The Nutritional Importance and Pharmacological Potential of Boron for Higher Animals and Human

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1. INTRODUCTION

At the Boron International Symposium, I presented an historical account of boron in food and nutrition, and reviewed findings indicating that boron is nutritionally important for animals and humans (Nielsen, 1997). Those findings came mainly from the Grand Forks Human Nutrition Research Center in North Dakota, USA, and showed that physiological amounts of boron can affect the metabolism or utilization of numerous other substances involved in life processes including macrominerals, energy substrates such as triglycerides and glucose, nitrogen-containing substances such as amino acids and proteins, reactive oxygen species, and estrogen. Through these effects, boron can affect several body systems, including the brain, skeleton and immune system, generally in a beneficial fashion. The review also presented evidence that resulted in the hypothesis that boron has an essential role in cell membrane function or stability such that it influences the response to hormone action, transmembrane signaling, or transmembrane movement of regulatory cations or anions. Since that symposium, the number of research findings, now from several research groups, supporting the contention that boron is of nutritional and pharmacological importance have increased markedly.

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The new findings establish boron as an essential element for animals, and thus, most likely for humans.

Many of the findings are consistent with the hypothesised cell membrane function for boron stated above. Boron has been found to be needed or beneficial for many of life processes, some of which, including reproduction and embryogenesis, bone growth and maintenance, eye development or structure, psychomotor skills, and cognitive functions, will be presented in the following.

Other processes in which boron plays a beneficial or essential function, including the immune response and inflammation, will be described in another presentation at this symposium (C. D. Hunt).

2. **BORON IN EMBRYOGENESIS AND REPRODUCTION**

The evidence that substantiates boron as an essential nutrient for animals is that boron is required to complete the life cycle for some species. The most extensive evidence for this requirement comes from studies using the African clawed frog, *Xenopus laevis*, but substantial evidence also has come from studies using zebrafish. Although experiments with mammals have not been able to show definitively that the life cycle is interrupted (interference with growth, development, or maturation such that procreation is prevented) by boron deprivation, these experiments found changes during reproduction that suggest boron is needed for optimal development of the fetus and reproductive competence.

2.1. **Boron in the Reproduction of the African Clawed Frog, *Xenopus laevis***

Fort et al. (1998, 1999a) found that boron deprivation (0.3 mg/kg diet) of maternal and paternal *Xenopus laevis* for 28 or 120 days resulted in a marked increase in necrotic eggs. Also, the fertilised eggs showed a high frequency of abnormal gastrulation characterised by bleeding yolk and exogastrulation, which suggested abnormal cell membrane structure or function. The adverse effects were more marked with the 120-day deprivation. More than 80% of the embryos from frogs fed the boron-deficient diet for 120 days died before 96 h of development; survival of embryos at 96 h from frogs fed boron-supplemented diets (1.85 mg/kg) exceeded 75%. Culturing of embryos from both boron-deficient and -adequate frogs in a culture medium containing less
than 0.4 μg/L of boron during organogenesis resulted in abnormal development of the gut, craniofacial region and eye, visceral edema, and kinking of the tail (muscular and skeletal). The rate of tail resorption was slower in boron-deprived than in boron-supplemented larvae (Fort et al., 1999b). Addition of 100 μg/L of thyroxine, a known enhancer of tail resorption reversed the delayed tail absorption in boron-deprived larvae, which suggests that boron deprivation impaired the ability of thyroxine/triiodothyronine to act, perhaps at the cell membrane level.

In a subsequent study, Fort et al. (2000) were able to culture and breed a limited number of progeny from boron-deprived frogs. Highly specific forelimb and hindlimb defects, including axial flexures resulting in crossed limbs and limb reduction deficits occurred in the boron-deficient F2 larvae, but not in the boron-adequate F2 larvae. Thyroxine or glutathione/glutathione peroxidase (0.25 U/ml) supplementation of the culture medium did not decrease the incidence or alter the type of limb malformations. Thus, the induction of the limb malformations was not caused by boron deprivation inducing oxidative damage or impaired thyroid hormone action. Boron deprivation apparently had a more immediate effect on a critical aspect in cartilage or bone development.

