The Effect of Tochu Bark on Bone Metabolism in the Rat Model with Ovariectomized Osteoporosis

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Summary In this study, Tochu bark extract was examined to find out whether it is effective in preventing bone loss after menopause. Six-week-old Sprague-Dawley female rats were ovariectomized (OVX) or were sham-operated (sham). The rats in the OVX and the sham groups were fed a low-calcium diet (Ca 0.01%, P 0.3%) for 33 days. Thereafter, the rats in the OVX group were subdivided into two groups: a control group and a Tochu group. The diet for the Tochu group contained 2% Tochu bark extract (Ca 0.3%, P 0.3%). The diet for the control group and the sham group was the control diet; it contained 0.3% Ca and 0.3% P. The rats in each group were fed each experimental diet for the next 31 days. The bone mineral density (BMD) and the breaking strength of the control group were lower than those of the sham group. However, the BMD and the bone strength had improved in the Tochu group. These results suggest that the Tochu bark extraction could be effective in the prevention of osteoporosis. Moreover, in the Tochu group, intestinal Ca absorption increased. This means the Tochu bark extract accelerated the intestinal Ca absorption. This could possibly affect the increase of the BMD and the bone strength. Moreover, the muscle weight of the Tochu group was higher than that of the control group. These results suggest that Tochu bark extract is also effective for the improvement of the bone and the muscle metabolism in sedentary people with back pains and/or joint pains.

Key Words Tochu bark, osteoporosis, bone mineral density, bone strength, intestinal Ca absorption, muscle weight

Osteoporosis is one of the involutional disorders with bone loss and fracture. The bone mass decreases rapidly because of the decrease of estrogen secretion at menopause. In view of the increasing number of elderly people in Japan, the number of female osteoporotic patients has increased: the number of patients was 3,000,000 in 1985 and 5,000,000 in 1989 (1). The causes and the mechanisms of osteoporosis, however, are still unclear. Moreover, treatment of osteoporosis is
very difficult. Consequently, it is important to prevent osteoporosis. It is a known fact that, not only high peak bone mass in young people but also the prevention of bone loss with aging people are essential for preventing osteoporosis. It is important for the prevention of osteoporosis to take enough nutrients, the nutrient balance, and to do moderate exercise (2-5). In most cases, nutrient intake is sufficient in Japan. The calcium (Ca) intake in particular, however, has never been adequate (6). This indicates that the Ca intake must be carefully observed. Moderate exercise is also important to utilize Ca in the body.

In many cases, elderly people have back pains and/or joint pains. Therefore, it is not possible to do enough physical activity. An investigation which evaluates new factors instead of physical activity must be carried out. Then, new factors have to be found. Recently, the effect of physical activity on bone and muscle has been reported. There is a positive correlation between bone mineral density and muscle power (7). Moreover the soleus weight of the rats in the exercise group increased (8). On the other hand, it has also reported that the power and the volume of the muscles decrease with aging (9,10). Consequently, the Tochu (Eucommia ulmoides Oliver, Eucommiaceae) bark extract was used in this study as a remedy which has some bioavailabilities for the skeleton and the muscle.

Tochu is a crude drug which is good for bone and muscles. In general, dried Tochu bark is called Tochu in China. Tochu is well known as a good medicine for refreshing and strengthening the body, and improving the skeleton (11). Accordingly, Tochu bark is used for patients who are weak or have back pains. In addition, from the results of the animal experiments, the Tochu leaf is effective for enhancing hormone secretion and the prevention of aging (12). Moreover, it has been reported that, the protein synthesis availability of the Tochu leaf is high, and the muscles are also strengthened by consumption of the Tochu leaf (13,14). However, the effect of Tochu bark, which has been used as a crude drug for a long time in China, has never been examined on bone and muscle metabolism. Therefore, in this experiment, the effect of Tochu bark extract on bone mineral density, bone strength, intestinal Ca absorption, and muscle volume were examined to clarify the bioavailability of Tochu bark extract.

