Effect of boron supplementation on blood and urinary calcium, magnesium, and phosphorus, and urinary boron in athletic and sedentary women[1–4]

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ABSTRACT It has been reported that boron may be beneficial for optimal calcium metabolism and, thus, optimal bone metabolism. Therefore, we designed a study to determine the effects of boron supplementation on blood and urinary minerals in athletic subjects and sedentary control subjects consuming self-selected typical Western diets. Serum phosphorus concentrations were lower in boron-supplemented subjects than in placebo-supplemented subjects. Compared with all other subjects, serum magnesium concentrations were greatest in the sedentary control subjects supplemented with boron and increased with time in all subjects. Exercise training diminished changes in serum phosphorus concentrations caused by boron supplementation. Calcium excretion increased over time in all groups combined, and boron excretion increased over time in all boron-supplemented subjects. The findings suggest that boron supplementation modestly affected mineral status, and exercise modified the effects of boron supplementation on serum minerals. *Am J Clin Nutr* 1995;61:341–5

KEY WORDS Female, athletes, calcium, phosphorus, magnesium

Introduction

Weight-bearing exercise increases bone hypertrophy and bone mineral density (1–3). However, female athletes may be at risk for developing exercise-induced menstrual dysfunction (2, 4, 5) and the consequences of hypoestrogenic status can include loss of bone mineral, an increased risk of developing osteoporosis, and increased musculoskeletal injuries (2, 4, 6–8).

The underlying physiological mechanisms for changes in bone mineral density induced by exercise have not been elucidated (2). Numerous studies have selectively characterized the effects of various nutritional patterns in estrogen-deficient females, all of whom were at risk for osteoporosis (6, 9–11).

Reports of the effectiveness of nutrient supplementation on bone mineral density in exercising individuals are conflicting (12). High calcium supplementation may be beneficial (2) although there is evidence that such supplementation causes metabolic disturbance of nutrients (13). In 1987 Nielsen et al (13) found that boron supplementation induced changes consistent with the prevention of calcium loss and bone demineralization in postmenopausal women. Additional work by these researchers indicated that boron is beneficial for optimal calcium metabolism and in the prevention of bone loss, which occurs in postmenopausal women and older men (14, 15). Nutritional approaches to the prevention or treatment of osteoporosis could be useful to women who are at risk for osteoporosis and are unable to take estrogen therapy.

The present study was designed to examine the effect of boron supplementation on mineral status in college female athletes consuming typical Western diets. Some of the effects of boron supplementation on serum and urinary mineral concentrations are reported.

Subjects and methods

Subjects and study design

Protocol for the recruitment and treatment of human subjects was reviewed by the Virginia Polytechnic Institute and State University Institutional Review Board for Research Involving Human Subjects at the University. Twenty-eight college females aged 18–25 y (26 white, 1 African-American, 1 Indian) were selected and completed the 10-mo study. Subjects were classified as either athletic or sedentary, depending on the level of activity in which they engaged. Athletic subjects (n = 17) were recruited mostly from University basketball, volleyball, tennis, and track and field programs. Sedentary individuals (n = 11) were selected from among their peers and initially identified through questionnaires as individuals who did not engage in any regular exercise as part of their daily routine. Subjects were excluded if they had a history of smoking, previous pregnancies, eating disorders, or orthopedic problems; had used recreational drugs, oral contraceptives, or anabolic steroids within the previous 6 mo; or had used oral contraceptives for >6 mo cumulatively. Menstrual status was classified

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as eumenorrheic if menses occurred every 25–35 d (10–13 menses/y). Menstrual status was confirmed with monthly calendar recordings of menses and weekly serum hormone assays during months 0, 6, and 10. Subjects also completed monthly menstrual records to accurately assess symptoms of their menses (ie, headaches, bloating, back and breast aches, and daily gauge of menstrual flow as heavy, medium, or light). All subjects received an oral and written explanation of the purpose of the study and procedures to be followed; all gave written consent. An additional questionnaire was completed at the end of the study to ascertain that subjects' health, exercise, and dietary habits had been consistent throughout the year.

