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Dietary Boron Supplementation Enhances the Effects of Estrogen on Bone Mineral Balance in Ovariectomized Rats

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ABSTRACT

The present study investigated whether boron would enhance the action of 17β-estradiol (E_2) or parathyroid hormone (PTH) on bone mineral balance in ovariectomized (OVX) rats. Forty-three days after OVX, the rats were treated for 5 wk with vehicle, boron (5 ppm as boric acid), E_2 (30 μg/kg/d, sc), PTH (60 μg/kg/d, sc), or a combination of boron and E_2 or PTH. Bone mineral balance was assessed by measuring apparent absorption, excretion, and retention of calcium (Ca), phosphorus (P), and magnesium (Mg). Serum Ca, P, Mg, and osteocalcin were also measured in this experiment. Boron alone had no effects on food consumption, weight gain, bone mineral balance, and serum levels of Ca, P, Mg, and osteocalcin. E_2 alone increased serum P and Mg and decreased serum osteocalcin, but it had no effect on bone mineral balance. The combination of boron and E_2 markedly improved apparent absorption of Ca, P, and Mg. In addition, the combination treatment increased the apparent retention of Ca and Mg (but not P) and also increased serum Ca and Mg but not

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serum P. On the other hand, boron cotreatment did not prevent the E_2-induced reduction in serum osteocalcin in OVX rats. PTH alone significantly increased serum Ca, P, Mg, and osteocalcin concentrations, although it had no effect on bone mineral balance. Contrary to the boron–E_2 combination treatment, the combination of boron and PTH did not enhance bone mineral balance. However, inasmuch as boron–PTH cotreatment did not enhance the stimulatory action of PTH on serum Ca, P, and osteocalcin, boron completely abolished the stimulatory effect of PTH on serum Mg. In conclusion, we have demonstrated for the first time that although boron by itself has no effect on bone mineral homeostasis, it appears to have synergistic enhancing effects on the action of E_2 on Ca and Mg homeostasis in OVX rats.

Index Entries: Boron; 17β-estradiol; parathyroid hormone; calcium; phosphorus; magnesium; ovariectomy; osteocalcin (rat).

INTRODUCTION

Boron is an essential ultratrace element in plants *(1).* There is circumstantial evidence that boron may also affect bone mineral homeostasis in various animal models, including humans. Accordingly, it has been reported that boron, when added in drinking water, corrected the deficits in calcium (Ca) and phosphorus (P) balance associated with fluoride intoxication in the rabbit *(2).* Dietary supplementation of boron in normal adult sheep for 11 d significantly increased Ca balance (without an effect on P balance), primarily through an increase in Ca absorption *(3).* In addition, dietary boron supplementation in vitamin-D-deficient rats for 12 wk increased not only apparent absorption of Ca but also apparent absorption of P, leading to an increase in Ca and P balance *(4).* However, despite the increased apparent absorption of Ca and P (without corresponding increases in Ca and P excretion), boron supplementation in vitamin-D-deficient rats did not affect serum Ca, Mg, and P levels. Thus, it may indicate that boron might have enhanced bone deposition or tissue uptakes of Ca and P in vitamin-D-deficient animals *(4).* Boron repletion in 12 postmenopausal women, who were previously on a low-boron diet for 119 d, markedly reduced the urinary excretion of Ca and magnesium (Mg) *(5).* This reduction was more pronounced in subjects who were on a low-Mg diet than those on a normal Mg diet. Boron also significantly reduced urinary P excretion, but only in postmenopausal women with low-Mg intake *(5).* These findings suggest that dietary boron repletion could have a positive effect on Ca, P, and Mg balance in "boron-deficient" postmenopausal women. However, it appears that this occurs only when their dietary Mg intake is inadequate. Nonetheless, the current evidence suggests that, under certain conditions of stress (e.g., fluoride intoxication, vitamin D deficiency, or Mg deficiency), boron could positively affect bone mineral homeostasis by increasing Ca and P

absorption and/or reducing Ca and P excretion. Because a positive balance of bone minerals, especially Ca and P, is essential for bone health, the positive effect of boron supplementation on bone mineral balance may help to improve bone mass and strength.

