Original Article

Intake of Calcium from Marine Resources for Prevention of Osteoporosis

— Bone and Lipid Metabolism in the Bone Modeling of Rats Administered with the Bonito Docosahexaenoic Acid and the Cuttlefish Calcium —

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Abstract

In order to fill up the shortage of calcium intake, particular attention was paid to the absorbable calcium derived from fishes and shellfishes. The diets prepared from cuttlefish shell calcium (C-Ca), bonito head oil (fish oil) were administered to rats, and the bone metabolism and the bone modeling were investigated the calcium and inorganic phosphate contents and the measurement of the trabecular bone mineral density (BMD), the cortical BMD and the stress strain index (SSI) of rat femur. From these results the bone modeling was accelerated more effectively by the administration of cuttlefish calcium and fish oil, compared to the administration of control diet calcium. It is suggested that cuttlefish shell calcium was effective in bone modeling, and these are available as new calcium resources.

Introduction

The calcium intake of the Japanese people, both males and females, is insufficient for the recommended allowance of calcium according to The National Health and Nutrition Survey in Japan (2005)[1]. To maintain this requirement, it may be necessary to take foods rich in calcium. Due to the aging Japanese population, the patients with osteoporosis have been increasing in number and calcium fortified foods have been in great demand. However not only addition of high dose of fortified calcium to foods, but also it is desirable to take absorbable calcium with high bioavailability. On the other hand, it is reported that n-3 polyunsaturated fatty acid (PUFA) is effective in bone modeling or preventing bone loss during aging[2]. For the compensation of the lack of calcium intake, we paid attention to taking absorbable calcium from fishery products[3]. The present study was carried out to clarify the effects of fishery products such as cuttlefish shell calcium (C-Ca), and bonito head oil (fish oil) on the bone metabolism and remodeling and evaluate the utility of these marine resources to compensate the lack of calcium intake.

Materials and Methods

The experimental diet of each group in the present study is shown in Table 1 and 2. Four week old male Wistar rats (Nippon Culea Co., Ltd.) after being provided with the standard diet[4] (AIN-93G, Oriental Yeast Industry Co., Ltd.) for 10 days were divided into 4 groups (A~D). The control diet was prepared to remove calcium and soybean oil from AIN-93G diet at Oriental Yeast Industry Co., Ltd. The number of animals in each group was six and each fed the following diets for 30days. Group A: control diet + AIN-93G calcium + corn oil; Group B: control diet + AIN-93G calcium + fish oil; Group C: control diet + cuttlefish calcium (Ca-C) + corn oil; Group D: control diet + cuttlefish calcium (Ca-C) + fish oil. Cuttlefish calcium and bonito head oil used in this experiment were obtained from Yaizu Meal Coop. AIN-93G calcium was prepared at

Table 1. The experimental diet of each group

Group A: removed soybean oil from AIN-93G diet and replaced corn oil
Group B: removed soybean oil from AIN-93G diet and replaced fish oil
Group C: removed calcium from Group A diet and replaced C-Ca
Group D: removed calcium from Group B diet and replaced C-Ca

Standard diet (AIN-93G diet): Oriental Yeast Industry Co., Ltd.
C-Ca: cuttlefish shell calcium (Ca extracted from cuttlefish shell powder)
Corn oil: Chemicals (Wako Chemicals Co.)
Fish oil: bonito head fish oil (Yaizu Meal Coop.)

Table 2. The composition of AIN-93G diet (g/kg diet)

Cornstarch	397.486
Casein (≧85% protein)	200.000
Dextrinized cornstarch	132.000
Sucrose	100.000
Soybean oil (no additives)	70.000
Fiber	50.000
Mineral mix (AIN-93G-MX)	35.000
Vitamin mix (AIN-93G-VX)	10.000
L-Cystine	3.000
Choline bitartrate	2.500
Tert- butylhydroquinone	0.014

Oriental Yeast Industry Co., Ltd. and the corn oil was purchased from Wako Chemicals Co. Calcium and bonito head oil were converted into AIN-93G diet composition based on the each analyzed values and administered with the correspondent content to the rats. The mixed diet in each group was given and deionized water was given ad libitum. The animal rooms were automatically maintained at a room temperature of 22±1℃, a relative humidity of 55±5% and lighted for 12 hours per day. The changes of the food consumption and the body weights in each group were measured on almost every 3 days for about 40 days.

