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Nutrition

# Boron supplementation improves bone health of non-obese diabetic mice

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ABSTRACT

Diabetes Mellitus is a condition that predisposes a higher risk for the development of osteoporosis. The objective of this study was to investigate the influence of boron supplementation on bone microstructure and strength in control and non-obese diabetic mice for 30 days. The animals were supplemented with 40 μg/0,5 ml of boron solution and controls received 0,5 ml of distilled water daily. We evaluated the biochemical parameters: total calcium, phosphorus, magnesium and boron; bone analysis: bone computed microtomography, and biomechanical assay with a three point test on the femur. This study consisted of 28 animals divided into four groups: Group water control - Ctrl (n = 10), Group boron control - Ctrl±B (n = 8), Group diabetic water - Diab (n = 5) and Group diabetic boron - Diab±B (n = 5). The results showed that cortical bone volume and the trabecular bone volume fraction were higher for Diab±B and Ctrl±B compared to the Diab and Ctrl groups ($p \leq 0,05$). The trabecular specific bone surface was greater for the Diab±B group, and the trabecular thickness and structure model index had the worst values for the Diab group. The boron serum concentrations were higher for the Diab±B group compared to non-supplemented groups. The magnesium concentration was lower for Diab and Diab±B compared with controls. The biomechanical test on the femur revealed maintenance of parameters of the bone strength in animals Diab±B compared to the Diab group and controls. The results suggest that boron supplementation improves parameters related to bone strength and microstructure of cortical and trabecular bone in diabetic animals and the controls that were supplemented.

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## 1. Introduction

The role of nutrition in the development of the bone tissue has been the focus of many studies, which investigate dietary components required for the maintenance of healthy bone functions, as well as there proper development. Nutrients such as calcium, phosphorus, magnesium, vitamin D, fluoride, zinc, copper and boron are known to promote normal development of bone functions, ensuring the gain of mass and strength. Inadequate consumption of these nutrients or changes in metabolism, can cause an increase of excretion and absorption losses due to the presence of the disease, can promote losses in bone structure, and consequently the development of related bone diseases like osteoporosis [1].

Osteoporosis is a systemic skeletal disease characterized by an increase in susceptibility to fracture. Most cases are associated with post menopause or aging, but it can also develop as a result of any pathological situation [2]. Diabetes Mellitus (DM) is a condition that predisposes a higher risk for the development of osteoporosis [3].

A chronic condition of DM can negatively affect several parts of the body, such as the bones, muscles, retina, kidneys, and the cardiovascular system. The effects of this disease in the bone cells are very complex, and many studies have been conducted in order to explore the exact mechanisms in which DM induces osteoporosis and, consequently the increase in the rate of bone fractures [4].

*Abbreviations:* DM, Diabetes Mellitus; NOD, non-obese diabetic mice; Ctrl, non-diabetic animals; Ctrl±B, non-diabetic animals supplemented; Diab, diabetic animals non-supplemented; Diab±B, diabetic animals supplemented; Fcfrp/USP, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo; ICP-MS, inductively coupled plasma mass spectrometry; μCT, bone computed microtomography; BV, bone volume; BV/TV, bone volume fraction; Ct.Th, cortical thickness; BS/BV, specific bone surface; SMI, structure model index; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D, connectivity density.

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Hyperglycemia deregulates the proper functioning of osteoblastic cells, and negatively regulates the function of osteoclastic cells, related to bone resorption. This condition can facilitate the process of the development of osteoporosis and cause dysfunction and failure in different organs [5–7].

The main complication of insulin deficiency is related to the bone formation. Experimental models of type 1 DM found a decrease in the number of osteoblasts and bone turnover occurs from a reduction in mineral content [5,8].

In diabetic patients decreases in levels of minerals, which exert important functions in the bone metabolism are common. Boron is an essential element for plants, but its role in the human body as well as in other mammals still needs to be further addressed. Studies are suggesting that this mineral is essential for maintaining bone health and has a vital role in embryogenesis, bone growth, immunity, and psychomotor functions [9]. Gorustovich et al. [10], through an experimental study with mice, reported that boron is beneficial for bone growth and maintenance. Hakki et al. [11], concluded that the deprivation of this mineral can affect bone growth by inhibiting its formation. Also, this mineral is linked to the formation of hormone steroids, and influences the metabolism of micronutrients such as calcium, magnesium and vitamin D. Therefore, it can be involved in preventing losses in calcium and bone demineralization [12,13].

Thus supplementation with minerals, as boron, is essential to bone metabolism and can be an important factor in preserving bone mass.

The available studies are still limited in their evaluation of the real participation of boron in bone metabolism and the effects of supplementation. Consequently, it has become necessary to obtain more results with regards to bone metabolism markers in the assessment of bone microarchitecture. Hence, the aim of this study was to investigate the influence of boron supplementation on bone microstructure and strength in control and non-obese diabetic mice.

