The Nutritional Evaluation of Globin on Maintenance of Bone Metabolism in Ovariectomized Osteoporotic Rats

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Summary In our previous study, globin was found to be an effective dietary source for increasing bone mineral density (BMD) and mechanical strength. In this study, the bioavailability of the globin preparation was examined to clarify the mechanism of increase in bone density and strength. Six-week-old Sprague-Dawley female rats were ovariectomized and were fed on a low Ca diet for 30 days to produce the experimental osteoporotic rats. Thereafter they were divided into two groups. The BMD and the mechanical strength of bone of the rat group, whose diet was supplemented with globin, were significantly higher than those of the control group. The levels of the serum calcitonin and the bone-type alkaline phosphatase (Alp) activity in serum and bone were also higher, and the tartrate-resistant acid phosphatase (Tr-Acp) activity in serum and bone was lower in the globin group. Moreover, the bone morphogenetic protein activity in bone in the globin group was found to be greater. From these results, it is concluded that alimentary globin is effective for the acceleration of bone formation and the prevention of bone resorption.

Key Words globin powder, bone mineral density, breaking strength, bone morphogenetic protein activity

The number of osteoporotic patients is increasing along with the aging population in Japan (1). It is well established that a sufficient amount of calcium (Ca) intake is beneficial for the prevention of osteoporosis (2). Even though the recommended Ca intake in Japan is lower than in Europe and America, Ca consumption in Japan is still thought to be insufficient (3). An adequate intake of Ca for Japanese is difficult because of their dietary habits.

There have been many reports on the means to increase Ca intake. We have reported that skimmed milk (4), cow bone powder (5), whey Ca (6), and lobster shell powder (7) are effective sources of Ca to prevent osteoporosis. In addition,
food components which affect Ca metabolism are also under investigation. Particular attention is being paid to the influence of some polypeptides on Ca absorption. It has been reported that a casein-derived phosphopeptide (8) and a peptide present in bovine bone (9) enhanced intestinal Ca absorption.

We have reported that a dietary globin preparation is effective for increasing bone mineral density (BMD) and physical bone strength in experimental animals (10). The globin powder is thought to be effective in increasing intestinal Ca absorption (10). However, the detailed mechanism of the effect is still unclear.

In this study, the nutritional value of globin is evaluated an attempt to clarify the mechanism of increasing the BMD and bone strength. The experimentally osteoporotic rats were fed on a diet containing globin powder and their bone metabolism was investigated. The levels of serum calcitonin and tartrate-resistant acid phosphatase (Tr-Acp) activity; the bone resorption markers, and serum and bone alkaline phosphatase (Alp) activity; the mineralization parameters, were determined. Moreover, osteoinductive activity in the bone of globin-fed animals was also examined. The osteoinductive activity is probably due to bone morphogenetic protein (BMP), which induces bone formation in vivo. BMP was discovered by Urist in 1965 (11) and is a factor which is present in decalcified bone matrix and induces cartilage and bone in various extraskeletal sites. In this study, it was also found that the BMP activity was also high in the bone of the globin group.

**MATERIALS AND METHODS**

**Experimental animals and feeding protocol.** Sixteen 6-week-old Sprague-Dawley (SD) female rats were ovariectomized and were fed on a low Ca diet for 30 days to produce experimental osteoporosis. The low Ca diet consisted of 18% casein, 0.01% Ca, and 0.3% phosphorus (P). Thereafter, the rats were divided into two groups. The experimental group (n = 8), or the “globin group,” was fed on a globin powder diet of 17.52% casein, 0.48% globin, 0.3% Ca, and 0.3% P. The control group (n = 8) was fed on a control diet of 18% casein, 0.3% Ca, and 0.3% P. The total nitrogen, Ca, and P contents of the respective diets were identical. The experimental period lasted for the following 29 days. The details of the compositions of the low Ca diet, the globin powder diet and the control diet are shown in Table 1.

The globin powder, which is a polypeptide mixture having an average molecular weight of 8,000, was prepared by dissociating the heme from hemoglobin using 0.8% Subtilisin-A (EC 3.4.21.14, alkalase 0.6L, Novo Co.) as previously reported (10). The rats were kept in separate cages (15 × 25 × 19.5 cm) and allowed free access to food and ion-exchanged distilled water. The conditions in the animal cages of both groups were as follows: temperature was kept at 23 ± 1°C, humidity at 50 ± 5%, and the fluorescent lighting from 7:00 a.m. to 7:00 p.m.

