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Studies on the Relationship between Boron and Magnesium Which Possibly Affects the Formation and Maintenance of Bones¹

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Abstract. Recent findings are reviewed indicating that changes in dietary boron and magnesium affect calcium, and thus bone, metabolism in animals and humans. In animals, the need for boron was found to be enhanced when they needed to respond to a nutritional stress which adversely affected calcium metabolism, including magnesium deficiency. A combined deficiency of boron and magnesium caused detrimental changes in the bones of animals. However, boron deprivation did not seem to enhance the requirement for magnesium. In two human studies, boron deprivation caused changes in variables associated with calcium metabolism in a manner that could be construed as being detrimental to bone formation and maintenance; these changes apparently were enhanced by low dietary magnesium. Changes caused by boron deprivation included depressed plasma ionized calcium and calcitonin as well as elevated plasma total calcium and urinary excretion of calcium. In one human study, magnesium deprivation depressed plasma ionized calcium and cholesterol. Because boron and/or magnesium deprivation causes changes similar to those seen in women with postmenopausal osteoporosis, these elements are apparently needed for optimal calcium metabolism and are thus needed to prevent the excessive bone loss which often occurs in postmenopausal women and older men.

Introduction

A complete understanding of the mechanisms operant in the loss of bone mass with aging has not been achieved in spite of exten-

sive research efforts. Moreover, recommendations for the prevention of bone loss often seem to be based more on conjecture than on scientific findings. For example, although most evidence reported to date indicates

¹ This paper represents the material presented by the author at the Fifth Annual Meeting of the American Society for Magnesium Research on September 26, 1989, in Norfolk, Va., USA.

that massive intakes of calcium do not prevent bone loss in postmenopausal women [1], calcium intakes difficult to achieve through a balanced diet, or up to 1,500–2,000 mg/day [2], are being recommended to these women. Attempts to prevent bone loss with various amounts and combinations of cholecalciferol and fluoride also have been extensive and apparently have been no more successful than the consumption of high amounts of calcium [3]. Therefore, I initiated a series of experiments that utilized human volunteers and which examined the possible effect on major mineral metabolism, and thus on bone, of some dietary substances other than the usual calcium, cholecalciferol and fluoride. In the following, the basis for studying and the findings from experiments involving two of these substances, boron and magnesium, are described.

Animal Studies

About 1980 in my laboratory, an experiment designed to examine the importance of arsenic in nutrition resulted in control chicks exhibiting poor growth and leg abnormalities, although all known essential nutrients were apparently provided in sufficient quantity by the diet. This experiment was followed by a study designed to determine whether the lack of some unrecognized essential nutrient was the cause of the poor growth and leg abnormalities. The data from this study indicated that boron stimulated growth and partially prevented the leg abnormalities in chicks [4]. Subsequent to that study, it was found that the diet contained inadequate cholecalciferol, because the true potency of the cholecalciferol supplement was less than indicated. This led to studies

examining the possible interaction between boron and cholecalciferol, and whether boron affects macromineral metabolism in animals. In one of the first studies using chicks [4], it was found that boron deprivation depressed growth; the effect was seemingly more marked when cholecalciferol was low. The most convincing evidence that an interaction was occurring between boron and cholecalciferol came from plasma alkaline phosphatase findings. The elevation in plasma alkaline phosphatase caused by cholecalciferol deprivation was more marked in boron-deprived than boron-supplemented chicks [4]. Also, rachitic long bones were found in 17 of 21 boron-deprived and in 9 of 22 boron-supplemented chicks fed the cholecalciferol-deficient diet [4]. Subsequently, it was found that boron was highest in the growing and calcifying areas of long bones [5].

