

# Relation of Boron to the Composition and Mechanical Properties of Bone

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A review of the experimental studies relating boron to biological effects on appendicular and axial bones in animal models suggests that numerous influences, known and unknown, affect the responsiveness of bone to dietary boron. Degrees of skeletal response to boron are modified by other nutritional variables that include calcium, magnesium, vitamin D, and fluoride. Evidence suggests that appendicular and axial bones may differ in their responses. Tests of the mechanical properties of bones may provide useful criteria for assessing the impacts of boron status on bone. These tests might resolve questions about optimal intakes of boron because mechanical properties sometimes respond to boron when composition of bones does not. Difficulty in interpreting some of the existing research arises because of the incipient state of knowledge regarding boron nutrition, to analytical problems associated with determining accurately the small quantities of boron in feed and tissues, and to technological difficulties in controlling extraneous exposure of experimental animals to boron. Yet there is considerable evidence that both compositional and functional properties of bone are affected by boron status. — *Environ Health Perspect* 102(Suppl 7):49–53 (1994)

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## Introduction

Bone loss with aging in humans can begin by the third decade for the axial skeleton and the fifth decade for the appendicular skeleton (1). Forming greater bone mass during growth protects against the effects of bone loss (2), and high calcium intake during young adulthood may improve axial and appendicular bone mass in women (3). Calcium, phosphorus, and vitamin D are established nutritional determinants of bone mineralization; but other nutrients, including boron, may modify use of these nutrients. Nielsen and co-workers (4) reported that a 3 mg/day supplement of dietary boron reduced urinary losses of Ca and magnesium (Mg) by postmenopausal women and increased serum estradiol-17 $\beta$  and testosterone, especially when dietary intake of Mg was low. Later studies from this laboratory showed that indicators of Ca status, including plasma ionized Ca, serum 25-hydroxycholecalciferol, calcitonin and osteocalcin, were affected by the boron intakes of postmenopausal women and men of comparable age (5).

Noninvasive methods to estimate bone mass in humans can assess mineral content of bone, but they cannot assess its total composition, physical structure, and functional properties (6) and may lack sensitivity to stages of change in aging bone that occur slowly, but cumulatively, over time. Rats are suitable models for studies of bone growth and modeling (7,8). These and other animal models can provide useful information about how dietary and physiologic conditions affect the total composition, physical structure, and functional qualities of bones. Times for peak growth and mineralization differ between trabecular and cortical bone (9). Thus, in addition to differences in the proportions of cortical and trabecular bone (10) and rates of resorption (11), vertebrae have a longer potential growth period than long bones in rats and humans because closure of the growth plate occurs later in the axial skeleton than in the appendicular skeleton (12,13). Therefore, bones in the axial and appendicular skeletal areas may respond differently to the same nutritional factors.

Because of boron's demonstrated relationship to aspects of metabolism that are known to affect the formation of bone mass in humans, we will review the relationship of boron to the composition and functioning of the two main types of bone in animal models under controlled conditions.

## Effects of Boron on Appendicular and Axial Bone

### Appendicular Bone

**Size and Composition.** That bone responds to boron is evident from both toxicologic and nutritional studies. Seal and Weeth (14) reported 15.6% lower Ca and 10% less phosphorus ( $P < 0.10$ ) in dry, fat-free femurs of rats receiving 300 mg boron/l drinking water, and 53% less fat in femurs of those receiving 150 or 300 mg boron/l water. Both groups were compared to controls receiving no supplemental boron; the chow fed to all groups contained an additional 54 mg boron/kg diet. The highest intake of boron reduced plasma alkaline phosphatase activity by 31.0%, so osteoblasts may have been inhibited. Calcium:phosphorus ratios and percent ash were not affected by boron intake.

Administration of boron reportedly corrected negative Ca and phosphorus balances and the hypocalcemia and secondary hyperparathyroidism associated with fluorosis in rabbits, although skeletal Ca was not significantly affected (15–18). Boron administration reduced or prevented the fluoride-induced cortical thickening in tibias (16). Uncharacterized boron-fluoride complexes and parathyroid hormone were postulated as mediators of these effects.

Daily doses of 4 or 8 mg boron/kg body weight given to castrated male pigs for 13 months decreased bone mineral content and the cortical area relative to bone surface area in femurs and metacarpal bones II

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**Table 1.** Effects of 3, 6, or 12 mg boron supplementation/kg calcium-deficient (CD) diet, experiment 1.

