# Metabolic responses of postmenopausal women to supplemental dietary boron and aluminum during usual and low magnesium intake: boron, calcium, and magnesium absorption and retention and blood mineral concentrations[1-4]

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**ABSTRACT**    Findings from animal studies indicate that dietary boron affects several aspects of mineral metabolism, especially when animals are subjected to nutritional stressors. Eleven postmenopausal volunteers living on a metabolic ward for 167 d (one 23-d equilibration period and six 24-d treatment periods) were fed a conventional basal diet that supplied a daily average intake of 0.36 mg B, 109 mg Mg, and < 0.10 mg Al/8400 kJ. They were given supplements of 0 (BB) or 3 mg B (SB, last two periods only), 0 (BMg) or 200 mg Mg (SMg) (with magnesium supplements held constant during the last two periods), or 0 (BAl) or 1000 mg Al (SAl)/d. The SB treatment, compared with the BB treatment, provided a 9.0-fold increase in dietary boron but yielded only a 1.5-fold increase in plasma boron concentrations. Regardless of boron dietary treatment, fecal plus urinary excretion of boron accounted for nearly 100% of dietary boron intake with no evidence of boron accumulation over time. Lack of boron accumulation and relatively small changes in blood boron values during a substantial increase in dietary boron support the concept of boron homeostasis. In subjects fed BMg, SB decreased the percentage of dietary calcium lost in the urine but increased that percentage in volunteers fed SMg, a relation that may be important in understanding metabolic mineral disorders that perturb calcium balance. Reduced calcium absorption during SAl suggests that aluminum supplementation should be limited or at least monitored in postmenopausal women prone to excessive calcium loss. Decreased total urinary oxalate during SB in BMg subjects indicates a possible role for boron in the control of urolithiasis during low-magnesium nutriture.    *Am J Clin Nutr* 1997;65:803–13.

**KEY WORDS**    Boron, aluminum, calcium, magnesium, postmenopause, humans, blood pressure, boron absorption, boron metabolism, urinary boron, blood urea nitrogen, oxalate, plasma boron, red blood cell boron

## INTRODUCTION

In 1981, Hunt and Nielsen (1) reported that vitamin $D_3$ (cholecalciferol)-deficient chicks responded to boron supplementation with improved growth and reduction in abnormally elevated plasma alkaline phosphatase activity. The findings suggested that boron positively affected calcium metabolism and prompted further investigation into the influence of dietary boron on bone and mineral metabolism. A subsequent group of studies showed that boron affected tibial growth plate morphology in these chicks (2) and bone magnesium concentrations in rats (3). The effects were more pronounced when the diets were manipulated to cause nutritional stress. Those findings prompted the first known investigation of the effects of dietary boron in women with a typical physiologic stressor (low circulating estrogen, postmenopause) compounded by concurrent nutritional stressors (low dietary magnesium and high dietary aluminum) for calcium metabolism.

Initial findings from the investigation with postmenopausal women were reported in 1987 (4). The findings indicated that urinary calcium and magnesium excretion were decreased in the women when their low-boron diet (0.36 mg B/d) was supplemented with boron (3 mg B/d). The decrease seemed more marked when dietary magnesium was low.

Complete boron, calcium, and magnesium balance data from the investigation are reported here with the successful development and validation of an analytical method for concurrent boron analysis (5). Urinary calcium and magnesium excretion data reported earlier are corrected here for the influence of fluctuating energy intake over the 6-mo course of the study. Thus, this report provides an in-depth analysis of boron, calcium, and magnesium absorption and retention in postmeno-

pausal women maintained in a highly controlled metabolic unit.

## SUBJECTS AND METHODS

### Volunteer selection

Healthy postmenopausal women were selected on the basis of medical (normal bone, kidney, and liver function; normal blood pressure; no chronic medication; negative lung scan), psychologic [free of psychopathology as determined by the Minnesota Multiphasic Personality Inventory (NCS Assessment, Minneapolis), an extensive in-house psychologic history questionnaire and clinical interview], and nutritional (no pertinent food allergies or refusal to eat required foods) data.

After selection, volunteers were admitted to the study after being informed of its purpose and associated risks. The project was approved by the Institutional Review Board of the University of North Dakota and the Human Studies Committee of the US Department of Agriculture, Agricultural Research Service. Informed consent and experimental procedures were consistent with the Declaration of Helsinki. All subjects were chaperoned when they left the metabolic unit to prevent ingestion of unauthorized foods or loss of excreta samples.

Fifteen postmenopausal women began the study initially. Two subjects left the study for personal reasons; one replacement volunteer did not complete all experimental phases. Data from one volunteer on estrogen therapy were not included because previous findings from this study indicated that boron supplementation affects serum $17\beta$-estradiol concentrations (4).

