Boron as a Dietary Factor for Bone Microarchitecture and Central Nervous System Function

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Introduction

Studies by several different research groups using different experimental animals indicate that nutritional amounts of boron beneficially affect bone histomorphological and gross physical characteristics. One of the first studies suggesting that boron is essential for higher animals found that boron improved bone calcification in chicks fed a diet deficient but not completely lacking in vitamin D (Hunt and Nielsen, 1981). At the microscopic level, boron deprivation (0.465 mg/kg diet) exacerbated the distortion of marrow sprouts (location of calcified scaffold erosion and new bone formation) and the delay in initiation of cartilage calcification in bones during marginal vitamin D deficiency (Hunt, 1996). Boron deprivation alone decreased chondrocyte density in the zone of proliferation in the bone growth plate (Hunt, 1996). Nutritional amounts of boron (5 mg/kg) supplemented to a basal AIN-76 diet containing 0.3 mg boron/kg enhanced the beneficial effects of 17β-estradiol on trabecular bone volume and plate density in tibias of ovariecctomized rats (Sheng et al. 2001). Low dietary boron (0.98 mg/kg compared to 5 mg/kg) decreased the bone strength variable bending moment in femurs of pigs (Armstrong et al. 2000). In a factorial arranged experiment where the variables were dietary boron at 0.07 or 3 mg/kg, dietary oil as either canola or palm oil at 75 g/kg and sex, femur bending moment was decreased by boron deprivation in female rats, particularly when they were fed canola oil (Nielsen, 2004). Femur breaking stress also was decreased by boron deprivation (most markedly in females) when the diet contained canola oil. Boron deprivation (0.6 mcg/kg instead of the usual 310 mcg/kg diet) of the African clawed frog (Xenopus laevis) resulted in abnormal limb development in offspring (grown in water containing 0.6 mcg boron/L instead of the usual 100 mcg boron/L) (Fort et al. 2002).
Supranutritional amounts of boron also may be beneficial to bone mechanical properties and microarchitecture. A boron supplement of 5 mg/kg to a diet containing 9.4 mg boron/kg increased bone strength in chicks (Rossi et al. 1993). In rats exposed to strenuous treadmill exercise, femur and vertebra bone mineral content and density were decreased and trabecular separation was increased. A supplement of 50 mg boron/kg diet increased bone mineral content and density, trabecular volume, and trabecular thickness in the exercised rats (Rico et al. 2002).

Brain function and eye histomorphology also are affected by dietary boron. In rats, boron deprivation resulted in decreased brain electrical activity similar to that observed in nonspecific malnutrition (Penland, 1998). In humans, boron supplementation after boron deprivation yielded changes in encephalograms that suggested improved behavior activation (e.g., less drowsiness) and mental alertness, improved psychomotor skills of motor speed and dexterity, and elicited improvements in the cognitive processes of attention and short-term memory (Penland, 1998). Dietary boron modified the effect of replacing dietary palm oil with canola oil on eye mitochondrial morphology of rats (Nielsen et al. 2004). In a factorial arranged experiment where the variables were dietary boron at 0.07 or 3 mg/kg and dietary oil as either canola or palm oil at 75 g/kg, rats fed the boron-supplemented diet with canola oil had the lowest number of rod inner segment mitochondria with a high abundance of crystal folds. Rats fed the boron-supplemented diet with palm oil had the lowest number of hydropic (swollen) mitochondria. In zebrafish, boron deficiency induced photoreceptor dystrophy; the photoreceptor cells were shortened because of reduction in myoid and outer segment regions (Eckhert and Rowe, 1999). These changes apparently were the reason boron-deficient zebrafish developed photophobia.

