Bioavailability of Magnesium Contained in Roasted and Ground Soybean (Kinako) as Evaluated by Serum and Bone Magnesium Contents, Kidney Calcification, and Magnesium Absorption

Miho Hanai and Takatoshi Esashi

Division of Applied Food Research, National Institute of Health and Nutrition, Shinjuku-ku, Tokyo 162-8636, Japan

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Summary The bioavailability of roasted and ground soybean (kinako) magnesium (Mg) in Fischer 344 strain male rats with respect to serum Mg level, bone Mg contents, kidney calcification, and Mg absorption was evaluated. Four-week-old male rats were divided into four groups of six rats each. The four groups were the control (20SC), Mg-deficient diet (1/3 Mg20SC), 20SCK diet, and 20SCDK diet. The 20SCK and 20SCDK diets were prepared to contain amounts of Mg equal to that in the 20SC diet with kinako or defatted kinako as the Mg source, respectively. After a 4-week experimental period, rats were decapitated and blood serum, right femur, and right kidney were collected, and Mg, calcium (Ca), and phosphorus (P) concentrations in those tissues were determined. The Mg balance was also investigated. The serum Mg concentration in the 1/3 Mg20SC group was half the level in the 20SC group, and the serum Ca concentration was higher than in the 20SC group, indicating apparent hypercalcemia. Serum Mg and Ca concentrations in the 20SCK and 20SCDK groups did not significantly differ from those in the 20SC group. Femur Mg concentration in the 1/3 Mg20SC group was lower than in the 20SC group. Femur Mg concentrations in the 20SCK and 20SCDK groups were lower than in the 20SC group, but significantly higher than in the 1/3 Mg20SC group. The kidney Ca concentrations in the 20SCK and 20SCDK groups were significantly higher than those in the 20SC and 1/3 Mg20SC groups, and there was also kidney calcification. These results indicated that kinako and defatted kinako Mg were used effectively as a serum and femur Mg source, but that kinako and defatted kinako carry a risk of kidney calcification when used as the only Mg source.

Key Words magnesium bioavailability, kinako, soybean magnesium, kidney calcification, femur magnesium
Although the importance of magnesium (Mg) has been indicated, Mg ingestion by Japanese is below average at 300 mg/d (1). Therefore it is necessary to obtain sufficient information about foods that are good Mg sources and about the absorption and utilization of Mg. The authors previously reported the bioavailability of Mg in purple layer (asakusa-nori) (2) and yeast (3). In the present study, the bioavailability of Mg in roasted and ground soybean (kinako), a traditional Japanese food, was evaluated.

Soybeans or soybean products are thought to be a useful Mg source because of their high mineral content (4). However, the bioavailability of minerals in soybeans and soybean products have rarely been reported. In the study using kinako as a calcium (Ca) source, Sato (5) reported that the rate of Ca absorption from kinako was superior to that from skimmed milk, but the study did not report on Mg absorption.

Thus an investigation of the bioavailability of Mg contained in kinako being supplied as the only Mg source was made.

Besides a determination of intake and excretion of minerals, increases in body weights, bone growth, and mineral accumulation in various organs are used as indices for evaluating the bioavailability of minerals in foods (6). In the present study, therefore, the levels of serum Mg and Ca, Mg concentration in the femur, Ca precipitation in the kidneys, and absorption of Mg were evaluated as indices of Mg bioavailability as previously described in the reports on purple-layer (asakusa-nori) (2) and yeast (3).

To facilitate the evaluation of the effects of Ca precipitation in the kidneys, Fischer 344 strain rats were used as experimental animals (7) and sucrose as the carbohydrate source in their diet (8). Furthermore, defatted kinako was also examined to clarify whether the removal of fat influenced the bioavailability of Mg.

This study was approved by the National Institute of Health and Nutrition Animal Experimental Committee, and animals were maintained in accordance with the Guideline for the Care and Use of Laboratory Animals.