The 11 subjects (not receiving estrogen therapy) who completed the study were aged $61.4 \pm 9.7$ y ($\bar{x} \pm$ SD) (range: 48–82 y); all were white. They were $165 \pm 7$ cm tall and weighed $66.3 \pm 8.4$ kg at the beginning of the study. All subjects had plasma calcium and magnesium concentrations within the reference range of healthy subjects for our laboratory at admittance into the study (6). All subjects completed 3-d food diaries before admission into the study. On the basis of dietary interview and computerized nutrient-intake calculations (GRAND, Grand Forks Research Analysis of Nutrient Data; USDA/ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND) from the food dietary data (7–9), average calcium and magnesium intakes for these 11 volunteers were $800 \pm 288$ and $280 \pm 94$ mg/d, respectively, before their entry into the study. Smoking, alcohol consumption, and

drug use were prohibited and random screening was done to monitor compliance. Various initial (cannabinoid, cocaine, phencyclidine, and methadone) and monthly (opiate, barbiturate, amphetamine, benzodiazepine, and alcohol) urine drug screens were all negative.

### Experimental design

The experimental design is summarized in **Table 1**. Subjects were fed a basal diet that supplied an average of 0.36 mg B, 109 mg Mg, and $< 0.10$ mg Al/8400 kJ. The experimental treatments were daily supplements (described below) of 0 or 3 mg B, 0 or 200 mg Mg, and 0 or 1000 mg Al.

The volunteers lived on a metabolic unit under close supervision for 167 d (divided into dietary periods that were subdivided into 6-d excreta collection periods). After an equilibration period of 23 d (basal diet supplemented with 200 mg Mg/d), all women participated in four 24-d dietary periods: *1)* basal diet only, *2)* basal diet supplemented with 1000 mg Al/d, *3)* basal diet supplemented with 200 mg Mg/d, and *4)* basal diet supplemented with 1000 mg Al and 200 mg Mg/d. The treatments were arranged in a Latin-square design.

Completion of these four 24-d periods and the equilibration period meant that the volunteers were fed a diet low in boron for 119 d. After completing this phase of the study, all volunteers participated in two additional 24-d dietary periods in which the basal diet was supplemented with 3 mg B/d. Six women were fed the boron basal diet only and the boron basal diet supplemented with 1000 mg Al/d; thus, these six women were fed a diet low in magnesium for the full 48 d. The other five women were fed the boron basal diet supplemented with 200 mg Mg/d and the boron basal diet supplemented with 200 mg Mg and 1000 mg Al/d. All dietary supplements throughout the study were fed in a double-blind fashion (except for boron) and given in divided doses at mealtimes.

### Diet

The experimental treatments were daily supplements of 0 or 3 mg B (as $Na_2B_4O_7 \cdot 10H_2O$; JT Baker Inc, Phillipsburg, NJ) in gelatin capsules that each provided $1.0 \pm 0.02$ mg B/capsule); 0 or 200 mg Mg (as $C_{12}H_{26}MgO_{16}$; Freeda Vitamins, Inc, New York) in gelatin capsules that each provided $25 \pm 0.5$ mg Mg/capsule, or 0 or 1000 mg Al [as $Al(OH)_3$; Fisher Scientific, Pittsburgh] in gelatin capsules that each provided $167 \pm 3.3$

**TABLE 1**
Experimental design[1]

| Dietary period and length | Treatments | | | | |
|---|---|---|---|---|---|
| | B | Mg, Al | Mg, Al | Mg, Al | Mg, Al |
| | *mg/d* | | *mg/d* | | |
| 0, 23 d | 0.36 | 309, <0.1 [3][2] | 309, <0.1 [3] | 309, <0.1 [3] | 309, <0.1 [3] |
| 1, 24 d | 0.36 | 109, <0.1 [3] | 109, 1000 [3] | 309, <0.1 [3] | 309, 1000 [2] |
| 2, 24 d | 0.36 | 309, <0.1 [3] | 309, 1000 [3] | 109, 1000 [3] | 109, <0.1 [2] |
| 3, 24 d | 0.36 | 109, 1000 [3] | 109, <0.1 [3] | 309, 1000 [3] | 309, <0.1 [2] |
| 4, 24 d | 0.36 | 309, 1000 [3] | 309, <0.1 [3] | 109, <0.1 [3] | 109, 1000 [2] |
| 5, 24 d | 3.23 | 109, <0.1 [2] | 109, 1000 [4] | 309, <0.1 [2] | 309, 1000 [3] |
| 6, 24 d | 3.23 | 109, 1000 [2] | 109, <0.1 [4] | 309, 1000 [2] | 309, <0.1 [3] |

[1] Period 0 was an equilibration period. For periods 1–4, the magnesium and aluminum treatments were arranged in a Latin-square design. For periods 5–6, the magnesium treatment remained constant and was the reverse of that fed in period 4.

[2] *n* in brackets.

mg Al/capsule (analyzed value: 176.5 mg Al/capsule). Placebos were indistinguishable from the paired supplements.

The basal diet was composed of conventional foods but low in fruit and vegetables to minimize dietary sources of boron and was planned on a 3-d rotating menu cycle (Table 2). In addition to the basal diet, volunteers were allowed to consume limited quantities of several low-energy foods containing no significant amounts of boron, magnesium, or aluminum. Salt, pepper, and coffee, if selected individually by each volunteer, were served in constant amounts throughout the study. Recorded quantities of sugar-free lemonade and tropical punch (sweetened with aspartame) were consumed ad libitum but limited to 0.96 L/d. Except for the lemonade and punch drinks, all dietary ingredients were weighed to within 1% accuracy. Unrecorded quantities of deionized water ($\approx$18.0 M$\Omega \cdot$ cm; Super Q system, Millipore Corp, Bedford, MA) were consumed ad libitum.

