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Dietary Boron Supplementation Enhanced the Action of Estrogen, But Not That of Parathyroid Hormone, to Improve Trabecular Bone Quality in Ovariectomized Rats

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ABSTRACT

This study investigated whether boron would enhance the ability of 17β-estradiol (E_2) or parathyroid hormone (PTH) to improve bone quality in ovariectomized OVX rats. Adult OVX 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, respectively. The E_2 treatment corrected many adverse effects of OVX on bone quality, increased bone Ca, P, and Mg contents, and decreased trabecular plate separation. Dietary boron supplementation had no effects on these bone parameters in OVX rats. When OVX rats were treated with boron and E_2 together, trabecular bone volume (Tb.BS/TV) and plate density were increased significantly more than that caused by E_2 alone. The boron and E_2 combination also increased trabecular bone surface (Tb.BV/TV) and decreased trabecular plate separation in OVX rats. In contrast, whereas daily PTH injection also increased bone Ca, Mg, and

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P contents, Tb.BV/TV, Tb.BS/TV, trabecular plate density and thickness, and decreased trabecular plate separation in OVX rats, the combination of boron and PTH had no additional improvement in bone quality over that achieved by PTH alone. In summary, this study shows for the first time that boron enhanced the action of E_2, but not that of PTH, to improve trabecular bone quality in OVX rats.

Index Entries: Boron; 17β-estradiol; parathyroid hormone; trabecular bone; bone quality; ovariectomy (rat).

INTRODUCTION

Boron, an essential ultratrace element in plants *(1),* may have an effect on bone and mineral metabolism in a number of animal models, including humans. Boron in drinking water corrected the deficits in calcium (Ca) and phosphorus (P) balance associated with fluoride intoxication in the rabbit *(2).* Boron also increased both Ca and P balance in vitamin D-deficient rats *(3).* Boron repletion in 12 postmenopausal women, who were previously on a low-boron diet for 119 d, markedly reduced the urinary excretion of Ca *(4).* This reduction was more pronounced in subjects who were on a low-Mg diet than those on a normal Mg diet *(4).* Therefore, under certain stresses (e.g., fluoride intoxication, vitamin D deficiency, or Mg deficiency), boron appeared to have a positive effect on bone mineral homeostasis.

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 *(4)*. A similar increase in serum E_2 levels was seen in healthy males after 4 wk of dietary boron supplementation *(5).* 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 (e.g., bone mineral deficits and reduced bone quality) *(6,7).* Accordingly, we recently tested whether boron could enhance the action of E_2 on bone mineral balance in the ovariectomized (OVX) rat model. We found that 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 to increase the apparent absorption and retention of several bone minerals (i.e., Ca, P, and Mg), resulting in a positive balance in these bone minerals *(8).*

A positive balance of bone minerals is essential for bone health *(9).* It has been speculated that boron might help to improve bone quality and strength *(10).* There is some circumstantial evidence that boron supplementation under certain experimental conditions could positively affect bone strength in a number of animal models. Boron treatment for up to 32 wk in rats increased significantly the compression strength of the lumbar vertebrae (but not long bones) by 5–10%, suggesting that dietary boron supplementation in rats could have a beneficial effect on the strength of

the axial skeleton *(11,12).* A long-term treatment of White Leghorn chickens with dietary boron also tended to increase shear fracture energy and shear force and stress in long bones *(13).* More recently, it was reported that dietary boron increased bone strength in pigs fed a semipurified diet low in boron content *(14).*

Because dietary boron supplementation enhanced the action of E_2 on bone mineral balance in OVX rats, we hypothesized that boron might also enhance the action of E_2 to improve bone quality in OVX rats. Consequently, the primary objective of the present study was to evaluate whether the 5-wk dietary boron supplementation (at a dose of 5 ppm) could also interact with E_2 to improve bone quality in OVX rats. Because the enhancing interaction of boron on bone mineral balance was not seen with parathyroid hormone (PTH) *(8),* and because the effect of E_2 on bone is mediated through a suppression of bone resorption *(15),* whereas intermittent PTH injection is a potent stimulus of bone formation *(16),* we also determined if dietary boron would interact with PTH to improve bone quality in OVX rats. Because there is compelling evidence that E_2 and PTH each act primarily on trabecular bones *(17,18)*, this study focused on trabecular bones in the tibia. In this study, bone quality was assessed with histomorphometry in the tibia.

