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 D_3)-deficient chicks, but does not markedly affect growth in chicks receiving adequate vitamin D_3 nutriture. The finding suggests that boron affects some aspect of vitamin D_3 metabolism or is synergistic with vitamin D_3 in influencing growth. Vitamin D_3 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 D_3 deficiency. In chicks, PSB substantially alleviated or corrected vitamin D_3 deficiency-induced elevations in plasma glucose concentrations. The influence of vitamin D_3 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 D_3 deficiency. In rachitic chicks, PSB alleviated distortion of the marrow sprouts of the proximal tibial epiphysial plate, a distortion characteristic of vitamin D_3 deficiency. *In ovo* injections of boron or 1,25-$(OH)^2$-vitamin D_3 reduced the abnormal height of the growth plate of 1-day-old chicks hatched from vitamin D_3-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 D_3-deficiency by enhancing utilization or sparing minimal supplies of an active vitamin D_3 metabolite. Also, boron and vitamin D_3 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 D_3. — 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 D_3-deficient (125 IU/kg) chicks. Boron supplementation did not markedly affect growth in chicks that received ample vitamin D_3 nutriture of 2500 IU/kg (Table 1) (*1*). Vitamin D_3 must be converted to metabolically active forms before it can function. The first obligatory enzymatic conversion to 25-hydroxyvitamin D_3 occurs in the liver. In the kidney, a major site of 25-hydroxyvitamin D_3 metabolism, an enzymatic reaction converts some of the circulating 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. 1,25-Dihydroxyvitamin D_3, the hormonal form of the vitamin, is degraded to calcitroic 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 D_3 metabolism or was synergistic with vitamin D_3 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 D_3. Magnesium deficiency was

Table 1. Effect of boron, vitamin D_3, and their interaction on body weight and plasma alkaline phosphatase in chicks (*1*).

Treatment[a]			Plasma alkaline
Boron mg/kg	Vitamin D_3 IU/kg	Body weight[b] g	phosphatase activity, units[c]
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		0.0002	0.000
Vitamin D_3		0.0001	0.0001
Boron × vitamin D_3		NS	0.0005

[a] Amounts of boron (orthoboric acid) and vitamin D_3 supplemented to the basal diet (0.28 mg boron/kg). [b] At age 32 days. [c] Units were μmoles of *p*-nitrophenyl phosphate split/min/ml of plasma.

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Table 2. Effect of boron, vitamin D_3 and their interaction on selected variables in chicks fed elevated amounts of calcium (*3*). [a]

Treatment [b]			Plasma				
Boron mg/kg	Vitamin D_3 IU/kg	Body weight [c] g	Alkaline phosphatase activity units [d]	Ca µg/ml	Mg µg/ml	P [e] µm/gml	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	1.61	74	10.1	128	311
			Analysis of variance–*p* values				
Boron		NS [f]	NS	0.0008	0.003	0.04	0.001
Vitamin D_3		0.0001	0.0001	0.0001	NS	0.0001	NS
Boron × Vitamin D_3		0.03	NS	NS	NS	NS	NS

[a] 20 g Ca as $CaCO_3$/kg diet; 500 mg Mg as $Mg(C_2H_3O_2)_2 \times H_2O$/kg diet. [b] Amounts of boron (orthoboric acid) and vitamin D_3 supplemented to the basal diet (~0.3 mg boron/kg). [c] At age 29 days. [d] Units were µmoles of *p*-nitrophenyl phosphate split/min/ml of plasma. [e] Total (inorganic + organic) phosphorus. [f] NS, nonsignificant; $p > 0.05$

Table 3. Effect of boron, vitamin D_3, and their interaction on selected variables in magnesium-deficient chicks (*3*). [a]

Treatment [b]			Plasma				
Boron mg/kg	Vitamin D_3 IU/kg	Body weight [c] g	Alkaline phosphatase activity, units [d]	Ca µg/ml	Mg µg/ml	P [e] µm/gml	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 [f]	NS	0.01	0.0001
Vitamin D_3		0.0001	0.0001	0.0001	NS	NS	0.04
Boron × vitamin D_3		NS	0.01	NS	NS	NS	NS

[a] 10 g Ca as $CaCO_3$/kg diet; 300 mg Mg as $Mg(C_2H_3O_2)_2 \times 4H_2O$/kg diet. [b] Amounts of boron (orthoboric acid) and vitamin D_3 supplemented to the basal diet (~0.3 mg boron/kg). [c] At age 29 days. [d] Units were µmoles of *p*-nitrophenyl phosphate split/min/ml of plasma. [e] Inorganic phosphorus only. [f] NS, nonsignificant; $p > 0.05$

chosen as a stressor of vitamin D_3 metabolism because the element is a cofactor for the hydroxylation of 25-hydroxycholecalciferol (OH) vitamin D_3 (*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 D_3 intake. However, the lowest amount of vitamin D_3 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 foodstuffs. 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*).

