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# The Effect of Boron Supplementation on Bone Strength in Ovariectomized Rats Fed with Diets Containing Different Calcium Levels

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**Abstract** The effect of calcium and boron supplementation on bone strength was determined in growing and ovariectomized (OVX) Sparague-Dawley rats. Rats were divided into 9 groups and fed diet with different intake levels of calcium and boron for 4 weeks. About fifty percentages of rats in each group were OVX and the others were sham-operated. The rats were fed same diets after operation for 8 weeks. The feed intake, body weight gain, and FER were significantly higher in OVX rats than those in sham-operated ones. Serum osteocalcin, bone formation biomarker, was significantly increased with increment in calcium and boron intakes. Serum estradiol was lower in OVX rats than in sham-operated ones. Bone mineral density of femur was significantly lower in OVX rats than in other group. The breaking forces of bones were not significantly different among the groups. The urinary excretion of deoxypyridinoline, osteolytic marker was significantly increased with increment in calcium intake and ovariectomy. The urinary calcium excretion was significantly increased with increment in calcium intake, but decreased with increment in boron intake. According to theses results, the boron supplementation resulted in higher serum osteocalcin and lower urinary calcium excretion. Therefore, it could be suggested that the boron supplementation may be complementary and useful to calcium nutrition for bone health.

**Keywords:** boron, calcium, bone strength, ovariectomized rat

## Introduction

Incidence of osteoporosis is increasing with increment in elderly population in Korea. And thus, developments of strategies to prevent this disease and maintain bone health are on the rising trend. For instance, the research for isoflavone as phytoestrogen was raised as a goal for preventing osteoporosis (1). Boron is an essential ultratrace element in plants (2). There are circumstantial evidences to prove that boron may also affect bone mineral homeostasis in various animal models, including humans. Hunt and Nielsen (3) reported that boron deprivation depressed growth and elevated plasma alkaline phosphatase activity in chicks fed with inadequate levels of cholecalciferol. Working with boron as a micronutrient, Nielsen *et al.* (4) found that adding boron to boron-deficient diets had salutary effects on indices of bone metabolism and mineral homeostasis in humans. These and other data have contributed to the preliminary conclusion that boron is 'probably essential' for human nutrition (5).

Some of the reported observation could be due to changes in macromineral metabolism. Hegsted *et al.* (6) found that apparent absorption and balance of calcium, magnesium, and phosphorus were higher in boron-supplemented than in boron-deprived rats fed with cholecalciferol-deficient diet. Brown *et al.* (7) observed that supplemental boron increased the apparent absorption and retention of calcium in sheep. Nielsen *et al.* (8) found that boron repletion in 12 postmenopausal women, who were previously on a low-boron diet for 119 days, had markedly reduced levels of urinary excretion of calcium. Although the preceding studies indicate that boron affects calcium metabolism, only limited reports are available on the interaction between boron and calcium.

Past studies in postmenopausal women, who were previously on a low-boron diet, showed that dietary boron repletion increased serum 17β-estradiol ($E_2$) and testosterone levels (8). A similar increase in serum $E_2$ levels was seen in healthy males after 4 weeks of dietary boron supplementation (9). Because boron increases the serum $E_2$ level, it has been suggested that boron may reduce some of the adverse effects of $E_2$ deficiency associated with menopause and/or cessation of ovarian function (4, 10).

The purpose of the present work was to confirm whether boron supplementation affects the bone status of the rats fed on diets with different calcium levels. Therefore, we designed a study to determine the effects of boron supplementation on bone strength and bone metabolism indicators in OVX rats and sham-operated control rats consuming diets with different calcium and boron intakes. The experiments were part of an overall plan to obtain further evidence that boron might be involved in bone metabolism, thus indicating that boron is an essential nutrient for bone health.

## Materials and Methods

**Animals and diets** One hundred-and eighty 6-wk-old Sprague-Dawley female rats were purchased from Chungang Experimental Animal Inc. (Seoul, Korea). After

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7 days of acclimatization, the animals were systematically assigned to 9 weight-matched treatment groups (20 rats per group). The animals were housed in a room maintained at 24°C on 12 hr light/12 hr dark cycles and were fed diets, *ad libitum* containing different content of calcium (0.1, 0.5, 1.5%) and boron (0.5, 50, 100 ppm) for 4 weeks. The chow diet fed to the animals in this experiment was the AIN-93 diet (11) and its ingredients are as shown in Table 1. At 12 weeks of age, the half of rats in each group were bilateral OVX and the others were sham operated under anesthesia with 15 mg of ketamine per kg of body weight (Ketalar, Yuhan Co., Korea). Deionized water was supplemented as drinking water. Feed cups and drinking bottles were soaked overnight in conc. $HNO_3$ prior to each use to remove trace metal contamination.