The basal diet was planned as a weighed metabolic diet that provided 6700–10 000 kJ/d (1600–2400 kcal/d) at 830-kJ (200-kcal) intervals. The 8400-kJ (2000-kcal) diet was the baseline from which other energy levels were derived by varying the amounts of all foods but not the amounts of specific vitamin and mineral supplements described below (Table 3). The energy intake of each volunteer was based on her energy needs as calculated with the Harris-Benedict equation (10) plus an additional 50% of basal energy expenditure for normal activity. Initial body weight was maintained ($\pm$ 2%) by adjusting energy intake.

To ensure adequacy, the menu was supplemented with some nutrients (Table 3) in constant amounts: 630 mg K/d as 1.2 g KCl (USP grade; JT Baker Chemical Co); an average of 135 mg Ca/d given as one or two tablets on alternate days as calcium gluconate (90 mg Ca/tablet; Eli Lilly & Co, Indianapolis), one at lunch and one at the evening meal; 0.8 mg Cu/d as $CuSO_4$ (USP grade; JT Baker Chemical Co) solution prepared on site, dispensed into a breakfast beverage on day 1, an evening-meal beverage on day 2, and a noon-meal beverage on

day 3; an average of 18 mg Fe/d as one tablet of ferrous gluconate (36 mg Fe/tablet, Fergon; Winthrop Consumer Products, New York) at breakfast on alternate days; an average of 200 $\mu$g folic acid/d given as one tablet (400 $\mu$g folic acid/tablet; Nature's Bounty, Bohemia, NY) on alternate days at breakfast; and 400 IU (10 $\mu$g) cholecalciferol/d as one tablet (Natures's Bounty) at the noon meal. Cholecalciferol was supplemented in amounts greater than the recommended dietary allowance (RDA; 11) to approximate typical US consumption. Total calcium intake was provided in amounts less than the RDA to help magnify the potential effects of dietary boron on calcium and to maximize the effect of excess aluminum supplementation on calcium absorption. Total folic acid intake reflected partial fulfillment of the 1980 RDA (12).

## Blood sampling

After a 10-h fast, blood samples were obtained from the volunteers between 0600 and 0700 on days 7 (60 mL), 16 (60 mL), and 24 (90 mL) in each 24-d dietary period for analyses that included mineral concentrations. To diminish carryover artifact between treatment periods, all reported blood variables represent data obtained from the third draw of each 24-d dietary period only. Blood was drawn from the cubital vein with a butterfly needle into 20-mL polypropylene syringes and an aliquot for serum calcium and magnesium analyses was immediately transferred to evacuated glass tubes with no additive (Vacutainer; Becton Dickinson and Co, Lincoln Park, NJ) and allowed to clot. For plasma copper, iron, and zinc analyses, another aliquot was transferred to polypropylene tubes containing 0.2 mL 10% potassium oxalate (Certified; Fisher Scientific, Fair Lawn, NJ). Both samples were centrifuged (1800 $\times$ g for 10 min at 4 °C) and the serum or plasma was removed by plastic transfer pipette and stored at 0 °C in sealed 5-mL polypropylene tubes (Becton Dickinson and Co). For plasma boron analysis, blood was drawn into an ultraclean polypropylene syringe (Sarstedt, Inc, Newton, NC) that contained heparin (20 000 U heparin in normal saline/L whole

**TABLE 2**
Three-day rotating menu

|  | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| Breakfast | Orange drink mix | French toast | Corn flakes |
|  | Pork sausage | Syrup | Sugar |
|  | White bread | Margarine | 2%-fat milk |
|  | Strawberry jelly | 2%-fat milk | Coffee cake with topping |
|  | Margarine |  | Margarine |
| Dinner | Barbecued beef | Breaded pork | Orange drink mix |
|  | Steamed rice | Parsley potatoes | Hamburger-cheese casserole |
|  | Lettuce | White bread | White bread |
|  | French dressing | Margarine | Margarine |
|  | Pound cake | Shortbread cookies | Vanilla wafers |
| Supper | Vegetable beef stew | Lemon-lime carbonated beverage | Crispy pork |
|  | White bread | Chicken rice soup | Steamed rice |
|  | Cheese | Crackers | Lime gelatin with peaches |
|  | Margarine | Angel food cake | Shortbread cookies |
|  | Peach gelatin | Cherries |  |
|  | Vanilla wafers |  |  |
| Snack | Shortbread cookies | Cherry gelatin with pears | Pound cake with lemon glaze |
|  | 2%-fat milk | Vanilla wafers |  |