In a single blind, random assignment, the 28 subjects were given daily either 3 mg boron (Tri-Boron; Twin Laboratories, Inc, Ronkonkoma, NY) or a placebo consisting of cornstarch in gelatin capsules (Revco Pharmacy, Blacksburg, VA). Tri-Boron is a combination of three chelated sources of the trace mineral boron as boron citrate, aspartate, and glycinate. No dietary restrictions were imposed on any of the four experimental groups during the study.

Procedures

A Physical Work Capacity 170 Test (PWC_{170}) was performed on a bicycle ergometer to verify that the athletes differed from the sedentary control subjects in physical condition. The PWC_{170} is a submaximal exercise test designed to predict subjects' maximal oxygen consumption by assessing their aerobic work capacity when a submaximal heart rate of 170 beats/min is obtained.

Triceps, suprailiac, and midthigh skinfold-thickness measurements were determined with Lange calipers (10/mm, constant pressure; Cambridge Scientific Instruments, Inc, Cambridge, MD). The average of five trial measurements was used as the representative score for each site to calculate the percent fat for each subject (16).

Before supplementation, the subjects provided a daily food record and a duplicate-plate collection of all food and beverages consumed for 3 d, including one weekend day.

Twelve- to 14-h fasting blood samples (30 mL) were drawn by qualified personnel at 0 and 10 mo. Blood was taken from a subject's antecubital vein into mineral-free Vacutainers (Becton-Dickinson, Sparks, MD). Vacutainers with anticoagulant additives were centrifuged on a clinical centrifuge at a low setting for 10 min. Plasma samples for ionized calcium were analyzed immediately; the remaining plasma was stored (−20 °C) in duplicate in Sarstedt (Newton, NC) tubes. The sera from additive-free Vacutainers was refrigerated and allowed to clot, and several hours later separated from cells in a clinical centrifuge, transferred to plastic Sarstedt tubes, and stored (−20 °C) until analyzed at the completion of the study.

Baseline 24-h urine samples were collected by the subjects into 1-L acid-washed polypropylene jugs during the 3-d period in which food collections were made and food records kept; a single-day collection was made at 10 mo on a day that also coincided with food collections and records. Daily volumes were recorded, aliquots were taken, and a 3-d composite was stored at 20 °C for analysis at the end of the study. Urine pH was recorded when the samples were analyzed for ionized calcium by using the NOVA 7/7 + 7 Electrolyte Analyzer (NOVA Biomedical, Waltham, WA).

Duplicate-plate food samples were analyzed for moisture, protein, and fat, and values for carbohydrate and total energy were calculated (Forage Testing Laboratory, Department of Dairy Science, Virginia Polytechnic Institute, Blacksburg, VA). For mineral determinations, 100-g wet-weight samples of homogenized composites were frozen, freeze-dried; and stored at −20 °C in plastic containers. All freeze-dried composites were digested at the completion of the study by a nitric-perchloric wet-ash procedure (Department of Human Nutrition and Foods, Virginia Polytechnic Institute) and analyzed for calcium and magnesium contents by a model 2100 atomic-absorption spectrometer (Perkin-Elmer Corp, Norwalk, CT). Serum magnesium and urine calcium and magnesium were also determined by atomic absorption. SeraChem clinical chemistry control serum (human) level 1 (Fisher Scientific, Orangeburg, NY) data sheets for the commercially supplied serum standards were used for reference. The determination of plasma ionized calcium in clinical samples is preferred over plasma total calcium values. Ionized calcium corrected for changes in plasma pH is referred to as normalized calcium (17). Normalized calcium was determined on the day of collection by using a NOVA 7/7 + 7 Electrolyte Analyzer. Dietary, serum, and urinary phosphorus were determined by a colorimetric procedure (#670-C; Sigma Diagnostics, St Louis). Samples for urinary boron determinations were digested by the wet-ash, low-temperature, tetrahydrofluoranamide tube procedure developed by Hunt and Shuler (18) and were analyzed by inductively coupled argon plasma spectroscopy (ICAP 9000; Thermo Jarrell Ash Corp, Franklin, MA).