MATERIALS AND METHODS

Animals and Diets

Seventy-five 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. Sixty-seven 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 rats were housed singly in stainless-steel rat cages at 22°C on 12-h light/12-h dark cycles and were fed the AIN-76 basal rodent diet, *ad libitum* containing 0.52% Ca, 0.4% P, 0.05% Mg, 0.00004% B, and 1000 IU/kg vitamin D_3. The ingredients of the AIN-76 diet have been described in our previous report *(8).* Deionized water (as drinking water) was available at all time. Although the boron content in the deionized water was not determined, feed cups and drinking bottles were soaked overnight in 2 *N* HC1 prior to each use to minimize boron contamination.

Experimental Protocol

Treatments were initiated on d 43 post-OVX. At the start of the experiment, the OVX rats were assigned to 6 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); and (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, Inc., 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* HC1. 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 injection (sc) of 30 µg/kg E_2 (Sigma Chemicals). Rat PTH was dissolved in acidic saline (0.001 *N* HC1) 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. The experiment was carried on for 5 wk.

At the end of the experiment, all rats were euthanized between 9 AM and 1 PM, a day after the final injection. Each animal was injected interperitoneally with pentobarbital sodium (25–40 µg/kg) and exposed to a carbon dioxide overdose. The tibiae of each animal were removed and defleshed. The right tibia was immediately bisected longitudinally, soaked in 70% ethanol in a glass vial, kept in the dark at room temperature, and was used for histomorphometric measurements. The left tibia was stored at –20°C for subsequent analyses. The animal protocol was reviewed and approved by the Animal Use Committee of the Virginia Polytechnic Institute and State University.

Analyses

Bone histomorphometry was measured at the right tibia at the Histopathology Laboratory of the College of Veterinary Medicine of the Virginia Polytechnic Institute and State University. The proximal right tibia of five to six randomly selected animals of each group was sawed off, soaked in Decalcifier II (Surgipath Medical Industries, Inc., Richmond, IL) overnight, dehydrated in graded ethanols, infiltrated with paraffin wax, and embedded in EM400 (Surgipath Medical Industries, Inc.). The embedded bone samples were then cut longitudinally into 5-µm sections using a Reichert–Jung microtome and stained with hematoxylin and eosin–phyloxin. Each slide was assigned a random number to blind its identity from the observer. All bone histomorphometric indices were measured in the area of secondary spongiosa at a site exactly 1 mm from the lowest point of the growth plate, with a total measuring area of 1.35 mm^2. Trabecular (cancellous) bone area and the perimeter were measured using the point and intercept counting method with a Mertz eyepiece graticule at ×200. Trabecular bone volume (Tb.BV/TV), bone surface to volume ratio (Tb.BS/TV), plate thickness, plate density, and plate separation were calculated according to Parfitt *(19).*

The left tibia was crushed and dried at 105°C for 48 h, weighed, and ashed in a muffle furnace at 600°C for 24 h. The bone ash residue was ground, weighed, and digested with nitric acid/perchloric acid (5/3, v/v) for measurements of bone Ca, P, and Mg contents. The wet-ashed samples were diluted with 1% lanthanum oxide *(20),* and the Ca and Mg concentrations were determined by atomic absorption spectroscopy (AAS, Perkin-Elmer Atomic Absorption Spectrophotometer Model 2100, Rockville, MD). The P content was determined by a colorimetric assay *(21)* using a microtiter plate reader (Ceres 900 HDI, Bio-Tek Instruments, Inc., Winooski, VT). For measurement of bone boron content, the right femur of each animal was 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 *(22).* The boron content of each wet-ashed bone sample was determined by inductively coupled plasma spectroscopy (ICP, Perkin-Elmer Plasma 400 ICP Emission Spectrometer, Rockville, MD).