**TABLE 4**
Effects of dietary boron, dietary aluminum, and their interaction on urinary and fecal mineral excretion in postmenopausal women fed different amounts of magnesium[1]

| | Treatments | | | | $P^2$ | | | |
| | < 0.1 mg Al/d | | 1000 mg Al/d | | | | | |
| Mineral excretion | 0.36 mg B/d | 3.23 mg B/d | 0.36 mg B/d | 3.23 mg B/d | B | Al | B × Al | RMSE[3] |
|---|---|---|---|---|---|---|---|---|
| Basal magnesium diet | | | | | | | | |
| (109 mg Mg/d total) | | | | | | | | |
| Boron (% of intake) | | | | | | | | |
| Urinary | 102 [6][4] | 89 [5] | 108 [6] | 87 [6] | NS | NS | NS | 30 |
| Fecal | 12 [6] | 3 [5] | 9 [6] | 2 [6] | 0.0001 | 0.03 | NS | 2 |
| Calcium (% of intake) | | | | | | | | |
| Urinary | 18.7 [6] | 17.5 [5] | 19.1 [6] | 17.6 [6] | 0.05 | NS | NS | 1.19 |
| Fecal (%) | 55.6 [6] | 48.6 [5] | 55.9 [6] | 55.2 [6] | NS | NS | NS | 7.67 |
| Magnesium, (% of intake) | | | | | | | | |
| Urinary | 61.2 [6] | 59.6 [5] | 62.0 [6] | 61.5 [6] | NS | NS | NS | 2.46 |
| Fecal | 31.1 [6] | 29.8 [5] | 28.0 [6] | 26.6 [6] | NS | NS | NS | 4.53 |
| Supplemental magnesium diet | | | | | | | | |
| (340 mg Mg/d total)[5] | | | | | | | | |
| Boron (% of intake) | | | | | | | | |
| Urinary | 111 [5] | 89 [5] | 104 [5] | 89 [5] | NS | NS | NS | 38 |
| Fecal | 17 [5] | 5 [5] | 24 [5] | 5 [5] | 0.004 | NS | NS | 10 |
| Calcium (% of intake) | | | | | | | | |
| Urinary | 23.4 [5] | 28.7 [5] | 24.5 [5] | 26.3 [5] | 0.05 | NS | NS | 3.63 |
| Fecal | 67.7 [5] | 65.4 [5] | 69.6 [5] | 82.1 [5] | NS | 0.03 | NS | 8.15 |
| Magnesium (% of intake) | | | | | | | | |
| Urinary | 36.3 [5] | 36.3 [5] | 34.8 [5] | 35.9 [5] | NS | NS | NS | 1.64 |
| Fecal | 45.4 [5] | 42.1 [5] | 42.6 [5] | 48.7 [5] | NS | NS | 0.04 | 4.51 |

[1] Urinary calcium and magnesium excretion data from the present study were published earlier (4) without corrections for changes in daily intake of calcium and magnesium and on the basis of individual daily urine samples instead of composites.

[2] Analyzed by repeated-measures ANOVA.

[3] Root mean square error.

[4] Group mean; $n$ in brackets.

[5] Mean daily amount of dietary magnesium; includes a 200-mg supplement of magnesium (per 8400 kJ) as magnesium gluconate.

percentage of dietary calcium lost in the urine in volunteers fed supplemental magnesium, which indicates that the effect of boron is modified by magnesium nutriture.

Supplemental aluminum substantially decreased apparent calcium absorption in volunteers fed supplemental magnesium (Table 4). The mean daily calcium intakes in volunteers fed no supplemental magnesium or supplemental magnesium were 580 ± 53 and 616 ± 69 mg/d, respectively; respective mean daily calcium urinary losses were 121 ± 49 and 165 ± 108 mg/d and mean daily calcium fecal losses were 314 ± 127 and 430 ± 148 mg/d.

*Magnesium*

An interaction between dietary boron and aluminum affected fecal magnesium in volunteers fed supplemental magnesium (Table 4). Supplemental boron decreased apparent magnesium absorption when the diet contained supplemental aluminum. The mean daily magnesium intakes in volunteers fed no supplemental magnesium or supplemental magnesium were 109 ± 15 and 340 ± 19 mg/d, respectively; respective mean daily urinary magnesium losses were 70 ± 12 and 123 ± 33 mg/d and mean daily fecal magnesium losses were 32 ± 13 and 150 ± 51 mg/d.

**Blood mineral content**

*Boron*

RBC boron concentrations were not affected by boron or aluminum intake nor by an interaction between boron and

aluminum (**Table 5**). The effect of the dietary treatments on plasma boron concentrations could not be assessed because there were too few complete data sets, the result of randomly insufficient plasma volumes. However, by collapsing the magnesium and aluminum treatments, it was determined (by paired $t$ test) that the 9.0-fold increase in dietary boron increased plasma boron concentrations only 1.5-fold (8.79 ± 5.18 compared with 5.92 ± 4.16 µmol B/L; $P < 0.025$). Plasma concentrations of copper, iron, and zinc were not affected by dietary boron, dietary aluminum, or an interaction between those two treatments regardless of magnesium nutriture (data not shown).

*Calcium, magnesium, and potassium*

In volunteers fed no supplemental magnesium, supplemental boron decreased serum magnesium concentrations (Table 5). However, in volunteers fed supplemental magnesium, supplemental boron tended to increase RBC magnesium concentrations ($P < 0.07$). Dietary boron did not affect serum calcium (Table 5) or potassium (data not shown) concentrations in volunteers fed either diets without or with supplemental magnesium.

