NUTRITION

Boron and fish oil have different beneficial effects on strength and trabecular microarchitecture of bone☆, **☆

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Abstract

An experiment was performed to determine whether boron deprivation would adversely affect vertebra (trabecular) bone microarchitecture, and whether any adverse effect would be modified by dietary fatty acid composition. Female rats were fed diets containing 0.1 mg (9 μmol) boron/kg in a factorial arrangement with variables of supplemental boron at 0 (boron-deprived) or 3 (boron-adequate) mg (278 μmol)/kg and fat sources of 75 g safflower oil/kg or 65 g fish (menhaden) oil/kg plus 10 g linoleic acid/kg. After 6 weeks, six females per treatment were bred. Dams and pups continued on their respective diets through gestation, lactation, and after weaning. At age 21 weeks, the microarchitecture of the fourth lumbar vertebrae from 12 randomly selected pups from each treatment was determined by microcomputed tomography. Boron deprivation decreased bone volume fraction and increased trabecular separation and structural model index. Boron deprivation decreased trabecular thickness when the dietary oil was safflower. A three-point bending test for bone strength found that boron deprivation decreased the maximum force needed to break the femur. Feeding fish oil instead of safflower oil decreased connectivity density in vertebrae of boron-deficient but not in boron-adequate rats. Fish oil instead of safflower oil increased the maximum force to break and the bending moment of the femur, especially in rats fed adequate boron. The findings confirm that boron and fish oil are beneficial to cortical bone strength, and show that nutritional intakes of boron are beneficial for trabecular bone microarchitecture and influence the beneficial effects of fish oil on bone.

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Keywords: Bone; Boron; Fatty acids; Fish oil; Nutrient interaction

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Introduction

One of the first findings suggesting that boron is beneficial, perhaps essential, for higher animals was that boron improved bone calcification in chicks fed a diet deficient but not completely lacking in vitamin D [1]. At the microscopic level, boron deprivation (0.465 mg kg\(^{-1}\) diet) exacerbated the distortion of marrow sprouts (location of calcified scaffold erosion and new bone formation) caused by marginal vitamin D deficiency in chicks [2]. Among the other findings showing that boron influenced factors affecting bone formation or turnover was that nutritional amounts of boron (5 mg or 0.46 mmol/kg) supplemented to a basal AIN-76 diet containing 0.3 mg (28 μmol) boron/kg enhanced the beneficial effects of 17β-estradiol on trabecular bone volume and plate density in tibias of ovariectomized rats [3]. However, compared to common nutritional intakes, low dietary boron alone has not been shown to have adverse effects on trabecular bone architecture, which could affect bone strength. Boron deprivation alone may have an effect on architecture because it has been found detrimental to long (cortical) bone morphological and gross physical characteristics in experimental animals. Boron deprivation decreased chondrocyte density in the zone of proliferation in long bone growth plate in chicks [4] and decreased the bone strength variable bending moment in femurs of pigs [5,6].

The long-chain omega-3 polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), have beneficial effects similar to boron on bone. EPA supplementation enhanced the ability of estrogen to inhibit bone loss [7] and omega-3 PUFAs supplementation decreased bone loss in ovariectomized mice [8] and rats [9]. Repletion with omega-3 PUFAs reversed bone structural and strength deficits in omega-3-deficient rats [10].

Boron [11,12] and long-chain omega-3 PUFAs [13] affect many of the same biological processes apparently through influencing cell membrane function that affects cellular responses to cytokines and hormones, including those that are involved in bone growth and turnover. Thus, the primary aim of the present study was to determine whether boron deprivation alone detrimentally affects trabecular bone microarchitecture, strength, and composition. A secondary aim was to determine whether changing cell membrane function by changing the omega-3 PUFA content of the diet influences any effect of boron deprivation on bone.

