The Bioavailability of Magnesium in Spinach and the Effect of Oxalic Acid on Magnesium Utilization Examined in Diets of Magnesium-Deficient Rats

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Summary Spinach was evaluated for its bioavailability of magnesium in the experiment with magnesium-deficient rats. The effect of oxalic acid on absorption of dietary magnesium was also examined in the same experiment. After there were significant differences in the body weight of the rats between the control group and the magnesium-deficient group, and after the number of dead rats increased, the magnesium-deficient rats were divided into six groups. They were pair-fed for 8 days on the magnesium-deficient diet, magnesium-deficient diet supplemented with raw powdered spinach (R-sp), boiled powdered spinach (B-sp), or fried powdered spinach (F-sp), control diet supplemented with oxalic acid (Ox-C), and control diet (+Mg). On the 10th day, there was no significant difference in the food intake of the rats between the control group and magnesium-deficient group. However, the body weight, and body weight gain of the rats increased more significantly in the control group than in those of the magnesium-deficient group. Also, the contents of calcium and phosphorus in the liver and kidneys, and serum calcium content increased significantly in the magnesium-deficient rats compared with those of the control rats. However, the serum magnesium content decreased significantly in the magnesium-deficient rats. An especially large amount of calcium was accumulated in the kidneys of the magnesium-deficient rats. At the end of the experimental period, there were no significant differences in the food intake, body weight and body weight gain of the rats among the control group and each of the spinach-added groups. The body weight and body weight gain of the Ox-C rats decreased significantly in comparison with those of the control group and each of the spinach-added groups. Although, there were no significant differences in the concentrations of serum minerals (Mg, Ca and P) among each of the groups, kidney magnesium, calcium and phosphorus, and liver magnesium and phosphorus were significantly higher in each of the spinach-added groups than those of the control, Ox-C and +Mg groups. A large
amount of calcium was accumulated in the kidneys of the rats fed on the R-sp, B-sp, F-sp and Ox-C diets. However, the kidney calcium of each of the spinach-added groups markedly decreased in comparison with kidney calcium of the magnesium-deficient rats on the 10th day, when the magnesium-deficient rats were separated. There was no significant difference in the magnesium content of the left tibiae among each of the spinach-added groups. Also, the magnesium contents of the left tibiae of each of the additional groups did not reach the level of those of the control rats. The contents of calcium and phosphorus of the left tibiae were not significantly different among any of the groups except for both the R-sp and Ox-C groups, and decreased significantly in the R-sp and Ox-C groups compared with those of the other groups. A highly positive correlation between bone calcium and bone strength was not observed in this study; the breaking force of the left femurs of the B-sp and F-sp rats increased significantly in comparison with that of the Ox-C group. The rate of magnesium absorbed by the rats receiving the control, R-sp, B-sp, F-sp, Ox-C, and +Mg diets was 88.9, 80.2, 88.4, 90.4, 88.1, and 87.7%, respectively. The rate of apparent absorption of calcium from the control, Mg-deficient, R-sp, B-sp, F-sp, Ox-C and +Mg diets was 87.0, 84.1, 57.3, 66.4, 66.2, 53.3 and 83.5%, respectively. The data indicate that oxalic acid remained in spinach after cooking of boil or frizzle was not deleterious to magnesium availability, and that spinach is one of the most promising sources of magnesium.

Key Words  magnesium, magnesium bioavailability, spinach, magnesium-deficient rats, oxalic acid, kidney calcification, hypercalcemia, calcium apparent absorption

Magnesium deficiency in humans can cause many diseases of the heart (1-4). The death rates of coronary heart disease (5,6) and of ischemic heart disease (7,8) have been observed to correlate with the ratio of dietary calcium to magnesium, the magnesium concentration in drinking water, and the content of exchangeable magnesium in the soil. On the other hand, magnesium influences both matrix and mineral metabolism in bone, and magnesium depletion causes cessation of bone growth, decreased osteoblast and osteoblastic activity, osteopenia, and bone fragility (9). Trabecular bone from osteoporotic women also has a reduced magnesium content and larger bone crystal formation than controls (10). For these reasons, a safe and adequate daily dietary intake of magnesium by an adult in Japan has been set at 300mg (11). Nuts, unrefined cereals and seaweeds are the richest in magnesium, but the consumption of these foods per day is small compared with green leaf vegetables which are also rich in magnesium. Among the green leaf vegetables, spinach is one of the most commonly consumed vegetables throughout the year. spinach contains oxalate acid which can bind magnesium as well as...
BIOAVAILABILITY OF MAGNESIUM IN SPINACH

Calcium (12, 13) and other minerals. We have already reported that the oxalic acid concentration in spinach was the highest of those vegetables analyzed, and that the calcium absorption ratio by rats from spinach was lower than that from calcium oxalate (14). We have also reported that the greater part of calcium in spinach did not exist as calcium oxalate and that the solubility of magnesium was not affected by oxalic acid in vitro (15). It is suggested that spinach is a good source of magnesium. The apparent absorption of magnesium from raw spinach, boiled spinach, and fried spinach was examined through the experiments with magnesium-deficient rats. The effect of oxalic acid on the apparent absorption of magnesium was also investigated in the experiments with the magnesium-deficient rats. The bioavailability of magnesium in spinach and the effect of oxalic acid on the utilization of magnesium in the diets were measured according to the growth rate, magnesium balance, magnesium content of left tibiae, liver and kidney, and breaking forces of left femur.

