Biochemical and Physiologic Consequences of Boron Deprivation in Humans

Forrest H. Nielsen

United States Department of Agriculture, Agricultural Research Service, Grand Forks, North Dakota

Boron deprivation experiments with humans have yielded some persuasive findings for the hypothesis that boron is an essential nutrient. In the first nutritional study with humans involving boron, 12 postmenopausal women first were fed a diet that provided 0.25 mg boron/2000 kcal for 119 days, and then were fed the same diet with a boron supplement of 3 mg boron/day for 48 days. The boron supplementation reduced the total plasma concentration of calcium and the urinary excretions of calcium and magnesium, and elevated the serum concentrations of 17β-estradiol and testosterone. This study was followed by one in which five men over the age of 45, four postmenopausal women, and five postmenopausal women on estrogen therapy were fed a boron-low diet (0.23 mg/2000 kcal) for 63 days, then fed the same diet supplemented with 3 mg boron/day for 49 days. The diet was low in magnesium (115 mg/2000 kcal) and marginally adequate in copper (1.6 mg/2000 kcal) throughout the study. This experiment found higher erythrocyte superoxide dismutase, serum enzymatic ceruloplasmin, and plasma copper during boron repletion than boron depletion. The design of the most recent experiment was the same as the second study, except this time the diet was adequate in magnesium and copper. Estrogen therapy increased plasma copper and serum 17β-estradiol concentrations; the increases were depressed by boron deprivation. Estrogen ingestion also increased serum immunoreactive ceruloplasmin and erythrocyte superoxide dismutase; these variables also were higher during boron repletion than depletion for all subjects, not just those ingesting estrogen. Dietary boron had no effect on those variables in the men and women not ingesting estrogen. These findings suggest that boron can both enhance and mimic some effects of estrogen ingestion. The findings from all three studies are consistent with the hypothesis that boron has an essential function that affects macromineral and cellular metabolism at the membrane level. — Environ Health Perspect 102(Suppl 7):59–63 (1994)

Key words: boron calcium, estrogen, hemoglobin, platelets, macrominerals, bone, membrane

Results—Human Experiments 1 and 2

Boron deprivation experiments with animals and humans have yielded some persuasive findings for the hypothesis that boron is nutritionally essential. In experimental animals, boron deprivation apparently affects the function or composition of several body systems, including the skeleton, kidney, and brain (*1,2*). The variables affected by boron deprivation are associated with the metabolism of several other nutrients including calcium, copper, and nitrogen (*1,2*). Responses to low dietary boron have been most marked when an experimental animal has had to respond to a stressful situation that adversely altered hormonal or cellular membrane status such as the deprivation of calcium, cholecalciferol, magnesium, or potassium (*1,2*). Therefore, the first nutritional study of boron (B) with humans used postmenopausal women (*3,4*); they undergo hormonal changes during perimenopause associated with an increased loss of calcium from bone or the body.

Twelve postmenopausal women first were fed a diet that provided 0.25 mg B/2000 kcal for 119 days and then were fed the same diet with a boron supplement of 3 mg/day for 48 days. During the experiment, seven of the women were fed a diet low in magnesium (116 mg/2000 kcal); the other five had their diet supplemented with 200 mg Mg/day. The boron supplementation of the boron-deprived women decreased the urinary excretion and plasma concentration of calcium and magnesium, decreased the urinary excretion and plasma concentration of phosphorus in the women fed the magnesium-low diets, and increased plasma testosterone and 17β-estradiol concentrations. Generally, the effects of boron deprivation, or supplementation, seemed more marked in the women fed the magnesium-low diet.

