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Fructose consumption does not worsen bone deficits resulting from high-fat feeding in young male rats

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ABSTRACT

Dietary-induced obesity (DIO) resulting from high-fat (HF) or high-sugar diets produces a host of deleterious metabolic consequences including adverse bone development. We compared the effects of feeding standard rodent chow (Control), a 30% moderately HF (starch-based/sugar-free) diet, or a combined 30%/40% HF/high-fructose (HF/F) diet for 12 weeks on cancellous/cortical bone development in male Sprague–Dawley rats aged 8 weeks. Both HF feeding regimens reduced the lean/fat mass ratio, elevated circulating leptin, and reduced serum total antioxidant capacity (tAOC) when compared with Controls. Distal femur cancellous bone mineral density (BMD) was 23–34% lower in both HF groups ($p < 0.001$) and was characterized by lower cancellous bone volume (BV/TV, $p < 0.01$), lower trabecular number (Tb.N, $p < 0.001$), and increased trabecular separation versus Controls ($p < 0.001$). Cancellous BMD, BV/TV, and Tb.N were negatively associated with leptin and positively associated with tAOC at the distal femur. Similar cancellous bone deficits were observed at the proximal tibia, along with increased bone marrow adipocyte density ($p < 0.05$), which was negatively associated with BV/TV and Tb.N. HF/F animals also exhibited lower osteoblast surface and reduced circulating osteocalcin ($p < 0.05$). Cortical thickness ($p < 0.01$) and tissue mineral density ($p < 0.05$) were higher in both HF-fed groups versus Controls, while whole bone biomechanical characteristics were not different among groups. These results demonstrate that "westernized" HF diets worsen cancellous, but not cortical, bone parameters in skeletally-immature male rats and that fructose incorporation into HF diets does not exacerbate bone loss. In addition, they suggest that leptin and/or oxidative stress may influence DIO-induced alterations in adolescent bone development.

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1. Introduction

Historically, obesity and body mass index (BMI) have been positively associated with bone mineral density (BMD) in adults, likely a result of higher body mass increasing mechanical strain on the weight bearing portions of the skeleton and/or an altered hormonal milieu resulting from increased adiposity that may positively influence bone acquisition in adulthood [1]. However, the influence of obesity on adolescent bone development requires further clarification because an inverse relationship exists between total body fat mass and total body, lumbar spine, and ultradistal radius areal BMD in children, despite greater lean mass in obese children [2] and because obese adolescents (assessed by BMI) exhibit higher fracture rates than their age-matched normal weight non-obese counterparts [3], suggesting that obesity may be detrimental to adolescent skeletal health. Consuming a high-fat and/or high-sugar diet is one factor that predisposes children to obesity [4]. Similarly, high-fat [5–10] or high fructose diets [11,12] lead to dietary-induced obesity (DIO) in skeletally-immature rodents, along with reduced BMD, diminished bone strength, and adverse microarchitectural changes in cancellous bone compartments that persist into adulthood.

Alterations in peripheral [13] and/or central leptin signaling [14] may be factors influencing DIO-induced bone loss, given that rodents fed high-fat [15] or high-fructose diets [16] exhibit chronically elevated circulating leptin and that leptin is negatively associated with BMD in

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mice with DIO resulting from high-fat feeding [10]. In this regard, leptin is an adipocyte-derived hormone that increases proportionally with adiposity and which influences bone accrual via central [14] and peripheral manners [17]. The central influence of leptin on bone accrual appears to occur indirectly via suppression of CNS serotonin release and subsequent inhibition of serotonergic regulation of bone accrual [14]; although, the role of this central pathway remains controversial with other groups demonstrating hypothalamic leptin administration [18] or leptin gene therapy [17,19] increasing bone formation. In contrast, peripheral leptin is known to directly stimulate bone formation [17] after binding leptin receptors present on osteoblasts and chondrocytes. As evidence, subcutaneous leptin replacement increases longitudinal bone growth, osteoblast number, and mineral apposition rate in *ob*/*ob* (leptin deficient) mice [17].

Increased oxidative stress has also been proposed as one factor underlying adverse skeletal development and the pathogenesis of osteoporosis in a variety of conditions, including diabetes [20], aging, and sex-steroid deficiency syndromes [21]. As evidence, oxidative stress is associated with increased bone resorption and low BMD in elderly men [22] and postmenopausal women [23]. Furthermore, incubation of human or rodent bone marrow cells with H_2O_2 (an inducer of oxidative stress) stimulates development of osteoclast-like cells, increases bone resorptive activity [23], and inhibits bone formation *in vitro* [24], effects that are reversed by co-incubation with antioxidants or reactive oxygen species (ROS) inhibitors. In this regard, DIO increases oxidative stress and leptin stimulates ROS generation in endothelial cells [20], suggesting that increased oxidative stress resulting from ROS generation and/or reduced activity of the antioxidant systems may influence the adverse skeletal development resulting from high-fat or high-fructose diets.

