The Biochemical Effects of Physiologic Amounts of Dietary Boron in Animal Nutrition Models

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This review summarizes evidence that supports working hypotheses for the roles of boron in animal model systems. It is well established that vascular plants, diatoms, and some species of marine algal flagellates have acquired an absolute requirement for boron, although the primary role of boron in plants remains unknown. Recent research findings suggest that physiologic amounts of supplemental dietary boron (PSB) affect a wide range of metabolic parameters in the chick and rat model systems. Much of the current interest in boron animal nutrition began with the initial finding that PSB stimulates growth in cholecalciferol (vitamin D₃)-deficient chicks, but does not markedly affect growth in chicks receiving adequate vitamin D₃ nutrition. The finding suggests that boron affects some aspect of vitamin D₃ metabolism or is synergistic with vitamin D₃ in influencing growth. Vitamin D₃ regulates energy substrate utilization, and current research findings indicate that dietary boron modifies that regulatory function. The concentration of circulating glucose, the most thoroughly investigated metabolite to date, responds to PSB, especially during concomitant vitamin D₃ deficiency. In chicks, PSB substantially alleviated or corrected vitamin D₃ deficiency-induced elevations in plasma glucose concentrations. The influence of vitamin D₃ on cartilage and bone mineralization is mediated in part through its role as a regulator of energy substrate utilization; calcification is an energy-intensive process. There is considerable evidence that dietary boron alleviates perturbations in mineral metabolism that are characteristic of vitamin D₃ deficiency. In rachitic chicks, PSB alleviated distortion of the marrow sprouts of the proximal tibial epiphysial plate, a distortion characteristic of vitamin D₃ deficiency. In ovo injections of boron or 1,25(OH)₂-vitamin D₃ reduced the abnormal height of the growth plate of 1-day-old chicks hatched from vitamin D₃-deficient eggs. Also, in vitamin D-deficient rats, PSB improved the apparent absorption and retention of calcium and phosphorus, and increased femur magnesium concentrations. Current findings lend support to the hypothesis that boron alleviates the symptoms of vitamin D₃ deficiency by enhancing utilization or sparing minimal supplies of an active vitamin D₃ metabolite. Also, boron and vitamin D₃ have the same overall effect on the local utilization of energy substrates. A corollary of the hypothesis is that some of the effects of dietary boron will be overshadowed by the effects of adequate amounts of dietary vitamin D₃. — Environ Health Perspect 102(Suppl 7): 35–43 (1994)

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Introduction

Dietary Boron and Body Growth

Much of the current interest in boron animal nutrition began in 1981 with the finding that physiologic amounts of boron (3 mg/kg diet), when added to diets low in boron content (≤0.3 mg/kg diet), stimulated growth in vitamin D₃-deficient (125 IU/kg) chicks. Boron supplementation did not markedly affect growth in chicks that received ample vitamin D₃ nutriture of 2500 IU/kg (Table 1) (J). Vitamin D₃ must be converted to metabolically active forms before it can function. The first obligatory enzymatic conversion to 25-hydroxyvitamin D₃ occurs in the liver. In the kidney, a major site of 25-hydroxyvitamin D₃ metabolism, an enzymatic reaction converts some of the circulating 25-hydroxyvitamin D₃ to 1,25-dihydroxyvitamin D₃. 1,25-Dihydroxyvitamin D₃, the hormonal form of the vitamin, is degraded to calcitriol acid, which is thought to represent a major inactivation route of the hormone (2). The findings from the boron feeding study suggested that boron affected some aspect of vitamin D₃ metabolism or was synergistic with vitamin D₃ to influence growth.

In a subsequent series of experiments, the dietary concentrations of calcium or magnesium were manipulated to examine the interaction between dietary boron and vitamin D₃. Magnesium deficiency was

| Table 1. Effect of boron, vitamin D₃, and their interaction on body weight and plasma alkaline phosphatase in chicks (J). |
| --- |
| Treatment* | Vitamin D₃ mg/kg | Body weight g | Phosphatase activity, units |
| Boron | IU/kg | | |
| 0 | 125 | 561 | 4.65 |
| 3 | 125 | 775 | 2.93 |
| 0 | 2500 | 896 | 1.77 |
| 3 | 2500 | 994 | 1.75 |

Analysis of variance—p values

| Boron | Vitamin D₃ | Boron × vitamin D₃ |
| --- | --- | --- |
| 0.0002 | 0.0001 | NS |
| 0.0005 |

*Values are means of 7 birds per treatment. *Amounts of boron (orthoboric acid) and vitamin D₃ supplemented to the basal diet (0.28 mg boron/kg). *At age 32 days. **Units were μmol of p-nitrophenyl phosphate split/min/ml of plasma.
chosen as a stressor of vitamin D₃ metabolism because the element is a cofactor for the hydroxylation of 25-hydroxycholecalciferol (OH) vitamin D₃ (2). Findings from boron–calcium studies (Table 2) indicated that the effect of boron supplementation on growth in chicks was eliminated when dietary calcium was increased from 10 to 20 g/kg of diet (3). Findings from boron–magnesium studies (Table 3) indicated that growth in magnesium-deficient chicks (300 mg Mg/kg diet) increased with boron supplementation (3). The response was independent of vitamin D₃ intake. However, the lowest amount of vitamin D₃ supplementation (250 IU/kg) was higher than in earlier experiments. In general, comparison of the findings on growth from the boron–calcium and boron–magnesium studies suggested that the relationship between magnesium and boron was stronger than that between calcium or phosphorus and boron. However, the boron:magnesium molar ratio was quite low in both plasma and diet. Therefore, a direct effect of boron on magnesium metabolism was not suggested. Apparently, boron indirectly influences magnesium metabolism, and ultimately, calcium and phosphorus metabolism, by influencing an enzyme or hormone system.

