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Effect of Boron Depletion and Repletion on Blood Indicators of Calcium Status in Humans Fed a Magnesium-Low Diet

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A study was performed with the objective of extending the evidence that indicates that dietary boron affects calcium metabolism in humans. Five men, five women on estrogen therapy, four postmenopausal women not on estrogen therapy, and one premenopausal woman were fed a mixed Western basal diet that supplied 706 mg of calcium, 115 mg of magnesium, and 0.23 mg of boron with a 2,000-kcal intake. After an equilibration period of 14 days when dietary boron was about 3.23 mg/day, the subjects were fed the basal diet only for 63 days and then fed the basal diet plus 3 mg/day of boron as sodium borate for 49 days. When compared between the last 42 days of depletion and the last 35 days of repletion, several blood variables associated with calcium metabolism or status were significantly different ($P < 0.05$). When all 15 subjects were used in the comparisons, plasma-ionized calcium (4.91 vs. 4.98 mg/dl) and serum 25-hydroxycholecalciferol (29.1 vs. 32.3 ng/ml) were lower, and serum calcitonin (74.1 vs. 59.0 pg/ml) and serum osteocalcin (3.4 vs. 2.5 ng/ml) were higher, during boron depletion than boron repletion. The postmenopausal women on estrogen therapy exhibited higher plasma-ionized calcium and serum 25-hydroxycholecalciferol and lower serum calcitonin and osteocalcin than did the men or postmenopausal women not on estrogen therapy. Boron supplementation apparently changed or tended to change those variables in a manner similar to that caused by estrogen therapy. Thus, the findings indicate that boron is beneficial, or essential, for optimal calcium metabolism and in the prevention of bone loss which occurs in postmenopausal women and older men.

Key words: calcitonin, 25-hydroxycholecalciferol, osteocalcin

INTRODUCTION

In 1981, Hunt and Nielsen [1] reported that boron deprivation depressed growth and elevated plasma alkaline phosphatase activity in chicks fed inade-

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quate cholecalciferol. Subsequent experiments suggested that cholecalciferol deficiency enhanced the need for boron and that boron affected calcium or magnesium metabolism [2]. After those early experiments, it was found in both chicks and rats that the response to changes in dietary boron was markedly influenced by the methionine, potassium, magnesium, cholecalciferol, aluminum, and calcium status of the animal [3–7]. Generally, the studies on boron showed that when the diet was manipulated to cause possible changes in cellular membrane integrity (potassium or magnesium deficiency) or in hormone responsiveness (magnesium or cholecalciferol deficiency, aluminum toxicity), a large number of responses to dietary boron occurred. On the other hand, when the animal was fed a diet apparently optimal in all respects, the response to dietary boron was not very marked. In other words, there was an enhanced need for boron when the animal was under some form of nutritional or metabolic stress.

A recent study [8,9] has indicated that the response to dietary boron by humans may be similar to that of animals. This study involved women who had gone through menopause, a stage of life that involves changes in hormone metabolism which often leads to a loss of bone mineral. After the women had consumed a conventional diet supplying about 0.25 mg B/day for 119 days, a boron supplement of 3 mg/day for 48 days markedly reduced the urinary excretion of calcium and magnesium; the depression seemed more marked when dietary magnesium was low [8]. Boron supplementation also markedly elevated the serum concentrations of estradiol-17β [8] and ionized calcium [9]; the elevation seemed more marked in the magnesium-low women.

Because the preceding findings suggest that boron may be important in the prevention of calcium loss and bone demineralization in older people, a follow-up study was performed with the objective of finding further evidence indicating that dietary boron affects calcium metabolism during the time of life when bone loss is often experienced. Some of the findings from that study follow.

MATERIALS AND METHODS

Subjects

The subjects were five men over the age of 45, five postmenopausal women on estrogen therapy, four postmenopausal women not on estrogen therapy, and one woman who was thought to be postmenopausal but whom estrogen analyses during the study showed was not. The subjects were Caucasian and ranged in age from 44 to 70 years. After medical, psychological, and nutritional evaluation had established that they were in good health, each subject signed an informed consent after receiving both oral and written presentations of the nature of the research. The study protocol was approved by the Institutional Review Board of the University of North Dakota and the Human Studies Committee of the U.S. Department of Agriculture. The protocol followed the guidelines of the Department of Health and Human Services and the Helsinki Doctrine regarding the use of human subjects. During the study, the subjects resided at their homes in the vicinity of Grand Forks, ND.