Statistical Analyses

Results are shown as mean±SEM. Statistical significance was determined with two-way analysis of variance (ANOVA) followed by the Tukey's Studentized range (HSD) post hoc test. A least-squares mean (LSMEANS) test was used for unequal sample sizes. Analyses were performed with a personal computer using the SAS™ software (SAS Institute Inc., Cary, NC 27512). The difference was significant when $p<0.05$.

RESULTS

Effects of OVX on Bone Histomorphometry and Bone Mineral Parameters in Young Adult Rats

The serum E_2 concentration of OVX rats at 11 wk after the surgery was significantly lower than that of sham-operated animals (16±3 vs 30±6 pg/mL, $p=0.0001$). The body weight of OVX rats was also significantly ($p<0.05$) higher than that of sham-operated animal (Table 1), as a result of increased food assumption *(8).* These findings confirmed the success of the OVX.

Because E_2 is a potent physiological inhibitor of bone resorption *(15),* it has long been recognized that the OVX would cause a marked increase in bone resorption, resulting in a decrease in trabecular bone mass and, subsequently, leading to the deterioration of bone quality *(23).* Table 1 shows that although the trabecular plate thickness of OVX rats was not significantly different from that of sham-operated controls, the plate density was reduced (by 70%, $p<0.05$) and plate separation increased (by 4.75-fold, $p<0.05$) in OVX rats compared to sham-operated control rats, supporting the premise that OVX caused an increase in bone resorption. In addition, OVX markedly decreased tibial Tb.BV/TV and Tb.BS/TV to

Table 1
Effect of OVX on Body Weight and Tibial Bone Parameters in Young Adult Rats

	Sham operated (n=7)	11 weeks post-OVX (n=8)
Body weight, g	269±13	343±12*
Bone weight, g	0.47±0.02	0.50±0.02
Bone weight/body weight, g/kg	1.75±0.05	1.47±0.05*
Total bone ash, g	0.29±0.01	0.30±0.01
Ash/BW, g/kg	1.08±0.03	0.87±0.03*
Ash/bone, g/kg	0.62±0.01	0.59±0.01*
Wall thickness, mm	0.45±0.01	0.48±0.01*
Tb.BV/TV, %	25.4±2.8	7.7±3.4*
Tb.BS/TV, mm^2/mm^3	11.37±0.70	3.30±0.86*
Tb.plate density, mm^{-1}	5.68±0.35	1.65±0.43*
Tb.plate thickness, μm	44.97±6.58	45.74±8.06
Tb.plate separation, μm	136±58	646±72*

* $p<0.05$.

approx 30% of each corresponding parameter of sham-operated animals ($p<0.05$ for each). The bone ash (normalized against either body weight or dried bone weight) of the tibia was also significantly decreased compared to that of control animals ($p<0.05$ for each), suggesting that the OVX rats have indeed lost a significant amount of bone mass.

Table 2 shows that the bone Ca, P, and Mg contents (normalized against either body weight or dried bone weight) of the tibia in OVX rats were significantly ($p<0.05$ for each) lower than that in sham-operated rats. However, when bone Ca, P, and Mg were each normalized against bone ash weight, there was no difference between the OVX group and the Sham group, suggesting that although the OVX caused a significant reduction in bone mass, the surgery probably had no effect on the mineral content of the bone crystal structure.