Another study was performed with five men over the age of 45, nine postmenopausal women (five postmenopausal women on estrogen therapy) and one premenopausal woman (originally thought to be postmenopausal). They were fed a boron-low diet, or 0.23 mg/2000 kcal for 63 days (*5,6*). Then they were fed the same diet supplemented with 3 mg B/day for 49 days. The diet was low in magnesium, 115 mg/2000 kcal, and marginally adequate in copper, 1.6 mg/2000 kcal, throughout the study. When all 15 subjects were in the comparison, serum calcitonin, 25-hydroxycholecalciferol and ceruloplasmin concentrations, plasma copper concentration and erythrocyte superoxide dismutase activity were lower whereas serum creatinine, glucose, and blood urea nitrogen concentrations were all higher during the boron depletion period than the boron repletion period. Boron supplementation of the boron-deprived women on estrogen therapy, but not the other two groups, also tended to increase serum 17β-estradiol concentrations. Table 1 shows the nature of the changes induced by dietary boron in erythrocyte superoxide dismutase, serum 17β-estradiol, plasma copper, and serum glucose.

Discussion—Human Experiments 1 and 2

The two human experiments described above yielded a bewildering and surprising array of significant findings, considering that boron has a biochemical role apparently so subtle it was considered nutritionally unimportant until the 1980s. However, a close analysis of the findings from these two experiments indicates that boron affects many of the same variables as calcium. Analysis suggests this similarity occurs because both elements affect a similar sys-

This paper was presented at the International Symposium on Health Effects of Boron and Its Compounds held 16–17 September 1992 at the University of California, Irvine, California.

Address correspondence to Dr. Forrest H. Nielsen, USDA, ARS, GFHNRC, PO Box 9034, University Station, Grand Forks, ND 58202-9034. Telephone (701) 795-8456. Fax (701)795-8395.

Table 1. Effect of boron on erythrocyte superoxide dismutase (SOD) activity, and plasma copper, serum glucose, and serum 17β-estradiol concentrations in subjects fed a diet low in magnesium and copper. [a]

Dietary boron [b]	SOD, U/g Hb	17β-estradiol, pg/ml	Copper, μg/dl	Glucose, mg/dl
Men over age 45 (n = 5)				
0	2287	33	91	94
3	2552	35	93	90
p value	0.41	0.50	0.52	0.21
Postmenopausal women (n = 4)				
0	2213	23	127	94
3	2386	18	128	88
p value	0.57	0.26	0.75	0.007
Postmenopausal women on estrogen therapy (n = 5)				
0	2160	107	141	91
3	2736	145	152	86
p value	0.04	0.09	0.03	0.009
Above combined plus one premenopausal woman (n = 15)				
0	2257	52 [c]	117	93
3	2578	64 [c]	122	88
p value	0.03	0.13	0.03	0.0004

[a] There was a depletion period of 63 days when no boron supplement was given. This was followed by a repletion period of 49 days when the boron supplement was given. Values obtained during the last 42 days of depletion and last 35 days of repletion were compared (*5,6*). [b] Amount of boron (B) as sodium borate supplemented per day to a diet containing 0.25 mg B, 115 mg Mg and 1.6 mg Cu / 2000 kcal. [c] Values do not include the premenopausal woman.

Table 2. Effect of boron on mean corpuscular hemoglobin content (MCH), hemoglobin concentration, hematocrits, and platelet counts in subjects fed a diet apparently adequate in all nutrients. [a]

Dietary boron [b]	MCH, pg	Hemoglobin, g/dl	Hematocrit, %	Platelets, 10^9/l
Men over age 45 (n = 4)				
0	30.4	14.7	43.4	280
3	31.9	14.8	42.5	256
p value	0.003	0.45	0.03	0.008
Postmenopausal women (n = 4)				
0	29.6	12.9	38.2	312
3	30.6	13.1	37.8	278
p value	0.008	0.26	0.42	0.007
Postmenopausal women on estrogen therapy (n = 5)				
0	30.3	13.0	38.7	297
3	31.8	13.3	37.5	268
p value	0.01	0.02	0.003	0.007
Above combined plus one premenopausal woman (n = 14)				
0	30.1	13.4	39.8	297
3	31.3	13.6	38.9	269
p value	0.0001	0.007	0.0001	0.0001

