weight gain (▲) with c or d, and the serum Mg concentration (■) with e, f or g.
at that level throughout the experimental period. Therefore, we confirmed that 50% of the amount of Mg recommended in the AIN-93G standard diet might be sufficient for restoring serum Mg concentrations to normal levels. Total amounts of Mg intake during the recovery period were the same for the 3 groups. However, in rats that were intermittently fed Mg (Mg500 and Mg750), the serum Mg concentrations markedly decreased when they were fed a Mg-deficient diet but increased when they were fed the Mg-containing diet. Furthermore, the serum Mg concentrations, particularly in the Mg500 group, fluctuated every other day with Mg feeding. We suppose that these rats were potentially Mg deficient (insufficient body stores of Mg). These results suggest that a potential Mg deficiency may be assessed by investigating variations in serum Mg concentration after feeding a Mg-deficient diet, though further studies are required.

In rats that were intermittently fed a Mg-containing diet, Mg absorption and retention were decreased, and urinary Mg excretion was increased. Previous studies have reported that Mg homeostasis is primarily maintained by the renal handling of Mg [17, 18]. The renal tubular reabsorption of Mg regulates urinary excretion of Mg, while Mg is rapidly excreted in the urine when it is in excess [23, 24]. When serum Mg levels are over the threshold, i.e., approximately 1.5 mg/dL, the urinary excretion of Mg increases [25]. A higher intake of Mg may lead to a transient increase in serum Mg concentration and prolong the time for which the serum Mg concentration remains over the threshold level. Further, Mg may be rapidly excreted in the urine. These results indicated that the decreased Mg retention in rats that were intermittently fed with high amounts of Mg led to an increase in the urinary excretion of Mg.

Half the total amount of Mg is contained in soft tissues; the other half is contained in the bone. Several studies have confirmed that, though Mg concentrations in soft tissues remain unchanged or decreased, their decrease is not as remarkable as that in the bone during Mg depletion, even if the Mg concentration in serum and the bone has decreased [26-29]. In this study, the Mg concentration in

### Table 2. Intake, fecal and urinary excretion, absorption, absorptivity, retention, and retentivity of Mg of rats in Mg-recovery period.

<table>
<thead>
<tr>
<th></th>
<th>Mg250 (n = 7)</th>
<th>Mg500 (n = 7)</th>
<th>Mg750 (n = 8)</th>
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<tbody>
<tr>
<td>Mg intake, mg/12d</td>
<td>66.5 ± 5.2</td>
<td>61.0 ± 3.6</td>
<td>59.8 ± 6.2</td>
</tr>
<tr>
<td>Fecal Mg, mg/12d</td>
<td>13.1 ± 2.4</td>
<td>14.2 ± 3.3</td>
<td>16.4 ± 3.7</td>
</tr>
<tr>
<td>Urinantly Mg, mg/12d</td>
<td>13.7 ± 1.8</td>
<td>18.7 ± 1.7</td>
<td>15.8 ± 2.2</td>
</tr>
<tr>
<td>Mg absorption, mg/12d</td>
<td>53.4 ± 4.8</td>
<td>46.9 ± 3.5</td>
<td>43.4 ± 4.2</td>
</tr>
<tr>
<td>Mg absorptivity, %</td>
<td>80.3 ± 3.3</td>
<td>76.8 ± 4.7</td>
<td>72.8 ± 4.4</td>
</tr>
<tr>
<td>Mg retention, mg/12d</td>
<td>39.7 ± 4.6</td>
<td>28.2 ± 2.7</td>
<td>26.6 ± 2.4</td>
</tr>
<tr>
<td>Mg retentivity, %</td>
<td>59.7 ± 4.6</td>
<td>46.2 ± 3.5</td>
<td>44.7 ± 3.8</td>
</tr>
</tbody>
</table>

Values are means ± SDs. Values with different superscript letters in the same row are significantly different (p < 0.05), when analyzed by Tukey-Kramer test.