## 2. Materials and methods

### 2.1. Animals

For this study, we used female non-obese diabetic (NOD) mice weighing between 18 and 20 g and 16 weeks old, from the Center for the Breeding of Special Mice, at the Faculty of Medicine of Ribeirão Preto (FMRP) –University of São Paulo (USP). The diabetes model used a model system. The development of diabetes in this animal model are similar to that observed in humans, since these mice exhibit spontaneous autoimmunity which leads to progressive destruction of insulin-producing pancreatic cells. This process of cell destruction begins at 3 to 4 weeks and extends to 4 to 6 months of age. The onset of diabetes is characterized by moderate glycosuria and blood glucose greater than 250 mg/dL. The glycosuria and hyperglycemia became progressively more severe around the 34th week of weight loss; polydipsia and polyuria both occurred. Without treatment with exogenous insulin, the diabetic mice became severely hyperglycemic and ketosis, but they did not become ketoacidosis, and had a survival rate of 3–4 weeks after the first detection of glycosuria.

The animals were kept in the USP Animal Testing Laboratory, staying there for about 16 weeks for observation, with follow-ups from the first day of accommodation with water and feed ad libitum. There was no therapeutic intervention to control glycemic animals because the objective was to maintain the high glucose to induce the bone loss process. Diabetic animals were a group selected from the moment their blood glucose levels were equal to or greater than 250 mg/dL. The animals that showed no changes in their blood sugar levels after 16 weeks, were selected for the control groups. After the development of diabetes these animals were divided into four groups: Water Control – Ctrl (n = 10): animals that received 0.5 ml/day of distilled water, Boron Control – Ctrl±B (n = 8): animals that received 40 μg/0.5 ml of boron, Diabetic Water – Diab (n = 5): diabetic animals that received 0.5 ml of distilled water, and Diabetic Boron – Diab±B (n = 5): diabetic animals that received 40 μg/0.5 ml of boron. Based on literature reports about NOD mice, we selected a sample of 40 animals. After 16 weeks half of the sample developed monitoring glucose levels above 250 mg/dL. Diabetic animals (n = 10) were allocated to each group, but due to high blood sugar levels, only five animals survived until the end of the experiment. The sample may not have the paired n, but it is in line with the minimum established n sample size calculation. The administration of distilled water and boron solution was held once a day by gavage for 30 days.

### 2.2. Experiment

The animals were kept according to the recommendations of the Commission of ethics in the use of Animals, according to the precepts of the law 11.794/2008 Resolution n° 879/2008 of the FMRP/USP. The project was approved by the Ethics Committee on Animal Research, FMRP registration number 074/2013.

For this study a boron solution was prepared with 40 μg of boron in 0.5 ml of solution using boron chelate H 5% (chelating glycine). The boron solution was prepared at the Laboratory of the Pharmacy Course at the Ribeirão Preto Medical School/USP. Chelated minerals, like boron, have the advantage of being better bioavailable (90% absorption), without interfering with the absorption of other nutrients and without having side effects.

The diet given to the animals (Nuvital Nutrients S/A) contained in its composition ground whole corn, soybean meal, wheat bran, calcium carbonate, dicalcium phosphate, sodium chloride, mineral and vitamin premix amino acids (lysine and methionine). The minerals present in the diet were: iron (50 mg), boron (0.5 mg), zinc (60 mg), copper (10 mg), iodine (2 mg), manganese (60 mg), selenium (0.05 mg), cobalt (1,50 mg) and for the supplemented animals 40 μg of boron was also added, which were administrated by gavage.

Throughout the experiment a bi-weekly monitoring of the glucose levels and the weight of control and diabetic animals were conducted. The blood glucose was measured through the use of a blood-glucose Monitor Accu-Chek Advantage II (Roche Diagnostics, São Paulo, Brazil) with the utilization of the test strip and weight on the scale Filizola ® Star precision (São Paulo, Penha, Brazil) of 0.5 g.

After the trial period of 30 days the animals, were euthanized by decapitation for the 0.5 ml of blood collection. The blood sample was collected in a tube Eppendorf® of 2 ml and centrifuged at 2.500 rpm, for twenty minutes. The serum used to determine the concentrations of total calcium, phosphorus, magnesium, and boron were obtained from the Laboratory of Toxicology and Essentiality of Metals – Faculty of Pharmaceutical Sciences of Ribeirão Preto (FCFRP). Blood samples were manipulated immediately after collection in a sterile environment, with sterile tips so that there was no contamination of the sample. The realization of mineral concentrations was frozen, as the serum was stored at −80 °C. The laboratory used for the concentrations has a sterile environment and is suitable for contamination of the sample not to occur. The determination of the concentration of minerals was performed according to Batista et al. [14], by an Inductively Coupled Plasma Mass Spectrometry (ICP-MS), fitted with a dynamic reaction cell (DRC) (Perkin Elmer Sciex Norwalk, CT USA) and operated with high purity argon (99.999% Praxaair, Brazil). Samples were diluted to the ratio 1:50 with a solution containing Triton X-100 0.01%

(v/v), HNO3 0.05% (v/v), and 10 mg/L-1 rhodium (Rh) as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 μg/L.