Statistical analysis

A one-way analysis of variance (ANOVA) was performed on age, body weight, skinfold thickness, and workload achieved (**Table 1**). Repeated-measures ANOVA was performed for the following variables: dietary information and blood and urinary minerals (Table 1 and **Tables 2** and **3**). Tukey's post hoc test was computed to locate differences (19). Significance was set, a priori, at $P = 0.05$.

TABLE 1
Physical characteristics and daily dietary intakes of athletic and sedentary women (month 0)[1]

	Athletic (n = 17)	Sedentary (n = 11)
Age (y)	19.8 ± 1.4	20.3 ± 1.1
Body weight (kg)	61.8 ± 9.1	59.6 ± 10.5
Skinfold thickness (mm)	52 ± 20	69 ± 21[2]
Workload (W)	159 ± 32	109 ± 18[2]
Energy (kJ/d)	6166 ± 2113	5931 ± 2453
Protein (%)	14.1 ± 5.1	14.9 ± 5.2
Fat (%)	28.7 ± 7.7	30.8 ± 6.3
Carbohydrate (%)	57.3 ± 9.8	54.3 ± 6.3
Calcium (mg/d)	650 ± 558	714 ± 442
Phosphorus (mg/d)	915 ± 616	840 ± 330
Magnesium (mg/d)	103 ± 107	73 ± 30
Boron (mg/d)	1.5 ± 1.3	0.7 ± 0.3

[1] $\bar{x}$ ± SD.
[2] Significantly different from athletes, $P < 0.05$.

TABLE 2
Effects of dietary boron supplementation on blood calcium, phosphorus, and magnesium concentrations in athletic and sedentary women[1]

	Athletic		Sedentary	
	Boron (n = 10)	Placebo (n = 7)	Boron (n = 6)	Placebo (n = 5)
Plasma calcium, normalized (mmol/L)				
0 mo	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0
10 mo	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1
Serum phosphorus (mmol/L)[2-4]				
0 mo	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.2	1.7 ± 0.1
10 mo	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.0	1.4 ± 0.1
Serum magnesium (mmol/L)[2,4,5]				
0 mo	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
10 mo	0.8 ± 0.0	0.8 ± 0.0	1.0 ± 0.2	0.8 ± 0.0

[1] $\bar{x}$ ± SD.
[2] Significant difference over time (combined over activity and supplementation), $P < 0.05$.
[3] Significant difference between supplement groups (combined over time and activity), $P < 0.05$.
[4] Significant group-supplement interaction, $P < 0.05$.
[5] Significant difference between activity groups (combined over time and supplementation, $P < 0.05$.

Results

All subjects completed the 10-mo supplementation period. Frequent interactions, inherent in the study design, and informal conversations with subjects provided assurance that subjects complied with the treatment regimen. The athletic subjects did not differ from the sedentary group in age or body weight (Table 1). Baseline skinfold thicknesses were lower in athletes than in sedentary individuals. Average workload capacity, assessed by the PWC_{170} test, was greater in the athletes than in the sedentary subjects.

Daily percentages of energy consumed as protein, fat, and carbohydrate and intakes of calcium, phosphorus, and magnesium did not differ between activity groups. Baseline dietary boron intakes were also determined and found to be 0.7 ± 0.3 and 1.5 ± 1.3 mg/d for sedentary and athletic control subjects, respectively (Table 1). This information is presented in more detail in a subsequent publication (20).

Plasma normalized calcium concentrations did not differ with time, activity, or supplementation (Table 2). However, final serum phosphorus concentrations, summed over all groups, were lower than baseline values ($P < 0.05$).