A possible explanation for the broad and varied response to changes in dietary boron is that it has a role that influences the physicochemical characteristics of cell membranes. This influence may alter the activity of membrane-bound enzymes such as oxidoreductase enzymes that have the ribose moiety (which binds boron) of NAD⁺, and the response to hormones and cytokines or transmembrane signaling. The long-chain omega-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), that have broad and varied beneficial effects, including on bone turnover and neurological function, are also hypothesized to act through influencing the physicochemical characteristics of cell membranes. Thus, EPA and DHA may affect cell-to-cell interactions and the expression of various receptors for hormones and cytokines. Cell membrane changes may explain the findings that EPA supplementation of ovariectomized rats inhibited bone loss (Sakaguchi et al. 1994) and enhanced the ability of estrogen to inhibit bone loss (Schlemmer et al.
1999). DHA has been shown to promote visual acuity (e.g., brightness discrimination) and development in rats (Okuyama et al. 2001) and humans (Hoffman, 2000). Also, diets enriched with the long-chain omega-3 PUFA enhanced cognitive functions in rats (Becker and Kyle, 2001; Takeuchi et al. 2002; Moriguchi and Salem, 2003).

Because dietary boron and long-chain omega-3 PUFA affect a large number of the same biological processes apparently through influencing the responses to hormones and cytokines at the cell membrane level, a reasonable hypothesis is that the fatty acid composition of the diet will alter the response to boron deprivation, or vice versa, boron deprivation will affect the response to diets containing differing amounts of omega-3 PUFA. A corollary to this hypothesis is that a finding that the omega-3 PUFA content of the diet affects the response to boron deprivation would support the contention that boron is beneficially bioactive at the cell membrane level. This hypothesis and corollary were the stimulus for the study described below.

Materials and methods

Female Sprague-Dawley rats (Charles River/SASCO, Wilmington, MA) weighing about 115 grams were fed diets in a factorial arrangement with variables being supplemental boron as boric acid at 0 or 3 mg/kg and either 75 g safflower oil/kg or 65 g fish (menhaden) oil plus 10 g linoleic acid/kg of diet. The composition of the ground corn-casein based diet has been reported (Nielsen, 2004). The basal diet contained about 0.10 mg boron/kg as determined by inductively coupled plasma atomic emission spectroscopy after low-temperature, acid-digestion in Teflon tubes (Hunt and Shuler, 1990). Standard reference material (National Institute of Standards and Technology, Gaithersburg, MD) #1515 (apple leaves) was used for quality control purposes in the diet analysis. The diets were not pelleted and were stored at -16°C in tightly capped plastic containers.

The females 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. After 5 weeks on 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 that were fed a commercial rat chow. Dams and pups continued having free access to their respective diets and deionized water through gestation, lactation and post-weaning. At about age 21 days, 15 male pups from each dietary group were placed individually in single stainless steel cages in a room maintained at 23°C and 50% relative humidity and with a reversed 12 h white and red light cycle. Absorbent paper under the wire mesh cages were changed daily. Rats were weighed and provided clean cages weekly. Repeated humane handling of the males after weaning was performed to minimize distress caused by behavioral testing.
At 6 and 19 weeks of age, behavioral reactivity, more specifically anxiety, was evaluated with the elevated plus-maze procedure described by Pellow et al. (1985). The apparatus consisted of a plus-shaped maze constructed of white opaque Plexiglas with two open arms opposite each other, crossed by two enclosed arms; the maze was elevated 50 cm from the floor. More entries into the open arms indicate a rat is less anxious about falling from the maze. Numbers of entries and times spent in open and closed arms of the maze, and total numbers of arm entries (movements), were determined during two consecutive 5-min trials.

At 15 weeks of age, visual function was evaluated with a brightness discrimination procedure adapted from Tang and Ho (1998). The apparatus consisted of an enclosed Y-shaped maze constructed of black opaque Plexiglas with a black lid covering one arm (dark arm) and transparent lids covering the other two arms (light arms). Rats were placed in one of the light arms and entry into either the dark or other light arm was recorded. Number of entries and times spent in light and dark arms, and total number of arm entries (movements), were determined during six consecutive 1-minute trials; light and dark arms and starting arm were varied across trials. Ambient lighting was set to 10 or 100 lux to ascertain any dietary differences in visual ability to distinguish light and dark arms (brightness discrimination) as indicated by a preference for either the light or dark arm.

