Dietary Boron Decreases Peak Pancreatic In Situ Insulin Release in Chicks and Plasma Insulin Concentrations in Rats Regardless of Vitamin D or Magnesium Status[1-3]

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ABSTRACT Because dietary boron deprivation induces hyperinsulinemia in vitamin D–deprived rats, the influence of dietary boron on insulin metabolism as modified by nutritional stressors was examined in two animal models. Male weanling Sprague-Dawley rats were assigned to each of four (Experiment 1) or 8 (Experiment 2) dietary groups for 35 d: the basal diet (< 0.2 mg B; <1.0 mg Mg/kg) was supplemented with boron (as orthoboric acid) to contain <0.2 or 2.0 (a physiologic amount) mg B/kg; with magnesium (as magnesium acetate), at 100 (inadequate) or 360–400 (adequate) mg/kg; and with cholecalciferol [vitamin D-3; 25 μg/kg for study length (Experiment 2), or, depleted for 16–17 d then repleted until end of experiment (Experiments 1 and 2)]. In the rat model, boron reduced plasma insulin (Experiment 1, $P < 0.002$; Experiment 2, $P < 0.03$), but did not change glucose concentrations regardless of vitamin D-3 or magnesium status. Cockerels (1 d old) were fed a ground corn, high protein casein and corn oil–based basal diet (low boron; 0.3 mg B/kg) supplemented with boron as orthoboric acid to contain 0.3 or 1.65 mg/kg (a physiologic amount) and vitamin D-3 at 3.13 (inadequate) or 15.60 (adequate) μg/kg. In the chick model, boron decreased ($P < 0.045$) in situ peak pancreatic insulin release at 26–37 d of age regardless of vitamin D-3 nutriture. These results suggest that physiologic amounts of boron may help reduce the amount of insulin required to maintain plasma glucose. J. Nutr. 133: 3577–3583, 2003.

KEY WORDS: • *boron* • *vitamin D* • *insulin* • *chicks* • *rats*

The trace element boron is essential for all higher plants (1,2) and is beneficial or established as essential (3–11) for four animal models of human nutrition. It appears to be beneficial to humans (12,13) and to be under homeostatic control (14). Dietary boron influences energy substrate metabolism in a wide variety of biological species including humans. At the molecular level, boron influences the activities of at least 26 enzymes (15), and many of these enzymes are essential in energy substrate metabolism. For example, in plants, a serious outcome of boron deficiency is the accumulation of starch in chloroplasts and acceleration of the pentose phosphate cycle (2). In vitamin D–deficient chicks, dietary boron decreases the abnormally elevated plasma concentrations of pyruvate, β-hydroxybutyrate and triglycerides that are typically associated with vitamin D deficiency (16). Vitamin D–deprived rats exhibited significant decreases in plasma triglyceride concentrations and increases in plasma pyruvate concentrations when they were deprived of boron (8). In older volunteers (men and women) fed a low magnesium, marginal copper diet, dietary boron deprivation induced a modest but significant increase in fasting serum glucose concentrations (17).

It has been demonstrated repeatedly in the chick model that physiologic amounts of dietary boron can attenuate the rise in plasma glucose concentration induced by vitamin D deficiency (6,9,16). However, it is not understood how boron deprivation perturbs energy substrate metabolism in humans and animal models, particularly when other nutrients are provided in suboptimal amounts. Even so, there is evidence that dietary boron affects insulin metabolism. For example, our group reported hyperinsulinemia in vitamin D–deprived rats that were concurrently deprived of boron (8).

Because boron deprivation can increase fasting serum glucose concentrations in volunteers fed a low magnesium diet (17) and can induce hyperinsulinemia in the vitamin D–de-

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prived rat model (8), we hypothesized that boron deprivation will increase the amount of insulin required to maintain plasma glucose concentrations when either magnesium or vitamin D nutriture is perturbed. The goal of this study was to test this hypothesis in a rat model of boron deprivation. Furthermore, a search was initiated for the mechanism by which boron affects insulin metabolism. The chick model is highly responsive to dietary boron manipulation and, contrary to rats, presents with a pancreas well-suited for in situ perfusion. Therefore, the effects of boron, either as a dietary component or as a direct secretagogue, on pancreatic insulin release were examined in chicks challenged with dietary boron and vitamin D deficiency.

MATERIALS AND METHODS

In vivo rat model

Experimental design. All procedures involving animals were reviewed and approved by the USDA-ARS Grand Forks Human Nutrition Research Center Animal Care Committee.

Experiment 1. A blocked randomization scheme based on weight was used to assign male weanling Sprague-Dawley rats (35.1–57.6 g; Harlan Sprague Dawley, Indianapolis, IN) (8) to each of the four following dietary groups (n = 12/group). The basal diet[6] for Experiments 1 and 2 was based on acid-washed [to remove mineral contamination (18)] ground corn, high-protein casein and corn oil. The basal diet contained adequate amounts of all vitamins (except vitamin D) and minerals (except boron and magnesium) considered essential for growing rats. Supplemental dietary magnesium (as magnesium acetate) was provided at either 100 (inadequate) or 400 (adequate) mg/kg (by analysis). The basal diet (low boron) contained 0.2 mg B/kg and was supplemented with boron (as orthoboric acid) so that the finished diet contained either 0.2 or 2.0 (physiologic amount) mg B/kg (by analysis). Thus, the rats were assigned to a fully crossed, two-factor design with a completely randomized factorial arrangement of treatments. All rats were depleted of vitamin D for 17 d then repleted with dietary cholecalciferol (vitamin D-3)[7] (as 25 μg vitamin D-3/kg diet) until the end of the experiment (d 35). There were no significant differences in weight among the groups.