The mechanism whereby boron influences bone mineral balance has not been determined. However, past studies by Nielsen et al. *(5)* indicate that dietary boron repletion in postmenopausal women, who were previously on a low-boron diet, increased their serum 17β-estradiol (E_2) and testosterone levels, particularly in those whose dietary Mg intake was low *(5).* Similar increase in serum E_2 levels was found in healthy males after 4 wk of dietary boron supplementation *(6).* Accordingly, it is possible that boron may modify bone mineral balance by increasing the synthesis and/or actions of sex hormones. Nevertheless, because boron treatment appears to increase serum E_2 level in postmenopausal women, it has been suggested that dietary boron supplementation may reduce some of the adverse effects of E_2 deficiency that is associated with menopause and cessation of ovarian function, such as bone mineral deficits and bone loss *(7).* On the other hand, inasmuch as the possibility that the effect of boron on bone mineral homeostasis is mediated through interaction between boron and sex hormones (particularly E_2) is attractive, supporting evidence for this hypothesis is currently lacking.

To address the hypothesis that boron might affect bone mineral homeostasis through interaction with E_2, the present study sought to evaluate whether dietary supplementation of boron alone or in combination with E_2 would affect bone mineral balance in ovariectomized (OVX) rats. Because the effect of E_2 on bone mineral homeostasis is mediated through suppression of bone resorption *(8),* we also included PTH, a potent bone-forming agent *(9),* in this study for comparison. In this study, bone mineral balance was monitored by measuring the apparent rate of absorption, excretion, and retention of Ca, P, and Mg. Bone turnover rate was assessed by measuring serum osteocalcin concentrations.

MATERIALS AND METHODS

Animals and Diets

A total of 84 12-wk-old Sprague–Dawley female rats were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). After 7 d of acclimation, the animals were systematically assigned to various weight-matched groups. Seventy-six rats were OVX with the dorsal approach, and the remaining eight rats were sham-operated (Sham group). One sham-operated animal died during the surgery, and four OVX rats exhibited normal serum E_2 concentrations and were excluded. The remaining rats were housed singly in stainless-steel rat cages at 22°C on 12-h light/12-hr dark cycles and were fed *ad libitum* the AIN-76 basal rodent diet (Table 1), containing 0.52% Ca, 0.4% P, 0.05% Mg, 0.00004% boron, and

Table 1
Composition of the AIN-76 Basal Rodent Diet

Ingredient	Amount (%)
Dextrose	54
Corn Starch	17
Casein, vitamin-free, 90%	14
Corn oil	5
Alphacel	5
AIN 76 minerals*	3.5
AIN vitamins#	1
DL-methionine	0.3
Choline bi-tartrate	0.2

*AIN-76 minerals (final concentration in the diet): calcium, 0.52%; phosphorus, 0.40%; sodium, 0.102%; potassium, 0.36%; magnesium, 0.05%; manganese, 54 mg/kg; iron, 35 mg/kg; copper, 6 mg/kg; zinc, 30 mg/kg; iodine, 0.2 mg/kg; selenium, 0.1 mg/kg; chromium, 2 mg/kg; chloride, 0.156%; and sulfate, 0.10%.

#AIN-76 vitamins (final concentrations in the diet): Thiamin mononitrate, 6 mg/kg; riboflavin, 6 mg/kg; pyridoxine hydrochloride, 7 mg/kg; nicotinic acid, 30 mg/kg; D-calcium pantothenate, 16 mg/kg; folic acid, 2 mg/kg; D-biotin, 0.2 mg/kg; cyanocobalamin (vitamin B_{12}), 10 µg/kg; retinyl palmitate (vitamin A), 4 IU/gm; tocopheryl acetate (vitamin E), 50 IU/kg; cholecalciferol (vitamin D_3), 1 mg/kg; menadione sodium bisulfite complex (vitamin K), 1.52 mg/kg.

1000 IU/kg vitamin D_3. Deionized water was available at all time. Food consumption and body weight were recorded every 2 d and once a week, respectively. Feed cups and drinking bottles were soaked overnight in 2*N* HCl prior to each use to remove trace metal contamination.

Experimental Protocol

Treatments were initiated on d 43 post-OVX. At the start of the experiment, a group of nine OVX rats was sacrificed to establish baseline values. The remaining OVX rats were assigned to six weight-matched treatment groups (9–10 rats per group): (1) vehicle control group (Veh); (2) boron group (B, 5 ppm); (3) E_2 group (E_2, 30 µg/kg/d); (4) PTH group (PTH, 60 µg/kg/d); (5) boron and E_2 group (B+E_2); (6) boron and PTH group (B+P). The sham-operated (Sham), Veh, PTH, and E_2 groups were maintained on the AIN-76 basal rodent diet and the boron groups (i.e., B, B+PTH, and B+E_2 groups) were fed the AIN basal diet supplemented

with 5 ppm boron in the form of boric acid (Sigma Chemicals, St. Louis, MO). The Sham, Veh, and boron groups were injected (sc) with the vehicle solvent, containing 5% ethanol in corn oil and 0.001*N* HCl. The PTH groups (i.e., PTH and B+PTH) received daily injection (sc) of rat PTH 1–34 fragment (Bachem California Inc., Torrance, CA) at a dose of 60 µg/kg. The E_2 groups (i.e., E_2 and B+E_2) received daily injections (sc) of 30 µg/kg E_2 (Sigma Chemicals, Inc.). Rat PTH was dissolved in acid saline (0.001*N* HCl) with 2% heat-inactivated rat serum, and E_2 was dissolved in 5% ethanol and 95% corn oil. The volume of injection was based on 1 mL/kg body weight and adjusted periodically for 50-g increments in body weight. The experiment was carried on for 5 wk.

