

# Effects of Boron Supplementation on Bone Mineral Density and Dietary, Blood, and Urinary Calcium, Phosphorus, Magnesium, and Boron in Female Athletes

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The effects of boron supplementation on blood and urinary minerals were studied in female college students—17 athletes and 11 sedentary controls—over a one-year period. The athletes had lower percent body fat and higher aerobic capacities than sedentary controls. Athletic subjects consumed more boron in their normal diets than sedentary subjects; all other dietary measures were similar between the two groups. The athletes showed a slight increase in bone mineral density, whereas the sedentary group showed a slight decrease. Serum phosphorus concentrations were lower in boron-supplemented subjects than in subjects receiving placebos, and were lower at the end of the study period than during baseline analysis. Activity depressed changes in serum phosphorus in boron-supplemented subjects. Serum magnesium concentrations were greatest in the sedentary controls whose diets were supplemented with boron, and increased with time in all subjects. A group × supplement interaction was observed with serum magnesium; exercise in boron-supplemented subjects lowered serum magnesium. In all subjects, calcium excretion increased over time; in boron-supplemented subjects, boron excretion increased over time. In all subjects, boron supplementation affected serum phosphorus and magnesium, and the excretion of urinary boron. — Environ Health Perspect 102(Suppl 7):79–82 (1994)

Key words: boron supplementation, female athletes, serum minerals, urinary mineral excretion, calcium, phosphorus, magnesium

## Introduction

We performed a study to ascertain the effects of boron supplementation on bone mineral density (BMD) and the mineral status of college female athletes. The results of selected diet and mineral analyses will be reported here (1,2).

Previous studies have linked vigorous exercise with amenorrhea, and associated this condition with the likelihood of increased incidence of stress fractures and increased risk of osteoporosis, particularly among young female athletes. Early studies by Drinkwater et al. (3), Lindberg et al. (4), and Marcus et al. (5) have reported that the BMD of the vertebrae is lower in amenorrheic athletes than in eumenorrheic or normal subjects (Table 1).

Researchers at the United States Department of Agriculture, Agricultural

Research Service (USDA, ARS), Grand Forks Human Nutrition Research Center in Grand Forks, ND have conducted a number of human studies on boron supplementation (6–9). Their findings can be briefly stated as follows: *a*) High dietary boron lowered urinary calcium and total calcium, and increased blood-ionized calcium, 17 $\beta$  estradiol, and testosterone. *b*) Blood-ionized calcium was higher and serum calcitonin was lower with boron repletion than with boron depletion. *c*) The researchers concluded that changes seen with boron supplementation induced changes in postmenopausal women consistent with the prevention of calcium and bone loss.

Thus, boron may have a preventive or therapeutic effect that helps to diminish

bone mineral loss in susceptible populations.

## Methodology

In the present study, 28 female students were recruited from Virginia Polytechnic Institute and State University, and Ferrum College. The subjects were screened for such factors as oral contraceptive use and smoking. The selected subjects were then assigned to four treatment groups in a single-blind study; ten athletic subjects received the boron supplement, while seven received a cornstarch placebo, and six sedentary subjects received the boron supplement, while five received the placebo. All subjects were instructed to take daily either a boron supplement, 3 mg/day (Tri-Boron, Twin Laboratories, Inc.,

**Table 1.** Amenorrhea and bone mineral density.

Measurement	Assessment	Reference
Amenorrheic = 1.12 Eumenorrheic = 1.30	DPA-L1-L4	Drinkwater et al. 1984 (3)
Amenorrheic = 1.08 Normal = 1.2–1.6	DPA-vertebrae	Lindberg et al. 1984 (4)
Amenorrheic = 1.51 Eumenorrheic = 1.81	CT-L1-L2	Marcus et al. 1985 (5)

Abbreviations: DPA, dual photon absorptiometry; CT, calorimetric test.

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Ronkonkoma, NY), or a placebo (Revclo Pharmacy, Blacksburg, VA) for 10 months.

Several descriptive tests were performed to assist with characterization of subjects. A physical work performance test (PWC<sub>170</sub>) for aerobic capacity was performed on a Monark bicycle ergometer (Monark-Crescent AB, Vargerg, Sweden) to confirm the athletic status of the subjects. Skinfold measurements of the triceps, suprailiac, and thigh were used to determine the subjects' body fat percentages (10).