[a] There was a depletion period of 63 days when no boron supplement was given followed by a repletion period of 49 days when the boron supplement was given. Values obtained during the last 35 days of depletion and the last 35 days of repletion were compared (*8*). [b] Amount of boron (B) as sodium borate supplemented per day to a diet containing 0.25 mg B / 2000 kcal.

tem, or systems (which indirectly may influence many other variables). Based on plant studies involving boron, the chemical properties of boron, and the known biochemical functions of calcium, a plausible hypothesis is that boron and calcium are both involved in maintaining cell membrane structure and function and also affect cellular signal transduction. When modified by a change in the presence of one or both of the elements, cellular signal transduction would alter hormone action, or transmembrane signaling. These alterations would affect a large array of variables including those associated with hematopoiesis, erythropoiesis, macromineral and electrolyte metabolism, and even the response to estrogen therapy. Unfortunately, in the first two human studies, examination of the specific effect of boron on these processes was prevented because the studies were complicated by diets low in magnesium and marginal in copper, both dietary substances that also affect these processes. To rectify this oversight, a third experiment recently was completed with the subjects consuming a diet apparently adequate in all nutrients (including copper and magnesium).

Experimental Design and Methods—Human Experiment 3

In this third study, the subjects were four men over the age of 45, nine postmenopausal women (five postmenopausal women on estrogen therapy) and—once again—one women who was thought to be postmenopausal but estrogen analyses during the study revealed that she was not. The subjects were fed a 3-day menu rotation diet that contained conventional foods including beef, pork, rice, bread, and milk, but was low in fruits and vegetables. The energy in the diet was 11% protein, 54% carbohydrate, and 35% fat. During the first 32 days of the experiment, the diet provided only 1.7 mg copper per 2000 kcal; this was lower than intended. So, from day 33 onward, the diet was supplemented so as to contain 2.4 mg of copper per 2000 kcal. Also, at an intake of 2000 kcal, the diet provided 680 mg of calcium, 300 mg of magnesium, 450 IU of cholecalciferol and only 0.25 mg of boron. After a 14-day equilibration period, there was a 63-day depletion period during which the basal low-boron diet was fed; this was followed by a 49-day repletion period when the diet was supplemented with 3 mg of boron per day. To limit the influence of dietary factors other than boron depletion (including the increase in dietary copper at day 32) and to assure boron repletion had occurred, only the last 35 days of each dietary period were used in the statistical analyses. For each variable, a mean was computed for the dietary period of each volunteer. Paired t-tests were then used to test for dietary effects (*7*). In these tests, each individual served as his or her own control.

Results and Discussion—Human Experiment 3

Dietary boron affected indices associated with erythropoietic or hematopoietic activity (*8*). That is, boron supplementation after boron depletion decreased hematocrit, red blood cell count, and platelet count, but increased total hemoglobin and mean

Table 3. Effect of boron on erythrocyte superoxide dismutase (SOD) activity, and serum 17β-estradiol, plasma copper, and serum immunoreactive ceruloplasmin concentrations in subjects fed a diet apparently adequate in all nutrients. [a]

Dietary boron [b]	SOD, U/g Hb	17β-estradiol, pg/ml	Copper, μg/dl	Ceruloplasmin, mg/day
Men over age 45 ($n = 4$)				
0	3091	20	83	25
3	3231	17	86	28
p value	0.79	0.12	0.14	0.06
Postmenopausal women ($n = 4$)				
0	2666	11	107	30
3	3169	11	108	33
p value	0.04	0.86	0.71	0.06
Postmenopausal women on estrogen therapy ($n = 5$)				
0	2520	99	146	42
3	3327	157	159	50
p value	0.03	0.02	0.04	0.05
Above combined plus one premenopausal woman ($n = 14$)				
0	2735 [c]	48 [c]	115 [c]	33
3	3243 [c]	69 [c]	121 [c]	38
p value	0.04	0.06	0.02	0.002

[a] There was a depletion period of 63 days when no boron supplement was given followed by a repletion period of 49 days when the boron supplement was given. Values obtained during the last 35 days of depletion and the last 35 days of repletion were compared (*9*). [b] Amount of boron (B) as sodium borate supplemented per day to a diet containing 0.25 mg B/2000 kcal. [c] Values do not include the premenopausal woman.