Eighteen weeks after weaning, the male 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 brain was immediately removed and quickly frozen in liquid nitrogen. The left femur and vertebra with some attached flesh were collected for measuring bone physical characteristics. The right femur with all flesh removed was collected for mineral analysis. Bones, brains and plasma were stored at -70°C until analysis.

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

Plasma 8-iso-prostaglandin F$_2$α (8-iso-PGF$_2$α) was determined by using a Correlate-EIA™ Immunoassay Kit (#900-010, Assay Designs, Ann Arbor, MI). For mineral analysis, femurs were cleaned to the periosteal surface with cheese cloth. Both the femurs and brains were lyophilized then subjected to a wet-ash, low-temperature digestion in Teflon tubes (Hunt and Shuler, 1990). Boron, calcium, copper, iron, magnesium and zinc were determined by coupled argon plasma atomic emission spectroscopy. Standard reference material (National
Institute of Standards and Technology, Gaithersburg, MD) #1515 (apple leaves) was used for quality control.

The bone strength variable maximum force (force in kg needed to break the bone) was determined on the right femur after thawing and removing the remaining flesh. A custom-designed and built apparatus that performed a three-point bending test the same as that performed by commercially available machines was used as described previously (Nielsen, 2004). Vertebra microarchitecture of seven randomly selected rats from each treatment was examined by microcomputed tomography (μCT40, Scanco Medical AG, Zurich, Switzerland). The vertebrae were scanned from the proximal to distal growth plate. Contours were placed on a volume of interest beginning and ending 10 slices (20.67 μm thickness per slice) away from the growth plate in order to include in the volume of interest only the secondary spongiosa within the two growth plates. Bone morphometric variables including trabecular bone volume fraction (BV/TV) and trabecular thickness, in addition to structural model index (SMI) and connectivity density were determined. SMI indicates whether the trabeculae are more plate-like or rod-like.

Data were statistically compared by 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

Boron deprivation significantly depressed final weight and femur boron concentration (Table 1); dietary oil did not affect either of these variables. Both boron deprivation and safflower oil instead of fish oil in the diet increased plasma concentration of 8-iso-PGF$_{2\alpha}$, an indicator of increased oxidative stress (Table 1).

Femur strength was reduced by both boron deprivation and feeding safflower oil instead of fish oil. As a result, the maximum force to break the femur was lower in the boron-deficient rats fed safflower oil than in boron-supplemented rats fed fish oil (Table 1). Neither dietary oil nor boron affected the concentration of calcium in the femur (Table 2). However, the concentrations of several elements associated with the organic matrix were altered by the dietary treatments (Table 2). Boron deprivation decreased the concentrations of iron and magnesium, and safflower oil compared to fish oil increased the concentration of zinc in femur. An interaction between dietary oil and boron affected the femur copper concentration. The femur copper concentration was lower in boron-deficient than boron-supplemented rats with the effect most marked when the diet contained fish oil.
**Table 1.** Effect of dietary boron, oil, and their interaction on boron status indicators

<table>
<thead>
<tr>
<th>Boron (mg/kg)</th>
<th>Oil</th>
<th>Final Weight (g)</th>
<th>Plasma 8-iso-PGF₂α (ng/mL)</th>
<th>Plasma Boron (ng/g)</th>
<th>Femur Maximum Force (Newton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fish</td>
<td>447±7**</td>
<td>10.6±1.1</td>
<td>190±28</td>
<td>153±4</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>476±7</td>
<td>8.2±0.6</td>
<td>978±81</td>
<td>165±4</td>
</tr>
<tr>
<td>0</td>
<td>Safflower</td>
<td>441±9</td>
<td>12.7±0.6</td>
<td>115±26</td>
<td>146±4</td>
</tr>
<tr>
<td>3</td>
<td>Safflower</td>
<td>487±9</td>
<td>11.8±0.6</td>
<td>920±96</td>
<td>155±4</td>
</tr>
</tbody>
</table>