**Experimental protocol** The rats were divided into 18 groups (10 rats per group) as: (1) low calcium (0.1%) + adequate boron (0.5 ppm); (2) low calcium + high boron (50 ppm); (3) low calcium + very high boron (100 ppm); (4) adequate calcium (0.5%) + adequate boron; (5) adequate calcium + high boron; (6) adequate calcium + very high boron; (7) high calcium (1.5%) + adequate boron; (8) high calcium + high boron; (9) high calcium + very high boron. 9 groups were OVX rats and 9 groups were sham-operated animals. Treatment diet with adequate nutrition as recommended by the American Institute of Nutrition (11) except calcium and boron was available *ad libitum* to the rats. The daily food intake in each group was measured and the rats were weighed weekly. The experiment was continued for 8 weeks. For 3 days before the termination of the experiment, the rats were placed in metabolic cages, and 24 hr urine was collected. At the termination of the experiment, animals were anesthetized with ether and blood was collected. The scapulae, tibiae, and femurs of each animal were dissected and defleshed by sterile saline. The right bones were immediately soaked in 70% ethanol in a glass vial, kept in the dark at room temperature, and was used for histomorphometric measurements. The left bones were stored in a freezer at -70°C until examination. The animals were treated in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Table 1. Composition of diets**

| Ingredients[1)] | Low Ca | Adequate Ca | High Ca |
|---|---|---|---|
| | | g/kg | |
| Corn starch | 517.1504 | 507.2597 | 475.3273 |
| Casein | 200.0000 | 200.0000 | 200.0000 |
| Sucrose | 100.0000 | 100.0000 | 100.0000 |
| Soybean oil | 70.0000 | 70.0000 | 70.0000 |
| Cellulose | 50.0000 | 50.0000 | 50.0000 |
| Mineral mixture (Ca/P/B free)[1)] | 35.0000 | 35.0000 | 35.0000 |
| Vitamin mixture[2)] | 10.0000 | 10.0000 | 10.0000 |
| *L*-Cystine | 3.0000 | 3.0000 | 3.0000 |
| Choline bitartrate | 2.5000 | 2.5000 | 2.5000 |
| *t*-butylhydroquinone | 0.0140 | 0.0140 | 0.0140 |
| Calcium phosphate | 3.4000 | 6.8770 | 33.3040 |
| Calcium carbonate | 0.0000 | 7.4200 | 12.9254 |
| Potassium phosphate | 2.7156 | 0.0000 | 0.0000 |
| Potassium citrate | 6.2200 | 7.9293 | 7.9293 |
| Boron, Adequate | 0.0005 | 0.0005 | 0.0005 |
| High | 0.05 | 0.05 | 0.05 |
| Very high | 0.1 | 0.1 | 0.1 |

[1)]Low Ca: 0.1% Ca, 0.3% P; Adequate Ca: 0.5% Ca, 0.3% P; High Ca: 1.5% Ca, 0.9% P.
[2)]AIN-93 mineral mixture (Ca/P/B free).
[3)]AIN-93 vitamin mixture.

**Analytical procedures** Serum concentrations of total protein and albumin were measured spectrophotometrically by using commercial kits in a dry-chemistry analyzer (Fuji Dri-Chem 3000, Fujifilm, Japan). Serum alkanine phosphatase (ALP), osteocalcin, estradiol, parathyroid hormone-intact (PTH-intact), and sex hormone binding globulin (SHBG) were measured with commercial radioimmunoassay at gamma counter (Cobra II, Packard, USA). For histological observation of femur, the specimens were unfrozen before biomechanical tests and kept moist during all handling and test procedures. Microphotographs of a cross-section of the femoral neck were determined by scanning electron microscope (PSEM-75 MICGOPSM, RJ Lee Instruments Ltd., USA). Bone mineral density (mg/cm$^3$) was calculated by dividing the bone wet weight from bone volume. Bone volume was measured by the principle of Archimedes (12). The 'three-point bending test' at fracture was used to determine the breaking force of bone. The force applied to the midpoint of the bone until breaking, was determined with a universal testing machine (CR-100D, Sun Scientific Co., Japan). Urinary deoxypyridinoline (DPD) excretions were measured with commercial radioimmunoassay in an immulite analyzer (DPC, USA). Food and urine were hydrolyzed with nitric acid by microwave digestion system (Ethos touch control, Milestone Inc., Italy). The calcium and boron concentrations of food and urine were measured by inductively coupled plasma spectrometer (Atomscan advantage axial sequential plasma spectrometer, Thermo Jarrell Ash Co., USA).