MATERIALS AND METHODS

Experimental animal and study protocol. In this study, 6-week-old Sprague-Dawley female rats were used (n=28). They were divided into an ovariectomized (OVX) group (n=20) and a sham-operated (sham) group (n=8). The rats in both groups were fed a low calcium (Ca) diet for 33 days. The low Ca diet consisted of 0.01% Ca and 0.3% phosphorus (P). Thereafter, the rats in the OVX group were sub-divided into control and Tochu groups. In the control group (n=10), the rats were fed a control diet of 0.3% Ca and 0.3% P. Ca source of the control diet was Ca carbonate (CaCO3) only. In the Tochu group (n=10), the rats were fed a Tochu diet of 0.3% Ca and 0.3% P; this diet contained 2% extract of

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Tochu bark. Thus, the Ca, P, protein, and oil content of each diet was identical. Then, the rats in the sham group were fed the control diet, which was the same as that of the control group. The experimental diet period lasted for the next 31 days. During the low-Ca diet period and the experimental diet period, all the rats were allowed ad libitum feeding and deionized distilled water. The compositions of the low-Ca diet, the control diet, and the experimental Tochu diet are shown in Table 1. The composition of the Tochu bark extract is shown in Table 2. The process of

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

| Components                        | Low Ca (0.01% Ca, 0.3% P) | Control (0.3% Ca, 0.3% P) | Tochu (0.3% Ca, 0.3% P) |
|-----------------------------------|---------------------------|---------------------------|-------------------------|
| Glucose monohydrate               | 65.1                      | 64.9                      | 62.9                    |
| Casein (vitamin-free)             | 18.0                      | 18.0                      | 18.0                    |
| Cottonseed oil                    | 10.0                      | 10.0                      | 10.0                    |
| Roughtage                         | 3.0                       | 3.0                       | 3.0                     |
| Ca- and P-free salt mixture¹      | 2.0                       | 2.0                       | 2.0                     |
| Equimolar mixture of K2HPO4 and K3HPO4 | 1.39                     | 0.86                      | 0.85                    |
| CaCO₃                             | 0.005                     | 0.74                      | 0.73                    |
| Cystine                           | 0.2                       | 0.2                       | 0.2                     |
| Water-soluble vitamin mixture²    | 0.1                       | 0.1                       | 0.1                     |
| Fat-soluble vitamin mixture³      | c                         | c                         | c                       |
| Choline chloride                  | 0.2                       | 0.2                       | 0.2                     |
| Tochu bark extract                | —                         | —                         | 2.0                     |

¹The 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. ²The 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 B₁₂, 0.002; biotin, 0.01; glucose monohydrate, 73.7. ³The rats received a supplement of fat-soluble vitamins in cottonseed 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 IU of vitamin D₃.

Table 2. Main composition of Tochu bark extract.

| Components       | %     |
|------------------|-------|
| Moisture         | 74.8  |
| Protein          | 1.3   |
| Lipid            | 0.7   |
| Fiber            | 0.2   |
| Carbohydrate     | 20.7  |
| Ash              | 2.3   |
| Ca               | 168 mg|
| P                | 85 mg |
the manufacturing of Tochu bark extract is also shown in Fig. 1. During the low-Ca diet and the experimental diet period, all the rats were housed in separate cages (15×25×19.5 cm). The conditions in the animal laboratory were as follows: the temperature was maintained at 23±1°C, the relative humidity was maintained at 50±5% and the lighting remained constant for all 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 fasted for one night (7:00 p.m. to 9:00 a.m.). The next day, after anesthetizing with ether, blood samples were taken from the abdominal aorta. The blood samples were centrifuged at 2,500 rpm for 15 min to extract the serum.

The level of the serum Ca was measured by atomic absorption spectrophotometry (using Shimadzu’s AA-640-12 atomic absorption spectrophotometer). P was determined by the Fiske-SubbaRow method (15), and total-protein was measured using the Biuret method (16).

**Measurement of bone mineral density.** In this study, the dual-energy X-ray absorptiometry (DXA (Hologic QDR-1000 X-ray bone densitometer)) was used to determine the bone mineral density (BMD). In comparison with the BMD of humans, the BMD of a small animal, such as rat, is remarkably low in density. Therefore, all scans were performed using the ultra high resolution scan mode (rat mode, Version 2.0 software) as previously reported (17). A detector collimator with a single slit was put on the X-ray generator.

