The Effects of Dietary Boric Acid on Bone Strength in Rats

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ABSTRACT

The effects of dietary boron (B) (from boric acid [BA]) on bone
strength were evaluated using male F344 rats. B was administered by
dietary admixture of BA to NIH-07 feed at concentrations of 200,
1000, 3000, and 9000 ppm. The latter two levels were found in previ-
ous studies to be reproductively toxic to both males and the devel-
oping fetus. The first two levels are below and just at, respectively,
the levels for producing fetal malformations, and are below the dose
required to produce male reproductive toxicity.

Resistance to destructive testing was measured on femora, tibiae,
and lumbar vertebrae. Although femur and tibia resistance to bending
force were not affected by any amount of dietary B, vertebral resis-
tance to a crushing force was increased by ≈10%, at all dose levels
(200–9000 ppm). These data show that even levels of BA that are not
reproductively toxic can affect the strength of the axial skeleton in rats.

Index Entries: Dietary boron; vertebra; strength; male rat.

INTRODUCTION

Boron (B) is known to affect bone physiology (1–3). In previous stud-
ies of the reproductive toxicity of boric acid (BA) in rats, we found that
B reached equilibrium in bone slower than in other tissues (4). Given the
known effects of B on bone physiology, and the likely accumulation of B
in bone to levels significantly greater than those found in serum, it
seemed prudent to evaluate the effects of BA consumption on bone
strength, as measured by resistance to destructive testing. The rationale

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and the full data set have been published *(5)*; key methods and results are summarized here.

METHODS

Adult male and female Fisher-344 rats were housed in standard laboratory animal housing conditions, and all procedures were approved by the Institutional Animal Care and Use Committee. The animals were fed a diet of powdered NIH-07 control feed, to which boric acid (BA) was added to levels of 200, 1000, 3000, or 9000 ppm. From measures of body weight and feed consumption, ingested doses were calculated to be <0.2 (control), 1.7, 8.5, 26, and 68 mg B/kg/d, respectively.

Forty-two males and 6 females were used/dose level. After 1, 2, 3, 4, 5, 8, and 12 wk of consuming the diet, 6 males/dose level were weighed, asphyxiated with CO_2, and necropsied. Females ($n = 6$) were evaluated only at week 5 to compare for any gross differences between genders. These times were chosen to capture the onset of any strength changes during the putative rise in bone B levels that should occur during the first 4 wk *(5)*.

Levels of serum total Ca, P, and Mg were determined using a Monarch 2000 Chemistry System analyzer using kits from Sigma Chemical Co. (St. Louis, MO). Both tibiae and femora were removed, cleaned of grossly adherent tissue, and placed in cold saline until being measured and tested for strength (<1 wk). This has been found previously not to alter measures of bone strength *(6)*. The right humerus was placed into 10% formalin, sectioned in paraffin, and evaluated for light-level structure. The first four lumbar vertebrae were removed and frozen in saline until being cleaned and tested; this has been shown not to affect bone strength *(6)*. The long bones were placed between supports and destructively tested by an Instron 1000 using a bending force applied to the middle of the bone. Vertebrae were thawed, cleaned of adherent tissue, and placed between the base plate and the striker of the Instron. Thus, these two bone types were subject to different forces: long bones were bent, whereas vertebrae were crushed. For details of the mechanical testing, *see (5)*.

After strength testing, tibia and femora were microwave acid-digested, and the B levels determined using the inductively coupled plasma emission spectroscopy techniques described previously *(7)*.

Statistical methods were as described *(5)*.

RESULTS

Consumption of BA at levels of 200, 1000, and 3000 had no adverse effect on body weight gain; males consuming 9000 ppm BA showed slight reductions in weight gain, and weighed ≈8% less than

Table 1
Effect of BA Acid on Male F344 Rat Bone Strength**

Dietary BA (ppm)	Femur Break Stress (MPa) [a]	Vertebral Yield Load (kg)	Vertebral Break Load (kg)	Vertebral Yield Stress (MPa)	Vertebral Break Stress (MPa)	Vertebral Modulus of Elasticity (MPa)
0	185 ± 2 0 [b] (42)	32 2 ± 1 2 (36)	40 7 ± 0 9 (36)	18 4 ± 0 8 (36)	23 2 ± 0 7 (36)	662 ± 36 (36)
200	185 ± 2 0 (40)	35.4 ± 1 1* (38)	42 4 ± 0.8* (38)	20 3 ± 0 7* (38)	24 3 ± 0 6* (38)	709 ± 35 (38)
1000	184 ± 2 0 (42)	36 5 ± 1 0* (39)	43 1 ± 0.8* (39)	21 2 ± 0 7* (39)	25 0 ± 0 6* (39)	720 ± 35 (39)
3000	189 ± 2 0 (42)	35.1 ± 1 1* (35)	42.8 ± 0 9* (35)	20.7 ± 0 7* (35)	25 2 ± 0 6* (35)	687 ± 38 (35)
9000	178 ± 2 4* (41)	34 9 ± 1 4* (35)	42 6 ± 1 1 (35)	20 6 ± 0 9* (35)	25 0 ± 0 8* (35)	744 ± 42* (35)