Analysis of variance – p Values

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron</td>
<td>0.0001</td>
<td>0.04</td>
<td>&lt;0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>Oil</td>
<td>0.72</td>
<td>0.0005</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>Boron x Oil</td>
<td>0.28</td>
<td>0.31</td>
<td>0.90</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* The dietary treatments were boron supplements of 0 or 3 mg/kg and either safflower oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg. The basal diet contained about 0.1 mg boron/kg. **Mean ±SE.

**Table 2.** Effect of dietary boron, oil and their interaction on femur mineral concentrations

<table>
<thead>
<tr>
<th>Boron (mg/kg)</th>
<th>Oil</th>
<th>Calcium (g/kg)</th>
<th>Copper (mg/kg)</th>
<th>Iron (mg/kg)</th>
<th>Magnesium (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fish</td>
<td>188±1**</td>
<td>0.84±0.05abcdef</td>
<td>76±4</td>
<td>3308±62</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>189±2</td>
<td>1.47±0.09b</td>
<td>122±6</td>
<td>4292±170</td>
</tr>
<tr>
<td>0</td>
<td>Safflower</td>
<td>189±3</td>
<td>1.04±0.05a</td>
<td>108±4</td>
<td>3436±96</td>
</tr>
<tr>
<td>3</td>
<td>Safflower</td>
<td>191±2</td>
<td>1.35±0.09b</td>
<td>136±8</td>
<td>3948±215</td>
</tr>
</tbody>
</table>

Analysis of Variance – p Values

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron</td>
<td>0.46</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oil</td>
<td>0.52</td>
<td>0.63</td>
<td>0.0002</td>
<td>0.48</td>
</tr>
<tr>
<td>Boron x Oil</td>
<td>0.89</td>
<td>0.04</td>
<td>0.10</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* The dietary treatments were boron supplements of 0 or 3 mg/kg and either safflower oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg. The basal diet contained about 0.1 mg boron/kg.

**Mean ±SE. **Values in the same column not followed by the same letter are significantly different (p < 0.05) according to Tukey’s contrasts.

The data in Table 3 show that vertebral microarchitecture was altered by the dietary treatments. Trabecular thickness was greater in boron-supplemented than
boron-deficient rats. Fish oil compared to safflower oil tended (p < 0.06) to increase trabecular thickness. The interaction between dietary boron and oil differently affected trabecular connectivity and SMI. Trabecular connectivity was increased by boron deficiency in rats fed the safflower oil; dietary boron had no effect on this variable in rats fed fish oil. SMI was significantly decreased (more plate-like than rod-like) by feeding fish oil instead of safflower oil to the boron-supplemented rats; dietary oil did not significantly affect SMI in the boron-deficient rats. BV/TV was highest in the boron-supplemented rats fed fish oil and lowest in boron-supplemented rats fed safflower oil, but this difference was not significant (boron x oil interaction < 0.07).

Table 3. Effect of dietary boron, oil and their interaction on vertebral trabecular microarchitecture.

<table>
<thead>
<tr>
<th>Diet*</th>
<th>Trabecular</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness</td>
<td>Connection Density</td>
</tr>
<tr>
<td>Boron</td>
<td>(mg/kg)</td>
<td>(mm)</td>
</tr>
<tr>
<td>0</td>
<td>Fish</td>
<td>0.082±0.002**</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>0.085±0.002</td>
</tr>
<tr>
<td>0</td>
<td>Safflower</td>
<td>0.078±0.001</td>
</tr>
<tr>
<td>3</td>
<td>Safflower</td>
<td>0.083±0.001</td>
</tr>
</tbody>
</table>

Analysis of Variance – p Values

<table>
<thead>
<tr>
<th></th>
<th>Boron</th>
<th>Oil</th>
<th>Boron x Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.04</td>
<td>0.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*The dietary treatments were boron supplements of 0 or 3 mg/kg and either safflower oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg. The basal diet contained about 0.1 mg boron/kg.