Table 4. Effect of boron on serum 25-hydroxycholecalciferol (25-OH-D_3) concentration, and urinary hydroxyproline (HP) and calcium excretion in subjects fed a diet apparently adequate in all nutrients. [a]

Dietary boron [b]	25-OH-D_3, ng/ml	HP, mmole/day	HP/creatinine, ratio	Calcium, g/day
Men over age 45 ($n = 4$)				
0	18	0.0418	0.0038	0.263
3	26	0.0470	0.0044	0.256
p value	0.34	0.36	0.19	0.74
Postmenopausal women ($n = 4$)				
0	18	0.0546	0.0082	0.182
3	20	0.0706	0.0101	0.204
p value	0.75	0.05	0.09	0.10
Postmenopausal women on estrogen therapy ($n = 5$)				
0	18	0.0520	0.0077	0.162
3	30	0.0609	0.0092	0.163
p value	0.06	0.05	0.16	0.94
Above combined plus one premenopausal woman ($n = 14$)				
0	18	0.0495	0.0069	0.198
3	25	0.0601	0.0082	0.205
p value	0.04	0.001	0.006	0.36

[a] There was a depletion period of 63 days when no boron supplement was given followed by a repletion period of 49 days when the boron supplement was given. Values obtained during the last 35 days of depletion and the last 35 days of repletion were compared (*8,9*). [b] Amount of boron (B) as sodium borate supplemented per day to a diet containing 0.25 mg B/2000 kcal.

corpuscular hemoglobin concentration and content. Table 2 shows the nature of some of these changes. Mean corpuscular hemoglobin content (MCH) slightly decreased between the start of the study and the end of boron depletion, and then steadily increased during boron repletion. Whether the statistical analysis was by each group, i.e., men, women, and women on estrogen therapy, or as a group of 14, the difference in MCH between the last 35 days of each dietary period was significant. For instance, when all 14 subjects were used in the comparison, the difference was 30.1 vs 31.3 and was significant at the 0.0001 level. In contrast to the MCH and hemoglobin concentration, hematocrits stayed fairly constant throughout boron depletion, but decreased after boron supplementation began. The difference between the last 35 days of each period was 39.8 vs 38.9, and was significant at the 0.0001 level. Also, during boron depletion, platelet counts were elevated. Two subjects actually had platelet counts of over 400×10^9 per liter. After boron supplementation began, all platelet counts dropped below 300×10^9 per liter.

The changes in blood variables that have been presented are all within normal ranges for adults and are not of clinical significance. But the changes are statistically significant and, because of their magnitude, they are most likely indirectly the result of boron altering some cellular function or structure which can influence blood cell or hemoglobin formation.

In the third and latest experiment, boron supplementation after boron deprivation increased erythrocyte superoxide dismutase activity in postmenopausal women on estrogen therapy, and (as shown in Table 3) in postmenopausal women not ingesting estrogen. Table 3 also shows that estrogen therapy increased serum 17β-estradiol and plasma copper concentrations in the postmenopausal women. For both variables, the elevation was significantly enhanced by boron supplementation. These changes were similar to those found with these two variables in the second human boron experiment when the diet was low in magnesium and marginal in copper. In both experiments, serum 17β-estradiol and plasma copper concentrations were not significantly affected by the dietary boron changes in men and those postmenopausal women not on estrogen therapy.

The finding that physiologic amounts of boron supplementation after boron deprivation causes higher peak serum 17β-estradiol concentrations in women ingesting estrogen suggests that boron can enhance estrogen absorption or decrease 17β-estradiol breakdown or excretion. This effect of boron on serum 17β-estradiol was the most likely reason for the significant increase in plasma copper concentration. In other words, because estrogen increases plasma copper, and boron increases estrogen retention or concentration in blood, boron—through estrogen—affected plasma copper concentrations.