One week prior to termination of the experiment, five rats from each treatment group were randomly selected and placed in metabolic cages. After 3 d of acclimation, 3-d urine and feces were collected into acid-washed plastic containers and stored at –20°C until analyses. Between 9 AM and 1 PM of the final day of the experiment, the animals were anesthetized with pentobarbital sodium (25–40 µg/kg body weight, ip). A blood sample was collected from each animal through cardiac puncture into ice-chilled tubes, and the animals were euthanized with carbon dioxide overdose. After blood clotting for 1 h at 4°C, serum samples were prepared by centrifugation at 4°C and were stored in aliquots at –80°C until analyses. This protocol was reviewed and approved by the Animal Use Committee of the Virginia Polytechnic Institute and State University.

Biochemical Analyses

Food and feces were individually dried at 105°C for 48 h, weighed, and hydrolyzed with nitric acid/perchloric acid (5 : 3 v/v). For measurements of Ca and Mg, the wet-ashed samples were diluted with 1% lanthanum oxide *(10),* and the Ca and Mg concentrations were measured by Atomic Absorption Spectroscopy (AAS; Atomic Absorption Spectrophotometer Model 2100; Perki-Elmer, Rockville, MD). P contents were determined by a colorimetric assay *(11)* using a microtiter plate reader (Ceres 900 HDI, Bio-Tek Instruments, Inc. [Winooski, VT]).

For the measurement of boron, food, and feces were individually dried at 105°C for 48 h, weighed, and wet-ashed with 16.1*N* nitric acid containing 30% hydrogen peroxide, according to the open-vessel wet-ash, low-temperature, Teflon-tube (WALTTT) procedure *(12).* The B content was determined by inductively coupled plasma spectroscopy (ICP; Plasma 400 ICP Emission Spectrometer; Perkin-Elmer, Rockville, MD).

Serum E_2 concentrations were measured with a commercial radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA) and serum osteocalcin was also determined with a commercial radioimmunoassay (Biomedical Technologies Inc., Stoughton, MA). Urine creatinine was determined colorimetrically by a modified Jaffe reaction *(13).*

Statistical Analyses

Results are shown as mean±SEM. The statistical significance was determined with two-way analysis of variance (ANOVA), followed by Tukey's Studentized Range (HSD) post hoc test. The Least Square Mean (LSMEANS) test was used for unequal sample sizes. Analyses were performed with a personal computer using SAS™ (SAS Institute Inc., Cary, NC). The difference is considered significant when $p < 0.05$.

RESULTS

Effects of OVX on Food Consumption, Weight Gain, and Food Efficiency in Adult Female Rats

Table 2 shows that serum E_2 level of OVX rats was significantly lower than that of sham-operated animals (16 ± 3 vs 30 ± 6 pg/mL, $p = 0.0001$), confirming the success of the surgery. The initial body weight at baseline between the Sham and OVX group was not significantly different (224 ± 4 vs 221 ± 2 g). However, OVX rats consumed significantly more food and had better feed utilization (or food efficiency) compared to sham-operated animals. Accordingly, OVX rats also had greater weight gain and, therefore, were heavier than sham-operated rats (e.g., 304 ± 5 g for OVX rats vs 253 ± 14 g for sham-operated rats, at 6 wks after the surgery). This finding is consistent with previous reports that OVX animals frequently gained more body weight as a result of overeating *(14).*

Effects of Boron Alone or in Combination with E_2 or PTH on Food Consumption, Weight Gain, and Food Efficiency in OVX Rats

The OVX rats were treated with boron alone or in combination with E_2 or PTH for 5 wk. E_2 treatment, as expected, significantly raised serum E_2 concentrations (57 ± 4 pg/mL for E_2-treated rats vs 16 ± 3 pg/mL for vehicle-treated rats, $p < 0.001$), which was even higher than that of the sham-operated rats (30 ± 6 pg/mL). Boron treatment slightly, but not significantly, increased serum E_2 (21 ± 3 pg/mL in the B group vs 12 ± 3 pg/mL in the Veh group, p = N.S.). Treatment with PTH alone also did not affect serum E_2 (13 ± 3 pg/mL for PTH-treated rats vs 12 ± 3 pg/mL for vehicle-treated rats). There was no significant difference in urinary creatinine excretion among any of the test groups (data not shown), suggesting that none of the treatments had an adverse effect on renal functions of OVX rats.