The concentration of boron in the supplemented diets cited was similar to that found in a variety of diets that contained more natural than purified foods. Therefore, the growth response to boron supplementation was probably physiologic and not pharmacologic in nature. For example, the concentration of boron in alfalfa was reported to be as high as 42 mg boron/kg dry material (4). In a 10% alfalfa ration, the amount of boron contributed by the alfalfa component alone would increase the total boron concentration to 4.2 mg boron/kg dry material. Samples of Ralston Rodent Laboratory Chow #5001, a common diet for the laboratory rat, were found to contain 12.1 to 13.7 mg boron/kg (5). In several fruits and vegetables typically consumed by humans, the concentration of boron is at least 2 mg/kg wet weight of material (6). Therefore, it is reasonable to assume that a physiologic amount of boron was present in the experimental chick diets, an amount defined as one within a range of concentrations typically present in animal or human diets.

**Animal Boron Nutrition Methodology**

Prior to the findings published in 1981, all other animal boron nutrition studies were published between 1939 and 1947. Unfortunately, all of the earlier findings were confounded by the use of basal diets that were either nutritionally inadequate or supplemented with excessive amounts of boron (100–2200 mg/kg) (7–11). Most findings from the earlier studies suggested that supplemental boron did not affect measured variables, such as growth, even though most of the basal diets fed reportedly contained only 0.16 to 0.45 mg boron/kg. In one unconfirmed study (10), supplemental dietary boron (100–1000 mg boron/kg) enhanced survival and maintenance of body fat, and elevated liver glycogen in severely potassium-deficient rats. The unusual technical difficulties encountered in the execution of boron nutrition studies, which may have compromised the findings of the earlier studies, justify a brief summary of techniques used to overcome these difficulties. The physical and chemical characteristics of boron put special constraints upon diet formulations, boron kinetic studies, and verification of dietary boron content.

**Diet Formulations**

Care must be taken to ensure that basal diets for boron nutrition research are nutritionally balanced, but low in boron content. Because typical commercial diets often contain appreciable amounts of boron, additional supplements of dietary boron should have negligible impact on general metabolism. On the other hand, balanced animal diets low in boron content (<0.2 mg/kg) are not difficult to formulate. Animal muscle and milk products, and certain plant species within the subclass *Monocotyledoneae*, such as the gramineous grain crops (corn, rice, wheat, barley), contain low (<0.2 mg/kg) or negligible amounts of boron. Plant products from species within the subclass *Dicotyledoneae*, which includes fruits, nuts, and vegetables, are major sources of dietary boron (6,12).

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**Table 2. Effect of boron, vitamin D₃ and their interaction on selected variables in chicks fed elevated amounts of calcium (3).**

| Treatment | Boron mg/kg | Vitamin D₃ IU/kg | Body weight g | Alkaline phosphatase activity units | Ca μg/ml | Mg μg/ml | P μm/g/ml | B ng/ml |
|-----------|-------------|-----------------|---------------|-----------------------------------|--------|--------|----------|--------|
| 0         | 125         | 730             | 4.04          | 72                                | 12.4   | 114    | 191      |        |
| 3         | 125         | 666             | 4.41          | 64                                | 10.5   | 101    | 263      |        |
| 0         | 2500        | 785             | 1.77          | 81                                | 11.1   | 134    | 225      |        |
| 3         | 2500        | 800             | 1.81          | 74                                | 10.1   | 128    | 311      |        |

Analysis of variance—p-values

| Boron     | NS*       | NS              | 0.0008       | 0.003   | 0.04   | 0.001   |        |
| Vitamin D₃| 0.0001    | 0.0001          | NS           | NS      | NS     | NS      |        |
| Boron × Vitamin D₃ | 0.03 | NS              | NS           | NS      | NS     | NS      |        |

*20 g Ca as CaCO₃/kg diet; 500 mg Mg as Mg(H₂O)₆ × H₂O/kg diet. *Amounts of boron (orthoboric acid) and vitamin D₃ supplemented to the basal diet (-0.3 mg boron/kg). At age 29 days. *Units were μmoles of p-nitrophenyl phosphate split/min/ml of plasma. Total (inorganic + organic) phosphorus. NS, nonsignificant; p > 0.05