Shortly after the above findings had been reported, evidence was obtained which indicated that, in addition to cholecalciferol, dietary magnesium and calcium affected the response to boron deprivation [6]. However, chicks are difficult experimental animals to use in studies involving magnesium deficiency; the deficiency does not have to be very severe before they convulse and die. Thus, I began to use the rat as an experimental animal to study the relationship between boron and magnesium in their effect on calcium, and thus bone, metabolism. Several experiments were performed to examine the interaction between boron and magnesium. It soon became apparent that dietary magnesium had a greater effect on the response to boron deprivation than dietary boron had on the response to magnesium deprivation. For example, feeding low boron to a marginally magnesium-deficient rat did not exacerbate or induce signs of magnesium deficiency [7–

9]. Changes in bone magnesium and boron with changes in dietary boron and magnesium also support the suggestion that dietary magnesium affects boron metabolism more than dietary boron affects magnesium metabolism. The data in table 1 show that changes in dietary boron only slightly affected bone magnesium concentrations. On the other hand, magnesium deprivation markedly increased the concentration of boron in bone when dietary calcium was adequate but decreased the concentration when dietary calcium was inadequate. Table 2 shows that boron did not affect femur magnesium in another experiment. However, in this experiment magnesium deficiency increased the concentration of boron in bone when potassium was marginal but decreased the concentration when potassium was adequate.

The numerous findings from animal studies probably can be best summarized by the statement that they showed a relationship between boron and magnesium, and that relationship apparently affected calcium metabolism. Moreover, the findings suggested that the need for boron was not crucial, or was quite low, when the animal was not under any nutritional stress; but there was an enhanced need for boron when the animal needed to respond to a nutritional stress, such as magnesium deprivation, which adversely affected calcium metabolism.

Human Studies

The findings from the animal studies were a stimulus to study the nutritional importance of boron for humans under metabolic or nutritional stress affecting calcium metabolism, for example a suboptimal di-

Table 1. Effect of dietary boron, calcium and magnesium and their interaction on boron and magnesium concentrations in bone of rats fed a marginal potassium diet (1,800 mg/kg)

Treatment, $\mu\text{g/g}$ diet			Boron	Magnesium
B	Mg	Ca	$\mu\text{g/g}$ dry femur ¹	mg/g dry femur ²
0	100	2,500	ND	1.47
0	400	2,500	0.006	4.22
0	100	5,000	0.903	1.12
0	400	5,000	0.101	4.39
3	100	2,500	ND	1.59
3	400	2,500	0.077	4.11
3	100	5,000	1.038	1.19
3	400	5,000	0.223	4.51

From Shuler and Nielsen [10]; rats were on treatment for 7 weeks. ND = Not detected.

¹ Significant effects: Ca, 0.0001; Mg, 0.0001; Ca \times Mg, 0.0001.

² Significant effects: B, 0.04; Ca, 0.004; Mg, 0.0001; Ca \times Mg, 0.0001.

Table 2. Effect in rats of dietary boron, magnesium and potassium and their interaction on femur concentrations of boron and magnesium

Treatment, $\mu\text{g/g}$ diet			Boron	Magnesium
B	Mg	K	$\mu\text{g/g}$ dry femur ¹	mg/g dry femur ²
0	100	1,800	0.59	1.13
0	400	1,800	0.48	4.23
0	100	3,600	0.54	0.98
0	400	3,600	1.16	3.92
3	100	1,800	0.83	1.13
3	400	1,800	0.59	4.25
3	100	3,600	0.88	1.03
3	400	3,600	1.18	4.08

From Shuler and Nielsen [11] and Nielsen et al. [12]; rats were on treatment for 7 weeks.

¹ Significant effects: B, 0.02; Mg, 0.06; K, 0.0001; Mg \times K, 0.0001.

² Significant effects: Mg, 0.0001; K, 0.0001.

Table 3. Effect of magnesium, boron and their interaction on serum sodium, plasma cholesterol and ionized calcium (iCa) in postmenopausal women

Dietary treatment		Serum sodium mmol/l	Plasma			
Mg, mg/day	B, mg/day		cholesterol mg/dl	LDL cholesterol, mg/dl	iCa, mg/dl	iCa, %
115	0.23	148.6	248	163	4.97	53.5
115	3.23	148.4	248	165	5.01	53.9
315	0.23	148.0	256	171	4.99	53.9
315	3.23	147.8	255	171	5.04	54.3
Analysis of variance: p values						
Boron		NS	NS	NS	0.06	NS
Magnesium		0.05	0.02	0.09	0.08	0.02
B × Mg		NS	NS	NS	NS	NS