To assess the effect of each treatment on weight gain, each group of OVX rats was matched in initial body weight at the beginning of treatment (Table 3). The boron treatment did not significantly affect weight

Table 2
Baseline Values of Serum 17β-Estradiol Concentration, Food Intake, Weight Gain, and Food Efficiency in Sham-Operated and OVX Rats

	Sham-operated (n=7)	OVX rats (n=63)
Serum E_2 concentration (pg/ml)	30±6	16±3***
Initial body weight (g)	224±4	221±2
Food intake (g/d)[#]	14.9±0.9	17.8±0.2*
Weight gain (g/d)[#]	0.68±0.19	1.97±0.05*
Food efficiency (g/g)[#@]	0.044±0.010	0.108±0.002*

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared to sham-operated.
[#]Average from 1 wk after the surgery to 6 wk after the surgery.
[@]Determined by dividing the weight gain against the amount of food consumed.

Table 3
Effect of Boron, 17β-Estradiol, and PTH, Each Alone or in Combination on Body Weight, Food Consumption, and Food Efficiency in Sham-Operated and OVX Rats after 5 wk of Respective Treatment

	N	Initial Body Weight (g)	Final Body Weight (g)	Weight gain (g/day)	Food intake (g/day)	Food efficiency (g/g)
Sham	7	253±13*	269±13*	0.44±0.14*	14.9±0.7*	0.029±0.008
OVX+Veh	9	314±12	343±12	0.81±0.13	18.2±0.6	0.044±0.008
OVX+B	10	298±11	318±11	0.57±0.12	17.0±0.6	0.033±0.007
OVX+E_2	9	298±12	283±11*	-0.42±12*	15.5±0.6*	-0.028±0.007*
OVX+PTH	8	306±12	328±12	0.64±0.13	17.5±0.6	0.037±0.008
OVX+B+E_2	10	298±11	283±11*	-0.43±0.12*,[#]	15.1±0.6*,[#]	-0.029±0.007*,[#]
OVX+B+PTH	7	319±13	338±13	0.52±0.14	17.8±0.7	0.029±0.008

*$p < 0.05$ compared to the OVX+Veh group.
[#]$p < 0.05$ compared to the OVX+B group.
Note: Veh = Vehicle; B = 5 ppm boron; E_2 = 30 μg/kg/d 17β-estradiol; PTH = 60 μg/kg/d PTH.

gain, food intake, or food utilization efficiency in OVX rats. Similarly, the PTH treatment also had no effect on weight gain, food consumption or efficiency, regardless of whether or not they were cotreated with boron.

In contrast, OVX rats receiving E_2 (with or without boron) exhibited a significant loss in body weight ($p = 0.0007$ for each). This loss is probably the result of the reduction in food consumption and feed utilization ($p = 0.0016$ for each). The fact that the higher weight-gain rate in OVX rats was reversed by the E_2 treatment is further consistent with the conclusion that the greater weight gain in OVX rats was the result of E_2 deficiency.

Effects of OVX, E_2, or PTH Treatment on Boron Metabolism in Adult Female Rats

Table 4 shows that virtually all (>99.5%) of the consumed boron was absorbed in each group of rats. The OVX did not appear to have effects on apparent absorption, excretion, and retention of boron, as there was no significant differences in any of the test parameters between sham-operated and vehicle-treated OVX rats. As expected, OVX rats receiving dietary boron supplementation absorbed approx 15-fold more and excreted 15–20-fold more boron than those animals without the supplementation. The boron-treated OVX rats also showed a significant increase in percent boron retention (i.e., approx 80% vs approx 60%) compared to vehicle-treated OVX rats. Five weeks treatment of E_2 or PTH did not affect the absorption, retention, and excretion of boron in OVX rats. Therefore, it is concluded that OVX, E_2 treatment, or PTH treatment had no effect on boron metabolism in OVX rats.

