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Effects of fructose-induced metabolic syndrome on rat skeletal cells and tissue, and their responses to metformin treatment

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ABSTRACT

Aims: Deleterious effects of metabolic syndrome (MS) on bone are still controversial. In this study we evaluated the effects of a fructose-induced MS, and/or an oral treatment with metformin on the osteogenic potential of bone marrow mesenchymal stromal cells (MSC), as well as on bone formation and architecture.

Methods: 32 male 8 week-old Wistar rats were assigned to four groups: control (C), control plus oral metformin (CM), rats receiving 10% fructose in drinking water (FRD), and FRD plus metformin (FRDM). Samples were collected to measure blood parameters, and to perform pQCT analysis and static and dynamic histomorphometry. MSC were isolated to determine their osteogenic potential.

Results: Metformin improved blood parameters in FRDM rats. pQCT and static and dynamic histomorphometry showed no significant differences in trabecular and cortical bone parameters among groups. FRD reduced TRAP expression and osteocyte density in trabecular bone and metformin only normalized osteocyte density. FRD decreased the osteogenic potential of MSC and metformin administration could revert some of these parameters.

Conclusions: FRD-induced MS shows reduction in MSC osteogenic potential, in osteocyte density and in TRAP activity. Oral metformin treatment was able to prevent trabecular osteocyte loss and the reduction in extracellular mineralization induced by FRD-induced MS.

1. Introduction

Metabolic syndrome (MS) is defined as the group of risk factors that predispose individuals to the development of type 2 Diabetes and cardiovascular disease [1]. This syndrome was first described by Reaven in 1988 and initially named "X Syndrome" [2]. Since then, MS has been subjected to several revisions with the intention of providing a more suitable definition worldwide. Following this objective, in 2009 a harmonized definition of MS was published [3]. According to this joint statement, a diagnosis of MS is made when at least 3 of the 5 following risk factors are present: central obesity,

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elevated triglycerides, decreased HDL-cholesterol, elevated blood pressure, and elevated fasting glucose, with the inclusion of patients taking medication to manage hypertriglyceridemia, low HDL-cholesterol, hypertension and/or hyperglycemia [3].

The negative effect of Diabetes mellitus (DM) on the skeleton and its association with bone fractures is well established [4]. Patients with type 2 DM and high bone mineral density, also show an increased rate of osteoporotic bone fractures [5]. However, less is known about the effects of MS on bone metabolism. Different reports reveal conflicting results regarding MS or its individual components, with bone mineral density and/or fracture risk [6,7].

Western diets are rich in carbohydrates (e.g. fructose and sucrose) and saturated fats and are associated with MS and cardiovascular disease. Many MS animal models are based in the administration of high-carbohydrate or high-fat diets, to develop one or more of the characteristics of this syndrome [8]. In particular, high-fructose diets have been successfully used in animal models to develop MS, displaying classical clinical and metabolic changes that include: hypertension, glucose intolerance, hypertriglyceridemia, insulin resistance and obesity [9–11]. Rats fed a high-fructose diet show a systemic accumulation of advanced glycation end-products (AGEs) secondarily to the presence of hyperglycemia [12]. Our group and others have shown that AGEs trigger oxidative stress and inflammatory reactions, both of which are related to metabolic disorders as well as to impaired bone cell development and survival [13,14]. More recently, we have shown that fructose-induced MS rats display alterations in metaphyseal bone microarchitecture and defective bone fracture healing, possibly as a result of a deviation in the adipogenic/osteogenic commitment of MSC due to an imbalance in the Runx2/PPARγ ratio [9].

Metformin is one of the most widely used agents for treatment of insulin resistance associated with type 2 DM and MS. We have shown that metformin also enhances osteoblast proliferation, differentiation and mineralization in the UMR 106 and MC3T3E1 osteoblastic cell lines [15], and induces bone formation *in vivo* and *ex vivo* in normal rats and in partially-insulin-deficient diabetic rats [16].

Based on these previous observations, we hypothesize that fructose-induced MS alters osteoblast differentiation and thus, maintenance of normal bone architecture and that these effects can be prevented by metformin treatment.

2. Materials and methods

2.1. Animals and experimental design

A schematic representation of the experimental design is shown in Fig. 1. Thirty-two 8 week-old male Wistar rats (200–220 g) were housed in a temperature-controlled room at 23 ± 3 °C, with average humidity of 30–70%, a 12:12 h light: dark cycle and continuous access to standard rat laboratory chow (Asociación de Cooperativas Argentinas, Buenos Aires, Argentina) and beverage *ad libitum*. The experiments were carried out in conformity with the Guidelines on Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare [17]. All experiments including animals were approved by the Institutional Ethics Committee (N° 001-05-15).

Animals were randomly divided into two groups of 16 animals. One of the groups received sterile water *ad libitum*. The other group was given a 10% w/v fructose solution (Biopack, Buenos Aires, Argentina) *ad libitum* until the end of the study [9,18,19]. After 14 days of treatment, half the rats from each group also received 100 mg/kg/day metformin (Química Montpellier, Buenos Aires, Argentina) added to their drinking water for the last 3 weeks of the study. Thus, four experimental groups of eight animals per group were set: control (C); control plus metformin (CM); fructose rich diet (FRD); and fructose rich diet plus metformin (FRDM). Treatment times were chosen based on previous reports showing that fructose administration to rats for at least 2 weeks induces metabolic changes that resemble human MS [10,18,20–25], as well as bone tissue alterations [9]. The metformin dose of 100 mg/kg/day that we employed in the *in vivo* experiments was within the human therapeutic range as modified by the Guidance of FDA-CDER [26]. In addition, Choi and colleagues [27] have shown that plasma half-life for oral metformin in rats is 2 min (versus 5 h in humans), underscoring the need for significantly higher oral dosing in rats. We have also demonstrated that oral administration of metformin for 2 weeks induces significant changes in *in vivo* and *in vitro* osteogenic parameters [15,16,28]. Total treatment time was not prolonged any further to avoid insulin secretion impairment, as explained in Section 4.

Half of the rats from each group received subcutaneous fluorochromes injections to perform dynamic histomorphometry [29]. Briefly, ten days prior to sacrifice rats received

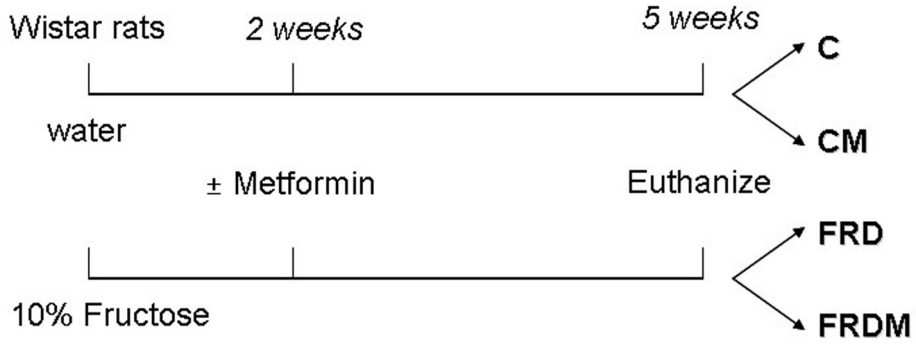

Fig. 1 – Schematic representation of the experimental design. C: control group; CM: control plus metformin treatment group; FRD: group of rats receiving fructose rich-diet and FRDM: group of rats receiving fructose rich-diet plus metformin treatment.