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Magnesium Supplementation through Seaweed Calcium Extract Rather than Synthetic Magnesium Oxide Improves Femur Bone Mineral Density and Strength in Ovariectomized Rats

Yun Jung Bae · So Young Bu · Jae Young Kim · Jee-Young Yeon · Eun-Wha Sohn · Ki-Hyo Jang · Jae-Cheol Lee · Mi-Hyun Kim

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Abstract Commercially available seaweed calcium extract can supply high amounts of calcium as well as significant amounts of magnesium and other microminerals. The purpose of this study was to investigate the degree to which the high levels of magnesium in seaweed calcium extract affects the calcium balance and the bone status in ovariectomized rats in comparison to rats supplemented with calcium carbonate and magnesium oxide. A total of 40 Sprague–Dawley female rats (7 weeks) were divided into four groups and bred for 12 weeks: sham-operated group (Sham), ovariectomized group (OVX), ovariectomized with inorganic calcium and magnesium supplementation group (OVX-Mg), and ovariectomized with seaweed calcium and magnesium supplementation group (OVX-SCa). All experimental diets contained 0.5% calcium. The magnesium content in the experimental diet was 0.05% of the diet in the Sham and OVX groups and 0.1% of the diet in the OVX-Mg and OVX-SCa groups. In the calcium balance study, the OVX-Mg and OVX-SCa groups were not significantly different in calcium absorption compared to the OVX group. However, the femoral bone mineral density and strength of the OVX-SCa group were higher than those of the OVX-Mg and OVX groups.

Y. J. Bae
Department of Food and Nutritional Sciences, Hanbuk University, Dongducheon 483-120, South Korea

S. Y. Bu
Division of Food Science, Kyungil University, Gyeongsan 712-701, South Korea

J. Y. Kim
Research Center for Biophamaceutical Lead Molecule, Bucheon 420-743, South Korea

J.-Y. Yeon
Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, South Korea

E.-W. Sohn
Department of Herbal Medicine Resource, Kangwon National University, Samcheok 245-711, South Korea

K.-H. Jang · J.-C. Lee · M.-H. Kim (✉)
Department of Food and Nutrition, Kangwon National University, Samcheok 245-711, South Korea
e-mail: mhkim1129@kangwon.ac.kr

Seaweed calcium with magnesium supplementation or magnesium supplementation alone did not affect the serum ALP and CTx levels in ovariectomized rats. In summary, consumption of seaweed calcium extract or inorganic calcium carbonate with magnesium oxide demonstrated the same degree of intestinal calcium absorption, but only the consumption of seaweed calcium extract resulted in increased femoral bone mineral density and strength in ovariectomized rats. Our results suggest that seaweed calcium extract is an effective calcium and magnesium source for improving bone health compared to synthetic calcium and magnesium supplementation.

Keyword Seaweed calcium · Magnesium · Bone status · Calcium balance · Ovariectomy

Introduction

For postmenopausal women, osteoporosis is one of the most critical age-related disorders. Menopause results in elevated bone turnover through an imbalance between bone formation and bone resorption [1]. Also, estrogen deficiency decreases intestinal calcium (Ca) absorption and body Ca availability for bone formation [2]. Therefore, the intake of the proper amount of Ca and the type of Ca supplementation that increases bio-availability is necessary for the prevention of osteoporosis. The Ministry of Health and Welfare of Korea recommends that female Koreans over 19 years old have a Ca intake of 650 ~ 700 mg/day [3]. However, the average Ca intake of Koreans is less than this recommendation [4]. Especially, in the age of postmenopause, the percentage of subjects consuming less than the estimated average requirement of Ca for Koreans was 74.9% for women aged 50–64 years and 86.8% for women aged over 65 years. Also, a multi-ethnic study reported that only 16.4% of four ethnicities of middle-aged and older Americans met the adequate intake (AI) for Ca [5]. Hence, it is important to study and identify a good source of Ca to increase Ca availability in the older population. Several studies have attempted to determine an excellent Ca supplement source for bone metabolism [6–9]. Kenny and colleagues [8] compared the efficacy of between calcium citrate and calcium carbonate for Ca supplementation in postmenopausal women and found that the decrease of the bone resorption marker in calcium citrate consumption was significantly higher than in calcium carbonate consumption. Also, Ca supplementation through algae extract compared to calcium citrate or calcium carbonate increases cell proliferation, alkaline phosphatase (ALP) activity, and Ca deposition in human osteoblastic cells [9].

