Effects of Dietary Boron in Rats Fed a Vitamin D-deficient Diet

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Although boron has long been known to be a required nutrient for plants since the early part of the 20th century (1). Early studies with dietary boron in rats had equivocal results and further investigation of boron as a required nutrient for animals was ignored (1). It was not until 1981 when Hunt and Nielsen discovered a possible need for boron in the diet of the chick that the study of boron in animals received further interest (2). Hunt and Nielsen observed that vitamin D-deficient chicks responded with improved growth and lowered concentrations of serum alkaline phosphatase when boron was added to the diet. Hunt later demonstrated that in the vitamin D-deficient chick, boron decreased body growth but enhanced initiation of cartilage calcification (3). In chicks with concomitant magnesium deficiency, boron had the opposite effect (3). However, most effects of low boron diets were seen when vitamin D in the diet was also low. In growing male rats, Brommage (R. Brommage personal communication, 1989) found no differences in calcium, magnesium, and phosphorus apparent balance as well as serum concentrations of 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D, when the only difference between dietary treatment groups was supplemental boron. Although both diets contained adequate vitamin D, it was not clear whether the diet unsupplemented with boron provided a low amount of the element since the diet was never analyzed for boron. Boron has been reported to have an effect in rats when other nutrients such as magnesium, calcium, or methionine are fed in amounts below recommended dietary guidelines (4-7). Researchers in our laboratory recently investigated the effects of supplemental (2.72 ppm) or low (0.16 ppm) dietary boron on rats fed a vitamin D-deprived diet for 12 weeks (8).

Table 1. Composition of rat diet for initial boron study.

| Ingredient | Amount, g/kg dry diet |
|------------|-----------------------|
| Casein, high protein a | 160.00 |
| Ground corn, acid washed a | 708.00 |
| Corn oil a | 75.00 |
| Methionine, L c | 3.00 |
| Mineral mix a | 42.00 |
| Choline bitartrate c | 2.00 |
| AIN-76A vitamin mix a | 10.00 |

*United States Biochemical Corporation, Cleveland, OH. "Mazola corn oil." Sigma Chemical, St. Louis, MO. *Mineral mix contained (in g/kg of diet): sodium chloride, 2.0; magnesium acetate, 3.5; manganese acetate, 0.1125; copper sulfate, 0.03; potassium iodide, 0.0004; zinc acetate, 0.05; sodium selenite, 0.0003; ammonium molybdate, 0.004; chromic chloride, 0.002; ammonium vanadate, 0.0003; nickel chloride, 0.002; sodium arsenate, 0.005; potassium chloride, 3.5; potassium acetate, 4.53; sodium metasilicate, 0.05; potassium fluoride, 0.0025; ferrous sulfate, 0.2; calcium phosphate (dibasic), 17; acid-washed ground corn, 11.011. This mineral mix yielded a dietary calcium, magnesium, and phosphorus content of 0.5%, 0.04%, and 0.39% respectively. The boron-supplemented diet contained 0.017 g boric acid/kg diet, to provide 3 mg B/g diet.
were magnesium, phosphorus, and boron. Univariate procedure of SAS was used to distribute the data quartiles and indicate the mean (#), median (central horizontal line), and the 25th and 75th percentiles (designated by the lower and upper horizontal lines, respectively). The zero above the extending "whiskers" of the box plot in the first panel indicates that the data point is outside of the 1.5 interquartile range, which is equal to the distance between the 25th and 75th percentiles.

**Table 2. Table of significant effects of boron on tissue calcium and phosphorus.**

| Tissue      | Treatment | Total P, mg / g | mg P / g a |
|-------------|-----------|-----------------|------------|
| Liver       | 2.72      | 23.9 ± 2.16     | 2.64 ± 0.23 |
|             | 0.16      | 29.8 ± 1.74     | 3.21 ± 0.10 |
| Cerebellum  | 2.72      | 0.645 ± 0.063   | 2.78 ± 0.25 |
|             | 0.16      | 0.873 ± 0.063   | 3.48 ± 0.19 |
| Brain cortex| 2.72      | 0.053 ± 0.002   | 0.043 ± 0.007 |
|             | 0.16      | 0.082 ± 0.008   | 0.065 ± 0.000 |

aSee Hegsted et al. (8). bWet weight basis. cAmount of boron in diet per analysis (ppm). dBoron effect, p<0.05. eBoron effect, p<0.02.