MATERIALS AND METHODS

The spinach (smooth leaved) used for this study was obtained from a grocer’s shop in Okayama city during the period between June and July 1994. The spinach was washed with tap water sufficiently in order to remove the soil, and was then washed several times with distilled and deionized water (in the following description of water means distilled and deionized water). The spinach was treated with several cooking operations as follows: boiled in 10 volumes of water for 3 min, fried with a final weight of 3% of salad oil for 2 min after it was boiled in 10 volumes of water for 1 min. The raw, boiled, and fried spinach were powdered with a mill after drying in a vacuum oven at 40°C. A portion of each spinach preparation was digested with a mixture of concentrated HNO₃-concentrated H₂O₂ (5:1, v/v) in a high-performance microwave digestion unit (MILESTONE), after which undigested substance was completely digested with a mixture of concentrated HNO₃-concentrated HClO₄ (1:1, v/v) on a hot plate. The ash was dissolved into a small volume of 3 M HCl, and water was added to make a final concentration of 0.1 M HCl. This solution was used for the estimation of minerals (Ca, Mg) using an atomic absorption spectrophotometer, as described previously (12), and the phosphorus concentration in this solution was determined by the method of Gomori (16). The amount of oxalic acid was estimated by isotochophoresis, as described previously (14). Water-soluble dietary fiber (SDF), and water-insoluble dietary fiber (IDF) in the raw, boiled, and fried spinach were prepared according to the method of Prosky et al. (17). The contents of the analyzed substances in spinach are shown in Table 1.

Weaned male Wister rats were purchased commercially (from Charles River Japan Co., Kanagawa), and were housed individually in stainless-steel wire cages in a temperature-controlled room (21–23°C) with a 12-h light-dark cycle (lights on 6:00–18:00). The rats were pair-fed on either the control diet or the magnesium-
deficient diet for 9 days. Water was given *ad libitum*. Although, there were no differences in food intake between the control group and the magnesium-deficient group, significant differences in body weight and body weight gain appeared between the two groups. Eight control rats and 6 magnesium-deficient rats were killed by decapitation on the 10th day. As the number of rats that died from magnesium deficiency increased rapidly on the 9th day, the magnesium-deficient rats were divided on the 10th day into 6 dietary groups consisting of 11 rats for the magnesium-deficient group and 6 rats for the other groups. They were pair-fed for 8 days on: 1) magnesium-deficient diet, 2) magnesium-deficient diet supplemented with raw powdered spinach (R-sp), 3) magnesium-deficient diet supplemented with boiled powdered spinach (B-sp), 4) magnesium-deficient diet supplemented with fried powdered spinach (F-sp), 5) control diet supplemented with oxalic acid (Ox-C) which is the same amount of Ox-C as R-sp, or 6) magnesium-deficient diet supplemented with an equimolar amount of magnesium in the control diet (+ Mg). The composition of diets for each of the groups, which was prepared according to the component of Bieri et al. (18), is shown in Table 2. The contents of sucrose and cellulose powder in these diets were adjusted by the amounts of spinach, IDF, and Ox-C which was added to the diets. Food consumption was recorded daily, and body weight was measured every fourth day. During the last 4 days of the experimental period (from 5th day to 8th day after separation of the magnesium-deficient rats into 6 groups), feces and urine of each rat were collected and analyzed quantitatively, and feces were freeze-dried, weighed and homogenized. At the end of the experimental period, the animals were killed by decapitation after blood was collected from the heart with a syringe, and their organs, tibiae and femurs were removed quickly and weighed. Those organs and serum were stored at −20°C until analyzed. The left femurs of the rats were used for the measurement of breaking force using a rheodynamacorde (Iio Electric Co., Tokyo). The left tibiae were dried at 105°C for 24 h and defatted in a mixture of chloroform and methanol (2:1, v/v) (19) for 24 h and in ethylether for 24 h. They were then dried at 105°C until they obtained a constant weight. Diets, feces, urine, organs, serum, and left tibiae were completely digested with the same manner when spinach was digested. The ash was dissolved in HCl, and water was added to make a final concentration of 0.1 M HCl. This solution was used for the determination of minerals using an atomic absorption spectrophotometer. The phosphorus content in this solution was estimated by the method of Gomori (16). The concentration of inorganic phospho-