Fort et al. (1999c) also found that both 28 and 120 days of boron deprivation affected the reproductive system of the adult male and female frogs; both ovaries and testes were atrophied. Boron-deprived males exhibited decreased sperm counts and sperm dysmorphology. Sperm abnormalities were primarily neck lesions and a flexure or kinking of the tail. In the females, boron deprivation impaired oocyte maturation. Culturing stage 1 and 2 oocytes from boron-adequate frogs in a medium containing progesterone resulted in successful maturation to stage 5 or 6 oocytes. In contrast, oocytes from boron-deprived frogs did not respond to the hormone and did not mature in vitro. Further study of the maturation process (Fort and Stover, 2000) revealed that the boron-deprived oocytes were capable of producing progesterone and maturation-promoting factor (involved in binding progesterone to its receptor on the plasma membrane), and responding to this factor. The impaired maturation process apparently was caused by progesterone not being bound efficiently to the membrane receptor because of changes in its structural homology.
2.2 Boron in the reproduction of the zebrafish

When zebrafish fed boron-depleted brine shrimp and maintained for 6 months in water containing 1 µg B/L were compared to those maintained in water containing about 500 µg B/L, differences occurred in embryogenesis (Rowe and Eckhart, 1999). Within 10 days after fertilisation of eggs, 92% of the embryos from the boron-deprived zebrafish had died; less than 35% of the boron-supplemented controls died during this time. The early cleavage stage of development was most sensitive to boron deficiency; 46% of the fertilised embryos did not complete the blastula stage compared to 2% for boron-supplemented controls. During this time of rapid cell division, the boron-deficient zygotes exhibited blebbing of the cell and yolk membranes. This was followed by cytoplasmic and yolk extrusion. Eckhart and Rowe (1999) stated that the membrane disruption was consistent with membrane alterations found with boron-deprived cyanobacteria and vascular plants, and suggested that boron had a function that was needed for the maintenance of membrane shape and integrity. Repletion of boron-deprived embryos during the first hour after fertilisation rescued them from death.

2.3 Boron in the reproduction of mammals (rats, mice, and pigs)

Studies with mammalian species have not been as definitive as those with frogs and zebrafish. Nonetheless, there are some findings indicating that boron deficiency impairs early development of rodents, and that boron is beneficial to the reproductive process in pigs.

Lanoue et al. (1998) fed rat dams diets containing either 0.04 or 2.00 µg B/g for 6 weeks before breeding and throughout pregnancy. Dietary boron had no marked effects on fetal growth and development. However, the average number of implantation sites and fetuses was slightly higher in the rats fed 2.00 µg B/g diet. Keehr and Hunt (2000) fed rat dams diets containing either 0.1 or 2.0 µg B/g for 8 weeks before breeding and during gestation. The average number of implantation sites tended to be lower and fetal resorptions higher in the boron-low rats. The low dietary boron also accelerated the rate of vertebral ossification; a change of unknown desirability, but one that possibly affects postnatal vertebral structure or function.

Although the rat findings were not marked, they encouraged Lanoue et al. (1999a, b) to use a pre-implantation embryo system to determine whether boron status affected early mouse development. They performed two
experiments with embryos collected from mice fed either 0.04 or 2.05 μg B/g diet. In experiment one, they cultured the embryos in media containing 10 μg B/L; in experiment two the media contained <0.05 μg B/L. In experiment one, when compared to embryos from the boron-supplemented mice, embryos from the boron-deprived mice had a moderate reduction in blastocyste formation, 83.5% vs 90.1%, and an increased number of degenerates, 13.0% vs 8.0%. In experiment 2, the boron-deficient culture media enhanced the differences in the development of the embryos from boron-deprived and supplemented mice. The rate of blastocyst formation was lower, 39.7% vs 54.5%, and the number of degenerate embryos was higher, 57% vs 20% in embryos from boron-deprived mice than in those from the boron-supplemented mice. Boron deprivation was associated with reduced glycine uptake by embryos, which suggests that boron influences preimplantation embryo membrane functions (Laneoue et al., 2000).