Because of the effects on copper and 17β-estradiol, other variables that seemed responsive to estrogen therapy were examined to see if dietary boron affected the response in the same way. It was found that boron affected some other variables responsive to estrogen therapy. However, for most

of these, the boron supplementation seemed to mimic, rather than enhance, the effects of estrogen therapy. For example, as shown in Table 3, serum immunoreactive ceruloplasmin apparently was increased by estrogen. Boron supplementation after boron deprivation increased this variable in all groups in a similar fashion. Unlike with plasma copper, the change did not occur only in the postmenopausal women ingesting estrogen. A similar effect was obtained with plasma triglycerides (*9*). Thus, boron supplementation of boron-deprived people apparently can cause changes in some variables similar to those induced by estrogen therapy. (The purpose of estrogen therapy is to prevent calcium loss and bone loss which can lead to osteoporosis, bone fractures, and crushed vertebrae.) Some further evidence from this experiment that boron, like estrogen, is a substance that can affect calcium or bone metabolism includes the finding that, as in the second experiment, boron supplementation after boron deprivation increased serum 25-hydroxycholecalciferol concentrations (Table 4).

Urinary hydroxyproline is used as an indicator of changes in bone metabolism. As shown in Table 4, the urinary excretion of hydroxyproline significantly increased during the boron repletion period. This change suggests that boron increased collagen turnover. Because increased urinary hydroxyproline usually is associated with bone loss, this finding is difficult to explain in light of the fact that most of the other changes related to calcium metabolism induced by boron supplementation after boron deprivation suggest boron has a positive effect on calcium and bone metabolism. However, urinary hydroxyproline is also derived from collagen synthesis during the breakdown of the procollagen *N*-terminal extension peptides and of neosynthesized collagen molecules (*10,11*). Therefore, it may be possible that boron supplementation increases active bone formation and causes a greater need for collagen (on which calcification is initiated). As a result, urinary hydroxyproline is slightly increased. The urinary excretion of calcium (Table 4) certainly does not suggest bone breakdown; dietary boron did not significantly affect this variable.

Because boron apparently influences calcium and bone metabolism, and because some of the effects of boron supplementation after a period of boron deprivation are similar to estrogen therapy, boron may be acting in a manner similar to estrogen in affecting calcium and bone metabolism. Unfortunately, the mechanism through which estrogen affects calcium metabolism has not been clearly established (*12,13*), but estrogen apparently can modify the actions of hormones that affect calcium metabolism. Some of this modification probably occurs at the cell membrane level. The possibility that boron has a similar effect is supported by findings with plants. They indicate boron has a regulatory role involving hormones such as auxin, gibberellic acid, and cytokinin, and the control of a second messenger such as calcium at the cell membrane level (*14,15*).

Some recent work with rats in my laboratory also supports the possibility that boron can alter membrane function. In this experiment, weanling rats were fed marginally deficient, adequate, and luxuriant amounts of potassium (0.18%, 0.36% and 0.72% of the diet) and two amounts of boron—0 and 3 mg/kg of diet. After 12 weeks on these diets, blood platelets were obtained. And, through the use of the fluorescent marker FURA-2, cellular ionized calcium concentrations were measured in the resting stage and after activation with 0.25 units per ml thrombin with and without the presence of 1.0 mM external calcium. Preliminary results suggest that the amount of calcium released from stores after activation with thrombin may be decreased by dietary boron. However, when the platelets were activated by thrombin in the presence of 1.0 mM external calcium, the response suggested that boron caused changes in the cell membrane that altered calcium transport in and out of the cell. Increasing potassium increased the transport of calcium into platelets from boron-deprived rats (454 to 548 to 586 nM). In boron-supplemented rats, the dietary potassium changes did not have much of an effect (501 to 501 to 536 nM). These cellular ionized calcium concentration findings need to be confirmed and extended before any conclusive statement can be made about their meaning. Nonetheless, the hypothesis that boron and calcium interact to influence hormone action, transmembrane signaling and/or membrane function or stability is made more attractive by these preliminary findings. Moreover, if this hypothesis is true, it provides a simple explanation for the large variety of effects of varying characteristics when boron and calcium are altered in the diet, including those found in the human experiments described above.