### Table 1. Contents of minerals, oxalic acid and dietary fiber in spinach added to diets.

|                      | Mg (mg/g) | Ca (mg/g) | P (mg/g) | Oxalic acid (mg/g) | IDF (%) | SDF (%) |
|----------------------|-----------|-----------|----------|-------------------|---------|---------|
| Raw powdered spinach | 8.96      | 7.91      | 3.62     | 146.3             | 23.3    | 15.3    |
| Boiled powdered spinach | 7.59     | 6.87      | 3.19     | 74.1              | 32.0    | 12.9    |
| Fried powdered spinach   | 5.75     | 4.65      | 2.14     | 39.1              | 27.1    | 12.1    |

J. Nutr. Sci. Vitaminol.
rus in the serum after treatment with trichloroacetic acid was determined by the method of Chen et al. (20).

The values for the analyzed substances in this work are given as the means and standard deviations. Statistical significance was evaluated by analysis of variance, followed by the UM test.

RESULTS

The contents of the spinach were found to be as follows: minerals (mg/g: Mg, 8.96; Ca, 7.91; P, 3.62), Ox-C (146.3 mg/g) and dietary fiber (%: IDF, 23.3; SDF, 15.3) in R-sp; minerals (mg/g: Mg, 7.59; Ca, 6.87; P, 3.19), Ox-C (74.1 mg/g) and dietary fiber (%: IDF, 32.0; SDF, 12.9) in B-sp; and minerals (mg/g: Mg, 5.75; Ca, 4.65; P, 2.14), Ox-C (39.1 mg/g) and dietary fiber (%: IDF, 27.1; SDF, 12.1) in F-sp. The amounts of minerals and Ox-C in the spinach were decreased by cooking. Especially, when spinach was boiled and fried, Ox-C content in the R-sp and F-sp decreased by 50% and 73%, respectively. As Ox-C is well known to impair calcium absorption (12,13), this reduction is desirable to improve absorption of dietary calcium.

Weaned rats, weighing 46.4 ± 1.4 g, were divided into the control group (17 rats) and the magnesium-deficient group (58 rats). The rats of both groups were fed for 9 days on the control or magnesium-deficient diets which are shown in Table 2. From the 7th to the 10th days, eleven out of 58 magnesium-deficient rats died. On the 10th day, the magnesium-deficient rats were divided into six groups (magnesium-deficient, R-sp, B-sp, F-sp, Ox-C, and + Mg groups) and the rats of each of the groups were fed for an additional 8 days on each of the diets shown in Table 2. The results of chemical analysis of each of the diets are also shown in Table 2.

Table 3 shows the food consumption and changes in the body weight of the rats receiving various diets before and after separation of magnesium-deficient rats. At the time of separation of the magnesium-deficient rats, there was no difference in the food intake of the rats for 9 days between the control group and the magnesium-deficient group, but the body weight and body weight gain of the control rats were significantly higher than those of the magnesium-deficient rats. There was no significant difference in the body weight of the rats at the time of separation of the magnesium-deficient rats among each group. During the 8 days after separation of the magnesium-deficient rats into 6 groups, three out of eleven magnesium-deficient rats died before the period of magnesium balance test, four out of eight magnesium-deficient rats died within the period of magnesium balance test, and two out of four magnesium-deficient rats died of magnesium deficiency at the final day of the experimental period. No significant differences were observed in food intake of the rats for 8 days after separation of the magnesium-deficient rats among the dietary groups. Although, there were no significant differences in the body weight and body weight gain of the rats between the control and each of the
Table 2. Composition of the experimental diets (g/100 g diet).

| Ingredient                      | Control | Mg-defi. | R-sp | B-sp | F-sp | Ox-C | + Mg |
|--------------------------------|---------|----------|------|------|------|------|------|
| Milk casein (vitamin free)     | 20.0    | 20.0     | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| L-Methionine                   | 0.3     | 0.3      | 0.3  | 0.3  | 0.3  | 0.3  | 0.3  |
| Sucrose                        | 65.0    | 65.0     | 60.55| 60.43| 58.6 | 64.17| 65.0 |
| Fiber                          | 5.0     | 5.0      | 3.7  | 2.9  | 2.6  | 5.0  | 5.0  |
| Soybean oil                    | 5.0     | 5.0      | 5.0  | 5.0  | 5.0  | 5.0  | 5.0  |
| AIN-76 Mineral mix.            | 3.5     | —        | —    | —    | —    | —    | —    |
| AIN-76 Mineral mix. (without Mg)| —       | 3.5      | 3.5  | 3.5  | 3.5  | —    | —    |
| AIN-76 vitamin mix.            | 1.0     | 1.0      | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  |
| Choline bitartrate             | 0.2     | 0.2      | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Spinach                        | —       | —        | 5.65 | 6.67 | 8.80 | —    | —    |
| Oxalic acid                    | —       | —        | —    | —    | —    | 0.827| —    |