[a]MPa = megapascals.
[b]Data are presented as body-weight adjusted means ± SEM (*n*).
*$p < 0.05$ compared to controls.
**Six rats were killed per dose level at each of seven time-points; since there was no difference across time, all data were collapsed within dose-groups. Where *n* is < 42, samples were damaged during removal or preparation.

controls by the end of the exposure period (12 wk). This was accounted for in the analysis by covarying bone strength values by body weight, since larger animals have stronger bones to help support the greater mass.

Serum Ca was reduced in a dose-dependent manner to a maximum of 10% less than control. Serum P was dose-dependently reduced by 4, 6, 10, and 16% compared to controls (all reductions significant at $p < 0.05$). Serum Mg was also reduced by ≈4, 5, 7, and 19% compared to controls ($p < 0.05$, all).

The microscopic structure of the humerus was unchanged: the growth plate, the cortical density, and the central cavity were all the same in controls and in BA-consuming rats.

For both tibia and femur, there were no differences across weeks, so all data were pooled within groups. Irrespective of the method of statistical analysis, no effects were seen on weight, length, cross-sectional area, yield load, peak load, yield stress, break stress, or the elasticity of the long bones (not shown) in either males or females. In vertebrae, also, there were no differences across weeks, so all data were pooled within treatment groups (Table 1). Although there were no effects on the physical measures of vertebral size (not shown), yield load, break load, and yield and break stresses were significantly increased by any amount of B consumption (Table 1). Elasticity was variably increased and significantly only at the highest level evaluated (9000 ppm BA). The effects seen in males were also seen in females, but generally did not reach statistical significance owing to the much smaller number of females providing the data (6 as opposed to 42 males/dose level). Nonetheless, the trend toward an increase in crush resistance is much the same in females as in males (not shown).

Long-bone levels of B in the 0, 200, 1000, 3000, and 9000 ppm BA groups were (in µg B/g bone) 0.78, 3.03, 9.79, 27.2, and 66.2.

DISCUSSION

The significant reductions seen in serum electrolytes can clearly be ascribed to B consumption, but the unfortunate lack of urinary and bone determinations of these same elements leaves us with uncertainty about whether there is increased excretion or movement of these ions into bone. Mass balance studies are required to address this issue. Either an increase or a decrease in bone Mg reduces bone strength *(8)*, suggesting that the vertebral strength increase seen in this study is coincident with, but may not be directly related to, any changes in Mg in either bone or serum.

Given the increases in strength seen in vertebrae, it would have been more satisfying to have an analysis of the microscopic structure of the vertebrae, rather than the humerus. This will be addressed in future studies.

Long bone resistance to bending was not affected by B consumption, but vertebral resistance to a crush force was increased by ≈10% at all dose levels. The most intriguing aspect of this effect is the lack of any apparent dose–response. In previous studies *(5)*, it was found that even 32 wk after the end of exposure, there was increased B in bones of animals that had earlier consumed diets containing between 3000 and 9000 ppm BA. The B values appeared to be descending to a plateau of B that was approximately two- to threefold more than control. This is effectively the same level as seen in the 200 ppm animals during consumption of the BA-containing feed. Thus, we hypothesize that the lack of a dose–response effect on bone strength is owing to the saturation of all the pertinent sites in the bones by all doses used in this study. Lower doses, if yielding lower levels of bone B, are likely to produce a graded increase in strength. This is the subject of studies being planned.

In conclusion, consumption of BA added to diets of F344 rats did not affect the ability of long bones to resist a bending force, whereas the ability of vertebrae to resist a crush force was increased by ≈10% at all dose levels. Further studies will evaluate different administration paradigms (gavage) and much lower doses, and will focus on the ovariectomized female rat model, in an attempt to define a dose–response relationship and the level of B consumption at which no increase in vertebral strength is observed.

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