Experimental Protocol

The subjects were fed a 3-day menu rotation shown in Table I. As shown in Table II, the diet contained 11% protein, 54% carbohydrate, and 35% fat. To

TABLE I. Food Items in the 3-Day Menu Rotation Diet

	Day 1	Day 2	Day 3
Breakfast	Tang, orange drink; pork sausage; bread, white; butter	Tang, orange drink; Cream of Wheat; milk, 2%; sugar; bread, white; margarine	Coffee cake; cornflakes; sugar; milk, 2%
Dinner	Lemonade, beef stew, Ritz cracker, cherry jello, pound cake	Lemonade; crispy pork; Tater Tots; cauliflower, steamed; shortbread cookie	Tang, orange drink; taco, hot sauce; shortbread cookie; lemon jello
Supper	7-Up, hamburger & bun, ketchup, mayonnaise, cheddar cheese, potato chips, vanilla wafers	Chicken rice dish, Ritz crackers, cream cheese, angelfood cake	Lemonade; chicken cheese dish; corn; bread, white; butter; vanilla wafers
Snack	Angelfood cake; milk, 2%	Peach jello, vanilla wafers	Pound cake with lemon glaze

TABLE II. Calculated Composition of the 3-Day Menu Rotation Diet

	Amount		
Component	1,400–3,600 kcal	2,000 kcal	2,000 kcal plus supplements
Protein, g	39–99	55	
Protein, % of energy	11	11	
CHO, g	193–497	276	
CHO, % of energy	54	54	
Crude fiber, g	0.9–2.3	1.3	
Fat, g	55–140	78	
Fat, % of energy	35	35	
Linoleic acid, g	7.8–20	11.1	
TSFA, g	17.9–45.9	25.5	
Linoleic/TSFA, P/S	0.44	0.44	
Cholesterol, mg	165–423	235	
Potassium, mg	872–2,241	1,245	1,875
Phosphorus, mg	509–1,309	727	
Calcium, mg[a]	400–1,028	571	706
Iron, mg[a]	7.2–18.5	10.3	28.3
Magnesium, mg[a]	81–207	115	
Zinc, mg[a]	3.83–9.85	5.47	9.47
Copper, mg[a]	0.29–0.74	0.41	1.61
Manganese, mg[a]	0.65–1.67	0.93	
Boron, mg[a]	0.16–0.41	0.23	
Vitamin A, I.U.	5,297–13,621	7,567	
Thiamin, mg	0.85–2.20	1.22	
Riboflavin, mg	0.82–2.11	1.17	
Niacin, mg	10.2–26.1	14.5	
Ascorbic acid, mg	141–364	202	
Vitamin B_6, mg	0.7–1.8	1.0	2.0
Vitamin B_{12}, mcg	2.54–6.53	3.63	
Folacin, mcg	74–191	106	306
Vitamin D, I.U.			400

[a]Actual amount determined by inductively coupled plasma emission spectrometry [3].

ensure nutritional adequacy, the diet was supplemented daily as follows: potassium, 630 mg as potassium chloride; calcium, 135 mg as calcium gluconate; iron, 18 mg as ferrous gluconate; zinc, 4 mg as zinc sulfate; copper, 1.2 mg as copper sulfate; pyridoxine hydrochloride, 1.0 mg; folacin, 200 μg; and cholecalciferol, 400 IU. All supplements were consumed at mealtimes. At an energy intake of 2,000 kcal per day, the diet provided (as determined by analysis): 706 mg calcium, 115 mg magnesium, and 0.23 mg boron. For the elemental analysis the diet samples were prepared by our usual methods using inductively coupled plasma emission spectrometry [3]. The energy requirements for each volunteer were based on energy needs determined by the Harris and Benedict equation [10] plus an additional 50% of basal energy expenditure for normal activity. The subjects were encouraged not to change their exercise habits during the study. During the study, energy intake was adjusted to maintain body weight within ±2% of admission weight; there were no major adjustments. To achieve the appropriate energy intake, all menu ingredients were increased or decreased proportionally in 200-kcal increments. The range of energy intakes among the subjects was 1,800–3,600 kcal. The subjects consumed one meal each weekday at the Grand Forks Human Nutrition Research Center. The other two meals and evening snack each weekday, and all weekend meals, were prepared at the Center but consumed by the subjects in their homes.

After a 14-day equilibration period during which the basal low-boron diet supplemented with 3 mg B/day as sodium borate was fed, there was a 63-day depletion period during which the basal diet was fed followed by a repletion period during which the basal diet was supplemented with 3 mg B/day as sodium borate. The supplement was given in three divided doses at mealtimes. The diet was low in magnesium, 115 mg/2,000 kcal, throughout the study.