Commercially available seaweed Ca extract is a widely used food supplement in Asian countries due to their high Ca but low phosphorus content which helps the efficiency of Ca absorption [10, 11]. A randomized study looking at seaweed Ca and bone health was conducted by Fujita and colleagues [10]. After they supplied for 30 months heated oyster shell-seaweed Ca to an elderly group whose average age was 80, the ratio of lumbar spine bone mineral density to the basal pretest value of the subject group was significantly higher than that of the placebo group. However, there was no difference in the ratio of lumbar bone mineral density (BMD) between the placebo control group and the positive control group, which were supplied inorganic Ca through $CaCO_3$. The study reported that heated oyster shell-seaweed Ca had a significant effect on increasing bone density [10].

Relative to Ca content in terms of grams in seaweed Ca extract, seaweed Ca extract contain significant amounts of Mg. Mg is the second most abundant intracellular cation in vertebrates [12], and a Mg-deficient diet has been implicated as a risk factor for osteoporosis [13]. Some researchers reported that Mg supplementation significantly

increased bone density or delayed bone loss in postmenopausal osteoporosis [14–16]. Mg supplementation also promoted bone formation and prevented bone loss in OVX rats [17]. Moreover, in our previous research, Ca and Mg supplementation improved the serum OPG and RANKL ratio in Ca-deficient OVX rats [18]. However, it has not been clearly established whether Mg supplementation by different sources (food extract vs. inorganic forms) have the same effect on Ca and bone metabolism. Therefore, the purpose of this study was to evaluate the effects of seaweed Ca extract which also contain high Mg, on Ca balance and bone metabolism (e.g., bone mineral density, bone mineral content, bone strength, and bone metabolism markers) by comparing them with inorganic Mg and Ca intake in OVX rats.

Materials and Methods

Animals and Study Protocols

Forty 6-week-old Sprague–Dawley female rats were purchased from Orient Inc. (Gyeonggi-do, Korea). The animals were housed in a room maintained at 22±3°C on 12-h light/12-h dark cycles. After 7 days of adaptation period, 30 of the rats were ovariectomized and 10 were subjected to sham operations (Sham). Then, the OVX rats were separated into three experimental groups. The experimental group design is shown in Table 1 ((1) Sham (0.5% Ca, 0.05% Mg diet), (2) OVX (0.5% Ca, 0.05% Mg diet), (3) OVX-Mg (0.5% Ca, 0.1% Mg diet), (4) OVX-SCa whose Ca content was adjusted and equivalent to Ca^{2+} content in Calcium carbonate in OVX-Mg group). Seaweed Ca was provided by a commercial product (Aquacal, CelticSea Minerals, Carrigaline, Ireland), which contains 33.0% Ca, 3.2% Mg, 0.3% S, 0.08% Fe, 0.06% P, 0.04% K, 0.007% Mn, and 0.0025% B along with other minor quantities of minerals. The basic diet was based on AIN-93, which already has 0.05% Mg. Therefore, the source of Ca and Mg for the Sham, OVX, and OVX-Mg group was $CaCO_3$ and MgO, while the source of Ca for the OVX-SCa group was seaweed Ca extract and the source of Mg was MgO (50%) and seaweed Ca extract (50%). Deionized water was used for drinking. Feed cups and drinking bottles were soaked overnight in conc. HNO_3 and 0.4% EDTA solution prior to each use to remove trace metal contamination.