**Mean ±SE, n=7/group.

*Values in the same column not followed by the same letter are significantly different (p < 0.05) according to Tukey’s contrasts.

Table 4 shows that neurological function and brain composition were affected by the dietary treatments. In the brightness discrimination test, boron-deficient rats made more entries into the 100 lux light arm of the test apparatus than did the boron-supplemented rats. The boron effect occurred mainly when the diet contained fish oil. Boron had no apparent effect when the diet contained safflower oil. In the plus maze, the boron-deficient rats entered the open arms more often than the boron-supplemented rats fed fish oil. When safflower oil was fed, there was a tendency for boron to have an opposite effect. Interestingly, the highest brain boron concentration was found in rats fed the
boron-deficient, fish oil diet. Unlike with femur, boron deprivation did not decrease the boron concentration in brain. The rats fed the boron-deficient fish oil diet also had the lowest brain copper and zinc concentrations.

Table 4. Effect of dietary boron, oil and their interaction on selected brain mineral concentrations and brightness discrimination and plus maze variables

<table>
<thead>
<tr>
<th>Boron</th>
<th>Diet*</th>
<th>Boron</th>
<th>Brain Copper</th>
<th>Zinc</th>
<th>Lux 100 Light Entries</th>
<th>Maze Open Arm Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/kg)</td>
<td>(ng/g)</td>
<td>(mcg/g)</td>
<td>(mcg/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Fish</td>
<td>323±10**</td>
<td>9.0±0.2a#</td>
<td>52±1a</td>
<td>1.2±0.07</td>
<td>5.0±0.6a</td>
</tr>
<tr>
<td>3</td>
<td>Fish</td>
<td>229±14</td>
<td>11.9±0.2b</td>
<td>66±1b</td>
<td>0.9±0.05</td>
<td>3.7±0.4b</td>
</tr>
<tr>
<td>0</td>
<td>Safflower</td>
<td>233±23</td>
<td>11.2±0.4b</td>
<td>60±1c</td>
<td>1.1±0.06</td>
<td>3.5±0.3b</td>
</tr>
<tr>
<td>3</td>
<td>Safflower</td>
<td>204±26</td>
<td>12.1±0.3b</td>
<td>65±1b</td>
<td>1.1±0.06</td>
<td>4.5±0.5ab</td>
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</table>

Analysis of Variance – p Values

<table>
<thead>
<tr>
<th></th>
<th>Boron</th>
<th>Oil</th>
<th>Boron x Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>p Value</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>0.004</td>
<td>0.0001</td>
<td>0.006</td>
<td>0.53</td>
</tr>
<tr>
<td>0.09</td>
<td>0.0005</td>
<td>0.0002</td>
<td>0.07</td>
</tr>
<tr>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The dietary treatments were boron supplements of 0 or 3 mg/kg and either safflower oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg. The basal diet contained about 0.1 mg boron/kg.

**Mean ±SE.

Values in the same column not followed by the same letter are significantly different (p < 0.05) according to Tukey's contrasts.

