Effect of Egg-Shell Ca on Preventing of Bone Loss after Ovariectomy

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Egg-shell calcium (Ca) is now being commonly used as a Ca supplement in many health foods and Ca tablets. However, there is not enough data for evaluating the effect of egg-shell Ca on bones. We have already reported that some Ca sources were effective for increasing the bone mineral density (BMD) and mechanical bone strength, or for delaying bone loss when testing on rats. Therefore, in this study, the effect of the egg-shell Ca on bone metabolism was determined in ovariectomized rats by using the same technique to evaluate the effect of several other Ca sources.

The BMD values for the extracted lumbar spine and the tibial proximal metaphysis in the egg-shell Ca group were found to be significantly higher than those of the control group. The content of each nutrient between the two groups was identical, but the source of Ca was different. The other indices for evaluating bone in the egg-shell Ca group were higher than those of the control group. These data suggest that egg-shell Ca could be effective for preventing bone loss after ovariectomy.

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Keyword: egg-shell calcium, bone mineral density, rat.

INTRODUCTION

It is a well known that taking enough calcium (Ca) and doing moderate physical activity are necessary for preventing osteoporosis (Bess et al. 1990; Aloia et al. 1978). While the intake of most nutrients has been sufficient in Japan, the Ca intake in particular has never been sufficient (Ministry of Health and Welfare 1997). The Ca intake has nearly reached the Ca daily allowance, although the Ca intake remains still inadequate. This indicates that an adequate intake of Ca from the daily Japanese diet is difficult; therefore, there have been many studies to find the excellent Ca source for bone metabolism. Egg-shell Ca is now being commonly used as a Ca supplement in health foods and for medical treatment. However, there is not enough data for evaluating the effect of egg-shell Ca on bones.

We have already reported that BMD values for the lumbar spine, tibial proximal metaphysis and tibial diaphysis were increased significantly after consuming diets with different Ca sources, such as whey-Ca (Igarashi et al. 1990), lobster shell Ca (Omi et al. 1992a) and oyster shell Ca (Omi and Ezawa 1993). Moreover, the mechanical bone strength (breaking force applied) of the femoral diaphysis was also greater after eating these kinds of diet. Based on our previous work, in this study, the effect of egg-shell Ca on bone metabolism was examined by using the same technique to evaluate the effect of several other Ca sources on bone.

MATERIALS AND METHODS

Experimental animals and feeding protocol

Six-week-old SD strain female rats were used (n = 16). All the rats were ovariectomized (OVX) and were then divided into two groups: a control group and an egg-Ca group. The experimental period lasted for 57 days, on during which the rats in the control group (n = 8) were fed a control diet containing 0.3% calcium (Ca) and 0.3% phosphorus (P), Table 1. The sole Ca source for the control diet was calcium carbonate (CaCO₃). The rats in the egg-Ca group were fed on an egg-shell Ca diet containing 0.3% Ca and 0.3% P. The sole Ca source for this diet was egg shell (Q.P. Corporation). The content of such nutrients as Ca, P, protein and oil in the two diets was identical. During this experiment, all the rats were allowed ad libitum access to the diets and to ion-exchanged distilled water, and were kept in individual cages (15×25×19.5 cm). The conditions in the cages of both animal groups were as follows.
temperature at 23±1°C, humidity at 50±5% and fluorescent lighting from 7:00 a.m. to 7:00 p.m.

**Bone and serum sampling**

At the end of this experiment, all the rats were deprived of food for one night (7:00 p.m.-9:00 a.m.). The following day, after inducting of anesthesia with ether, blood samples were taken from the abdominal aorta. These blood samples were each centrifuged at 2,500 rpm for 15 min to extract the serum, all the serum samples being kept in a deep freezer (−90°C).

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**Table 1. Composition of the experimental diets (%)**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Low Ca</th>
<th>CaCO₃</th>
<th>Egg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0%</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td></td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>65.1</td>
<td>64.4</td>
<td>63.9</td>
</tr>
<tr>
<td>Casein (vitamin-free)</td>
<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Cotton seed 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</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Equimolar mixture of K₂HPO₄ and K₂HPO₄</td>
<td>1.39</td>
<td>1.39</td>
<td>1.38</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>0.005</td>
<td>0.73</td>
<td>—</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</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Egg-shell Ca powder</td>
<td>—</td>
<td>—</td>
<td>0.78</td>
</tr>
</tbody>
</table>

The lumbar spines, tibiae, and femurs were isolated, and the musculas and connective tissues carefully removed. The lumbar spine and both of the tibiae were kept in 70% ethanol.

