Magnesium (Mg) is an important cofactor for various basic metabolic reactions in the body. Mg is necessary for stabilizing calcium phosphate in osseous tissue, and Mg deficiency causes osteoporosis. In terms of chemical properties, both Mg and calcium (Ca) are bivalent ions and alkaline-earth metals (class IIA). Among various tissues in the body, most Ca and Mg atoms are contained in osseous tissue, and these elements have a close chemical relationship.

While many studies have shown the effects of Mg deficiency in various tissues, there have been few reports on the effects of Mg deficiency in hard tissues, and the relationship between Mg intake and bone strength has not been fully clarified yet. However, it has been reported that when rats were fed with a 0.008% Mg diet, the bone strength decreased without affecting the calcium concentration of the femur. Additional studies using rats which investigated the effect of different concentrations of Mg diet (0.09%, 0.009%, 0.006% or 0.003%) found that the bone strength decreased without a decrease in the bone mineral content (BMC). These studies suggest that the bone strength in this model is not affected by the BMC but is strongly affected by the trabecular structure. The low-Mg diet model is considered to be an excellent model for examining bone quality.

Key words: magnesium, osteoporosis, rat, bone strength, bone quality
due to decrease in bone density and/or quality. Bone quality refers to architecture, turnover, damage accumulation and mineralization. In other words, when diagnosing osteoporosis and assessing therapeutic effects, it is necessary to evaluate not only bone mineral density, but also bone quality. Various experimental models, such as the ovariectomized model or the steroid-induced osteoporosis model, have been utilized to simulate osteoporosis, but there is no experimental model to assess bone quality alone. It is expected that the low-Mg diet model may serve as a model of low bone quality unaccompanied by decreased bone density.

The purpose of the present study is to clarify the relationship between bone strength and bone quality, in particular the trabecular structure, using rats fed with a low-Mg diet and to determine whether this low-Mg diet model could serve as a model for low bone quality.

Materials and Methods

1. Experimental animals

Twenty male Wistar rats (CLEA Japan, Inc., Tokyo, Japan) aged 4 weeks were divided into a control group (n = 10) and a low-Mg diet group (n = 10). Each group was fed with a conventional diet (Mg content: 90 mg/100 g diet) or low-Mg diet (Mg content: 6 mg/100 g diet), respectively. Diets with Mg concentrations of 0.09% or 0.006% were prepared using a purified diet (CLEA Japan, Inc.) having a Ca content of 0.5% and inorganic phosphate (Pi) content of 0.66% and were given to the control or low-Mg diet group, respectively. Intake of the diet for the two groups was restricted to 80% of the ordinary food intake of Wistar rats of the same age. The animal room was subjected to a 12-h light-dark cycle and maintained at 23 ± 3 °C and 55 ± 10% humidity. During the experiments, the animals were housed individually in stainless steel cages and given deionized water at libitum. The body weight of the rats was measured weekly. After 8 weeks, the animals were sacrificed under pentobarbital (Nembutal, Dinabot, Osaka, Japan) anesthesia, and blood samples were obtained from the abdominal aorta with heparinization. After blood sampling, the lumbar vertebra was removed. The BMC, bone strength and three-dimensional (3D) trabecular structure were analyzed in the lumbar vertebra. Fig. 1 shows the experimental protocol of this study.

2. Plasma components

The blood samples were centrifuged at 4 °C and 3000 rpm for 10 min, and the Ca level, Mg level, alkaline phosphatase (ALPase) activity and osteocalcin content (OC) were measured using the plasma obtained. The Ca and Mg levels were measured by the atomic absorption method (atomic absorption spectrophotometer AA-6200, Shimadzu, Kyoto, Japan), ALPase activities by the phenylphosphate substrate method (Alkaline Phospha K-Test Wako, Wako Pure Chemicals, Osaka, Japan), OC by ELISA (BIOTRAK, Amersham Biosciences Corp., Piscataway NJ, USA).