Materials and methods

Experimental design

Female Sprague-Dawley rats (Charles River/SASCO, Wilmington, MA) weighing about 115 g were fed diets in a factorial arrangement with dietary variables being supplemental boron as boric acid at 0 and 3 mg (278 μmol)/kg and either 75 g kg\(^{-1}\) safflower oil, or 65 g kg\(^{-1}\) fish (menhaden) oil plus 10 g kg\(^{-1}\) linoleic acid. Linoleic acid was added with fish oil to assure that the diet provided the requirement of 6 g kg\(^{-1}\) diet set for rats by the National Research Council [14]. The omega-6/omega-3 fatty acid ratio of the diet with safflower oil was about 250 and with fish oil was less than 0.5. The composition of the basal diet, which met all nutrient requirements set by the National Research Council [14], is shown in Table 1. The basal diet contained about 0.1 mg (9 μmol)/kg boron as determined by inductively coupled plasma atomic emission spectroscopy (ICPAES) using a Teflon cyclonic spray chamber and Miramist nebulizer (Optima 3100XL, Perkin Elmer, Shelton, CA) after low-temperature, acid digestion in Teflon tubes [15]. The line of 208.958 was used with a limit of quantification of 5.14 ng/mL. Standard reference materials (National Institute of Standards and Technology, Gaithersburg, MD) NIST #1548a (typical diet) and NIST #1515 (apple leaves) were used for quality control.

Table 1. Composition of the basal diet meeting all nutrient requirements set by the National Research Council [14]a

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn, acid washed</td>
<td>713.5</td>
</tr>
<tr>
<td>Casein, vitamin free</td>
<td>160.0</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>75.0</td>
</tr>
<tr>
<td>dl-α-Tocopherol</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>4.0</td>
</tr>
<tr>
<td>Macro-mineral mix</td>
<td>29.3</td>
</tr>
<tr>
<td>Trace mineral mix</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>1000.0</td>
</tr>
</tbody>
</table>

a Analyzed concentration of boron was about 0.1 mg (9 μmol)/kg.

b Dietary variable – 75 g kg\(^{-1}\) safflower oil [approximately 10% saturated, 23% monounsaturated, 77% polyunsaturated (linoleic acid) fatty acids, and only a trace of α-linolenic acid]; or 65 g kg\(^{-1}\) fish oil [30% saturated, 27% monounsaturated, and 34% polyunsaturated fatty acids (13.1% eicosapentaenoic acid and 8.6% docosahexaenoic acid)] plus 10 g kg\(^{-1}\) linoleic acid to meet the 6 g kg\(^{-1}\) requirement set by the National Research Council [19].

c Composition of the vitamin mix (in mg): vitamin A palmitate (500,000 IU/g), 16; thiamine·HCl, 10; pyridoxine·HCl, 15; nicotinic acid, 30; dl-pantothenic acid, 48; vitamin B₁₂ (0.1% in mannitol), 50; folic acid, 2; biotin, 1; riboflavin, 27; vitamin K (phyloquinone), 1; inositol, 50; paraaminobenzoic acid, 5; vitamin D₃ (400,000 IU/g), 2.5; and dextrose, 3742.5.

d Composition of the macro-mineral mix (in g): CaHPO₄, 17.0; KCl, 7.0; and Mg(C₂H₃O₂)₂·4H₂O, 5.3.

e Composition of the trace element mix (in mg): NaCl, 2000; Mn(C₂H₃O₂)₂·4H₂O, 45; CuSO₄·5H₂O, 30; Zn(C₂H₃O₂)₂·2H₂O, 84; iron powder (dissolved in HCl), 75; NaHAsO₄·7H₂O, 5; KI, 0.4; Na₂SiO₃·9H₂O, 1.4; Cr(C₂H₃O₂)₃·2H₂O, 2; NH₄VO₃, 0.3; (NH₄)₂MoO₄, 1; NaF, 2; NiCl·6H₂O, 3.7; NaSiO₃·9H₂O, 5, and ground corn (acid washed), 12,700.2.
purposes in the diet analysis. Analyzed values obtained for typical diet and apple leaves, respectively, were 4.12 ± 0.29 and 26.9 ± 1.6 versus certified values of 4.16 ± 0.04 and 27 ± 2. The unpelleted diets were stored at −16 °C in tightly capped plastic containers.