**Biochemical assays of serum.** At the end of the feeding experiment, all the rats
Table 1. Compositions of the experimental diets (%).

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Low-Ca diet</th>
<th>Control diet</th>
<th>Globin diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.01% Ca, 0.3% P</td>
<td>0.3% Ca, 0.3% P</td>
<td>0.3% Ca, 0.3% P</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>65.1</td>
<td>64.7</td>
<td>64.7</td>
</tr>
<tr>
<td>Casein (vitamin-free)</td>
<td>18.0</td>
<td>18.0</td>
<td>17.52</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Roughage</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Ca- and P-free salt mixture&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Equimolar mixture of KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; and K&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.39</td>
<td>1.03</td>
<td>1.05</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.005</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Water-soluble vitamin mixture&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Fat-soluble vitamin mixture</td>
<td>(³)</td>
<td>(³)</td>
<td>(³)</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Globin powder</td>
<td>—</td>
<td>—</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<sup>1</sup>Ca- and P-free salt mixture (in %): KCl, 57.7; NaCl, 20.9; MgSO<sub>4</sub>, 17.9; FeSO<sub>4</sub>•7H<sub>2</sub>O, 3.22; CuSO<sub>4</sub>•5H<sub>2</sub>O, 0.078; NaF, 0.113; CoCl<sub>2</sub>•6H<sub>2</sub>O, 0.004; KI, 0.01; MnSO<sub>4</sub>•5H<sub>2</sub>O, 0.06; ZnSO<sub>4</sub>•7H<sub>2</sub>O, 0.44; and (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>•4H<sub>2</sub>O, 0.005.  
<sup>2</sup>The water-soluble vitamin mixture (in %): thiamine, 0.5; riboflavin, 0.5; pyridoxine, 0.5; calcium pantothenate, 2.8; nicotinamide, 2.0; inositol, 20.0; folic acid, 0.02; vitamin B<sub>12</sub>, 0.002; biotin, 0.01; and glucose monohydrate, 73.7.

The rats received a supplement of the following fat-soluble vitamins in cottonseed oil three times a week: β-carotene, 70 µg; 2-methyl-1,4-naphthoquinone, 105 µg; α-tocopherol, 875 µg; and vitamin D₃, 525 IU.

were deprived of food overnight (7:00 p.m.—9:00 a.m.). Blood samples were taken from the abdominal aorta under ether anesthetic. The blood was centrifuged at 2,500 rpm for 15 min to extract the serum. Serum Ca was measured by atomic absorption spectrophotometry (Shimadzu AA-640-120 Atomic Absorption Spectrophotometer). Phosphorous was determined by the Fiske-SubbaRow method (12) and the total protein was measured by the biuret method (13). Serum calcitonin (CT) was determined by Takami's method (14).

The alkaline phosphatase (Alp: EC 3.1.3.1) activity was determined at 37°C with 10 mmol/liter p-nitro-phenylphosphate as a substrate in 100 mmol/liter 2-amino-2-methyl-1,3-propanediol-HCl buffer, pH 10.0, containing 5 mmol/liter MgCl₂, as previously reported (15). Alp activity was expressed in terms of units according to the rate of hydrolysis of p-nitrophenylphosphate (µmol p-nitrophenol formed/min). Thermal inhibition of Alp activity was also assayed. The serum preparation was pretreated at 56°C for 10 min and then reacted with the substrate.

The tartrate-resistant acid phosphatase (Tr-Acp, EC 3.1.3.2) activity was also measured at 37°C with 100 mmol/liter p-nitrophenylphosphate as a substrate in 200 mmol/liter sodium citrate buffer, pH 5.5, containing 200 mmol/liter sodium chloride, and 80 mmol/liter L(+)-sodium tartrate. Before the measurement, each
serum was diluted with 10 mmol/liter sodium citrate, pH 5.5. Tr-Acp activity was expressed in terms of units according to the rate of hydrolysis of p-nitrophenylphosphate (μmol p-nitrophenol formed/min).