**Table 3. Effect of boron, vitamin D₃, and their interaction on selected variables in magnesium-deficient chicks (3).**

| Treatment | Boron mg/kg | Vitamin D₃ IU/kg | Body weight g | Alkaline phosphatase activity units | Ca μg/ml | Mg μg/ml | P μm/g/ml | B ng/ml |
|-----------|-------------|-----------------|---------------|-----------------------------------|--------|--------|----------|--------|
| 0         | 250         | 444             | 3.91          | 81                                | 11.9   | 71     | 35       |        |
| 3         | 250         | 510             | 2.62          | 85                                | 12.0   | 78     | 126      |        |
| 0         | 2500        | 765             | 1.11          | 109                               | 11.8   | 73     | 60       |        |
| 3         | 2500        | 811             | 1.05          | 107                               | 12.9   | 76     | 149      |        |

Analysis of variance—p-values

| Boron     | 0.03       | 0.007           | NS*          | NS      | 0.01   | 0.0001  |        |
| Vitamin D₃| 0.0001     | 0.0001          | NS           | NS      | NS     | 0.04    |        |
| Boron × vitamin D₃ | NS | NS              | NS           | NS      | NS     | NS      |        |

*10 g Ca as CaCO₃/kg diet; 300 mg Mg as Mg(C₂H₃O₂)₂ × 4H₂O/kg diet. *Amounts of boron (orthoboric acid) and vitamin D₃ supplemented to the basal diet (-0.3 mg boron/kg). At age 29 days. *Units were μmoles of p-nitrophenyl phosphate split/min/ml of plasma. *Inorganic phosphorus only. NS, nonsignificant; p > 0.05
**Table 4. Composition of balanced basal diet for chicks low in boron and vitamin D**  

| Ingredient         | g/kg diet |
|--------------------|-----------|
| Corn, ground *     | 681.96    |
| Casein, high protein * | 160.00    |
| Corn oil           | 75.00     |
| CaHPO₄ 25.00      | 25.00     |
| Mineral mix 4      | 27.26     |
| CaCO₃ 6.59        | 6.44      |
| Iron mix 7         | 8.64      |
| Glycine, free base  | 5.00      |
| Vitamin mix 5      | 4.98      |
| L-arginine, free base | 4.00      |
| L-methionine 2.50  |           |
| Choline chloride 1.30 |             |
| Dl-α-tocopheryl 0.06 |             |

*Basal diet contained about 0.180 mg boron on an air-dried basis. *Supplemented with orthoboric acid (H₃BO₃), Puratronic Johnson Matthey Chemicals Ltd., Aesar, Seabrook, NH) and vitamin D, vitamin D3, powder in corn endosperm carrier: 400,000 IU/g; ICN Biochemicals, Cleveland, Ohio) in separate mixes of anhydrous dextrose (ICN Biochemicals). *ICN Pharmaceuticals. *Corn was acid washed with HCl. *Teklad, Division of Harlan Industries, Inc. (Madison, WI). *Baker Analyzed. "J. T. Baker, Phillipsburg, NJ." *See Table 6. *Reagent, low in alkalis (MCB, Manufacturing Chemists Inc., Cincinnati, OH). *14.50 g iron sponge, 22 mesh (Puratronic) dissolved in 157.3 ml of 6M HCl ("double distilled from Vycor.") GFS Chemicals, Columbus, OH) then mixed to dryness in 1610.45 g acid washed ground corn (see footnotes c and d). When fed at 0.644% of the diet, the iron mix will supply 59 mg iron/kg diet. *Sigma (St. Louis, MO). *See Table 5. *GIBCO (Grand Island, NY).

For chick or rat breeding studies, a well-balanced basal diet low in boron can be made from ground corn, high protein casein, and corn oil supplemented with certain minerals and vitamins (Tables 4–6). Sucrose is probably an acceptable carbohydrate source in boron test diets because it contains negligible amounts of boron (<0.015 mg/kg) (6).

Because boron leaches readily from most laboratory glassware, neither drinking water nor liquid reagents used in boron biological research should come in contact with glassware for any length of time. For example, the brief time taken to quantify deionized water volume with a standard glass volumetric pipet is sufficient exposure to glass to increase the boron content of the water sample from background concentrations to concentrations found in plasma (0.025 µg/ml) (Hunt, unpublished observations).

The specification of boron in foods has not been determined but is probably complex and dependent upon the nature of the integral ligands. If boron absorption mechanisms in plants and animals are analogous, the organic forms of boron per se are probably unavailable to animals (13). On the other hand, the strong association between polyhydroxyl ligands and boron is easily and rapidly reversed by dialysis, change in pH, heat, or the excess addition of another low-molecular-weight polyhydroxyl ligand (4). In all animal nutrition studies with boron conducted in this laboratory, boron was added to the experimental diets as orthoboric acid, because it is a common inorganic form of boron of high purity (99.9999%) that is absorbed well from the gastrointestinal tract (15).

**Boron Kinetics**

The determination of boron uptake, turnover, and excretion in animal model systems is hampered by the radiochemical properties of boron. The radioisotopes 18B, 11B, and 11B all have half-lives of less than one second. However, the two stable boron isotopes, 10B and 11B, are distributed unequally in nature (19.8 and 80.2%, respectively). As discussed in more detail elsewhere in this symposium, attempts are being made to exploit this phenomenon for the determination of boron body pool size and distribution by isotopic dilution and mass spectrometry.

