The Effect of Spiny Lobster Shell Powder on Bone Metabolism in Ovariectomized Osteoporotic Model Rats

Naomi OMI, Naomi MORIKAWA, and Ikuko EZAWA

Department of Food and Nutrition, School of Home-economics, Japan Women's University, Bunkyo-ku, Tokyo 112, Japan

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Summary Calcium has been found to be indispensable in the prevention of osteoporosis. Recently, there has been a great deal of research into the best way to consume calcium. In this study, the effect of "powdered lobster shell" on bone metabolism was examined in ovariectomized osteoporotic model rats. This powder has a good flavor and taste, and contains high quantities of calcium. Six-week-old SD-strain female rats were ovariectomized and were fed a low Ca diet (0.01% Ca and 0.3% P) for 32 days. Thereafter, the rats were divided into two groups; the control group was fed a control diet (0.3% Ca and 0.3% P) and an experimental group, the lobster group, was fed a lobster shell powder diet (0.3% Ca and 0.3% P) ad libitum for 30 days. The results were as follows: in comparison with the control group, the lobster group had significant increases in (1) bone mineral density [BMD (DEXA Hologic's QDR-1000)] of lumbar spines and tibial proximal metaphyses, which are mainly trabecular bones, and BMD of tibial diaphyses, which is a mainly cortical bone, (2) the breaking force and energy of femur. These results suggest the lobster shell powder could be a valuable source of dietary calcium in increasing BMD, breaking force and energy in osteoporotic model rats.

Key Words bone mineral density, breaking force, breaking energy, spiny lobster shell powder, dietary calcium source, osteoporosis, ovariectomy

Calcium has been found to be indispensable in the prevention of osteoporosis (1). In Japan, our food habits have changed significantly. We have a large variety of food in abundance. However, it has been shown that specifically the calcium intake has never been sufficient according to the national nutrition survey in 1990 conducted by the Ministry of Health and Welfare (2). Japan is becoming an aging society. It is aging more rapidly than all other countries (3). As a result, the number of patients with osteoporosis is increasing and the higher frequency of bone fractures due to osteoporosis is also increasing (4). Accordingly, there has been a great deal of research recently into the best way to consume calcium. In this study, "powdered spiny lobster shell" was used. This powder has a good flavor and taste,
and contains high quantities of calcium. We examined whether the "powdered spiny lobster shell" was an effective source of dietary calcium for bone metabolism in osteoporotic model rats which had been ovariectomized.

MATERIALS AND METHODS

The experimental animals and protocol. In this study, six-week-old SD-strain female rats were used \( (n = 16) \). These were ovariectomized and were fed a low calcium diet for 32 days. The low calcium diet consisted of 0.01% calcium (Ca) and 0.3% phosphorus (P). Thereafter, the rats were divided into two groups. The control group \( (n = 8) \) was fed a control diet of 0.3% Ca and 0.3% P. Calcium source of the control diet was calcium carbonate (CaCO\(_3\)). The experimental group \( (n = 8) \), which was called the "lobster group," was fed a lobster shell powder diet of 0.3% Ca and 0.3% P. Calcium source of this diet was spiny lobster shell powder only. Thus, the calcium content of each group was identical but the source was different. The composition of the spiny lobster shell powder and the experimental diets are shown in Tables 1 and 2. The experimental period lasted for the next 30 days. During this time, all the rats were allowed ad libitum feeding and ion-exchanged distilled water. These were kept in separate cages \( (15 \times 25 \times 19.5 \text{ cm}) \). The conditions in the animal laboratory were as follows: the temperature was kept at 23±1°C, the humidity was maintained at 50±5% and the lighting remained constant for both groups (fluorescent lighting from 7:00 a.m. to 7:00 p.m. and darkness from 7:00 p.m. to 7:00 a.m.).

Biochemical assays of serum. At the end of this experiment, all the rats were deprived of food for one night \( (7:00 \text{ p.m.} - 9:00 \text{ a.m.}) \). The next day, after induction of anesthesia with ether, blood samples were taken from the aorta. The blood samples were centrifuged (at 2,500 rpm for 15 min) to extract the serum.

The level of serum calcium was measured by atomic absorption spectrophotometry (using Shimadzu's AA-640-12 Atomic absorption spectrophotometer).