During the 2-day period before starting the experimental diet period, the BMD of the fourth and the fifth lumbar vertebrae of each rat was measured under
anesthesia. The BMD of the fourth and the fifth lumbar vertebrae was an example of the BMD of the lumbar spine. Moreover, during the last 2 days of the experimental diet period, the BMD of the lumbar spine was also determined. At the end of this experiment, the lumbar spines and all the tibial bones were isolated. These muscles and connective tissues were carefully removed. Thereafter, the BMD of the extracted lumbar spine was measured by DXA. The whole tibias were also measured by DXA.

Bone has two different structures: trabecular and cortical bone. Different parts of the tibia have different structures. Analysis of the tibial BMD was performed as previously reported (18). The tibial proximal metaphysis is an example of the trabecular bone. The tibial diaphysis is an example of the cortical bone.

Measurement of the breaking force. During the dissection, the right and the left femoral bones were isolated. Then the muscles and connective tissues were carefully removed. Thereafter, the breaking force of the femoral diaphysis (the center of the femur) was tested and analyzed using the breaking properties test as previously reported (19). The measurement conditions were as follows: the sample space was 1.0 cm, the plunger speed was 100 mm/min, the load range was 50.0 kg, and the chart speed was 120.0 cm/min.

Measurement of the wet weight of the muscles. During the dissection, the femoral quadriceps and the right soleus, which is situated behind the tibia were isolated from each rat. Afterwards, the wet weight of each muscle was measured. In this study, the body weight of the OVX group was significantly higher than that of the sham group. Accordingly, the results indicated that the muscle weight was proportional to the body weight.

Intestinal calcium absorption. In this study, four balance studies were carried out to determine the Ca absorption. The first phase was carried out for 2 days before starting the experimental diet period. The second phase was carried out during the first 2 days after starting the experimental diet period. The next one was carried out during a 2-day period in the middle of the experimental diet period. The final phase was carried out for 2 days at the end of the experimental diet period. At each phase, feces and urine were collected over two 24-h periods. Urine was collected under acidic conditions by using 1 ml 6 N hydrochloric acid, thus preventing Ca precipitation and putrefaction. In the fecal determination, all daily feces' were burnt to ash at 550–600°C for approximately 18 h, and than these were dissolved in 1 N nitric acid. The urinary and fecal Ca excretions were measured using the same method as the biochemical assay of the serum.

Statistical method. The t-test was used to analyze the differences between the sham group and the control group, and between the control group and the Tochu group, within each parameter. $p < 0.05$ was considered statistically significant.

RESULTS

The body weight gain, the food intake, and the food efficiency are shown in
Table 3. As far as the body weight gain and the food intake were concerned, there were significant differences between the sham and the control groups \((p < 0.05, p < 0.01, \text{respectively})\). However, there was no difference between the control and the Tochu groups. The food efficiency of the control group was higher than that of the sham group. The food efficiency of the Tochu group, however, was the same as the control group. The level of serum Ca, P, and total protein, which were normal, showed no differences among the three groups (data not shown).

The change in the lumbar spine BMD over a period of 1 month, in the experimental period, is shown in Fig. 2. In the first phase (phase I), which lasted for 2 days before the experimental period, the BMD of the control group was

![Graph showing change in lumbar spine BMD](image)

Fig. 2. The change of the bone mineral density of the lumbar spine. The bone mineral density of the lumbar spine was measured twice by dual-energy X-ray absorptiometry. Phase I was carried out before starting the experimental diet period, and Phase II was carried out during the last 2 days of the experimental diet period. The lumbar BMD of the OVX groups (control and Tochu groups) was significantly lower than that of the sham group in phase I and II. In phase II, the BMD of the Tochu group had a tendency to be higher than that of the control group. \(\Delta\), the sham group; \(\bullet\), the OVX-control group; \(\bigcirc\), the OVX-Tochu group. Significant differences between the sham and the OVX-control groups: \(*p < 0.05, **p < 0.01, ###p < 0.001\).
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significantly lower than that of the sham group \((p<0.001)\). Between the control and the Tochu groups, there was no difference. After feeding the experimental diet for approximately 1 month, there was a significant difference between the sham group and the control group \((p<0.001)\). Detecting a small change in BMD in situ is difficult, however, in comparison with the control group the BMD of the Tochu group had a tendency to be higher. Moreover, the measurement of the extracted lumbar spine and the tibia is shown in Fig. 3. The BMD of the lumbar spine (Fig. 3, upper part) and the tibial proximal metaphysis (Fig. 3, middle part) in the