3. Bone mass parameters

The bone mass of the fifth lumbar vertebra was measured using peripheral quantitative computed tomography (pQCT, XCT-microscope, Stratec, Birkenfeld, Germany) at the central region of the lumbar vertebra. The tomography conditions were: diameter 15 mm, voxel size 0.08 × 0.08 × 0.77 mm, CT speed 8 mm/sec, block number 1. After a scout scan was obtained, the transverse image of a slice was measured at a point of half of the bone length. The total area of the region was...
measured using an algorithm (contour mode 2 and peer mode 2)\textsuperscript{12}, and then the BMC of the total region (Total BMC) and the BMC of the trabecular bone region (Trab BMC) were measured. The cortical region was extracted from the total region using the threshold level (690 mg/cm\textsuperscript{3}), and the BMC of the cortical bone region (Cort BMC) and the cortical thickness (Ct.Th) were measured.

4. Mechanical properties of bone

The bone strength of the fifth lumbar vertebra was measured by a bone compression test using a tester (AG-500E, Shimadzu, Kyoto, Japan). The elastic modulus, maximum load and stiffness were obtained using software for the measurement of bone strength (Shikibu, Shimadzu).

5. Three-dimensional trabecular structure analysis

A 3D image of the fourth lumbar vertebra was acquired using micro CT equipped with a microfocus X-ray tube (focus size 8 × 8 μm, MCT-100MF, Hitachi Medico, Tokyo, Japan). The image information from 201 slices of the fourth lumbar vertebra was obtained under the following exposure conditions: tube voltage 60 kV, tube current 100 μA, magnification ×3, and voxel size 43.0 × 43.0 × 43.0 μm. The 3D trabecular structure parameters were calculated by trabecular structure analysis software (TRI/3D BON, Ratoc System Engineering Co., Ltd., Tokyo, Japan) from the image information of 50 slices at the center of the fourth lumbar vertebra. As for the procedure, cortical bone and trabecular bone regions were separated by 3D space filtration of the bone marrow cavity. Data from the trabecular bone region of each slice were binarized using a threshold obtained by discriminant analysis\textsuperscript{12}. In other words, the histograms of pixel value of background and bone were supposed to be two normal distributions. The threshold was set as the intermediate pixel value that divided the two normal distributions.

The trabecular structure parameters of the bone volume fraction (bone volume per tissue volume; BV/TV), the trabecular number (Tb.N), the trabecular separation (Tb.Sp), the trabecular spacing (Tb.Spac) and the structure model index (SMI) were measured from the binarized images. Tb.N, Tb.Sp and Tb.Spac were measured according to the parallel plate model\textsuperscript{13}. SMI was measured using the method proposed by Hildebrand and Ruegsegger\textsuperscript{14}. The node-strut analysis\textsuperscript{15} that evaluates connectivity of trabecular bone was performed. The parameters of the number of nodes per tissue volume (N.Nd/TV), the number of cortices per tissue volume (N.Ct/TV), the number of struts between Ct and Nd (N.CtNd), the number of struts between Ct and Ct (N.CtCt), the total strut length per tissue volume (TSL/TV), the strut length between Ct and Nd per tissue volume (CtNd/TV) and the strut length between Ct and Ct per tissue volume (CtCt/TV) were obtained by node-strut analysis.

6. Statistics

Values in the tables are shown as the mean ± standard deviation (SD). Significant differences between the two groups were analyzed with Student’s t test.

Results

1. Changes in body weight and plasma components

Table 1 shows changes in body weight and plasma components. There was a significant increase in the plasma Ca level in the low-Mg diet group to 106% of that in the control group. The plasma Mg level and ALPase activity in the low-Mg diet group were significantly decreased to 26% and 55%, respectively, of that in the control group. The plasma OC level was not significantly different between the control and low-Mg diet groups.

2. Changes in bone mass

Table 2 shows changes in the bone mass in the fifth lumbar vertebra. Total BMC, Trab BMC and Cort BMC showed almost the same values between the control and low-Mg diet groups. There were no significant differences. There was also no significant difference in Ct.Th between the control and the low-Mg diet groups.