**Boron Analysis**

The lack of affordable analytical capabilities of acceptable sensitivity is a major deterrent to boron nutrition research. Accurate determinations of low concentrations of boron in biological substances have proven exceptionally difficult. The analytical difficulty is exemplified by the fact that there is no current US boron certified reference biometaliferous. Analytical procedures commonly employed for trace element analysis are inappropriate for the determination of low concentrations of boron (<5.0 µg/g). Most forms of glassware and chemical reagents with background boron contamination must be avoided. Furthermore, many boron compounds volatilize at temperatures far below those required for most dry-as-hush procedures, and the extreme volatility of boron–halide compounds exacerbates the problem. This author has developed an economical procedure for the digestion of biological substances prior to boron analysis by inductively-coupled argon plasma spectrometry. The procedure circumvents many of the problems associated with boron analysis (16).

**Guideposts to the Roles of Boron in Animal Species**

It is well established that vascular plants, diatoms, and some species of marine algal flagellates have acquired an absolute requirement for boron (12,17). Even so, the primary role of boron in those organisms remains unknown. The diverse effects of boron deficiency on plant anatomy, physiology, and biochemistry suggest that the element has multiple functions. There is considerable evidence from in vitro studies
C. D. HUNT

with both plant and animal tissues that an important role of boron is metabolic regulation, because it complexes with a variety of substrate or reactant compounds in which there are hydroxyl groups in favorable positions (18). For example, two classes of enzymes are competitively inhibited in vitro by borate or its derivatives. One class, the oxidoreductase enzymes, which require pyridine or flavin nucleotides, are inhibited as borate competes for the NAD, or flavin cofactor. Some examples are aldehyde dehydrogenase (19), xanthine oxidase (20), and cytochrome b, reductase (21). Borate apparently complexes with the ribosyl cis-hydroxy groups of NAD (22). The other class of enzymes forms transition state analogues with borate and boronic acid derivatives (23). Important examples are the serine proteases, several of which are key regulators of the normal inflammatory process. The coagulation factors Xa, IXa, Xla, XIIa, activated Hageman factor, and thrombin are serine proteases within the coagulation cascade, an integral component of the inflammatory process. There are at least three boronic acids which are highly effective, slow-binding inhibitors of thrombin (24).

Key pathways of energy substrate metabolism contain members of both classes of enzymes that are inhibited by boron. In the glycolytic pathway, glyceraldehyde-3-phosphate dehydrogenase [EC 1.2.1.12] (GPD), which is composed of four identical subunits, converts D-glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. ATP and NAD+ regulate GPD activity as the former dissociates the enzyme into dimers and/or monomers (25), and the latter promotes reassociation (26). Boron also may regulate the enzyme, as there is evidence from in vitro experiments that borate binds to a specific site(s) on the enzyme that triggers structural changes and dissociation of the tetramer (27). In addition to forming a transition-state-analogue with the enzyme, boron also interacts with the NAD cofactor associated with GPD (28). Competitive inhibition with respect to NAD+ is also observed for another NAD-requiring enzyme of the glycolytic pathway, lactate dehydrogenase.

The pentose–phosphate pathway in mammalian liver generates a major part of the NADPH required for, among other things, fatty acid synthesis (29). In leukocytes, the same pathway generates the NADPH required for respiratory burst, the process by which the cell produces oxidants for attack on malignant cells, invading organisms too large to be ingested, and certain soluble mediators. The NADPH requirement is met by the oxidation of glucose-6-phosphate in the pentose–phosphate pathway. In plants, one substrate of the pentose–phosphate pathway, 6-phosphogluconate, is known to complex with boron, which thereby inhibits 6-phosphogluconate dehydrogenase. Thus, in boron-deficient plants, there is an increase in the amount of substrate metabolized via the pentose–phosphate pathway, and a decrease in that metabolized via the Krebs cycle (30).

The in vitro evidence for the inhibitory, and possibly regulatory, effects of boron on certain enzymes in energy substrate metabolism pathways, coupled with the finding that boron exerts an apparently beneficial effect on body growth, prompted further investigation of the possible role of boron in energy metabolism, especially during concomitant vitamin D3 deficiency. The well-known relationship between vitamin D3 and mineral/bone metabolism prompted investigation of the role of boron in bone morphology and metabolism as well. Thus, the effects of boron on various aspects of energy and mineral metabolism were examined simultaneously throughout the course of several experiments conducted in the author's laboratory. To facilitate discussion, those findings are reviewed separately here.

**Boron, Vitamin D3, and Energy Substrate Utilization**

**Vitamin D3**

Classical definitions of functional vitamin D deficiency do not adequately account for the dissociation between plasma-calcium concentrations, deficiency of vitamin D metabolites, and/or bone pathology under certain conditions (31). The complexity of vitamin D-deficiency disease is exemplified by the heterogeneity of pathologic conditions that characterize the disease, from diabetics to rickets, and by the number of intrinsic and extrinsic factors which either enhance or alleviate expression of vitamin D-deficiency. It was therefore important to determine whether dietary boron is a factor in the expression of the disease.