Table 4. Composition of balanced basal diet for chicks low in boron and vitamin D_3.[a,b]

Ingredient	g/kg diet
Corn, ground[c], acid washed[d]	681.96
Casein, high protein[e]	160.00
Corn oil[c]	75.00
$CaHPO_4$[f]	25.00
Mineral mix[g]	27.26
$CaCO_3$[h]	6.59
Iron mix[i]	6.44
Glycine, free base[j]	5.00
Vitamin mix[k]	4.89
L-arginine, free base[j]	4.00
L-methionine[j]	2.50
Choline chloride[l]	1.30
DL-α-tocopherol[c]	0.06

[a] Basal diet contained about 0.180 mg boron on an air-dried basis. [b] Supplemented with orthoboric acid (H_3BO_3, Puratronic; Johnson Matthey Chemicals Ltd., Aesar, Seabrook, NH) and vitamin D_3 (vitamin D_3 powder in corn endosperm carrier; 400,000 IU/g; ICN Biochemicals, Cleveland, Ohio) in separate mixes of anhydrous dextrose (ICN Biochemicals). [c] ICN Pharmaceuticals. [d] Corn was acid washed with Hcl. [e] Teklad, Division of Harlan Industries, Inc. (Madison, WI). [f] "Baker Analyzed," J. T. Baker, Phillipsburg, NJ. [g] See Table 6. [h] Reagent, low in alkalies (MCB, Manufacturing Chemists Inc., Cincinnati, OH). [i] 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. [j] Sigma (St. Louis, MO). [k] See Table 5. [l] GIBCO (Grand Island, NY).

For chick or rat boron feeding 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 speciation 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 (*14*). 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 (99.9995%) 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 ^8B, ^{12}B, and ^{13}B all have half-lives of less than one second. However, the two stable boron isotopes, ^{10}B and ^{11}B, 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 biomaterial. 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 volatize at temperatures far below those required for most dry-ash 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 spectroscopy. The procedure circumvents many of the problems associated with boron analysis (*16*).

Table 5. Composition of vitamin mix used in basal diet for chicks.

Ingredient	g/kg vitamin mix[a]
D-dextrose, anhydrous[b]	943.5583
Nicotinic acid[c]	20.4499
DL-pantothenic acid, calcium salt[b]	8.1800
Riboflavin[c]	6.5440
Thiamine·HCl[c]	6.5440
Pyridoxine·HCl[c]	4.9080
Vitamin B_{12} (0.1% in mannitol)[b]	4.0900
Retinyl palmitate (250,000 IU/g)[b]	3.2720
Retinyl acetate (500,000 IU/g)[b]	1.6360
Folic acid[c]	1.2270
Biotin[c]	0.2045
Menadione[c]	0.2045

[a] Vitamin mix, fed at 0.489% of diet, provides (per kilogram of diet): niacin, 100 mg; Ca *d*-pantothenic acid, 40 mg; riboflavin, 32 mg; thiamine, 32 mg; pyridoxine, 24 mg; vitamin B_{12}, 0.05 mg; retinyl palmitate (250,000 IU/g), 16 mg; retinyl acetate (500,000 IU/g), 8 mg; folic acid, 6 mg; biotin, 1 mg; menadione, 1 mg. [b] ICN Pharmaceuticals, Inc., Life Sciences Group (Cleveland, OH). [c] GIBCO, (Grand Island, NY). New evidence suggests that the requirement for vitamin K should be met by vitamin K_1 (1 mg/kg) instead of menadione as recommended by the AIN-93 diet.

Table 6. Composition of mineral mix used in basal diet for chicks.[a]

Ingredient	g/kg mineral mix[a]
Corn, ground, [b] acid-washed	472.5000
KCl[c]	209.8000
$Mg(C_2H_3O_2)_2 \times 4H_2O$[c]	159.6000
NaCl[c]	137.6000
$Mn(C_2H_3O_2)_2 \times 4H_2O$[d]	12.3808
$ZnC_2H_3O_2)_2 \times 2H_2O$[e]	3.0814
$CuSO_4 \times 5H_2O$[f]	2.2010
$Na_2HAsO_4 \times 7H_2O$[g]	0.2935
$(NH_4)_6Mo_7O_{24} \times 4H_2O$, grade 1[d]	0.1467
$Cr(C_2H_3O_2)_2 \times H_2O$, purified[h]	0.0734
KI, ultrapure[i]	0.0147
Na_2SeO_3[i]	0.0110
NH_4VO_3[f]	0.0073