Variable	Control (CTL)	CD	CD + 3B	CD + 6B	CD + 12B	P		
						CTL vs CD	Among B levels	CD vs CD + B
<b>Femur</b>								
Dry wt, mg	414	277	257	248	264	0.001	ns	0.054
Calcium content, mg/bone	92.7	43.6	39.6	39.8	40.0	0.001	ns	0.037
Magnesium content, mg/bone	1.79	1.15	0.83	0.85	0.88	0.001	ns	0.001
Length, mm	32.14	30.57	31.49	31.55	31.78	0.001	ns	0.001
Inside diameter, mm	2.20	2.24	2.30	2.32	2.37	ns	ns	0.039
Wall thickness, mm	0.67	0.63	0.52	0.54	0.53	0.042	ns	0.001
Inside/outside diameter	0.62	0.64	0.69	0.68	0.69	0.090	ns	0.001
Yield load, kg	6.10	2.11	1.78	1.99	1.46	0.001	ns	0.077
Yield/ultimate stress	0.566	0.537	0.516	0.503	0.423	ns	0.048	0.049
Strain	0.0288	0.0310	0.0317	0.0286	0.0245	ns	0.076	0.068
<b>Vertebra</b>								
Magnesium content, mg/bone	0.351	0.180	0.156	0.159	0.163	0.001	ns	0.014
Peak load, kg	16.6	5.6	8.3	7.9	7.6	0.001	ns	0.017

**Table 2.** Consistent effects<sup>a</sup> of calcium-deficient (CD) or CD + 12B diets (12 mg boron supplement) in 3 experiments, compared with controls (CTL).

Variable	CTL	CD	CD + 12B
<b>Femur</b>			
Dry wt, mg	0.418	0.277	0.272
Calcium content, mg/bone	91	46	44
Outside diameter, mm	3.43	3.26	3.25
Inside diameter, mm	2.15 <sup>x</sup>	2.27 <sup>xy</sup>	2.36 <sup>y</sup>
Ultimate stress, MPa	132	69	71
Yield/ultimate stress	0.576 <sup>x</sup>	0.523 <sup>xy</sup>	0.469 <sup>y</sup>
Breaking extension	15.6	22.0	21.4
Elasticity, MPa	3128	1483	1487
<b>Vertebra</b>			
Dry wt, mg	95	53	53
Calcium content, mg/bone	19.8	8.5	9.0
Magnesium content, mg/bone	0.37	0.18	0.18
Peak load, kg	21.7	8.2	9.6

<sup>a</sup> Except as noted, CD and CD + 12B differed from CTL but not from each other. Where superscripts are shown, values not sharing a common superscript differ.

and III, and diminished bone and osteoid mass (corresponding to osteoporosis) in the iliac crest (19). (We estimate these dosages to have been equivalent, initially, to about 80 and 160 mg boron/kg diet.) The higher dose of boron also markedly reduced the volume of spongy bone in the iliac crest, with some reduction in the volume of osteoid. Boron did not significantly reduce the ash content of the iliac crest, although both levels of boron reduced rib ash. There was no evidence that boron was stored in any of the bones, possibly because of reduced femoral and metacarpal mineral content. Compared to controls, both boron levels significantly decreased

parathyroid activity. The authors concluded (19) that boron led to osteoporosis because of direct action of boron on bone, as loss of bone mass was associated with reduced parathyroid activity in their pigs. In contrast to the pigs, adult male rats (weight 200–220 g) fed 1575 mg boron/kg diet for up to 7 days accumulated boron progressively in the tibias and fibulas for the duration of the experiment (20). Bone from a human accident victim contained a higher concentration of boron than other tissues (21); boron concentrations in parietal, rib, and femoral bone were similar in a series of 33 cadavers ranging in age from 5 months to 75 years (22).

Boron content of bone did not change with age in that human study (22) nor in boron-unsupplemented, chow-fed mice studied at 30 to 1000 days of age (23).

We examined bone size and composition in mineral-depleted, female Sprague-Dawley rats that had been given added dietary boron for 6 weeks (24,25). Purified diets were patterned after energy sources in the American diet; control rations were planned to be adequate in all nutrients (26). Experimental diets provided 100% or 30% recommended Ca and 100% or 20% recommended Mg (26). Boron supplements were added at 3 to 12 mg/kg diet, and all diets were intended to provide a safe and adequate range of boron for well-nourished rats. Diets and glass-distilled water were provided *ad libitum*.

