Dietary Boron Decreases Peak Pancreatic In Situ Insulin Release in Chicks and Plasma Insulin Concentrations in Rats Regardless of Vitamin D or Magnesium Status¹⁻³

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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 nutrition. 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-
proved 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 nutrition is perturbed. The goal of the 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 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. 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 following anterior cardiac puncture (with a sodium heparin-coated needle) and decapitated. Plasma insulin was determined by RIA by a preprecipitated second 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 precipitated 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. 125I-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 glucose-6-phosphatase and Mg2+. NADH production of glucose light at 340 nm, which was detected in an automated clinical chemistry analyzer (COBAS-FARA; Roche Diagnostics Systems, Hoffmann-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) 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 (Germinfree 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.** 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 gland 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; Ca2+, 5.08; Mg2+, 2.36; Cl−, 109.7; PO43−, 1.11; SO42−, 1.18; HCO3−, 24.9; pyruvate, 4.9; glutamate, 4.9; and fumarate, 5.4. Heparin (LiphoMed, 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); osmolality, 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-xylotrose, anhydrous (ICN Pharmaceuticals, Life Sciences Group, Cleveland, OH) and boron as H3BO3 (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-D3, 1,25-dihydroxycholecalciferol; 25-OH-D3, 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.
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 [125I] RIA Kit; Inestar,
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 (Glucocet glucose test;
Sclavo Diagnostics, Wayne, NJ) with the glucose oxidase-peroxidase
reagent containing hydroxybenzoate-4-aminoantipyrine. The inten-
sity of the color of this complex was directly proportional to the
concentration of d-glucose in the sample. The absorbance of stan-
dards and unknown collection fractions were measured against a
blank at 510 nm on a Biomek 1000 Automated Laboratory Work-
station 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 con-
sidered significant at the P < 0.05 level.

RESULTS

In vivo rat model

Dietary boron. In both experiments, rats deprived of bo-
ron 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 1

Composition of basal perfusion solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5.767</td>
</tr>
<tr>
<td>Dextran</td>
<td>2.500</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.092</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.900</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>0.539</td>
</tr>
<tr>
<td>Heparin</td>
<td>0.500</td>
</tr>
<tr>
<td>Glutamic acid monosodium salt</td>
<td>0.458</td>
</tr>
<tr>
<td>KCl</td>
<td>0.442</td>
</tr>
<tr>
<td>Sodium fumarate di-basic</td>
<td>0.432</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.373</td>
</tr>
<tr>
<td>Na₃H₂PO₄</td>
<td>0.158</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.142</td>
</tr>
</tbody>
</table>

1 The basal perfusion solution contained ≤0.93 μmol B/L by anal-
ysis.
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.

Experimental and control solutions (aerated with a mixture of
95% O₂ and 5% CO₂) 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, Dick-
inson 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 eval-
uate recovery from the glucose load. Thus, five phases comprised each

### TABLE 2

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

<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Plasma</th>
<th>Body weight d 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron Mg Vitamin D status</td>
<td>Insulin pmol/L</td>
<td>Glucose mmol/L</td>
</tr>
<tr>
<td>0.2 100</td>
<td>Depleted/Repleted</td>
<td>44.6 ± 3.1 (11)³</td>
</tr>
<tr>
<td>2.0 100</td>
<td>Depleted/Repleted</td>
<td>37.0 ± 3.2 (10)</td>
</tr>
<tr>
<td>0.2 400</td>
<td>Depleted/Repleted</td>
<td>50.4 ± 3.2 (10)</td>
</tr>
<tr>
<td>2.0 400</td>
<td>Depleted/Repleted</td>
<td>36.3 ± 3.2 (10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>ANOVA P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>Boron × Magnesium</td>
<td>1</td>
<td>0.31</td>
</tr>
</tbody>
</table>

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.
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Boron</th>
<th>Vitamin D</th>
<th>Peak insulin secretion4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>μg/kg</td>
<td>pmol/(kg pancreas · min)</td>
</tr>
<tr>
<td>Phase2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.29</td>
<td>3.13</td>
<td>0.64 ± 1.00 (6)5</td>
</tr>
<tr>
<td>Glucose: 5.5 mmol/L</td>
<td>0.29</td>
<td>15.60</td>
<td>0.91 ± 0.93 (7)</td>
</tr>
<tr>
<td>Boron: ≤0.9 μmol/L</td>
<td>1.65</td>
<td>3.13</td>
<td>0.31 ± 1.00 (6)</td>
</tr>
<tr>
<td>2</td>
<td>0.29</td>
<td>3.13</td>
<td>4.11 ± 1.00 (6)</td>
</tr>
<tr>
<td>Glucose: 38.9 mmol/L</td>
<td>0.29</td>
<td>15.60</td>
<td>1.04 ± 0.93 (7)</td>
</tr>
<tr>
<td>Boron: ≤0.9 μmol/L</td>
<td>1.65</td>
<td>3.13</td>
<td>0.34 ± 1.00 (6)</td>
</tr>
<tr>
<td>3</td>
<td>0.29</td>
<td>3.13</td>
<td>1.81 ± 0.93 (7)</td>
</tr>
<tr>
<td>Glucose: 38.9 mmol/L</td>
<td>0.29</td>
<td>15.60</td>
<td>0.63 ± 0.99 (6)</td>
</tr>
<tr>
<td>Boron: 13.2 μmol/L</td>
<td>1.65</td>
<td>3.13</td>
<td>0.70 ± 1.00 (6)</td>
</tr>
<tr>
<td>4</td>
<td>0.29</td>
<td>3.13</td>
<td>0.24 ± 0.93 (7)</td>
</tr>
<tr>
<td>Glucose: 38.9 mmol/L</td>
<td>0.29</td>
<td>15.60</td>
<td>2.02 ± 1.00 (6)</td>
</tr>
<tr>
<td>Boron: ≤0.9 μmol/L</td>
<td>1.65</td>
<td>3.13</td>
<td>0.99 ± 1.00 (6)</td>
</tr>
<tr>
<td>5</td>
<td>0.29</td>
<td>3.13</td>
<td>0.30 ± 1.00 (6)</td>
</tr>
<tr>
<td>Glucose: 5.5 mmol/L</td>
<td>0.29</td>
<td>15.60</td>
<td>2.60 ± 1.09 (5)</td>
</tr>
<tr>
<td>Boron: ≤0.9 μmol/L</td>
<td>1.65</td>
<td>3.13</td>
<td>0.45 ± 1.00 (6)</td>
</tr>
<tr>
<td></td>
<td>1.65</td>
<td>15.60</td>
<td>0.32 ± 0.99 (6)</td>
</tr>
</tbody>
</table>

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.

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-hydroxycalciferol (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-dihydroxyvitamin D$_3$ and 24,25-dihydroxyvitamin D$_3$ do not play 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 islet 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 (35). 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 magnesium also significantly affects plasma insulin concentrations but in a manner opposite that of boron or vitamin D$_3$, 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 or glucose-octreotide 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 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


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