![Graph showing bone mineral density](image)

**Fig. 3.** The bone mineral density of the extracted bones. During the dissection, the lumbar spine and the right and left tibias of each rat were isolated. Then the muscle and the connective tissue were carefully removed. The bone mineral density (BMD) of the lumbar spine and the tibias were measured by dual-energy X-ray absorptiometry (DXA). The upper part of this graph shows the BMD of the lumbar spine, the middle part shows the BMD of the tibial proximal metaphysis, and these sites are mainly trabecular bone. The lower part shows the BMD of the tibial diaphysis, this site is mainly cortical bone. In the trabecular sites, the BMDs of the control group were significantly lower than those of the sham group. However, in the Tochu group, the BMD of the trabecular sites was significantly higher than those of the control group. However, in the cortical site (tibial diaphysis) there were no significant differences among the three groups. Significant differences between the sham and the OVX-control groups or the OVX-control and the OVX-Tochu groups: \(*p<0.05, \**p<0.01, \text{ and } ***p<0.001\).
Fig. 4. Breaking force of the femoral diaphysis. During the dissection, the right and left femurs of each rat were isolated. Then, the muscle and the connective tissue were carefully removed. Therefore, the breaking force of the femur was tested using the breaking properties test. The breaking force of the control group was significantly lower than that of the sham group. That of the Tochu group had a tendency to be higher than the control group. Significant difference between the sham and the OVX-control groups: *p<0.05.

Fig. 5. The intestinal calcium absorption. In this experiment, four balance studies were carried out to examine the intestinal calcium (Ca) absorption. Phase I was carried out during a 2-day period before starting the experimental diets. Phase II was carried out during the first 2 days in the experimental diet period. Phase III lasted for 2 days in the middle of the experimental diet period, and phase IV was done during the last 2 days of this experiment. The Ca intake, the fecal Ca excretion, and the urinary Ca excretion were measured. Then, the intestinal Ca absorption was calculated. The Ca absorption in the phase II and III of the Tochu group was significantly higher than that of the control group. ▲, the sham group; ●, the OVX-control group; ○, the OVX-Tochu group. Significant differences between the OVX-control and the OVX-Tochu groups: **p<0.01, and ***p<0.001.
control group were significantly lower than that of the sham group ($p < 0.001, p < 0.001$). These bone sites are mainly trabecular bone. However, the BMD of the trabecular bone sites in the Tochu group significantly increased ($p < 0.05, p < 0.01$). The bottom part of Fig. 3 shows the BMD of the tibial diaphysis. In comparison with the sham group, the BMD of the tibial diaphysis of the control group was slightly lower. However, in the Tochu group the BMD of the tibial diaphysis had a tendency to be higher than that of the control group, but there was no significant difference.

As shown in Fig. 4, the breaking force of the femoral diaphysis in the control group was significantly lower than that of the sham group ($p < 0.05$). The breaking force of the Tochu group was higher than that of the control group, however there was no significant difference.

Intestinal Ca absorption is shown in Fig. 5. That of the sham group was lower than that of the control group because of the difference in the food intake. However, the intestinal Ca absorption of the Tochu group was significantly greater than that of the control group in the phase II and IV balance studies. Moreover, the levels of intestinal Ca absorption in the Tochu group were higher during the experimental diet period.

The weight of the muscles are shown in Fig. 6. The weight of the soleus and the
femoral quadriceps of the control group was lower than that of the sham group. In the Tochu group, however, the weight was significantly higher ($p < 0.05$, $p < 0.05$).

**DISCUSSION**

In this experiment, the Tochu bark extract was examined to find out whether it is an effective source for improving derangement in bone and muscle metabolisms in an ovariectomized osteoporotic rat model.