Chemical analysis:

| Mineral | Control | Mg-defi. | R-sp | B-sp | F-sp | Ox-C | + Mg |
|---------|---------|----------|------|------|------|------|------|
| Mg (mg) | 51.0    | 0.43     | 56.6 | 57.0 | 59.2 | 55.4 | 51.0 |
| Ca (mg)  | 488     | 459      | 538  | 543  | 537  | 478  | 488  |
| P (mg)   | 411     | 419      | 435  | 430  | 428  | 414  | 411  |

1 Demineralized cellulose was prepared by stirring them in 3 M HCl, and then washing them in water. This treatment was repeated several times until no magnesium was detected by an atomic absorption spectrophotometer. 2 Fat-soluble vitamin dissolved in soybean oil.

Table 3. Food intake and bood weight of the rats receiving various diets.

| Beginning state | On the 10th day | Weight gain |
|-----------------|-----------------|-------------|
|                 | Food intake (g) | Body weight (g) | (g) |
| Control         | 46.3±1.3        | 61.7±0.6     | 73.9±4.0* | 12.2±1.3* |
| Mg-defi.        | 46.4±1.5        | 59.1±2.3     | 70.4±2.5* | 11.3±0.8* |

At the time of separation

| At the time of separation | On the 9th day after separation | Weight gain |
|---------------------------|--------------------------------|-------------|
|                           | Food intake (g) | Body weight (g) | (g) |
| Control                   | 73.9±4.0        | 61.4±0.3      | 95.5±4.8bc | 21.6±3.6bc |
| R-sp                      | 69.2±2.5        | 61.0±1.6      | 95.7±3.1de | 26.5±3.4cd |
| B-sp                      | 73.1±2.6        | 61.0±1.4      | 95.4±3.8fg | 22.3±3.0ef |
| F-sp                      | 72.1±2.4        | 60.6±1.7      | 94.5±2.7hi | 22.4±3.2f  |
| Ox-C                      | 70.7±5.4        | 60.6±1.4      | 85.6±4.6dng | 14.9±1.5gfg |
| +Mg                       | 69.9±2.2        | 61.0±1.5      | 88.9±1.6egj | 19.0±2.5fg |

Results are shown as M±SD. Having the same superscript letters in a column means a significant difference at the level of p<0.05 between the control and magnesium-deficient rats on the 10th day at the time of separation of the magnesium-deficient rats, and also at p<0.05 among each of the groups on the 9th day after separation of the magnesium-deficient rats.
spinach-added groups, the body weight and body weight gain of the control, R-sp, B-sp and F-sp rats increased significantly in comparison with those of the Ox-C rats. The body weight gain of the Ox-C rats was the lowest among each of the dietary groups.

The weight of organs and the contents of magnesium, calcium and phosphorus in the liver, kidneys and serum of the rats receiving various diets are shown in Table 4. On the 10th day at the time of separation of the magnesium-deficient rats, the liver weight of the magnesium-deficient rats was significantly higher than that of the control rats. Then, on the 9th day after separation of the magnesium-deficient rats, the liver weight of the R-sp and B-sp rats was significantly higher than that of the control, F-sp, Ox-C and +Mg rats. On the other hand, there was no difference in the weight of the kidneys on the 10th day, at the time of separation of the magnesium-deficient rats between the control and magnesium-deficient groups. However, the weight of the kidneys of the R-sp, B-sp and F-sp rats was significantly higher than that of the control rats. The weight of the kidneys was also significantly higher in the R-sp and B-sp rats than that of the control rats. Although, on the 10th day, at the time of separation of the magnesium-deficient rats, the liver magnesium content was not different between the control and each of the magnesium-deficient rats, the contents of calcium and phosphorus in the liver and kidneys and the concentration of serum calcium were significantly higher in the magnesium-deficient rats than in those of the control rats. An especially large amount of calcium was accumulated in the kidneys of the magnesium-deficient rats. The concentration of serum magnesium of the magnesium-deficient rats was significantly lower than that of the control rats. On the 9th day after separation of the magnesium-deficient rats, there was no difference in the content of liver calcium among the groups, but the content of liver magnesium was significantly higher in the each of the spinach-added groups than that in the Ox-C group, and was also significantly higher in the R-sp group than that of the +Mg group. The content of liver phosphorus was significantly higher in each of the spinach-added groups than those of the other groups. The contents of magnesium and phosphorus in the liver did not differ significantly among the control, Ox-C and +Mg groups. The magnesium content in the kidneys was significantly higher in each of the spinach-additional rats than that of the control and Ox-C rats. The contents of calcium and phosphorus in the kidneys were significantly higher in each of the spinach-added groups than those of the control and +Mg rats. The calcium content in the kidneys increased drastically in each of the spinach- or Ox-C added groups than that in the control. However, the calcium content in the kidneys of each of the additional groups decreased significantly in comparison with that of the magnesium-deficient group on the 10th day, at the time of separation of the magnesium-deficient rats. The magnesium concentration in the serum of the rats fed on each of the additional diets reached the level of the control rats. There were no differences in the concentration of calcium and phosphorus in the serum of the rats among any of the groups.
Table 4. Weight of organs and contents of magnesium, calcium and phosphorous in liver, kidneys and serum of the rats receiving various diets.