Analyses

Blood was drawn by using standard phlebotomy techniques between 7:00 and 9:00 A.M. after 12 hr of fasting. Serum and plasma were obtained weekly for the determination of alkaline phosphatase activity and ionized calcium. Serum and plasma were obtained in weeks 3, 6, and 9 of the depletion period and weeks 2, 4, and 7 of the repletion period for the determination of total calcium, calcitonin, 25-hydroxycholecalciferol, osteocalcin, magnesium, and phosphorus. Serum alkaline phosphatase activity and phosphorus were determined by using standard techniques of the Cobas Fara Robotic Analyzer (Roche Diagnostics Systems, Nutley, NJ). Plasma-ionized calcium was determined with an ion-specific electrode adjusted to physiological pH [11–13]. Serum was diluted with a 0.5% solution of lanthanum chloride and analyzed by atomic absorption spectrometry for calcium and magnesium [14]. Standard radioimmunoassay kits (Incstar Corp., Stillwater, MN) were used for the determination of calcitonin, 1,25-dihydroxycholecalciferol, and osteocalcin. Radioimmunoassay techniques were also used to determine serum 25-hydroxycholecalciferol [15].

Statistics

Although the volunteers were allowed an equilibration period of 14 days, all variables being examined did not appear to stabilize into a steady change or

plateau until about the fourth week. Thus, to assure that the comparisons between boron depletion and repletion were not affected by the change from the usual diet to the experimental diet, only the values from the last 42 days of depletion were used in the statistical comparisons. The response to boron supplementation varied with individual and variable examined. However, the majority of the responses for all individuals and variables occurred during the first 14 days of supplementation. Thus, the values obtained during the last 35 days of repletion were used in the statistical comparisons. Values from the five men, four postmenopausal women, and five postmenopausal women were first compared separately; then values obtained from all 15 subjects were compared between the depletion and repletion periods. For each variable, a mean was computed for each dietary period for each volunteer. Paired *t*-tests were then used to test for dietary effects [16]. In this test, each individual was his or her own control.

RESULTS

After being fed a boron-low diet for 63 days, a boron supplement of 3 mg/day affected several variables associated with calcium metabolism in the 15 subjects. Although the total plasma calcium concentration was not affected by dietary boron, plasma-ionized calcium, whether expressed as mg/dl or percent of total, was higher during boron repletion than boron depletion (Table III). The difference was significant for each group separately and when all 15 subjects were combined. The data in Table IV show that serum magnesium and phosphorus

TABLE III. Effect of Boron on Plasma Total and Ionized Calcium in Subjects Fed a Low-Magnesium Diet*

Dietary boron[a] (mg/day)	Total calcium[b] (mg/dl)	Ionized calcium[b]	
		mg/dl	%
Men over age 45 (n = 5)			
0.23	9.3 ± 0.09[c]	4.86 ± 0.04	52.1 ± 0.1
3.23	9.2 ± 0.07	4.92 ± 0.04	53.2 ± 0.2
P value	0.40	0.06	0.004
Postmenopausal women (n = 4)			
0.23	9.3 ± 0.08	4.90 ± 0.04	52.4 ± 0.2
3.23	9.2 ± 0.10	4.97 ± 0.03	53.6 ± 0.6
P value	0.35	0.008	0.06
Postmenopausal women on estrogen therapy (n = 5)			
0.23	9.3 ± 0.10	4.95 ± 0.04	53.2 ± 0.3
3.23	9.3 ± 0.10	5.04 ± 0.02	54.3 ± 0.3
P value	0.78	0.02	0.02
Above combined plus one premenopausal woman (n = 15)			
0.23	9.3 ± 0.05	4.91 ± 0.02	52.6 ± 0.2
3.23	9.3 ± 0.05	4.98 ± 0.02	53.8 ± 0.2
P value	0.14	0.0001	0.0001

* Dietary magnesium was 115 mg/2,000 kcal throughout the study.
[a]After an equilibration period of 14 days when dietary boron was about 3.23 mg/day there was a depletion period of 63 days when dietary boron was 0.23 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3 mg B/day as sodium borate.
[b]Values obtained during the last 42 days of depletion and last 35 days of repletion were compared.
[c]SEM.