Measurement of bone mineral density. The right tibial bone of the rat was dissected and adhering muscles and connective tissues were carefully removed. The bone mineral density (BMD) was measured by dual X-ray absorptiometry (DXA, Hologic's QDR-1000 X-ray bone densitometer) as reported previously (10). The BMD of rat bone is remarkably low, therefore, all scans were performed in an ultra-high resolution mode (rat mode, Version 2.0 software), with a line spacing of 0.0254 cm and a point resolution of 0.0127 cm. A detector collimator with a single slit was applied on the X-ray generator. Analysis of the tibial bone was separated into three parts: proximal metaphysis, distal metaphysis and diaphysis, and the BMD of the proximal metaphysis and diaphysis was examined for examples of trabecular and cortical bone, respectively (7).

Measurement of mechanical strength of bone. The right and left femurs were isolated and the muscles and connective tissues were carefully removed. The mechanical strength of the bone was determined by the breaking test using an instrument (Iio Co., type DYN-1255) as reported previously (16). The force and energy necessary for the break at the center of the femur were measured under the conditions of 1.0 cm sample space, 100 mm/min plunger speed, and 50.0 kg load range.

Measurement of alkaline phosphatase and tartrate-resistant acid phosphatase activity in humerus. The right and left humeri were isolated and the muscles and connective tissues were carefully removed. The proximal and distal metaphyses were removed and the bone marrow in the humeral diaphysis was cleaned with saline solution. One side of the humeral diaphyses was used to determine the Alp activity. Humeral diaphysis was mechanically crushed and homogenized in Tris-buffered saline (TBS; 10 mmol/liter Tris-HCl, pH 7.4, containing 0.9% NaCl and 1% Triton X-100) with a Polytron homogenizer (Kinematic, Switzerland). The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was used for the determination of total protein and Alp activity. The total protein was measured by Lowry's method (17) and Alp and Tr-Acp activities were determined by the same method as the biochemical assay of serum.

Bone induction experiment. The other side of the humeral diaphysis was used for the bone induction experiment. After freeze-drying, the bone was weighed and a soft X-ray photograph was taken at 38 kV and 4.5 mA for 12 min on Konika CS 100 E film (Sofron M-40, Sofron Co.). Then, the humeral diaphysis was decalcified with 0.6 N HCl for 48 h at 4°C and excess acid was washed out exhaustively with distilled water at 4°C until pH 7.0. A soft X-ray photograph of the decalcified diaphyses was taken to check the decalcification by the same method as above. The bone was dried by lyophilization and weighed. The decalcified and dried humeral diaphyses (6, each group) was used to determine the osteoinductive activity. Two samples from each of the globin and the control groups, total four
samples, of decalcified-dried humeral diaphysis was implanted beneath the skin on the back of the host rat of 7-week-old SD males. The host rats were kept in a cage and allowed free access to food and ion-exchanged distilled water. After 39 days, the implants were extirpated and the soft tissues were carefully cleaned to determine the bone formation. A soft X-ray picture of the implanted humeral diaphysis was taken and the photographic density was determined with a densitometer (Immunomedica Co., TIF-64), then the weight was measured.

Each implanted humeral diaphysis was cut into two parts. One part was weighed and used for the determination of total protein, Alp activity, and Ca and P contents. The sample was homogenized and centrifuged, and the total protein and Alp activity in the supernatant were measured by the above-mentioned method. The precipitate was incinerated at 550–600°C for 24 h and the ash was dissolved in 1 N nitric acid. The Ca and P contents were measured using the same methods as described above.

The other part was used for the histological examination. The histological specimens were fixed with 10% phosphate-buffered formaldehyde solution. They were dehydrated by increasing ethanol concentration up to 100% and embedded in polyester resin. Cross-sections of 15–20 μm in thickness were cut with a low speed saw (ISOMET: BUEHLER Co.). One side of the section was stained with hematoxylin-eosin or toluidine blue.

Statistical methods. Student's t-test was used to analyze the difference between the globin and the control groups. p<0.05 was considered to be statistically significant.