**Statistical analysis** Results are shown as means ± standard deviation. Statistical significance was determined with three-way analysis of variance (ANOVA) using the SAS software. When the ANOVA indicated significant difference among the means, the differences were evaluated using Duncan's multiple range test. The difference was significant when $p<0.05$.

## Results and Discussion

**Feed consumption, weight gain, and feed efficiency** The initial body weight at baseline among the groups was not significantly different. However, OVX rats fed with high-calcium diet consumed significantly more feed and had greater weight gain when compared to sham-operated ones fed with low-calcium diet (Table 2). Feed efficiency of OVX rats was significantly higher than that of sham-operated animals. Dietary boron supplementation had no effects on body weight gain and feed efficiency. This finding is consistent with previous reports (13, 14), that OVX or calcium supplemented animals frequently gained more body weight as a result of overeating.

**Table 2. Feed intake, body weight gain, and feed efficiency of the experimental rats**

| Groups | | | Feed intake | Body weight gain | Feed efficiency |
|---|---|---|---|---|---|
| | Calcium | Boron | g/day | g/wk | g/g |
| Sham operation | 0.1% | 0.5 ppm | 12.19±0.18$^{ij}$[1)] | 3.80±1.46$^{d}$ | 0.04±0.02$^{b}$ |
| | 0.1% | 50 ppm | 12.34±0.17$^{j}$ | 5.06±2.41$^{d}$ | 0.06±0.02$^{b}$ |
| | 0.1% | 100 ppm | 11.22±1.05$^{k}$ | 4.09±2.00$^{d}$ | 0.05±0.02$^{b}$ |
| | 0.5% | 0.5 ppm | 12.66±0.08$^{ij}$ | 4.01±1.35$^{d}$ | 0.05±0.02$^{b}$ |
| | 0.5% | 50 ppm | 12.91±0.28$^{i}$ | 4.73±0.85$^{d}$ | 0.05±0.01$^{b}$ |
| | 0.5% | 100 ppm | 13.17±0.63$^{hi}$ | 5.19±1.45$^{d}$ | 0.06±0.02$^{b}$ |
| | 1.5% | 0.5 ppm | 13.64±0.23$^{gh}$ | 4.40±1.01$^{d}$ | 0.05±0.01$^{b}$ |
| | 1.5% | 50 ppm | 13.93±0.49$^{fg}$ | 4.34±1.11$^{d}$ | 0.04±0.01$^{b}$ |
| | 1.5% | 100 ppm | 14.11±0.15$^{fg}$ | 4.75±1.44$^{d}$ | 0.05±0.01$^{b}$ |
| Ovariectomy | 0.1% | 0.5 ppm | 14.35±0.65$^{ef}$ | 10.38±4.51$^{c}$ | 0.10±0.05$^{a}$ |
| | 0.1% | 50 ppm | 14.75±0.79$^{de}$ | 12.28±3.63$^{abc}$ | 0.12±0.04$^{a}$ |
| | 0.1% | 100 ppm | 14.67±0.43$^{de}$ | 11.45±4.46$^{bc}$ | 0.11±0.04$^{a}$ |
| | 0.5% | 0.5 ppm | 15.07±0.36$^{cd}$ | 12.95±2.56$^{abc}$ | 0.12±0.03$^{a}$ |
| | 0.5% | 50 ppm | 14.73±0.63$^{de}$ | 12.69±4.20$^{abc}$ | 0.12±0.04$^{a}$ |
| | 0.5% | 100 ppm | 15.35±0.86$^{c}$ | 12.39±2.44$^{abc}$ | 0.12±0.02$^{a}$ |
| | 1.5% | 0.5 ppm | 15.94±0.32$^{b}$ | 13.34±2.69$^{ab}$ | 0.12±0.02$^{a}$ |
| | 1.5% | 50 ppm | 16.51±0.50$^{a}$ | 14.61±2.37$^{a}$ | 0.13±0.01$^{a}$ |
| | 1.5% | 100 ppm | 16.47±0.71$^{a}$ | 13.97±2.17$^{ab}$ | 0.12±0.02$^{a}$ |
| Significance | | | p<0.001 | p<0.001 | p<0.001 |
| Calcium | | | p<0.001 | p<0.05 | N.S. |
| Boron | | | p<0.01 | N.S. | N.S. |
| Operation | | | p<0.001 | p<0.001 | p<0.001 |
| Calcium×Boron | | | p<0.001 | N.S. | N.S. |
| Calcium×Operation | | | p<0.001 | p<0.05 | N.S. |
| Boron×Operation | | | N.S.[2)] | N.S. | N.S. |
| Calcium×Boron×Operation | | | N.S. | N.S. | N.S. |

[1)]Values with different superscripts within a column are significantly different.
[2)]Not significant.