Summary and Conclusions

In summary, boron most likely is an essential element in the human diet. However, the exact biochemical function of boron has not been elucidated. Many findings to date suggest that boron and calcium action are interrelated, or that these two elements affect similar systems that influence many variables, including the modification of hormone action, the alteration of cell membrane characteristics, and/or transmembrane signaling. Because of its apparent nutritional importance in calcium metabolism and utilization, humans should consume foods luxuriant in boron (fruits, vegetables, legumes, and nuts).

REFERENCES

1. Nielsen FH. Nutritional requirements for boron, silicon, vanadium, nickel, and arsenic: current knowledge and speculation. FASEB J 5:2661-2667 (1991).
2. Nielsen FH. The saga of boron in food: from a banished food preservative to a beneficial nutrient for humans. Curr Top Plant Biochem Physiol 10:274–286 (1991).
3. Nielsen FH, Hunt CD, Mullen LM, Hunt JR. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. FASEB J 1:394-397 (1987).
4. Nielsen FH, Hunt CD, Mullen LM, Hunt JR. Dietary boron affects calcium, phosphorus and magnesium metabolism of postmenopausal women fed low or adequate magnesium. Proc ND Acad Sci 41:48 (1987).
5. Nielsen FH, Mullen LM, Gallagher SK. Effect of boron depletion and repletion on blood indicators of calcium status in humans fed a magnesium-low diet. J Tr Elem Exp Med 3:45–54 (1990).
6. Nielsen FH. Dietary boron affects variables associated with copper metabolism in humans. In: 6th International Trace Element Symposium 1989. As, B, Br, Co, Cr, F, Fe, Mn, Ni, Sb, Sc, Si, Sn and Other Ultratrace Elements (Anke M, Baumann W, Bräunlich H, Brückner C, Groppel B, Grün M, eds). Jena, Germany:Friedrich-Schiller-Universität, 1989; 1106–1111.
7. Fleiss JL. The Design and Analysis of Clinical Experiments. New York:John Wiley 1986.
8. Nielsen FH, Mullen LM, Nielsen EJ. Dietary boron affects blood cell counts and hemoglobin concentrations in humans. J Tr Elem Exp Med 4:211–223 (1991).
9. Nielsen FH, Gallagher SK, Johnson LK, Nielsen EJ. Boron

enhances and mimics some effects of estrogen therapy in postmenopausal women. J Tr Elem Exp Med 5:237–246 (1992).
10. Prockop DJ. Isotopic studies on collagen degradation and the urine excretion of hydroxyproline. J Clin Invest 43:453–460 (1964).
11. Hörlein D, Fietzek PP, Fuhn K. Pro-gln: the procollagen peptidase cleavage site in the alpha l (i) chain of dermatosporatic calf skin procollagen. FEBS Lett 89:279–282 (1978).
12. Nordin BEC, Morris HA. The calcium deficiency model of osteoporosis. Nutr Rev 47:65–72 (1989).
13. Rude RK, Singer FR. Hormonal modifiers of mineral metabolism other than parathyroid hormone, vitamin D, and calcitonin. In: Disorders of Mineral Metabolism, vol 2 (Bronner F, Coburn JW, eds). New York:Academic Press, 1982;481–556.
14. Parr AJ, Loughman BC. Boron and membrane function in plants. Ann Proc Phytochem Soc Eur 21:87–107 (1983).
15. Duggar, WM. Boron in plant metabolism. Encycl Plant Physiol New Ser 15B:626–650 (1983).