Experiment 2. Experiment 1 was repeated and expanded: another four treatment groups were supplemented with adequate vitamin D-3 (25 μg/kg vitamin D-3/kg diet) from d 0 to 35 (end of experiment). The finished diets contained either 0.1 or 2.0 mg B/kg and either 100 or 360 mg Mg/kg (by analysis). Thus, in Experiment 2, the rats were assigned to a fully crossed, three-factor design with a completely randomized factorial arrangement of treatments. The number, strain and weight range of rats were similar in Experiments 1 and 2.

Environment. All rats were maintained in a temperature- (22–25°C) and humidity- (42–55%) controlled room with a 12-h light:dark cycle and housed in all plastic false-bottomed cages placed inside a laminar flow rack (Becton Dickinson, Carworth Division, New York, NY). Fresh food and distilled, deionized water (18 MΩ-cm; Super Q system, Millipore, Bedford, MA) were provided daily in plastic containers and consumed ad libitum.

Biochemical analyses. After overnight food deprivation, all rats were weighed, injected with Ketamine and Rompun, exsanguinated by anterior cardiac puncture (with a sodium heparin-coated needle and syringe) and decapitated. Plasma insulin was determined by RIA (Insulin ^{125}I RIA Kit; Incstar, Stillwater, MN), using the double-antibody technique according to established methods (19). Samples, standards and controls were combined with the first antibody and tracer and incubated for 20 h at 2°C. A preprecipiated second antibody complex was added and then incubated at 24°C for 15 min, centrifuged for 20 min at 24°C at 760 × g, and decanted. ^{125}Iodine-labeled insulin was measured with an Auto-Gamma scintillation counter (Cobra, Downer's Grove, IL). Plasma glucose concentration was determined with hexokinase in the presence of ATP and magnesium ions to produce glucose-6-phosphate and ADP. The NADH product absorbed light at 340 nm, which was detected in an automated clinical chemistry analyzer (COBAS-FARA; Roche Diagnostic Systems, Hoffman-LaRoche, Nutley, NJ).

In situ chick model

Experimental design. A blocked randomization scheme based on weight was used to assign 1-d-old, Jumbo Cornish Cross Broiler cockerels (32–46 g; Hoovers Hatchery, Rudd, IA) to each of the following four dietary groups (n = 20/group). The basal chick diet (9) was based on acid-washed ground corn, high protein casein and corn oil and contained adequate amounts of all vitamins and minerals considered essential for growing chicks (20), except for vitamin D. The basal diet (low boron) contained ≤0.29 mg B/kg (by analysis) and was supplemented with boron (as orthoboric acid) so that the finished diet contained either ≤0.29 or 1.65 (regarded as a physiologic amount) mg B/kg (by analysis). The basal diet was also supplemented with vitamin D-3 [as a powder in corn endosperm carrier (ICN Biomedicals, Aurora, OH); 9984 μg/kg] at either 3.13 (inadequate) or 15.6 (adequate) μg/kg. Thus, the chicks were assigned to a fully crossed, two-factor design with a completely randomized factorial arrangement of treatments.

Environment. Chicks were housed in all plastic environmental chambers (Germfree Laboratories, Miami, FL) with a raised plastic grating floor and provided 24 h of fluorescent lighting filtered through acrylic plastic and 6.4-mm plate glass (6). All chicks had free access to fresh food and demineralized, deionized water provided daily in acrylic plastic containers. Oxytetracycline (Sigma Chemical, St. Louis, MO) was added to the drinking water (0.1 g/L) on d 1–10. The relative humidity was maintained at 50% and environmental chamber temperatures were decreased (from an initial 34°C down to a final 22°C) in ~0.4°C/d increments throughout the experiment.

Pancreas perfusions. *Pancreas preparations.* Pancreatic perfusions were performed on d 26–37. Pancreata were isolated from randomly selected, fed chicks weighing 513-1723 g under intravenously administered sodium pentobarbital anesthesia (64.8 g/L, Anpro Pharmaceutical, Arcadia, CA) at a dose of 45 mg/kg body weight. The cannulation method of King and Hazelwood (21) was used with minor modifications. In brief, the pancreaticoduodenal loop was exposed and the two main vessels to the gizzard and colon were ligated. The pancreaticoduodenal artery and vein were cannulated in rapid succession, and the pancreaticoduodenal loop was wrapped in saline-soaked gauze and maintained at 42°C using a 250-W infrared heat lamp. Body temperature was maintained within physiologic range by placing the anesthetized chick on a warmed pad.

Glucose-boron solution preparation and perfusion. Three perfusate solutions were prepared from the basal perfusion solution (**Table 1**), a Krebs-Ringer bicarbonate buffer supplemented with sodium salts of pyruvate, fumarate and glutamate. Dextran (molecular weight 40,000, Sigma Chemical ; final concentration 2.5 g/L) and albumin (Teklad, Division of Harlan Industries, Madison, WI; final concentration 0.9 g/L) were added as colloidal sources. Final concentrations (mmol/L) of constituents were: Na^+, 141; K^+, 5.93; Ca^{2+}, 5.08; Mg^{2+}, 2.36; Cl^-, 109.7; PO_4^-, 1.11; SO_4^-, 1.18; HCO_3^-, 24.9; pyruvate, 4.9; glutamate, 4.9; and fumarate, 5.4. Heparin (LyphoMed, Rosemont, IL) was added (200 USP U/L) to prevent clotting during the cannulation procedure; the pH was adjusted to 7.4 with 6 mol/L HCl ("Baker Analyzed," J. T. Baker, Phillipsburg, NJ). This solution was used to prepare the control solution [glucose, 5.55 mmol/L; boron, ≤0.93 μmol/L (by analysis); osmolarity, 286 mmol/L] (G5.5-B0.9), and the first [glucose, 38.9 mmol/L; boron, ≤0.93 μmol/L by (analysis)] (G38.9-B0.9), and second experimental solutions [glucose, 38.9 mmol/L; boron, 13.2 μmol/L (by analysis)] (G38.9-B13.2). The supplemental glucose was supplied as D-dextrose, anhydrous (ICN Pharmaceuticals, Life Sciences Group, Cleveland, OH) and boron as H_3BO_3 (Puratronic, Johnson Matthey Chemicals, Aesar, Seabrook, NH).