Final serum magnesium values were significantly higher than baseline values ($P < 0.05$). Serum magnesium concentrations were different between activity groups. The athletes given boron supplements had lower serum magnesium concentrations than did the sedentary subjects. No differences were identified between activity groups receiving the placebo.

Average daily urinary excretion of calcium, phosphorus, magnesium, and boron are reported in Table 3. At 10 mo, the amount of calcium excreted in the urine was higher ($P < 0.05$) than that determined at the beginning of the study for all groups combined. The athletes tended to have elevated amounts of total urinary calcium compared with sedentary subjects. Urinary excretion of phosphorus and magnesium was not affected by time, activity, or supplementation. Compared baseline, urinary boron excretion increased fourfold in athletes fed supplemental boron for 10 mo. An increase was also seen in sedentary control subjects. The increase in urinary boron excretion was not as dramatic (1.6-fold) over time in sedentary subjects fed supplemental boron for 10 mo. Urinary excretion of boron did not increase over time in subjects receiving placebos.

TABLE 3
Effects of dietary boron supplementation on urinary calcium, phosphorus, magnesium, and boron concentrations in athletic and sedentary women[1]

	Athletic		Sedentary	
	Boron (n = 10)	Placebo (n = 7)	Boron (n = 6)	Placebo (n = 5)
Calcium (mmol/d)[2]				
0 mo	1.3 ± 1.1	1.8 ± 1.3	1.6 ± 0.9	1.2 ± 0.8
10 mo	2.7 ± 1.0	2.2 ± 0.7	1.9 ± 1.0	1.3 ± 0.4
Phosphorus (mmol/d)				
0 mo	24.5 ± 25.8	23.2 ± 12.3	24.4 ± 11.8	15.5 ± 10.2
10 mo	19.4 ± 9.0	19.6 ± 5.8	24.7 ± 10.1	21.7 ± 7.8
Magnesium (mmol/d)				
0 mo	2.2 ± 1.2	2.8 ± 1.8	2.5 ± 1.0	2.2 ± 1.4
10 mo	4.9 ± 4.0	3.0 ± 1.4	2.9 ± 1.5	2.1 ± 1.4
Boron (mmol/d)[2-5]				
0 mo	0.07 ± 0.06	0.07 ± 0.03	0.07 ± 0.03	0.05 ± 0.03
10 mo	0.26 ± 0.15	0.07 ± 0.07	0.10 ± 0.10	0.07 ± 0.06

[1] $\bar{x}$ ± SD.
[2] Significant difference over time (combined over supplementation and activity), $P < 0.05$.
[3] Significant difference between activity groups (combined over time and supplementation), $P < 0.05$.
[4] Significant difference between supplement groups (combined over time and activity), $P < 0.05$.
[5] Significant time-supplementation interaction, $P < 0.05$.

Discussion

Physical characteristics

The greater workload capacity and leaner body composition of the athletic group confirmed physical differences between them and the sedentary groups.

Although female athletes may be at risk for developing exercise-induced menstrual dysfunction (2, 4, 5), this was not evident in the current study. The bone mineral density and hormone-status data reported indicate that activity, and not

boron supplementation, attributed to the differences observed in these variables (21). Additional analyses, discussed in more detail in a subsequent publication, indicated greater bone mineral density and greater final plasma 1,25-dihydroxycholecalciferol observed in the athletic group than in the sedentary group ($P < 0.05$) (21).

Dietary-intake analysis

Dietary boron was the only variable nutrient in the diets of study participants; no differences were found between subjects for average intakes of other minerals or energy. Average baseline dietary boron intakes were within expected intake ranges according to Nielsen et al (13).