The daily food intake in each group was measured, and the rats were weighed on a weekly basis. The experimental diet was maintained for 12 weeks. For the last 2 days before the end of the experiment, the rats were placed in metabolic cages, and we collected feces and urine from all the rats for 24 h. All urine samples were centrifuged at 2,500 rpm for 15 min at room temperature to eliminate contaminating sediments, and supernatants were stored at −20°C until they were measured. All experimental procedures followed the guiding principles in *Guide for the Care and Use of Laboratory Animals* [19] and were

Table 1 Experimental design

Group	OVX	Feed composition
Sham	–	0.5% Ca, 0.05% Mg
OVX	+	0.5% Ca, 0.05% Mg
OVX-Mg	+	0.5% Ca, 0.1% Mg
OVX-SCa	+	0.5% Ca, 0.1% Mg

approved by the Institutional Animal Care and Use Committee of Kangwon National University.

Bone and Blood Sampling

At the termination of the experiment, animals were anesthetized with ether. Blood samples from anesthetized rats were collected via the abdominal aorta and centrifuged at 4,000 rpm for 20 min. Blood samples were store at −80°C until the time of assay. The lumbar vertebrae, right femur, and tibia of each animal were dissected and stored at −70°C until examination.

Serum Analysis

Serum ALP was measured with a commercial radioimmunoassay and gamma counter (Boëhringer Mannheim, Mannheim, Germany). And the serum levels of C-telopeptide crosslinks of type I collagen (CTx) were measured with an ELISA kit (Nordic Bioscience Diagnostics, Copenhagen, Denmark) by an ELISA reader (ELx808, BioTek Instruments Inc., Winooski, VT, USA). The intra- and inter-assay coefficients of variation (CV) were less than 10% for CTx according to the manufacturer, as well as in our lab. Serum calcium levels were determined by auto-analyzer (Hitachi 747, Tokyo, Japan) using a commercial kit (Daiichi pure chemicals, Tokyo, Japan).

Calcium and Magnesium Balance Studies

Feces and urine were hydrolyzed with nitric acid by a microwave digestion system (Ethos touch control, Milestone Inc., Italy). The amounts of calcium and magnesium in the feces and urine were determined by an inductively coupled plasma spectrometer (Ultra mass-700, Varian, Australia). The absorption and retention of calcium and magnesium were determined by the following equations:

$$\text{Retention}(\text{mg/d}) = \text{intake} - (\text{fecal excretion} + \text{urinary excretion})$$
$$\text{Retention ratio}(\%) = (\text{retention/intake}) \times 100$$
$$\text{Apparent digestibility}(\%) = \{(\text{intake} - \text{fecal excretion})/\text{intake}\} \times 100$$

Measurement of Bone Status

The rats' lumbar vertebrae, right femur, and tibia were dissected, eliminating the soft tissue [20]. BMD, bone mineral content (BMC), and bone area for the lumbar vertebra (L2–L4), right femur, and tibia were measured by dual-energy X-ray absorptiometry using a PIXImus2 (Lunar, Madison, WI, USA) adapted for measuring small animals. Our percent coefficient of variation (%CV) for this measurement, determined from three measurements made in 15 rat lumbar spine, femur, and tibia at intervals of 2 to 3 days, were 0.10%, 0.05%, and 0.10%, respectively. The instrument was calibrated daily. The biomechanical property (bone strength) was assessed by a three-point bending test of the femoral bone and tibia (the center of the femur and tibia). A rheometer (Universal testing machine, CR-100D, Sun Scientific Co., Tokyo, Japan) with a spherical plunger (3 mm in diameter) was applied to analyze bone strength. The crosshead loaded at a speed of 20 mm/min until bones were fractured. Data were collected at the maximum load (kgf).

Statistical Analyses

Results are shown as means±standard deviation. Statistical significance was determined with one-way analysis of variance (ANOVA) using the SAS^{TM} (version 9.1; SAS Institute Inc., Cary, NC, USA). When the ANOVA indicated significant difference among the means, the differences were further evaluated using Duncan's multiple range tests. The difference was considered significant when $p<0.05$. Student's t test was used to determine the individual group difference at $\alpha=0.05$.

Results

Food Consumption, Weight Gain, and Food Efficiency

The initial body weight at baseline among the four groups was not significantly different (Table 2). However, OVX rats consumed significantly lower feeds ($p<0.001$), but had greater weight gain ($p<0.05$) than the sham-operated group. The feed efficiency ratio of OVX rats was significantly higher than that of the sham-operated group ($p<0.01$). Mg supplementation by MgO or seaweed Ca had no effect on body weight gain and feed efficiency ratio.