Rats fed 0.1 mg (9 μmol) boron/kg diet in the present study were deemed boron deprived. This judgment was based on experiments showing that chicks responded to boron supplementation when fed diets containing 0.44 mg (40 μmol) boron/kg or less but not 0.66 mg (59 μmol) boron/kg and higher [4], and that rats fed 0.3 (27 μmol) mg boron/kg diet or less responded to physiological amounts of boron supplementation [3,16]. The same experiments [3,4,16], in addition to a pig experiment [5], indicate that boron adequacy is achieved by feeding a boron supplement of 3 mg (278 μmol)/kg diet. The supplement was not considered supranutritional because commonly used rat chows contain 13–14 mg (1.2–1.3 mmol) boron/kg diet [17,18].

Animal handling

The female rats were housed individually in double stainless-steel cages in a room maintained at 23 °C and 50% relative humidity with a normal 12-h light and dark cycle. Five weeks after consuming their respective diets and deionized water (Super Q, Millipore, Bedford, MA) provided in plastic food and water cups, six females in each treatment were bred by males from an in-house rat colony fed a commercial rat chow. Dams and pups continued with free access to their respective diets and deionized water through gestation, lactation, and post-weaning. At about age 21 days, 15 seemingly male pups from three dams (5 pups per dam) in each dietary group were placed individually in a single stainless-steel cage on a rack in a room maintained at 23 °C and 50% humidity and with a reversed 12 h white and 12 h red light cycle. Absorbent paper under the wire mesh cages was changed daily. The assignment of 15 rats per group was based on a power analysis that indicated this number was needed to ascertain behavior changes, which were also assessed and reported elsewhere [19]. Rats were weighed and provided with clean cages weekly.

During the course of the experiment, excessive tooth growth occurred in one rat in the boron-deficient, fish oil group and in two rats in the boron-deficient, safflower group. The tooth problem resulted in an inability to thrive; so the rats were euthanized. Eight weeks into the experiment, it was determined that two rats in the boron-adequate, safflower group were female. These rats were removed from the study. Thus, the number of rats in each group was: boron-deficient, fish oil – 14, boron-deficient, safflower oil – 13, boron-adequate, fish oil – 15, and boron-adequate, safflower oil – 13.

At age 21 weeks, the rats were anesthetized with ether for the collection of blood from the vena cava with a heparin-coated syringe and needle. After euthanasia by decapitation, the tibia with flesh removed was collected for prostaglandin E2 (PGE2) release determination, and the left femur and vertebra with some attached flesh were collected for measuring bone physical characteristics. Plasma obtained by centrifugation and bones were stored at −70 °C until analysis.

The Animal Care Committee of the Grand Forks Human Nutrition Research Center approved the study, and the lawfully acquired animals were maintained in accordance with National Institute of Health guidelines for the care and use of laboratory animals.

Plasma and tibia biochemical analyses

A commercially available assay kit was used to determine plasma alkaline phosphatase (Cat. # 83604, Raichem, San Diego, CA), a measure of bone formation. Because increased plasma homocysteine has been associated with an increased risk of decreased bone mineral density [20] and bone quality [21], plasma total (reduced plus oxidized) homocysteine was determined by using the HPLC procedure of Durand et al. [22].

Prostaglandins formed from long-chain PUFAs are primary mediators of bone cell function [23]. PGE2, a major prostaglandin affecting bone, stimulates bone formation at low concentrations but inhibits formation at high concentrations [23]. Thus, PGE2 in bone was measured to ascertain whether this variable might be related to changes in bone microarchitecture. The tibias were cleaned of flesh before removing the ends without shattering the shaft by using a bone cutter. The interior of the shaft was cleared of cellular debris by rinsing with cold 0.89% NaCl delivered by a peristaltic pump fitted with tubing attached to a blunt needle. After blotting dry, the tibia shafts were weighed and immersed in 2 ml of cold Hank’s buffered saline at pH 7.31 (Sigma H-287, Sigma, St. Louis, MO) in 5 ml plastic falcon tubes. Within 30 min after collection, the samples were incubated for 2 h in a gently shaking water bath before the sample solutions were removed, transferred to micro-centrifuge tubes and immersed in liquid nitrogen. The samples were stored at −70 °C for later PGE2 analysis by using a commercially available kit (Kit #900-001, Assay Designs, Ann Arbor, MI).