**Serum bone metabolism indicators** There were no significant difference in serum ALP and PTH levels among any of animals in the test groups (Table 3). However, serum osteocalcin, osteogenic marker, was significantly higher in high-calcium, boron supplementation, and OVX groups than those in other groups. Serum $E_2$ level of OVX rats was significantly lower than that of sham-operated ones, confirming the success of the surgery. One hundred ppm of boron supplementation significantly increased serum SHBG in low-calcium fed rats.

The mechanism whereby boron influences bone status has not been determined. However, some studies (14, 15) have shown that dietary boron supplementation increases serum $E_2$ and testosterone levels. Accordingly, it is possible that boron may modify bone status by increasing the synthesis mechanism of sex hormones. Unlike past studies, we observed that dietary boron supplementation does not affects serum level of $E_2$, but increases osteocalcin in low-calcium/sham-operated group and SHBG in low-calcium/OVX group to improve bone quality in OVX rats. Osteocalcin is the most abundant noncollagenous protein in bone, and serum concentrations of osteocalcin can provide a measure of osteoblast activity or bone remodeling (16). Therefore, the reported data suggests that boron supplementation may prevent bone loss through osteogenic effect in OVX rats.

**Bone measures** Figure 1 shows histological evidence on the difference in femur loss in OVX rats and sham-

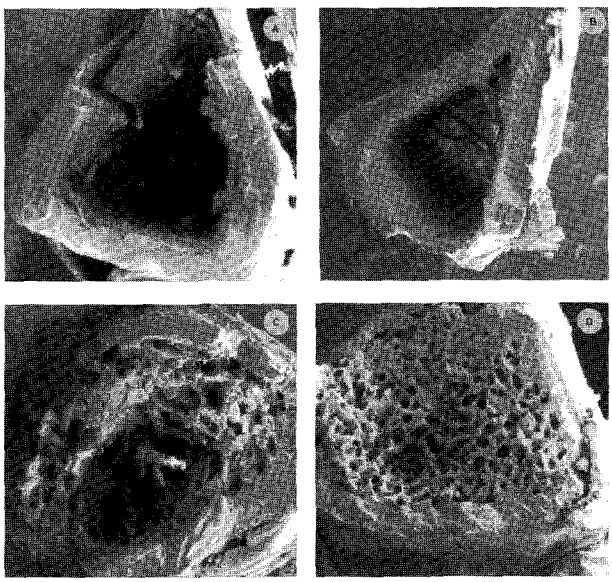

**Fig. 1. Histological observation of the tibia and femur in ovariectomized rat by scanning electron microscope.** A: Tibia ovariectomized. B: Tibia sham-operated. C: Femur ovariectomized. D: Femur sham-operated.