METHODS

I. Animals. Twenty-four Fischer strain (F344) male rats (purchased from Charles River Japan Inc., Kanagawa, Japan, at 3 weeks of age) were preliminarily maintained for a week on a 20SC diet (Table 1), then subdivided into 4 groups so that the mean body weight in each group was equivalent. These 4 groups consisted of the control diet group (20SC), the low-Mg diet group (1/3 Mg20SC), the kinako-supplemented diet group (20SCK), and the defatted kinako-supplemented diet group (20SCDK).

Each rat was maintained for 4 weeks in an individual stainless steel metabolic cage (product of Shinano Manufacture, Laboratory of National Institute of Health and Nutrition specification) at a room temperature of 22 ± 1°C and humidity of 55 ± 5% with a 12-h light/dark cycle (light from 07:00 to 19:00). All rats were
allowed free access to food and drinking water (distilled water).

2. Diet composition. The composition of the diets is shown in Table 1.

The composition of the AIN-76 diet (9) was partially modified, and sucrose was used as the carbohydrate source in each diet. The level of Mg in the 1/3 Mg20SC group was 1/3 what it was in the control diet group (20SC). The 20SCK group and the 20SCDK group obtained Mg from kinako or defatted kinako as the only Mg sources. The kinako-supplemented diet and the defatted kinako-supplemented diet were prepared to contain amounts of Mg equal to that in the 20SC diet. Moreover, levels of protein, lipids, fiber, Ca, and P in the 20SCK and 20SCDK diets were maintained equivalent to those in the 20SC diet.

The defatted kinako used in this study was obtained from kinako treated with ether by using a large Soxhlet extractor.

3. Balance study. During 4d on the 4th week of the study, feces and urine were collected separately, and the absorption and excretion of minerals were determined. As a marker for the collected feces, 0.1% of carmine was added to each diet. Feces collected were freeze-dried, weighed, and crushed, and admixtures

Table 1. Composition of the experimental diets (/100 g diet).

| Ingredients                  | 20SC | 1/3 Mg20SC | 20SCK | 20SCDK |
|------------------------------|------|------------|-------|--------|
| Milk casein (g)              | 20   | 20         | 10.98 | 10.81  |
| l-Methionine (g)             | 0.3  | 0.3        | 0.3   | 0.3    |
| Sucrose (g)                  | 63.01| 63.07      | 58.24 | 58.21  |
| Fiber (g)                    | 5    | 5          | 1.3   | 1.4    |
| Soybean oil (g)              | 5    | 5          | 1.4   | 5      |
| Vitamin mix. (g)             | 1    | 1          | 1     | 1      |
| Mineral mix. (g)             | 3.5  | 3.5        | 3.5   | 3.5    |
| Choline bitartrate (g)       | 0.2  | 0.2        | 0.2   | 0.2    |
| MgO (mg)                     | 83.0 | 27.7       | 0     | 0      |
| CaHPO₄ (g)                   | 1.770| 1.770      | 1.642 | 1.640  |
| KH₂PO₄ (mg)                  | 0    | 0          | 218.2 | 237.9  |
| KCl (mg)                     | 134.5| 134.5      | 14.9  | 4.0    |
| Kinako (g)                   | 0    | 0          | 21.2  | 0      |
| Defatted kinako (g)          | 0    | 0          | 0     | 17.7   |
| Analyzed Ca (mg)             | 463.4| 488.7      | 484.4 | 482.8  |
| Mg (mg)                      | 42.7 | 16.1       | 47.5  | 45.4   |
| P (mg)                       | 506.0| 496.1      | 583.3 | 578.0  |

1 AIN-76™ vitamin mixture (9).
2 AIN-76™ mineral mixture (9) omitted Mg, Ca, and P; contains K (289.5 mg/100 g mineral mixture).
including hairs were removed by filtering through an 80-mesh plastic nylon sieve to obtain samples for analysis. Urine was collected in a glass flask containing 10 mL of 5 N HCl and diluted with distilled water up to 250 mL, followed by filtering with No. 7 filter paper to obtain samples for analysis.