Effects of Boron Alone or in Combination with E_2 or PTH on Body Weight, Dried Bone Weight, and Bone Ash in OVX Rats

Table 3 reveals that a 5-wk treatment with 30 μg/kg/d E_2 effectively reduced the body weight of OVX rats to a weight approaching that of the age-matched sham-operated animals (i.e., 269±13 g vs 283±11 g, p=N.S.). Although the E_2 treatment had no apparent effect on the absolute values of dried bone weight, mean wall thickness, and bone ash, the bone ash and dried bone weight after normalization against the body weight of OVX

Table 2
Effect of OVX on Bone Mineral Contents in Tibia of Young Adult Rats

	Sham operated (n=7)	11 weeks post-OVX (n=8)
Total bone Ca, mg	100±3	104±3
Bone Ca/BW, g/kg	0.38±0.01	0.31±0.01*
Bone Ca/Bone, g/g	0.215±0.002	0.208±0.002*
Bone Ca/Ash, g/g	0.348±0.003	0.353±0.003
Total bone P, mg	53±2	53±2
Bone P/BW, g/kg	0.20±0.01	0.16±0.01*
Bone P/Bone, g/g	0.113±0.003	0.107±0.002*
Bone P/Ash, g/g	0.183±0.004	0.181±0.003
Total bone Mg, mg	2.01±0.07	1.99±0.06
Bone Mg/BW, g/kg	7.50±0.30	5.84±0.27*
Bone Mg/Bone, g/g	4.28±0.11	3.97±0.11*
Bone Mg/Ash, g/g	6.94±0.16	6.75±0.13

* $p<0.05$.

rats was each significantly increased compared to that of sham-operated animals, as a result of the significant reduction in body weight. The boron treatment alone had no significant effect on body weight, dried bone weight, mean wall thickness, and bone ash content of the tibia. However, it appears that boron enhanced the effect of E_2 to increase dried bone weight and bone ash weight normalization against body weight (Table 3). Consistent with the premise that PTH is a stimulator of bone formation, daily PTH injection (60 μg/kg/d) for 5 wk, although it had no significant effects on the body weight of the OVX rats, tended to increase the bone weight, mean wall thickness, and bone ash content (the total level or the level normalized against body weight or dried bone weight). The combination treatment of boron and PTH had no additional effect on these parameters compared to the PTH treatment alone (Table 3).

Effects of Boron Alone or in Combination with E_2 or PTH on Bone Mineral Content in OVX Rats

Table 4 shows the bone mineral and bone boron contents of these OVX rats after a 5-wk treatment with boron alone or in combination with E_2 or PTH. As expected, the boron treatment alone or in combination with E_2 or PTH each markedly increased the bone boron content, confirming the increased intake of boron from the diet. With respect to bone mineral content, the boron treatment alone had no significant effects on bone Ca, P, or

Table 3
Effect of 5 wk Dietary Boron Supplementation Alone or in Combination with E_2 or PTH on Body Weight, Dried Bone Weight, and Bone Ash Weight in Tibia of OVX Rats

	Veh (n=8)	B (n=10)	E_2 (n=9)	PTH (n=8)	B+E_2 (n=10)	B+PTH (n=7)
Body Weight, g	343±12	318±11	283±11*	328±12	283±11*	338±13
Bone Weight, g	0.50±0.02	0.48±0.01	0.47±0.01	0.53±0.02	0.49±0.01	0.54±0.02#
Bone/BW, g/kg	1.47±0.05	1.54±0.04	1.67±0.05*	1.63±0.05*	1.73±0.05*	1.60±0.05*
Wall Thickness, mm	0.48±0.01	0.47±0.01	0.47±0.01	0.51±0.01*	0.46±0.01	0.50±0.02*
Total Ash, g	0.30±0.01	0.28±0.01	0.28±0.01	0.33±0.01*	0.29±0.01	0.33±0.01*#
Ash/BW, g/kg	0.87±0.03	0.90±0.03	1.00±0.03*	1.01±0.03*	1.02±0.03*#	0.99±0.03*
Ash/Bone, g/g	0.59±0.01	0.59±0.00	0.59±0.00	0.62±0.01*	0.59±0.01	0.62±0.01*#