**Table 3. Biochemical values of serum indicators of the bone metabolism in the experimental rats**

| Groups | | | Total protein | Albumin | ALP | Osteocalcin | Estradiol | PTH | SHBG |
|---|---|---|---|---|---|---|---|---|---|
| | Calcium | Boron | g/dL | g/dL | U/L | ng/mL | pg/mL | pg/mL | nmol/dL |
| Sham operation | 0.1% | 0.5 ppm | 5.66±0.15$^{ab1)}$ | 3.98±0.04$^{ab}$ | 33.86±12.92 | 0.12±0.03$^{bcd}$ | 35.70±11.46$^{a}$ | 8.73±2.85 | 0.06±0.01$^{d}$ |
| | 0.1% | 50 ppm | 6.04±0.48$^{a}$ | 4.20±0.33$^{a}$ | 57.18±32.58 | 0.12±0.02$^{abcd}$ | 33.00±5.99$^{a}$ | 6.06±1.31 | 0.06±0.03$^{cd}$ |
| | 0.1% | 100 ppm | 4.38±0.39$^{cde}$ | 2.94±0.30$^{c}$ | 29.76±7.68 | 0.20±0.05$^{a}$ | 31.24±5.00$^{ab}$ | 8.91±1.92 | 0.11±0.03$^{ab}$ |
| | 0.5% | 0.5 ppm | 5.52±0.97$^{abc}$ | 3.78±0.73$^{abc}$ | 26.08±10.53 | 0.13±0.08$^{abcd}$ | 32.42±6.93$^{a}$ | 7.99±1.56 | 0.11±0.05$^{ab}$ |
| | 0.5% | 50 ppm | 5.00±0.38$^{abcde}$ | 3.56±0.30$^{abc}$ | 34.16±8.03 | 0.13±0.04$^{abcd}$ | 27.08±4.52$^{abc}$ | 8.01±2.15 | 0.09±0.01$^{abcd}$ |
| | 0.5% | 100 ppm | 4.94±0.84$^{abcde}$ | 3.68±0.50$^{abc}$ | 31.32±21.02 | 0.13±0.04$^{abcd}$ | 26.92±3.29$^{abc}$ | 5.55±1.27 | 0.09±0.02$^{abcd}$ |
| | 1.5% | 0.5 ppm | 4.26±0.96$^{de}$ | 3.20±0.81$^{bc}$ | 40.22±17.98 | 0.16±0.04$^{abc}$ | 33.40±8.29$^{a}$ | 8.42±3.15 | 0.07±0.01$^{cd}$ |
| | 1.5% | 50 ppm | 4.20±0.73$^{e}$ | 3.08±0.48$^{c}$ | 29.94±21.12 | 0.16±0.11$^{abc}$ | 33.48±13.22$^{a}$ | 8.23±2.54 | 0.07±0.01$^{cd}$ |
| | 1.5% | 100 ppm | 4.50±0.72$^{bcde}$ | 3.42±0.53$^{abc}$ | 41.22±23.37 | 0.16±0.03$^{abc}$ | 36.82±10.94$^{a}$ | 5.64±1.75 | 0.06±0.01$^{cd}$ |
| Ovariectomy | 0.1% | 0.5 ppm | 5.28±0.78$^{abcde}$ | 3.55±0.54$^{abc}$ | 37.85±12.32 | 0.14±0.05$^{abcd}$ | 22.52±3.93$^{bcd}$ | 9.20±3.53 | 0.07±0.00$^{cd}$ |
| | 0.1% | 50 ppm | 5.44±0.70$^{abcd}$ | 3.50±0.51$^{abc}$ | 34.18±19.01 | 0.10±0.04$^{cd}$ | 18.18±2.45$^{cd}$ | 8.91±1.76 | 0.06±0.01$^{cd}$ |
| | 0.1% | 100 ppm | 4.65±1.02$^{bcde}$ | 2.95±0.85$^{c}$ | 23.62±9.35 | 0.09±0.02$^{cd}$ | 21.64±10.13$^{cd}$ | 8.61±2.57 | 0.12±0.02$^{a}$ |
| | 0.5% | 0.5 ppm | 5.32±0.91$^{abcde}$ | 3.54±0.67$^{abc}$ | 35.08±30.79 | 0.08±0.06$^{d}$ | 21.36±4.23$^{cd}$ | 8.57±1.38 | 0.07±0.03$^{cd}$ |
| | 0.5% | 50 ppm | 4.55±0.60$^{bcde}$ | 2.92±0.40$^{c}$ | 30.92±14.04 | 0.10±0.04$^{cd}$ | 18.86±2.38$^{cd}$ | 7.40±2.24 | 0.10±0.03$^{abc}$ |
| | 0.5% | 100 ppm | 5.50±1.22$^{abc}$ | 3.73±0.78$^{abc}$ | 36.32±25.32 | 0.15±0.05$^{abcd}$ | 18.72±2.58$^{cd}$ | 8.89±3.35 | 0.09±0.03$^{abcd}$ |
| | 1.5% | 0.5 ppm | 4.98±0.86$^{abcde}$ | 3.52±0.59$^{abc}$ | 41.82±20.60 | 0.15±0.06$^{abcd}$ | 18.82±5.10$^{cd}$ | 5.64±1.39 | 0.08±0.03$^{bcd}$ |
| | 1.5% | 50 ppm | 5.12±1.13$^{abcde}$ | 3.53±0.75$^{abc}$ | 40.10±19.52 | 0.11±0.06$^{cd}$ | 19.70±4.36$^{cd}$ | 6.07±3.94 | 0.07±0.03$^{cd}$ |
| | 1.5% | 100 ppm | 4.92±1.04$^{abcde}$ | 3.47±0.71$^{abc}$ | 31.67±16.58 | 0.20±0.05$^{ab}$ | 15.70±2.17$^{d}$ | 8.25±2.59 | 0.07±0.01$^{cd}$ |
| Significance | | | p<0.05 | p<0.05 | N.S.$^{2)}$ | p<0.05 | p<0.001 | N.S. | p<0.001 |
| Calcium | | | p<0.05 | N.S. | N.S. | p<0.05 | N.S. | N.S. | p<0.01 |
| Boron | | | N.S. | N.S. | N.S. | p<0.05 | N.S. | N.S. | N.S. |
| Operation | | | N.S. | N.S. | N.S. | N.S. | p<0.001 | N.S. | N.S. |
| Calcium×Boron | | | p<0.05 | p<0.01 | N.S. | N.S. | N.S. | N.S. | p<0.001 |
| Calcium×Operation | | | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |
| Boron×Operation | | | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |
| Calcium×Boron×Operation | | | N.S. | N.S. | N.S. | p<0.05 | N.S. | N.S. | N.S. |