### 2.3. Bone computed microtomography

This method evaluated the left femur, as suggested by De Paula et al. [15]. The left femur of the animals was preserved in 70% ethanol, for 21 days after the end of the experiment. During this period the bones remained in this period in alcohol to facilitate removal of soft tissue in order to carry out the analyses. Later the bones were cleaned, from the withdrawal of all the muscle that surrounded them and stored again in 70% alcohol until the completion of the analyses, which were held at the Faculty of Dentistry of the University of São Paulo in Ribeirão Preto.

The bone samples were scanned by a high resolution 1172 model Microtomography, brand SkyScan (Belgium, Bruker Corporation), consisting of a tube of a Micro Focus x-ray high voltage source (100 kV), each sample had precision handling by a CCD camera detector with 11Mp connected to a computer data acquisition system and controlled by a cluster of networked computers with software for the reconstruction, visualization and quantification of 2D and 3D images (software CT Analyser v.1.13).

The scan was performed at a low resolution, a 55k Vp energy level, and intensity of 145 μA. The unit was calibrated weekly with Phantom supplied by the manufacturer. Two regions of interest were delimited, one at the distal femoral metaphysis, which mainly contains trabecular bone, and the other at the mid-diaphysis, which mainly includes cortical bone. The reconstruction of the metaphysis was selected manually starting at approximately 0.6 mm from the distal growth plate for an extension of 2.5 mm (Fig. 1). The reconstruction of the diaphysis was defined by a 1 mm region starting at the third throcanter. Cortical and trabecular bone were isolated using manually drawn contouring. CTAn software (Bruker-micro CT), version 2.2.1, was used for the determination of the optimal threshold from the image histograms and was set to exclude soft tissue, but did include poorly mineralized bone. The same threshold was used in all of the samples but differed between trabecular and cortical bone. The parameters analyzed for trabecular, and cortical bone are in Table 1. All bone-morphometric measurements and nomenclature are in agreement with recommendations of the ASBMR [16].

### 2.4. Biomechanical assay at three point test in femur

The right femur of the animals was used for the three point biomechanical assay test done through an analysis performed by the material resistance equipment (Universal Testing Machine) of the Bioengineering Laboratory at the FMRP (USP).

The load on the bone was applied in the central region in the anterior-posterior direction, through an accessory attached to the load cell. The bone was placed next to the device so that the extremety of the femur was put against the supports, and the sides would aim at the base of the machine (Fig. 2). A dial indicator gauge Mitutoyo®, with an accuracy of 0.01 mm was used for the collection of the deflection intervals during all mechanical testing. The deflection values were measured every 0.05 mm at the same time as the measurement of the value of the applied load on the bridge of the extensometer.

The vertical load was applied until the bone fractured. Load and deformation curves were recorded in real time. The Tesc® software (EMIC, São José dos Pinhais, PR, Brazil) was used to obtain ultimate load, stiffness, and resilience.