Effects of Boron Alone or in Combination with E_2 or PTH on Ca, P, and Mg Balance in OVX Rats

To assess the effect of boron treatment alone or in combination with E_2 or PTH on bone mineral balance in OVX rats, we measured apparent absorption, retention, and urinary excretion of Ca, P, and Mg after 5 wk of each respective treatment (Table 5). There were no significant differences in percent apparent absorption and retention of Ca, P, or Mg nor were there significant differences in urinary P and Mg excretion between the OVX rats and the sham-operated rats. In contrast, urinary Ca excretion appeared to be lower in OVX rats than in sham-operated rats. Because of small sample size ($n = 5$), the reduction barely missed the significant level ($p = 0.054$). Thus, these findings indicate that, perhaps with the exception of urinary Ca excretion, the OVX had no significant effect on bone mineral balance.

Compared to vehicle-treated rats, the boron treatment alone had no significant effect on percent apparent absorption, percent retention, and excretion of Ca, P, and Mg. Similarly, the PTH treatment alone also did not lead to statistically significant increases in percent apparent absorption, percent retention, and excretion of Ca, P, or Mg. OVX rats that were treated

Table 4
Effect of Boron, 17β-Estradiol, and PTH, Each Alone or in Combination on Apparent Rate of Absorption, Retention, and Urinary Excretion of Boron in Sham-Operated (Sham) and OVX Rats After 5 wk of Treatment (n = 5 for Each Group)

	Absorption (μg/day)	Absorption (%)	Retention (μg/day)	Retention (%)	Excretion[#] (μg/mg Cr)
Sham	12.9±6.0	99.8±0.1	7.6±5.6	58.9±3.6	0.75±0.25
OVX+Veh	13.9±6.0	99.5±0.1	8.9±5.6	64.9±3.6	0.77±0.25
OVX+B	214.8±6.0*	99.9±0.1*	175.8±5.6*	81.6±3.6*	5.30±0.25*
OVX+E_2	12.6±6.7	99.7±0.1	7.7±6.3	61.6±4.0	0.72±0.25
OVX+PTH	13.6±6.7	99.5±0.1	8.3±6.3	60.6±4.0	0.72±0.25
OVX+B+E_2	183.8±6.0*,@,&	99.9±0.1*,&	140.0±5.6*,@,&	76.0±3.6*,&	6.52±0.25*,@,&
OVX+B+PTH	205.7±6.0*,+	99.9±0.1*,+	159.7±5.6*,+	77.7±3.6*,+	6.06±0.25*,+

[#]Rate of urinary excretion of boron was normalized by urinary creatinine excretion.
*$p < 0.05$ compared to the OVX + Veh group.
@$p < 0.05$ compared to the OVX + B group.
&$p < 0.05$ compared to the OVX + E_2 group.
+$p < 0.05$ compared to the OVX + PTH group.

with boron and PTH together exhibited a highly significant ($p < 0.032$) increase in both percent apparent Ca absorption and retention compared to vehicle-treated rats. Thus, these findings suggest that boron might have a small enhancing effect on the actions of PTH in apparent absorption and retention of Ca but not those of P and Mg.

17β-Estradiol alone also had no significant effect on apparent absorption, retention, and excretion of Ca, P, and Mg in OVX rats. Intriguingly, the boron and E_2 combination treatment markedly and significantly increased Ca absorption and Ca retention, compared to the Veh group as well as to the E_2 group. The combination treatment also markedly and significantly increased Mg absorption and retention as well as P absorption, compared to OVX rats treated with solvent vehicle or E_2 alone. These observations raise the interesting possibility that there might be synergistic interaction between E_2 and boron on absorption and retention of bone minerals. On the other hand, the combination treatment with boron and E_2 did not appear to have a significant effect on the excretion rate of Ca, P, and Mg in OVX rats, suggesting that the increased tissue retention of these bone minerals are primarily the result of an increased absorption.

Table 5
Effect of Boron, 17β-Estradiol, and PTH, Each Alone or in Combination on Apparent Absorption, Retention, and Urinary Excretion of Ca, P, and Mg in Sham-Operated (Sham) and OVX Rats After 5 wk of Respective Treatment (n = 5)

	Ca			P			Mg		
	Absorption (%)	Retention (%)	Excretion (μg/mg Cr)	Absorption (%)	Retention (%)	Excretion (mg/mg Cr)	Absorption (%)	Retention (%)	Excretion (μg/mg Cr)
Sham	17.08±4.9	17.3±4.9	47±7*	52.2±3.2	25.8±3.8	2.44±0.10	49.5±3.7	49.4±3.7	1.22±0.24
OVX+Veh	12.7±4.4	12.6±4.4	11±6	47.6±2.8	27.3±3.4	2.31±0.09	42.7±3.3	42.6±3.5	0.25±0.11
OVX+B	23.5±4.4	23.4±4.4	10±6	49.6±2.8	27.3±3.4	2.18±0.09	48.1±3.3	48.0±3.5	0.16±0.11
OVX+E_2	15.4±4.9	15.3±4.9	14±7	52.7±3.2	28.4±3.8	2.29±0.10	54.9±3.7	54.9±3.9	0.40±0.12
OVX+PTH	26.9±4.9	26.8±4.9	9±7	57.3±3.2	32.8±3.8	2.40±0.10	49.3±3.7	49.3±3.9	0.43±0.12
OVX+B+E_2	34.3±4.9*#	34.2±4.9*#	9±7	60.6±3.2*#	32.3±3.8	2.44±0.10	61.9±3.7*$	61.9±3.9*$	0.15±0.12
OVX+B+PTH	29.8±4.4*	29.7±4.4*	8±6	54.4±2.8	28.2±3.4	2.33±0.09	50.9±3.3	50.8±3.5	0.11±0.11