**Electrocardiograms**

The data collected from the 12-lead ECGs indicated that for all volunteers for all measurement intervals, there was no

**TABLE 5**
Effects of dietary boron, dietary aluminum, and their interaction on blood mineral concentrations in postmenopausal women fed different amounts of magnesium

| | Treatments | | | | $P^1$ | | | |
|---|---|---|---|---|---|---|---|---|
| | < 0.1 mg Al/d | | 1000 mg Al/d | | | | | |
| Blood mineral | 0.36 mg B/d | 3.23 mg B/d | 0.36 mg B/d | 3.23 mg B/d | B | Al | B × Al | RMSE² |
| Basal magnesium diet (109 mg Mg/d total) | | | | | | | | |
| Boron, red blood cell (μmol/kg dry wt) | 14.6 [5]³ | 16.1 [5] | 16.9 [5] | 18.3 [5] | NS | NS | NS | 5.8 |
| Calcium, serum (mmol/L) | 2.44 [5] | 2.42 [5] | 2.48 [5] | 2.48 [5] | NS | NS | NS | 0.08 |
| Magnesium, serum (mmol/L) | 0.87 [5] | 0.83 [5] | 0.90 [5] | 0.86 [5] | 0.02 | 0.07 | NS | 0.03 |
| Magnesium, red blood cell (mmol/kg dry wt) | 5.30 [4] | 5.59 [4] | 5.47 [4] | 5.51 [4] | NS | NS | NS | 0.34 |
| Supplemental magnesium diet (340 mg Mg/d total)⁴ | | | | | | | | |
| Boron, red blood cell (μmol/kg dry wt) | 18.2 [4] | 16.2 [4] | 29.8 [4] | 18.8 [4] | NS | NS | NS | 13.2 |
| Calcium, serum (mmol/L) | 2.42 [4] | 2.44 [4] | 2.40 [4] | 2.46 [4] | NS | NS | NS | 0.09 |
| Magnesium, serum (mmol/L) | 0.91 [4] | 0.86 [4] | 0.88 [4] | 0.86 [4] | NS | NS | NS | 0.06 |
| Magnesium, red blood cell (mmol/kg dry wt) | 5.26 [4] | 5.47 [4] | 5.38 [4] | 5.92 [4] | 0.07 | NS | NS | 0.36 |

[1] Analyzed by repeated-measures ANOVA.
[2] Root mean square error.
[3] Group mean; n in brackets.
[4] Mean daily amount of dietary magnesium; includes a 200-mg supplement of magnesium (per 8400 kJ) as magnesium gluconate.

interruption of normal sinus rhythm. Supplemental boron decreased the width of the QRS complex in volunteers fed no supplemental magnesium but not in those fed supplemental magnesium (**Table 6**). In general, a QRS duration is considered to be prolonged when it lasts > 0.08 s (17). By this criterion, supplemental boron slightly improved electrical transmission. Supplemental aluminum increased the width of the QRS complex in volunteers fed supplemental magnesium but not in

**TABLE 6**
Effects of dietary boron, dietary aluminum, and their interaction on variables associated with circulatory functions in postmenopausal women fed different amounts of magnesium

| | Treatments | | | | $P^1$ | | | |
|---|---|---|---|---|---|---|---|---|
| | < 0.1 mg Al/d | | 1000 mg Al/d | | | | | |
| Circulatory function | 0.36 mg B/d | 3.23 mg B/d | 0.36 mg B/d | 3.23 mg B/d | B | Al | B × Al | RMSE² |
| Basal magnesium diet (109 mg Mg/d total) | | | | | | | | |
| Blood pressure (mm Hg) | | | | | | | | |
| Diastolic | 69 [6]³ | 76 [6] | 69 [6] | 75 [6] | 0.0001 | NS | NS | 2 |
| Systolic | 114 [6] | 122 [6] | 113 [6] | 120 [6] | 0.0005 | NS | NS | 4.20 |
| QRS complex (S) | | | | | | | | |
| Lead 1 | 0.078 [4] | 0.075 [4] | 0.085 [4] | 0.073 [4] | 0.05 | NS | NS | 0.007 |
| Lead 2 | 0.088 [4] | 0.075 [4] | 0.085 [4] | 0.080 [4] | 0.03 | NS | NS | 0.007 |
| Lead 3 | 0.085 [4] | 0.075 [4] | 0.083 [4] | 0.080 [4] | NS | NS | NS | 0.007 |
| Supplemental magnesium diet (340 mg Mg/d total)⁴ | | | | | | | | |
| Blood pressure (mm Hg) | | | | | | | | |
| Diastolic | 69 [5] | 73 [5] | 71 [5] | 72 [5] | 0.03 | NS | NS | 2 |
| Systolic | 113 [5] | 119 [5] | 112 [5] | 119 [5] | 0.03 | NS | NS | 5.56 |
| QRS complex (S) | | | | | | | | |
| Lead 1 | 0.072 [5] | 0.068 [5] | 0.080 [5] | 0.076 [5] | NS | 0.05 | NS | 0.008 |
| Lead 2 | 0.080 [5] | 0.084 [5] | 0.094 [5] | 0.092 [5] | NS | 0.01 | NS | 0.008 |
| Lead 3 | 0.084 [5] | 0.084 [5] | 0.096 [5] | 0.088 [5] | NS | 0.01 | NS | 0.006 |

[1] Analyzed by repeated-measures ANOVA.
[2] Root mean square error.
[3] Group mean; n in brackets.
[4] Mean daily amount of dietary magnesium; includes a 200-mg supplement of magnesium (per 8400 kJ) as magnesium gluconate.

those fed no supplemental magnesium. Thus, the data suggest that supplemental aluminum delayed conduction of the electrical impulse through the ventricles.