RESULTS

The body weight gain, food intake, food efficiency, levels of serum Ca, P, and total protein are shown in Table 2. There were no significant differences between

<table>
<thead>
<tr>
<th></th>
<th>Control group (8)</th>
<th>Globin group (8)</th>
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<tbody>
<tr>
<td>Body weight gain (g/day)</td>
<td>2.22±0.11</td>
<td>2.04±0.11</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>15.02±0.49</td>
<td>15.16±0.45</td>
</tr>
<tr>
<td>Food efficiency</td>
<td>0.15±0.04</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Levels in serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.6±0.1</td>
<td>6.8±0.0</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>10.4±0.1</td>
<td>10.6±0.1</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>5.3±0.2</td>
<td>5.7±0.3</td>
</tr>
<tr>
<td>Calcitonin (pg/ml)</td>
<td>106.4±12.5</td>
<td>142.1±25.0</td>
</tr>
<tr>
<td>Tr-Acp (mU/ml)</td>
<td>0.023±0.003</td>
<td>0.017±0.002**</td>
</tr>
</tbody>
</table>

Data are M±SE. ¹Number. ²p<0.01: Significant difference between the control and globin groups.
Fig. 1. Bone mineral density of the tibia. The bone mineral density (BMD) of right tibial bone was measured by dual X-ray absorptiometry (DXA). The proximal metaphysis (trabecular bone) is the upper 1/3 from the tibiofibular junction. The diaphysis (cortical bone) is the middle 2/3 between the proximal epiphysis and the tibiofibular junction. There are significant differences between the globin and control groups \((p<0.05\) and \(p<0.05\)). Data are M±SE.

the globin group and the control animals. The biochemical data of both groups was within the normal range.

The result of tibial BMD determination is shown in Fig. 1. The BMD values of proximal metaphysis and diaphysis of the globin group were significantly higher than those of the control group \((p<0.05, p<0.05\). Furthermore, with regard to the mechanical strength of the bone, as shown in Fig. 2, there were significant differences between the globin and the control groups \((p<0.01, p<0.05\).

In Table 2, the serum CT level is also shown. In the globin group, the serum CT level had a tendency to be elevated. Moreover, the Tr-Acp activity in the globin group was significantly lower than that of the control group. Concerning serum Alp activity, there was no difference in the total activity of the two groups as shown in Fig. 3. However, heat-labile Alp activity in serum shown in the upper part of the bar, which is assumed to be bone-type Alp activity \((I8\), was found to be higher in the globin group than in the control group.

Total protein, Alp activity, and Tr-Acp activity in the humerus are shown in Table 3. There was no difference in total protein between the globin and the control
Fig. 2. Mechanical strength of the femur. The breaking force and breaking energy of the femurs were determined at the point of the cortical bone area. There were significant differences between the globin and the control groups \( p < 0.01 \) and \( p < 0.05 \). Data are \( \text{M±SE} \).

Fig. 3. Serum alkaline phosphatase activity. The serum total alkaline phosphatase (Alp) activity and the heat-stable Alp activity (after treatment at 56°C for 10 min) were measured and the heat-labile Alp activity was calculated. The upper part of each bar shows the heat labile Alp activity. There is no difference in total Alp activity but there is a tendency for the heat-labile Alp activity to increase in the globin group. Data are \( \text{M±SE} \).
Table 3. The alkaline phosphatase and the tartrate-resistant acid phosphatase activities in the humerus.

<table>
<thead>
<tr>
<th></th>
<th>Control group (8)</th>
<th>Globin group (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-protein (mg/dl)</td>
<td>0.68±0.04</td>
<td>0.72±0.05</td>
</tr>
<tr>
<td>Alp activity (U/min)</td>
<td>0.77±0.06</td>
<td>0.88±0.05</td>
</tr>
<tr>
<td>Specific Alp activity (U/mg protein)</td>
<td>1.13±0.03</td>
<td>1.24±0.07</td>
</tr>
<tr>
<td>Tr-Acp activity (mU/min)</td>
<td>2.71±0.34</td>
<td>1.65±0.20*</td>
</tr>
<tr>
<td>Specific Tr-Acp activity (mU/mg protein)</td>
<td>4.24±0.64</td>
<td>2.34±0.31*</td>
</tr>
</tbody>
</table>

Data are M±SE. ¹Number. *p<0.05: Significant difference between the control and globin groups.