[6] Chick and rat diets are included as supplemental data in the online posting of this article at www.nutrition.org.

[7] Abbreviations used: 1,25-$(OH)_2$-D_3, 1α,25-dihydroxycholecalciferol; 25-OH-D_3, 25-hydroxycholecalciferol; G5.5-B0.9, G38.2-B0.9, or G38.9-B13.2, perfusion solutions containing the corresponding amount of glucose (mmol/L) and boron (μmol/L); vitamin D-3, cholecalciferol.

TABLE 1

Composition of basal perfusion solution[1]

Ingredient	g/L
NaCl[2]	5.767
Dextran[2]	2.500
$NaHCO_3$ [2]	2.092
Albumin[3]	0.900
Sodium pyruvate[4]	0.539
Heparin[5]	0.500
Glutamic acid monosodium salt[4]	0.458
KCl[6]	0.442
Sodium fumarate dibasic[4]	0.432
$CaCl_2 \cdot 2H_2O$[2]	0.373
Na_2HPO_4 [2]	0.158
$MgSO_4$ [2]	0.142

[1] The basal perfusion solution contained ≤0.93 μmol B/L by analysis.
[2] Sigma Chemical, St. Louis, MO.
[3] Teklad, Division of Harlan Industries, Madison, WI.
[4] Aldrich Chemical, Milwaukee, WI.
[5] 10,000 USP U/mL, LyphoMed, Rosemont, IL.
[6] "Baker Analyzed," J. T. Baker, Phillipsburg, NJ.

Experimental and control solutions (aerated with a mixture of 95% O_2 and 5% CO_2) were placed in cleaned intravenous bags and hung in a heated perfusion chamber (CBC Industries, Philadelphia, PA). A peristaltic pump ("WIZ," Isco, Lincoln, NE) pumped each solution through the pancreaticoduodenal artery at a flow rate of 0.9 mL/min for 10 min each, and the venous effluent was collected in 1-min fractions into chilled 5-mL polypropylene tubes (Becton, Dickinson Labware, Lincoln Park, NJ) and put on ice.

To examine the effect of boron as a direct secretagogue on in situ insulin output of the pancreatic β-cells during a glucose load, the control solution (G5.5-B0.9) was perfused first to establish baseline values. The first experimental solution (G38.9-B0.9) introduced the glucose load and the second (G38.9-B13.2), both glucose and boron. Subsequently, the G38.9-B0.9 solution (low boron) was reintroduced to determine the residual effects of boron as a direct secretagogue. Finally, the control solution (G5.5-B0.9) was reintroduced to evaluate recovery from the glucose load. Thus, five phases comprised each perfusion, and a complete perfusion cycle lasted 50 min. After each in situ perfusion, the pancreas was removed, weighed, lyophilized and weighed again.

Collection fraction analyses. Immediately after each perfusion, all collection fractions were centrifuged at 0°C for 15 min (760 × *g*) to remove erythrocytes, and the supernatant was decanted and frozen at −77°C in plastic vials until assayed. Samples were then thawed and 20- and 100-μL aliquots were taken for measurement of glucose and immunoreactive insulin, respectively, as described below.

Biochemical analyses. Insulin concentrations in each collection fraction were determined by RIA (Insulin ^{125}I RIA Kit; Incstar, Stillwater, MN) as described for the rat experiments. Insulin was expressed as pmol insulin secreted/(kg dry pancreas · min). Glucose concentrations in each collection fraction were determined by a commercially available colorimetric kit (Glu-cinet glucose test; Sclavo Diagnostics, Wayne, NJ) with the glucose oxidase-peroxidase reagent containing hydroxybenzoate-4-aminoantipyrine. The intensity of the color of this complex was directly proportional to the concentration of D-glucose in the sample. The absorbance of standards and unknown collection fractions were measured against a blank at 510 nm on a Biomek 1000 Automated Laboratory Workstation with built-in spectrophotometer (Beckman Instruments, Schaumburg, IL).

Statistical analyses. For all analyses, values beyond two SD of groups means were removed as outliers. In cases in which Bartlett's test for homogeneity of variance indicated that the homogeneity assumption for ANOVA was violated (22), data were transformed (23) into the natural log (plasma insulin and glucose, rat model Experiment 2). The means of transformed data reported in the tables represent appropriate back-transformations. The chick in situ insulin data were analyzed by using a 2 × 2 × 5 mixed model ANOVA; the phase was a repeated measure. All other data were analyzed by 2 × 2 or 2 × 2 × 2 ANOVA as appropriate (24). Differences were considered significant at the $P < 0.05$ level.