Table 1. Composition of spiny lobster shell powder.

| Elements     | %  |
|--------------|----|
| Moisture     | 4.7|
| Protein      | 42.2|
| Lipid        | 6.0|
| Fiber        | 10.4|
| Carbohydrate | 0.5|
| Ash          | 36.2|
| Ca           | 12.0|
| P            | 0.75|
| K            | 0.58|
| Na           | 1.23|
| Chitin       | 12.2|
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Table 2. Composition of the experimental diets (%).

| Constituents                        | Low Ca (0.01% Ca, 0.3% P) | CaCO₃ (0.3% Ca, 0.3% P) | Lobster |
|-------------------------------------|---------------------------|------------------------|---------|
| Glucose monohydrate                 | 65.1                      | 64.4                   | 64.1    |
| Casein (vitamin-free)               | 18.0                      | 18.0                   | 17.0    |
| Cotton seed oil                     | 10.0                      | 10.0                   | 10.0    |
| Roughage                            | 3.0                       | 3.0                    | 2.7     |
| Ca and P-free salt mixturea         | 2.0                       | 2.0                    | 2.0     |
| Equimolar mixture of KH₂PO₄ and K₂HPO₄ | 1.39                      | 1.39                   | 1.31    |
| CaCO₃                               | 0.005                     | 0.73                   | —       |
| Cystine                             | 0.2                       | 0.2                    | 0.2     |
| Water-soluble vitamin mixtureb      | 0.1                       | 0.1                    | 0.1     |
| Fat-soluble vitamin mixturec        | —                         | —                      | —       |
| Choline chloride                    | 0.2                       | 0.2                    | 0.2     |
| Lobster powder                      | —                         | —                      | 2.44    |

*a* Ca- and P-free salt mixture (in %): KCl, 57.7; NaCl, 20.9; MgSO₄, 17.9; FeSO₄·7H₂O, 3.22; CuSO₄·5H₂O, 0.078; NaF, 0.113; CoCl₂·6H₂O, 0.004; KI, 0.01; MnSO₄·5H₂O, 0.06; ZnSO₄·7H₂O, 0.44; (NH₄)₆Mo₇O₂₄·4H₂O, 0.005. bThe water-soluble vitamin mixture consisted of (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_{12}, 0.002; biotin, 0.01; glucose monohydrate, 73.7. cThe rats received a supplement of fat-soluble vitamins in cotton seed oil three times a week which was supplied with 70 μg of β-carotene, 105 μg of 2-methyl-1,4-naphthoquinone, 875 μg of α-tocopherol, and 525 I.U. of vitamin-D₃.

Phosphorus was determined by the Fiske-Subbarow methods (5), and total-protein was measured by the biuret method (6).

**Measurement of bone mineral density.** At the dissection, lumbar spines and all tibial bones were isolated. The muscles and connective tissues were carefully removed. Thereafter the bone mineral density (BMD) of the fourth and fifth lumbar vertebrae (L4, L5) were measured by dual energy X-ray absorptiometry [DEXA (Hologic's QDR-1000 X-ray bone densitometer)] as previously reported (7). Moreover, whole tibias were measured by DEXA. In comparison with the BMD of a human, the BMD of a small animal is remarkably low in density. Therefore all scans were performed using the ultra high resolution scan mode (rat mode, Version 2.0 software). And, a detector collimator with a single slit was put on the X-ray generator.

Analysis of tibial BMD was practiced as follows. The tibial bones were separated into three parts. The first part (A) is the tibial proximal metaphysis, which is a mainly trabecular bone. The second part (B) is the tibial diaphysis; it is mainly a cortical bone. The third part is the tibial distal metaphysis. These are shown in Fig. 1. In this study, the BMDs of the tibial proximal metaphyses and diaphyses were examined. As well, the lumbar vertebrae were measured as...
trabecular bones.

**Measurement of breaking properties.** At this dissection, femoral bones were isolated and muscles and connective tissues were carefully removed. Then the breaking properties of the femurs were tested by the breaking properties test as previously reported (8) (Iio's DYN-1255). The center of the femoral diaphyses were broken. The breaking force and energy were analyzed. The measurement conditions were as follows: the sample space was 1.0 cm, the plunger speed was 100.0 mm/min, the load range was 50.0 kg, and the chart speed was 120.0 cm/min.