Values used were those obtained the last 21 days in each dietary period. NS = Not significant; LDL = low-density lipoprotein.

etary intake of magnesium or the presence of hormonal changes which cause an increased loss of calcium from bone or the body (e.g. menopause). Three experiments with human subjects fed low-boron diets (0.25 mg B/2,000 kcal) have been completed. The boron depletion periods in these three experiments were for 42, 63 or 119 days. Although 42 days of depletion induced a depression in serum ionized calcium (see table 3) similar to that when depletion was for either 63 or 119 days, it did not induce the numerous significant changes found with the longer depletion periods. However, the 42-day depletion study also included periods of low magnesium intake (115 mg/2,000 kcal). Thus, in the following, all three experiments are discussed. The 63- and 119-day boron depletion experiments demonstrate the importance of boron in calcium and bone metabolism; the 42-day depletion experiment indicates the possible importance of magnesium in bone maintenance.

The first human study [13–15] was designed to examine the effects of aluminum and magnesium, in addition to boron, on macromineral metabolism. Thirteen postmenopausal women, who lived in a metabolic ward under strict supervision for 167 days, were fed a 3-day menu rotation diet composed of conventional foods, including beef, pork, rice, bread and milk, but low in fruits and vegetables. The diet provided 600 mg calcium, 870 mg phosphorus, 115 mg magnesium and 0.25 mg boron per 2,000 kcal. After an equilibration period of 23 days during which the basal low-boron diet supplemented with 200 mg of magnesium per day was fed, all women participated in four dietary periods of 24 days in which magnesium supplemented at 0 or 200 mg/day and aluminum supplemented at 0 or 1,000 mg/day were varied in a latin-square design. Completion of these four 24-day periods and the equilibration period resulted in the subjects consuming a diet low in boron

for 119 days. After completing this phase of the experiment, 12 of the women participated in two additional 24-day dietary periods in which the basal diet was supplemented with 3 mg of boron as sodium borate per day in divided doses at meal times. Seven women were fed the low-magnesium diet, and 5 women were fed the diet supplemented with 200 mg of magnesium per day during the 48 days of boron supplementation. For 24 days in this phase of the experiment, the women were fed 1,000 mg of aluminum per day.

As shown in table 4, findings were obtained from this experiment which indicated that dietary boron had a marked effect on calcium metabolism. The data show that boron supplementation reduced the urinary excretion of calcium and serum concentration of total calcium and increased the serum concentration of ionized calcium. Although the experimental design prevented a direct examination of the effect of magnesium status on the response to changes in dietary boron, the difference in urinary excretion of calcium caused by dietary boron seemed more significant in the low-magnesium than in the adequate-magnesium women. Other findings from this experiment which indicated that boron affects calcium metabolism included an elevation of serum 17β -estradiol and testosterone with boron supplementation [14].

The findings from the first human study were a stimulus to do a follow-up study in which additional variables related to calcium metabolism were determined [16–18]. Because magnesium deprivation seemed to enhance the effects of dietary boron, the diet was kept low in magnesium (115 mg/2,000 kcal) throughout the study. The subjects were 5 men over the age of 45 years, 5 post-

Table 4. Effect in postmenopausal women of boron and aluminum on the plasma concentrations of calcium and ionized calcium (iCa) and the urinary excretion of calcium

Dietary treatment mg/day		Urinary excretion of Ca g/24 h ¹	Serum calcium	
B	Al		total mg/dl ²	iCa % ³
<i>Mg = 115 mg/day</i>				
0.25	0	0.117	9.9	49.7
0.25	1,000	0.124	10.1	49.1
3.25	0	0.065	9.6	52.1
3.25	1,000	0.073	9.7	51.6
Analysis of variance: p values				
Boron		0.0004	0.02	0.002
Aluminum		NS	NS	NS
B × Al		NS	NS	NS
<i>Mg = 315 mg/day</i>				
0.25	0	0.132	9.9	49.5
0.25	1,000	0.128	9.9	49.1
3.25	0	0.104	9.7	51.0
3.25	1,000	0.113	9.6	51.5
Analysis of variance: p values				
Boron		0.001	0.03	0.02
Aluminum		NS	NS	NS
B × Al		NS	NS	NS

NS = Not significant.