Other research showed that vitamin D3 influences energy substrate utilization as well as mineral metabolism. Rachitic chick bone that was incubated aerobically in vitro consumed more glucose and released more lactate than normal bone. When the rachitic bone was pretreated 48 hr with vitamin D3, the rate of glycolysis returned to normal (32). This effect of vitamin D3 on glycolysis may have been mediated through calcium because calcium is one of the main inhibitors of phosphofructokinase (33), a rate-limiting enzyme in the glycolytic pathway. In rats, cellular glycolysis was doubled in rachitic cartilage compared to normal cartilage. There also was a corresponding increase in the activity of phosphofructokinase, aldolase, pyruvate kinase, and lactate dehydrogenase (34). In vitro consumption of glucose in rachitic chick bone was reduced markedly by the addition of vitamin D to the culture media (35). Patients with chronic renal failure, compared to age- and sex-matched controls, exhibited impaired glucose tolerance and hyperlipoproteinemia prior to therapy. Treatment with a synthetic analogue to active vitamin D reduced fasting blood-glucose concentrations and serum triglycerides, and improved glucose tolerance (36).

There is further evidence that vitamin D3 is important in energy substrate utilization. Findings from several studies with animals and humans indicate that vitamin D3 is essential for insulin secretion (37–40). Various vitamin D3 metabolites stimulated creatine kinase BB activity in kidney and long bone diaphyses (41). Also, dietary vitamin D3 deficiency reduced hepatic glycogen content in rats (42). In the same study, designed to reexamine the hypothesis that lipid-carbohydrate conversions do not occur in mammalian liver, liver slices from vitamin D3-deficient rats had a 35% increase in glycogen content after incubation with palmitate. Similar treatment of slices from the vitamin D3-deficient rats yielded no change in glycogen content. Other findings from the study indicated that vitamin D3 treatment of rachitic animals produced a 5- and 4-fold increase in the activity of two enzymes unique to the glyoxylate cycle, isocitrate lyase, and malate synthase.

**Boron and Glucose Utilization**

Because vitamin D3 regulates indices of energy substrate utilization, and boron improves body growth in vitamin D3-deficient chicks, several studies were conducted to determine whether an interaction between dietary boron and vitamin D3 modifies energy substrate utilization. There is now considerable evidence that glucose, the most thoroughly investigated metabolite to date, responds to physiologic supplements of dietary boron, especially during concomitant vitamin D3 deficiency. In an experiment designed to test interactions between boron, magnesium, and vitamin D3, (Table 7) (43), dietary boron (3.04 vs 0.04 mg/kg diet) decreased the abnormally elevated plasma-glucose concentrations by...
Table 7. Effect in chicks of dietary boron, magnesium, and vitamin D₃ and their interactions on selected variables in chicks (43).

| Treatment* | Plasma |
|------------|--------|
| Boron mg/kg | Mg mg/kg | Vitamin D₃ IU/kg | Body weight g | Uric acid, mg/dl | Glucose, mg/dl | Albumin, g/dl | Boron ng/ml |
| 0 | 300 | 125 | 560 | 6.24 | 327 | 2.42 | 40 |
| 3 | 300 | 125 | 512 | 7.78 | 418 | 2.24 | 93 |
| 0 | 500 | 125 | 582 | 6.63 | 463 | 2.36 | 32 |
| 3 | 500 | 125 | 655 | 5.64 | 329 | 2.29 | 160 |
| 0 | 300 | 625 | 835 | 6.33 | 327 | 0.31 | 34 |
| 3 | 300 | 625 | 771 | 7.07 | 315 | 2.25 | 125 |
| 0 | 500 | 625 | 825 | 7.43 | 337 | 2.36 | 33 |
| 3 | 500 | 625 | 761 | 6.08 | 317 | 2.13 | 150 |

Analysis of variance—p values

| Boron | NS* | NS | NS | 0.0006 | 0.0001 |
| Mg | NS | NS | NS | 0.0002 |
| Vitamin D₃ | 0.0001 | NS | 0.0001 | NS | NS |
| Boron × Mg | NS | 0.0003 | 0.0001 | NS | NS |
| Mg × vitamin D₃ | NS | NS | NS | NS |
| Boron × vitamin D₃ | NS | NS | NS | NS |
| Boron × Mg × vitamin D₃ | NS | NS | 0.0002 | NS |

*Amounts of boron (orthoboric acid), magnesium (magnesium acetate) and vitamin D₃ supplemented to the basal diet (−0.04 mg B/kg). *At age 29 days. *NS, nonsignificant; p>0.05.

Table 8. Effect of dietary boron, magnesium, and molybdenum and their interactions on selected variables in vitamin D₃-deficient chicks (44).

| Treatment* | Plasma |
|------------|--------|
| Boron mg/kg | Mg mg/kg | Mo mg/kg | Body weight g | Uric acid, mg/dl | Glucose, mg/dl | MS to CEM μm |
| 0 | 300 | 0 | 418 | 7.99 | 398 | 53 |
| 3 | 300 | 0 | 426 | 8.48 | 375 | 134 |
| 0 | 300 | 20 | 431 | 9.59 | 404 | 123 |
| 3 | 300 | 20 | 481 | 6.94 | 334 | 260 |
| 0 | 500 | 0 | 552 | 8.25 | 453 | 131 |
| 3 | 500 | 0 | 491 | 6.44 | 389 | 64 |
| 0 | 500 | 20 | 559 | 6.88 | 367 | 871 |
| 3 | 500 | 20 | 503 | 6.45 | 385 | 252 |

