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Effects of a metabolic syndrome induced by a fructose-rich diet on bone metabolism in rats

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ABSTRACT

Objective. The aims of this study were: first, to evaluate the possible effects of a fructose rich diet (FRD)-induced metabolic syndrome (MS) on different aspects of long bone histomorphometry in young male rats; second, to investigate the effects of this diet on bone tissue regeneration; and third, to correlate these morphometric alterations with changes in the osteogenic/adipogenic potential and expression of specific transcription factors, of marrow stromal cells (MSC) isolated from rats with fructose-induced MS.

Materials/Methods. MS was induced in rats by treatment with a FRD for 28 days. Halfway through treatment, a parietal wound was made and bone healing was evaluated 14 days later. After treatments, histomorphometric analysis was performed in dissected femoral and parietal bones. MSC were isolated from the femora of control or fructose-treated rats and differentiated either to osteoblasts (evaluated by type 1 collagen, Alkaline phosphatase and extracellular nodule mineralization) or to adipocytes (evaluated by intracellular triglyceride accumulation). Expression of Runx2 and PPARγ was assessed by Western blot.

Results. Fructose-induced MS induced deleterious effects on femoral metaphysis microarchitecture and impaired bone regeneration. Fructose treatment decreased the osteogenic potential of MSC and Runx2 expression. In addition, it increased the adipogenic commitment of MSC and PPARγ expression.

Conclusions. Fructose-induced MS is associated with deleterious effects on bone microarchitecture and with a decrease in bone repair. These alterations could be due to a deviation in the adipogenic/osteogenic commitment of MSC, probably by modulation of the Runx2/PPARγ ratio.

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Abbreviations: AB, Alcian Blue; ALP, alkaline phosphatase; DMEM, Dulbecco's modified essential medium; FBS, fetal bovine serum; FRD, fructose rich diet; H&E, Haematoxylin and Eosin; IBMX, 3-isobutyl-1-methylxanthine; MS, metabolic syndrome; MSC, marrow stromal cells; NBF, Neutral Buffered Formalin; p-NP, *p*-nitrophenol; p-NPP, *p*-nitrophenylphosphate; TRAP, Tartrate Resistant Acid Phosphatase.

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1. Introduction

Metabolic Syndrome (MS) is a heterogeneous and multifactorial human disorder associated with increased cardiovascular risk [1]. Common associated comorbidities observed in MS patients include insulin resistance, dyslipidemia (particularly hypertriglyceridemia and low levels of HDL), central obesity, hypertension, glucose intolerance, diabetes mellitus (DM), and a high proportion of atherosclerotic disease. MS affects approximately 25% of adults in Latin America, ranging from 18.8% to 43.3% depending on the country of origin [2]. A similar prevalence has been observed in the US population, and an even higher prevalence was reported in certain ethnic groups around the world [3]. Importantly, the prevalence of MS is correlated with an increased prevalence of obesity, age, and is also associated with an increased risk for the development of DM with its associated cardiovascular complications [4].

Western diets contain substantial amounts of refined sugars including sucrose, fructose and glucose. Fructose intake has been associated with higher levels of plasmatic triglycerides (VLDL) and a decrease in the HDL-cholesterol [5]. According to USDA Nationwide Food Consumption Survey reports, the average daily fructose intake in the US population was 37 g in 1978. Sugar-sweetened non-alcoholic beverages, such as soft drinks, appeared as the major source of fructose, and adolescents and young adults (19–22 years) of both genders are the main consumers. Recent data from the NHANES 1999–2004 study, have estimated an average fructose intake of 49 g/day, which corresponds to a 30% increase in daily intake [5]. Several studies performed in rats that received fructose or sucrose-rich diets reproduced the severe and adverse metabolic and cardiovascular effects observed in MS patients [5–8], suggesting that this could be an interesting animal model to investigate different aspects of MS.