At the end of the experiments, both femurs and two vertebrae were removed and cleaned (27,28). After testing (described subsequently), bones were dried, ashed, and analyzed for Ca and Mg. In experiment 1, feeding a Ca-deficient diet (CD) reduced the weight, Ca and Mg contents, length, and cortical thickness of femurs (Table 1). Thinning of femoral wall was related to an increased ratio of inside:outside diameter. Animals fed an additional 3, 6, or 12 mg boron/kg CD diet generally responded similarly but often differed from CD rats. Adding boron to the CD diet reduced even more substantially the dry bone weight and Ca and Mg contents, while it increased bone length. Boron also increased the inside diameters of femurs without changing outer diameters, further thinning the shaft walls beyond that produced by CD alone. This change paralleled a decrease in femur mass.

Two additional experiments contained groups fed diets of the same formulations as the control (CTL), CD, and CD with 12 mg boron/kg (CD + 12B) diets in experiment 1. CD affected weight, Ca content, and outside diameter of femurs when all three experiments were evaluated (Table 2), but effects of boron on CD rats were less apparent in experiments 2 and 3 than in experiment 1 (Table 1). Inside diameters of femurs were not significantly increased by CD alone, but adding boron led to a clear increase compared with controls (Table 2).

One notable difference among these experiments was the markedly reduced serum Ca concentration in CD rats in experiment 1; the control concentration was 9.5 mg Ca/dl compared to 4.8 g/dl in CD. This hypocalcemia was completely normalized by addition of 3 to 12 mg

boron/kg diet. In experiments 2 and 3, slight reductions in serum Ca ( $-0.2$  and  $-0.3$  mg/dl) were not significant, and boron did not affect serum Ca. Thus boron effects on bone were greatest in the experiment in which CD animals exhibited low serum Ca concentrations. Others (29–31) have reported hypocalcemia and boron responsiveness in vitamin D-deficient chicks and rats. Vitamin D deficiency seems an unlikely explanation for boron effects in experiment 1 because the same lot of vitamin mix was used in experiments 1 and 2. Wherever possible, in fact, diet ingredients from the same lots were utilized. It was necessary to use a new lot of CD mineral mix in experiments 2 and 3; however, analyses revealed similar Ca, Mg, and boron contents in both lots. Boron in CD diets in experiments 2 and 3 was approximately 0.8 mg/kg, perhaps slightly less than the 1.0 mg/kg determined for experiment 1. (All boron analyses were courtesy of C.D. Hunt, Grand Forks Human Nutrition Research Center, Grand Forks, ND) All animals were of the same strain and similar initial ages (22–24 days), but those from experiment 3 were obtained from another supplier. Rats that were not expressly supplemented with boron in our experiments might have been ingesting marginal amounts of boron. Extraneous nondietary sources of boron, such as from drinking water from a glass still, may have sufficiently increased boron intake to meet boron requirements, with the extraneous extra boron reducing the likelihood that modest supplements would have significant effects. Genetic or early dietary differences between animals at different times or from different colonies (experiments 1 and 2 versus 3) could have led to different reserves of boron initially and also influenced adequacy of diets during the experiment to maintain sufficient body boron content. Dietary boron and other minerals may greatly alter tissue-boron content (32), especially in bone (20). Low initial boron reserves in experiment 1 could have enhanced effects of the supplements given. Intakes of 12 mg boron/kg were not expected to be toxic, but pigs given an estimated 80 to 160 mg boron/kg diet initially (19) exhibited thinning of walls and reduced mass of femurs described as osteoporotic changes. Qualitatively, some of these changes resembled the boron effects we observed with 12 mg/kg purified rations. It is not possible to conclude with any certainty that the range of intakes with 0 to 12 mg/kg supplements was, in fact, either always adequate or never toxic. The various intrinsic and

**Table 3.** Effects on femur of adding boron to a diet low in magnesium (MD) or in both magnesium and calcium (CMD), experiment 2.