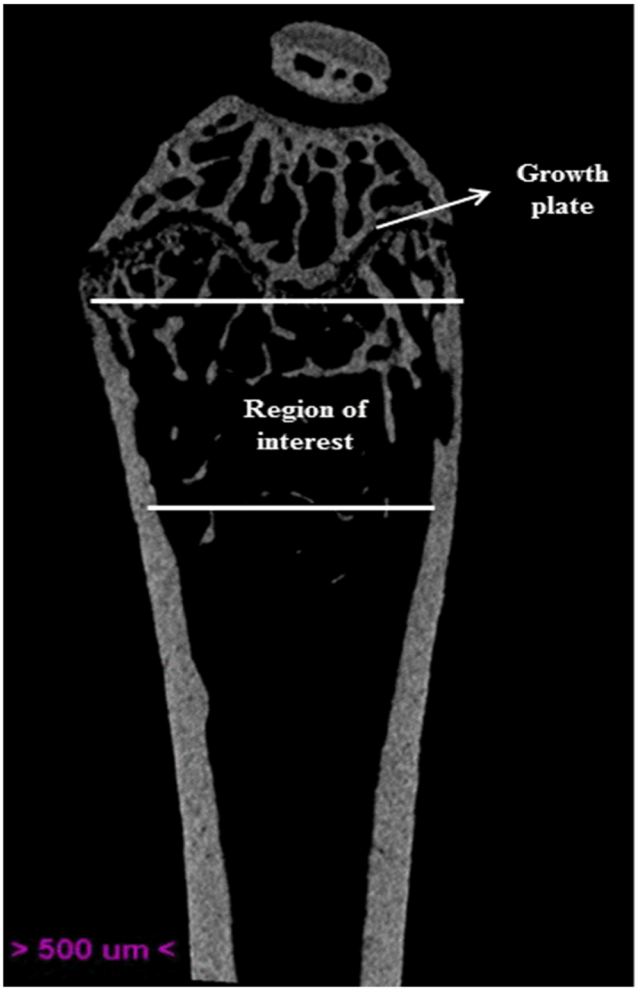


**Fig. 1.** Reconstruction of the metaphysis (femur), starting at approximately 0.6 mm from the distal growth plate for an extension of 2.5 mm.

### 2.5. Statistic analysis

Exploratory analysis of data for an overview of the features of the variables were obtained, describing them through tables with descriptive measures. Median, minimum, and maximum statistics were calculated with a reliability rate of 95%. Statistical tests were performed to test the equality between groups.

We used the SPSS Statistics software version 22.0 for statistical analysis. For a comparison between all groups, we used the two-way nonparametric ANOVA test with Tukey as a post hoc test. The statistical significance was defined as $p \leq 0.05$.

## 3. Results

The measurements of weight and blood glucose of groups Ctrl, Ctrl±B, Diab and Diab±B, are presented in Table 2. The analysis revealed that there was no difference in body weight of diabetic animals and controls in the first (one day before the start of the experiment) and second measurements (fifteen days afther the first measurement), but at the end of the experiment there was significant weight loss in the diabetic animals (Diab and Diab±B) compared to the Ctrl group ($p < 0.05$).

The glycemic values revealed that Diab and Diab±B groups had glucose values significantly higher compared to Ctrl and Ctrl±B at the first, second, and third measurements showing evidence of diabetes.

**Table 1**
Definition and description of outcomes for cortical and trabecular bone morphology.

| BONE PARAMETERS | VARIABLE DESCRIPTION | STANDARD UNIT |
|---|---|---|
| Trabecular Bone | | |
| BV (bone volume) | Volume of the region segmented as bone | $mm^3$ |
| BV/TV (bone volume fraction) | Ratio of the segmented bone volume to the total volume of the region of interest | % |
| BS/BV (specific bone surface) | Ratio of the segmented bone volume to the total volume of the region of interest | $mm^3$ |
| SMI (structure model index) | An indicator of the stucture of trabeculae; SMI will be 0 for parallel plates and 3 for cylindrical rods | – |
| Tb.Th (trabecular thickness) | Mean thickness of trabeculae, assessed using direct 3D methods | mm |
| Tb.N (trabecular number) | Measure of the average number of trabeculae per unit length | 1/mm |
| Tb.Sp (trabecular separation) | Mean distance between trabeculae, assessed using direct 3D methods | mm |
| Conn.D (connectivity density) | A measure of the degree of connectivity of trabecular normalized by total volume | $1/mm^3$ |
| Cortical Bone | | |
| Ct.Th | Average cortical thickness | mm |
| Ct.Ar/Tt.Ar | Cortical area fraction | % |
| Ct.V | Cortical volume | $mm^2$ |