4. Analytical methods. After 4 weeks, the rats were decapitated, and the blood, kidney, and femur were collected. Blood was centrifuged (3,000 rpm, 15 min, 1,811 g, 4 °C) and the serum obtained was stored at −20 °C until analysis. The right kidney, food samples, feces, and urine were wet-digested by using a dry block bath. Details of the degradation process were previously reported (2).

The right femur was ashed by the method described by Calvo et al (10) and then dissolved in 0.5 N HCl to obtain samples for analysis.

Magnesium and Ca levels were determined by atomic absorption spectrophotometry (model Spectr AA-40, Varian, Victoria, Australia), with strontium added at the final concentration of 2,500 ppm to the samples. The concentration of strontium was appropriately increased according to the P/Ca ratio in samples containing a higher level of P (11). Phosphorus was determined by Gomori’s method (12). The accuracy of these mineral determinations was assessed by using bovine liver 1577b obtained from the National Institute of Standards & Technology (USA).

5. Statistical analysis. The results of the experiment were analyzed by one-way ANOVA and subsequently by Duncan’s multiple comparison test (13). Differences among mean values were considered significant at p < 0.05.

RESULTS

1. Serum levels of Mg, Ca, and P

Serum levels of Mg, Ca, and P are shown in Table 2. Serum Mg levels were significantly decreased in the 1/3 Mg20SC group compared with that in the control group (20SC). There was no significant difference observed among the 20SCK, 20SCDK, and 20SC groups. Serum Ca level was increased in the 1/3 Mg20SC group compared with the other groups, indicating hypercalcemia. Decreased serum P level was observed in both the 1/3 Mg20SC and the 20SCDK groups compared with that in the 20SC group.

| Table 2. Serum magnesium, calcium, and phosphorus content. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 20SC            | 1/3 Mg20SC      | 20SCK           | 20SCDK          |
| Mg (µg/mL)     | 13.0 ± 0.6a     | 5.5 ± 0.9b      | 12.1 ± 0.9a     | 13.7 ± 1.3c     |
| Ca (µg/mL)     | 107 ± 1a        | 112 ± 2b        | 109 ± 2a        | 109 ± 3a        |
| P (µg/mL)      | 86.0 ± 10.4a    | 73.1 ± 4.1b     | 78.6 ± 2.5ab    | 76.4 ± 4.8b     |

Values are the means ± SD (n = 6); those having different superscripts within a row are significantly different at p < 0.05.

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Table 3. Femur weight and magnesium, calcium, and phosphorus content in defatted dry femur.

|                        | 20SC  | 1/3 Mg20SC | 20SCK  | 20SCDK |
|------------------------|-------|------------|--------|--------|
| Dry femur wt. (mg)     | 187 ± 10 | 199 ± 10 | 199 ± 7 | 194 ± 15 |
| Defatted dry femur wt. (mg) | 176 ± 12 | 189 ± 10 | 191 ± 6 | 186 ± 16 |
| Ash (mg)               | 111 ± 8 | 116 ± 6   | 118 ± 4 | 114 ± 10 |
| Mg (mg/g DDF*)         | 3.98 ± 0.08 \( ^a \) | 2.17 ± 0.15 \( ^b \) | 3.74 ± 0.15 \( ^c \) | 3.60 ± 0.11 \( ^c \) |
| Ca (mg/g DDF)          | 238 ± 6 | 241 ± 32  | 225 ± 3.6 | 223 ± 1  |
| P (mg/g DDF)           | 113 ± 1 \( ^a \) | 110 ± 3 \( ^b \) | 112 ± 2 \( ^ab \) | 110 ± 1 \( ^b \) |

Values are the means ± SD (n = 6); those having different superscripts within a row are significantly different at \( p < 0.05 \).

* Defatted dry femur.