## Blood pressure

Supplemental boron induced a modest increase in systolic pressure in volunteers fed either the no supplemental magnesium or supplemental magnesium diets (Table 6). Because the volunteers received boron supplementation during the last two treatment periods only (Table 1), we examined whether the apparent effects of boron on systolic pressure were the result of time. In comparing individual systolic blood pressures with the day of experiment (data not shown), the slope of the regression line changed between the periods without and with supplemental boron in the plots of three of the six volunteers fed no supplemental magnesium ($P <$ 0.0006, 0.04, 0.04). This finding indicates that boron supplementation affected blood pressure in at least three of the six volunteers fed basal amounts of magnesium. One of five volunteers fed supplemental magnesium had a change ($P < 0.0001$) in slope between the no supplemental boron and supplemental boron periods and three exhibited a trend toward ($P < 0.06, 0.06$), or a significant increase in ($P < 0.007$), systolic pressure as a function of time over the entire length of the study. Thus, the apparent effect of boron on systolic blood pressure in volunteers fed supplemental amounts of magnesium was probably more the result of time.

The observed effect of supplemental boron on diastolic blood pressure may have been an artifact of time. For example, only 1 volunteer in the group of 6 fed no supplemental magnesium had a slope change between the no supplemental boron and supplemental boron periods ($P < 0.02$); 5 of 11 volunteers fed either no supplemental magnesium or supplemental magnesium showed trends toward, or significant increases in, diastolic blood pressure as a function of time over the entire length of the study (no supplemental magnesium: $P < 0.07, 0.03,$ 0.09; supplemental magnesium: $P < 0.02, 0.1$), or a trend toward decreased diastolic blood pressure (no supplemental magnesium: $P < 0.06$).

## Kidney-related variables

Supplemental boron decreased blood urea nitrogen (BUN) in volunteers consuming no supplemental magnesium (**Table 7**). However, supplemental boron did not affect BUN in volunteers consuming supplemental magnesium. Supplemental boron decreased urinary oxalate excretion in volunteers consuming no supplemental magnesium but had no effect on oxalate excretion in volunteers consuming supplemental magnesium. Urine volume was not affected by supplemental boron during either magnesium regimen. Two determinations of serum creatinine over the course of the study for purposes of general health monitoring were insufficient for appropriate statistical analysis of that variable.

## DISCUSSION

Analyses of food and personal care products (18–20) indicate that usual adult human dietary consumption of boron in the United States is in the range of 1–2 mg (0.092–0.185 mmol/d). Increased consumption of specific foods with high boron content increases boron intake significantly; one serving of avocado provides 1.11 mg (0.102 mmol) B (18). The findings from the present study indicate that a low-boron diet [0.36 mg (0.033 mmol)/d] supplemented with boron in amounts [3.00 mg (0.277 mmol)/d] equivalent to those found in diets with plenty of fruit, vegetables, and nuts is sufficient to affect several aspects of human physiology. Therefore, the findings on the effects of dietary boron on human physiology are relevant to at least postmenopausal woman and need to be considered when mineral status assessments are conducted.

## Boron absorption and excretion

All mineral excretion data were presented as a percentage of mineral intake because those intakes fluctuated as energy intakes were adjusted to maintain body weight, a common concern in human metabolic studies (21, 22). The current report includes the first summary of boron balance data collected from humans maintained in a highly controlled environment.

## TABLE 7
Effects of dietary boron, dietary aluminum, and their interaction on kidney-related variables in postmenopausal women fed different amounts of magnesium

| | Treatments | | | | $P^1$ | | | |
| | < 0.1 mg Al/d | | 1000 mg Al/d | | | | | |
| Kidney-related variables | 0.36 mg B/d | 3.23 mg B/d | 0.36 mg B/d | 3.23 mg B/d | B | Al | B × Al | RMSE² |
|---|---|---|---|---|---|---|---|---|
| Basal magnesium diet (109 mg Mg/d total) | | | | | | | | |
| Oxalate, urine (μmol/d) | 130 [6]³ | 89 [6] | 104 [6] | 87 [6] | 0.03 | NS | NS | 29 |
| Urea nitrogen, serum (mmol/L urea) | 6.36 [5] | 5.30 [5] | 6.55 [5] | 5.72 [5] | 0.003 | NS | NS | 0.58 |
| Urine volume (L/d) | 2.31 [6] | 2.29 [6] | 2.40 [6] | 2.31 [6] | NS | NS | NS | 0.22 |
| Supplemental magnesium diet (340 mg Mg/d total)⁴ | | | | | | | | |
| Oxalate, urine (μmol/d) | 100 [5] | 104 [5] | 93 [5] | 89 [5] | NS | NS | NS | 15 |
| Urea nitrogen, serum (mmol/L urea) | 5.28 [4] | 5.88 [4] | 5.58 [4] | 5.75 [4] | NS | NS | NS | 0.83 |
| Urine volume (L/d) | 2.89 [5] | 3.04 [5] | 2.86 [5] | 2.88 [5] | NS | NS | NS | 0.41 |

¹ Analyzed by repeated-measures ANOVA.

² Root mean square error.

³ Group mean; $n$ in brackets.