3. Changes in bone strength

Fig. 2 shows changes in the compressive strength of the fifth lumbar vertebra. The maximum load, elastic modulus and stiffness of the lumbar vertebra were 169.2 ± 17.8 N, 280 ± 78.2 N/mm\textsuperscript{2} and 712.3 ± 221.7 N/mm, respectively, in the control group, but were reduced to 84%, 53% and 64% of respective values in the low-Mg diet group.
Changes in trabecular structure

Table 3 summarizes changes in the 3D trabecular structure of the fourth lumbar vertebra, and Fig. 3 shows typical reconstructed 3D images of lumbar vertebra in the control and low-Mg diet groups. In the low-Mg diet group, BV/TV and Tb.N were significantly decreased, and Tb.Sp and Tb.Spac were significantly increased compared to the control group. The SMI value in the low-Mg group was also significantly higher than that of the control group. Furthermore, the results of the node-strut analysis showed that N.Nd/TV, N.Ct/TV, N.CtNd, N.CtCt, TSL/TV, CtNd/TV and CtCt/TV were significantly decreased in the low-Mg diet group compared to the control group.

Discussion

The National Institutes of Health (NIH) consensus meeting suggests that bone strength primarily reflects the integration of bone density and bone quality. Bone quality refers to architecture, turnover, damage accumulation (e.g., microfractures) and mineralization. In fact, several clinical studies have reported that increased bone mineral density does not always decrease the occurrence of new fractures. These reports sug-

Table 1: Effects of low-Mg diet intake on body weight, plasma Ca level, plasma Mg level, alkaline phosphatase (ALP) activity, and osteocalcin (OC) content in rats.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body weight (g)</th>
<th>Ca (mg/dL)</th>
<th>Mg (mg/dL)</th>
<th>ALP (KA-U/dL)</th>
<th>OC (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>305 ± 10</td>
<td>9.21 ± 0.11</td>
<td>1.62 ± 0.06</td>
<td>24.7 ± 6.6</td>
<td>78.0 ± 6.3</td>
</tr>
<tr>
<td>low-Mg</td>
<td>10</td>
<td>254 ± 10**</td>
<td>9.77 ± 0.21**</td>
<td>0.42 ± 0.06**</td>
<td>13.5 ± 5.6**</td>
<td>80.0 ± 14.4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD (n = 10).
Control: Mg 0.09%, low-Mg: Mg 0.006%.
**P < 0.01 vs. control (Student’s t test).

Table 2: Effect of low-Mg diet intake on bone mass parameters of lumbar vertebra in rats.

<table>
<thead>
<tr>
<th></th>
<th>Total BMC (mg/mm)</th>
<th>Trab BMC (mg/mm)</th>
<th>Cort BMC (mg/mm)</th>
<th>Ct. Th (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>4.74 ± 0.44</td>
<td>0.68 ± 0.11</td>
<td>3.35 ± 0.40</td>
<td>0.384 ± 0.037</td>
</tr>
<tr>
<td>low-Mg</td>
<td>4.73 ± 0.59</td>
<td>0.73 ± 0.06</td>
<td>3.36 ± 0.48</td>
<td>0.353 ± 0.044</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD (n = 10).
Control: Mg 0.09%, low-Mg: Mg 0.006%.

Fig. 2: Effects of low-Mg diet intake on mechanical properties of lumbar vertebra in rats. The bone strength of lumbar vertebra was measured using a compression test. Control: 0.09% Mg, low-Mg: 0.006% Mg. Each value represents the mean ± SD (n = 10). **P < 0.01 vs. control (Student’s t test). The number in each column indicates the percentages relative to the control group.
suggest that the bone strength cannot be accurately predicted based on bone density alone; however, an experimental model for the study of bone quality has not been established. It is expected that a model using rats fed with the low-Mg diet could be applicable for examining bone quality.

While there have been many studies on the effects of Mg deficiency, few studies have investigated its effects on bone structure. In the present study, the changes in bone quality, in particular the trabecular structure, of the rats fed with the low-Mg diet were evaluated, and the usefulness of the low-Mg diet model as a model for low bone quality was investigated.

Several studies have reported that Mg deficiency decreased the level of osseous Mg, reducing osteoblast and osteoclast activity and bone formation. On the other
hand, Mg-ATP complexation is necessary for enzyme activity\textsuperscript{20}. Therefore, Mg deficiency induces lower collagen biosynthesis\textsuperscript{20} and reduces ALPase activity in blood and bone\textsuperscript{18, 21}. Furthermore, it is known that Mg deficiency suppresses cellular metabolic activities and bone matrix synthesis for calcification. In the present study, the plasma ALPase activities in the low-Mg diet group were significantly decreased as compared to the control group, suggesting reduced osteoblast function. Osteoblasts are cells responsible for the synthesis of matrix proteins, such as collagen, OC in addition to bone mineralization.