*$p < 0.05$ compared to the OVX + Veh group.
#$p < 0.05$ compared to the OVX + E_2 group.
$$p < 0.05$ compared to the OVX + B group.

Table 6
Effect of Boron, 17β-Estradiol, and PTH, Each Alone or in Combination on Serum Ca, P, Mg, and Osteocalcin in Sham-Operated (Sham) and OVX rats after 5 wk of Respective Treatment

	N	Ca (mg/dl)	P (mg/dl)	Mg (mg/dl)	Osteocalcin (ng/ml)
Sham	7	9.6±0.2	6.7±0.4	2.45±0.10*	33.5±3.2*
OVX+Veh	9	9.4±0.1	6.3±0.4	2.24±0.10	47.6±3.0
OVX+B	10	9.6±0.1	7.1±0.4	2.34±0.09	42.7±2.7
OVX+E_2	9	9.5±0.1	7.4±0.4*	2.54±0.09*	25.1±2.9*
OVX+PTH	8	9.9±0.1*	7.6±0.4*	2.66±0.10*	67.7±3.0*
OVX+B+E_2	10	9.8±0.1*$^{\#}$	7.1±0.4	2.52±0.09*	28.6±2.7*$^{\&}$
OVX+B+PTH	7	9.9±0.2*	7.3±0.4*	2.24±0.10$^{@}$	64.9±3.2*$^{\&}$

*$p < 0.05$ compared to the OVX + Veh group.
$^{\&}p < 0.05$ compared to the OVX + B group.
$^{\#}p < 0.05$ compared to the OVX + E_2 group.
$^{@}p < 0.05$ compared to the OVX + PTH group.

Effects of Boron Alone or in Combination with E_2 or PTH on Serum Ca, P, and Mg in OVX Rats

To further evaluate the effect of boron alone or in combination with E_2 or PTH on bone mineral homeostasis, the effect of each respective treatment on serum levels of Ca, P, and Mg was measured (Table 6). OVX appeared to slightly decrease the serum levels of Ca, P, and Mg. However, only the decrease in serum Mg was statistically significant ($p = 0.042$). Boron alone increased only serum levels of Ca, P, and Mg very slightly, but not significantly. In contrast, PTH treatment alone significantly elevated the serum levels of Ca, P, as well as Mg in OVX rats to a level that was even higher than that in sham-operated animals. Boron in combination with PTH also markedly and significantly ($p < 0.05$ for each) increased the serum levels of Ca, and P, but the increases were not significantly different from those elicited by PTH treatment alone. Coadministration of boron not only did not enhance but also completely abolished the stimulatory effect of PTH on serum Mg level (i.e., brought the serum Mg level back to that of the vehicle-treated OVX rats).

The 5-wk treatment of E_2 alone also significantly elevated serum P and Mg levels in OVX rats compared to vehicle-treated animals. Contrary to the PTH treatment, E_2 alone had no effect on serum Ca. More

interestingly, combination treatment of boron and E_2 markedly and significantly ($p < 0.022$ for each) raised the serum Ca level when compared to both the Veh group and the E_2 group, further supporting the premise that boron might have an enhancing effect on the action of E_2 on Ca homeostasis. This enhancing effect was not apparent with respect to serum Mg, as there is no statistically significant difference between the boron–E_2 combination treatment and E_2 alone on serum Mg level. In addition, the combination treatment of boron and E_2 appeared to block the stimulatory effect of the E_2 treatment on the serum P level. Consequently, the interaction between boron and E_2 on serum bone minerals in OVX rats is complicated.