All the rats were anesthetized with diethyl ether, dissected, and their femoral bones were removed. After cutting the diaphysis of the femoral bone, the marrow was washed out, and the wet weight of the femoral bone was weighed, which was dried and the dry weight was weighed. Next it was laid in ashes and the ash content was calculated, which was resolved in 5N nitric acid and the sample solution was obtained. The calcium and the inorganic phosphate contents in the femoral bone were determined by the o-CPC (o-cresolphthalein complexone) method using the autoanalyzer (COBAS-FARA II; F. Hoffman La-Roche Ltd.) and the enzymatic method, respectively.

The femoral bone after being removed was examined for the trabecular bone mineral density (BMD) in the regions of 3mm (metaphysis) and the cortical BMD in the regions of 12mm (diaphysis) from the distal growth cartilage using pQCT (XCT-960A, Norland-Stratec Inc.) and SSI (Stress Strain Index) was calculated.

Statistical analysis of the data obtained for each test. Data analysis was performed by SPSS 15.0J and differences in calcium and inorganic phosphate contents and the total bone mineral density (BMD), the trabecular BMD, the cortical BMD and the stress strain index (SSI) of rat femur bone was determined by the ANOVA and Tukey's multiple comparative test. Values are the means ± SD. Differences of $p<0.01$ and $p<0.05$ were considered to be significant.

Results and Discussion

1. The calcium and inorganic phosphate contents in the femoral bone

The changes in the body weights of the rats in each group are shown in Fig.1. There was no significant difference in the food consumption rate of all groups. The calcium and inorganic phosphate contents in the femoral bone are shown in Figure 2. In the calcium content, Group B (6.7%) fed with AIN-93G calcium and fish oil showed no significance ($p>0.01$), compared to Group A (6.4%) fed with AIN- 93G calcium and corn oil. And the content of calcium in Group C (7.2%) fed with cuttlefish calcium and corn oil showed a high value significantly ($p<0.05$), compared to Group A. Group D (8.6%) administered cuttlefish calcium and fish oil showed a high value significantly ($p<0.01$), compared to Groups A, B and C. Further, the inorganic phosphate content showed the same tendency with the calcium content in all groups and the ratio of calcium to inorganic phosphate in each group was approximately 2:1. Regarding the calcium content in the femoral

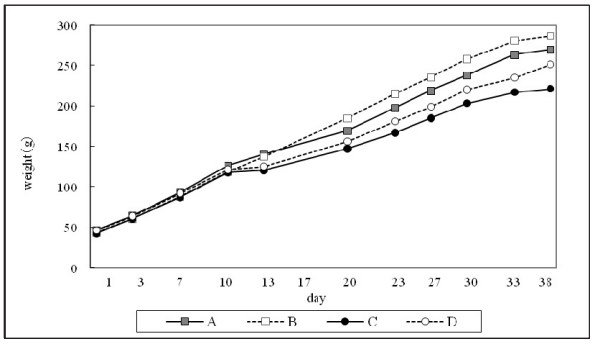

Fig. 1. Changes in the body weight of rats in each group

The body weight in each group is the mean value of 6 rats.
■ A, Ca (AIN-93G Ca)+corn oil; □B, Ca (AIN-93G Ca)+fish oil, ● C, C-Ca (cuttlefish Ca)+corn oil; ○D, C-Ca (cuttlefish Ca)+fish oil.
C-Ca: cuttlefish shell calcium (Ca extracted from cuttlefish shell powder, Yaizu Meal Coop.)
Corn oil: Chemicals (Wako Chemicals Co.)
Fish oil: bonito head fish oil (Yaizu Meal Coop.)

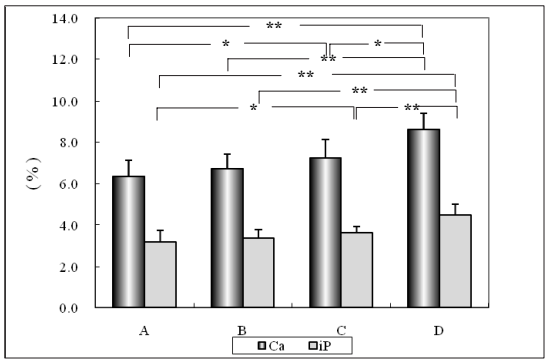

Fig. 2. Content of calcium and inorganic phosphate in femoral bone of rats.