Fig. 4. Soft X-ray radiograph of the implants of decalcified humeral diaphyses. I shows non-decalcified humeral diaphyses and II is humeral diaphyses after decalcification. III shows decalcified humeral diaphyses 39 days after implantation in the bone induction experiment. Distinct radiopaque calcification in all implants is detected. Relative radiopacity of the globin group was 8.27±0.42 and that of the control group was 4.58±0.78. There was a significant difference between the two groups.

groups but Alp activity of the globin group, expressed either in total or specific activity, was higher than that of the control group. On the other hand, the total or specific Tr-Acp activity in the globin group was significantly lower than that of the control group (p<0.05, p<0.05).

Figure 4 shows the radiological appearance of the soft X-ray of the humeral diaphyses. The top of the figures (Fig. 4–I) represents the humeral diaphyses (before decalcification), and Fig. 4–II shows the decalcified humeral diaphyses.
Fig. 5. Histological section of an implant of decalcified humeral diaphysis from a globin-fed rat 39 days after implantation. Osteoinduction is shown by the presence of osteoblasts and new bone matrix (A) near the implant (B). Bone marrow formation (C) is also seen (Toluidine blue staining, ×200).

(before the bone induction experiment). Figure 4–III shows that the decalcified humeral diaphyses recovered after 39 days of hypodermic implantation into host rats. A distinct radiopaque calcification pattern due to the bone induction is observed in all samples of the implants. There was a significant difference in radiopacity between the two groups when measured quantitatively using a densitometer, the relative radiopacity of the globin group was $8.27 \pm 0.42$ (M±SE) and that of the control group was $4.58 \pm 0.78$ (M±SE).

The implant specimens in the bone induction experiment were also examined histologically. Both in the globin and control groups, new bone formation was evident by the appearance of osteoblasts and bone matrix. Figure 5 shows an example of new bone formation in an implant from the globin group animal 39 days after implantation.

The weights of the implanted samples are shown in Fig. 6. The bottom part of each bar shows the weight before implantation and the upper part of the bar shows the weight 39 days after implantation. In comparison with the control group, the weight of the implants in the globin group was heavier.

Total protein and the level of Alp activity in the implanted diaphysis in the bone induction experiment are shown in Fig. 7. Total protein per weight in the implant was higher in the globin group than in the control group. The total or specific Alp activity in the samples of the globin group was also significantly higher
Fig. 6. The weight of the decalcified humeral diaphyses before and after the implantation. The lower part of the column represents the weight before implantation and the upper part the weight 39 days after implantation. The weight of the globin group after implantation (upper part) is larger than that of the control group. Data are M±SE.

Fig. 7. Total protein and alkaline phosphatase activity in the implants. The specific Alp activity was calculated as the total Alp activity per total protein. Total protein, total Alp activity, and specific activity in the implant from the globin group are higher than those of the control group (p<0.01 and p<0.05). Data are M±SE.
Fig. 8. Calcium and phosphorus contents in the implants. Calcium and phosphorus contents in the implants from the globin group are significantly higher than those from the control group ($p<0.01$ and $p<0.001$). Data are M±SE.

DISCUSSION

The number of patients with osteoporosis is increasing and the frequency of bone fracture due to this disease is also increasing (1). It is well known that a sufficient intake of Ca is one of the important means of preventing osteoporosis (2). The Ca intake, however, has never been sufficient in Japan according to the National Nutrition Survey in 1991 conducted by the Ministry of Health and Welfare (3). Recently, researchers have started to pay more attention to the alimentary factors which affect Ca metabolism. We have reported that globin powder (10) is an excellent food component of nitrogen source for increasing BMD and mechanical strength. The globin powder could be an effective additive for the prevention and the treatment of osteoporosis. In this study, the mechanism of the nutritional effect of globin was investigated in terms of the changes in bone metabolic markers and osteoinductive activity.

The BMD of the tibial proximal metaphysis and diaphysis increased significantly in the globin group. Moreover, the physical strength, breaking force and breaking energy, of the femoral diaphysis were also significantly greater in the globin group. The globin powder was confirmed to be an excellent aliment which affects Ca metabolism.