Table 4. Kidney magnesium, calcium, and phosphorus content.

|                       | 20SC | 1/3 Mg20SC | 20SCK | 20SCDK |
|-----------------------|------|------------|-------|--------|
| Mg (\( \mu \)g)       | 224 ± 12 \( ^ac \) | 191 ± 10 \( ^a \) | 351 ± 36 \( ^b \) | 268 ± 96 \( ^c \) |
| Ca (mg/g)             | 2.76 ± 0.61 \( ^a \) | 3.15 ± 0.26 \( ^c \) | 9.78 ± 1.83 \( ^b \) | 9.51 ± 1.28 \( ^b \) |
| P (mg/g)              | 3.97 ± 0.09 \( ^a \) | 4.02 ± 0.08 \( ^a \) | 7.45 ± 0.78 \( ^b \) | 7.20 ± 0.56 \( ^b \) |

Values are the means ± SD (n = 6); those having different superscripts within a row are significantly different at \( p < 0.05 \).

2. Levels of Mg, Ca, and P in the right femur

The weight of the right femur and levels of Mg, Ca, and P per gram of defatted femur are shown in Table 3. There was no significant difference among the four groups with regard to the weight of the dry or defatted femur and the amount of ash.

A significant decrease in the Mg level per gram of defatted femur was observed in the 1/3 Mg20SC group. The levels of Mg in the 20SCK and 20SCDK groups were significantly higher than in the 1/3 Mg20SC group, but did not reach the level of Mg in the 20SC group.

There was no difference in Ca concentrations among the four groups. Decreased P levels were observed in the 1/3 Mg20SC and 20SCK groups compared with that in the 20SC group.

3. Levels of Mg, Ca, and P in the kidney

Levels of Mg, Ca, and P in the kidney are shown in Table 4. Mg levels in the 20SCK and 20SCDK groups were significantly higher than in the 1/3 Mg20SC group.

Ca levels in the 20SCK and 20SCDK groups were higher than those in the 20SC and 1/3 Mg20SC groups, and P levels showed a similar tendency.

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4. Absorption of Mg

The amount of Mg intake, rate of absorption, and amounts of absorption and retention are shown in Table 5. The amount of Mg absorption or retention in the 1/3 Mg20SC group was lower than in the other groups. The rate of Mg absorption and the amount of absorption or retention in the 20SCK group were higher than in the 20SC group. No significant differences were observed in the rate of Mg absorption and in the amounts of absorption or retention between the 20SC group and the 20SCDK group.

5. Levels of urinary excretion of Mg, Ca, and P

The urinary excretion levels of Mg, Ca, and P are shown in Table 6. The urinary excretion of Mg was decreased in the 1/3 Mg20SC group and increased in the 20SCK and 20SCDK groups.

The excretion of Ca was significantly decreased in the 1/3 Mg20SC, 20SCK, and 20SCDK groups compared with that in the 20SC group.

The excretion of P was significantly increased in the 20SCK and 20SCDK groups compared with that in the 20SC group.

DISCUSSION

Rats in the 1/3 Mg20SC group showed apparent hypercalcemia, and the serum
Mg level in this group was decreased to less than 1/2 of that in the control (20SC) group. Moreover, Mg contents in the femur decreased to almost 1/2 of that in the 20SC group. These results for the Mg-deficient diet were similar to those previously reported (14). The serum levels of Ca and Mg in the 20SCK or 20SCDK groups demonstrated levels similar to that in the control. Although the Mg content in the femur was lower in the 20SCK and 20SCDK groups than in the control group, it was significantly higher than in the Mg-deficient group. No difference was observed in the weight of the femur and Ca contents between the groups maintained on a diet supplemented with kinako (20SCK) or defatted kinako (20SCDK) and the control group (20SC). Therefore it was thought that the Mg in kinako or defatted kinako was effectively used to maintain normal levels of serum Ca and Mg and normal bone formation in the femur.