RESULTS

In vivo rat model

Dietary boron. In both experiments, rats deprived of boron had significantly higher plasma insulin concentrations than rats supplemented with boron (Experiment 1, **Table 2**; Experiment 2, **Table 3**). The higher plasma insulin was not accompanied by a change in plasma glucose concentrations in

TABLE 2

Effects of dietary boron, magnesium, and their interaction on body weight, and plasma glucose and insulin concentrations in rats (Experiment 1)[1]

Treatment[2]			Plasma		
Boron	Mg	Vitamin D status	Insulin	Glucose	Body weight d 35
mg/kg			*pmol/L*	*mmol/L*	*g*
0.2	100	Depleted/Repleted	44.6 ± 3.1 (11)[3]	12.8 ± 0.8 (10)	199 ± 4 (12)
2.0	100	Depleted/Repleted	37.0 ± 3.2 (10)	11.3 ± 0.8 (11)	197 ± 4 (12)
0.2	400	Depleted/Repleted	50.4 ± 3.2 (10)	10.9 ± 0.8 (11)	234 ± 4 (12)
2.0	400	Depleted/Repleted	36.3 ± 3.2 (10)	11.1 ± 0.7 (12)	245 ± 4 (11)
		df	ANOVA *P*-values		
Boron		1	0.002	0.40	0.28
Magnesium		1	0.43	0.20	0.0001
Boron × Magnesium		1	0.31	0.28	0.12

[1] Weanling male rats were fed their respective diets for 35 d and then killed after overnight feed-deprivation.
[2] Total mean amounts of boron and magnesium in diets (by analysis) after supplementation of basal diet (boron, 0.2 mg; magnesium, <1.0 mg/kg) with boron (as orthoboric acid) and magnesium (as magnesium acetate). Rats were depleted of vitamin D for 17 d then repleted with dietary vitamin D-3 (25 μg cholecalciferol/kg) on experimental d 18 until the end of the experiment.
[3] Values are group means ± pooled SEM (*n*). The number varied according to availability, integrity and/or variability of individual samples.

TABLE 3

Effects of dietary boron, magnesium, vitamin D status and their interactions on body weight, and plasma glucose and insulin concentrations in rats (Experiment 2)[1]

Treatment[2]			Plasma		Body weight d 35
Boron	Mg	Vitamin D status	Insulin[3]	Glucose[3]	
mg/kg			*pmol/L*	*mmol/L*	*g*
0.1	100	Depleted/Repleted	[43.4] 3.77 ± 0.09 (9)[4]	[6.17] 1.82 ± 0.08 (9)	201 ± 7 (9)
2.0	100	Depleted/Repleted	[40.8] 3.71 ± 0.09 (10)	[6.97] 1.94 ± 0.07 (12)	201 ± 6 (11)
0.1	360	Depleted/Repleted	[59.2] 4.08 ± 0.08 (12)	[8.45] 2.13 ± 0.07 (12)	277 ± 6 (11)
2.0	360	Depleted/Repleted	[53.6] 3.98 ± 0.08 (12)	[8.93] 2.19 ± 0.07 (12)	287 ± 6 (11)
0.1	100	Adequate	[42.2] 3.74 ± 0.09 (10)	[6.56] 1.88 ± 0.07 (10)	216 ± 6 (10)
2.0	100	Adequate	[37.0] 3.61 ± 0.08 (11)	[6.96] 1.94 ± 0.07 (11)	216 ± 6 (11)
0.1	360	Adequate	[47.0] 3.85 ± 0.08 (12)	[9.07] 2.21 ± 0.07 (12)	285 ± 6 (12)
2.0	360	Adequate	[37.2] 3.62 ± 0.08 (11)	[8.04] 2.08 ± 0.07 (12)	256 ± 6 (11)

	df	ANOVA *P*-values		
Boron	1	0.03	0.55	0.26
Magnesium	1	0.005	0.0001	0.0001
Vitamin D Status	1	0.004	0.90	0.66
Boron × Mg	1	0.56	0.22	0.30
Boron × Vitamin D Status	1	0.40	0.23	0.03
Mg × Vitamin D Status	1	0.06	0.63	0.003
B × Mg × Vitamin D Status	1	0.79	0.57	0.03

[1] Weanling male rats were fed their respective diets for 35 d and then killed after overnight feed-deprivation.
[2] Total mean amounts of boron and magnesium in diets (by analysis) after supplementation of basal diet (boron, 0.1 mg; magnesium, <1.0 mg/kg) with boron (as orthoboric acid) and magnesium (as magnesium acetate). Rats were supplemented with vitamin D (25 μg cholecalciferol/kg throughout the study or depleted of vitamin D for 18 d) then repleted with dietary vitamin D (25 μg cholecalciferol/kg) on experimental day 19 until end of experiment.
[3] Data transformed to natural log to conform to the homogeneity assumption for ANOVA. Bracketed number represents back-transformed mean.
[4] Values are group means ± pooled SEM (*n*). The number varied according to availability, integrity, and/or variability of individual samples.

either experiment. The effect of dietary boron on plasma insulin concentrations was not dependent upon either magnesium nutriture or dietary vitamin D status because no significant interactions were detected between boron and magnesium or vitamin D that affected plasma insulin concentration.

Dietary magnesium. In Experiment 2 (Table 3), plasma insulin concentrations were significantly higher in magnesium-adequate rats than in magnesium-inadequate rats; a similar but nonsignificant trend was observed in Experiment 1 (Table 2). Magnesium-adequate rats, compared with magnesium-inadequate rats also exhibited significantly higher plasma glucose concentrations in Experiment 2 (Table 3), in which the effect of vitamin D status on plasma glucose was tested. As expected, adequate compared with inadequate dietary magnesium appeared to improve growth in both experiments.