Armstrong et al. (2001) studied the effect of a 5 mg/kg diet boron supplement on reproductive characteristics of pigs fed a diet that was, at best, marginal in boron (2.1 mg/kg during gestation and 2.9 mg/kg during lactation). Gilts were fed the diet with or without the boron supplement throughout the nursery phase, growing-finishing phase, sexual maturity, breeding, gestation, and lactation. Although the basal boron-low diet was relatively high in boron compared to diets used in the study of other animal species, some trends and differences were found between the two groups. Boron supplementation tended to increase the number of live embryos in the uterus at day 35 of gestation, and decreased the number of mummified fetuses at parturition. Boron supplementation tended to increase birth weight and significantly increased pig weaning weight. Boron supplementation decreased the calcium concentration in the embryo, oviduct and uterus and phosphorus concentration in the ovary, uterus and liver.

3. **BORON IN BONE GROWTH AND MAINTENANCE**

At the Boron Symposium, I presented considerable evidence indicating that boron can affect bone formation and maintenance including that showing boron affects the response to hormones involved in bone turnover such as estrogen and vitamin D (Nielsen, 1997). Since that time, additional findings given below have appeared showing that both nutritional and supra nutritional or pharmacologic amounts of boron have a beneficial effect on bone development, composition, and strength characteristics. These effects apparently are the result of boron influencing the presence or action of
hormones involved in bone mineral metabolism, and affecting organic matrix components involved in the formation and maturation of the matrix upon which calcification occurs.

3.1 Boron effects on bone organic matrix components

Relatively high or pharmacologic amounts of boron were found to affect components of bone growth cartilage; when added to the culture medium they decreased the synthesis of proteoglycans, collagen and total proteins by pelvic cartilage of cultured chick embryo (Benderdour et al., 1997). On the other hand, boric acid stimulated the release of proteoglycans, collagen, and total proteins into the culture medium. Nutritional amounts of boron were found to alleviate defects in growth cartilage maturation and calcification (Keeler and Hunt, 1996) and gait abnormalities (Bai and Hunt, 1996) induced by vitamin D deficiency in chicks.

The main pathological characteristics of Kashin-Beck disease that is endemic in certain parts of China are a focus necrosis in the deep zone of the growth plate and articular cartilage caused by impaired cartilage and bone development and secondary osteoarthropathy. The necrotic chondrocytes lose the ability to synthesise collagen and proteoglycan. Kashin-Beck disease has been associated with a low boron status by the finding that diseased individuals have markedly lower hair boron concentrations than healthy individuals in both non-Kashin-Beck disease and Kashin-Beck disease areas (Peng et al., 2000).

3.2 Boron effects on bone composition

Both nutritional and supra nutritional intakes of boron have been found to affect bone ash content and calcium concentration. Bai and Hunt (1996) found that boron deprivation of chicks decreased the dry weight and calcium concentration in femurs; the effect was more marked when dietary vitamin D was marginal than when it was very deficient or adequate. Boron deficiency also decreased the copper and zinc concentrations in femurs of chicks fed marginal but not adequate or very deficient vitamin D. These changes are consistent with boron alleviating growth cartilage abnormalities in chicks fed marginally inadequate vitamin D. Wilson and Ruszler (1997) found that a supra nutritional boron supplement of 50 mg/kg added to a basal diet containing 14.7 mg/kg fed to chickens from hatching to 16 weeks of age increased the per cent of ash in bone. A supplement of 200 mg B/kg diet did not further increase bone ash; a supplement of 400 mg B/kg diet returned the bone ash percentage to that found in chicks fed the basal diet. Hunt (1998)
found that in exercise-trained boron-deprived (0.15 mg B/kg diet) rats, femur concentrations of calcium, phosphorus, and magnesium were increased by supplementing the diet with 1.0 mg B/kg.

In addition to ash and mineral element content, changes in lipid content possibly occurs with altered boron status. Armstrong et al. (2000) found that boron supplements of 5 and 15 mg/kg to a diet containing 0.98 mg B/kg decreased the lipid concentration in femurs of male pigs.