TABLE IV. Effect of Boron on Serum Magnesium, Phosphorus, and Alkaline Phosphatase in Subjects Fed a Low-Magnesium Diet*

Dietary boron[a] (mg/day)	Magnesium[b] (mg/dl)	Phosphorus[b] (mg/dl)	Alkaline phosphatase[b] (IU/L)
Men over age 45 (n = 5)			
0.23	1.98 ± 0.07[c]	3.8 ± 0.2	72 ± 2
3.23	2.00 ± 0.06	3.9 ± 0.3	71 ± 3
P value	0.54	0.20	0.58
Postmenopausal women (n = 4)			
0.23	2.04 ± 0.06	4.1 ± 0.2	108 ± 17
3.23	2.01 ± 0.07	4.0 ± 0.2	105 ± 15
P value	0.18	0.60	0.55
Postmenopausal women on estrogen therapy (n = 5)			
0.23	1.97 ± 0.08	3.5 ± 0.1	76 ± 6
3.23	1.89 ± 0.08	4.0 ± 0.2	73 ± 3
P value	0.003	0.04	0.38
Above combined plus one premenopausal woman (n = 15)			
0.23	1.99 ± 0.04	3.8 ± 0.1	83 ± 6
3.23	1.96 ± 0.04	3.9 ± 0.1	82 ± 6
P value	0.06	0.17	0.27

*Dietary magnesium was 115 mg/2,000 kcal throughout the study.
[a]After an equilibration period of 14 days when dietary boron was about 3.23 mg/day there was a depletion period of 63 days when dietary boron was 0.23 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3 mg B/day as sodium borate.
[b]Values obtained during the last 42 days of depletion and last 35 days of repletion were compared.
[c]SEM.

were apparently affected by dietary boron only in the postmenopausal women on estrogen therapy; boron supplementation decreased the concentration of magnesium but increased the concentration of phosphorus in serum. Serum magnesium and phosphorus concentrations were not affected by dietary boron in the men or women not on estrogen therapy. Serum alkaline phosphatase was not significantly affected by dietary boron in any of the groups, nor in all subjects combined.

Table V indicates that dietary boron has an influence on hormones which affect calcium metabolism. When all 15 subjects were used in the comparison, serum calcitonin and osteocalcin concentrations were lower, and the serum 25-hydroxycholecalciferol concentration was higher during boron repletion than boron depletion. Similar changes were found when each group was examined separately. However, significance at the 0.05 level was achieved for calcitonin only with the postmenopausal women and for osteocalcin only with the postmenopausal women on estrogen therapy. Dietary boron did not significantly affect serum 1,25-dihydroxycholecalciferol.

DISCUSSION

The findings support the contention that boron has a biological function that affects calcium metabolism, and thus bone formation and maintenance in humans. After 63 days of boron depletion, a boron supplement of 3 mg/day changed

TABLE V. Effect of Boron on Serum Hormones in Subjects Fed a Low-Magnesium Diet*

Dietary boron[a] (mg/day)	Calcitonin[b] (pg/ml)	25-hydroxy cholecalciferol (ng/ml)	1,25-dihydroxy cholecalciferol (pg/ml)	Osteocalcin[b] (ng/ml)
Men over age 45 (n = 5)				
0.23	71 ± 14[c]	25 ± 4	25.5 ± 2.6	3.7 ± 0.2
3.23	60 ± 9	29 ± 6	25.9 ± 1.2	3.6 ± 0.6
P value	0.16	0.15	0.86	0.74
Postmenopausal women (n = 4)				
0.23	78 ± 8	29 ± 5	24.4 ± 1.0	3.8 ± 0.2
3.23	52 ± 9	34 ± 6	25.3 ± 1.8	3.5 ± 0.4
P value	0.02	0.23	0.57	0.58
Postmenopausal women on estrogen therapy (n = 5)				
0.23	61 ± 6	36 ± 5	28.4 ± 3.4	2.8 ± 0.5
3.23	55 ± 7	37 ± 5	28.7 ± 3.2	1.8 ± 0.3
P value	0.02	0.13	0.88	0.08
Above combined plus one premenopausal woman (n = 15)				
0.23	74 ± 7	29 ± 3	26.0 ± 1.2	3.3 ± 0.2
3.23	59 ± 5	32 ± 3	26.3 ± 1.0	2.8 ± 0.3
P value	0.0008	0.01	0.72	0.06

* Dietary magnesium was 115 mg/2,000 kcal throughout the study.
[a]After an equilibration period of 14 days when dietary boron was about 3.23 mg/day there was a depletion period of 63 days when dietary boron was 0.23 mg/2,000 kcal followed by a repletion period of 49 days when the basal diet was supplemented with 3 mg B/day as sodium borate.
[b]Values obtained during the last 42 days of depletion and last 35 days of repletion were compared.
[c]SEM.

several variables associated with calcium metabolism in a manner that apparently was beneficial to the maintenance of bone mass.