|                  |       |       |       |       |
|------------------|-------|-------|-------|-------|
|                  | Weight (g) | Mg     | Ca     | P     |
|                  | mg/liver | µg/liver | mg/liver |       |
| **Liver**        |         |         |         |       |
| On the 10th day  |         |         |         |       |
| Control          | 2.87±0.12* | 1.01±0.10 | 178±18* | 9.83±1.03* |
| Mg-defi.         | 3.36±0.22* | 1.07±0.09 | 238±22* | 14.2±1.4* |
| On the 9th day after separation |         |         |         |       |
| Control          | 3.49±0.42bc | 1.03±0.20 | 136±17 | 8.25±2.31bcd |
| R-sp             | 4.18±0.27bcdef | 1.24±0.15bc | 118±10 | 14.6±1.3bcf |
| B-sp             | 4.18±0.31cghi | 1.05±0.10d | 130±11 | 14.3±1.2cgh |
| F-sp             | 3.56±0.10g | 1.01±0.02e | 119±10 | 15.0±1.5gij |
| Ox-C             | 3.55±0.27h | 0.84±0.07bcde | 140±19 | 7.72±0.82ghi |
| + Mg             | 3.61±0.14g | 0.94±0.08c | 126±18 | 8.33±0.87ghij |
| **Kidneys**      |         |         |         |       |
| On the 10th day  |         |         |         |       |
| Control          | 0.85±0.10 | 257±35 | 40.0±4.5a | 2.24±0.28a |
| Mg-defi.         | 1.17±0.26 | 226±56 | 10,735±1,961* | 7.72±2.51* |
| On the 9th day after separation |         |         |         |       |
| Control          | 0.95±0.06abc | 182±11abcd | 34.6±3.7bcdef | 1.64±0.46bcdef |
| R-sp             | 1.42±0.26ad | 290±11bcdef | 7,831±1,268gh | 6.09±2.28bcf |
| B-sp             | 1.39±0.27bc | 279±27gh | 6,482±1,733i | 6.84±1.82gij |
| F-sp             | 1.19±0.10f | 299±18cghi | 5,600±1,036dij | 5.94±0.78fh |
| Ox-C             | 1.03±0.12de | 197±14gij | 6,827±1,470gk | 6.23±1.30fhij |
| + Mg             | 1.16±0.21 | 251±13dij | 2,661±417hijkl | 2.00±0.25ghi |
| **Serum**        |         |         |         |       |
| On the 10th day  |         |         |         |       |
| Control          | 3.47±0.45a | 9.90±0.80a | 3.87±0.54 |       |
| Mg-defi.         | 0.96±0.20a | 11.0±0.3a | 3.77±0.23 |       |
| On the 9th day after separation |         |         |         |       |
| Control          | 1.33±0.09 | 10.1±0.7 | 3.58±0.28 |       |
| R-sp             | 1.39±0.11 | 10.3±0.7 | 3.61±0.45 |       |
| B-sp             | 1.41±0.18 | 10.0±0.5 | 3.85±0.78 |       |
| F-sp             | 1.36±0.17 | 10.0±0.5 | 3.31±0.49 |       |
| Ox-C             | 1.35±0.12 | 10.2±0.7 | 3.63±0.64 |       |
| + Mg             | 1.28±0.14 | 10.2±0.8 | 3.17±0.67 |       |