Ca and Mg Balance

The results of Ca and Mg balance are shown in Table 3. In the Ca balance study, Ca intake ($p<0.001$), and fecal ($p<0.05$) and urinary ($p<0.01$) Ca excretion in the sham group were higher than in all OVX groups. Among the OVX rats, the OVX-Mg and OVX-SCa groups tended to be high in Ca retention and Ca absorption compared to the OVX group, but no statistical significance was achieved. In the Mg balance study, fecal Mg excretion was not different among the four groups, while urinary Mg excretion of the OVX-Mg and OVX-SCa groups was higher than in the OVX group ($p<0.05$, $p<0.001$). Also, with regard to the Mg retention ratio, there was no difference in the Mg retention ratio between the OVX group and the OVX-Mg group, while the OVX-SCa group had a significantly higher Mg retention ratio than that of the OVX group ($p<0.05$).

Table 2 Feed intake, body weight gain, and feed efficiency ratio (FER) of the experimental rats

Variable	Sham	OVX	OVX-Mg	OVX-Seaweed Ca	Significance
Initial weight(g)	231.18±13.85	235.79±12.51	230.75±21.92	240.11±16.29	NS
Final weight(g)	332.36±29.50b	387.41±50.07a**	377.92±59.39a*	383.57±34.30a**	$p<0.05$
Body weight gain(g/wk)	7.68±1.81b	12.08±3.50a**	11.84±4.42a**	11.85±2.89a**	$p<0.05$
Feed intake(g/day)	20.12±0.38a	18.13±0.24b***	18.15±0.23b***	17.95±0.35b***	$p<0.001$
FER(%)	0.05±0.01b	0.10±0.03a**	0.09±0.03a**	0.09±0.02a***	$p<0.01$

Data are presented as mean±SD ($n=10$). Means in a row with unlike letters differ significantly (Duncan's multiple range test)

NS not significant

*$p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to Sham group

Table 3 Ca and Mg balance of the experimental rats

Variable	Sham	OVX	OVX-Mg	OVX-Seaweed Ca	Significance
Ca balance					
Intake (mg/day)	100.62±1.90a	90.62±1.20b***	90.73±1.14b***	89.75±1.73b***	$p<0.001$
Feces Ca excretion (mg/day)	38.12±12.84a	33.83±15.40ab	23.88±14.25b*	22.49±9.44b*	$p<0.05$
Urine Ca excretion (mg/day)	0.77±0.15a	0.41±0.32b*	0.44±0.22b**	0.58±0.20ab*	$p<0.01$
Ca retention (mg/day)	61.74±14.49	56.39±14.65	66.40±13.92	67.03±10.36	NS
Ca retention ratio (%)	61.16±13.32	62.32±16.49	73.25±15.62	74.3±10.58*	NS
Apparent Ca digestibility (%)	61.92±13.27	62.77±16.67	73.73±15.62	74.93±10.73*	NS
Mg balance					
Intake (mg/day)	10.06±0.19b	9.06±0.12c***	18.15±0.23a***,*****	17.95±0.35a***,*****	$p<0.001$
Feces Mg excretion (mg/day)	2.79±1.07	2.40±0.99	3.37±2.06	2.93±1.13	NS
Urine Mg excretion (mg/day)	0.92±0.20bc	0.76±0.49c	1.21±0.22b**,****	1.56±0.28a***,******,******	$p<0.001$
Mg retention (mg/day)	6.35±1.32b	5.91±0.77b	13.57±2.12a***,*****	13.61±1.39a***,*****	$p<0.001$
Mg retention ratio (%)	62.90±11.91b	65.24±9.09ab	74.81±11.85a*	75.54±6.87a*,****	$p<0.05$
Apparent Mg digestibility (%)	72.11±11.05	73.58±10.68	81.45±11.28	75.53±6.85	NS

Data are presented as mean±SD ($n=10$). Means in a row with unlike letters differ significantly (Duncan's multiple range test)

NS not significant

*$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to Sham group; ****$p<0.05$, *****$p<0.001$ compared to OVX group; ******$p<0.01$ compared to OVX-Mg group

Bone Status

The BMC and bone area of the lumbar vertebrae and tibia were not significantly different among the four experimental groups (Table 4). However, the BMD of the lumbar vertebrae, femur, and tibia were significantly lower in the OVX rats compared to the sham group. The femur BMC and bone area of the OVX group and the OVX-Mg group were significantly lower than those of the sham group. However, the OVX-SCa group was not significantly different from the sham group. Also, femur bone strength was significantly higher in the OVX-SCa group compared to the OVX ($p<0.05$) and OVX-Mg groups ($p<0.05$).