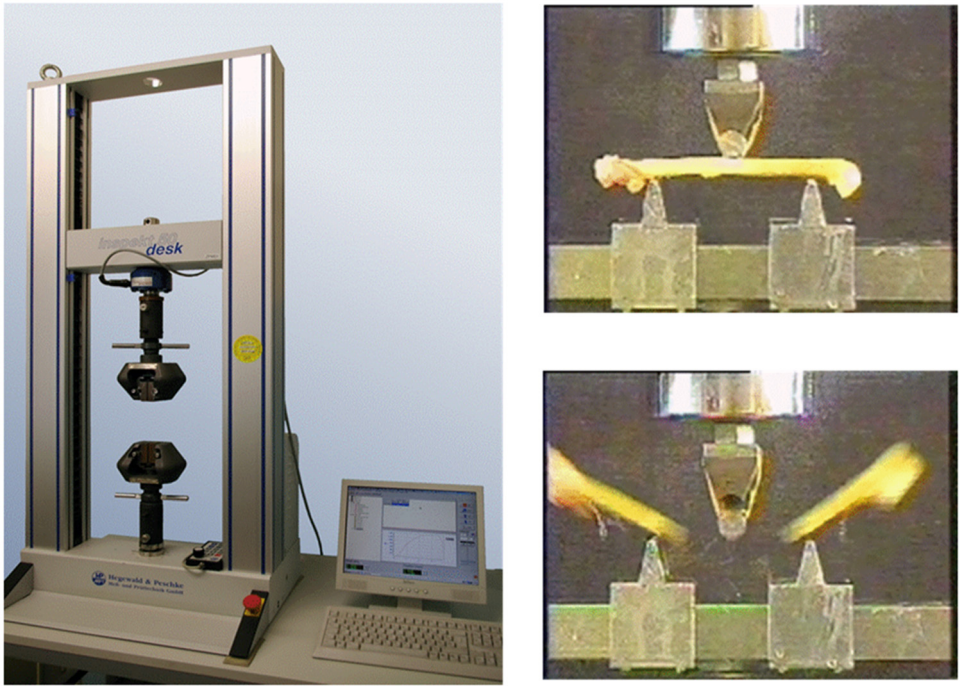

**Fig. 2.** Universal Machine strength and biomechanical simulation test three-point bending. The picture illustrates the correct positioning of the bone (colon of the femur and tibia are placed up against a machine support with a dedicated front face to the base of the machine) to the test.

**Table 2**
The effect of boron on body weight and blood glucose in control and diabetic mice supplemented and non-supplemented with boron during the experimental period of 30 days.

| | Crtl (n = 10) | Crtl±B (n = 8) | Diab (n = 5) | Diab±B (n = 5) |
|---|---|---|---|---|
| Weight (g) | | | | |
| Day 01 | 26 [24–26] | 25 [22–28] | 26 [26–28] | 24 [24–26] |
| Day 15 | 24 [24–26] | 25 [24–28] | 24 [22–24] | 22 [20–26] |
| Day 30 | $26^{a}$ [24–26] | $24^{ab}$ [22–28] | $22^{b}$ [20–24] | $20^{b}$ [16–24] |
| Blood Glucose (mg/dl) | | | | |
| Day 01 | $113.5^{a}$ [96–128] | $108^{a}$ [98–117] | $360^{b}$ [308–500] | $390^{b}$ [320–550] |
| Day 15 | $114.5^{a}$ [94–154] | $118.5^{a}$ [111–130] | $585^{b}$ [440–593] | $551^{b}$ [545–593] |
| Day 30 | $109.5^{a}$ [98–161] | $120^{a}$ [101–140] | $556^{b}$ [403–560] | $580^{b}$ [550–593] |

Note: Two way nonparametric ANOVA. The values are expressed in median, minimum and maximum. Different letters (a, b, ab) $p \leq 0{,}05$. Crtl = control animal non-supplemented; Crtl±B = control animal supplemented; Diab = diabetic animal non-supplemented; Diab±B = diabetic animal supplemented.

The results of a determination of minerals in the serum of diabetic animals and controls are in Table 3. The determination of boron showed that the Diab±B group presented greater values than the Ctrl group. There was no difference for levels of calcium and phosphorus. Magnesium was higher for the Ctrl and Ctrl±B groups compared with Diab and Diab±B ($p < 0.05$).

The results of the three point biomechanical assay test on the femur of diabetics and controls animals are in Table 4. The Diab group showed less strength than the Ctrl, Ctrl±B and Diab±B group ($p < 0.05$). The stiffness and energy absorption in the Diab group were lower than Ctrl, and the Diab±B group maintained the values similar to the control groups.

The results of the microtomography of the cortical bone are in Table 5. Cortical bone from Ctrl±B and Diab±B groups displayed more cortical volume (Ct.V) than Ctrl and Diab groups. Animals of the Ctrl±B group showed higher values of cortical area fraction

**Table 3**
The effect of boron on concentrations of calcium, phosphorus, boron and magnesium in the serum of control and diabetic mice supplemented and non-supplemented with boron during the experimental period of 30 days.

| | Crtl (n = 10) | Crtl±B (n = 8) | Diab (n = 5) | Diab±B (n = 5) |
|---|---|---|---|---|
| Boron (μg/l) | 81.5[a] [53.3–97.7] | 98.7[ab] [74.1–126.4] | 83.4[ab] [37.2–107.1] | 114.9[b] [93.3–160.8] |
| Calcium (mg/dl) | 25.0 [22.9–28.9] | 25.6 [18.7–32.5] | 24.3 [17.4–25.6] | 22.2 [18.1–28.3] |
| Phosphorus (mg/dl) | 17.2 [15.4–20.0] | 18.4 [13.8–21.9] | 16.1 [13.4–21.4] | 18.4 [13.5–23.1] |
| Magnesium (mg/dl) | 2.4[a] [2.2–2.8] | 2.6[a] [2.2–2.9] | 1.8[b] [1.8–1.9] | 2.1[b] [1.9–2.3] |

Note: Two way nonparametric ANOVA. The values are expressed in median, minimum and maximum. Different letters (a, b, ab) $p \leq 0,05$. Crtl = control animal non-supplemented; Ctrl±B = control animal supplemented; Diab = diabetic animal non-supplemented; Diab±B = diabetic animal supplemented.

