Bioavailability of Magnesium Contained in Purple Laver (Asakusa-Nori) by Rats with Scarce Magnesium, Being Evaluated from Serum Magnesium, Kidney Calcification, and Bone Magnesium Contents

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Summary An experiment was designed to evaluate the bioavailability of purple laver (Asakusa-Nori, Porphyra tenera Kjellman) magnesium (Mg) in Mg-scarcity Fischer 344 male rats from serum Mg level, kidney calcification and bone Mg contents. Male rats of 4 weeks of age were divided into four groups of six rats. The four groups were control (20SC), Mg-restricted (−Mg20SC), −Mg20SC plus purple layer (−Mg20SCP), and 20SC plus purple laver (20SCP) group respectively. To −Mg20SC, 1/10 Mg of the 20SC diet was added. −Mg20SCP diet was prepared to contain equal amount of Mg as in the 20SC diet with purple laver as a Mg source. 20SCP diet was designed to contain double amount of Mg. After a 3-week experimental period, rats were decapitated. Blood serum, right kidney, and right femur were collected and Mg, calcium (Ca), phosphorus (P) were determined. Serum Mg concentration of the −Mg20SC was 1/3 of the 20SC, indicating apparent hypomagnesemia. Serum P also showed lowered concentration. On the other hand, the serum Ca indicated higher value than the other groups, indicating hypercalcemia. Addition of purple laver to −Mg20SC diet resulted in a normal serum Mg, Ca, and P level. The Mg-scarcity (−Mg20SC) rats accumulated much amount of kidney Ca. Whereas, there was no significant difference in kidney Ca between control (20SC) group and purple laver-supplemented (−Mg20SCP) rat group. The −Mg20SC rats showed lowered ash content and reduced Mg and P concentrations in the femur. Purple laver supplementation increased the ash, Mg, and P. All of the results indicated that the purple laver Mg was used as a Mg source.

Key Words magnesium, magnesium bioavailability, magnesium deficient, purple laver, Asakusa-Nori, serum magnesium, kidney calcification, nephrocalcinosis, bone magnesium, femur magnesium
Depletion of magnesium (Mg) levels in blood serum, calcification of kidney and bone of rats fed a Mg-deficient diet have been reported by many investigators (1–4). Rats fed a diet containing sucrose, as compared to starch, as the dietary carbohydrate exhibited increased concentration of Ca in the kidneys (5). Also, histopathological examinations on nephrocalcinosis among the three strains of rats, Fischer 344 (F344), Wistar, and Sprague-Dawley, F344 rats showed the most severe nephrocalcinosis (6).

Due to an increased consumption of polished and/or refined food items and decreased use of grain and the flour in recent years, Mg intake of the people are being given attention (7–9). But, there are very few data on the bioavailability of Mg in the natural feeds (10).

The experiment reported here was designed to evaluate the bioavailability of purple layer (Asakusa-Nori, Porphyra tenera Kjellman) Mg in Mg-restricted F344 male rats from serum Mg level, kidney calcification and bone Mg contents (10–12). Purple layer is one of the traditional food items in Japan and contains comparatively higher amount of Mg (13).

METHODS

1. Animals. Male F344 rats of 3 weeks of age were purchased (Charles River Japan Inc., Atsugi, Kanagawa) and an experiment was conducted on those rats after they were fed control (20SC) diet (shown in Table 1) for a week as preliminary feeding. They were housed in an individual stainless steel, wire-mesh-bottomed cages at 22±1°C and humidity of 55±5%, in a room free from specific pathogens with a 12h light/dark cycle (lighted time from 7:00 through 19:00). After 1 week of feeding, those rats were divided into 4 groups consisting of six rats so that body weight of each group could be almost equivalent. They are as follows: control group (20SC), Mg-scarcity group (−Mg20SC), Mg-scarcity group with feeding purple layer (−Mg20SCP), and control group with feeding purple layer (20SCP).

2. Diets. The composition of diets for each group is listed in Table 1. Nephrocalcinogenic sucrose was prepared as diets to each group as carbohydrate source. To Mg-scarcity diet (−Mg20SC) group, 1/10 Mg of the 20SC diet was added. Purple layer was supplemented to the diet of −Mg20SCP group to provide the same amount of Mg contents to the diet of control group (20SC) as Mg source. Double amount of Mg content of those of −Mg20SCP group was supplemented to the diet of control group with feeding purple layer (20SCP). The protein and fiber from the purple layer were deducted from milk casein and fiber, and total amount of the diets was adjusted at the expense of sucrose. Analyzed mineral contents are also shown in Table 1.

Food and distilled water were provided to all rats ad libitum.

3. Analysis. After a 3-week experimental period, rats were decapitated and blood serum, right kidney, and right femur were collected to measure the concen-
Table 1. Composition of the diets (/100 g diet).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet group</th>
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<tbody>
<tr>
<td></td>
<td>20SC</td>
</tr>
<tr>
<td>Milk casein (g)</td>
<td>20</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>0.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>63.3</td>
</tr>
<tr>
<td>Fiber</td>
<td>5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mix.(^1)</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mix.(^2)</td>
<td>3.5</td>
</tr>
<tr>
<td>Calcium phosphate, dibasic</td>
<td>1.77</td>
</tr>
<tr>
<td>Magnesium oxide (mg)</td>
<td>83</td>
</tr>
<tr>
<td>Purple layer(^3)</td>
<td>0</td>
</tr>
<tr>
<td>Analyzed Ca (mg)</td>
<td>481</td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>45.9</td>
</tr>
<tr>
<td>P (mg)</td>
<td>521</td>
</tr>
</tbody>
</table>