Values are shown as the mean±SD of 6 rats per group.
Significantly different in comparison with each group; *$p<0.05$, **$p<0.01$.

bone, the groups administered cuttlefish calcium and the group administered both cuttlefish calcium and fish oil had high calcium content, compared to the groups administered AIN-93G calcium. Thus it is suggested that cuttlefish calcium might act effectively in bone modeling.

2. Bone mineral density (BMD) and the stress strain index (SSI)

For the bone metabolism, the pQCT images are shown in Figure3. Next, the bone mineral density (BMD) and the stress strain index (SSI) of the rat femoral bone measured by pQCT are shown in Figure 4. In the total BMD in the metaphysis region, compared to Group A (252.2 (mg/cm^3)) administered AIN-93G calcium, Group B (277.30 (mg/cm^3)) showed a little high value ($p>0.01$). And also Group D (259.47 (mg/cm^3)) administered cuttlefish calcium and fish oil showed a significant high value, compared to Group C (205.82 (mg/cm^3)) administered cuttlefish calcium ($p<0.01$). In the trabecular BMD, compared to Group A (85.8 (mg/cm^3)), Group D (118.03 (mg/cm^3)) showed significant difference ($p<0.05$), and between Groups C (70.20 (mg/cm^3)) and D there was also significant difference ($p<0.05$). In the cortical BMD in the region of diaphsis, compared to Group A (811.37 (mg/cm^3)), Groups B (830.07 (mg/cm^3)), C (824.55 (mg/cm^3)) and D (887067 (mg/cm^3)) showed almost the same values. Significant difference between Groups A and D showed ($p>0.05$). In a stress strain index, compared to Group A (3.10), Group B (3.29) showed a little high value ($p>0.01$) and both Groups C (6.11) and D (6.62) showed high values. There was significant difference between Groups A and C and Groups A and D ($p>0.01$).

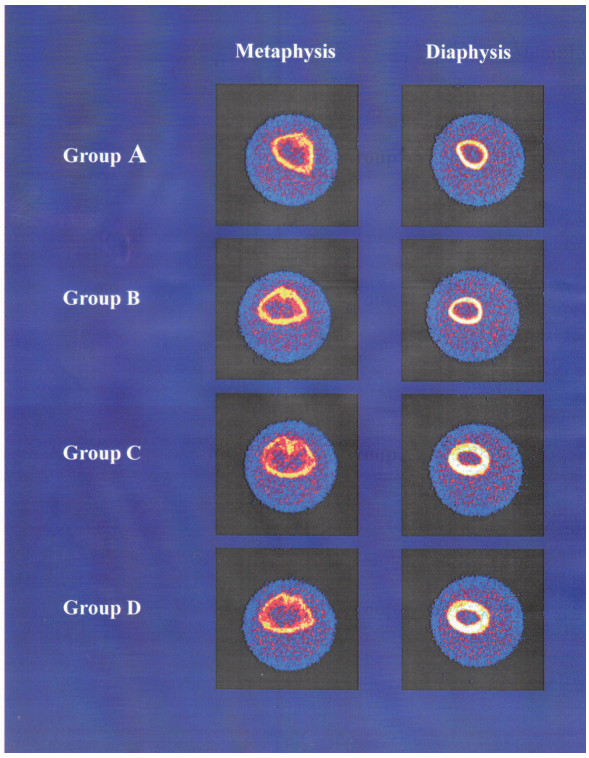

Fig. 3. Bone mineral density of rat femur pQCT image.

Trabecular and total BMD are metaphysis at the region of 3mm and cortical BMD and SSI are diaphysis at the region of 12mm from the distal growth cartilage.