Vitamin D status. The effect of vitamin D status on plasma insulin was tested in Experiment 2 (Table 3). Vitamin D-adequate controls, compared with rats depleted then repleted with vitamin D, exhibited significantly lower plasma insulin concentrations, although vitamin D dietary status did not affect plasma glucose concentrations.

Boron × magnesium × vitamin D. Plasma insulin and glucose concentrations were not affected by any of the treatment interaction terms in either experiment. In Experiment 2 (Table 3), which tested the effects of vitamin D status on growth, body weight was affected by an interaction among all three dietary treatments and this interaction was far greater than any other interaction. Thus, when dietary magnesium and vitamin D were adequate, physiologic amounts of dietary boron reduced body weight but did not induce any sign of physiologic impairment; bone mineral content and biochemical indices were normal.

In situ chick model

Dietary boron. Dietary boron, regardless of vitamin D nutriture, affected peak insulin release (defined as the highest concentration of insulin secreted during a perfusion phase) (**Table 4, Fig. 1**). Overall, chicks deprived of dietary boron had significantly higher peak insulin values compared with those fed physiologic amounts of this element (**Fig. 1**). Boron, as a direct secretagogue (added during the third phase of perfusion), did not affect peak insulin release ($P > 0.05$; data not shown). Peak insulin release was not related to pancreatic weight; dietary boron had no effect on pancreatic weight (data not shown). Neither dietary vitamin D alone nor an interaction among dietary variables affected peak insulin release from isolated, perfused chick pancreata.

DISCUSSION

Boron and insulin

The findings indicate that dietary boron may be important when biological responses to insulin and related insulin-like growth factors are impaired, a condition referred to as insulin resistance. Individuals with insulin resistance (decreased insulin sensitivity) can remain glucose tolerant if the pancreas compensates for this defect by secreting large amounts of insulin (25). However, sustained compensatory hyperinsulinemia predisposes such individuals to several abnormalities including type-2 diabetes, stroke and cardiovascular disease (26). Lower plasma insulin during boron supplementation without a change in plasma glucose suggests that boron reduces the amount of insulin required to maintain plasma glucose concentrations. Further research on the mechanism by

which boron affects peak insulin release and fasting plasma insulin concentrations should include determination of glucose disappearance rates (27).

The chick experiment represents the first attempt to elucidate the mechanism whereby boron affects insulin metabolism. Given the variability in the data, we did not have the statistical power to detect a significant effect of boron as a direct secretagogue of insulin. However, boron as a dietary ingredient decreased overall peak insulin release, a finding that may be relevant in understanding diabetes mellitus. It was hypothesized earlier (25,28) that β-cell "exhaustion" might explain the β-cell deterioration that occurs during excessive insulin demand. That is, β-cells that are too easily induced to secrete mass quantities of insulin are more readily damaged, which eventually can cause them to stop functioning and result in diabetes mellitus.

TABLE 4

Effects of dietary boron, vitamin D and their interactions on peak insulin secretion by isolated, perfused chick pancreata[1]

	Treatment[3]		
Phase[2]	Boron	Vitamin D	Peak insulin secretion[4]
	mg/kg	*μg/kg*	*pmol/(kg pancreas · min)*
1	0.29	3.13	0.64 ± 1.00 (6)[5]
Glucose: 5.5 mmol/L	0.29	15.60	0.91 ± 0.93 (7)
Boron: ≤0.9 μmol/L	1.65	3.13	0.31 ± 1.00 (6)
	1.65	15.60	0.45 ± 0.93 (7)
2	0.29	3.13	4.11 ± 1.00 (6)
Glucose: 38.9 mmol/L	0.29	15.60	1.04 ± 0.93 (7)
Boron: ≤0.9 μmol/L	1.65	3.13	0.34 ± 1.00 (6)
	1.65	15.60	0.61 ± 0.93 (7)
3	0.29	3.13	1.81 ± 1.00 (6)
Glucose: 38.9 mmol/L	0.29	15.60	0.63 ± 0.99 (6)
Boron: 13.2 μmol/L	1.65	3.13	0.70 ± 1.00 (6)
	1.65	15.60	0.24 ± 0.93 (7)
4	0.29	3.13	2.73 ± 1.00 (6)
Glucose: 38.9 mmol/L	0.29	15.60	2.02 ± 1.00 (6)
Boron: ≤0.9 μmol/L	1.65	3.13	0.99 ± 1.00 (6)
	1.65	15.60	0.36 ± 0.93 (7)
5	0.29	3.13	0.30 ± 1.00 (6)
Glucose: 5.5 mmol/L	0.29	15.60	2.60 ± 1.09 (5)
Boron: ≤0.9 μmol/L	1.65	3.13	0.45 ± 1.00 (6)
	1.65	15.60	0.32 ± 0.99 (6)

	df	ANOVA *P*-values
Boron	1	0.045
Vitamin D	1	0.58
Boron × Vitamin D	1	0.78
Phase	4	0.28
Boron × Phase	4	0.48

[1] Cockerels (1 d old) were fed their respective diets for 26–37 d and then the pancreata were perfused in situ.

[2] Phase, the time during which a particular perfusion solution was pumped through the pancreas. Glucose and boron refer to the respective concentrations of glucose and boron in the perfusate. Each complete pancreas perfusion consisted of five 10-min phases.

[3] Total mean amount of boron in diet (by analysis) after supplementation of basal diet (0.29 mg/kg) with boron (as orthoboric acid). Amount of vitamin D-3 (cholecalciferol powder in corn endosperm carrier) supplemented to the basal diet.

[4] The highest concentration of insulin secreted during each phase.

[5] Values are group means ± pooled SEM (*n*). The number varied according to availability, integrity, and/or variability of individual samples.