Interestingly, typical "westernized" diets that are high in both fat and fructose are known to produce greater elevations in both leptin and oxidative stress, when compared with high-fat starch-based diets [25]. However, we are unaware of any study examining whether a combined high-fat/high-fructose (HF/F) diet produces greater bone deficits than that of a calorically-matched high-fat (HF) starch-based diet. Our primary purpose was to compare bone development in skeletally-immature rats consuming a moderately HF diet to that of an isocaloric HF/F diet. We hypothesized that the HF/F diet would produce deleterious skeletal adaptations in comparison to the HF (starch-based/sugar-free) diet and that both aforementioned groups would exhibit adverse skeletal development in comparison to animals fed standard low-fat/sugar-free rodent chow. A secondary purpose was to determine whether skeletal outcomes were associated with circulating leptin, systemic oxidative stress, or bone marrow adipocytes (a source of leptin in close proximity to cancellous bone).

2. Materials and methods

2.1. Animals

Barrier-raised and specific pathogen-free male Sprague Dawley rats aged 7 weeks were obtained from Harlan Labs (Indianapolis, IN). Upon arrival, rats were examined and remained in quarantine for one week during which they were provided standard rodent chow and water *ad libitum*. Rats were maintained on a 12:12 h light–dark cycle. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals and protocols were approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Experimental design

We acquired bones from a larger experiment that evaluated cardiovascular and body composition responses to HF and combined HF/F feeding in skeletally-immature rats [25]. Rats were randomized to receive one of three diets: 1) standard rodent chow (Control, n = 10; Harlan Teklad Global 18% Protein Rodent Diet, Harlan Laboratories Inc., Madison, WI), 2) a moderately HF (sugar-free) chow with 30% of kcal from fat (n = 8; Harlan TD08703), or 3) a moderately HF chow (30% kcal from fat) with 40% kcal from fructose (HF/F, n = 8; Harlan TD08702) and water *ad libitum* (see Supplemental Table 1 for nutrient composition of diets). No glucose was present in either HF diet. Total kcal and macronutrient/micronutrient distributions were matched in the HF and HF/F groups, with the source of carbohydrate (starch *vs.* fructose) serving as the sole difference between these dietary regimens, which allowed us to distinguish the direct impact of fructose on bone metabolism in the presence of a high-fat diet. All diets exceeded the calcium threshold (2.5 g/kg) necessary to produce normal growth in Sprague–Dawley rats [26] and met the daily phosphorus requirements. Both HF dietary regimens contained 30% kcal from fat, a value that is near the average percentage fat intake of children and adolescents in the United States [27] and within the accepted macronutrient distribution range defined in the 2015–2020 Dietary Guidelines for America recommendations for children and adolescents [28]. Rats were euthanized with an overdose of isoflurane at week 12 and blood was collected via cardiac puncture. The circulatory system was perfused with 20 ml of ice-cold saline and the right and left femurs and tibiae were excised and cleaned of surrounding soft-tissue, weighed, and measured. Blood samples were centrifuged at 4000 rpm for 20 min and serum aliquots were separated and stored at −80 °C until analysis. Femurs were wrapped in saline-soaked gauze to prevent dehydration and stored at −20 °C for microcomputed tomography (μCT) analysis and assessment of bone mechanical properties. Tibiae were cut in half, cross-sectionally, placed in 10% phosphate-buffered formalin for 48 h tissue fixation, dehydrated in ethanol, and embedded undecalcified in methyl methacrylate for subsequent sectioning and histologic analysis.

2.3. μCT analysis of bone structure

The left distal femoral metaphysis and diaphysis were scanned by μCT using a Bruker Skyscan 1172 (Kontich, Belgium), as previously described [29–31]. Images were acquired using the following parameters: 80 kVP/120 μA, 0.5 mm aluminum filter, 1k camera resolution, 19.2 μm voxel size, 0.5° rotation step, and 180° tomographic rotation. The cancellous regions of interest (ROI) at the distal femoral metaphysis began 1.5 mm proximal to the growth plate and encompassed 4 mm, including the sponge-like trabecular (cancellous) bone spicules in the medullary cavity and excluding the dense compact (cortical) bone that surrounds the medullary cavity. The cortical ROI at the distal femur began 3 mm proximal to the growth plate (in order to completely avoid residual growth plate) and encompassed a total of 2 mm, excluding all cancellous bone. The cortical ROI at the femoral diaphysis encompassed a 2 mm region beginning at 55% of the femur length in order to avoid the third trochanter. Cross-sectional images were reconstructed using a filtered back-projection algorithm (NRecon, Kontich, Belgium). 2D and 3D morphometric measurements were calculated using CTan software (Bruker Skyscan, Kontich Belgium). Measurements at the distal femur include: cancellous bone volume (as a percentage of bone tissue area, BV/TV, %), trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, 1/mm), trabecular separation (Tb.Sp, mm), trabecular pattern factor (Tb.Pf), and structural model index (SMI). Cortical measurements at the distal femur and femoral diaphysis include: total cross-sectional (bone plus medullary) area (Tt.Ar, mm^2), cortical bone area (Ct.Ar, mm^2), cortical area fraction (Ct.Ar/Tt.Ar, %), and three-dimensional cortical thickness (3D Ct.Th, mm). Additionally, medullary volumetric bone mineral density (vBMD, mg/cm^3) and cortical volumetric tissue mineral density (vTMD, mg/cm^3) were evaluated in the previously defined distal femur and femoral diaphysis ROIs, respectively. Densities were determined following calibration with hydroxyapatite phantoms.