Discussion

Finding markedly lower boron concentrations in the femur of rats fed the low-boron diet indicates that they were boron-deficient. Further evidence that the rats fed the low-boron diet were deficient is that they exhibited depressed final weights and increased plasma 8-iso-PGF_{2α} (an indication of increased oxidative stress). Fish oil compared to safflower oil did not affect these responses to boron deprivation but did decrease plasma 8-iso-PGF_{2α}. The decrease was independent of boron deprivation. However, the lowest plasma concentration of 8-iso-PGF_{2α} was found in boron-supplemented rats fed fish oil, which indicates that fish oil and boron were complementary in reducing oxidative stress. This finding plus the lack of a significant interaction affecting weight and 8-iso-PGF_{2α} suggest that although boron and long-chain omega-3 PUFA affect many similar biological processes (see Introduction), they apparently do this through different biochemical mechanisms and not through directly affecting the metabolism of each other. Nonetheless, the numerous significant interactive findings in the present study indicate that boron has in vivo effects that can alter some responses to changes in the dietary intake of omega-3 PUFA, and vice versa.
The 8-iso-PGF$_{2\alpha}$ data suggest that some of the interaction between dietary boron and fatty acid composition may occur because both affect redox metabolism. That is, changing oxidative stress by one of these bioactive substances may change the magnitude of the oxidative metabolism response to the other. Support for this suggestion is that boron deprivation increased plasma glutathione in rats when their diet fat source was palm oil (Nielsen, 2004), fish oil or safflower oil (Nielsen, 2005), but had no effect when diet fat source was canola oil (Nielsen, 2004). Hunt and Idso (1999) have reviewed the evidence suggesting that boron hastens the destruction of reactive oxygen species that are scavenged and destroyed by defense mechanisms employing glutathione, superoxide dismutase and catalase. Among the evidence was the finding that boron deprivation decreased erythrocyte superoxide dismutase in humans (Nielsen, 1996). DHA has been suggested to be a biodevice to combat oxidative stress in the brain (Yavin et al. 2002). This finding is consistent with the 8-iso-PGF$_{2\alpha}$ results in the present study. Other reports, however, indicate that fish oil may increase oxidative stress. For example, fish oil was found to enhance lipid oxidation in hearts and livers of rats (Yuan and Kitts, 2003). Additionally, glutathione concentrations were reduced in red blood cells but increased in livers of the rats fed fish oil. Another study found that fish oil decreased hepatic α-tocopherol and retinol concentrations, and hepatic and spleen superoxide dismutase activity in rats (Miret et al. 2003). Nonheme iron concentration was decreased and the expression of iron regulatory protein-1 was increased by fish oil in the spleen and liver of rats. Miret et al. (2003) concluded that their findings indicated that fish oil increased oxidative stress in the liver and spleen of rats.

As indicated in the introduction, both boron and omega-3 PUFA have been shown to beneficially affect bone histomorphometric measurements in rats. Thus, it was not surprising that both enhanced femur strength as assessed by the maximum force needed for breaking. It was mildly surprising, however, to find that neither dietary boron nor fatty acid composition significantly affected the calcium concentration in the femur. Thus, the change in bone strength apparently was the result of changes in the physical characteristics of the bone through a modification of the organic matrix upon which calcification occurs. Moreover, because dietary boron and oil did not interact to affect maximum force, it is likely that they affected bone strength through different mechanisms. The femur copper, iron and magnesium findings support this suggestion. Copper and iron are involved in the formation of the organic matrix. The enzymes prolyl and lysyl hydroxylase need iron to catalyze the ascorbate-dependent hydroxylation of select prolyl and lysyl residues in collagen (Tuderman et al. 1977) before crosslinking by the copper-dependent enzyme lysyl oxidase (Siegel, 1978). Magnesium deficiency has been found to decrease collagen formation and
sulfate incorporation in glycosaminoglycans in the organic matrix (Wallach, 1990). The concentrations of copper, iron and magnesium were higher in boron-supplemented than boron-deficient rat femurs. In contrast to the effect of boron supplementation, increasing the omega-3 PUFA content of the diet by feeding fish oil decreased the iron concentration in femur. Fish oil compared to safflower oil did not affect the femur concentration of magnesium or copper.