In comparison with the sham group, the BMD of the lumbar spine and the tibial proximal metaphysis in the control group were significantly lower. The lumbar spine and the tibial proximal metaphysis contain mainly trabecular bone. Moreover, the tibial diaphysis which is mainly cortical bone, was measured in this study. The BMD of the tibial diaphysis of the control group was slightly lower than that of the sham group. The femoral diaphysis breaking force was also significantly decreased. The low BMD and bone strength in the control group was caused by a decrease in the estrogen secretion due to ovariectomy. However, the BMD of the lumbar spine and the tibial proximal metaphysis in the Tochu group were significantly higher than those of the control group. These results suggest that the Tochu bark extract is effective for increasing the BMD in trabecular bone. In the BMD of the tibial diaphysis, there was no difference between the Tochu and the control groups. Moreover, the breaking strength of the cortical bone site was slightly improved by consuming Tochu bark extract; however, there was no significant difference. The tibial diaphysis and the femoral diaphysis are examples of cortical bone. Metabolism of cortical bone is slower compared with trabecular bone (20). As a result, it is more difficult to increase the BMD and bone strength in cortical bone. Consequently, the BMD and the bone strength of the cortical bone site did not change significantly.

In comparison with the sham group, the intestinal Ca absorption of the control group was significantly higher or had a tendency to be higher. That is to say that the intestinal Ca absorption was increased due to an increase in the food intake in the control group. Moreover, in the Tochu group the intestinal Ca absorption showed a significant increase compared with the control group. These results indicate that the Tochu bark extract could be effective for increasing the intestinal Ca absorption. This effect is thought to influence the increase in bone metabolism.

In this study, in comparison with the sham group the weights of the quadriceps femoris and the soleus were lower than those of the sham group. However, those of the Tochu group were significantly higher than those of the control group. These results indicate that the Tochu bark extract is thought to be effective for muscle metabolism.

It was reported, that in the experimental chicken which ate Tochu leaf, the intrafusal fiber become more elastic (13). The protein in the fibrous tissue was also stronger in the group given Tochu leaf (14). Moreover, the other experiments which used mice (21) or eels (22), showed that Tochu leaf is effective for muscle metabolism.
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strengthening muscles. These results caused an acceleration of the protein synthesis by Tochu feeding. The mechanism of the effect the Tochu bark, however, is still unknown. On the other hand, the components of the Tochu bark are reported to be the same as those of the Tochu leaf (23). Accordingly, it is possible to consider that the Tochu bark might have the same effective function as the Tochu leaf. In this study, the muscle weight of the Tochu group was higher than that of the control group. These results indicate that the muscular protein synthesis would be accelerated by some of the components in the Tochu bark.

There are few studies which have investigated the relationship between the BMD and the skeletal muscle. Nilsson and Westlin reported that there was a positive correlation between the BMD and the power of the femoral muscle (7). It was reported that the weight of the soleus increased using the voluntary running exercise (8). During this study, the experimental animals (the rats in the sham, the OVX-control, and the OVX-Tochu groups) did not exercise, and the weights of the soleus and the femoral quadriceps were significantly higher in the Tochu group. These results suggest that Tochu bark is possibly effective for preventing muscle reduction and for increasing muscle in people who are not able to do enough physical activity.

In China Tochu bark has been used for a long time as an excellent medicine to strengthen the skeleton and the muscle. The present experimental results showed that Tochu bark extract could be effective for increasing the BMD and the bone strength on the ovariectomised osteoporotic model rats. Moreover, Tochu bark might be effective for increasing muscle weight. From the present finding, the Tochu bark extract may be effective for preventing postmenopausal osteoporosis. Moreover, the Tochu bark extract is thought to be effective for bone and muscle metabolism in those who have back and joint pains. However, the functional mechanism of Tochu bark is still unknown, and it is not clear which component of the Tochu bark extract affects the bone and the muscle metabolism. Henceforth, we are going to examine what sort of extra components in the Tochu bark affect the bone and the muscle, and we are also going to examine the mechanism of these components and their effective function in Tochu bark.

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