Variable	CTL	MD	MD + 12B	CMD	CMD + 12B	P		
						CTL vs MD	CTL vs CMD	B x Ca interactions <sup>a</sup>
Ca content, mg/g bone	127	124	125	69	78	ns	.001	0.061 [ns,0.01]
Outside diameter, mm	3.60	3.47	3.61	3.55	3.45	ns	ns	0.024 [0.13,0.09]
Inside diameter, mm	2.27	2.02	2.14	2.51	2.34	0.004	0.004	0.020 [0.08,0.12]
Peak load, kg	9.58	9.50	9.20	3.12	3.85	ns	0.001	0.056 [ns,.07]
Yield stress, MPa	54.9	74.0	61.3	19.5	20.3	0.003	0.001	0.064 [0.08,ns]
Ultimate stress, MPa	114.9	124.9	110.7	46.5	57.3	ns	0.001	0.018 [.12,.07]

<sup>a</sup>P for boron effects with MD and CMD, respectively, are given in brackets; ns = >.15.

extrinsic factors that influenced nutritional and environmental conditions in these experiments were too difficult to control. Such uncertainty, familiar to others who have studied nutritional properties of boron, is due in large part to the limited existence of resources to control extraneous sources of boron and to the incipient state of knowledge in this relatively new area of nutritional study.

In experiment 2, additional groups were fed diets deficient in Mg (MD) or in both Ca and Mg (CMD) with or without 12 mg boron/kg diet (25). Among these groups, boron had no effect on the amount of femur Ca in MD animals, but it increased the bone Ca in CMD rats (Table 3). MD decreased the inside femur diameters, while CMD (like CD in experiment 1 above) increased them; because neither deficiency significantly affected outside diameters, femur shafts in MD rats were thick and those in CMD animals were thin. In a significant Ca by boron interaction, boron tended to increase both inner and outer femur diameters in MD rats and to decrease them in CMD animals. Actions of boron frequently have been associated with Mg metabolism (32,33). In rat femur, Ca and Mg concentrations were decreased by boron when dietary Mg was limited, but Ca tended to increase when Mg intake was adequate (33).

Serum Ca concentrations were altered by CD in only one of our experiments, whereas serum Mg was elevated by adding boron in all experiments. Serum Mg was unaffected by CD but was significantly elevated by boron in experiment 1 ( $p < 0.01$ ). The effect was less pronounced in other experiments, but, overall, the serum Mg for CD + 12B exceeded the concentration in controls ( $P = 0.03$ ), and boron also increased the serum Mg in Mg-depleted animals ( $p = 0.03$ ), particularly the CMD group ( $p = 0.02$ ).

**Mechanical Properties.** Although mechanical properties have often been used to assess functional characteristics of bone in relation to other nutritional factors (34,35), we are the first investigators (24,25) to examine effects of boron on mechanical properties of bone. Peak load required to break the femur was measured in a 3-point flexure test using an Instron testing machine as described elsewhere (24,25,27,28). Various bone characteristics were measured from the load-deformation curves plotted during the flexure test. The yield load, corresponding to the force at the elastic limit of femurs in our experiment 1 was reduced by CD, and tended to be further reduced by boron ( $p < 0.08$ ; Table 1). With the larger boron supplements, a decline in strain, or limit of elastic bending, was also suggested. The ultimate stress, or force-per-unit bone cross section, was not significantly affected by boron, but the ratio of stresses at yield and breaking points decreased progressively with increasing boron additions. Because there was no corresponding difference in femoral weight or Ca among the three levels of boron supplementation, this difference among boron levels may reflect a qualitative alteration of bone mineral or a change in matrix properties. A similar progressive decline in strain, or limit of elastic bending, was also suggested. When control, CD and CD + 12B groups in all three experiments were examined together, the yield:ultimate stress ratio was significantly less in CD + 12B than in controls, with the CD value intermediate (Table 2). Investigation to determine the significance of, and basis for, a difference in that ratio of stresses may help elucidate a physiological role for boron. Because the only measurements that differed among the three levels of boron in experiment 1 were mechanical properties, they may be especially useful parameters in resolving questions of optimal intakes.

Femoral ultimate stress was reduced by CMD (Table 3) but not by MD or CD alone. In these Mg-depleted rats, the effect of boron depended on Ca intake. Boron tended to decrease stress tolerated by bones of MD group, but to increase that of the CMD group.

### Axial Bone

Administration of 4 and 8 mg boron/kg body weight for 13 months to castrated male pigs reduced the ash in ribs as compared to controls (19). Vertebrae (as well as femurs) from female rats fed an adequate ration and from ovariectomized rats fed a low-Mg diet were compared with those from similar groups supplemented with 40 mg boron/kg diet for 12 weeks (36). Ca concentration in dry femur was significantly reduced by boron in ovariectomized, Mg-depleted rats; a similar trend with vertebral Ca concentration was not significant. Boron had no significant effects in intact animals fed an adequate diet.

