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Effects of Water-Soluble Calcium Supplements Made from Eggshells and Oyster Shells on the Calcium Metabolism of Growing Rats

– Research Note –

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Abstract

This study investigated the effects of water-soluble calcium supplements manufactured with eggshells and oyster shells on growing rats. The aim was to review the potential use of food wastes as materials for water-soluble calcium supplements as compared to water-soluble calcium supplements made from imported seaweed powder. When experimental animals were administered three types of water-soluble calcium supplements orally for six weeks, the serum calcium level of the seaweed calcium supplement group were significantly higher than that of eggshell or oyster shell-derived calcium, but blood alkali phosphatase activity, osteocalcin and urine crosslink levels were not different in the three types of calcium supplements. Bone mineral density and bone mineral content in spine, femur and tibia also were not significantly different among the groups. However, when considering body weight of each group, bone mineral density and bone mineral content of the femur were significantly higher in the oyster shell calcium supplement group. These results suggest that at least on a short-term basis, the effect of calcium supplements prepared from eggshell and oyster shell are similar to the effects of seaweed calcium supplements.

Key words: calcium supplements, water-soluble calcium, eggshell, oyster shell, calcium absorption

INTRODUCTION

According to a 2005 Korea national health & nutrition examination survey (1), only 79.0% of the recommended average calcium intake per day (553.1 mg) was taken by Koreans. Calcium is a main component of bones and teeth and is related to neurotransmission, muscle movement, blood clotting and hormone secretion. A long-term calcium deficiency can increase the likelihood of hypertension (2), stroke (3) and colon cancer (4), as well as skeletal diseases (5). Although milk, cheese and anchovies contain a lot of calcium, the majority of calcium intake by Koreans comes from vegetables (6). Therefore many Koreans, especially adolescents, the elderly and osteoporosis patients tend to supplement the lack of calcium by using calcium supplement or calcium enriched foods.

Calcium carbonate, a water-insoluble calcium has been widely used as a representative calcium supplement as it can be produced on a large scale at low cost. However, as some reports found that more soluble calcium supplements had more effective absorption into the body (7-10), the calcium absorption rate as well as calcium content has been recognized as critical factors in calcium supplement usage. Commercial calcium supplements are divided into synthetic supplements such as calcium carbonate, calcium lactate and calcium citrate, and natural supplements including whey calcium and seaweed powder. In a previous study, the authors had studied the uses of water-soluble calcium supplement produced with seaweed powders in growing rats (11). The seaweed powder based, water-soluble calcium supplement was expected to be more beneficial to bone mineral density in terms of weight bearing on the skeleton than the insoluble calcium supplement. However, the cost of seaweed powder-based calcium is relatively high because most of it is imported, so other natural calcium materials are being developed as potential substitutes.

This study investigated the effects of intake of water-soluble calcium supplements produced with eggshells and oyster shells to determine the possibility of using these waste resources as cost-effective materials for calcium supplements.

MATERIALS AND METHODS

Experimental animals and diet

In this study, growing female rats, for which the ad-

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ministration of liquid calcium supplement was feasible and calcium metabolism was active, were selected as experimental animals. Twenty one female Sprague-Dawley rats weighing an average of 90 g were purchased (Danhan Biolink Co., Seoul, Korea) and adapted on rat chow (Sam Yang Co., Seoul, Korea) for one week. Then they were randomly divided into 3 groups according to body weight, 7 animals per group, and fed experimental diets for 6 weeks. During the experiment period, the animal room was maintained 22±2°C and 55±5% relative humidity with a 12 hr light/dark cycle. Experimental diets and deionized water were offered *ad libitum*. A calcium-free AIN-93G (Ca free) diet was prepared (Table 1) and fed to all experimental rats. The three experimental groups consisted one that was supplied water-soluble calcium supplement made from commercial seaweed powder (Aqua-Ca, Marigot Group Celtic Sea Minerals Ltd., Cork, Ireland), one that was supplied water-soluble calcium supplement made from eggshells (ES-Ca), and one that was supplied water-soluble calcium supplement made from oyster shells (OS-Ca). The control group in this study was the one receiving the seaweed calcium supplement because the effect of this supplement has been previously documented (11).

Administration of calcium supplements

Three water-soluble calcium supplements (60 mg/mL, over 90% ionizing ratio) which were prepared from calcium materials (seaweed powder, eggshells and oyster shells) by soaking in vinegar (12) were provided by Kyemyeong Foodex Co. (Daegu, Korea). Each calcium supplement was administered once a day at a constant time, 10 mL/kg body weight.

Dietary consumption rate and weight measurement

Dietary consumption rate was measured once a day, and body weight was measured two times a week at the same times, and the diet efficiency was calculated by dividing the weight gain during the experimental period by the diet consumption amount.