¹ From Nielsen et al. [13–15].

² From Nielsen et al. [13].

³ From Nielsen et al. [13, 15].

menopausal women on estrogen therapy, 4 postmenopausal women not on estrogen therapy and 1 woman assumed to be postmenopausal, but estrogen analysis during the study revealed that she was not. The subjects, who resided at their homes during the study, ate a conventional diet similar to that used in the first human experiment; it supplied 706 mg calcium and 0.23 mg boron per 2,000 kcal. After an equilibration period of

Table 5. Effect of boron on plasma ionized calcium and serum calcitonin in subjects fed a low-magnesium diet

Dietary Boron mg/day	Plasma ionized calcium		Serum calcitonin pg/ml
	mg/dl	%	
Men over 45 years (n = 5)			
0.23	4.86	52.1	72
3.23	4.92	53.2	60
p value	0.06	0.004	0.20
Postmenopausal women (n = 4)			
0.23	4.90	52.4	77
3.23	4.97	53.6	52
p value	0.008	0.06	0.02
Postmenopausal women on estrogen therapy (n = 5)			
0.23	4.95	53.2	61
3.23	5.04	54.3	55
p value	0.02	0.02	0.02
Above combines + 1 premenopausal woman (n = 15)			
0.23	4.91	52.6	74
3.23	4.98	53.8	59
p value	0.0001	0.0001	0.001

From Nielsen et al. [17].

14 days during which the basal low-boron diet supplemented with 3 mg of boron per day as sodium borate was fed, there was a depletion period of 63 days when the basal diet was fed. This was followed by a 49-day repletion period when the basal diet was supplemented with 3 mg of boron per day.

When comparisons were made between the last 42 days of depletion and the last 35 days of repletion, several variables associated with calcium metabolism in the 15 subjects were significantly altered by dietary boron. As shown in table 5, plasma ionized calcium, whether expressed as mg/dl or as percent of total calcium, was higher during boron repletion than boron depletion. The

difference was significant for each group separately and when data from all 15 subjects were combined. Table 5 also shows that when all 15 subjects combined were used in the comparison, serum calcitonin concentrations were lower during boron repletion than boron depletion. Similar changes were found when each group was examined separately; however, significance was achieved for calcitonin with only the postmenopausal women. Other findings in this experiment included that serum 25-hydroxycholecalciferol was lower and serum osteocalcin was higher in all 15 subjects combined during boron depletion than during boron repletion [16, 17].

Subjects that were receiving estrogen therapy had the lowest serum calcitonin, and highest serum ionized calcium, concentrations. Boron supplementation changed, or tended to change, these variables in the men and postmenopausal women not on estrogen therapy in a manner that made them more like the women on estrogen therapy. This suggests that boron and estrogen were having similar effects. Thus, if estrogen is beneficial, boron must be beneficial to calcium metabolism and may aid in the prevention of bone loss related to aging.

The finding that boron repletion depressed serum calcitonin seems contrary to the suggestion that boron aids in maintaining bone integrity. Calcitonin is generally viewed as a hormone that prevents the loss of calcium from bone. However, a recent report indicates that serum calcitonin is elevated in women with postmenopausal osteoporosis [19]. Moreover, calcitonin administration has been found to cause a transient increase in calcium excretion in humans [20–22]. If serum calcitonin is directly related to the loss of calcium via the urine, then the increased serum calcitonin ob-

served during depletion can be construed as an indication that the lack of dietary boron results in the loss of body or bone calcium.

The mechanism through which boron affects calcium metabolism is unknown. However, the calcitonin findings suggest that boron has a role in the kidney which prevents the urinary loss of calcium. Further support for this suggestion is that both blood urea nitrogen and serum creatinine were found to be elevated during boron depletion [18]. Although the elevated blood urea nitrogen and serum creatinine concentrations were still in the normal range and therefore nowhere near those indicating renal failure, the elevations may be an indication of detrimental changes in the kidney. Elevations in blood urea nitrogen and serum creatinine often are signs of impaired kidney function.