⁴ Mean daily amount of dietary magnesium; includes a 200-mg supplement of magnesium (per 8400 kJ) as magnesium gluconate.

The boron excretion data do not indicate that a dietary intake of 3.23 mg B/d causes abnormal boron accumulation. Furthermore, the data suggest that postmenopausal women ingesting very low amounts of boron (0.36 mg/d) are prone to net boron loss.

The boron excretion data support earlier findings that boron is highly absorbable and excreted primarily by the kidneys. Urinary excretion data collected from rats indicated that the absorption of an intrinsically labeled $^{10}B$ dose from broccoli was 100% (23). In heifers (24) or sheep (25), only 30% or 41%, respectively, of dietary boron was excreted in the urine. However, the boron load (from natural typical foodstuffs) on a body weight basis was much higher in those animal studies (0.715 mg B in the heifers and 0.667 mg B/kg body wt in the sheep) than in the present study (0.005 mg B in the no supplemental boron period and 0.048 mg B/kg body wt in the supplemental boron period). Other boron excretion data from the present study also support earlier findings (23) that the rate of boron excretion is extremely rapid. For example, within 24 h after initiation of the boron supplementation regimen, urinary boron rose to amounts similar to those present in the supplement (data not shown).

A range of boron concentrations in whole blood of apparently healthy humans with unknown dietary histories was reported (26). It was the opinion of the investigators that the range was much narrower than that expected for a nonessential ultratrace element. A finding from a study with yearling beef heifers indicated that as the amount of filtered boron increased, the percentage of filtered boron that was reabsorbed decreased (27). Also, in another animal study, female rats consuming water high in boron (100 mg/L) for 21 d showed increased plasma boron concentrations although some mechanism concurrently eliminated any excess boron from the liver and brain against their own concentration gradients (28). A finding from the present study indicates that there is an obligatory boron loss. During the dietary regimen providing 340 mg Mg/d, 89% of dietary boron was excreted in the urine in volunteers fed supplemental boron, a percentage that increased to 111% in volunteers deprived of boron. Further study with concurrent measures of urinary creatinine and plasma boron concentrations is needed to determine whether increased renal boron clearance during boron supplementation reflects decreased tubular reabsorption and, therefore, homeostatic control of boron.

### Effects on calcium and magnesium utilization

A previous report on the present study concluded that boron supplementation reduced urinary calcium and magnesium loss (4). That report did not take into account adjustments in energy intake as described above. As now reported, boron supplementation did not affect urinary magnesium excretion but did induce a minor decrease or modest increase in the percentage of dietary calcium lost in the urine as magnesium intake changed from amounts considered appreciably lower than, to slightly more than, the current RDA for magnesium, respectively (11). Similar findings were reported from an animal study in which boron supplementation (2.46 compared with < 0.06 mg B/kg diet) increased total 24-h urinary calcium (0.91 ± 0.38 compared with 0.72 ± 0.22 mg) in 8-wk-old, cholecalciferol-deprived rats (exhibiting few signs of cholecalciferol deficiency) fed adequate magnesium (29). However, the findings from two separate studies of female volunteers differ

from those of the present study. In a 10-mo study of premenopausal women consuming ≈83 mg Mg and 680 mg Ca/d, supplemental boron did not affect urinary calcium loss (30). In a short 6-wk study of postmenopausal volunteers consuming ≈298 mg Mg and 927 mg Ca/d, supplemental dietary boron did not affect urinary excretion of either calcium or magnesium (31). However, the composition of the basal diet seemed to elevate urinary excretion of calcium, which may have inhibited or obscured any effect of boron.

Assuming a magnesium sweat loss of ≤ 15 mg/d (32, 33), a dietary intake of 109 mg Mg/d resulted in essentially zero magnesium balance in the postmenopausal women. Earlier findings indicate that a magnesium intake of 100 mg/d for ≥ 14 d is sufficient to maintain positive magnesium balance (34). Whether zero magnesium balance can be maintained for periods longer than 24 d in postmenopausal women fed 109 mg Mg/d remains unknown.

Other findings from this study indicate that dietary aluminum decreases calcium absorption. In a different study with adult men, small doses of aluminum-containing antacids (for example, between 90 and 450 mL antacid/d containing between 1530 and 7650 mg Al/d) increased fecal calcium when they received an average of 252 mg Ca/d (35). During a calcium intake of 800 mg/d, these doses of antacids did not affect calcium excretion. Therefore, the present findings indicate that aluminum supplementation should probably be limited or at least monitored in postmenopausal women prone to excessive calcium loss and who are consuming low amounts of calcium.

### Blood mineral content

#### Boron

The mean plasma boron concentrations reported in the current study are higher than limited published values. The newest study available reported a median plasma boron concentration of 2.31 μmol/L (range: 1.30–3.61 μmol/L) for 12 subjects with detectable boron concentrations but unknown dietary histories (36). Boron volatizes at high temperatures (5) and it is unknown whether boron was lost during vessel venting, a necessary step in the microwave digestion method used in that study. The method used to analyze for boron in the present study provides satisfactory recovery of boron from spiked samples (99.7 ± 0.5%) (5). In a different study, the plasma boron concentration (determined by neutron activation and mass spectrometry) of one individual with an unknown dietary history was reported as 3.03 ± 0.15 μmol/L (26). Other investigators reported a median value of 2.06 μmol/L and a range of 0.77–4.45 μmol/L for serum boron (37). The amount of boron (18.6 μmol/kg dry wt) found in washed, dried RBCs in the present study was approximately twice that reported in whole, dried blood analyzed by neutron irradiation (9.0 μmol/kg dry wt) (38).

