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NUTRITION

Dietary boron does not affect tooth strength, micro-hardness, and density, but affects tooth mineral composition and alveolar bone mineral density in rabbits fed a high-energy diet

Sema S. Hakki[a,c,*], Siddik Malkoc[b], Niyazi Dundar[c], Seyit Ali Kayis[d], Erdogan E. Hakki[e], Mehmet Hamurcu[e], Nuri Baspinar[f], Abdullah Basoglu[g], Forrest H. Nielsen[h], Werner Götz[i]

[a] Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya 42079, Turkey
[b] Inonu University, Faculty of Dentistry, Department of Orthodontics, Malatya, Turkey
[c] Selcuk University, Research Center of Faculty of Dentistry, Konya, Turkey
[d] Selcuk University, Faculty of Agriculture, Department of Animal Science, Konya, Turkey
[e] Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Konya, Turkey
[f] Selcuk University, Faculty of Veterinary Medicine Department of Biochemistry, Konya, Turkey
[g] Selcuk University, Faculty of Veterinary Medicine, Department of Internal Medicine, Konya, Turkey
[h] USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202, USA
[i] Bonn University, Medical Faculty, Department of Orthodontics, Oral Biology Lab, Bonn, Germany

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ABSTRACT

The objective of this study was to determine whether dietary boron (B) affects the strength, density and mineral composition of teeth and mineral density of alveolar bone in rabbits with apparent obesity induced by a high-energy diet. Sixty female, 8-month-old, New Zealand rabbits were randomly assigned for 7 months into five groups as follows: (1) control 1, fed alfalfa hay only (5.91 MJ/kg and 57.5 mg B/kg); (2) control 2, high energy diet (11.76 MJ and 3.88 mg B/kg); (3) B10, high energy diet + 10 mg B gavage/kg body weight/96 h; (4) B30, high energy diet + 30 mg B gavage/kg body weight/96 h; (5) B50, high energy diet + 50 mg B gavage/kg body weight/96 h. Maxillary incisor teeth of the rabbits were evaluated for compression strength, mineral composition, and micro-hardness. Enamel, dentin, cementum and pulp tissue were examined histologically. Mineral densities of the incisor teeth and surrounding alveolar bone were determined by using micro-CT. When compared to controls, the different boron treatments did not significantly affect compression strength, and micro-hardness of the teeth, although the B content of teeth increased in a dose-dependent manner. Compared to control 1, B50 teeth had decreased phosphorus (P) concentrations. Histological examination revealed that teeth structure (shape and thickness of the enamel, dentin, cementum and pulp) was similar in the B-treated and control rabbits. Micro CT evaluation revealed greater alveolar bone mineral density in B10 and B30 groups than in controls. Alveolar bone density of the B50 group was not different than the controls. Although the B treatments did not affect teeth structure, strength, mineral density and micro-hardness, increasing B intake altered the mineral composition of teeth, and, in moderate amounts, had beneficial effects on surrounding alveolar bone.

Introduction

Boron (B) has been shown to have an osteogenic effect and thus can influence bone, especially trabecular and alveolar type, bone growth and maintenance [1,2]. We have demonstrated that B, especially at 1 and 10 ng/mL concentrations compared to a control not treated with B, increased mineralized nodules and regulated mineralized tissue-associated gene expressions in cultured osteoblastic cells [3]; increased mRNA expressions including collagen type I (COL I), osteopontin (OPN), bone sialoprotein (BSP), osteocalcin (OCN) and runX2. In addition, we also found increased BMP-4, -6 and -7 protein levels with B treatment [3]. Ying et al. [4] subsequently reported that 10 and 100 ng B/mL culture solution stimulated osteogenic differentiation-related genes (COL I, OCN, BMP-7, alkaline phosphatase; ALP) during the proliferation and

* Corresponding author at: Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya 42079, Turkey. Tel.: +90 332 2231221; fax: +90 332 2410062.
E-mail addresses: sshakki@selcuk.edu.tr, sshakki@yahoo.com (S.S. Hakki).

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differentiation phase of human bone marrow stromal cells. Based on these in vitro studies, it was suggested that B could promote osteogenic capacity in the bio-engineering of bone [4]. Recently, Tasli et al. [5] found that human tooth germ stem cells treated with sodium pentaborate pentahydrate had the highest ALP activity and expression of osteo- and odontogenic-related genes and proteins, and increased in vitro odontogenic and osteogenic differentiation capacity of the cells [5]. Boron-containing scaffolds/biomaterials or boron doped films may emerge as a new generation of biomaterial for bone regeneration because B in these substances enhanced the adhesion, growth, and osteogenic differentiation of bone-derived cells [6–8], and bone formation in the intramedullary canal of rat tibiae [9].

Numerous reports indicate that B beneficially influences the formation and maintenance of cartilage and bone [1,10–20]. However, whether B affects teeth that contain the unique biomaterials enamel, dentin and cementum, remain unclear. Boron is present in dental enamel of primary and permanent human [21] and rat [22] teeth. Some early studies found that B did not markedly affect the incidence of dental caries [23,24]. Haro-Durand et al. [25] used the continuously erupting incisor of the rat to investigate the effect of B deficiency on the thickness and chemical structure of dental enamel. Compared to rats fed 3 mg B/kg diet, rats fed a B-deficient diet (0.07 mg B/kg) for 14 days exhibited a reduction in enamel thickness but no significant alterations in developing dental enamel mineralization.