The vertebral microarchitecture data also indicate that boron and fatty acid composition each affect bone physical characteristics such as strength through different mechanisms, but the response induced by a change in the dietary intake of one of these dietary components influences the response to a change in the other. The increased trabecular thickness in boron-supplemented rats and a tendency for a similar effect in rats fed fish oil suggests that a change in thickness was partly responsible for these two dietary treatments increasing bone strength. However, trabecular thickness was not affected by an interaction between dietary boron and oil, but trabecular connectivity and SMI were. Greater trabecular connectivity usually is considered advantageous for bone strength. Thus, it was surprising that the boron-deficient rats fed safflower oil had the highest vertebra trabecular connectivity. Perhaps this was in response to having the lowest trabecular thickness. The SMI findings are prime examples for showing that boron influences the response to a change in dietary fatty acid composition or vice versa. Feeding fish oil instead of safflower oil resulted in the more preferred plate-like structure (low SMI) of trabecular bone in the vertebra only in the boron-supplemented rats. Based on the maximum force, trabecular thickness, SMI and BV/TV findings, boron and fish oil are complementary in enhancing bone strength and structure.

Brain mineral composition and visual and cognitive function tests also indicate that both boron and omega-3 PUFA have bioactivities that affect the response to changes in each other’s intakes. Data from boron-deficient rats fed fish oil shown in Table 4 were noticeably different than the data from rats in the other three treatments. The boron-deficient rats fed fish oil apparently were less anxious based on more entries into open arms of the plus maze. This finding may be confounded by the results from the brightness discrimination test which indicated that visual acuity was altered by an interaction between dietary oil and boron. In contrast to boron-deficient zebrafish that become photophobic (Eckhart and Rowe, 1999), the boron-deficient rats, especially those fed fish oil, were more willing to enter areas of bright light (100 lux). The boron-supplemented rats fed fish oil was more normal in preferring to enter the bright light areas less times. The changes in the brightness discrimination and plus maze tests may be related to changes in the concentrations of minerals that affect brain function. Penland & Prohaska (2004) demonstrated lasting effects of early copper deprivation on brain copper and iron, and behavior of repleted
adult rats. Johnson (2005) reviewed the role of copper in dopamine-
β-monoxygenase for neurotransmitter synthesis and copper-zinc superoxide
dismutase as an antioxidant; mechanisms by which changes in brain copper
likely affect behavior. Sandstead et al. (2000) reviewed the detrimental effects of
zinc deficiency on brain biochemistry and function, and behavior in rats. Both
zinc and copper concentrations were lowest in the boron-deficient rats fed fish
oil. Interestingly, this group had the highest brain boron concentration, a finding
difficult to explain. Apparently, boron deficiency alters the effect of fish oil on
the ability of copper, zinc and boron to enter and exit the cell. Perhaps the
transport of boron into the cell by a boron transporter (Park et al. 2004) is
increased by boron deficiency and the transporter efficiency is promoted by
increasing the long-chain omega-3 fatty acid composition of the cell membrane.

To summarize, feeding a boron-deficient diet decreased weight gain, femur
strength, and femur concentrations of copper, iron and magnesium (minerals
associated with the organic matrix), and increased the plasma concentration of
8-iso-PGF$_{2\alpha}$ (an indicator of oxidative stress) in rats. A change in the omega-3
PUFA content in the diet did not alter these boron deficiency responses. A diet
high (fat provided by fish oil) compared to one low (fat provided as safflower oil)
in long-chain omega-3 PUFA did not affect weight gain, but decreased the
plasma concentration of 8-iso-PGF$_{2\alpha}$, increased femur strength, and decreased
the femur concentration of iron in rats. Boron deprivation did not alter these
responses to the change in dietary fatty acid composition. Thus, dietary boron
and long-chain, omega-3 PUFA affect similar processes (bone growth and
composition, oxidative metabolism), but apparently through affecting different
biochemical systems. However, through changing the activity or function of
some respective systems they affect, dietary boron and omega-3 PUFA each
influenced the effect of the other on vertebral trabecular physical characteristics,
brightness discrimination, plus maze activity, and brain composition. The basis
for the relationship between boron and fatty acid bioactivity is still unclear.
However, the findings to date do not eliminate the possibility that alterations of
the beneficial actions of boron and omega-3 PUFA by each other occur through
changes at the cell membrane level and may involve changes in redox
metabolism.

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