Estrogen replacement generally is accepted as the only proven therapy which prevents the loss of bone in postmenopausal women [17,18]. The concentrations of plasma-ionized calcium and serum 25-hydroxycholecalciferol were higher, and the concentrations of serum calcitonin and osteocalcin were lower, in the postmenopausal women on estrogen therapy than in the women not on estrogen therapy or the men. Boron supplementation changed, or tended to change, these variables in the men and postmenopausal women not on estrogen in a manner that made them more like the women on estrogen therapy. This indicates that boron and estrogen were having similar effects. Thus, if estrogen is beneficial to calcium metabolism and in the prevention of bone loss which occurs in postmenopausal women and older men, boron must be beneficial through similar processes.

Findings by other investigators indicate that, depending upon one's point of view, boron deprivation causes, or boron supplementation prevents, changes in calcium-regulation hormones in a manner detrimental to maintaining bone mass in older people. Osteocalcin is regarded as a 1,25-hydroxycholecalciferol-regulated protein that inhibits osteoid mineralization [19]. Thus, increased amounts of osteocalcin in serum or plasma may be an indicator of conditions enhancing the loss of bone mass; this is supported by the findings of an inverse relationship between plasma osteocalcin and bone mineral content in postmenopausal wo-

men [20]. Moreover, urinary osteocalcin was found to be elevated in women with postmenopausal osteoporosis [21]. In the current study, the highest serum concentrations of osteocalcin occurred during boron depletion. Lips et al. [22] found that serum 25-hydroxycholecalciferol concentrations were lower in patients with hip fracture than in elderly control subjects. Aloia et al. [23] found that serum 25-hydroxycholecalciferol was depressed in women with postmenopausal osteoporosis. Thus, the increase in serum 25-hydroxycholecalciferol concentration caused by boron supplementation apparently is beneficial.

The finding that boron repletion depressed serum calcitonin seems contrary to the suggestion that boron helps maintain bone mass. Calcitonin is generally viewed as a hormone which prevents the loss of calcium from bone. This notion is based on findings which show that calcitonin diminishes the ruffled borders of osteoclasts and inhibits net bone resorption in acute experiments [24–26]. However, calcitonin has been found to cause a transient increase in calcium excretion in humans [27–30]. Moreover, Tiegs et al. [31] found that calcitonin was elevated in women with postmenopausal osteoporosis. In people with metastatic bone disease, the failure of the kidney to excrete the calcium from bone breakdown apparently is a major contributor to hypercalcemia in these patients [32]. Calcitonin administration decreases the hypercalcemia in these patients, perhaps through a calciuria effect [33]. If serum calcitonin is directly related to the loss of calcium from the kidney, then decreased serum calcitonin may help prevent the loss of calcium from the body. Once again, it is possible to construe boron supplementation as beneficial to conserving calcium in the body.

Because boron affects several hormones involved in calcium metabolism in humans [8], and because the response to boron seems to be enhanced in nutritional disorders in animals characterized by secondary hyperparathyroidism (e.g., magnesium deficiency [3] and aluminum toxicity [4]), it seems possible that boron affects macromineral metabolism via a regulatory role involving a hormone. In plants boron is suspected of having a regulatory role involving such hormones as auxin, gibberellic acid, and cytokinin, perhaps through control of second messengers such as calcium at the cell membrane level [34].

The possibility that boron has a function at the cell membrane level is supported by a variety of different findings. Some recent reviews [35,36] have presented evidence consistent with the view that boron is directly associated with membranes and is involved in their functional efficiency in plants. In other words, many symptoms of boron deficiency in plants are secondary effects caused by changes in membrane permeability. In animals, boron affects the response to magnesium [3,4] and potassium deficiencies [5]; these deficiencies affect membrane integrity [37]. Perhaps boron has a role at the cell membrane level in the kidney which prevents the urinary loss of calcium. Prevention of loss of calcium from the body most likely is beneficial to conserving the bone mineral content in older people.

CONCLUSIONS

Findings were obtained which indicate that boron deprivation depresses plasma-ionized calcium and serum 25-hydroxycholecalciferol and elevates serum calcitonin and osteocalcin in men over the age of 45 and postmenopausal women.

Because these changes are similar to those seen in women with postmenopausal osteoporosis, boron apparently is needed for optimal calcium metabolism and thus is needed to prevent the excessive bone loss which often occurs in postmenopausal women and older men. The findings suggest that boron is an essential trace element for humans.

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