Femur boron analysis

Femurs were cleaned to the periosteal surface with cheesecloth, lyophilized, and subjected to wet-ash, low-temperature digestion in Teflon tubes [15]. Boron was determined by ICAPES as described in the Experimental Design section. Standard reference material NIST #1515
(apple leaves) was used for quality control; analyzed value obtained was 27.1 ± 0.5 versus a certified value of 27 ± 2 for boron.

**Femur strength determination**

Bone strength variables were determined on femurs after thawing and removing the remaining flesh. A custom-designed and -built apparatus that performed a three-point bending test was used to determine bone breaking variables. The fulcra length and point of force application were determined by femur length. Two adjustable supports were placed as near as possible without touching the soft cartilaginous tissue at the ends of the bone. The point of force (crosshead) was centered over the greatest possible distance between the two fulcra below the femur placed in a stable position with the ventral side up and the knee joint to the left while facing the instrument. The rate of deformation was a constant 5 mm/min. The terms used for the assessment of bone strength have been described [24]. Briefly, the definitions of the variables given are as follows. Maximum force is the force in newtons needed to break the bone. Bending moment is force times fulcra length. Area moment of inertia is the effect of bone geometry on resistance to bending.

**Vertebra microarchitecture determination**

The fourth lumbar vertebrae from 12 randomly selected rats from each treatment were examined by microcomputed tomography (μCT) (μCT 40, Scanco Medical AG, Zurich, Switzerland). Twelve were used because previous experiments [16,25,26] indicated that this number would be adequate to ascertain microarchitectural changes. The vertebrae were scanned from the cephalic to caudal growth plate by an operator who was blinded to the treatments associated with the specimen. The volume of interest included only the secondary spongiosa and was identified by placing contours beginning and ending 10 slices (20.67 μm thickness per slice) away from the growth plate. Vertebral morphometric variables, including bone volume fraction in the volume of interest (BV/TV), trabecular number (average number in a given distance), thickness (average in defined region), and separation (average between trabeculae), connectivity density (average number of connections between trabeculae in specified volume), and structural model index (SMI) were determined. SMI is a measure of the rod-like or plate-like properties of bone. A value of 0 designates pure plates and a value of 3 designates perfect rods. A lower value or more plate-like property is preferable. Coefficients of variation for the trabecular morphometric measurements were 2.0% for BV/TV, 1.1% for number, 0.66% for thickness, 1.3% for separation, 4.6% for connectivity density, and 2.7% for SMI.

**Vertebra biomechanical testing using finite-element analysis by μCT**

Data from the μCT analyses were used with finite-element software to simulate compression of a region of interest in the vertebrae. Validation of this method is provided by the work of Bagi et al. [27]. The bone sample used for the determination of microarchitecture was subjected to a high friction compression test in the z direction. Force is the force in newtons required to compress (completely crush) the vertebra. Size-independent stiffness is the force for compression divided by the average cross-sectional area (accounts for larger bones being able to resist more force). Von Mises stress (VMS) is the average level of von Mises forces that exist in the finite-element model created; a higher value indicates a greater propensity to break.

**Statistical analysis**

Data were statistically compared using two-way analysis of variance (SAS/STAT, Version 9.02, SAS Institute, Inc., Cary, NC) followed by Tukey’s contrasts when appropriate. A p value of ≤0.05 was considered significant.

**Results**

The significantly depressed final body weight and femur boron concentration exhibited by rats fed the diet without boron supplementation confirmed that these rats were boron deprived (Table 2); dietary oil did not affect either of these variables. Only three possible indicators of changes in bone metabolism (shown in Table 2) were measured in this experiment. Neither dietary boron nor oil significantly affected plasma alkaline phosphatase activity. Boron deprivation significantly increased plasma homocysteine concentration, which was not affected by dietary oil. Feeding fish oil instead of safflower oil markedly decreased tibia PGE2 release; boron did not significantly affect PGE2 release.