\(^1\)AIN-76 vitamin mixture (19). \(^2\)AIN-76 mineral mixture (19) omitted Mg, Ca, and P. \(^3\)Powdered purple layer (80 mesh).

|tration of Mg, calcium (Ca), and phosphorus (P). Blood was centrifuged and the serum was kept at -20°C until analysis. Kidney was digested by a method combining acid hydrolysis and dry heat. Briefly, right kidney was digested and dried up with analytical grade nitric acid (80–120°C). Then the residue was heated to 270–300°C. After cooling to room temperature, the residue was digested and dried up with hydroperoxide (80–120°C). Following 0.5 N HCl digestion, dried-up (80–100°C) residue was dissolved in 0.5 N HCl. Right femur was ashed by the method of Calvo et al. (14) and was dissolved in 0.5 N HCl.

Magnesium and Ca were analyzed by atomic absorption spectrometry (model Spectre AA-40, Varian, Victoria, Australia) with a 2.500 ppm final concentration of the strontium in the sample. In the case of high phosphorus samples, the concentration of the strontium was elevated (15). Phosphorus was determined by Gomori method (16). Bovine liver 1577a from the National Bureau of Standards Reference Material was digested and analyzed along with samples to confirm accuracy.

4. Statistics. After confirming homogeneity of variance of the group data, t-test was conducted for these data and Welch t-test method was applied on other data which did not indicate homogeneity variance.

RESULTS AND DISCUSSION

1. Serum magnesium, calcium, and phosphorus

Serum Mg, Ca, and P concentrations are shown in Table 2. Serum Mg
concentration of the $-\text{Mg20SC}$ was 1/3 of the 20SC indicating apparent hypomagnesemia. Also, serum P showed lowered concentration. On the other hand, concentration of the serum Ca was higher than that of the other groups, indicating hypercalcemia. All of these results are characteristics of Mg-deficient rats (1).

Addition of purple layer to $-\text{Mg20SC}$ diet resulted in an approximately normal serum Mg, P level, and the Ca concentration maintained in normal level. There was no significant difference between control (20SC) and double amount of Mg diet (20SCP) rats.

These results indicate that purple layer Mg is useful for maintaining serum Mg, P, and Ca concentration in a normal range. Hypercalcemia due to Mg-deficient diet are well documented (1,17,18). Therefore, the restoration of the hypercalcemia by purple layer feeding seemed to indicate that purple layer Mg was used as Mg source.

2. Kidney magnesium, calcium, and phosphorus

Kidney Mg, Ca, and P concentrations are shown in Table 3. The Mg-scarcity rats ($-\text{Mg20SC}$) accumulated much amounts of kidney Ca. Whereas, there was no significant difference in kidney Ca between control (20SC) and purple layer-supplemented ($-\text{Mg20SCP}$) rats group. These results suggest that purple layer Mg was used as Mg source. Calcium content of the kidney of animals served a diet (20SCP) containing double amount of Mg was below 1/2 of the control group.
Table 4. Femur magnesium, calcium, and phosphorus content (/defatted dry right femur).

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Ash (mg)</th>
<th>Mg (mg/g)</th>
<th>Ca (mg/g)</th>
<th>P (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20SC</td>
<td>111.7±4.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.01±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>224.4±5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.4±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>−Mg20SC</td>
<td>93.8±3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.51±0.07&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>233.9±15.6</td>
<td>118.5±1.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>20SCP</td>
<td>108.0±4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.91±0.30&lt;sup&gt;de&lt;/sup&gt;</td>
<td>234.5±6.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>125.0±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>−Mg20SCP</td>
<td>104.3±6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.39±0.16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>248.0±4.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>122.1±0.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Having the same superscript letters within a column means significantly different to at least 95% probability.

(20SC). Diets used in this experiment contain nephrocalcinogenic sucrose as a sole carbohydrate source. Therefore, significant reduction of the kidney Ca in the 20SCP indicated that purple layer would prevent sucrose-induced nephrocalcinosis. Dietary Mg is known to prevent development of nephrocalcinosis (2). Therefore, the protective effect of 20SCP diet on nephrocalcinosis could be explained by double amount of Mg in the diet. But further experiments are needed before decisive conclusion.

Kidney Mg concentrations of the Mg-restricted rats (−Mg20SC) were lower than the control rats; whereas, there was no significant difference between control and purple layer-supplemented (−Mg20SCP) rats group. These results also seemed to indicate that the purple layer Mg was utilized as a Mg source.

3. Femur magnesium, calcium, and phosphorus

Ash, Mg, Ca, and P concentrations in defatted dry right femur are shown in Table 4.

Rat groups with scarcity of magnesium (−Mg20SC) showed lower value in the amount of ash, Mg, and P per one gram of defatted dry right femur than those of control rat group (20SC). In −Mg20SCP rat group supplemented with purple layer, amount of ash, Mg, and P concentration were increased and showed higher values than those of −Mg20SC. These results clearly indicate that supplemented amounts of Mg of purple layer to Mg-scarce diet were utilized as Mg supply source. That the amount of Mg of femur of rat group (20SCP) fed a double Mg amount in the diet was equivalent to the amount of those of control rat group (20SC) suggests the existence of saturation point in the amount of magnesium in rat femur. On the other hand, it was found that Ca concentration per gram of the defatted femur of −Mg20SCP rat group showed the highest value. These findings seemed interesting, according to my intuition, on the development of the further study on purple layer and its effect on mineral metabolism.

All of these results clearly indicate that magnesium contained in purple layer performs proper action or functions as Mg supply source.
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REFERENCES