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

Mg content in OVX rats, regardless of whether these bone minerals were shown in total amounts or in amounts normalized against body weight, dried bone weight, or bone ash. The E_2 treatment alone also had no effect on total amounts of bone Ca, P, and Mg. However, because of the significant reduction in bone weight by the E_2 treatment, the bone content of these bone minerals, after normalization against body weight, was significant increased by the E_2 treatment in these OVX rats. Combination treatment with boron and E_2 did not produce any additional effect on bone content of these minerals over that obtained by the E_2 treatment alone. In contrast, the PTH treatment significantly increased the total amount (and the amount normalized against body weight or dried bone weight) of bone Ca, P, and Mg (Table 4). The combination treatment with boron and PTH did not significantly increase the total or normalized bone contents of Ca, P, or Mg over those obtained by PTH treatment alone in OVX rats. When each bone mineral was normalized against bone ash, none of the treatments had a significant effect, suggesting that these treatments probably did not alter the ratios of Ca, P, and Mg in bone mineral crystals in OVX animals.

Effects of Boron Alone or in Combination with E_2 or PTH on Bone Histomorphometry in OVX Rats

Dietary boron alone had no significant effect on any of the measured histomorphometric indices in the tibia of OVX rats (Table 5). The E_2 treat-

Table 4
Effect of 5 wk Dietary Boron Supplementation Alone or in Combination with E_2 or PTH on Bone Mineral and Boron Contents in Tibia of OVX Rats

	Veh (n=8)	B (n=10)	E_2 (n=9)	PTH (n=8)	B+E_2 (n=10)	B+PTH (n=7)
Total bone Ca, mg	104±3	99±3	99±3	115±3*	100±3	117±3*#
Ca/BW, g/kg	0.31±0.01	0.31±0.01	0.35±0.01*	0.35±0.01*	0.36±0.01*	0.35±0.01*#
Ca/Bone, g/g	0.208±0.002	0.205±0.002	0.211±0.002	0.217±0.002*	0.206±0.002	0.217±0.002*#
Ca/Ash, g/g	0.353±0.003	0.348±0.003	0.355±0.003	0.351±0.003	0.348±0.003	0.351±0.003
Total bone P, mg	53±2	51±2	50±2	59±2*	48±2	60±2*#
P/BW, g/kg	0.16±0.01	0.16±0.01	0.18±0.01*	0.18±0.01*	0.17±0.01	0.18±0.01*#
P/Bone, g/g	0.107±0.002	0.105±0.002	0.107±0.002	0.111±0.002*	0.101±0.002	0.112±0.002*#
P/Ash, g/g	0.181±0.003	0.179±0.003	0.180±0.004	0.180±0.004	0.170±0.004	0.181±0.003
Total bone Mg, mg	1.99±0.06	1.86±0.06	1.86±0.06	2.22±0.06*	1.81±0.06	2.20±0.06*#
Mg/BW, µg/kg	5.84±0.27	5.95±0.24	6.62±0.25*	6.80±0.27*	6.50±0.25	6.54±0.29*
Mg/Bone, g/kg	3.97±0.11	3.87±0.10	3.95±0.11	4.17±0.11*	3.78±0.11	4.09±0.12#
Mg/Ash, g/kg	6.75±0.13	6.57±0.13	6.65±0.14	6.74±0.14	6.39±0.14	6.62±0.13
Total bone B, µg	0.39±0.03	0.61±0.02*	0.39±0.02	0.43±0.03	0.59±0.02*@	0.65±0.03*^
B/BW, µg/kg	1.13±0.10	1.95±0.08*	1.37±0.09	1.31±0.10	2.09±0.08*@	1.94±0.16*^
B/Bone, µg/g	0.66±0.04	1.08±0.04*	0.66±0.04	0.67±0.04	1.02±0.04*@	1.02±0.04*^

* $p<0.05$ compared to Veh group.
\# $p<0.05$ compared to B group.
@ $p<0.05$ compared to E_2 group.
^ $p<0.05$ compared to PTH group.