Boron deprivation decreased L4 vertebrae trabecular BV/TV (Fig. 1A) and increased the average separation between trabeculae (Fig. 1C) and trabecular SMI (Fig. 1D). Boron deprivation decreased L4 vertebrae trabecular thickness (Fig. 1B) when dietary oil was safflower. Feeding fish oil instead of safflower oil did not significantly affect any of these microarchitectural variables. Feeding safflower oil to boron-deprived rats decreased trabecular thickness (Fig. 1B) and increased
connectivity density (Table 3) in vertebrae. The interaction between dietary oil and boron resulted in the boron-deprived rats fed safflower oil having the thinnest trabeculae.

The dietary treatments did not show as many significant effects on the simulated compression as they did on the microarchitecture of the vertebrae (Table 3). Feeding fish oil instead of safflower oil increased force for compression with the effect most marked in boron-adequate rats.

Femur length was decreased by feeding fish oil instead of safflower oil and by boron deprivation (Table 4). The differences in femur length did not correspond to the differences in body weight, which suggest that the

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**Table 2.** Effect of dietary boron, oil, and their interaction on indicators of boron status and bone metabolism

<table>
<thead>
<tr>
<th>Dietb</th>
<th>Final weight (g)</th>
<th>Femur boron (ng/g)</th>
<th>Plasma AP (mU/mLc)</th>
<th>Plasma homocysteine (nmol/mL)</th>
<th>Tibial PGE2 release (ng/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron (µg/g)</td>
<td>Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Fish</td>
<td>447 ± 7</td>
<td>190 ± 28</td>
<td>57 ± 3</td>
<td>2.81 ± 0.21</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>476 ± 7</td>
<td>978 ± 81</td>
<td>54 ± 2</td>
<td>2.36 ± 0.18</td>
</tr>
<tr>
<td>0</td>
<td>Safflower</td>
<td>441 ± 9</td>
<td>115 ± 26</td>
<td>59 ± 4</td>
<td>3.02 ± 0.18</td>
</tr>
<tr>
<td>3</td>
<td>Safflower</td>
<td>487 ± 9</td>
<td>920 ± 96</td>
<td>52 ± 2</td>
<td>2.59 ± 0.16</td>
</tr>
</tbody>
</table>

*Values presented are mean ± SEM. Abbreviations used: AP – alkaline phosphatase activity; PGE2 – prostaglandin E2.

**Analysis of variance – p values**

- Boron: 0.0001
- Oil: 0.72
- Boron x Oil: 0.0001

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**Fig. 1.** Effects of inadequate and adequate dietary boron and feeding fish oil instead of safflower oil on vertebral (A) bone volume fraction, (B) trabecular thickness, (C) trabecular thickness, and (D) trabecular structural model index (SMI) determined by μCT. Data shown are mean ± SEM from male rats on treatments from conception to age 21 weeks.
dietary treatments had differential effects on bone growth as opposed to general growth. Both safflower oil and boron deprivation decreased the maximum force needed to break the femur (Table 4). As a result, mean maximum force was the lowest in boron-deprived rats fed safflower oil, and the highest in boron-adequate rats fed fish oil. Bending moment was increased by feeding fish oil instead of safflower oil (Table 4). Area moment of inertia (Table 4) and modulus elasticity (data not shown) were not significantly affected by either boron or oil. In addition, bone measurements of outside lateral width and vertical depth at the point where the bones broke were not significantly affected by the dietary treatments (data not shown).

### Table 3. Effect of dietary boron, oil, and their interaction on selected vertebral trabecular microarchitectural variables assessed by μCT and selected vertebral strength variables determined by finite-element analysis