Analysis of variance—p values

| B | NS* | 0.02 | 0.03 | NS |
| Mg | NS | 0.004 | NS | 0.03 |
| Mo | 0.0003 | NS | NS | NS |
| B × Mg | NS | NS | NS | NS |
| B × Mo | NS | NS | NS | NS |
| Mo × Mo | NS | NS | NS | NS |
| B × Mg × Mo | NS | 0.009 | 0.02 | NS |

*Amounts of boron (orthoboric acid), magnesium (magnesium acetate-4 hydrate), and molybdenum (ammonium molybdenum oxide [para]) supplemented to the basal diet (−0.47 mg B, 25.0 mg Mg, and 0.42 mg Mo/kg). *At age 25 days. *Distance between proximal end of the marrow sprouts and the proximal edge of the calcified extracellular matrix. Negative values occur when calcification begins proximal to the tip of the sprouts. *NS, nonsignificant; p>0.05.

29% in the vitamin D₃-deficient animals but only by 6% in the vitamin D₃-adequate control group. Further physiologic stress, introduced as magnesium deficiency, reversed the effect of dietary boron in the vitamin D₃-deficient chicks. Similar effects of dietary boron on glucose metabolism in vitamin D₃-deficient animals were noted in three other studies. In a study designed to test interactions among boron, magnesium, and molybdenum in vitamin D₃-deficient chicks only (44), boron (3.47 vs 0.05 mg/kg diet) decreased plasma glucose by 21% in chicks fed either adequate amounts of magnesium and no molybdenum supplementation (29%) or inadequate magnesium and supplemental molybdenum (27%) (Table 8). Findings from a preliminary study (45) (Table 9) with vitamin D₃-deficient chicks only indicated that the percent of decrease in plasma glucose concentration varied according to the amount of boron supplementation. Compared to a basal dietary boron concentration of 0.20 mg/kg, boron concentrations of 0.25, 0.33, 0.48, 1.23, 2.10, and 3.97 decreased glucose concentrations by 9, 30, 34, 29, 35, and 21%, respectively. Second-order regression analysis of the glucose values vs boron intake described a parabola whose critical value (slope equals 0) occurred around the point where the milligram of boron per kilogram of diet equaled 1.00. In vitamin D₃-deprived rats, there was a trend for boron supplementation to decrease plasma glucose concentrations (149 vs 134 mg/dl) (46). Finally, a study with humans who were fed a low-magnesium diet showed that a daily dietary intake of 3.23 mg boron for 49 days, compared to a daily intake of 0.23 mg for 63 days, decreased serum glucose concentrations (in the normal range) approximately 6% (88 vs 94 mg/dl; p<0.007) in postmenopausal women (47). In the same study, male volunteers exhibited no response to supplemental dietary boron.

Other findings suggest that boron modulates hepatic glycolysis, particularly when vitamin D₃ intake is inadequate. Dietary boron (2.25 vs 0.16 mg/kg) lowered concentrations of the glycolytic metabolites fructose-1,6-diphosphate-P₂, glyceraldehyde-3-phosphate, and (OH)₂-acetone P in freeze-clamped chick liver (Table 10) (48). Furthermore, the vitamin D₃-deprived rat that is fed supplemental boron (2.0 mg/kg) exhibits reduced plasma pyruvate concentrations (46). In summary, the evidence to date suggests that boron acts as a regulator of energy substrate utilization by quenching the activity of some enzymes and/or stabilizing reactive compounds.

**Boron, Vitamin D₃, and Mineral/Growth Cartilage Metabolism**

**Bone Metabolism**

There is evidence that initiation of cartilage calcification is dependent upon the energy charge of the chondrocyte. For example, in chick epiphyseal growth cartilage, creatine kinase activity is related to chondrocyte maturation because activity increases with cell hypertrophy. Creatine phosphate concentrations are highest in the proliferative zone and are nondetectable in calcified cartilage as compared to amounts present in
Table 9. Effects of dietary boron on growth and plasma glucose in the vitamin D$_3$-deficient chick (45).

| Treatment $^*$ | Body weight $^*$ | Glucose, mg/dl | Boron, pg/ml |
|---------------|-----------------|----------------|--------------|
| Boron, mg/kg  | g               |                |              |
| 0.200         | 604             | 496            | 58           |
| 0.248         | 662             | 452            | 60           |
| 0.334         | 613             | 349            | 58           |
| 0.481         | 695             | 327            | 62           |
| 1.231         | 710             | 352            | 95           |
| 2.095         | 725             | 330            | 131          |
| 3.973         | 509             | 400            | 218          |

Second order regression analysis—p-values

| Boron, mg/kg | Vitamin D$_3$, IU/kg | Fructose-1,6-biphosphate, µmole/g | Glycerate-2-phosphate, µmole/g | Dihydroxy-acetone phosphate, µmole/g |
|--------------|-----------------------|-----------------------------------|-------------------------------|-----------------------------------|
| 0            | 125                   | 0.069                             | 0.065                         | 0.057                             |
| 0            | 625                   | 0.074                             | 0.075                         | 0.070                             |
| 3            | 125                   | 0.038                             | 0.055                         | 0.051                             |
| 3            | 625                   | 0.062                             | 0.064                         | 0.052                             |

Analysis of variance—p-values

| Boron, mg/kg | Vitamin D$_3$, IU/kg | Fructose-1,6-biphosphate, µmole/g | Glycerate-2-phosphate, µmole/g | Dihydroxy-acetone phosphate, µmole/g |
|--------------|-----------------------|-----------------------------------|-------------------------------|-----------------------------------|
| 0            | 125                   | 0.001                             | 0.0003                        | 0.02                              |
| 3            | 125                   | 0.02                              | 0.0009                        | NS $^*$                           |
| Boron × vitamin D$_3$ | NS | NS                                  | NS                           | NS                                |

*Amount of boron in diet by analysis. Supplemental boron supplied as orthoboric acid. *At age 28 days.