In our previous report [26], we found that an increased boron intake improved strength and altered mineral composition of bone (femur and tibia) of rabbits with increased adiposity; femur maximum breaking force was highest in rabbits gavaged with 50 mg B/kg body weight/96 h. Tibia compression strength was highest in rabbits receiving a gavage of 30 or 50 mg B/kg body weight/96 h; these treatments also increased tibia phosphorus. A gavage of 10, 30, or 50 mg B/kg body weight/96 h increased tibia calcium and magnesium concentrations, The 30 mg treatment significantly increased calcium, phosphorus and magnesium concentrations in the femur.

Increased adiposity might act as a stressor that exacerbates signs of boron deprivation and thus heighten the response to boron supplementation. Numerous studies have shown that obesity is associated with chronic low-grade inflammation characterized by abnormal cytokine production and increased circulating C-reactive protein (CRP) [27]. Low-grade Inflammation stimulates osteoclastic bone resorption leading to bone loss [28]. Boron in the form commonly found in fruits and vegetables (calcium fructoborate) has been found to ameliorate low-grade inflammation indicated by elevated levels of serum CRP [29,30]. These findings suggest that increased adiposity may exacerbate diminished bone health exhibited by boron-deprived animals. Thus, the objective of the study presented in the following was to determine whether dietary B affects the strength, histological structure, mineral density and mineral composition of teeth, and the mineral density of alveolar bone in rabbits with increased adiposity induced by a high-energy diet.

Materials and methods

Experimental procedure

Sixty female New Zealand White rabbits, aged 8 months were housed in groups of five in cages located in a room maintained at 20–23 °C and with an automatic 12 h light and dark cycle. They were fed a low-energy diet (only alfalfa hay containing 5.1 MJ/kg metabolic energy) for four weeks then randomly divided into five groups with about equal mean body weight. One group of 15 (control 1) was fed a low-energy diet (alfalfa hay containing 57.5 mg B/kg) to determine whether the other four groups fed a high energy diet based on grains and containing 11.76 MJ/kg (Diet CP 5701, CP Turkey) showed increased adiposity [31]. The high energy diet contained 3.88 mg B/kg. The protein contents were 16% and 18% and the calcium contents were 1.9% and between 1 and 2% for the low-energy and high-energy diets, respectively. Three groups of rabbits fed the high energy diet were orally gavaged with a 1% borax solution (boron decahydrate, $Na_2B_4O_7{\cdot}10\ H_2O$) every 96 h (4 days) that provided 10 (B10, $n = 15$), 30 (B30, $n = 10$), or 50 (B50, $n = 10$) mg/kg body weight. The other group of 10 rabbits (control 2) fed the high energy diet received a sham gavage of water. A limited amount of alfalfa hay for microbial balance was provided weekly to the rabbits fed the high energy diet. Diet consumption and body weights were determined monthly. Within 10 min following oral acepromacine administration, blood was drawn from the ear central arteria into heparin-coated tubes after seven months of treatment, then the rabbits were euthanized by ether overdose to obtain maxillary incisor teeth with surrounding alveolar bone. Samples were stored at −80 °C until analysis [31]. The experiment was approved by the Committee on Use of Animals in Research of Selcuk University, Faculty of Veterinary Medicine.

Tooth strength determination

After removal of alveolar bone and soft tissues, 2 mm slices were cut a standardized distance (7 mm) from the incisal edge of the teeth (Fig. 1). This procedure resulted in each specimen coming from the same relative location (proportionally). The slices were obtained by using a low-speed diamond blade watering saw (Isomet 1000 Precision Saw, Buehler, USA) under continuous irrigation. Reduced-platen compression (RPC,) in N/mm was determined by using a universal testing machine (TSTM 02500, Elista Corp., Istanbul, Turkey). Each specimen, which was kept wet at room temperature throughout the measurement, was loaded between flat parallel platens and compressed. Quasi-static compression was applied at a displacement rate of 3 mm/min [26].

Histological evaluation

For creating undecalcified grinding sections, teeth were fixed by immersion in 4% buffered formaldehyde at room temperature for at least 3 days, and then embedded in plastic. After dehydration with ascending ethanol and 2-hydroxymethylmethacrylate (GMA) concentrations, an ultraviolet light-activated polymethylmethacrylate (PMMA, Technovit® 7200, VLC, Heraeus Kulzer, Wehrheim, Germany) was used as an infiltration medium. A 3-day immersion in a combination of GMA and embedding medium was followed by 100% embedding medium. After penetration of the whole specimen, Technovit® was photopolymerized in three steps by using high power UV-light in the last stage until full hardening. After hardening, parallel sections of 100–200 μm thickness were cut by using a micro-saw (Exakt, Norderstedt, Germany). The desired final thickness of 10–20 μm needed for light microscopic analysis was obtained using a micro-grinding system (Exakt). Toluidine blue stain was applied without removal of the plastic and sections analyzed under a light microscope (Axioskop 2, Zeiss, Jena, Germany).

Tooth mineral determination

Teeth were placed in a 70 °C incubator (Ventisell, Italy) for two days, which resulted in a constant weight. After recording the dry weights, the teeth were digested by placing them in a solution composed of 5 mL concentrated nitric acid (HNO_3), 3 mL 70% perchloric acid ($HClO_4$) and 2 mL 35% hydrogen peroxide (H_2O_2), and