ment alone significantly reduced trabecular plate separation in the OVX rats compared to vehicle-treated controls (Table 5). This treatment also appeared to increase Tb.BV/TV, Tb.BS/TV, and plate density. However, because of the relatively large variations in these measurements, the increases did not reach the statistically significant level. It is important to note that dietary boron produced a significant enhancement in the E_2-dependent increases in Tb.BV/TV, Tb.BS/TV, and plate density when compared to the E_2 treatment alone (Table 5). In addition, boron in combination with E_2 led to a further and significant reduction in the trabecular plate separation compared to the E_2 treatment alone. On the other hand, neither E_2 treatment alone nor in combination with boron appeared to

Table 5
Effect of 5 wk Dietary Boron Supplementation Alone or in Combination with E_2 or PTH on Trabecular Bone Histomorphometric Indices in Tibia of OVX Rats

	Veh (n=5)	B (n=6)	E_2 (n=6)	PTH (n=5)	B+E_2 (n=6)	B+PTH (n=4)
Tb.BV, %	7.7±3.4	6.5±3.4	12.2±2.8	29.4±3.4*	15.8±2.8*#	17.5±3.4*#
Tb.BS/TV, mm^2/mm^3	3.30±0.86	3.17±0.86	5.12±0.70	6.87±0.86*	7.39±0.7*#^	7.69±0.86*#
Tb.Plate Thickness, μm	45.74±8.06	42.45±8.06	49.08±6.58	81.52±8.06*	43.52±6.58	47.63±8.06
Tb.Plate Density, mm^{-1}	1.65±0.43	1.59±0.43	2.56±0.35	3.43±0.43*	3.69±0.35*#^	3.85±0.43*#
Tb.Plate Separation, μm	646±72	637±72	363±59*	219±72*	251±59*#	248±72*#

* $p<0.05$ compared to Veh group.
\# $p<0.05$ compared to B group.
^ $p<0.05$ compared to E_2 group.

increase trabecular plate thickness. These findings strongly suggest that boron could synergistically enhance the action of E_2 in improving the quality of trabecular bone in OVX rats.

Daily PTH injection alone also significantly increased Tb.BV/TV, Tb.BS/BV, and plate density of trabecular bone in the tibia of OVX rats (Table 5). The treatment also markedly reduced the trabecular plate separation. However, unlike the E_2 treatment, PTH also significantly increased the trabecular plate thickness. More importantly, the combination treatment of PTH and boron had no enhancing effect on the increase in Tb.BV/TV, Tb.BS/TV, plate thickness, and plate density, nor did it lead to further reduction in trabecular plate separation in OVX rats. In fact, the boron treatment might have even reduced the stimulatory effect of PTH on some of the parameters (e.g., Tb.BV/TV and plate density). Thus, it appears that dietary boron did not have an enhancing interaction with PTH in the improvement of the trabecular bone quality in OVX rats.

DISCUSSION

In this study, we confirmed that the OVX led to a significant loss of trabecular bone, as OVX rats had significant lower Tb.BV/TV, Tb.BS/TV, and bone mineral contents than sham-operated rats. The marked increase in trabecular plate separation and the decrease in trabecular plate density in OVX rats are consistent with the contention that OVX caused bone loss, primarily through an increase in bone resorption. This study also confirmed that E_2 treatment reversed many of the adverse effects of OVX on trabecular structure of long bones. For instance, E_2 repletion significantly increased Tb.BV/TV when compared to vehicle-treated OVX rats. This

increase in trabecular bone volume is presumably caused by an alleviation of the OVX-induced increase in bone resorption in response to E_2 repletion, as reflected by the large decrease in trabecular plate separation and the significant increase in trabecular plate density in E_2-treated compared to vehicle-treated OVX rats. The fact that E_2 treatment did not significantly increase trabecular plate thickness is consistent with the premise that E_2 did not increase trabecular bone formation *(15).* Consequently, this study confirmed the well-known facts that long-term E_2 deficiency (i.e., OVX) had deleterious effects on the quality of trabecular bones and that these effects could be reversed, at least in part, by E_2 repletion.