**Ca balance study.** In this study, four balance studies were carried out to determine the Ca absorption. Within each phase, feces and urine were collected for 24 h. Urine was collected under acidic conditions by using 1 ml 6N hydrochloric acid, thus preventing Ca precipitate. Phase I was carried out the last two days of the “Low Ca diet” period; Phase II, the first two days of the “experimental diet” period; Phase III, the 12th and 13th days of the “experimental diet” period; Phase IV, the last two days before the end of the “experimental diet” period. All urine was centrifuged (at 2,500 rpm, for 15 min) to extract the supernatant. In the fecal determination, all daily feces’ were burnt to ash (at 550–600°C, for approximately 14 h). Thereafter, the resulting ash was dissolved into 1N nitric acid. Urinary Ca excretion and fecal Ca excretion were measured using the same method as the biochemical assay of serum.

The intestinal Ca absorption was calculated by using Ca concentrations of each diet (“Low Ca diet,” 0.014%; “control diet,” 0.344%; “lobster shell powder diet,” 0.334%) and the fecal Ca excretions.

**Statistical methods.** The t-tests were used to analyze the differences between the control group and the lobster group, within each experiment. \( p < 0.05 \) was considered statistically significant.
RESULTS

The body weight gain, the food intake, and the food efficiency are shown in Table 3. In the body weight gain, there was a significant difference between the control group and the lobster group ($p < 0.05$). However, there were no significant differences in the food intake and the food efficiency. Not shown in the table, the level of serum calcium, phosphorus, and total-protein, which were normal, showed no differences between the two groups.

As shown in Fig. 2, the BMD of the lumbar spines of the lobster group was significantly greater than that of the control group ($p < 0.05$). Figure 3 shows that

Table 3. Body weight gain, food intake, and food efficiency.

| Groups   | $n$ | Body weight gain (g/day) | Food intake (g/day) | Food efficiency |
|----------|-----|--------------------------|---------------------|-----------------|
| CaCO$_3$ | 8   | 1.73±0.11                | 14.5±0.39           | 0.12±0.01       |
| Lobster  | 8   | 2.04±0.09*               | 15.5±0.30           | 0.13±0.01       |

*aMean±SD.  *$p<0.05$.

Fig. 2. BMD (bone mineral density) of lumbar spine. C, control group ($n=8$); L, lobster diet group ($n=8$). Lumbar spine of each rat was removed and its BMD was measured by DEXA (dual energy X-ray absorptiometry). Significantly different from control group, $p<0.05$.

Fig. 3. BMD (bone mineral density) of tibial proximal metaphysis. C, control group ($n=16$); L, lobster diet group ($n=16$). The right and left tibias of each rat were removed and their BMD were measured by DEXA (dual energy X-ray absorptiometry). The tibial proximal metaphysis was analyzed. Significantly different from control group, $p<0.001$.
Fig. 4. BMD (bone mineral density) of tibial diaphysis. C, control group \((n=16)\); L, lobster diet group \((n=16)\). The right and left tibias of each rat were removed and their BMD were measured by DEXA (dual energy X-ray absorptiometry). Significantly different from control group, \(p<0.001\).

Fig. 5. Breaking force of femur. C, control group \((n=16)\); L, lobster diet group \((n=16)\). The right and left femurs of each rat were removed, and the breaking force of the femurs were tested by breaking property test. Significantly different from control group, \(p<0.001\).

Fig. 6. Breaking energy of femur. C, control group \((n=16)\); L, lobster diet group \((n=16)\). The right and left femurs of each rat were removed, and the breaking energy of the femurs were tested by breaking property test. Significantly different from control group, \(p<0.01\).

the BMD of the tibial proximal metaphyses was also significantly greater \((p<0.001)\). The lumbar spines and the tibial proximal metaphyses are mainly trabecular bones. Furthermore, in the BMD of the tibial diaphyses, as shown in Fig. 4,
there was a significant difference between the control group and the lobster group ($p < 0.001$). Tibial diaphyses are mainly cortical bones. Not shown in the figure, also the BMD of whole tibial bones in the lobster group was significantly greater ($p < 0.001$).