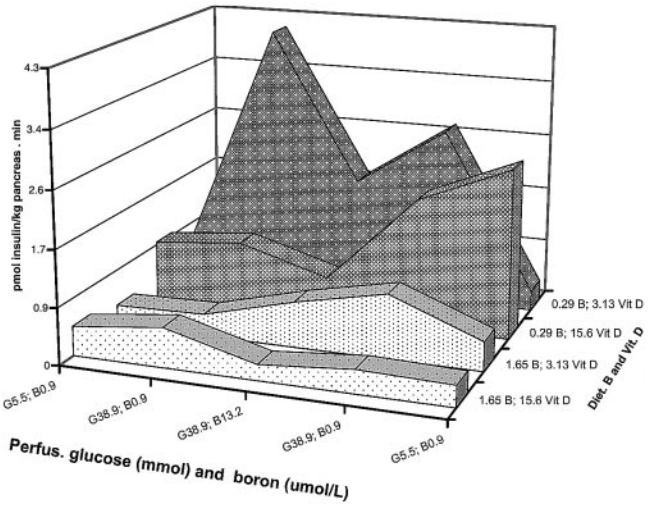

FIGURE 1 Alteration in peak insulin secretion from isolated, perfused pancreata from 1-d-old cockerels fed a diet containing 0.29 (boron-low) or 1.65 mg boron/kg and supplemented with cholecalciferol (Vit D) at 3.13 (inadequate) or 15.60 (adequate) μg/kg for 26–37 d. Perfusion phases varied in the total amount of glucose (G; 5.5 or 38.0 mmol/L) and boron (B; 0.9 or 13.2 μmol/L) in the perfusate. Data are group means given in Table 4 without SEM to enhance clarity.

That the effect of dietary boron on insulin is physiologic rather than pharmacologic in nature was indicated by the concentrations of dietary boron fed in our rat [2.0 μg (0.19 μmol) B/g] and chick [1.5 μg (0.14 μmol) B/g] studies. These concentrations were about one sixth of that found in Ralston Rodent Laboratory Chow #5001 [12.1–13.7 μg (1.12–1.27 μmol) B/g] (29), a common diet for laboratory rats. The concentration of boron in alfalfa has been reported to be as high as 42 μg (3.88 μmol) B/g dry material (30). In rations for chicks or rats containing 2 or 5% alfalfa, respectively, the amount of boron contributed by the alfalfa component alone would increase the total boron concentration to 0.84 μg (0.08 μmol) or 2.1 μg (0.19 μmol) B/g dry ration. Because physiologic amounts of boron were fed, it is reasonable to conclude that the findings are relevant to the normal function and metabolism of insulin.

Our group reported earlier (8) that vitamin D–deprived rats develop hyperinsulinemia when concurrently deprived of boron. That finding was the basis for the working hypothesis that boron deprivation will increase the amount of insulin required to maintain plasma glucose concentrations when either magnesium or vitamin D nutriture is perturbed. Contrary to our hypothesis, we report here that the effect of dietary boron on plasma insulin concentrations in rats is not dependent on either magnesium nutriture or vitamin D status. That is, boron reduced plasma insulin concentrations with no apparent relationship to vitamin D or magnesium dietary status.

Vitamin D and insulin

Examination of the effects of dietary boron and vitamin D on plasma insulin concentrations in the in vivo rat model demonstrated that these dietary components affect plasma insulin similarly: 47.5 (low boron groups) vs. 41.7 pmol/L (adequate boron groups), and 48.7 (vitamin D depletion/repletion groups) vs. 40.6 pmol/L (adequate vitamin D groups) (values are the collapsed and back-transformed group means in Experiment 2). The similar response of plasma insulin to

either boron or vitamin D nutriture suggests that both dietary factors may act independently at the same site of insulin metabolism. It is also possible that the effects of boron are mediated through a vitamin D metabolite. For example, our group reported earlier that physiologic amounts of supplemental boron increased concentrations of serum 25-hydroxycholecalciferol (25-OH-D_3) in chicks fed 3.13 μg (inadequate), but not 0 or 15.63 (adequate) μg vitamin D-3/kg diet (10). That is, boron apparently enhances the efficacy of vitamin D but does not substitute for the vitamin. Nielsen et al. (13) reported that serum 25-OH-D_3 concentrations were increased in volunteers when their low boron research diet was supplemented with boron.

The role of vitamin D in β-cell function is not yet clear but probably involves binding of active vitamin D metabolites to the plasma membrane and nuclear vitamin D receptors to induce nongenomic and genomic responses, respectively (31). Elucidation of the role is complicated by knowledge that 1α,25-dihydroxycholecalciferol [1,25-$(OH)_2$-D_3] not only has a role in bone and calcium metabolism, but also modulates the immune response in the pancreas via specific receptors expressed in antigen-presenting cells and activated T-cells to inhibit type I diabetes development (32). There were numerous earlier reports on the beneficial in vitro effects of vitamin D on insulin secretion from islets isolated from vitamin D–depleted rats (33). However, there are now several reports of an inhibitory effect of vitamin D metabolites compatible with the findings reported here. For example, the metabolite 1,25-$(OH)_2$-D_3 inhibited insulin secretion from the rat insulinoma β-cell line RIN 1046–38 and from islets isolated from vitamin D–replete rats (34). The same metabolite significantly decreased insulin release and insulin mRNA levels of cultured human islets and insulinoma cells (33). Additionally, hyperinsulinemic patients with insulinoma or insulin autoimmune syndrome exhibited a reduction in insulin concentrations after treatment with 1,25-$(OH)_2$-D_3 (a synthetic vitamin D analog) (35). In a separate study, hyperinsulinemic patients, compared with controls, exhibited significantly decreased 25-OH-D_3 and 1,25-$(OH)_2$-D_3 concentrations (36).