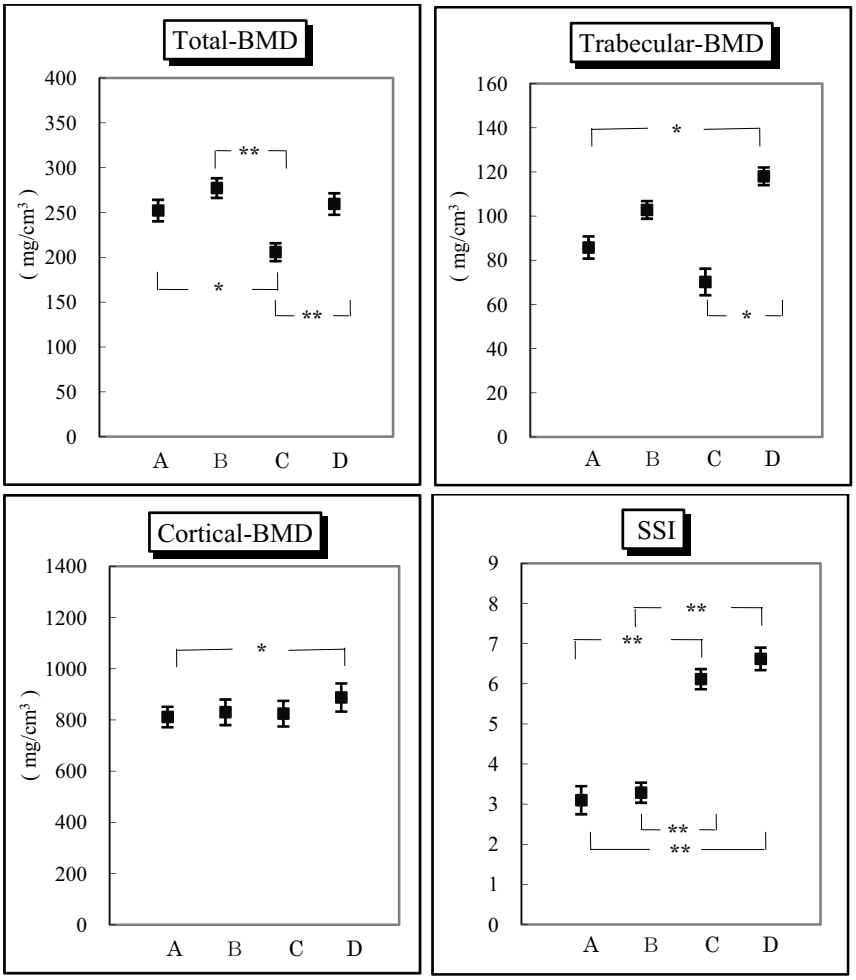

Fig. 4. Bone mineral density (BMD) and the strength strain index (SSI) of rat femur measured by pQCT.
Trabecular and total BMD are metaphysis at the region of 3mm and cortical BMD and SSI are diaphysis at the region of 12mm from the distal growth cartilage.
Values are shown as mean±SD of 6 rats per group.
pQCT: XCT-960A (Norland-Stratec Inc.)
Significant difference in comparison with each group; *$p<0.05$, **$p<0.01$

3. Effects of the combination of two kinds of calcium and oils

Figure 5. shows the interactive effect on calcium and oils. Regarding the effects of the combination of calcium and oils, there is significant difference in the calcium content of the femoral bone between two kinds of calcium ($p<0.01$) and between two kinds of oils ($p<0.01$). An interaction of calcium and oil was admitted in the calcium content with significant difference ($p<0.01$). Group D fed with cuttlefish calcium and fish oil and Group C fed with cuttlefish calcium and corn oil contained high calcium content. And also the same result was gained in inorganic phosphate ($p<0.01$). In total BMD, trabecular BMD, cortical BMD and SSI, there is no interaction in the combination of calcium and oils. Between calciums in total BMD and SSI, there was significant difference ($P<0.01$). Trabecular BMD and cortical BMD had significant difference in between oils ($p<0.01$). High values in the bone parameters were shown in Groups D and C in order. From these results, the total BMD, the trabecular BMD and the cortical BMD in Groups C and D, cuttlefish calcium diet groups were maintained and cuttlefish calcium acted the bone modeling effectively, and furthermore, it is suggested that by using together with fish oil the bone formation was accelerated.