[1)] Values with different superscripts within a column are significantly different.
[2)] Not significant.

operated animals. It can be seen that femur loss in OVX rats was greater than sham-operated animals. These findings confirmed the success of the OVX. The OVX rat has been considered as a useful animal model for studying the effects of different osteoporosis treatment diets on the skeleton (17, 18). The data on bone status with boron supplementation are provided in Table 4 and 5. There was no significant difference in bone mineral density of scapular, tibia, and femur among boron treatment groups. Breaking forces of these bones also were not significantly different with boron supplementation in OVX and sham-operated rats fed diets with various calcium levels. However, breaking force of scapular increased with increment in calcium intake and OVX. This result may be explained by the possibility that OVX or calcium supplemented animals gained more body weight and scapular weight as a result of overeating.

Past studies in postmenopausal women (8, 10) and animals (19, 20) showed that dietary boron supplementation independently could promote positive bone mineral balance. These findings led to the speculation that dietary boron might also have a beneficial effect on bone quality and strength in $E_2$-deficient subjects. However, the present study showed that 100 ppm of dietary boron supplementation did not improve bone mineral density and bone strength in OVX rats. Sheng *et al.* (15) emphasized that a positive response of boron on bone status was seen only in those postmenopausal women who were deficient in boron. Chapin *et al.* (21) reported that supplementation of higher levels of boron will not bring about any further changes in bone strength because of saturation of a relatively small pool of boron binding sites. Therefore, to address these findings, additional studies are needed to investigate the effects of higher boron intake levels.

**Urinary DPD and calcium excretion** Table 6 shows that urinary DPD excretion, osteolytic marker was significantly diminished with increasing levels of dietary calcium and was higher in OVX rats when compared to sham-operated control rats. However, boron supplementation had no effect on urinary DPD excretion. Urinary calcium excretion was significantly increased with increasing levels of dietary calcium, but diminished with boron supplementation, particularly in high-calcium/OVX group.

The effect of boron supplementation on calcium metabolism most likely involved some biochemical or endocrine mechanism. It seems unlikely that urinary calcium excretion was reduced through chemical or complexing actions of boron. That is, it suggests that boron supplementation prevents urinary calcium loss and bone demineralization through endocrine mechanism of osteocalcin. However, this hypothesis must be confirmed by further experiments.

In conclusion, the boron supplementation resulted in higher serum osteocalcin in low-calcium group and lower