In the present study both the athletic and sedentary subjects consumed slightly more than half of the recommended dietary allowance (RDA) for calcium (1200 mg/d) (22). Although slightly higher amounts of phosphorus than calcium were consumed, these intakes were also lower than the RDA (1200 mg/d) (22). There are other reports that female athletes in this same age range consume less energy, calcium, and phosphorus than recommended (2–4). In addition, the dietary intakes of magnesium in the subjects in the present study were markedly below the recommended amount of 280 mg/d for their age group (21).

Blood mineral analysis

In the present study daily supplementation with 3 mg boron for 10 mo affected only blood phosphorus concentrations. Plasma normalized calcium did not differ with time, supplementation, or activity. This finding does not support the findings of Nielsen et al (14, 15), who found that in postmenopausal women fed low amounts of boron (0.23 mg/d), boron supplementation elevated plasma normalized calcium and had no effect on plasma total calcium. These conflicting findings may reflect the difference in subject age (younger females are still accruing bone mineral mass), estrogen status, or possibly basal boron consumption.

In general, the blood values for calcium, phosphorus, and magnesium were within the expected blood concentration ranges. However, the magnesium concentrations in this study were at the lower end of normal range. Low serum magnesium concentrations may be related to disorders of bone mineral metabolism. Steidl et al (23) reported that some osteoporotic patients showed signs of chronic magnesium deficiency, whereas others showed a trend toward low serum calcium concentrations. Because magnesium plays an important role in metabolism, the young female athletes in this study who displayed low serum magnesium concentrations and who voluntarily consumed a low-magnesium diet may be at risk of developing disorders of bone metabolism. The blood magnesium values were influenced by activity, being higher in sedentary than in athletic subjects.

Of the three major minerals important for bone metabolism, phosphorus has received little attention except for concern that phosphorus intakes are too high (24–26). Blood phosphorus concentrations in this study were in the upper end of normal in relation to reference values (27). However, boron supplementation was associated with lower serum phosphorus concentrations ($P < 0.05$). Thus, increasing both dietary calcium and boron may be beneficial in balancing blood minerals, to optimize bone mineralization during this final developmental period.

Urine mineral analysis

The observations reported here in regards to urinary minerals do not coincide with those of Nielsen et al (13), who reported that a supplement of 3.0 mg boron d, added to a low-boron diet (0.23 mg/d), after 4 mo markedly depressed the urinary excretion of calcium and magnesium in postmenopausal women.

The inability to confirm the findings of Nielsen et al (13) in the present study may be attributed to differences in age, hormonal status, boron-deficient status, dietary boron intake, duration of supplementation, statistical power, and/or activity.

As would be expected, boron excretion rates were significantly influenced by boron supplementation. This would explain the significantly different boron excretion rates reported over time and between supplement groups. However, activity also appears to influence boron excretion. The higher boron excretion concentrations in the athletes cannot be explained by supplementation alone, because an increase of the same magnitude was not seen in the sedentary control subjects. Diet should not be discounted. Dietary intakes of boron are higher in the athletes' diets (1.5 ± 1.3 mg/d) than in the sedentary group's diet (0.7 ± 0.3 mg/d). Thus, athletes supplemented with boron were consuming, through diet and supplementation combined, possibly more than the body needed and the excess was being excreted. Whereas, in the sedentary group, the lower dietary boron intake was reflected in the lower boron excretion concentrations. Compliance with supplementation is assumed, as stated earlier, on the basis of the rapport developed between subjects and researchers.

The effects of low intakes of minerals important to normal bone metabolism need further investigation. An inadequate intake or imbalance of one or several of the minerals critical to bone development may jeopardize normal bone metabolism. There has been widespread interest over the years in assuring adequate calcium intakes at critical stages of the female life cycle (28). This interest should be extended to emphasize optimal intakes of all minerals known or suspected to affect bone mineral density, such as calcium, phosphorus, magnesium, and boron.

In summary, boron supplementation affected serum phosphorus and magnesium concentrations in young women and the effect was modified by exercise. Dietary patterns did not appear to differ between athletic and sedentary individuals, and both groups had low intakes of calcium, phosphorus, and magnesium.

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