<table>
<thead>
<tr>
<th>Diet&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Vertebral trabecular</th>
<th>Vertebral</th>
<th>Analysis of variance – p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron (µg/g)</td>
<td>Oil</td>
<td>Number (per mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>Connectivity (per mm&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>0 Fish</td>
<td>Safflower</td>
<td>2.78 ± 0.14</td>
<td>40 ± 3y</td>
</tr>
<tr>
<td>3 Fish</td>
<td>Safflower</td>
<td>3.09 ± 0.08</td>
<td>47 ± 2z</td>
</tr>
<tr>
<td>0 Safflower</td>
<td>Fish</td>
<td>2.93 ± 0.12</td>
<td>52 ± 3z</td>
</tr>
<tr>
<td>3 Safflower</td>
<td>Fish</td>
<td>3.01 ± 0.07</td>
<td>43 ± 2yz</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values presented are mean ± SEM. Values in the same column not followed by the same letter (y and z) are significantly different (p<0.05) according to Tukey’s contrast. Abbreviations used: μCT – micro-computed tomography; Size-ind. stiffness – size-independent stiffness; VMS – average von Mises stress.

<sup>b</sup>The dietary treatments were boron supplements of 0 and 3 mg (278 μmol)/kg and either safflower oil at 75 g kg<sup>-1</sup> or fish oil at 65 g kg<sup>-1</sup> plus linoleic acid at 10 g kg<sup>-1</sup>. The basal diet contained about 0.1 mg (9 μmol)/kg.

### Table 4. Effect of dietary boron, oil, and their interaction on femur strength determined by a three-point bending test

<table>
<thead>
<tr>
<th>Diet&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Femur</th>
<th>Analysis of variance – p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron (µg/g)</td>
<td>Oil</td>
<td>Length (mm)</td>
</tr>
<tr>
<td>0 Fish</td>
<td>Safflower</td>
<td>39.7 ± 0.3</td>
</tr>
<tr>
<td>3 Fish</td>
<td>Safflower</td>
<td>40.1 ± 0.2</td>
</tr>
<tr>
<td>0 Safflower</td>
<td>Fish</td>
<td>40.4 ± 0.2</td>
</tr>
<tr>
<td>3 Safflower</td>
<td>Fish</td>
<td>40.9 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values presented are mean ± SEM.

<sup>b</sup>The dietary treatments were boron supplements of 0 and 3 mg (278 μmol)/kg and either safflower oil at 75 g kg<sup>-1</sup> or fish oil at 65 g kg<sup>-1</sup> plus linoleic acid at 10 g kg<sup>-1</sup>. The basal diet contained about 0.1 mg (9 μmol)/kg.

### Discussion

#### Main effects of boron

The bone breaking findings confirm that boron promotes cortical bone strength. The vertebral morphometric findings indicate that boron also is beneficial, if not essential, for trabecular microarchitecture that promotes bone strength. Moreover, these beneficial bone effects of boron are independent of any other nutritional stressor. The mechanism through which boron affects trabecular microarchitecture and cortical bone strength is unclear. However, boron deprivation might be impairing bone formation through increasing...
plasma homocysteine. It has been hypothesized that homocysteine interferes with collagen cross-linking such that it results in less mineralized and more fragile bone [28,29], and that homocysteine interferes with development of microarchitecture of bone independently of the amount of mineral in bone [28]. The recent finding that boron deprivation impairs alveolar bone (primary support structure for teeth) repair [25], which is initiated immediately after tooth extraction, supports the suggestion that boron promotes normal trabecular bone formation or microarchitecture. Boron deprivation decreased bone volume fraction and osteoblast surface and increased quiescent bone-forming surface in the alveolus after tooth extraction in rats [25]. Boron deprivation also decreased osteoblast surface and increased quiescent bone-forming surface in both the lingual and buccal side of periodontal alveolar bone in growing mice [26]. In addition, the finding that boron deprivation decreases chondrocyte density in the growth plate of bone in chicks [30] supports the suggestion that the beneficial function of boron occurs mostly in the organic matrix of bone. Boron apparently does not markedly affect bone calcification because it did not affect plasma alkaline phosphatase activity, and other reports show that boron deprivation does not markedly affect calcium and phosphorus concentrations in bone [16,31]. The tibia PGE2 release results indicate that boron may not have much effect on the presence of eicosanoids, which influence bone turnover.

The effect of boron on plasma homocysteine might be the result of boron affecting the utilization or metabolism of its precursor, S-adenosylmethionine. S-adenosylmethionine and diadenosine polyphosphates (signaling molecules) have higher affinities for boron than any other currently recognized boron ligands present in animal tissues [32]. Boron deprivation decreases liver S-adenosylmethionine concentration [33], which would be consistent with increased circulating homocysteine.