[a] Mineral mix, fed at 2.776% of diet, provides (per kilogram of diet): K, 3 g; Cl, 5 g; Mg, 490 mg; Na, 1.47 g; Mn, 76 mg; Zn, 25 mg; Cu, 4.5 mg; As, 2 mg; Mo, 1.3 mg; Cr, 0.55 mg; I, 0.3 mg; Se, 0.1 mg; V, 90 ng. [b] ICN Pharmaceuticals, Inc., (Cleveland, OH). [c] Baker Analyzed (J T Baker Phillipsburg, NJ). [d] Johnson Matthey Chemicals Ltd. (Aesar, Seabrook, NH). [e] Certified ACS (Fisher Scientific Co., Fair Lawn, NJ). [f] Puratronic (Johnson Matthey Chemicals Ltd.). [g] Certified ACS (Fisher Scientific Co.). [h] Fisher Scientific Co. [i] Alfa Products (Morton Thiokol, Inc., Danvers, MA).

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

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_5 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, XIa, 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 D_3 deficiency. The well-known relationship between vitamin D_3 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 D_3, and Energy Substrate Utilization

Vitamin D_3

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 diabetes 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 D_3 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 D_3, the rate of glycolysis returned to normal (*32*). This effect of vitamin D_3 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 D_3 is important in energy substrate utilization. Findings from several studies with animals and humans indicate that vitamin D_3 is essential for insulin secretion (*37–40*). Various vitamin D_3 metabolites stimulated creatine kinase BB activity in kidney and long bone diaphyses (*41*). Also, dietary vitamin D_3 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 D_3-adequate rats had a 35% increase in glycogen content after incubation with palmitate. Similar treatment of slices from the vitamin D_3-deficient rats yielded no change in glycogen content. Other findings from the study indicated that vitamin D_3 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 D_3 regulates indices of energy substrate utilization, and boron improves body growth in vitamin D_3-deficient chicks, several studies were conducted to determine whether an interaction between dietary boron and vitamin D_3 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 D_3 deficiency. In an experiment designed to test interactions between boron, magnesium, and vitamin D_3 (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 D_3 and their interactions on selected variables in chicks (*43*).

Treatment[a]			Plasma				
Boron mg/kg	Mg mg/kg	Vitamin D_3 IU/kg	Body weight[b] 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	592	6.83	463	2.36	32
3	500	125	635	5.64	329	2.29	160
0	300	625	835	6.33	327	2.31	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[c]	NS	NS	0.0006	0.0001
Mg			NS	NS	NS	NS	0.0002
Vitamin D_3			0.0001	NS	0.0001	NS	NS
Boron × Mg			NS	0.0003	0.0001	NS	0.0001
Mg × vitamin D_3				NS	NS	NS	NS
NS							
Boron × vitamin D_3			NS	NS	NS	NS	NS
Boron × Mg × vitamin D_3			NS	NS	0.0002	NS	0.02

[a] Amounts of boron (orthoboric acid), magnesium (magnesium acetate) and vitamin D_3 supplemented to the basal diet (~0.04 mg B/kg). [b] At age 29 days. [c] NS, nonsignificant; $p>0.05$.

Table 8. Effect of dietary boron, magnesium, and molybdenum and their interactions on selected variables in vitamin D_3-deficient chicks (*44*).

Treatment[a]				Plasma		
Boron, mg/kg	Mg, mg/kg	Mo, mg/kg	Body weight[b], g	Uric acid, mg/dl	Glucose, mg/dl	MS to CEM[c] µm
0	300	0	418	7.99	388	~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	369	64
0	500	20	559	6.68	367	871
3	500	20	503	6.45	385	252
			Analysis of variance–*p* values			
B			NS[d]	0.02	0.03	NS
Mg			NS	0.004	NS	0.03
Mo			0.0003	NS	NS	NS
B × Mg			NS	NS	NS	0.02
B × Mo			NS	NS	NS	NS
Mg × Mo			NS	NS	NS	NS
B × Mg × Mo			NS	0.009	0.02	NS

[a] 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). [b] At age 25 days. [c] 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. [d] NS, nonsignificant; $p>0.05$.