Although boron supplementation did increase plasma boron concentrations, the data do not indicate that plasma boron content is a sensitive indicator of dietary boron intake. In the present study, the supplemental boron regimen provided a 9.0-fold increase in dietary boron, yet this increase caused only a 1.5-fold increase in plasma boron concentrations in postmenopausal women. In magnesium-adequate chicks, a ninefold increase in dietary boron (1.58 compared with 0.18 mg/kg)

increased plasma boron concentrations by only twofold (10.8 compared with 5.3 $\mu$mol/L). In another study with cholecalciferol–deficient chicks only, a 7.5-fold increase in dietary boron (from 0.465 to 3.465 mg/kg) yielded only a 2.0-fold increase (from 7.12 to 14.1 $\mu$mol/L) in plasma boron concentrations (39).

### Magnesium

The mechanism by which dietary boron supplementation reduced serum magnesium concentrations in postmenopausal women fed no supplemental magnesium in this study or similar amounts in a different study (40) remains unknown. Inorganic boron in concentrations found in blood or urine of normal pH (7.35–7.45 or 4.5–8, respectively) would exist almost entirely in the mononuclear uncharged species $B(OH)_3$ (41) and therefore would not complex with the portion of ultrafilterable plasma magnesium that complexes with anions (42). Furthermore, the disproportionate molar ratio between magnesium and boron in plasma (0.87:0.01) or urine (5.00:0.27) would preclude any significant direct chemical interaction. Thus, boron may influence magnesium metabolism through intermediate or parallel molecular mechanisms.

### Blood pressure and kidney-related variables

The finding that boron supplementation increased systolic blood pressure in postmenopausal women fed basal amounts of magnesium is reported because of no compelling reason to assume it to be artifactual. The finding was unexpected because there is strong evidence that lactoovovegetarian diets (high in boron content), compared with omnivorous diets (typically lower in boron), depress blood pressure (43, 44); although fruit and vegetables are important sources of many nutrients, they also have a relatively high boron content (typically 2–3 mg B/kg wet wt) (19). The total amount of boron consumed by the boron-supplemented women in the present study was similar to that typically consumed by the adult US population. Therefore, it seems highly unlikely that the affect of boron on blood pressure was pharmacologic or toxicologic in nature. Most importantly, supplemental boron did not increase systolic pressure to values outside of the normal range.

In a different study, BUN concentrations were reported to be lower at the end of a boron-repletion period of 49 d (4.1 mmol urea/L) compared with those determined at the end of the preceding boron depletion period of 63 d (4.8 mmol urea/L) (45). That finding was replicated in the present study. BUN concentrations, in the absence of disease states, reflect the degree of protein catabolism, whether produced by a high-protein diet or by factors that result in the mobilization of protein for energy purposes (46). There is previous evidence for a role of boron in energy substrate metabolism. In the cholecalciferol–deficient chick nutrition model, physiologic supplements of boron alleviated perturbation in plasma glucose and triacylglycerol concentrations and substantially improved food consumption (47). Furthermore, findings from a human study conducted after the one reported here indicated that dietary boron supplementation decreased serum glucose concentrations in postmenopausal women fed 115 mg Mg/d (45). Thus, the modest influence of boron on BUN concentrations may reflect an uncharacterized role for boron in energy substrate metabolism.

Characterization of the mechanism through which boron affects oxalate excretion may reveal a relation between boron nutriture and the formation of oxalate stones, one type of urolithiasis responsible for considerable pain, discomfort, and medical expense in the human population. Oxalate in the body is derived from dietary sources (rhubarb, spinach) and from glycine and ascorbic acid metabolism (48). It is thought that a decrease in urinary oxalate excretion would reduce the degree of urine supersaturation with respect to calcium oxalate and so diminish the tendency to form oxalate stones (49). Because supplemental boron reduced total urine oxalate and calcium in volunteers fed no supplemental magnesium (but not in those fed supplemental magnesium), postmenopausal women consuming diets low in magnesium may benefit from dietary boron in amounts normally found in diets with ample quantities of fruit and vegetables.

In summary, the findings indicate that volunteers fed 0.36 or 3.23 mg B/d did not accumulate boron. The boron supplement was within the range of normal dietary boron intake. Obligatory boron loss and relatively small changes in boron blood values during substantial increases in dietary boron support the concept of boron homeostasis and, therefore, a possible biological function for boron. Dietary boron directly affects magnesium metabolism because supplemental boron decreased serum magnesium concentrations in volunteers fed no supplemental magnesium. Decreased total urine oxalate during supplemental boron treatment in volunteers receiving no supplemental magnesium indicates a possible role for boron in the control of urolithiasis. The change in urinary calcium excretion as a function of boron and magnesium nutriture indicates a close interrelation among boron, calcium, and magnesium, a relation that may be important in understanding better the mineral metabolism disorders that perturb long-term calcium balance. Finally, aluminum supplementation should be limited or at least monitored in postmenopausal women prone to excessive calcium loss.

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