In our shorter experiments with rats given lower amounts of boron (24,25), the dry weights and Ca and Mg contents of two vertebrae examined (13th thoracic and first lumbar) were reduced by CD (Table 2). In experiment 1 only, addition of boron led to further reduction in Mg content. Vertebral weights and ash contents were not significantly affected by boron, compared with unsupplemented Ca-depleted animals, even though dry weights of femurs were reduced by boron in the same experiment. Vertebrae were compressed by using the same testing machine as for femurs, with flat anvil and striker to ascertain the load required.

In experiment 1, boron significantly increased the maximal compressive load withstood by vertebrae, compared with CD rats (Table 1); groups fed 3 to 12 mg boron/kg diet did not differ from one another. Like differences in composition already noted, this increased strength of vertebrae is in contrast to the reduced femur loads for the same boron-supplemented animals. The slightly greater mean peak load for CD + 12B groups from all three experiments, however, was not significant (Table 2). No effects of boron on physical properties of vertebrae from MD- or CMD-fed animals were observed. Such findings as these suggest a possible redistribution of Ca in the skeleton with addition of boron to the diet.

### Significance and Limitations of Nutritional Studies to Date

Boron has tended to normalize some abnormal values in Ca- and/or Mg-defi-

cient or fluoride-supplemented animals. For example, femur length, yield extension and strain, vertebral peak load values, and serum Ca were returned to or toward normal with boron feeding in our experiment 1. Measures that were made more abnormal by boron than by another underlying mineral deficit included: femur inside diameter and yield; ultimate stress in all experiments; and in our experiment 1 CD rats, femur dry weight, Ca and Mg, wall thickness, and vertebral Mg content. Some of these effects may be desirable but others may not. The increased inside diameter of femurs was consistent with the osteoporotic changes in appendicular bones from pigs (19) in response to supplemental amounts of boron higher than we used.

One difficulty in nutritional studies of boron is lack of certainty as to the boron status, requirements, and tolerance of experimental subjects. A boron deficiency syndrome is not recognized. It is difficult to ascertain whether analysis of tissues for low concentrations of boron accurately measures their boron content because of the complexity of the analysis and the high risk of contamination during handling of the specimens for analysis. Because of limited information about dietary and environmental sources (and variability in boron content), it is understandable that initial boron reserves and extraneous sources during experiments may lead to a variety of outcomes. The lower the levels of boron studied, the greater the impact of such background variation. Yet, the interest in boron as a nutrient or, at least, as a factor proven at low levels to influence the need for and metabolism of various nutrients, makes it particularly important to test within a range of low intakes. Lack of precise knowledge as to the actual boron requirement means there is risk at low levels that conditional boron deficiency may be induced by diets that, under other conditions, would contain adequate boron. A further limitation is the early stage of development that characterizes research in the area of bone cell regulation (37).

Research reviewed here suggests that many factors, identifiable and unknown, may affect sensitivity and responsiveness of bone to dietary boron. A similar conclusion has been drawn previously concerning boron metabolism in general (33). Nutritional state with regard to minerals (calcium, magnesium, fluoride, aluminum, manganese, potassium, copper) or other nutrients (methionine, vitamin D) (4, 15-19, 29, 32, 33, 36, 38-42) modifies the type or degree of response to boron.

Most of these nutrients have clear roles in skeletal metabolism. Both qualitative and quantitative differences in the response to boron in various experiments suggest that there are other factors not yet identified that may determine boron's effects.

We originally hypothesized that sensitivity of different types of bone to boron might vary, and available evidence from several laboratories supports this concept. Even in the same animals (24,36), femurs and vertebrae sometimes were affected differently by boron, both in composition and in mechanical properties. Such responses may have occurred because of the different composition of bone in various sites and the different growth patterns of these types of bone. The results indicate the need for further examination of components of bones to ascertain why they respond differently to boron.

Despite difficulty in interpreting some of the existing research because of the nascent state of knowledge and analytical difficulties, there is considerable evidence that skeletal changes can result from varying boron intake and that these skeletal effects involve both compositional and functional properties. This evidence, together with the tremendous societal cost of dealing with health problems related to skeletal deterioration in humans, should justify further investigation of the role of boron in relation to bone.

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