Bone is a highly dynamic tissue [9]. Marrow stromal cells (MSC) are found in the bone marrow microenvironment and present the ability to differentiate into various cell types such as osteoblasts, adipocytes, and chondrocytes [10–12]. In this context, bone marrow metabolic conditions are determinants of the biologic balance between osteoblast-mediated bone formation and marrow adipogenesis [11]. Diverse factors such as endogenous hormones and drug treatment can affect this delicate balance, modifying the osteoblast–adipocyte ratio in the bone marrow [11]. There is a growing body of clinical evidence reporting the association of skeletal abnormalities that include osteopenia, osteoporosis and/or an increased incidence of low-stress fractures, with MS [13–17] or some of its individual components such as hypertension [18,19] and obesity [20], in elderly patients of both sexes. However, this association has not been found in all studies [21], and its underlying mechanisms have not been elucidated to date.

The aims of this study were: first, to evaluate the possible effects of a fructose-rich diet-induced MS on different aspects of long bone histomorphometry in young male rats; second, to investigate the effects of this diet on bone tissue regeneration; and third, to correlate these morphometric alterations with changes in the osteogenic/adipogenic potential and expression of specific transcription factors, of marrow stromal cells (MSC) isolated from rats with fructose-induced MS.

2. Methods

2.1. *Animal Treatments*

Young adult male Sprague–Dawley rats initially weighing 200 to 220 g were used. They were maintained in a temperature-controlled room at 23 °C with a fixed 12 h light–dark cycle. All experiments on animals were carried out in conformity with the Guidelines on Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare (1992) [22]. Approval for animal studies was obtained from the institutional accreditation committee (INIBIOLP's Animal Welfare Assurance No A5647-01). The animals were divided into two groups of 6 animals per group: one group received standard chow and water *ad libitum* (control, C) and the other group received standard chow and water containing 10% w/v fructose (Biopack, Buenos Aires, Argentina) *ad libitum* (fructose-rich diet, FRD) [23]. After 28 days, all animals were sacrificed under anesthesia by cervical dislocation. This period of time was chosen because some authors have found that drinking 10% fructose solution for at least 3 weeks causes, in Sprague–Dawley rats, development of hypertension, hypertriglyceridemia and impaired glucose tolerance [24,25]. Prior to sacrifice, body weight was measured and non-fasting blood samples were obtained for determination of serum biochemical parameters. Glucose, triglycerides and cholesterol were measured by commercial kits (Wiener Laboratories, Rosario, Argentina) with a Metrolab 2300plus automated Chemistry Analyzer (Metrolab, Argentina). Insulin was determined by a rat-specific ELISA kit (ALPCO, New Hampshire, USA) and fructosamine was measured with a colorimetric kit (Biosystems, Barcelona, Spain).

2.2. *Bone reossification model*

The possible effects of a fructose-rich diet-induced MS on the process of bone repair were evaluated by a reossification model previously described by Santana et al. [26]. Briefly, half-halfway through the study (i.e., after 14 days) all animals were anesthetized by intraperitoneal/intramuscular injection of 0.12 mL/100 g body weight of 62.5 mg/mL ketamine hydrochloride and 6.25 mg/mL xylazine (Laboratorios Richmond, Buenos Aires, Argentina). Circular craniotomy defects of 1.0 mm diameter were performed in the right parietal bones of animals with a cylindrical low-speed carbide bur. Animals were then maintained in the previously described conditions for another 14 days to allow bone lesions to heal partially.

2.3. *Histological examination of femoral metaphysis*

After sacrifice, both femora of each rat were dissected. One femur was used for histomorphometric analysis and the other was used for isolation of MSC, as described below. The length of each femur was measured using a Vernier caliper. Femora were fixed in Neutral Buffered Formalin (NBF) for 72 h and decalcified by consecutive immersions in 10% EDTA (Biopack, Buenos Aires, Argentina), pH = 7.0. After decalcification, they were embedded in paraffin, and 5 μm sections were obtained using an SM 2000R Leica microtome (Leica Microsystems, Wetzlar, Germany). The sections were stained with Haematoxylin and

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