Table 1. Composition of experimental diet (g/kg of diet)

Ingredient	Calcium free diet
Casein	200
Corn starch	592.486
Sucrose	100
Soybean oil	70
Cellulose	50
t-Butylhydroquinone	0.014
Min-mix[1)]	35
Vit-mix[2)]	10
L-Cystine	3
Choline bitartrate	2.500

[1)]AIN-93G mineral mixture (Ca free).
[2)]AIN-93VX vitamin mixture.

Sample collection

After 6 weeks of oral administration, rats were fasted overnight and the urine collected, then they were anesthetized with ether and sacrificed. After the blood collected from the aorta was centrifuged at 3,000 rpm for 20 min, the serum was separated and stored frozen at -70°C.

Biochemical analysis

Serum calcium content was measured by an automatic absorption analyzer applying the TECHNICON CHEMTM system (Alliance Instruments, Paris, France) and alkaline phosphatase (ALP) activity was analyzed using a kit based on the Kind and King method (13). Serum osteocalcin content was analyzed by an osteocalcin radioimmuno-assay kit (Brahams Co., MI, USA). Deoxypyridinoline and creatinine content were analyzed using a collagen crosslink kit (Metra biosystems Inc., CA, USA) by ELISA. The crosslink value was the value obtained by dividing deoxypyridinoline content by creatinine content.

Bone mineral density and bone mineral content measurement

At 6 weeks of oral administration, the anesthetic pentobabobital sodium (Han Lim Pharmaceuticals, Seoul, Korea) was injected intraperitoneally, at 1 mL/kg dose. The bone mineral density (BMD) and bone mineral content (BMC) of the spine, the femur, and the tibia were measured using the dual energy x-ray absorptionmetry (DEXA) FIXImus (LUNAR, Madison, WI, USA).

Statistical analysis

Experiment results were presented as a mean and standard deviation using the SPSS program (13.0), the comparison among experiment groups was performed by one way ANOVA, and their significance was validated by Duncan's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

Diet and calcium intake, weight gain, and food efficiency ratio

Dietary intake, calcium intake, the amount of weight gain and dietary efficiency (food efficiency ratio, FER) of the experimental animals taking oral water-soluble calcium supplements made of seaweed powders, eggshells and oyster shells over the course of six weeks are shown in Table 2. While the average dietary intake per day was significantly high in the eggshell calcium supplement group ($p<0.05$), weight gain was not sig-

Table 2. Diet intake, total calcium intake, body weight gain and food efficient ratio (FER) of rats fed different calcium supplements

Group[1)]	Dietary intake (g/day)	Total calcium intake (g/6 weeks)	Weight gain (g/day)	FER[4)]
Aqua-Ca	13.63±0.46[b2)]	5.10±0.45[NS3)]	2.07±0.41[NS]	0.15±0.02[a]
ES-Ca	15.00±0.89[a]	4.95±0.42	2.01±0.36	0.13±0.02[ab]
OS-Ca	14.13±0.65[b]	5.05±0.38	1.82±0.32	0.12±0.02[b]

[1)]Aqua-Ca (control): rats supplied soluble calcium supplement made of commercial seaweed powder (AquaCal), ES-Ca: rats supplied soluble calcium supplement made of eggshell, OS-Ca: rats supplied soluble calcium supplement made of oyster shell.
[2)]Means with different superscripts within the column are significantly different at $p<0.05$.
[3)]Not significantly different.
[4)]FER=weight gain/dieary intake.

nificantly different among the three groups. The food efficiency ratio (FER) was significantly higher in the seaweed calcium supplement group than in oyster shell calcium supplement group. Dietary intake could be affected by trace components that might differ in each of the calcium supplements, causing a change in taste or appetite, however more research is needed. The total calcium intake was not significantly different among the experimental groups.

Serum and urine composition

The results for the serum calcium content and bone formation markers, alkaline phosphatase and osteocalcin, are shown in Table 3. Serum calcium in the seaweed calcium supplement group was 11.85 mg/dL and significantly different from the other groups. There was no significant difference in serum calcium between the eggshell calcium supplement group and the oyster shell calcium supplement group. The levels of alkaline phosphatase and osteocalcin among three groups were not significantly different. Alkaline phosphatase induces bone formation through osteoblast activation (14,15), leading to the secretion of osteocalcin, a specific protein of bone matrix, which is recognized as an index for bone turnover as well as bone formation (16). According to the previous study (11), supplementation with the water-soluble calcium manufactured with seaweed powder was expected to positively affect skeletal health, and both the eggshell and the oyster shell calcium supplements were thought to act similarly. However high concentration of serum calcium would protect bone resorption and accelerate calcium usage in cells such as osteoblasts (17). Considering the homeostasis of the serum calcium concentrations, a long-term effect of calcium supplementation is expected to be more pronounced for the seaweed calcium supplement. In addition, serum calcium could be affected by the ionization rate of the calcium supplement (18), however, more research on that is needed.