### Table 3. Bone magnesium pools and soft tissues of rats in Mg-deficient period and Mg-recovery period.

<table>
<thead>
<tr>
<th></th>
<th>Mg-depletion period</th>
<th>Mg-recovery period</th>
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<tbody>
<tr>
<td></td>
<td>C MgD</td>
<td>Mg250</td>
</tr>
<tr>
<td>mg/g ash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.46 ± 0.287</td>
<td>2.93 ± 0.165*</td>
</tr>
<tr>
<td>Residual</td>
<td>6.08 ± 0.278</td>
<td>2.41 ± 0.131*</td>
</tr>
<tr>
<td>Elutable</td>
<td>1.38 ± 0.064</td>
<td>0.52 ± 0.045*</td>
</tr>
<tr>
<td>μg/g tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>213 ± 7.3</td>
<td>211 ± 20.7*</td>
</tr>
<tr>
<td>Liver</td>
<td>195 ± 7.8</td>
<td>195 ± 22.5*</td>
</tr>
<tr>
<td>Kidney</td>
<td>198 ± 7.5</td>
<td>192 ± 30.3*</td>
</tr>
<tr>
<td>Duodenum</td>
<td>191 ± 3.9</td>
<td>180 ± 8.2</td>
</tr>
<tr>
<td>Muscle, soleus</td>
<td>216 ± 6.2</td>
<td>208 ± 22.6</td>
</tr>
</tbody>
</table>

Values are means ± SDs. *p < 0.05 significant difference as compared to the control group. Values with different superscript letters in the same row among all groups, excluding the control group are significantly different (p < 0.05), when analyzed by Tukey-Kramer test.
soft tissues remained mostly constant, irrespective of the amount of Mg retention. This result indicates that the body gives priority to the maintenance of Mg concentration in soft tissues as compared to that in the serum and in the bone. The Mg present in soft tissues is probably required for many physiological processes that occur therein [18, 19].

Bone Mg is not equally distributed in all the regions of the bone. Approximately 30% of bone Mg is present on the bone surface and is elutable, and this Mg is released from the bone surface in response to decreased serum Mg concentrations to maintain serum Mg levels [17, 30]. Further, in another study, the amount of elutable bone Mg was lower than that of residual bone Mg when the intake of Mg was restricted [30]. Therefore, an abundant amount of elutable bone Mg indicates that there is sufficient Mg for releasing. The increase in elutable bone Mg as well as total bone Mg may contribute to the maintenance of serum Mg levels during periods of Mg insufficiency. In the present study, we determined elutable and residual bone Mg by the method described by Alfrey and coworkers [22]. We observed that there is an insufficient increase in the amount of bone Mg in rats intermittently fed with Mg (Mg500, Mg750) as compared to that in rats fed on the Mg250 diet. After the Mg feeding period, the elutable bone Mg increased with an increase in the serum Mg concentration. The amounts of total and elutable bone Mg were higher in the Mg250 group than in the Mg500 and Mg750 groups. It was previously reported that the release of Mg from bone occurs within 5 minutes in vitro [22]. In the present study, the amount of elutable bone Mg was significantly lower in the Mg750 group than in the Mg500 group, although the degree of Mg retention was similar in both groups. Because lower serum Mg concentrations probably existed for a longer duration in the Mg750 group than in the Mg500 group, although the degree of Mg retention was similar in both groups. Because lower serum Mg concentrations probably existed for a longer duration in the Mg750 group than in the Mg500 group, the amount of elutable bone Mg did not increase or that elutable bone Mg released accumulated Mg. Indeed, we found that the elutable bone Mg inversely correlated with the variation of coefficient of serum Mg. This result agrees with the hypothesis that accumulated Mg is released from the elutable bone in response to low serum Mg concentrations during periods of intermittent Mg intake.

In conclusion, in rats that were intermittently fed with Mg, the serum Mg concentrations were not maintained within the normal range and fluctuated markedly according to the intake of Mg because the amount of Mg retention decreased due to an

\[ y = -19.69x + 45.742 \]
\[ r = -0.652 \ (p = 0.001) \]

**Figure 2.** The correlation of coefficient of variation of serum Mg concentration and elutable bone Mg concentration in recovery groups.
increase in urinary Mg excretion. Therefore, the variation of serum Mg concentration after feeding a Mg-deficient diet may reflect the body’s Mg status. We found that elutable bone Mg is utilized mainly when the serum Mg concentration decreases, since the variation of serum Mg concentration is related to the amount of elutable bone Mg, not to the amount of Mg in other soft tissues. We suggest that for the prevention of a potential Mg deficiency, it may be important to observe variations in serum Mg concentration and to frequently consume a Mg-containing diet for the accumulation of elutable bone Mg.

Disclosure
None of the authors has any conflict of interest to disclose.

References