Magnesium and insulin

Findings from the in vivo rat model indicate that dietary magnesium also significantly affects plasma insulin concentrations but in a manner opposite that of boron or vitamin D, i.e., 40.8 (inadequate Mg groups) vs. 48.5 (adequate Mg groups) pmol/L (values are the collapsed and back-transformed group means in Experiment 2). Reports from other laboratories also indicate that magnesium deficiency (37), especially long-term deficiency (27), increases basal glucose disposal without affecting fasting glucose or insulin concentrations. The role of magnesium nutriture in diabetes is still unclear, but several lines of evidence suggest that magnesium deficiency may increase insulin resistance (decrease insulin sensitivity) (38–41). The incomplete characterization of the role of magnesium in insulin metabolism is typified by the finding from a prospective study (42) that low serum magnesium is a strong independent predictor of incident type II diabetes, but that low dietary magnesium intake does not confer risk for that disease.

In summary, findings from the present study indicate that dietary boron can affect insulin metabolism. Physiologic amounts of boron appeared to reduce plasma insulin concentrations independently of vitamin D or magnesium nutrition, and to reduce peak pancreatic insulin release. These effects suggest that boron may help reduce the amount of insulin required to maintain plasma glucose. The similar response of plasma insulin to either boron or vitamin D nutriture suggests that both dietary factors may act independently at the same site of insulin metabolism.

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LITERATURE CITED

1. Lovatt, C. J. & Dugger, W. M. (1984) Boron. In: Biochemistry of the Essential Ultratrace Elements (Frieden, E., ed.), pp. 389–421. Plenum Press, New York, NY.
2. Goldbach, H. E. (1997) A critical review on current hypotheses concerning the role of boron in higher plants: suggestions for further research and methodological requirements. J. Trace Microprobe Tech. 15: 51–91.
3. Fort, D. J., Stover, E. L., Strong, P. L., Murray, F. J. & Keen, C. L. (1999) Chronic feeding of a low boron diet adversely affects reproduction and development in *Xenopus laevis*. J. Nutr. 129: 2055–2060.
4. Eckhert, C. D. (1998) Boron stimulates embryonic trout growth. J. Nutr. 128: 2488–2493.
5. Nielsen, F., Shuler, T. R., Zimmerman, T. J. & Uthus, E. O. (1988) Magnesium and methionine deprivation affect the response of rats to boron deprivation. Biol. Trace Elem. Res. 17: 91–107.
6. Hunt, C. D. (1989) Dietary boron modified the effects of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. Biol. Trace Elem. Res. 22: 201–220.
7. Hegsted, M., Keenan, M. J., Siver, F. & Wozniak, P. (1991) Effect of boron on vitamin D deficient rats. Biol. Trace Elem. Res. 28: 243–256.
8. Hunt, C. D. & Herbel, J. L. (1991–1992) Boron affects energy metabolism in the streptozotocin-injected, vitamin D_3-deprived rat. Magnesium Trace Elem. 10: 374–386.
9. Hunt, C. D. & Herbel, J. L. (1993) Physiological amounts of dietary boron improve growth and indicators of physiological status over a 20-fold range in the vitamin D_3-deficient chick. In: Trace Element Metabolism in Man and Animals-8, (Anke, M., Meissner, D. & Mills, C., eds.), pp. 714–718. Verlag Media Touristik, Gersdorf, Germany.
10. Bai, Y. & Hunt, C. D. (1996) Dietary boron enhances efficacy of cholecalciferol in broiler chicks. J. Trace Elem. Exp. Med. 9: 117–132.
11. Armstrong, T. A., Spears, J. W., Crenshaw, T. D. & Nielsen, F. H. (2000) Boron supplementation of a semipurified diet for weanling pigs improves feed efficiency and bone strength characteristics and alters plasma lipid metabolites. J. Nutr. 130: 2575–2581.
12. Travers, R. L., Rennie, G. C. & Newnham, R. E. (1990) Boron and arthritis: the results of a double-blind pilot study. J. Nutr. Med. 1: 127–132.
13. Nielsen, F. H., Gallagher, S. K., Johnson, L. K. & Nielsen, E. J. (1992) Boron enhances and mimics some effects of estrogen therapy in postmenopausal women. J. Trace Elem. Exp. Med. 5: 237–246.
14. Hunt, C. D., Herbel, J. L. & Nielsen, F. H. (1997) Metabolic response of postmenopausal women to supplemental dietary boron and aluminum during usual and low magnesium intake: boron, calcium, and magnesium absorption and retention and blood mineral concentrations. Am. J. Clin. Nutr. 65: 803–813.
15. Hunt, C. D. (1998) Regulation of enzymatic activity. One possible role of dietary boron in higher animals and humans. Biol. Trace Elem. Res. 66: 205–225.
16. Hunt, C. D., Herbel, J. L. & Idso, J. P. (1994) Dietary boron modifies the effects of vitamin D_3 nutriture on indices of energy substrate utilization and mineral metabolism in the chick. J. Bone Miner. Res. 9: 171–181.
17. Nielsen, F. H. (1989) Dietary boron affects variables associated with copper metabolism in humans. In: 6th International Trace Element Symposium 1989, vol. 4 (Anke, M., Baumann, W., Bräunlich, H., Brückner, C., Groppel, B. & Grün, M., eds.), pp. 1106–1111. Karl-Marx-Universitat, Leipzig and Friedrich-Schiller-Universitat, Jena, Germany.
18. Hunt, C. D. (1996) Dietary boron deficiency and supplementation. In: Trace Elements in Laboratory Rodents (Watson, R. R., ed.), pp. 229–253. CRC Press, Boca Raton, FL.
19. Simon, J. & Rosselin, G. (1978) Effect of fasting, glucose, amino acids and food intake on in vivo insulin release in the chicken. Horm. Metab. Res. 10: 93–98.
20. National Research Council (1994) Nutrient Requirements of Poultry. National Academy Press, Washington, DC.
21. King, D. L. & Hazelwood, R. L. (1976) Regulation of avian insulin secretion by isolated perfused chicken pancreas. Am. J. Physiol. 231: 1830–1839.
22. Snedecor, G. W. & Cochran, W. G. (1967) Statistical Methods. Iowa State University Press, Ames, IA.
23. Cohen, J. & Cohen, P. (1975) Applied Multiple Regression-Correlation Analysis of the Behavioral Sciences. Lawrence Erlbaum Associates, Hillsdale, NJ.