Bone Metabolism Indicators

Bone metabolism indicators are shown in Table 5. The levels of serum ALP of the OVX rats were significantly higher than those of the sham-operated group ($p<0.05$), but there was no significant difference among the ovariectomized groups. Also, there was no significant difference in the serum levels of C-telopeptide crosslinks of type I collagen (CTx) among the four groups. The serum Ca levels of the OVX-SCa group were significantly higher than those of the OVX and OVX-Mg groups ($p<0.01$, $p<0.001$), while they were not significantly different from those of the sham group.

Discussion

Although seaweed Ca extract contains significant amounts of Mg in addition to high amounts of Ca, a main ingredient, several previous studies [9, 10] which report positive effects of seaweed Ca extract on bone metabolism may underemphasize the role of Mg in seaweed Ca supplementation. This study addressed the presence of Mg in seaweed Ca extract and investigated the role of the Mg in seaweed Ca extract on bone metabolism. Our results showed that supplementing the diet of the OVX rats with Mg in the form of both seaweed Ca extract and inorganic minerals (MgO) significantly increased Mg retention without changing Ca absorption. A common transport mechanism of Ca and Mg was identified, and these two divalent cations interacted in the process of intestinal absorption, so Mg inhibited intestinal Ca absorption, while Ca interfered with intestinal Mg absorption [21, 22]. According to Toba and colleagues' research, supplying high amounts of Mg supplementation (0.15% of diet), three times over the adequate amounts (0.05% of diet), decreased calcium retention, because magnesium promoted the secretion of endogenous calcium into the intestine or inhibited intestinal calcium absorption [17]. Therefore, maintaining adequate body Ca retention is necessary before considering Mg supplementation for promoting bone health. In this study, Ca absorption in the OVX-Mg and OVX-SCa groups which had 0.1% Mg intake in their diet was not significantly different compared to the OVX group (0.05% Mg diet). This result suggests that two times of Mg supplementation (0.1% Mg) over the adequate amount (0.05% Mg) may not affect intestinal Ca absorption when diet Ca status is adequate.

The mineralization of bone is associated with the volume of bone matrix, the microstructure, hardness, and bone stiffness, which are crucial factors in determining bone strength. Toba and colleagues reported that the breaking force of the femur was increased in the OVX rats fed a high-magnesium diet (0.15%), although the bone mineral density and bone mineral contents of the femur were not affected by Mg

Table 4 Bone status of the experimental rats

	Variable	Sham	OVX	OVX-Mg	OVX-Seaweed Ca	Significance
Spine	BMD(mg/cm^2)	192.95±13.14a	173.99±13.92b*	174.00±9.66b**	178.61±7.48b*	$p<0.01$
	BMC(mg)	548.75±52.02	529.89±73.92	519.63±49.79	531.11±80.98	NS
	Area(cm^2)	2.84±0.09	3.06±0.44	2.99±0.28	2.97±0.38	NS
Femur	BMD(mg/cm^2)	211.08±5.39a	191.60±14.12b**	187.87±8.43b***	197.01±6.55b***,******	$p<0.001$
	BMC(mg)	479.50±32.99a	425.86±43.59b*	418.56±35.80b**	474.75±30.61a****,*******	$p<0.01$
	Area(cm^2)	2.27±0.10b	2.22±0.14b	2.23±0.14b	2.41±0.09a**,*****,*******	$p<0.05$
	Maximum load (kg)	11.71±0.89	11.49±0.59	11.69±0.35	12.09±0.44****,******	NS
Tibia	BMD(mg/cm^2)	173.14±9.26a	164.40±6.49b*	161.71±9.41b*	167.99±7.64ab	$p<0.05$
	BMC(mg)	364.60±29.25	348.57±17.43	346.22±25.82	362.10±20.24	NS
	Area(cm^2)	2.10±0.11b	2.12±0.06ab	2.14±0.10a	2.16±0.10a	NS
	Maximum load (kg)	10.50±0.99	10.62±0.29	10.29±0.67	10.72±0.87	NS