**Table 4. Bone mineral density of the experimental rats**

| Groups | | | Scapula | Tibia | Femur |
|---|---|---|---|---|---|
| | Calcium | Boron | mg/cm$^3$ | mg/cm$^3$ | mg/cm$^3$ |
| Sham operation | 0.1% | 0.5 ppm | 2.45±0.26 | 2.62±0.39 | 2.94±0.38$^{a1)}$ |
| | 0.1% | 50 ppm | 2.74±0.59 | 2.72±0.26 | 2.94±0.11$^{a}$ |
| | 0.1% | 100 ppm | 2.54±0.36 | 2.78±0.11 | 2.92±0.16$^{a}$ |
| | 0.5% | 0.5 ppm | 2.65±0.28 | 2.68±0.11 | 2.95±0.21$^{a}$ |
| | 0.5% | 50 ppm | 2.63±0.51 | 2.14±1.91 | 2.80±0.20$^{a}$ |
| | 0.5% | 100 ppm | 2.51±0.44 | 2.45±0.19 | 2.90±0.16$^{a}$ |
| | 1.5% | 0.5 ppm | 2.42±0.20 | 2.62±0.17 | 2.78±0.22$^{a}$ |
| | 1.5% | 50 ppm | 2.40±0.24 | 2.65±0.17 | 2.84±0.15$^{a}$ |
| | 1.5% | 100 ppm | 2.24±0.17 | 2.67±0.10 | 2.83±0.15$^{a}$ |
| Ovariectomy | 0.1% | 0.5 ppm | 2.67±0.41 | 2.67±0.25 | 2.69±0.26$^{ab}$ |
| | 0.1% | 50 ppm | 2.55±0.34 | 2.80±0.21 | 2.73±0.26$^{a}$ |
| | 0.1% | 100 ppm | 2.40±0.31 | 2.74±0.37 | 2.84±0.11$^{a}$ |
| | 0.5% | 0.5 ppm | 2.58±0.13 | 2.89±0.23 | 2.77±0.14$^{a}$ |
| | 0.5% | 50 ppm | 2.90±1.02 | 2.63±0.18 | 2.36±1.22$^{b}$ |
| | 0.5% | 100 ppm | 2.61±0.25 | 2.79±0.17 | 2.71±0.18$^{ab}$ |
| | 1.5% | 0.5 ppm | 2.48±0.13 | 1.63±3.09 | 2.69±0.09$^{ab}$ |
| | 1.5% | 50 ppm | 2.41±0.19 | 2.69±0.15 | 2.64±0.17$^{a}$ |
| | 1.5% | 100 ppm | 2.48±0.14 | 2.54±0.38 | 2.71±0.27$^{ab}$ |
| Significance | | | N.S.$^{2)}$ | N.S. | N.S. |
| Calcium | | | N.S. | N.S. | N.S. |
| Boron | | | N.S. | N.S. | N.S. |
| Operation | | | N.S. | N.S. | p<0.001 |
| Calcium×Boron | | | N.S. | N.S. | N.S. |
| Calcium×Operation | | | N.S. | N.S. | N.S. |
| Boron×Operation | | | N.S. | N.S. | N.S. |
| Calcium×Boron×Operation | | | N.S. | N.S. | N.S. |

$^{1)}$Values with different superscripts within a column are significantly different.
$^{2)}$Not significant.

**Table 5. Breaking force of bone in the experimental rats**

| Groups | | | Scapula | Tibia | Femur |
|---|---|---|---|---|---|
| | Calcium | Boron | kg | kg | kg |
| Sham operation | 0.1% | 0.5 ppm | 2.94±0.39$^{c1)}$ | 10.90±2.84 | 14.64±3.04 |
| | 0.1% | 50 ppm | 3.49±0.42$^{abc}$ | 10.19±3.61 | 12.71±2.13 |
| | 0.1% | 100 ppm | 3.03±0.50$^{bc}$ | 12.47±1.93 | 13.07±1.56 |
| | 0.5% | 0.5 ppm | 3.62±0.92$^{abc}$ | 12.62±2.13 | 14.85±1.74 |
| | 0.5% | 50 ppm | 3.60±0.54$^{abc}$ | 12.83±2.36 | 13.97±1.61 |
| | 0.5% | 100 ppm | 3.51±0.50$^{abc}$ | 11.08±2.74 | 12.63±1.44 |
| | 1.5% | 0.5 ppm | 3.60±0.68$^{abc}$ | 12.44±3.17 | 14.20±2.86 |
| | 1.5% | 50 ppm | 4.00±0.66$^{a}$ | 11.11±3.67 | 14.64±2.00 |
| | 1.5% | 100 ppm | 3.44±0.73$^{abc}$ | 10.62±3.28 | 13.43±1.01 |
| Ovariectomy | 0.1% | 0.5 ppm | 3.60±0.33$^{abc}$ | 13.64±3.35 | 14.57±1.80 |
| | 0.1% | 50 ppm | 3.48±0.63$^{abc}$ | 13.43±2.64 | 13.76±4.38 |
| | 0.1% | 100 ppm | 3.36±0.64$^{abc}$ | 12.09±2.44 | 14.20±2.33 |
| | 0.5% | 0.5 ppm | 3.95±1.24$^{a}$ | 13.21±2.43 | 15.67±1.46 |
| | 0.5% | 50 ppm | 3.80±0.63$^{abc}$ | 13.76±2.66 | 15.67±2.47 |
| | 0.5% | 100 ppm | 4.08±1.06$^{a}$ | 14.21±1.56 | 14.40±2.81 |
| | 1.5% | 0.5 ppm | 3.87±0.61$^{ab}$ | 12.51±2.66 | 13.74±1.93 |
| | 1.5% | 50 ppm | 3.99±0.48$^{a}$ | 13.42±2.70 | 14.76±2.29 |
| | 1.5% | 100 ppm | 3.92±0.96$^{a}$ | 12.68±2.67 | 13.19±3.96 |
| Significance | | | N.S.$^{2)}$ | N.S. | N.S. |
| Calcium | | | p<0.01 | N.S. | N.S. |
| Boron | | | N.S. | N.S. | N.S. |
| Operation | | | p<0.05 | N.S. | N.S. |
| Calcium×Boron | | | N.S. | N.S. | N.S. |
| Calcium×Operation | | | N.S. | N.S. | N.S. |
| Boron×Operation | | | N.S. | N.S. | N.S. |
| Calcium×Boron×Operation | | | N.S. | N.S. | N.S. |

$^{1)}$Values with different superscripts within a column are significantly different.
$^{2)}$Not significant.