Main effects of dietary oil

Although microarchitectural variables were not independently significantly affected by dietary oil, vertebrae simulated compression and cortical bone biomechanical findings indicated that fish oil was beneficial to bone strength.

The mechanism behind the beneficial effect of omega-3 PUFAs on bone strength is still open to conjecture, but is apparently different from that of boron based on the results of the present experiment. Based on plasma alkaline phosphatase results from the present study and the reported lack of an effect on femur calcium and phosphorus concentrations [31,34], feeding a diet high in omega-3 PUFAs (fish oil) versus one high in omega-6 fatty acids (safflower oil) does not markedly affect the incorporation of calcium into bone. One hypothesis is that the omega-3 PUFAs modulate the production or excretion of inflammatory cytokines that may be involved in bone loss [35]. This hypothesis is supported by the present finding of omega-3 fatty acids decreasing tibia PGE2 release. Endogenous PGE2 is synthesized from the omega-6 fatty acid arachidonic acid. PGE2 favors bone formation at low concentrations and bone resorption at high concentrations [36,37]. High PGE2 apparently increases pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α, which promote osteoclastogenesis and bone resorption [38]. However, a change in bone PGE2 content indicated by its release (Table 2) can be only a partial explanation of why dietary oil affected some bone strength and microarchitecture variables in the present study. This is evidenced by fish oil instead of safflower oil decreasing PGE2 release regardless of boron status of rats, but having different, sometimes opposite, effects on bone microarchitecture and strength in boron-deprived and boron-adequate rats. Hormone receptor modulation and function as a second messenger have also been suggested [7] as a mechanism behind the beneficial effects of omega-3 fatty acids on bone. The effect on hormone utilization may occur through the omega-3 PUFAs affecting bone cell membrane physicochemical characteristics [39].

Interactions between boron and dietary oil

In addition to having independent positive effects on vertebral morphometric measures, boron was a factor that determined whether dietary oil had an effect on some of these measures. The findings of the lowest mean SMI value (more desirable plate-like structure) and the highest mean force to compress the vertebrae and to break the femur in boron-adequate rats fed fish oil suggest that long-chain omega-3 PUFAs may complement the beneficial effects of boron. On the other hand, the findings of the lowest trabecular connectivity, bone volume fraction and number, and highest trabecular separation and SMI in boron-deficient rats fed fish oil suggest they had the highest bone turnover and the weakest bones. Thus, a diet high in omega-3 fatty acids provided by fish oil is apparently detrimental to rats if they have a low boron status. The suggestion that fish oil can be detrimental in some circumstances is supported by the finding that maternal fish oil supplementation during lactation apparently had adverse effects in children, especially in boys [40].

The mechanism through which dietary fatty acids interact with boron is unknown. A possible mechanism is that they interact at the cell membrane level. A change in cell membrane physicochemical characteristics resulting
from a different intake of omega-3 PUFAs [36] might alter the influence of boron on the utilization of S-adenosylmethionine or the signaling function diadenosine polyphosphates. Diadenosine polyphosphates, which avidly bind boron [32], are involved in the normal development of chondrocytes [38]. Definition of how fatty acids affect the response to boron deprivation, or vice versa, might help determine the mechanism through which boron is beneficial for bone.

**Conclusion**

The present findings show that boron is a bioactive element that has beneficial, if not essential, effects on trabecular microarchitecture and cortical bone strength. Feeding fish oil instead of safflower oil is beneficial to vertebral and cortical bone strength. Boron status determines whether feeding a diet high in long-chain omega-3 PUFAs instead of a diet high in omega-6 PUFAs has a beneficial or no effect on vertebral trabecular microarchitecture. Boron and fish oil apparently have beneficial effects through different mechanisms that sometimes appear complementary. The mechanisms through which boron and long-chain omega-3 PUFAs exert their beneficial effects need to be determined, but probably involves an influence on chondrocyte function or osteoblast and/or osteoclast activity.

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