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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.

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%