To characterize the dietary patterns of the free-living subjects, duplicate plates were collected over 3 days at the beginning of the study. The macronutrient composition of the subjects' diets was determined from aliquots of homogenized composites by proximate analysis. Concentrations of several selected dietary minerals were determined by either atomic absorptiometry (AA), colorimetry, or inductively coupled argon plasma spectroscopy (ICAP).

Bone mineral density analyses were performed on a LUNAR DP3 bone density absorptiometer at Montgomery Regional Hospital in Blacksburg, VA.

Fasting blood samples were collected in mineral-free tubes, and blood plasma and serum fractions were frozen in polyethylene tubes for subsequent analysis. Blood minerals were determined by several analytical procedures. Serum total calcium was determined by AA. Also, plasma total, and normalized and ionized calcium were determined immediately following collection, using a NOVA 7 electrolyte analyzer. Blood phosphorus was determined colorimetrically, and blood magnesium was determined by AA. Serum boron was determined by ICAP, applying a low-temperature, Teflon tube, wet-ashing digestion procedure (11).

Urinary calcium was analyzed by AA and ICAP; urinary phosphorus was determined colorimetrically and by ICAP; and urinary magnesium was determined using AA and ICAP. Urinary boron was determined by ICAP following the digestion procedures described by Hunt and Schuler (11).

## Results and Discussion

The athletic and sedentary subjects were similar in age and body weight (Table 2). However, their body fat and aerobic capacities differed significantly ( $p < 0.05$ ). The athletes' average percent body fat was 20.6, compared to the sedentary groups' percent body fat of 25.8 ( $p < 0.05$ ). The subjects' physical work capacity, determined by  $VO_2$  max, was higher in the athletic groups

**Table 2.** Age, body weight, body fat and  $VO_2$  max.

Parameter	Athletes (n = 17)	Sedentary (n = 11)
Age, years	19.8 ± 1.4 <sup>a</sup>	20.3 ± 1.1
Body weight, kg	61.8 ± 9.1	59.6 ± 10.5
Body fat, % <sup>b</sup>	20.6 ± 5.6	25.8 ± 6.5
$VO_2$ max, $LO_2$ /min <sup>b</sup>	2.9 ± 0.5	2.1 ± 0.4

<sup>a</sup> Values shown are means ± standard deviation.

<sup>b</sup> Significant difference between groups ( $p < 0.05$ ).

(2.9) than in the sedentary groups (2.1) ( $p < 0.05$ ).

The athletic and sedentary controls did not differ in their daily consumption of total kilocalories, percent protein, fat, and carbohydrate as determined by proximate analysis (Table 3). Also, the intake of milligrams of dietary calcium, phosphorus, and magnesium did not differ between activity groups. However, the athletes' dietary boron intake levels were higher than those for sedentary controls when expressed as either total mg boron/day or as mg boron/kg dry matter/day ( $p < 0.05$ ). Macromineral intakes fell markedly below the RDAs for both groups (12).

Bone mineral density determinations of the lumbar vertebrae were higher for athletes than for sedentary subjects ( $p < 0.05$ ) (Table 4). Also, over time, the differences in BMD between the two activity groups were significant ( $p < 0.05$ ); BMD increased by 0.03 g/cm<sup>2</sup> in the athletic group and decreased by -0.005 g/cm<sup>2</sup> in the sedentary group. However, bone mineral densi-

ties did not seem to be influenced directly by boron supplementation.

Other changes over time were observed in serum total calcium determinations. Concentrations of serum calcium, when determined by AA, and plasma-ionized calcium, when determined by the NOVA 7, increased significantly over time. This increase over time was not seen with plasma total calcium determinations from the electrolyte analyzer (Table 5).

In contrast, serum phosphorus decreased over time among all subjects. In addition, serum phosphorus was significantly lower in boron-supplemented subjects than in subjects receiving the placebo ( $p < 0.05$ ). A significant group × supplement interaction was also observed ( $p < 0.05$ ). At both testing times, the sedentary group fed the boron supplement had the lowest serum phosphorus levels; the sedentary placebo group had the highest serum phosphorus levels at both testing times ( $p < 0.05$ ). Activity depressed the effect of boron supplementation observed in sedentary controls ( $p < 0.05$ ) (Table 5).