**Table 4**
The effect of boron on bone strength parameters, by performing the biomechanical test of three-point bending on femurs of control and diabetic mice supplemented and non-supplemented with boron during the experimental period of 30 days.

| | Crtl (n = 10) | Crtl±B (n = 8) | Diab (n = 5) | Diab±B (n = 5) |
|---|---|---|---|---|
| Maximum Strengh (N) | 23.8[a] [2.03–24.7] | 21.8[a] [20.3–22.6] | 17.76[b] [14.8–19.1] | 21.9[a] [19.3–25.1] |
| Displacement (mm) | 0.4 [0.3–0.4] | 0.3 [0.2–0.5] | 0.3 [0.2–0.3] | 0.3 [0.3–0.3] |
| Stiffness (N/mm) | 109.0[a] [106.1–112.5] | 104.1[ab] [93.9–109.9] | 89.9[b] [64.4–99.6] | 100.9[ab] [93.2–112.7] |
| Energy Absorption (N.mm) | 7.8[a] [5.9–9.3] | 6.3[ab] [3.4–8.7] | 3.4[b] [1.8–4.5] | 5.3[ab] [4.1–9.8] |

Note. Two way nonparametric ANOVA. The values are expressed in median, minimum and maximum. Different letters (a, b, ab) $p \leq 0,05$. Crtl = control animal non-supplemented; Ctrl±B = control animal supplemented; Diab = diabetic animal non-supplemented; Diab±B = diabetic animal supplemented.

**Table 5**
The effect of boron on bone parameters computed microtomography of cortical bone on femurs of control and diabetic mice supplemented and non-supplemented with boron during the experimental period of 30 days.

| | Crtl (n = 10) | Crtl±B (n = 8) | Diab (n = 5) | Diab±B (n = 5) |
|---|---|---|---|---|
| Ct.V ($mm^2$) | 1.03[a] [1.02–1.06] | 9.02[b] [9.31–9.64] | 1.04[a] [1.03–1.10] | 9.61[b] [9.23–9.99] |
| Ct.Ar/Tt.Ar (%) | 40,6[a] [38.2–43.4] | 48.6[b] [44.1–57.9] | 37.4[ab] [35.9–43.1] | 42.5[b] [40.6–44.4] |
| Ct.Th (mm) | 1.96 [1.5–2.2] | 1.5 [1.4–1.9] | 1.7 [1.6–1.9] | 1.5 [1.6–1.7] |

Note: Two way nonparametric ANOVA. The values are expressed in median, minimum and maximum. Different letters (a, b, ab) $p \leq 0,05$. Crtl = control animal non-supplemented; Ctrl±B = control animal supplemented; Diab = diabetic animal non-supplemented; Diab±B = diabetic animal supplemented; BV = bone volume; BV/TV = bone volume fraction; Ct.Th = average cortical thickness.

(Ct. Ar/Tt.Ar) higher compared with the Ctrl and Diab ($p < 0.05$) and Diab±B showed values similar with Ctrl±B.

Table 6 presents the results of the analysis of the microtomography of the trabecular bone. Fig. 3 shows a representative image of the trabecular bone of each group of the study. The choice of the figures was based on the analysis of the qualitative variables of each animal and which profile was in common among diabetics and control groups. Therefore, a picture of an animal had been chosen that best represented the characteristics of the group. The bone volume fraction was higher for the Ctrl±B group than for the Ctrl, Diab and Diab±B. The specific bone surface (BS/BV) was greater in DB group compared to Ctrl. The trabecular thickness for the Ctrl and Ctrl±B groups presented values significantly higher than the Diab group ($p < 0.05$). There were no significant differences between the groups for the parameters of bone volume, SMI, trabecular number, trabecular separation, or connectivity density.

It is noted that the analysis parameters related to trabecular and cortical bone have a significant overall effect of boron in the diabetic state of animals and with boron supplementation for parameters Ct.V, Ct.Ar/Tt.Ar, BV/TV, BS/BV, SMI and Tb.Th ($p < 0.001$). The same can be observed for the three points bending test for parameters of maximum strengh, stiffness, and energy absorption ($p < 0.001$). Regarding the concentration of minerals and diabetic state, there was no significant overall effect of boron between groups ($p = 0.582$).

## 4. Discussion

The present study investigated the possible changes in bone metabolism between control and diabetic animals supplemented or not with boron through tests that enabled the evaluation of bone microstructure, strength and concentration of minerals related to bone health.

According to Olofsson [17], the bone complications arising from chronic hyperglycemia caused by diabetes show that in diabetic patients there is also the risk of deficiencies in vitamins and minerals. The literature shows that these patients have increases in urinary excretion of some minerals such as calcium, phosphorus and magnesium, which directly relate to bone health.