3.3 Boron effects on hormones involved in bone turnover and maintenance

At Boron\textsuperscript{97}, I reviewed findings showing that dietary boron alters the plasma or serum concentrations of several hormones involved in bone turnover in humans including calcitonin, 17β-estradiol, 25-hydroxycholecalciferol, and triiodothyronine (Nielsen, 1997). Since that review, only a few studies of the effect of boron on hormones associated with bone turnover have been performed. Naghii and Samman (1997a) provided male rats with an additional 2 mg of boron per day via the drinking water; their diet contained 10 mg B/kg. This supra nutritional amount of boron increased plasma and testicular testosterone concentrations and transiently increased the plasma concentration of 1,25-hydroxycholecalciferol. Providing 12.5 mg B·day\textsuperscript{-1}·rat\textsuperscript{-1} decreased the magnitude of the increase in plasma and testicular concentrations and 25 mg B·day\textsuperscript{-1} was toxic because it caused testicular atrophy (Naghii and Samman, 1996). Naghii and Samman (1997b) also found that a supra nutritional supplement of 10 mg B·day\textsuperscript{-1} for 4 weeks significantly increased plasma estradiol concentrations and tended to increase plasma testosterone in healthy male humans. Nielsen and Penland (1999) supplemented 43 perimenopausal women with a nutritional 2.5 mg B/day for 60 days followed (19 women) or preceded (24 women) by 90 days of receiving a placebo. Overall, the serum 17β-estradiol concentration was higher during the boron supplementation period than during the placebo period; this increase approached significance (P=0.07) when all subjects were included in the comparison, but was significant (P=0.04) when the comparison included only those subjects whose urinary boron excretion was <1.0 mg/day during the placebo period, which indicated a low dietary boron intake.

3.4 Boron Effects on Bone Mechanical Properties

Since 1997, there have been some reports indicating that both nutritional and pharmacological intakes of boron have beneficial effects on bone mechanical properties. Wilson and Ruszler (1997, 1998) supplemented a
diet containing 14.7 mg B/kg with supra nutritional amounts of boron (50, 100, 200 or 400 mg B/kg). When they fed chickens the diets for 16 weeks after being hatched, they found that shear force of the tibia and femur, shear stress of the tibia, and shear fracture energy of the femur were significantly increased by the 50 and 100 mg B/kg diet supplements. When started at 32 weeks of age and fed the supplements until 72 weeks of age, both the tibia and radius exhibited increased shear fracture energy in egg producing chickens; shear force, stress and fracture energy of both the tibia and radius were increased in non-egg producing chickens. Chapin et al. (1998) found that both pharmacologic (200 and 1000 mg B/kg diet) and toxic (3000 and 9000 mg B/kg diet) intakes of boron increased vertebral resistance to a crushing force by approximately 10% in rats. Boron provided to pigs in amounts that could be considered nutritional, 5 mg or 15 mg/kg of diet, also was beneficial to bone strength when a comparison was made to pigs fed a diet containing only 0.98 mg B/kg; the supplements increased the bending moment of bones (Armstrong et al., 2000).

4. BORON IN EYE DEVELOPMENT AND STRUCTURE

In the adult F1 zebrafish boron deficiency induced photophobia (Eckhart and Rowe, 1999). Histologic evaluation showed that the boron-deficient adults had photoreceptor dystrophy. Compared with boron-supplemented controls, the photoreceptor cells of the boron-deficient zebrafish were shortened because of a reduction in the myoid and outer-segment regions. These regions are characterised by the production of prodigious quantities of membrane. Eye development is affected by boron deficiency in the developing frog (Fort et al., 1999a). Boron-deficient Xenopus larvae exhibited maldevelopment of the pigmented retina and abnormal myotome assembly.

5. BORON EFFECTS ON PSYCHOMOTOR SKILLS AND COGNITIVE FUNCTION

Some of the strongest evidence for boron essentiality is that showing low intakes of boron affect brain function and cognitive performance in older men and women. Penland (1998) found that boron deprivation altered electroencephalograms (EEG) such that there was a shift toward more activity in the low frequencies and less activity in the high, dominant frequencies of the EEG spectrum. He also found a similar effect of boron
deprivation in rats. The EEG change induced by boron deprivation is similar to that found in non-specific malnutrition and heavy metal toxicity. Penland (1998) cited reports indicating that increased low frequency activity is typical of states of reduced behavioural activation (i.e., drowsiness) and mental alertness, and has been associated with reduced performance in vigilance and psychomotor tasks. Decreased high frequency activity has been related to impaired memory performance under some conditions.