**Measurement of bone mineral density**

During the experiment, in vivo measurements for the bone mineral density (BMD) of the lumbar spine and right tibia for each rat were carried out three times: before ovariectomy (phase I), 4 weeks after ovariectomy (phase II), and at the end of the experiment (phase III). The dual energy X-ray absorptiometry (DXA, using a Hologic QDR-1500 X-ray bone densitometer) was used to determine the BMD values. After dissection, in vitro measurements of BMD were taken on the fourth and fifth lumbar vertebrae and the right tibia of each rat. An analysis of the tibia was carried out as previously reported (Omi et al. 1992a), because different areas in the tibia had a different bone structure.

**Measurement of femur weight and bone strength**

The wet weight of both femurs in each rat was measured, and then the breaking force and energy for the right and left femurs were examined by taking a dynagragh of the bone strength (Iio type DYN-1255 apparatus) as previously reported (Ezawa et al. 1979). The load necessary to break the center of each femur was measured under the conditions of 1.0 cm sample space, 100 mm/min plunger speed, and 50.0 kg load range. After measuring the breaking force for each femur, all femurs were dried at 98°C for 24 h (Omi et al. 1992b), and then the dry weight of the femurs of each rat was measured. Thereafter, all samples were burnt to ash at 550-600°C for approximately 24 h (Omi et al. 1992b), and the ash weight for the femurs of each rat was measured.

**Biochemical assays**

The level of serum protein was measured by the biuret method (Gornal 1949). Ca in the serum was measured by atomic absorption spectrophotometry with a Shimadzu AA-640-12 atomic absorption spectrophotometer, while P in the serum was determined by the Fiske-SubbaRow method (Fiske and SubbaRow 1925).

**Statistical methods**

Student's t-test was used to analyze the difference between the control group and the egg-Ca group, p <0.05 being considered statistically significant.

**RESULTS**

There was no difference in the initial body weight of the control group and the egg-Ca group (148.6±...
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2.1, 148.6 ± 1.9). After the experimental diet has been fed for 57 days, there were not significant differences in the body weight gain, food intake, and food efficiency between the control group and the egg-Ca group (Table 2).

The biochemical data for the serum, such as Ca, P and total-protein, of the control group and the egg-Ca group were normal, and there were no differences between the two groups.

The change in the tibial BMD over the experimental diet period is shown in Fig. 1. There was no significant difference in BMD of the tibial proximal metaphysis and tibial diaphysis before ovariectomy (zero time measurement (phase I)) between the two groups. In both groups, BMD of the tibial proximal metaphysis tended to increase (p<0.2, p<0.2), and BMD of the tibial diaphysis significantly increased (p <0.001, p<0.001), during the first 1 month of the experimental diet (between phases I and II). Compared with the control group and the egg-Ca group, there was no significant difference in BMD of the tibial proximal metaphysis and diaphysis from the phase II measurements. However, BMD at these sites for the egg-Ca group tended to be higher than that of the control group (p<0.25, p<0.1). Between phases II and III for both groups, BMD of the tibial proximal metaphysis significantly increased (p<0.001, p <0.001), and BMD of the tibial diaphysis tended to increase (p<0.5, p<0.5). At the end of the experiment (phase III, 2 months after starting to eat the experimental diet), BMD of the tibial proximal metaphysis and diaphysis in the egg-Ca group was also higher than that in the control group (p<0.1). Data for the change in lumbar spine BMD is not shown; however, the lumbar spine BMD value for the two groups was significantly increased (p<0.001) after 57 days of feeding on the experimental diet, as with the tibial measurements with each measurement. BMD of the lumbar spine for the egg-Ca group was higher than that of the control group (p<0.1). BMD of the extracted bone was also determined after dissection. The BMD value of the extracted lumbar spine for the egg-Ca group was significantly greater than that of the control group (p<0.001), as shown in Fig. 2. In addition, BMD of the tibial proximal metaphysis for the egg-Ca group was also significantly higher than that of the control group (p<0.05, Fig. 3). BMD of the tibial diaphysis in the egg-Ca group tended to be higher than that of the control group (p <0.1, Fig. 3).

The breaking force at the center of the femur of the egg-Ca group tended to be higher than that of the control group (p<0.2, Table 3). The ash weight per dry weight for the egg-Ca group was also higher than that of the control group.