Serum magnesium levels, like calcium levels, increased over time among all subjects ( $p < 0.05$ ). Also, a significant group × supplement interaction was observed—sedentary subjects who were fed boron supplements exhibited higher serum magnesium levels than athletic subjects who were fed the boron supplement. Mean serum magnesium concentration was significantly

**Table 3.** Subjects' daily dietary intakes assessed by laboratory analyses.

	Athletes (n = 17)	Sedentary (n = 11)	RDAs
Energy, kcal	1468 ± 503 <sup>a</sup>	1417 ± 584	
Protein, %	14.1 ± 5.1	14.9 ± 5.2	12
Fat, %	28.7 ± 7.7	30.8 ± 6.3	(<30)
Carbohydrate, %	57.3 ± 9.8	54.3 ± 6.3	55–60
Calcium, mg	650 ± 558	714 ± 442	1200
Phosphorus, mg	915 ± 616	840 ± 330	1200
Magnesium, mg	103 ± 107	73 ± 30	280
Boron, μg <sup>b</sup>	1.5 ± 1.3	0.7 ± 0.3	—

<sup>a</sup> Values shown are means ± standard deviation. <sup>b</sup> Significant difference between groups ( $p < 0.05$ ).

**Table 4.** Bone mineral density for activity and supplement groups.

Time	Bone mineral density, g/cm <sup>2</sup>			
	Athletes		Sedentary	
	Boron (n = 10)	Placebo (n = 7)	Boron (n = 6)	Placebo (n = 5)
Month 0	1.27 ± 0.14 <sup>a</sup>	1.3 ± 0.08	1.25 ± 0.11	1.19 ± 0.11
Month 10	1.3 ± 0.16	1.34 ± 0.09	1.26 ± 0.13	1.17 ± 0.11
Difference <sup>b</sup>	+0.024 ± 0.049	+0.036 ± 0.038	+0.012 ± 0.044	-0.024 ± 0.029

<sup>a</sup> Values shown are means ± standard deviation. <sup>b</sup> Significantly greater for the athletes than for the sedentary group ( $p < 0.05$ ).

**Table 5.** Pre- and post-test blood calcium (mg/DI).

	Time	Athletes		Sedentary	
		Boron (n=10)	Placebo (n=7)	Boron (n=6)	Placebo (n=5)
TCa (AA) <sup>a</sup>	1	8.9 ± 0.4 <sup>b</sup>	8.8 ± 0.2	9.1 ± 0.2	9.0 ± 0.5
	2 <sup>f</sup>	9.6 ± 0.3	9.8 ± 0.3	9.8 ± 0.2	9.7 ± 0.4
TCa (NOVA)	1 <sup>g</sup>	9.4 ± 0.5	9.7 ± 0.6	9.4 ± 0.4	9.7 ± 0.2
	2	9.6 ± 0.2	9.7 ± 0.3	9.5 ± 0.2	9.4 ± 0.1
iCa (NOVA) <sup>a</sup>	1	4.6 ± 0.3	4.7 ± 0.2	4.7 ± 0.1	4.5 ± 0.0
	2	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.1	4.7 ± 0.2
P (C) <sup>a,c,d</sup>	1	4.7 ± 0.4	4.7 ± 0.6	4.3 ± 0.5	5.1 ± 0.3
	2	3.9 ± 0.2	4.0 ± 0.2	3.6 ± 1.0	4.0 ± 0.3
Mg (AA) <sup>a,d,e</sup>	1	1.5 ± 0.3	1.7 ± 0.2	1.9 ± 0.3	1.7 ± 0.2
	2	2.0 ± 0.0	2.0 ± 0.1	2.4 ± 0.4	2.0 ± 0.1

Abbreviations: AA, atomic absorptiometry; NOVA, NOVA 7 electrolytic analyzer; C, calorimetry. <sup>a</sup> Significant time effect ( $p < 0.05$ ). <sup>b</sup> Values shown are means ± standard deviation. <sup>c</sup> Significant supplement effect ( $p < 0.05$ ). <sup>d</sup> Significant group × supplement effect ( $p < 0.05$ ). <sup>e</sup> Significant group effect ( $p < 0.05$ ). <sup>f</sup> Time 2, tests performed at the end of the study. <sup>g</sup> Time 1, tests performed at the beginning of the study.