In conjection with the increased BMD and strength, the Tr-Acp activities in both the serum and the humerus, which is a bone resorption marker, were significantly lower in the globin group. These results suggest that the globin powder...
added to food may prevent bone resorption. Furthermore, serum CT levels in the
glovin group had a tendency to be higher than that of the control group. CT is a
hormone which inhibits bone resorption, and this result supports the possibility that
the alimentary globin prevents bone resorption. However, further investigation is
required to clarify the mechanism of elevating serum CT level in relation to the
globin preparation feeding. On the other hand, the humerus Alp activity and the
heat-labile bone-type Alp activity in serum were higher in the globin group. Alp
in rats are classified into two types: a tissue-nonspecific type, including a bone-type,
and an intestinal type (19). The bone-type Alp could play a role normal skeletal
mineralization. From this aspect, the globin powder in nutrition is thought to
accelerate the skeletal mineralization. This data had no significant difference.
However, a continually higher level of the Alp activity in serum and bone might
affect bone metabolism.

Osteoinductive activity of the bone matrix from the globin-fed animals was
also examined in this study. Several factors besides BMP (20), such as transform-
ing growth factor-β (21), are known to influence bone induction. However, the
only factor which was clearly shown to induce cartilage and bone formation in the
extraskeletal sites in the host animals was BMP (22). There are no techniques to
determine the BMP activity directly. Consequently, in this study, the BMP activity
was evaluated by histological and radiological examination and by the Alp activity,
the total protein and the Ca and P contents values in the implanted bone. The
radiological and the histological examinations in the present study showed definite
bone formation in the decalcified bone matrix. This bone formation could be
regarded as induction by BMP. In addition, the radiopacity and biochemical
parameters, such as Alp activity, total protein, Ca, and P contents, were either
significantly higher or tended to be higher in the globin group. This result indicates
that the bone-forming factors in the bone of the globin group were enhanced. BMP
activity was influenced by aging (23-25) and also by the vitamin D levels (26-28).
Several factors, hormones and nutrients may also affect the BMP activity. From
this aspect, globin powder could be one of the nutrients which enhances the BMP
activity. Increasing the radiopacity and the biochemical parameters shows that, in
the globin group, a larger amount of BMP is retained or synthesized in the bone.
Thus, the globin powder in food may be one of the effective nutrients for the
acceleration of BMP activity. The BMP may be one of the activators which
controls bone formation. Accordingly, a small change in the BMP activity level
could influence the bone metabolism. A continually high level of BMP activity
might lead to a dramatic change in BMD and mechanical bone strength. The high
level of the BMP activity in the bone from the globin group indicated that a large
amount of BMP was synthesized by the osteocytes in the bone from the globin-fed
animals because of the high quality bone; such as an increased level of BMD and
bone strength of the globin group. Another possibility is the large amount of BMP
which was accumulated in the bone from the globin group because of improving
bone quality. The high BMP activity might enhance the bone remodeling in the

globin group, therefore, the bone metabolism of the globin group might improve. It is possible that the globin preparation might be an effective nutrient for the acceleration of BMP activity in bone.

We have reported that the globin preparation might elevate intestinal Ca absorption \( (10) \). On the other hand, in the bone from vitamin D-deficient rats, either there is no osteoinductive activity or there is a smaller amount of osteoinductive activity. It is well known that vitamin D is an important factor influencing intestinal Ca absorption. A decrease in intestinal Ca absorption causes a smaller amount of Ca retention in the body. The reason for the lower level of osteoinductive activity in the bone in vitamin D-deficient animals might be due to an inadequate intestinal Ca absorption. Consequently, an increase of intestinal Ca absorption in the globin-fed animals, which was reported previously \( (10) \), might affect osteoinductive activity. The other possible reason for the acceleration of the BMP activity in the globin-fed rats could be that the globin affected the BMP synthesis and/or the BMP retention through another mechanism, for example by increasing the number of osteoblasts or by enhancing osteoblast activity. Further investigation is required to clarify the mechanism of the enhancement of BMP activity through globin in alimentation. Based on the results of the present study, it was concluded that the BMP content in the bone matrix of the globin-fed rat was higher than that of the control group.

It is possible that the mechanism for the increase of the bone mineral density and the physical strength of the bone in the globin-fed group was due to the acceleration of bone formation and the prevention of the bone resorption through globin alimentation.

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