Table 10. Effects of dietary boron, vitamin D$_3$ deficiency and their interaction on the concentration of selected glycolytic metabolites in chick liver (49).

| Treatment $^*$ | Fructose-1,6-biphosphate, µmole/g | Glycerate-2-phosphate, µmole/g | Dihydroxy-acetone phosphate, µmole/g |
|---------------|-----------------------------------|-------------------------------|-----------------------------------|
| Boron, mg/kg  |                                   |                               |                                   |
| 0            | 125                               | 0.069                         | 0.065                             |
| 0            | 625                               | 0.074                         | 0.075                             |
| 3            | 125                               | 0.038                         | 0.055                             |
| 3            | 625                               | 0.062                         | 0.064                             |

Analysis of variance—p-values

| Treatment $^*$ | Fructose-1,6-biphosphate, µmole/g | Glycerate-2-phosphate, µmole/g | Dihydroxy-acetone phosphate, µmole/g |
|---------------|-----------------------------------|-------------------------------|-----------------------------------|
| Boron, mg/kg  |                                   |                               |                                   |
| 0            | 125                               | 0.001                         | 0.0003                           |
| 3            | 125                               | 0.02                           | 0.0009                           |
| Boron × vitamin D$_3$ | NS | NS                                  | NS                                |

*Amounts of boron (orthoboric acid) and vitamin D$_3$, supplemented to the basal diet (0.16 mg B/kg). *NS, nonsignificant; p>0.05.

Table 11. Effect of dietary boron, magnesium, and molybdenum and their interactions on bone and mineral metabolism in the vitamin D$_3$-deficient chick (44).

| Treatment $^*$ | Calcium concentrations | Magnesium concentrations |
|---------------|------------------------|--------------------------|
| Boron, mg/kg  | Plasma, µg/ml | Femur, mg/kg | Plasma, µg/ml | Femur, µg/g |
| 0            | 300         | 0           | 77           | 110         | 9.5           | 1.91          |
| 3            | 300         | 0           | 85           | 116         | 10.0          | 1.94          |
| 0            | 300 20     | 74           | 108          | 11.6        | 2.05          |
| 3            | 300 20     | 87           | 107          | 14.4        | 2.33          |
| 0            | 500         | 0           | 108          | 111         | 21.2          | 2.91          |
| 3            | 500         | 0           | 96           | 100         | 19.2          | 2.71          |
| 0            | 500 20     | 107          | 104          | 21.6        | 2.73          |
| 3            | 500 20     | 103          | 99           | 20.7        | 2.75          |

Analysis of variance—p-values

| Treatment $^*$ | Calcium concentrations | Magnesium concentrations |
|---------------|------------------------|--------------------------|
| Boron, mg/kg  |                        |                          |
| 0            | NS                     | NS                       | NS                     | NS                     |
| 3            | NS                     | NS                       | NS                     | NS                     |
| Mg           | 0.0001                 | 0.0001                   | NS                     | NS                     |
| Mo           | NS                     | NS                       | NS                     | NS                     |
| Boron × Mg   | 0.01                   | 0.03                     | NS                     | NS                     |
| Boron × Mo   | NS                     | NS                       | NS                     | NS                     |
| Mg × Mo      | NS                     | NS                       | NS                     | NS                     |
| Boron × Mg × Mo | NS                  | NS                       | NS                     | NS                     |

*Amounts of boron (orthoboric acid), magnesium (magnesium acetate-4 hydrate), and molybdenum (ammonium molybdenum oxide [para]) supplemented to the basal diet (–0.47 mg B, 2.50 mg Mg, and 0.42 mg Mo/kg). *NS, nonsignificant; p>0.05.

Resting and hypertrophic zones (49). Mineralization of chick growth cartilage begins in the perivascular region of the marrow sprouts, a region of hypertrophic cartilage that has a higher level of oxidative metabolism than chondrocytes greater than 150 µm from the vascular channels (50). Also, redox studies of chick epiphyseal growth cartilage show that the NAD/NADH ratio is much higher in proliferative than hypertrophic cartilage (51).

Vitamin D$_3$ and Bone Metabolism

The rachitic chick, compared to the vitamin D$_3$-adequate chick, exhibits decreased creatine kinase activity in the hypertrophic cartilage of the growth plate (49). In both the proliferative and hypertrophic zones, the induction of rickets causes a large decrease in the actual concentration of NAD and NADH, as well as a perturbation in the ratio between the two. Administration of vitamin D to the rachitic birds induces a rapid increase in NAD and NADH in all zones of the growth cartilage (51).