**Table 6.** Pre- and post-test urinary calcium and phosphorus.

	Time	Athletes		Sedentary	
		Boron (n=10)	Placebo (n=7)	Boron (n=6)	Placebo (n=5)
Ca (mg/day) (AA) <sup>a</sup>	1 <sup>d</sup>	50 ± 45 <sup>b</sup>	72 ± 50	62 ± 37	47 ± 31
	2 <sup>e</sup>	106 ± 40	89 ± 27	74 ± 41	53 ± 16
P (mg/day) (C)	1	760 ± 791	719 ± 394	755 ± 364	480 ± 316
	2	601 ± 280	606 ± 178	767 ± 312	672 ± 240
Mg (mg/day) (AA)	1	53 ± 30	67 ± 44	60 ± 23	53 ± 33
	2	118 ± 98	72 ± 34	70 ± 36	52 ± 33
B (µg/day) (ICP) <sup>a,c</sup>	1	0.7 ± 0.6	0.7 ± 0.3	0.7 ± 0.3	0.5 ± 0.3
	2	2.8 ± 1.6	0.8 ± 0.8	1.1 ± 1.1	0.7 ± 0.6

Abbreviations: AA, atomic absorptiometry; C, calorimetry; ICP, inductively coupled argon plasma spectroscopy. <sup>a</sup> Significant time effect ( $p < 0.05$ ). <sup>b</sup> Values shown are means ± standard deviation. <sup>c</sup> Significant group, supplement and time × supplement effects ( $p < 0.05$ ). <sup>d</sup> Time 1, tests performed at the beginning of the study. <sup>e</sup> Time 2, tests performed at the end of the study.

higher ( $p < 0.05$ ) in athletes than in sedentary controls.

Boron analysis of subjects' serum was attempted. Following wet-ashing, low-temperature, Teflon tube (WALTTT) digestions, serum analysis revealed values in the 0.15 to 0.31 µg/ml range. Because of the small amount of the sample and the low concentration of boron typically expected in serum, the validity of these values warrants additional study to improve the standard error rate.

Urinary calcium, like serum calcium, increased over time ( $p < 0.05$ ) (Table 6). Urinary phosphorus and magnesium did not reflect any significant changes ( $p < 0.05$ ) over time, either between active and inactive groups or with supplementation (Table 6). Urinary boron, however, differed significantly over time, both between high- and low-activity groups and with supplementation ( $p < 0.05$ ). Boron excretion increased over time; excretion was higher among athletes than sedentary controls and was greater in boron-supple-

mented subjects than in those receiving the placebo ( $p < 0.05$ ). At the end of the experimental period, a time × supplement interaction was observed in which subjects who had received boron supplementation for 10 months exhibited greater boron excretion levels than subjects who received the placebos ( $p < 0.05$ ).

Here is a brief summary of the present findings: *a*) Athletic subjects and controls differed in aerobic capacity and percent body fat, but not in age or body weight. *b*) Dietary intakes between groups did not differ for selected nutrients measured, except for dietary boron, which was higher in athletes. *c*) Serum and plasma calcium increased over time in all subjects. *d*) Serum phosphorus decreased over time and decreased with boron supplementation in sedentary subjects. *e*) Serum magnesium increased over time, and increased with boron supplementation in sedentary subjects. *f*) Urinary calcium increased over time, but urinary phosphorus and magnesium did not change. *g*) Urinary

boron increased over time with dietary boron supplementation.

## Conclusion

Our 1-year study of athletic and sedentary female college students indicated that boron affected blood phosphorus and magnesium, an effect modified by exercise. The dietary patterns were similar between athletes and sedentary individuals (except for dietary boron), and all subjects had intakes below the RDA for calcium, phosphorus, magnesium, and total energy. Before preventive or therapeutic recommendations can be made with regard to boron supplementation for bone disorders, the effects of low- and unknown boron intakes and of boron supplementation need further investigation as regards bone metabolism in humans.

## Recommendations for Further Research

Recommendations for future research include studies on: *a*) the effects of various boron supplementation levels at various physical work capacities on dietary, blood, and urinary Ca, P, Mg, and calcitonin and parathyroid hormone levels; *b*) the comparison of effects of varied levels of boron supplementation on males and females of various ages, physical work capacities (PWCs), and hormonal conditions; *c*) the effects of interactions of various dietary intakes of Ca, phosphorus, Mg, and boron on bone, blood, and urine minerals; *d*) methods of analysis for Ca, phosphorus, Mg, and boron in food, blood, and urine; *e*) the mechanism of action for boron (in vitro and animal studies) as it relates to bone metabolism.

Human studies characterizing "normal" physiologic levels of boron are needed at this time to advance our understanding of boron's role in normal human nutrition—our ultimate goal. To accomplish this goal, reliable analysis techniques and digestion procedures for study of dietary boron are needed.

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