Also, in the present study, the plasma Mg level in the low-Mg diet group was lower, but the plasma Ca level in the low-Mg diet group was higher, than that of the control group. Classen et al.\textsuperscript{22} reported that Mg deficiency induced hypercalcemia, in agreement with the results of the present study, and this hypercalcemia is observed specifically in rats\textsuperscript{23}.

Boskey et al.\textsuperscript{24} and Kenny et al.\textsuperscript{6} reported that the low-Mg diet intake reduced the strength of rat femurs in terms of the elastic modulus and bending strength. In the present study, the bone strength in terms of the maximum load, elastic modulus and stiffness decreased 84\%, 53\% and 64\%, respectively in the low-Mg diet group as compared to the control group. These differences were significant (Fig. 2). However, the results of the pQCT showed almost the same values between the two groups in Total BMC, Trab BMC, Cort BMC and Ct.Th. There were no significant differences (Table 2). These findings suggest that a low-Mg diet intake causes bone strength to decrease without a decrease in BMC, in agreement with the study of Kobayashi et al.\textsuperscript{7}. In other studies using experimental models (e.g. ovariectomized model, steroid-induced osteoporosis model), the bone strength decreases as the BMC decreases\textsuperscript{5, 10}. The results from this study suggest that, unlike other models, the reduced bone strength in the low-Mg diet model is caused by reduced bone quality.

Furthermore, the low-Mg diet intake induced a significant decrease in trabecular structure. The results of the 3D trabecular structure analysis showed that BV/TV and Th.N of the low-Mg group were 15.2\% and 16.7\% lower than those of the control group, respectively. On the other hand, Tb.Sp and Tb.Spac in the low-Mg group were 26.0\% and 20.0\% higher than in the control group, respectively (Table 3). These findings indicate that the trabecular structure in the low-Mg diet group was rougher than that in the control group.

The SMI of the low-Mg diet group was significantly higher than that of the control group (Table 3). The SMI provides an estimation of plate or rod-like trabecular structure. The SMI was defined as a value between 0 and 3. In an ideal plate structure model, the SMI value was 0, and in an ideal cylindrical rod structure, the SMI value was 3. This result shows that the rod-like structure increased and the plate-like structure decreased in the low-Mg diet group. Ding et al.\textsuperscript{25} reported that the bone strength decreased with age, and the SMI as the rod-like structures increased. This suggests that the change of the SMI in the low-Mg diet model may be responsible for the decrease in bone strength.

In the results of node-strut analysis as the indicator of trabecular connectivity, the parameters of N.Nd/TV, N.Ct/TV, N.CtNd, N.CtCt, TSL/TV, CtNd/TV and CtCt/TV of the low-Mg diet group were significantly lower than those of the control group (Table 3). These results suggest that the trabecular connectivity of the low-Mg diet group was reduced. In particular, the parameters related to cortex (Ct), which indicates connectivity between cortical bone and trabecular bone, were markedly decreased. When load is applied, stress is concentrated at the boundary regions between the cortical bone and the trabecular bone and a decrease in these boundary regions may be closely associated with bone strength decrease.

Mg constitutes only 0.5-1\% of the bone minerals, yet it is an important element in bone matrix synthesis\textsuperscript{5}. In the present study, we hypothesize that the low-Mg intake induces a decrease in trabecular structure without markedly affecting BMC. In conclusion, the bone strength of the lumbar vertebra in rats fed with a low-Mg diet was significantly low. This was not due to a decrease in the BMC, but due to a decrease in the bone quality, particularly the trabecular structure. These findings indicate that the bone strength is strongly affected by the trabecular structure. It is suggested that the low-Mg diet model can be used as a model of low bone quality.

**Acknowledgments**

The authors express their sincere thanks to Masatoshi Kobayashi, Kuniko Hara, and Yasuhiro Akiyama, Department of Applied Drug Research, Eisai Co., Ltd., for their significant ad-
vice and support regarding this work.

This work was performed in the Kanagawa Dental College, Oral Health Science Research Center.

References