It is important to note that, inasmuch as the E_2 repletion in this study was effective in reducing trabecular plate separation and increasing Tb.BV/BV in OVX rats, E_2 at the test dose (30 μg/kg/d) did not restore fully the tibial trabecular parameters to the level of each respective parameter of sham-operated rats (i.e., Table 1 vs Table 4). The incomplete restoration of trabecular bone architecture in OVX by the E_2 treatment could suggest that the test dose of E_2 might be inadequate to fully correct the E_2-deficiency-induced defects in OVX rats. Consistent with this speculation is our recent observation that this dose of E_2 also failed to restore fully the apparent absorption efficiency and retention of bone minerals in OVX to respective level in sham-operated rats *(8).* On the other hand, because the primary action of E_2 with respect to bone metabolism is to inhibit bone turnover *(15),* the E_2 repletion in OVX rats would probably only prevent further bone loss and would not be able to rebuild the lost trabeculae. Moreover, the majority of bone loss due to E_2 deficiency in OVX rats occurs acutely during the first few weeks after the OVX. Thus, E_2 repletion must be instituted very early in the E_2-deficient state in order to preserve trabecular bone structure fully *(24).* In this regard, E_2 replacement therapy for postmenopausal osteoporosis is known to be most effective when the therapy is instituted during the early stage of E_2 deficiency (i.e., perimenopause) *(25).* Consequently, we cannot exclude the possibility that institution of E_2 repletion 6 wk after the OVX (as it was the case in this study) might be too late to allow for a full restoration of the lost trabecular bone structure as a result of the OVX by E_2 treatment alone. Nevertheless, because the architecture of trabeculae bone (i.e., the number, thickness, and connectivity of trabeculae) is a key determinant of bone quality *(26)* and because E_2 repletion in OVX rats appeared to improve trabecular bone architecture by increasing the number (but not the thickness) and density of trabeculae, the E_2 repletion would improve (or preserve) the trabecular structure and, thus, improve the quality of trabecular bones in OVX rats.

Past studies in postmenopausal women showed that dietary boron supplementation by itself could promote positive bone mineral balance *(4,6).* 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 the dietary boron supplementation by itself at the dose of 5 ppm did not improve any test

histomorphometric indices or bone mineral contents in OVX rats. Moreover, we recently showed that the same boron treatment also did not affect the bone mineral balance in OVX rats *(8).* Consequently, our findings do not support the contention that dietary boron supplementation alone would promote bone mineral balance or preserve trabecular bone architecture in E_2-deficient OVX rats. Although we could not preclude the possibility that the lack of a positive response by boron alone was the result of an inadequate dosage of boron or to a rapid urinary clearance of the absorbed boron, we are not in favor of this possibility because (1) the dosage used in this study was comparable to that in several past studies that showed a positive response *(4)* and (2) the boron-treated OVX rats in our studies retained significant amounts of boron *(8).* On the other hand, we could not rule out the possibility that this was a species-related difference. We should also emphasize that a positive response was seen only in those postmenopausal women who were deficient in boron *(4).* Thus, it is possible that boron by itself would elicit a response only under boron-deficient states. In this regard, the nutritional requirement of boron for normal growth is very low and, thus, even the smallest boron "contamination" in the diet, drinking water, or the environment might be sufficient to support the normal growth. The boron content in our diet was 0.4 ppm or 0.4 mg/kg diet, which was a considerable amount. Moreover, because we did not measure the boron content in the drinking water (deionized water), we cannot rule out the possibility that there might be sufficient boron in the drinking water and in the diet to meet the nutritional need of the OVX rats. Additional studies are needed to address these possibilities.

There are two noteworthy findings in this study that are relevant to the effect of dietary boron supplementation on bone quality. First, dietary boron supplementation at the dose of 5 ppm significantly enhanced the effect of E_2 on several trabecular bone parameters in the tibia of OVX rats. Specifically, the combination treatment of boron and E_2 produced increases in Tb.BS/TV and trabecular plate density that were significantly higher than did the E_2 treatment alone. More importantly, because dietary boron by itself at the test dose had no apparent effect on any of the test parameters, we conclude that the enhancing effect of boron on the action of E_2 on trabecular bone parameters in OVX is of a synergistic nature.