The influence of soybean products on the bioavailability of minerals was reported by Brink et al (15), who indicated that the rate of Mg absorption was decreased by the ingestion of soybean proteins. It was also reported that phytic acid in soybeans or soybean products inhibited mineral absorption (16). Forbes et al (17) reported that the ingestion of soybean powder reduced the availability of Zn, but did not influence the availability of Ca and Mg. Moreover, Fukui et al (18) reported that the rate of absorbing Ca, Mg, and Zn from a soybean protein diet was similar to that from a casein diet. In those experiments, various concentrations of MgSO₄, MgCO₃, MgO, ZnCO₃, and CaCO₃ were added to soybean proteins or soybean powders, and the bioavailability of Mg, Zn, and Ca was evaluated by comparing them with an egg white protein diet or with a casein diet. In the present study, although kinako and defatted kinako were used as the only Mg source, no inhibition of Mg, Ca, and P absorption was observed (data for Ca and P not shown), which is consistent with the results reported by Forbes et al (17) and Fukui et al (18).

It was reported that Ca content in the kidneys is increased by low-Mg, high-P, and low-protein diets (19–22). Therefore in this study we determined the amount of Ca precipitated in the kidneys as an index for evaluating the bioavailability of Mg. As shown in Table 4, decreasing the dietary Mg content to 1/3 of the control amount did not induce significant differences in Ca levels in the kidneys when compared with that in the control group. However, Ca levels in the kidneys significantly increased in the groups fed a diet supplemented with kinako or defatted kinako. Furthermore, the level of P in the kidneys was also increased. Although Mg in kinako or defatted kinako was used as shown by the results for serum and femur, increased levels of Ca content in the kidney were observed in rats fed kinako or defatted kinako diets. Furthermore, it was apparent from the results of Mg absorption (Table 5) that Mg in kinako or defatted kinako was absorbed at a higher or equivalent level, respectively, compared with that in the control group. Especially, kinako Mg showed higher levels in the rate of absorption and in the amounts of absorption and retention than those in the control group. Also, as shown in Table 4, Mg levels in the kidneys increased significantly in both kinako-based groups.
compared with that in the control group. Although data are not shown, the rate of Ca or P absorption significantly increased in these two groups. Therefore it was thought that an increased level of Ca content in the kidneys after being fed with kinako or defatted kinako was not due to Mg deficiency.

Zhang and Beynen (23) compared the amount of ash and concentrations of Ca, P, and Mg in the kidneys between a casein-diet group and a soybean protein-diet group and reported that calcification of the kidneys resulting from the ingestion of soybean protein was not observed. However, Zalups (24) demonstrated that kidney calcification occurred after young rats were fed diets containing soybean protein as the only protein source. Therefore it was thought that soybean protein may participate in Ca accumulation in the kidneys, as observed in this study.

However, because an increased Ca content in the kidneys was also observed after a defatted kinako supplemented diet was consumed, lipids or fat-soluble substances contained in kinako might not participate in calcification in the kidneys. Therefore other components were thought to be strongly related to the calcification. It was not clear from the results of this experiment whether the cause of calcification was due to a single component of kinako or to an interaction of many components.

Since decreased urinary excretion of Ca and increased Mg and P excretion were observed in the rats fed diets supplemented with kinako or defatted kinako, it was thought that there were perhaps reabsorption disorders of minerals in the kidneys. It was previously reported that when calcification of the kidneys occurred, a decreased urinary excretion of Ca and an increased excretion of Mg were observed (25), which is consistent with the present results.

These results indicated that when a diet containing kinako as the only Mg source is provided, kinako Mg is utilized effectively in the serum and femur, but it induces calcification in the kidneys. Therefore an excessive ingestion of kinako could cause renal dysfunction.

The results of this experiment suggest the necessity of evaluating the bioavailability of the various components and analyzing the ingredients during any nutritional evaluation of food components. That is, besides a determination of the absorption rate of the suitable dietary components, it is necessary to design an experiment that could also evaluate whether the components travel to the various target organs and are used there to maintain normal organ function.

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