Data are presented as mean±SD (n=10). Means in a row with unlike letters differ significantly (Duncan's multiple range test)

NS not significant

*$p<0.05$, **$p<0.01$, or *** $p<0.001$ compared to Sham group; ****$p<0.05$, *****$p<0.01$ compared to OVX group; ******$p<0.05$, *******$p<0.01$ compared to OVX-Mg group

Table 5 Serum bone metabolism indicators of the experimental rats

Variable	Sham	OVX	OVX-Mg	OVX-Seaweed Ca	Significance
ALP (U/L)	79.50±36.91b	136.17±31.78a*	117.25±32.14a*	121.11±26.54a*	$p<0.05$
CTx (ng/ml)	26.69±6.50	22.19±9.57	17.17±10.98	30.85±17.23	NS
Ca (mg/dl)	10.88±0.46a	10.06±0.50b**	9.94±0.15b***	10.79±0.35a****,*****	$p<0.001$

Data are presented as mean±SD ($n=10$). Means in a row with unlike letters differ significantly (Duncan's multiple range test)

NS not significant

*$p<0.05$, **$p<0.01$, *** $p<0.001$ compared to Sham group; **** $p<0.01$ compared to OVX group; *****$p<0.001$ compared to OVX-Mg group

supplementation [17]. In this study, the OVX group did not show any significant changes in bone strength when Mg (0.1% of diet) was supplied only from MgO. However, when the same amount (0.1%) of Mg was provided in the form of seaweed Ca extract, the OVX rats showed improved femur strength indicating that supplying Mg as a food extract has pronounced effects on bone mineralization compared to supplying Mg in an inorganic form.

In 1994, Strause and colleagues examined the effects of Ca supplementation alone, microminerals only (copper, manganese, and zinc), or both Ca and micromineral supplementation on bone status in postmenopausal women. They observed that lumbar bone loss was prevented only when Ca was combined with micromineral supplementation [23]. In addition to high amounts of Ca as a major ingredient and considerable amounts of Mg, seaweed Ca extract also contained other microminerals such as manganese (0.007%) and boron (0.0025%). Manganese (Mn) is an essential mineral in activating glycosyltransferase, the enzyme synthesizing glycosaminoglycan chain of proteoglycan, and Mn deficiency induces a delay in long bone development [24]. In our previous study, Mn supplementation in the OVX rats increased serum osteocalcin, a sensitive bone formation marker [25]. Boron is also known to enhance bone mineral balance, although the mechanism of action is uncertain. A study in the OVX rats showed that a combination of boron and estrogen improved the intestinal absorption of calcium, phosphorus, and magnesium, and supplementation of boron increased bone mass in spongy bones [26, 27]. Nielsen and colleagues [28] reported that boron supplementation in postmenopausal women after a boron-deficient diet produced positive results in bone metabolism and in maintaining calcium homeostasis. Therefore, additional investigation is warranted if a certain combination of other microminerals residing in seaweed Ca extract contributes to improved bone metabolism.

In this study, we could observe significantly improved bone mineralization and strength in femoral bone by use of the seaweed Ca extract diet. Also the supplementation of Mg in the form of seaweed Ca extract showed more pronounced effects on bone metabolism compared to the supplementation of inorganic Mg (MgO) and did not affect calcium balance. However, a caution is necessary before we propose a direct relationship between Mg in seaweed Ca extract and bone strength since this seaweed Ca extract contains other microminerals which may play an additional role in bone metabolism. Further investigation is required to understand how equal amounts of Mg, provided in different forms (a food extract or inorganic form) could affect bone metabolism differently and how these in vivo findings might be developed for clinical applications.

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