Results are shown as M±SD. Having the same superscript letters in a column means a significant difference at the level of p < 0.05 between the control and magnesium-deficient rats on the 10th day at the time of separation of the magnesium-deficient rats, and also at p < 0.05 among each of the groups on the 9th day after separation of the magnesium-deficient rats.
The bone weight and contents of minerals of the defatted dry left tibiae and the breaking force of the left femurs of the rats receiving various diets are shown in Table 5. On the 10th day, at the time of separation of the magnesium-deficient rats, the magnesium content of the left tibiae was significantly lower in the magnesium-deficient rats than that of the control rats, but there were no significant differences in the weight and the contents of calcium and phosphorus in the left tibiae and the breaking force of the left femurs between the control and magnesium-deficient rats. On the 9th day after separation of the magnesium-deficient rats, there were significant increases in the weight of the left tibiae of the control, F-sp and +Mg rats in comparison with that of the R-sp and Ox-C rats. Although, the contents of calcium and phosphorus of the left tibiae increased significantly in the control, B-sp, F-sp and +Mg rats compared with those of the R-sp and Ox-C rats, the breaking force of the left femurs was significantly higher in the B-sp and F-sp rats in comparison with that of the Ox-C rats. The magnesium content of the left tibiae of the rats increased significantly in each of the additional groups compared with that of the magnesium-deficient rats on the 10th day at the time of separation of the magnesium-deficient rats, and the magnesium-content of the left tibiae of each of the additional groups did not reach the level of the control rats.

Table 6 shows the daily magnesium intake, daily fecal and urinary magnesium excretion, magnesium apparent absorption, and magnesium retention of the rats receiving various diets. The magnesium intake was significantly higher in the spinach-additional groups and the Ox-C group than that of the control, magnesium-deficient and +Mg groups and did not differ among the spinach-additional groups.
Table 6. Magnesium apparent absorption and balance of the rats receiving various diets.

|                | Intake (µg/day) | Feces (µg/day) | Urine (µg/day) | Absorption (%) | Retention (µg/day) |
|----------------|----------------|----------------|----------------|----------------|-------------------|
| Control        | 4,078±100       | 453±139        | 2,197±144      | 88.9±3.3        | 1,428±154         |
| Mg-defi.       | 33±8           | 229±25         | 1.32±0.4       | -197±22         |                   |
| R-sp           | 4,500±166       | 890±149        | 1,794±189      | 80.2±3.4        | 1,816±134         |
| B-sp           | 4,548±173       | 527±73         | 858±147        | 88.4±1.6        | 3,163±129         |
| F-sp           | 4,309±198       | 413±38         | 896±151        | 90.4±0.7        | 3,000±122         |
| Ox-C           | 4,428±125       | 525±75         | 1,786±193      | 88.1±2.7        | 2,117±208         |
| +Mg            | 4,078±101       | 502±164        | 1,998±264      | 87.7±4.0        | 1,578±144         |

Results are shown as M±SD. Having the same superscript letters in a column means a significant difference at the level of p<0.05 among each of the groups on the 9th day after separation of the magnesium-deficient rats.

and the Ox-C group. Fecal magnesium excretion of the rats fed on each of the spinach-added diets was high in the order R-sp, B-sp and F-sp. Then, fecal magnesium excretion of the rats fed on the Ox-C diet which added the same amount of Ox-C as the R-sp diet was significantly higher than that of the F-sp rats. In the magnesium-deficient rats, magnesium excretion into the feces was about 7 times as much as daily intake. Urinary magnesium excretion was significantly higher in the control rats than that of each of the additional groups except the +Mg group, and increased significantly in the R-sp, Ox-C and +Mg rats compared with that of both the B-sp and F-sp rats. The apparent absorption of magnesium in the control, R-sp, B-sp, F-sp, Ox-C and +Mg diets was 88.9, 80.2, 88.4, 90.4, 88.1 and 87.7%, respectively; although, the apparent absorption of magnesium was the lowest in the R-sp diet. The utilization of magnesium in the diets supplemented with raw, boiled, and fried spinach was extremely high in the magnesium-deficient rats. The retention of magnesium of the rats fed on each of the spinach-added diets was significantly higher than the magnesium retention of the control rats.

Calcium apparent absorption and balance of the rats receiving various diets are shown in Table 7. The calcium intake did not differ among each of the groups except the R-sp and B-sp groups, and was significantly higher in the R-sp and B-sp groups than that of the other groups. Fecal calcium excretion of the rats was significantly higher in the spinach-added groups and the Ox-C group than that of the other groups without Ox-C. However, urinary calcium excretion was significantly lower in the B-sp and F-sp groups than that of the other groups. The apparent absorption of calcium in the control, magnesium-deficient, R-sp, B-sp, F-sp, Ox-C and +Mg diets was 87.0, 84.1, 57.3, 66.4, 66.2, 53.3 and 83.5%, respectively. The rate of calcium absorption of the rats fed on the diets containing a large amount of Ox-C was lower than that of the other groups. The retention of calcium of the rats fed on the spinach-added groups, and the Ox-C group was
Table 7. Calcium apparent absorption and balance of the rats receiving various diets.