The choice of this nutrient was based on studies that suggest the essentiality of this mineral in bone structure and mineralization, as well as the influence it exerts on the metabolism of some bone minerals matrix components [13,1]. The concentration of boron that was given in serum form to the control and diabetic animals showed that the Diab±B group presented boron concentrations significantly higher compared to the Ctrl group, suggesting that this increase could be due to supplementation. The analysis of the boron concentration in plasma or serum may be indicative of the nutritional status of the boron. A study in humans showed 1.5 times higher plasma levels in response to the increase of this mineral in the diet [18]. Boron supplementation for 60 days in perimenopause women showed an increase in their plasma levels [19,20].

In this study, we did not observe a significant difference in calcium levels in the serum of animals in both groups. Studies conducted by Derivan; Volpe [19] showed that boron supplementation reduced urinary excretion of calcium and increased plasma concentrations of this mineral in humans. However, some studies have shown that boron does not significantly affect the levels of calcium and phosphorus, even in cases of deprivation and in this sense where the bone changes relate to a lower bone turnover leading to insufficient strength and loss of bone structure in rodents [11,18,21].

The levels of magnesium in animals Diab and Diab±B showed a significant reduction compared to the Ctrl and Ctrl±B groups, possibly because of the diabetes. In diabetic animals, boron supplementation was ineffective for increasing magnesium levels,

**Table 6**
The effect of boron on bone parameters computed microtomography of trabecular bone on femurs of control and diabetic mice supplemented and non-supplemented with boron during the experimental period of 30 days.

| | Crtl (n = 10) | Crtl±B (n = 8) | Diab (n = 5) | Diab±B (n = 5) |
|---|---|---|---|---|
| BV ($mm^3$) | 0.03 [0.02–0.06] | 0.05 [0.03–0.10] | 0.03 [0.01–0.05] | 0.04 [0.03–0.06] |
| BV/TV(%) | 2.13[a] [1.93–2.28] | 4.58[b] [3.20–6.74] | 1.91[a] [0.98–2.69] | 2.3[a] [2.3–2.7] |
| BS/BV ($mm^3$) | 84.6[a] [62.6–92.9] | 86.7[ab] [80.2–93.2] | 94.7[ab] [77.6–99.3] | 95.8[b] [89.4–111.6] |
| SMI | 2.70 [2.60–2.80][a] | 2.52 [2.40–2.80][ab] | 2.34 [2.10–2.50][b] | 2.5 [2.1–2.8][ab] |
| Tb.Th (mm) | 0.04[a] [0.04–0.05] | 0.05[a] [0.04–0.06] | 0.03[b] [0.03–0.04] | 0.04[ab] [0.03–0.05] |
| Tb.N (1/mm) | 0.49 [0.37–0.68] | 0.59 [0.43–0.99] | 0.51 [0.21–0.70] | 0.54 [0.35–0.70] |
| Tb.Sp (mm) | 0.43 [0.40–0.52] | 0.41 [0.37–0.46] | 0.46 [0.38–0.66] | 0.54 [0.46–0.58] |
| Conn. D. ($1/mm^3$) | 43.2 [31.3–59.7] | 46.2 [34.4–64.6] | 30.4 [19.2–67.5] | 46.7 [30.9–93.1] |

Note: Two way nonparametric ANOVA. The values are expressed in median, minimum and maximum. Different letters (a, b, ab) $p \leq 0,05$. Crtl = control animal non-supplemented; Ctrl±B = control animal supplemented; Diab = diabetic animal non-supplemented; Diab±B = diabetic animal supplemented; BV = bone volume; BV/TV = bone volume fraction; BS/BV = specific bone surface; SMI = structure model index; Tb.Th = trabecular thickness; Tb.N = trabecular number; Tb.Sp = trabecular separation; Conn.D.= connectivity density.

possibly in response to diabetes that causes an increase in the urinary excretion of some minerals. Derivan; Volpe [19] studied the relation between magnesium and boron supplementation and the results showed the opposite because boron increases magnesium concentrations in plasma in animals supplemented and also attenuated urinary excretion. It must be pointed out that the animals mentioned in the study of Derivan; Volpe were not diabetic.

The results of the biomechanical test performed on the femur showed that diabetic supplemented animals presented higher bone strength compared to the Diab group and observed the maintenance parameters of stiffness and energy absorption for diabetic supplemented animals compared to controls. It was observed that diabetic animals have susceptibility to present loss of bone strength, which can be ameliorated by supplementation with boron and even animals that are not ill may benefit from supplementation.

Armstrong et al. [22] evaluated the boron supplementation ability to increase bone strength in femurs of pigs. The results showed that animals supplemented with boron had higher bone strength compared to the animals that received a non-supplemented diet. Sheng et al. [23] evaluated the effects of boron supplementation in femurs and tibias of rats and observed no increase in strength parameters in the supplemented animals however it was found that boron deprivation impairs bone strength parameters. Thus, a higher intake of boron for animals deprived of this mineral can result in improvements in bone strength.