As shown in Fig. 5, in comparison with the control group, the lobster group showed a significant increase in the breaking force necessary for the femurs ($p < 0.001$). Figure 6 shows the breaking energy of the femurs; here too there is a significant difference between the control group and the lobster group ($p < 0.01$).

Not shown in the figure, in this study the intestinal Ca absorption was examined. There was no significant difference between the control group and the lobster group.

**DISCUSSION**

Insufficient intake of calcium is one of the risk factors in osteoporosis (1). Furthermore, it is shown that various physiological functions decline due to aging (9). As a person grows older, the intestinal absorption of various nutrients decreases (10–12). Therefore, intestinal calcium absorption also decreases (12, 13). This suggests that it is more difficult for elderly people to absorb calcium. It is essential to increase BMD in young people for the prevention of osteoporosis (14). Researchers are constantly working on finding better ways to improve calcium consumption. It is known that milk contains a great deal of calcium, and that this calcium is absorbed more easily. Furthermore, it is easy to supplement one's diet with milk. However, the habit of drinking milk is not popular amongst elderly people, and some of them have a lactase deficiency. Insufficient intake of calcium is becoming a more serious problem for elderly people.

Recently, there has been a great deal of research about the development of valuable sources of calcium for the prevention of osteoporosis. It is known that the different types of calcium salt affect the intestinal calcium absorption (15) and various food components affect this as well (16, 17). We have shown that milk (18), cow-bone powder (19), and whey calcium (20) are valuable sources of calcium for the prevention of disturbances of bone metabolism.

In this study, the BMD of lumbar spines and tibial proximal metaphyses were measured as examples of trabecular bones. The trabecular bones metabolize rapidly because they have a large area. This suggests that it is easier to increase the bone mineral content (BMC) and the BMD (21). As shown in Figs. 2 and 3, the BMD of lumbar spines and tibial proximal metaphyses of the lobster group were significantly greater ($p < 0.05, p < 0.001$) than those of the control group. Moreover, the BMD of tibial diaphyses was measured as examples of cortical bone. The cortical bones metabolize slowly (21). The area of the cortical bone is smaller than the trabecular bone. This shows that it is difficult to increase the BMC and the BMD. However, in this study, the BMD of tibial diaphyses of the lobster group significantly increased ($p < 0.001$). These indicate that the lobster shell powder could be
effective for bone metabolism. The body weight gain in the lobster group significantly increased \((p < 0.05)\). However, the BMD is expressed by the fixed BMC per that area. The high level of the BMD in the lobster group was not simply due to the increased body weight of that.

Again the cortical bones were examined by the breaking properties test. In comparison with the control group the lobster group showed significant increases in the femoral breaking force and energy \((p < 0.001, p < 0.01)\). These results suggest that lobster shell powder can be considered an effective source of calcium.

Generally speaking, lobster shell powder has a specific component called "chitin" which is a dietary fiber. One of the previous reports examined chitin of lobster shell as a source of dietary fiber \((22)\). That diet consisted of approximately 5% chitin. It showed that, in comparison with the cellulose group, the chitin group experienced a decrease in mineral availability and calcium content in the bone \((22)\). However, lobster shell powder diet was composed of approximately 0.3% chitin. In this study, the intestinal Ca absorption was examined. It can been seen from the result of the Ca balance study that the intestinal Ca absorption has no significant difference between the lobster group and the control group. Moreover, the BMD and the breaking properties in the lobster group were significantly greater. These results indicate that the bone metabolism was not inhibited by a small quantity of chitin. It is known, however, that calcium availability is affected by the different types of calcium salt and other various food components \((15–17)\). Consequently, there is a possibility that some of the extra components in the lobster shell powder influence these results.

These results suggest that the "spiny lobster shell powder" is effective in increasing BMD, breaking force, and breaking energy. Spiny lobster shell powder could be a valuable, effective source of dietary calcium in the prevention of osteoporosis. This powder has a distinctive, pleasant flavor and taste. This indicates that it would be easy to supplement the diet with lobster shell powder.

Henceforth, we are going to examine what kind of extra components in the lobster shell powder relate to the bone metabolism, and we will also examine the mechanism of these components and their effective function in the lobster shell powder.

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