|           | Intake (mg/day) | Feces (mg/day) | Urine (µg/day) | Absorption (%) | Retention (mg/day) |
|-----------|-----------------|----------------|---------------|----------------|-------------------|
| Control   | 39.0±0.9<sup>ab</sup> | 5.07±1.36<sup>bcd</sup> | 1,809±613<sup>abc</sup> | 87.0±5.1<sup>abed</sup> | 32.1±2.2<sup>abcd</sup> |
| Mg-defi.  | 36.1±3.1<sup>ed</sup> | 5.70±1.59<sup>gh</sup> | 1,290±396<sup>deh</sup> | 84.1±4.7<sup>efgh</sup> | 29.0±2.3<sup>ef</sup> |
| R-sp      | 42.8±1.5<sup>ncdefg</sup> | 18.3±2.4<sup>anij</sup> | 1,093±126<sup>afgh</sup> | 57.3±5.7<sup>ani</sup> | 23.4±2.4<sup>anghi</sup> |
| B-sp      | 43.3±1.6<sup>bhij</sup> | 14.5±2.0<sup>bilm</sup> | 155±21<sup>bdeij</sup> | 66.4±5.4<sup>bdfk</sup> | 28.6±2.0<sup>bfk</sup> |
| F-sp      | 39.0±1.8<sup>ef</sup> | 13.2±1.4<sup>egino</sup> | 186±19<sup>efgkl</sup> | 66.2±3.8<sup>eglm</sup> | 25.7±1.4<sup>egm</sup> |
| Ox-C      | 38.2±1.1<sup>il</sup> | 17.8±1.8<sup>dhlm</sup> | 1,213±155<sup>ikkm</sup> | 53.3±5.1<sup>dhjlo</sup> | 19.2±1.8<sup>dhklm</sup> |
| +Mg       | 39.0±1.0<sup>ijkl</sup> | 6.4±0.6<sup>iknop</sup> | 1,789±220<sup>hjkm</sup> | 83.5±2.4<sup>ikno</sup> | 30.8±1.2<sup>ikmn</sup> |

Results are shown as M±SD. Having the same superscript letters in a column means a significant difference at the level of p<0.05 among each of the groups on the 9th day after separation of the magnesium-deficient rats.

significantly lower than that of the control group. Especially, the retention of calcium in the Ox-C rats was the lowest among the dietary groups.