Table 3. Serum calcium, alkaline phosphatase (ALP) and osteocalcin of rats fed different calcium supplements

Group[1)]	Serum calcium (mg/dL)	ALP (IU/L)	Osteocalcin (ng/mL)
Aqua-Ca	11.85±0.43[a2)]	431.83±61.82[NS3)]	0.75±0.51[NS]
ES-Ca	11.43±0.50[b]	404.66±82.76	0.74±0.29
OS-Ca	11.32±0.54[b]	404.50±77.22	0.62±0.38

[1)]Aqua-Ca (control): rats supplied soluble calcium supplement made of commercial seaweed powder (AquaCal), ES-Ca: rats supplied soluble calcium supplement made of eggshell, OS-Ca: rats supplied soluble calcium supplement made of oyster shell.
[2)]Means with different superscripts within the column are significantly different at $p<0.05$.
[3)]Not significantly different.

Table 4 reveals that there was no significant difference in the levels of urine deoxypyridinoline and creatinine among the three kinds of calcium supplemented groups. Deoxypyridinoline in the skeleton is excreted in the urine with collagen by-products as a result of osteoclast action (19). Because of the diurnal variations in urine deoxypyridinoline levels, crosslink values based on creatinine levels are used to monitor bone resorption (20). Considering the results for crosslink value, we speculate

Table 4. Urine deoxypyridinoline, creatinine and crosslink value of rats fed different calcium supplements

Group[1)]	Deoxypyridinoline (nM)	Creatinine (mM)	Crosslink value[4)] (nM/mM)
Aqua-Ca	1136.81±276.66[2)NS3)]	6.67±2.37[NS]	171.84±49.18[NS]
ES-Ca	1357.53±259.20	7.74±2.02	175.23±29.74
OS-Ca	1223.76±159.72	7.54±2.03	163.75±34.36

[1)]Aqua-Ca (control): rats supplied soluble calcium supplement made of commercial seaweed powder (AquaCal), ES-Ca: rats supplied soluble calcium supplement made of eggshell, OS-Ca: rats supplied soluble calcium supplement made of oyster shell.
[2)]Mean±SD.
[3)]Not significantly different.
[4)]Crosslink value=deoxypyridinoline/creatinine.

Table 5. Bone mineral density (BMD) and bone mineral content (BMC) of rats fed different calcium supplements

Bone	Group[1)]	BMD (g/cm^2)	BMD (g/cm^2)/100 g wt	BMC (g)	BMC (g/cm^2)/100 g wt
Spine	Aqua-Ca	0.138±0.018[NS2)]	0.057±0.010[NS]	0.341±0.046[NS]	0.142±0.021[NS]
	ES-Ca	0.136±0.014	0.057±0.006	0.345±0.056	0.146±0.019
	OS-Ca	0.136±0.009	0.059±0.004	0.344±0.071	0.159±0.017
Femur	Aqua-Ca	0.177±0.023[NS]	0.073±0.006[b3)]	0.332±0.042[NS]	0.138±0.010[b]
	ES-Ca	0.178±0.016	0.075±0.008[ab]	0.337±0.040	0.143±0.015[b]
	OS-Ca	0.183±0.093	0.080±0.006[a]	0.357±0.024	0.156±0.010[a]
Tibia	Aqua-Ca	0.120±0.009[NS]	0.050±0.005[NS]	0.284±0.041[NS]	0.118±0.018[NS]
	ES-Ca	0.118±0.007	0.050±0.004	0.284±0.031	0.120±0.010
	OS-Ca	0.117±0.007	0.051±0.004	0.281±0.041	0.122±0.015

[1)] Aqua-Ca (control): rats supplied soluble calcium supplement made of commercial seaweed powder (AquaCal), ES-Ca: rats supplied soluble calcium supplement made of eggshell, OS-Ca: rats supplied soluble calcium supplement made of oyster shell.
[2)] Not significantly different.
[3)] Means with different superscripts within the column are significantly different at $p<0.05$.

no significant difference of bone resorption among the three calcium supplemented groups.

Bone mineral density (BMD) and bone mineral content (BMC)

Table 5 shows the lack of a significant difference in the BMD and BMC of the spine, femur and tibia after six weeks of calcium supplementation across the groups. Therefore, the eggshell calcium supplement and the oyster shell calcium supplements were considered not to be significantly different from the seaweed calcium supplement in bone metabolism. In addition, when realistic skeletal health condition considering weight bearing was compared (21), both of bone density and bone content per 100 g of body weight in femur were high in the oyster shell group. Lee and Kim (22) reported that the amount of calcium intake affected the mineral composition of femur more than the spine. Schaafsma et al. (23) also reported that intake of enriched eggshell powder supplement increased BMD of the femoral neck in post-menopausal women. Hence the oyster shell calcium supplement might act on femur specifically.

When each calcium supplement was taken on a short-term basis, the calcium supplementation effect of the eggshell calcium supplement and the oyster shell calcium supplement were expected to be similar to the seaweed calcium supplement. Hereafter, studies on trace component and specificity of action of each calcium supplement are necessary.

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