The rats used in our study were from a colony supplemented with 3000 IU of vitamin D/kg of diet. We have previously used rats from a low-vitamin D Harlan Sprague-Dawley colony that had no supplemental vitamin D added to their customary, unrefined diet (13). The rats from the low-vitamin D colony had lower vitamin D stores than the rats from the regular (vitamin D-supplemented) Harlan Sprague-Dawley colony. Weanling rats from the low-vitamin D colony exhibited vitamin D deficiency signs earlier when fed a vitamin D-deficient diet (American Institute of Nutrition-76A); higher apparent calcium, magnesium, and phosphorus balance at the end of the study (64–72 days of age) when fed a vitamin D-adequate.

**Further Studies and Results**

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diet; and slower initial growth rate, followed by accelerated growth when fed a vitamin D-adequate diet. Therefore, use of low-vitamin D colony rats to investigate the effects of boron may enhance results. Our results from experiments with low-vitamin D and regular colony rats were similar to the results of Halloran (16). He also observed that low-vitamin D-fed rats and regular-diet rat colonies develop hypocalcemia at different rates.

Results from our laboratory support our hypothesis that a delay in depletion of variable vitamin D stores limits the response of rats to dietary boron (17). In the first of two studies with low-vitamin D-fed colony weaning rats, problems with the cornmeal-based diet (fed 1 week), resulted in a switch to rat chow (fed 12 hr) and then to a vitamin D-deficient, purified diet (17). (Table 3; diet is similar to the AIN-76A diet). The calculated value for the amount of boron added to the purified diet was 3 mg/kg diet or 3 ppm. However, the value from the analysis was 1.08 mg/kg diet (analyzed by Dr. Curtiss Hunt). The unsupplemented diet had an analyzed value of 0.23 mg boron/kg diet. Following 5 weeks on this diet, a small but significantly higher total plasma calcium (p<0.05) in the 8-week-old boron-supplemented rats indicated the need for a second study. In the second study, the rats fed supplemental boron had higher total plasma calcium at 8 (p<0.001), 10 (p<0.0005) and 12 (p<0.05) weeks of age. These results suggest that supplemental boron prevents the severe hypocalcemia of vitamin D deficiency. This condition occurs earlier in low-vitamin D-fed colony rats than in vitamin D-supplemented colony rats, during a time period associated with rapid bone growth.

Our most recent results also demonstrated effects of supplemental dietary boron using a purified (17) diet. Diet preparation time and effort are much reduced when feeding animals a purified diet. Although further work is needed to confirm that purified diets provide low bone content, use of a purified diet with an analyzed low-boron content instead of the acid-washed cornmeal-based diet may simplify future rat studies with boron.

Table 3. Purified diet fed to rats from low-vitamin D colony.

| Ingredient | Percent |
|------------|---------|
| Casein high nitrogen | 15.2 |
| dl-methionine | 0.3 |
| Cornstarch | 32.8 |
| Sucrose | 32.8 |
| Fiber-cellul | 5.6 |
| Corn oil * | 8.1 |
| AIN mineral mixture * | 4.0 |
| AIN-76A vitamin mixture | 1.0 |
| without cholecalciferol | 0.2 |

* BHT was added at 0.01% of corn oil. * Mineral mixture composition (g/kg): calcium phosphate, dibasic (CaHPO4), 500; sodium chloride, 74; potassium citrate monohydrate, 220; potassium sulfate (K2SO4), 52; magnesium oxide (MgO), 24; manganese carbonate (MnCO3) (43-48% Mn), 3.5; ferric citrate (16-17% Fe), 6; zinc carbonate (70% ZnO), 1.6; cupric carbonate (53-55% Cu), 0.3; K3D3, 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55. * Vitamin mixture composition (mg/kg): thiamine HCl, 600; riboflavin, 600; pyridoxine HCl, 700; nicotinic acid, 3000; d-calcium pantothenate, 1800; folic acid, 200; b-biotin, 20; cyanocobalamin, 1; retinyl palmitate premix (500,000 IU/g); 1; dl-tocopheryl acetate premix (250 IU/g), 20,000; menadione (vitamin K), 50.

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