**DISCUSSION**

The bioavailability of magnesium from spinach was evaluated by the degree of recovery from magnesium-deficiency of the rats fed on the diet containing raw, boiled or fried spinach. There was no difference in the body weight of the rats at the time of separation of the magnesium-deficient rats among each of the dietary groups. However, at the final day of the experimental period, the body weight and weight gain of the Ox-C rats was the lowest among the dietary groups (Table 3). The apparent absorption and retention of calcium of the Ox-C rats was the lowest among the dietary groups (Table 7). It is possible that the low body weight of the Ox-C rats resulted from an apparent absorption and retention of calcium which is the lowest among the dietary groups. It is known that Ox-C impairs calcium absorption (12,13). There is a possibility that this phenomenon observed among the Ox-C rats might have resulted from disturbed calcium absorption by Ox-C. Although, there was no significant difference in the body weight at the time of separation of the magnesium-deficient rats between the control and +Mg groups, the body weight of the +Mg rats at the final day of the experimental period was significantly lower than that of the control; however, there was no significant difference in the body weight gain between these two groups. These results suggested that +Mg rats did not completely recover from magnesium-deficiency. The body weight and body weight gain of the spinach-added groups at the final day of the experimental period reached the levels of the control group (Table 3). In an animal study by Whang and Welt (21) in which the effect of dietary magnesium depletion was examined on the levels of electrolytes in serum and muscle, the serum level of magnesium decreased and that of calcium increased, but there were no significant differences in serum levels of potassium, chloride, sodium, or phospho-
rus. However, the magnesium and potassium content of skeletal muscle decreased with magnesium depletion despite provision of potassium. On the other hand, it was reported that repletion with potassium alone resulted in less skeletal muscle potassium repletion than when both potassium and magnesium were replaced (21). Spinach contains a large quantity of potassium (mg/100 g: R-sp, 740; B-sp, 450) (22). The potassium in spinach added to the diets might be one of many factors which accelerated the recovery of growth of the R-sp, B-sp and F-sp rats. On the 10th day, at the time of separation of the magnesium-deficient rats, the weight and contents of calcium and phosphorus in the liver were significantly higher in the magnesium-deficient rats than those of the control rats (Table 4). At the final day of the experimental period, however, the calcium content in the liver was not different among each of the groups, and the liver weight of the R-sp and B-sp rats and the phosphorus content in liver of the spinach-added groups was significantly higher than those of the control group. It is possible that these results about the liver weight of the R-sp and B-sp rats and the phosphorus content in the liver of the spinach-added groups have no connection with intake in the diets containing Ox-C, because the weight and phosphorus content in the liver did not differ between the control and Ox-C rats. The magnesium content in the liver of the +Mg rats was significantly lower than that of the R-sp rats. However, there was no significant difference in the magnesium content in the liver between the control and R-sp rats. These results suggest that the +Mg rats did not reach the level of the control rats. On the 10th day, at the time of separation of the magnesium-deficient rats, the weight and content of calcium and phosphorus in the liver were significantly higher in the magnesium-deficient rats than those of the control rats. The calcinosis of the kidneys and hypercalcemia due to magnesium-deficient diet is one of the typical symptoms of magnesium-deficient animals (23, 24). However, the calcium content in the kidneys of each of the spinach-added groups was significantly lower than that of the magnesium-deficient rats on the 10th day. It may be assumed that this reduction of calcium in the kidneys of the rats of each of the spinach-added groups is due to the effect of magnesium in each of the additional diets. On the other hand, the calcium content of the kidneys of the rats fed on the Ox-C diet was about 2.5 times higher than that of the +Mg rats. Cook (25) and Hautman et al. (26) have reported that the majority of the stones in the kidney and urinary tract were formed by calcium-oxalate. An especially large amount of calcium accumulation in the kidneys of the rats fed on the Ox-C diet was about 2.5 times higher than that of the +Mg rats. Although the phosphorus content in the liver was not different between the control and Ox-C rats, the phosphorus content in the kidneys of the Ox-C rats was significantly higher than that of the control rats. From these results, it is suggested that the high content of phosphorus in the kidneys of the spinach-additional groups and Ox-C groups compared with that of the control and +Mg groups is associated with the accumulation of calcium in the kidneys of the spinach-added groups and Ox-C group. On the 10th day at the time of separation of the magnesium-deficient rats, serum magnesium decreased and that of calcium.
increased in the magnesium-deficient rats compared with the control rats. This phenomenon is clearly seen in animal studies in which magnesium was omitted from the diets (21, 23, 24). In another study, it was reported that a significant number of patients with low levels of serum potassium, phosphorus, sodium and calcium were found to have concurrent hypomagnesemia; 42% of those with hypokalemia, 29% of those with hypophosphatemia, 23% of those with hyponatremia, and 22% of those with hypocalcemia (27). The concentrations of serum magnesium and calcium of each of the additional groups reached the levels of the control group. These results indicate that spinach is useful for maintaining serum magnesium and calcium concentrations in a normal range. Thus, it is suggested that magnesium in the R-sp, B-sp and F-sp diets is absorbed sufficiently by the magnesium-deficient rats and that spinach is a good source of magnesium. The magnesium content in the defatted dry left tibiae was significantly lower in the magnesium-deficient rats than that of the control rats (Table 5). In each of the additional groups, the bone magnesium content did not reach the level of the control group. Thus, the contents of calcium and phosphorus in the defatted dry left tibiae of both the R-sp and Ox-C rats were significantly lower than those of the other groups. It was thought that the lower value in the amount of calcium and phosphorus in the defatted dry left tibiae of the R-sp and Ox-C rats resulted from low calcium absorption (Table 7) compared with the other groups. However, there was no difference in the breaking force of the left femurs between the control and each of the additional groups except the Ox-C group. Crenshaw has reported that there was a highly positive correlation between bone calcium content and bone strength (28). However, a highly positive correlation between bone calcium content and bone strength was not obtained from these results (Table 5). These results suggest that more time is required to reach the normal levels of minerals in the bone. Fecal magnesium excretion was the highest in the R-sp rats. The magnesium apparent absorption was the lowest in the R-sp rats (Table 6). On the other hand, the magnesium excretion of both feces and urine was the lowest in the B-sp and F-sp rat. These results suggested that a large amount Ox-C in the diets impaired magnesium absorption, and that it leads to the excretion of endogenous magnesium into the urine. The kidney is the major excretory pathway for absorbed magnesium. However, it is known that there is a considerable secretion of magnesium into the intestinal tract from the bile and from pancreatic and intestinal juices (29). About 200 μg per day of endogenous magnesium was lost into the feces in the magnesium-deficient rats. The magnesium retention of the spinach-added groups was significantly higher than that of the control group. This result suggested that the physiological requirement of magnesium was much higher in the spinach-added groups than the control group, and it does not mean that the quality of the magnesium source was higher in the raw, boiled and fried spinach than the control diet. These results (Table 6) also indicate that spinach is utilized as a good magnesium source. In the spinach additional groups and the Ox-C groups, fecal calcium excretion increased significantly in comparison with the other groups, and
apparent calcium absorption was lower than that of the other groups (Table 7). This result indicates that Ox-C impairs calcium absorption. From all of these results (Tables 3–6), it may be concluded that Ox-C in spinach affected magnesium absorption, but that removal of Ox-C in spinach by boiling or other cooking Ox-C in the spinach hardly affected the magnesium absorption of the magnesium-deficient rats. It is apparent that spinach is one of the most promising sources of magnesium.

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