29% in the vitamin D_3-deficient animals but only by 6% in the vitamin D_3-adequate control group. Further physiologic stress, introduced as magnesium deficiency, reversed the effect of dietary boron in the vitamin D_3-deficient chicks. Similar effects of dietary boron on glucose metabolism in vitamin D_3-deficient animals were noted in three other studies. In a study designed to test interactions among boron, magnesium, and molybdenum in vitamin D_3-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 D_3-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, 33, 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 D_3-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 D_3 intake is inadequate. Dietary boron (2.25 vs 0.16 mg/kg) lowered concentrations of the glycolytic metabolites fructose-1,6-diphosphate-P_2, glycerate-2P, and $(OH)_2$-acetone *P* in freeze-clamped chick liver (Table 10) (*48*). Furthermore, the vitamin D_3-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 D_3, 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 epiphysial 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 [a]		Plasma	
Boron, mg/kg	Body weight [b] g	Glucose, mg/dl	Boron, µg/ml
0.200	604	496	58
0.248	662	452	60
0.334	613	349	58
0.481	695	327	82
1.231	710	352	95
2.095	725	330	131
3.973	509	400	218
	Second order regression analysis–*p* values		
	0.010	0.001	
	Critical values, mg boron/kg		
	0.86	1.04	

[a] Amount of boron in diet by analysis. Supplemental boron supplied as orthoboric acid. [b] 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 (*48*).

Treatment[a]		Fructose-1,6-biphosphate	Glycerate-2-phosphate	Dihydroxy-acetone phosphate
Boron mg/kg	Vitamin D_3 IU/kg	µmole/g	µmole/g	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		0.001	0.0003	0.02
Vitamin D_3		0.02	0.0009	NS[b]
Boron × vitamin D_3		NS	NS	NS

[a] Amounts of boron (orthoboric acid) and vitamin D_3 supplemented to the basal diet (~0.16 mg B/kg). [b] 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 [a]			Calcium concentrations		Magnesium concentrations	
Boron, mg/kg	Mg, mg/kg	Mo, mg/kg	Plasma, µg/ml	Femur, mg/g	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			
Boron			NS[b]	NS	NS	NS
Mg			0.0001	NS	0.0001	0.0001
Mo			NS	NS	0.003	NS
Boron × Mg			0.01	NS	0.03	NS
Boron × Mo			NS	NS	NS	NS
Mg × Mo			NS	NS	NS	NS
Boron × Mg × Mo			NS	NS	NS	NS

[a] 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). [b] 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 D_3-deprived rats (*53*).

Treatment [a]		Heart			
Boron, mg/kg	Streptozotocin	Calcium, µg/g	Phosphorus, mg/g	Manganese, µg/g	Molybdenum µg/g
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[b]	0.03	NS	NS
Streptozotocin		0.04	0.0001	NS	NS
Boron × streptozotocin		0.05	NS	0.02	0.02

[a] 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. [b] NS, nonsignificant; $p>0.05$.

tion of cartilage calcification and reduced body growth. The findings indicated that physiologic amounts of boron may function to modify mineral metabolism in vitamin D_3 deficiency by suppressing bone anabolism in magnesium deficiency and bone catabolism in magnesium adequacy. The effects of boron on cartilage calcification seem beneficial in both magnesium inadequacy and adequacy because the vitamin D_3 deficiency-induced mortality was substantially reduced by dietary boron. Furthermore, supplemental boron alleviated distortion of the marrow sprouts, a distortion characteristic of vitamin D_3 deficiency. Other findings from a different laboratory indicate that *in ovo* injections of boron or 1,25-$(OH)_2$ vitamin D_3 reduced the abnormal height of the growth plate of one-day-old chicks hatched from vitamin D_3-deficient eggs (*52*).

Boron and Mineral Metabolism

Dietary boron also affects mineral metabolism in blood and tissues. In the vitamin D_3-deficient chick, an interaction between dietary boron and magnesium affected calcium and magnesium concentrations in plasma but not in femora (Table 11) (*44*). Thus, in the vitamin D_3-deficient chick, also stressed with magnesium inadequacy, 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 D_3-deprived rat, supplemental boron depressed cardiac calcium but elevated cardiac phosphorus concentrations. Supplemental boron also elevated cardiac manganese and molybdenum concentrations in vitamin D_3-deprived rats, but depressed those concentrations in littermates 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 D_3 (*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 vertebra (*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, fluorine, 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 D_3 regulates energy substrate utilization. Current research findings indicate that dietary boron modifies that regulatory function. The influence of vitamin D_3 on 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 D_3 deficiency. The findings described herein lend support to the hypothesis that boron alleviates the symptoms of vitamin D_3 deficiency by enhancing utilization or sparing minimal supplies of an active vitamin D_3 metabolite. Also, boron and vitamin D_3 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 D_3.

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