24. Kleinbaum, D. G. & Kupper, L. L. (1978) Applied Regression Analysis and Other Multivariable Methods. Duxbury Press, North Scituate, MA.
25. Reaven, G. M. (1999) Insulin resistance: a chicken that has come to roost. Ann. N.Y. Acad. Sci. 892: 45–57.
26. Zammit, V. A. (2002) Insulin stimulation of hepatic triacylglycerol secretion in the insulin-replete state: implications for the etiology of peripheral insulin resistance. Ann. N.Y. Acad. Sci. 967: 52–65.
27. Reis, M. A., Reyes, F. G., Saad, M. J. & Velloso, L. A. (2000) Magnesium deficiency modulates the insulin signaling pathway in liver but not muscle of rats. J. Nutr. 130: 133–138.
28. Sprietsma, J. & Schuitemaker, G. (1993) Diabetes can be prevented by reducing insulin production. Med. Hypotheses 42: 15–23.
29. Hunt, C. D., Halas, E. S. & Eberhardt, M. J. (1988) Long-term effects of lactational zinc deficiency on bone mineral composition in rats fed a commercially modified Luecke diet. Biol. Trace Elem. Res. 16: 97–113.
30. Pau, J.C.-M., Pickett, E. E. & Koirtyohann, S. R. (1972) Determination of boron in plants by emission spectroscopy with the nitrous oxide-hydrogen flame. Analyst 97: 860–865.
31. Kajikawa, M., Ishida, H., Fujimoto, S., Mukai, E., Nishimura, M., Fujita, J., Tsuura, Y., Okamoto, Y., Norman, A. W. & Seino, Y. (1999) An insulinotropic effect of vitamin D analog with increasing intracellular Ca2+ concentration in pancreatic beta-cells through nongenomic signal transduction. Endocrinology 140: 4706–4712.
32. Gregori, S., Giarratana, N., Smiroldo, S., Uskokovic, M. & Adorini, L. (2002) A 1 alpha, 25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes 51: 1367–1374.
33. Galbiati, F., Polastri, L., Gregori, S., Freschi, M., Casorati, M., Cavallaro, U., Fiorina, P., Bertuzzi, F., Zerbi, A., Pozza, G., Adorini, L., Folli, F., Christofori, G. & Davalli, A. M. (2002) Antitumorigenic and antiinsulinogenic effects of calcitriol on insulinoma cells and solid beta-cell tumors. Endocrinology 143: 4018–4030.
34. Lee, S., Clark, S. A., Gill, R. K. & Christakos, S. (1994) 1, 25-Dihydroxyvitamin D3 and pancreatic beta-cell function: vitamin D receptors, gene expression, and insulin secretion. Endocrinology 134: 1602–1610.
35. Gunal, A. I., Celiker, H., Celebi, H., Ustundag, B. & Gunal, S. Y. (1997) Intravenous alfacalcidol improves insulin resistance in hemodialysis patients. Clin. Nephrol. 48: 109–113.
36. Ishida, H., Seino, Y., Tsuda, K., Matsukura, S., Miyamoto, Y., Ishizuka, S. & Imura, H. (1984) Circulating levels of vitamin D metabolites in patients with hyperinsulinaemia. Hum. Nutr. Clin. Nutr. 38: 473–475.
37. Lowney, P., Hannon, T. S. & Baron, A. D. (1995) Magnesium deficiency enhances basal glucose disposal in the rat. Am. J. Physiol. 268: E925–E931.
38. Paolisso, G., Sgambato, S., Gambardella, A., Pizza, G., Tesauro, P., Varricchio, M. & D'Onofrio, F. (1992) Daily magnesium supplements improve glucose handling in elderly subjects. Am. J. Clin. Nutr. 55: 1161–1167.
39. Nadler, J. L., Buchanan, T., Natarajan, R., Antonipillai, I., Bergman, R. & Rude, R. (1993) Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. Hypertension 21: 1024–1029.
40. Balon, T. W., Gu, J. L., Tokuyama, Y., Jasman, A. P. & Nadler, J. L. (1995) Magnesium supplementation reduces development of diabetes in a rat model of spontaneous NIDDM. Am. J. Physiol. 269: E745–E752.
41. Humphries, S., Kushner, H. & Falkner, B. (1999) Low dietary magnesium is associated with insulin resistance in a sample of young, nondiabetic Black Americans. Am. J. Hypertens. 12: 747–756.
42. Kao, W. H., Folsom, A. R., Nieto, F. J., Mo, J. P., Watson, R. L. & Brancati, F. L. (1999) Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study. Arch. Intern. Med. 159: 2151–2159.

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