**DISCUSSION**

The effect of egg-shell Ca on bone metabolism was examined in this study by evaluating BMD and breaking strength. BMD values for the extracted lumbar spine and

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**Table 2. Body weight gain, food intake and food efficiency**

<table>
<thead>
<tr>
<th></th>
<th>Body weight gain (g/day)</th>
<th>Food intake (g/day)</th>
<th>Food efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.22±0.07</td>
<td>14.76±0.22</td>
<td>0.22±0.00</td>
</tr>
<tr>
<td>Egg-shell Ca</td>
<td>2.98±0.09</td>
<td>14.36±0.28</td>
<td>0.21±0.01</td>
</tr>
</tbody>
</table>

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*Fig. 1. Change in the tibial bone mineral density*

*In vivo* measurements on the bone mineral density (BMD) of the right tibia for each rat were taken three times: before ovariectomy (phase I), 4 weeks after ovariectomy (phase II), and at the end of the experiment (phase III) by using dual energy X-ray absorptiometry (DXA, Hologic QDR-1500).
tibial proximal metaphysis in the egg-shell Ca group were significantly higher than those of the control group. Kikuchi et al. (1992) have reported that egg-shell Ca was effective for maintaining bone mineral level in lactating rats. The purpose of Kikuchi's study was to evaluate the influence of the phosphorus level in different diets on bone maintenance. Therefore, the phosphorus level for each experimental diet was different. To evaluate the usefulness of different Ca sources, the nutrient content should be identical. The content of each nutrient in our present study is identical. Under this condition, BMD values for the lumbar spine and tibia of the egg-shell Ca group were significantly greater than those of the control group. This data suggests that the egg-shell Ca could be effective for preventing bone loss after ovariectomy.

There was no significant difference in the breaking strength and bone weights of the femur between the control and egg-shell Ca groups. However, the breaking force and ash/dry weight in the femur for the egg-shell Ca group tended to be higher than those of the control group. Diaphysis of the tibia and femur had a mainly cortical bone structure. The effect on cortical bone appeared slowly. BMD of tibial diaphysis and the breaking strength at the center of the femur in the egg-Ca group, however, tended to be higher than the control group. These results support the effect of egg-shell Ca on the bone.

In addition to this, we also preliminarily determined, the effect of egg-shell Ca on bone metabolism in a rat model with osteoporosis by OVX and a low-Ca diet (data are not shown). In the preliminary study, all the rats were OVX and were fed on a low-Ca diet containing 0.01% Ca and 0.3% P for 1 month to produce an experimental osteoporotic rat model. Thereafter, they were divided into the CaCO3-control diet group and the egg-shell Ca diet group. The diets in the preliminary study were the same as those given to the rats in this present study. After consuming each experimental diet for 1 month, BMD and the breaking strength for the egg-shell Ca group were greater than those of the control group. These data

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**Table 3.** Breaking force and bone ash in the femur

<table>
<thead>
<tr>
<th></th>
<th>Breaking force (×10^4 dyn/body wt.)</th>
<th>Ash/dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.87±0.11</td>
<td>57.9±0.00</td>
</tr>
<tr>
<td>Egg-shell Ca</td>
<td>5.23±0.09</td>
<td>59.7±0.00</td>
</tr>
</tbody>
</table>

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Fig. 2. Bone mineral density of the lumbar spine

*In vitro* measurements on BMD were taken after dissection of the fourth and fifth lumbar vertebrae of each rat by DXA. There was a significant difference in lumbar BMD between the control and the egg-Ca groups (p<0.001).

Fig. 3. Bone mineral density of the tibia

*In vitro* measurements on BMD were taken after dissection of the right tibia of each rat by DXA. There was a significant difference in the proximal metaphysis between the control and the egg-Ca groups (p<0.05).
also supported the results of this present study.

The prevention of osteoporosis has become of increasing social concern. The way to ensure sufficient Ca intake and effective Ca sources are actively under investigation. Many different types of Ca source have been used for increasing the Ca content in foods, egg-shell Ca being one of the most common. Insufficient research to evaluate the effect of egg-shell Ca on bone has been done. We thus determined in this study the effect of egg-shell Ca on bone. It is known that different types of Ca salt and various food components affect Ca metabolism (Goto 1979; Gerhard et al. 1990; Robert et al. 1989). The mechanism for the effect of egg-shell Ca on bone is still unclear, although these experimental results suggest that the egg-shell Ca could be beneficial for bone.

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Food Sci., 32, 329-335
卵巣摘出ラットの骨塩減少に対する卵殻カルシウムの効果

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卵殻カルシウムは、種々の健康食品やカルシウム剤にカルシウム源として広く使われている。しかしながら、卵殻カルシウムの骨に対する検討は未だ十分に行われていない。そこで、われわれは、これまでに様々な他のカルシウム剤の骨に対する効果を検討してきた方法を用いて、卵殻カルシウムの骨に対する効果を検討した。その結果、卵殻カルシウム食群の腰椎および股骨近位部骨密度は、コントロール群に比べ有意な高値を示した。また、卵殻カルシウム群の股骨幹部骨密度、骨強度、乾燥重量/乾燥重量が、コントロール群に比べ高値傾向を示した。これらの結果から、卵殻カルシウムは卵巣摘出による骨量減少を抑制する効果があると考えられ、骨粗鬆症の予防に有効なカルシウム源である可能性が示唆された。

キーワード：卵殻カルシウム、骨密度、ラット。