The findings that initiation of cartilage calcification is energy dependent, and that vitamin D$_3$ regulates the energy charge of the chondrocyte, support the thesis that the influence of vitamin D$_3$ on cartilage and bone mineralization is mediated through its role as a regulator of energy substrate utilization. There are two important corollaries to the vitamin D$_3$-energy thesis. First, calcification is an energy intensive process. Second, factors that modulate energy metabolism also affect bone and mineral metabolism.

Boron and Growth Cartilage Metabolism

There is evidence that dietary boron modulates growth-cartilage metabolism. In the vitamin D$_3$-deficient chick, an interaction between dietary boron and magnesium affected the histology of the tibial epiphysial growth plate (Table 8) (44). Calcification of growth-plate cartilage matrix normally begins distal to the tips of marrow sprouts that invade the hypertrophic zone of growth cartilage from the metaphysis as a parallel array of straight excavations. The distance between the tips of the marrow sprouts and the first appearance of calcified matrix is a convenient measure of the mineralization rate. In the vitamin D$_3$-deficient chick, also stressed with magnesium inadequacy, boron supplementation inhibited the initiation of cartilage calcification, but enhanced body growth. When the chicks were supplied with adequate dietary magnesium, supplemental boron enhanced initia-
Table 12. Effect of boron, streptozotocin injection and their interaction on heart-mineral concentrations in vitamin D3-deprived rats (53).

| Treatment | Calcium, µg/g | Phosphorus, µg/g | Manganese, µg/g | Molybdenum, µg/g |
|-----------|---------------|-----------------|----------------|-----------------|
| Boron, mg/kg | Streptozotocin |                 |                |                 |
| 0         | –             | 148             | 9.68           | 1.76            | 0.75            |
| 2.4       | –             | 160             | 10.1           | 1.91            | 1.04            |
| 0         | +             | 148             | 8.90           | 1.80            | 0.92            |
| 2.4       | +             | 144             | 9.32           | 1.67            | 0.65            |

Analysis of variance-p-values

| Boron   | NS* | 0.03 | NS | NS |
|---------|-----|------|----|----|
| Streptozotocin | 0.040 | 0.0001 | NS | NS |
| Boron x streptozotocin | 0.05 | NS | 0.02 | 0.02 |

*Amounts of boron (orthoboric acid) supplemented to the basal diet (0.06 mg B/Kg). All rats were injected with either 1 ml citrate buffer/kg body weight or 75 mg streptozotocin/ml citrate buffer/kg body weight 3 days before kill. *NS, nonsignificant, p > 0.05.

Boron supplementation elevated plasma calcium and magnesium concentrations. When the chicks were supplied with adequate dietary magnesium, supplemental boron had the opposite effect on those plasma mineral concentrations.

Heart mineral metabolism is also responsive to physiologic amounts of dietary boron (Table 12) (52). In the vitamin D3-deprived rat, supplemental boron depressed cardiac calcium but elevated cardiac phosphorus concentrations. Supplemental boron also elevated cardiac manganese and molybdenum concentrations in vitamin D3-deprived rats, but depressed those concentrations in littersmates stressed with an injection of streptozotocin. The streptozotocin injection induced an acute phase of experimental diabetes characterized by decreased intestinal absorption of calcium (31) and low concentrations of plasma 1,25-(OH)2 vitamin D3 (54). Dietary boron probably had an indirect effect on cardiac mineral metabolism because supplemental boron did not affect cardiac boron concentrations (not shown).

Boron bone content correlated with potassium and zinc concentrations in iliac cortical bone samples, obtained from men and women (62 ± 11 years) who suffered from severe, untreated osteoporosis, with at least one collapsed vertebral (55). The investigators reported a negative boron–potassium correlation and a positive boron–zinc correlation. Analysis of bone from age-matched normal controls showed no correlation between either element and boron. Except for a positive correlation between magnesium and boron in the normal subjects, there were no other correlations between any two elements, although several bone minerals were analyzed (aluminum, calcium, copper, fluoride, iron, potassium, magnesium, phosphorus, lead, silicon, strontium, and zinc). Finally, in vitamin D-deficient rats, supplemental dietary boron improved the apparent absorption and retention of calcium and phosphorus, and increased femur magnesium concentrations (55).

Summary

There is considerable evidence that physiologic amounts of dietary boron modulate both energy substrate utilization and mineral metabolism. Vitamin D3 regulates energy substrate utilization. Current research findings indicate that dietary boron modifies that regulatory function. The influence of vitamin D3, in growth-cartilage mineralization is mediated at least in part through its role as a regulator of energy substrate utilization; calcification is an energy-intensive process. There is considerable evidence that dietary boron alleviates perturbations in mineral metabolism that are characteristic of vitamin D3 deficiency. The findings described herein lend support to the hypothesis that boron alleviates the symptoms of vitamin D3 deficiency by enhancing utilization or sparing minimal supplies of an active vitamin D3 metabolite. Also, boron and vitamin D3 have the same overall effect on the local utilization of energy substrates. A corollary of the hypothesis is that some of the effects of dietary boron will be overshadowed by the effects of adequate amounts of dietary vitamin D3.

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