Some of the suggested behavioural consequences of a changed EEG spectrum induced by a low dietary boron intake were found by Penland (1998). He found that boron deprivation impaired performance in tasks that emphasise psychomotor skills, and the cognitive processes of attention, perception, and memory. Measures of cognitive processes showing impairment were Search-Count (attention) and Symbol-Digit (encoding skills and memory). Performance in Tapping, a simple task of tapping keys on a computer which measures manual dexterity and fatigue, was a psychomotor task impaired by boron deprivation. It seems possible that these boron deprivation effects could have been the result of changes in membranes that affected nerve-impulse transmission.

6. OTHER POSSIBLE BENEFICIAL EFFECTS OF BORON FOR HUMANS

6.1 Boron in wound healing

Treatment with a 3% boric acid solution significantly improves the healing of deep wounds (Blech et al., 1990). Doussset et al. (2000) reported that dosing nude mice with 11 ml of 3% boric acid greatly increased specific proteins in implanted sponges; these proteins included heat shock protein 70, tumor necrosis factor alpha, and vascular endothelium growth factor. They stated that boron potentiated the formation of new blood vessels during the formation of granulation in wound healing, and concluded that boron improves wound healing by promoting the synthesis and release of angiogenic factors.

6.2 Boron effects on cancer incidence and prevention

Limited epidemiologic evidence suggests that the incidence of some types of cancer is related to boron intake. In the county of Rangarvallasysla in Iceland, boron deficiency in pasture grass, drinking water and cow’s milk was associated with cancer of the stomach (Armstrong, 1990). Based on a
study of 76 cases and 7751 controls, low dietary boron intake was associated with increased prostate cancer risk (Zhang et al., 2001). The protective effect of boron became stronger with increasing amounts of boron consumed as a constituent of foods. In addition to these nutritional-type findings, boron as a component of the molecule contributes to the anti-neoplastic properties of some potential pharmaceuticals. For example, Ghosh et al. (1998) tested the anti-tumor properties of hydroxy biguanido hydrochloride monohydrate and salicyl hydroxamic acid against Ehrlich ascites carcinoma in mice. The mice showed enhanced survival time when boron was incorporated into the compounds. Hall and co-workers (1994, 1998) have found that substituted carbonizes and polyhedral hydorborate salts are potent anti-neoplastic agents inhibiting the growth of human leukemias, uterine carcinoma, colon adenocarcinoma, lung bronchogenic tumor, and gliomas.

7. NUTRITIONAL GUIDANCE FOR BORON

Considering the amount of evidence available indicating that boron is beneficial or essential for humans, it was somewhat surprising that the Food and Nutrition Board (2001) of the U.S. National Academy of Sciences did not establish an Adequate Intake level (AI) for boron. However, they did establish Upper Limit intake levels (UL) for different ages; for people aged 19 years or older the UL is 20 mg/day. Developmental and reproductive defects in animals exposed to high intakes of boron were used to establish the UL. In lieu of an AI to make people realise that consuming adequate amounts of boron most likely would help assure good bone, brain, eye, immune, psychomotor and reproductive function, perhaps dietary guidance given by a World Health Organization (1996) publication should be emphasised. This publication, based on an analysis of both human and animal data, suggested that an acceptable safe range of population mean boron intakes for adults is 1 to 13 mg/day.

Several reports indicate that many people consume <1 mg/day, the lower limit of the safe range given. For example, mean intakes of mature females in the United States have been reported to be 0.75 (Meacham and Hunt, 1998), 1.01 (Anderson et al., 1994), and 0.97 (Rainey et al., 1999) mg/day. In a study of 43 peri-menopausal women in the eastern North Dakota area, 2 women apparently consumed on average <0.5 mg/day of boron and 14 women consumed between 0.5 and 1.0 mg/day over a 90-day period, as determined by urinary excretion data (Nielsen and Penland, 1999). Thus, there apparently is a significant number of people who could benefit by increasing their intake of boron. Good sources of boron (Meacham and
Hunt, 1998; Anderson et al., 1994; Rainey et al., 1999) are foods of plant origin, especially fruits, leafy vegetables, nuts, pulses, and legumes. Wine, cider, and beer are also rich sources of boron.

REFERENCES


Nutritional Importance of Boron