The mechanism of this "synergistic" interaction between boron and E_2 on trabecular bone quality in OVX rats is not known. Previous studies indicate that boron significantly increased serum E_2 concentration in postmenopausal women *(4,6)* as well as in adult men *(5),* leading to the speculation that boron might exert effects on bone through increased biosynthesis of E_2. However, the 5-wk dietary boron supplementation only slightly, but not significantly, increased serum E_2 concentrations in OVX rats in our studies *(8).* Therefore, we do not believe that the apparent synergistic interaction between boron and E_2 on bone was the result of the boron-induced E_2 production. Conversely, boron exerts an enhancing inter-

action with E_2 to promote a positive balance in bone minerals, such as Ca, Mg, and P *(8).* A positive balance of Ca is known to be essential for bone health *(27).* Because Ca has been reported to potentiate the effect of E_2 on bone *(28),* it would be tempting to associate the positive Ca balance with the improvement in trabecular bone architecture in OVX rats. On the other hand, combination treatment with boron and PTH in these OVX rats also led to a significant enhancement in apparent absorption and retention of Ca *(8).* Because boron did not enhance the action of PTH on trabecular bone quality in OVX rats, we conclude that the "synergistic" interaction between boron and E_2 on trabecular bone quality is probably not the result of an increase in Ca balance. Nevertheless, because combination treatment of boron and E_2, but not that of boron and PTH, also promoted positive balances in P and Mg *(8)* and because both P and Mg balance might play a role in the maintenance trabecular bone quality *(27,29),* we cannot completely rule out the possibility that the enhancing interaction of boron and E2 on trabecular bone quality might be related to the positive balance of P or Mg.

The second noteworthy observation of this study is that the same dietary boron supplementation, although it enhanced the action of E_2, did not promote the action of PTH (60 μg/kg/d) in the improvement of trabecular bone quality. We should note that a daily injection of PTH alone for 5 weeks noticeably increased Tb.BV/TV, Tb.BS/TV, as well as trabecular plate thickness and density in OVX rats, findings that are consistent with the well-known anabolic action of intermittent PTH treatment on trabecular bone formation *(16,30).* Therefore, the lack of an enhancing effect on PTH, as opposed to E_2, was not the result of an ineffective PTH treatment. Nevertheless, the fact that dietary boron supplementation was able to enhance the effect of E_2, but not that of PTH, on bone mineral balance *(8)* and trabecular bone quality in OVX rats raises an interesting possibility that the enhancing interaction of boron might be unique for E_2.

The fact that boron could interact with E_2, but not with PTH, is intriguing. It may be speculated that the reason(s) why boron could synergistically interact with E_2, but not with PTH, to promote bone mineral metabolism and improve bone quality in OVX rats may be related to the mechanism of action of E_2 as opposed to PTH. Nevertheless, much further work is needed to allow a better understanding to this apparent differential interaction of boron with E_2 as opposed to PTH.

The effect of boron alone or in combination with E_2 or PTH on bone strength of OVX rats was not investigated in this study. However, although the issue of whether dietary boron supplementation would have a beneficial effect on the strength of cortical bone is controversial, there is abundance of evidence, at least in the rat, that dietary boron supplementation markedly increased the compression strength of the trabecular-bone-rich lumbar vertebrae *(11,12).* Consequently, it is reasonable to assume that the synergistic enhancement of boron on the action of E_2 to improve trabecular bone architecture and quality could most probably translate into an improved strength in trabecular bone in OVX rats.

In summary, we have demonstrated, for the first time, that dietary boron supplementation in OVX rats for 5 wk could significantly enhance the actions of E_2 to reverse the adverse actions of OVX on trabecular bone quality. This apparently synergistic enhancing interaction of boron on the action of E_2 on bone appeared to be unique in that it was not observed with PTH. Although the molecular mechanism of this interaction has not been determined, this apparent synergistic interaction, if confirmed and extended to humans, could be clinically relevant and could indicate an important role of dietary boron in treatment and/or prevention of postmenopausal osteoporosis.

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