Effects of Boron Alone or in Combination with E_2 or PTH on Serum Osteocalcin in OVX Rats

17β-Estradiol is a well-known suppressor of bone resorption and, therefore, OVX would lead to an increase in bone resorption. Under normal physiological conditions including OVX *(15),* bone resorption is coupled to bone formation *(16).* Consequently, an inhibition of bone resorption (e.g., E_2 treatment in OVX animals) is frequently followed by a decrease in bone formation. To monitor the effect of each respective treatment on bone formation, serum osteocalcin, which is a well-accepted serum marker of bone formation *(17)* was measured. As expected, OVX led to a significant ($p = 0.0001$) increase in serum osteocalcin level (Table 6). Also as expected, the E_2 treatment reduced bone turnover (resorption as well as formation) in OVX rats, as indicated by the marked and significant ($p = 0.0001$) decrease in serum osteocalcin. Treatment with boron alone in OVX rats for 5 wk had no effect on serum osteocalcin, and the combination treatment of boron and E_2 also did not result in a further change in serum osteocalcin compared to E_2 treatment alone. On the other hand, consistent with an anabolic action of PTH on bone formation, the 5-wk daily injection of PTH in OVX rats led to a highly significant ($p = 0.0001$) increase in serum osteocalcin. Combination treatment with boron and PTH also did not produce further enhancement in serum osteocalcin level compared to PTH treatment alone. These findings suggest that dietary boron supplementation did not affect bone turnover and that boron had no modulating effect on respective action of E_2 or PTH on bone turnover in OVX rats.

DISCUSSION

Past human studies indicate that dietary boron intake had significant impacts on bone mineral homeostasis in postmenopausal women *(5,7,18),* leading to the suggestion that boron may enhance and mimic some of the

actions of E_2 on bone mineral metabolism *(7)*. However, this suggestion remains merely a speculation, as direct evidence that boron could indeed interact with E_2 to modulate actions of the sex hormone on bone mineral metabolism in animals or humans is lacking. Accordingly, we sought in this study to test whether boron alone or in combination with E_2 would promote bone mineral balance in the OVX rat model. To our knowledge, this is the first attempt to address the important question as to whether boron interacts with E_2 to modulate bone mineral homeostasis in the OVX rat model.

In this study, we demonstrate for the first time that daily dietary boron supplementation (at a dose of 5 ppm) for 5 wk in OVX rats had an enhancing effect on the action of E_2 on apparent absorption and retention of several key bone minerals (i.e., Ca, Mg, and P). Accordingly, although E_2 treatment alone did not significantly improve bone mineral balance in OVX rats, the combination treatment of boron and E_2 produced marked increases in apparent absorption and retention of Ca, Mg, and P. These findings are consistent with the premise that boron could interact with E_2 to modulate bone mineral balance. In addition, because boron treatment by itself also did not significantly promote apparent absorption and retention of Ca, Mg, and P in OVX rats, we tentatively conclude that the interaction between boron and E_2 on bone mineral balance is of a synergistic nature.

The fact that boron treatment alone did not significantly increase apparent absorption and tissue retention of bone minerals in OVX rats is somewhat intriguing, because several past reports indicate that boron treatment produced a positive balance of bone minerals in several animal models *(2–5)*. Although we cannot rule out the possibility that the lack of a response to boron on bone mineral metabolism was the result of an inadequate dosage of boron, we should note that the dose of boron in this study was similar to or higher than many studies that showed a positive response *(4)*. It has been previously reported that over 99% of the absorbed boron from a single oral dose were excreted in the 24-h urine *(19)*. Thus, it is also possible that the lack of a response to boron might be the result of the rapid urinary clearance of the absorbed boron. However, our boron-treated OVX rats retained a significant amount (i.e., approx 150–200 μg/d) of boron and this amount of boron absorption is comparable to that in many past studies that showed a positive response *(5)*. Consequently, we do not favor the conclusion that an insufficient boron dosage or rapid urinary clearance was responsible for the lack of a positive response to boron on bone mineral balance in this study. On the other hand, we should emphasize that boron was able to elicit a positive bone mineral balance only if and when the animal was under certain types of stresses, such as fluoride intoxication, vitamin D deficiency, or Mg insufficiency *(2–5,20)*. Therefore, it is conceivable that E_2 deficiency by itself may not produce sufficient amounts or the right type of stress

to induce a positive bone mineral balance. Much further work is needed to confirm this possibility.

With respect to potential mechanism(s) of the interaction between boron and E_2, previous human studies indicate that boron significantly increased serum E_2 concentration in postmenopausal females *(5,7)* as well as in adult males *(6)*, raising the speculation that boron may exert effects on bone mineral metabolism through increased synthesis of E_2 *(5–7)*. In this regard, we also found that 5-wk daily dietary boron supplementation appeared to increase serum E_2 concentrations in OVX rats, albeit the increase did not reach a statistically significant level. Nonetheless, the increase in the serum E_2 concentration resulting from boron treatment was relatively small compared to that in response to E_2 replacement therapy. Moreover, the E_2 treatment alone did not produce a significant enhancement in apparent absorption and retention of bone minerals in OVX, in spite of a large increase in the serum E_2 concentration. Therefore, we do not believe that the apparent synergistic interaction between boron and E_2 on bone mineral metabolism was the result of the boron-induced E_2 production.