Hakki et al. [11] found a similar result in femurs of rabbits submitted to a biomechanical test after boron supplementation, and Ghanizadeh et al. [1] verified increases in maximum strength and resistance of rat femurs supplemented with boron and fluorine, sustaining the results of this study.

The analysis of bone computed microtomography was done to evaluate the bone microstructure, an essential study to assess the action of boron on the trabecular and cortical bone in the femur. Studies suggest that boron action is directed toward the improvement of the trabecular bone [13,24,25]. In this study, in addition to the improvements in the parameters for the trabecular bone, it can also be noted that there was a significant improvement in the parameters of the cortical bone of supplemented diabetic and control animals compared to those not supplemented, groups (Diab and Ctrl). The supplemented animals showed a significant gain in bone volume compared to the Diab and Ctrl groups and the Diab±B animals presented values of BV/TV similar to controls. The same was not observed in the Diab group. While analyzing the effects of diabetes and supplementation with boron, it was observed that diabetics animals are susceptible to presenting a change in the microstructure of the bone, leading to bone fragility and consequently increasing the probablility of a fracture to occur. Supplements with boron given to diabetic animals were able to improve the change in bone microarchitecture in comparison to the diabetic animals that received no supplementation. In addition, control animals that received the supplementation also showed improved bone health compared to the unsupplemented group. The gain in bone volume and maintenance of BV/TV is similar to the study conducted by Nielsen and Stoecker [24] and with the

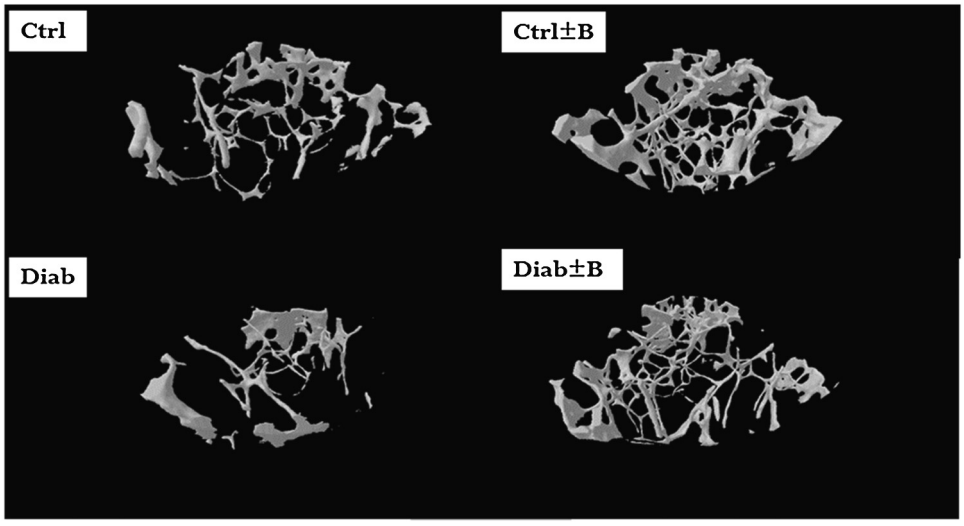


**Fig. 3.** μCT of trabecular bone, in the secondary spongy to 0.6 mm proximal growth plate up to 2.5 mm proximal of control animals non-suppplemented (Ctrl), supplemented (Ctrl±B) and diabetic animals non-supplemented (Diab) and supplemented (Diab±B).

results of the biomechanical, which showed that the femur of diabetic supplemented animals presented higher bone strength. With respect to the trabecular bone, we observed higher maintenance of parameter values of BV/TV and Tb. Th in control and diabetic supplemented animals compared to the controls, respectivaly. Also, the animals of the Diab±B group showed higher values of BS/BV compared to the Ctrl group. Similar results to the trabecular bone were observed in the study of Nielsen and Stoecker [24], by evaluating the influence of deprivation of this mineral. Microtomography analysis performed by these researchers at the fourth vertebra of the lumbar spine indicated that boron deprivation led to a reduction in the fraction of bone volume, reduced trabecular thickness, SMI and, increased the trabecular separation. This study shows that higher boron intakes improve bone microarchitecture.

From the results obtained, we concluded that boron supplementation improved parameters related to bone strength and microstructure of cortical and trabecular bone in supplemented diabetic and controlled animals. The bone abnormalities caused by diabetes can be reduced by the supplementation of boron and even in non disease conditions supplementation may be beneficial to bone health. However, more studies determining the relationship between boron and bone health in animals and humans are definitely needed.

## Conflict of interest

None.

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