**Table 6. Urinary deoxypyridinoline and mineral excretions in the experimental rats**

| Groups | | | Deoxypyridinoline | Ca | B |
|---|---|---|---|---|---|
| | Calcium | Boron | nmol/mmol Cr | mg/day | μg/day |
| Sham operation | 0.1% | 0.5 ppm | 114.05±21.81$^{ef1)}$ | 0.60±0.34$^{ab}$ | 0.13±0.14$^{b}$ |
| | 0.1% | 50 ppm | 104.43±28.61$^{f}$ | 0.33±0.18$^{b}$ | 0.34±0.39$^{ab}$ |
| | 0.1% | 100 ppm | 136.73±50.57$^{def}$ | 0.42±0.52$^{b}$ | 0.65±0.69$^{a}$ |
| | 0.5% | 0.5 ppm | 84.53±15.33$^{f}$ | 0.60±0.46$^{ab}$ | 0.39±0.44$^{ab}$ |
| | 0.5% | 50 ppm | 86.93±11.33$^{f}$ | 0.44±0.36$^{b}$ | 0.24±0.17$^{ab}$ |
| | 0.5% | 100 ppm | 99.88±6.32$^{f}$ | 0.49±0.47$^{b}$ | 0.35±0.36$^{ab}$ |
| | 1.5% | 0.5 ppm | 92.08±21.58$^{f}$ | 1.05±0.86$^{a}$ | 0.20±0.13$^{ab}$ |
| | 1.5% | 50 ppm | 93.18±20.49$^{f}$ | 0.40±0.17$^{b}$ | 0.41±0.52$^{ab}$ |
| | 1.5% | 100 ppm | 87.05±13.03$^{f}$ | 0.69±0.39$^{ab}$ | 0.35±0.33$^{ab}$ |
| Ovariectomy | 0.1% | 0.5 ppm | 198.13±33.74$^{bcd}$ | 0.34±0.09$^{b}$ | 0.11±0.12$^{b}$ |
| | 0.1% | 50 ppm | 261.23±86.71$^{a}$ | 0.40±0.20$^{b}$ | 0.23±0.17$^{ab}$ |
| | 0.1% | 100 ppm | 252.48±37.93$^{ab}$ | 0.35±0.08$^{b}$ | 0.48±0.41$^{ab}$ |
| | 0.5% | 0.5 ppm | 177.53±17.28$^{cd}$ | 0.52±0.23$^{b}$ | 0.48±0.79$^{ab}$ |
| | 0.5% | 50 ppm | 227.03±91.74$^{abc}$ | 0.60±0.48$^{ab}$ | 0.36±0.36$^{ab}$ |
| | 0.5% | 100 ppm | 220.63±31.12$^{abc}$ | 0.46±0.41$^{b}$ | 0.53±0.25$^{ab}$ |
| | 1.5% | 0.5 ppm | 167.15±22.74$^{cde}$ | 1.03±0.62$^{a}$ | 0.10±0.07$^{b}$ |
| | 1.5% | 50 ppm | 172.95±14.93$^{cde}$ | 0.78±0.51$^{ab}$ | 0.41±0.32$^{ab}$ |
| | 1.5% | 100 ppm | 187.80±53.73$^{cd}$ | 0.34±0.26$^{b}$ | 0.27±0.29$^{ab}$ |
| Significance | | | p<0.001 | p<0.01 | N.S.$^{2)}$ |
| Calcium | | | p<0.01 | p<0.01 | N.S. |
| Boron | | | N.S. | p<0.05 | N.S. |
| Operation | | | p<0.001 | N.S. | N.S. |
| Calcium×Boron | | | N.S. | N.S. | p<0.05 |
| Calcium×Operation | | | N.S. | N.S. | N.S. |
| Boron×Operation | | | N.S. | p<0.05 | N.S. |
| Calcium×Boron×Operation | | | N.S. | N.S. | N.S. |

[1)]Values with different superscripts within a column are significantly different.
[2)]Not significant.

urinary calcium excretion in high-calcium group. Therefore, it could be suggested that the boron supplementation may be complementary and also useful to calcium nutrition for bone health.

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