According to the Annual Report of Recommended Intake of Individual Food Group, the calcium intake from fishes and shellfishes is decreasing, though the one from

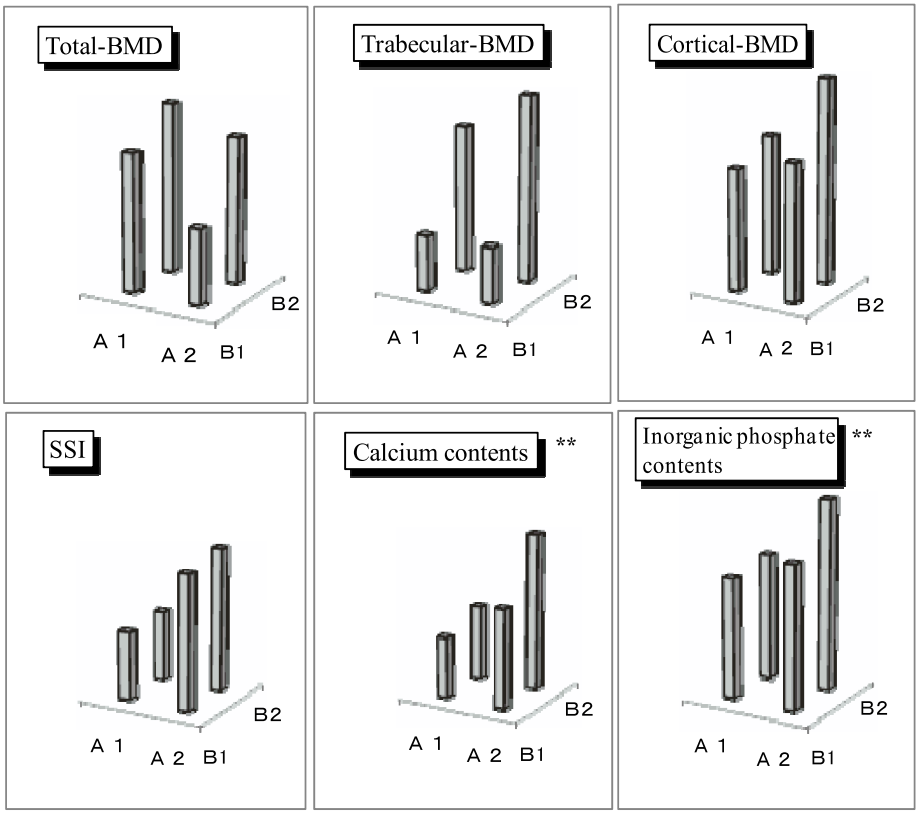

Fig. 5. Interaction of the combination with calcium and oils

A_1, Ca (AIN-93G Ca); A_2, C-Ca (cuttlefish Ca); B_1, corn oil; B_2, fish oil

** Interactions were effective in calcium content and inorganic phosphate content ($p<0.01$)

milk and dairy products is increasing. As the calcium intake has not yet attained the recommended calcium allowance, small fishes, seaweeds, green-yellow vegetables and beans as well as milk and dairy products are recommended to take. Today thirteen kinds of calcium compounds such as calcium carbonate, calcium citric acid, and calcium chloride are admitted to use as the calcium additives, according to the Japanese Standard of Food Additives. However, calcium is hard to be absorbed in the intestines compared to other nutrients and there is much variety found in the calcium absorption rate among foods[5]. For the calcium supply, as it is not always easy to take calcium only from food rich in calcium, it is thought that the calcium fortified foods are necessary more and more in the future. Kato, Takada and et al. reported that it is desirable for calcium to be absorbable and available, namely, to have a high bioavilablility[6].

Conclusion

From these results, it is suggested that cuttlefish calcium would be effective from the viewpoint of the histological observation of femoral bone, calcium content, inorganic content, the maintenance of bone mineral density and bone modeling in rats, and besides, it is expected the cuttlefish calcium would be used for the natural calcium derived from fishes and shellfishes in order to fortify calcium. On the other hand, the utility of the fish residues would be available for environmental conservation. Regarding the metabolism of calcium, it is expected to make clear how would these calcium compounds affect the absorbance of calcium in small intestines and the constancy of calcium in the body.

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