An additional observation that might be relevant to the potential interaction between boron and E_2 on bone mineral homeostasis is the finding of an apparent enhancing effect of boron on the action of E_2 to increase serum Ca in OVX rats. Accordingly, combination treatment of boron and E_2 significantly increased serum Ca concentrations, despite the fact that neither boron nor E_2 treatment alone significantly changes the serum concentration of Ca in OVX rats. We should note that the interaction between boron and E_2 on serum bone mineral homeostasis might be specific for serum Ca, because no additional enhancement of the effect of E_2 on serum P and Mg concentrations was observed with the combination treatment of boron and E_2. A major regulatory mechanism of serum Ca is the bone resorption, and E_2 is a known suppressor of bone resorption. We do not know at this time whether interaction between boron and E_2 in increasing serum Ca was the result of a reduction of the suppressive action of E_2 on bone resorption by boron or the result of an increase in Ca absorption and/or decrease in Ca excretion. However, because boron treatment by itself did not affect bone turnover, as reflected by the lack of an effect on serum osteocalcin, and because coadministration of boron and E_2 did not significantly alter the E_2-induced suppression of serum osteocalcin (or bone turnover), we tentatively conclude that the interaction between boron and E_2 in increasing serum Ca concentration (as well as in enhancing bone mineral balance) in OVX rats probably does not involve a boron-dependent modulation of the inhibitory action of E_2 on bone turnover.

Parathyroid hormone is another known regulator of bone mineral homeostasis. In contrast to E_2, which is a bone resorption suppressor, intermittent PTH injection is a potent stimulus of bone formation *(21)*. Thus, we were also interested in evaluating whether boron would have

a similar interaction with PTH on bone mineral homeostasis in OVX rats. In this regard, we found that the 5-wk daily injection of PTH alone, like E_2 or boron treatment alone, also had no appreciable effect on boron metabolism, apparent absorption, retention, and excretion of Ca, P, and Mg. This is consistent with the previous finding that intermittent PTH treatment of 21 osteoporotic women for 6–24 mo did not improve Ca, Mg, and P balance *(22).* However, contrary to the boron and E_2 combination treatment, coadministration of boron and PTH in OVX led to a significant enhancement only in apparent absorption and retention of Ca, but not those of P or Mg. Consequently, boron also seems to interact with PTH on Ca balance in OVX. The fact that the boron and PTH combination treatment, unlike the boron and E_2 combination, failed to improve P and Mg balance, suggesting that the interaction between boron and PTH on bone mineral balance, is probably different from that between boron and E_2. There are also significant differences between the interaction of boron and E_2 and that of boron and PTH in that whereas boron seemed to enhance the action of E_2 to increase serum Ca, boron appeared to completely abolish the PTH-induced increase in serum Mg. These observations further support our contention that boron may interact with E_2 differently than it interacts with PTH.

Recent studies suggest a tissue-specific concentration of boron in the parathyroid glands. The physiological significance of this finding is not clear at this time in that it is not known if boron would have an effect on the production and/or secretion of PTH, as there has been little or no reports on the effect of boron on serum PTH. Nonetheless, we cannot ignore the possibility that the effect of dietary boron supplementation on bone mineral balance could be secondary to its effect on PTH secretion. On the other hand, because a daily injection of PTH to the ovariectomized rats in this study did not abolish or mimic the effects of boron on bone mineral balance, we do not favor this possibility at this time. Further studies on the effect of boron on PTH secretion are required to better address this issue.

In summary, we have demonstrated that dietary supplementation of boron for 5 wk could significantly enhance the action of E_2 to promote bone mineral balance. Although the significance of these findings is not clear at this time, it is generally accepted that a positive bone mineral balance (i.e., increased absorption and retention) is necessary and essential for bone health *(23).* Accordingly, improved retention of Ca, P, and also, perhaps, Mg has been shown to lead to suppression of bone resorption and stimulation of bone formation, resulting in an increase in bone mass and strength *(24).* Consequently, it is conceivable that the enhancing effect of boron on the action of E_2 to promote a positive bone mineral balance would be beneficial to bone. Nevertheless, confirmation of this hypothesis would require much additional work, especially those concerning the effect of boron alone or in combination with E_2 on bone density, mass, and strength.

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