In summary, two human experiments have been completed which indicate that boron has a role in calcium metabolism, probably at the kidney level. Thus, boron most likely has an important role in the maintenance of normal bones. Moreover, this role seems to become more apparent with magnesium deprivation or under conditions in which increased calcium loss from the bone is quite likely.

The preceding suggests that magnesium is important for bone maintenance through affecting the response to changes in dietary boron. However, recent human studies (unpublished) performed at the Grand Forks Human Nutrition Research Center have yielded data suggesting that magnesium deprivation alone may be detrimental to bone health.

Although magnesium deficiency can be induced with relative ease in young experimental animals, deficiency is difficult to in-

duce in humans. In fact, efforts to produce symptomatic magnesium deficiency in healthy humans simply by restricting dietary intake have been generally unsuccessful, although some subjects became hypomagnesemic or retained large amounts of magnesium when repleted [23, 24]. Thus, it was not surprising that 24 days of magnesium depletion (115 mg/2,000 kcal) resulted in only a few significant changes in the experiment that showed an effect of boron (see above). These changes included depressed urinary excretion of magnesium in response to a decreased intake of magnesium [13, 14], thus again confirming that this is a major homeostatic mechanism for magnesium. The magnesium deprivation significantly depressed serum magnesium [15], but the change was very slight, thus also confirming that serum magnesium is not a sensitive indicator of magnesium intake or status. Other small but significant changes observed during the 24-day magnesium depletion included an elevation in plasma alkaline phosphatase [15], and a depression in serum phosphorus when dietary aluminum was low, but an elevation when dietary aluminum was high [15].

Although the findings were unexciting with the 24-day dietary magnesium deprivation periods, they encouraged further study, because the small effects were on variables associated with macromineral metabolism. In the second experiment with magnesium, 13 postmenopausal women who lived in a metabolic ward were fed a 3-day menu rotation diet similar to that described for the second boron experiment [17]. At an intake of 2,000 kcal, the diet provided per day 706 mg calcium, 701 mg phosphorus, 115 mg magnesium and 0.23 mg boron. After an equilibration period of 21 days during which the

basal low-magnesium, low-boron diet supplemented with 200 mg of magnesium and 3 mg of boron per day was fed, all women participated in four dietary periods of 42 days in which magnesium supplemented at 0 and 200 mg/day and boron supplemented at 0 and 3 mg/day were varied in a latin-square design.

Evidence that the women were responding to the changes in dietary magnesium was the finding that plasma cholesterol was depressed and serum sodium was elevated during the low-magnesium periods (table 3). Most of the changes in plasma cholesterol occurred in the low-density lipoprotein fraction. Magnesium deprivation also slightly, but significantly, increased plasma copper, mean corpuscular volume and mean corpuscular hemoglobin (data not shown). These changes were similar to those obtained in rat experiments in my laboratory. Finally, but most importantly for this discussion, magnesium deprivation depressed plasma ionized calcium whether expressed as mg/dl or percent of total plasma calcium (table 3). Like in the animal experiments, dietary boron apparently had very little effect on magnesium deprivation in humans.

Although the changes obtained with the magnesium deprivation were small, they probably represent the first time that experimentally significant effects other than on urinary magnesium have been obtained by the dietary restriction of magnesium in otherwise healthy adults. Furthermore, the findings are evidence that magnesium deprivation can affect indices associated with bone metabolism. Although the change in ionized calcium probably is the most direct evidence that dietary magnesium affects bone health, the alteration in plasma cholesterol should not be ignored. Cholesterol is

the precursor in the steroid cascade that leads to 17β -estradiol. Low circulating estrogen is often implicated in the etiology of osteoporosis [3]. Thus, the change in plasma cholesterol may be another indication that magnesium deprivation changes metabolism in a manner that is detrimental to bone structure and function.

Concluding Statement

Both boron and magnesium deprivation in animals and humans cause changes in biochemical indices associated with bone metabolism; most of these changes can be construed as being detrimental to bone health. This suggests that both boron and magnesium are important nutritional factors determining the incidence of osteoporosis in humans.

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