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Reduced size-independent mechanical properties of cortical bone in high-fat diet-induced obesity

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ABSTRACT

Overweight and obesity are rapidly expanding health problems in children and adolescents. Obesity is associated with greater bone mineral content that might be expected to protect against fracture, which has been observed in adults. Paradoxically, however, the incidence of bone fractures has been found to increase in overweight and obese children and adolescents. Prior studies have shown some reduced mechanical properties as a result of high-fat diet (HFD) but do not fully address size-independent measures of mechanical properties, which are important to understand material behavior. To clarify the effects of HFD on the mechanical properties and microstructure of bone, femora from C57BL/6 mice fed either a HFD or standard laboratory chow (Chow) were evaluated for structural changes and tested for bending strength, bending stiffness and fracture toughness. Here, we find that in young, obese, high-fat fed mice, all geometric parameters of the femoral bone, except length, are increased, but strength, bending stiffness, and fracture toughness are all reduced. This increased bone size and reduced size-independent mechanical properties suggests that obesity leads to a general reduction in *bone quality* despite an increase in *bone quantity*; yield and maximum loads, however, remained unchanged, suggesting compensatory mechanisms. We conclude that diet-induced obesity increases bone size and reduces size-independent mechanical properties of cortical bone in mice. This study indicates that bone quantity and bone quality play important compensatory roles in determining fracture risk.

Introduction

Obesity is an increasingly prevalent medical condition [1], which is associated with other medical problems such as diabetes and heart disease [2]. A number of public health studies have linked adult obesity with increased bone mineral density and content and reduced fracture risk in adults [3–10]. Despite this trend in adults, an increased fracture incidence has been seen in adolescents and children when compared to age-matched controls [11–13].[1] Children and adolescents who are overweight tend to also have poorer posture control and body position sense than their normal-weight peers [14–16], which is likely a contributing factor even when diabetes is not present. However, in the general context, the question arises whether obesity can be related to microstructural and mechanical behavior changes in bone, *i.e.*, to *bone quality*, in addition to higher bone mass (*bone quantity*), to explain the altered fracture risk, especially in adolescents and children who exhibit increased fracture incidence with obesity. Surprisingly, only a few studies have addressed this question using animal models. Rat studies have found reductions in yield and maximum stresses, energy absorption, structural rigidity, and failure loads, despite larger bone sizes as a result of high-fat and high-sugar diet-induced obesity [17–19]. Conclusions have not always been consistent; however, in general, a significant *decrease* in mechanical performance (reduced bone quality) concurrent with an *increase* in bone size (increased bone quantity) has been reported.

Most prior studies have focused on properties such as failure load and energy absorption which do not account for changes in the bone cross-section area, thereby confounding the effects of bone quality and quantity. To fully understand the mechanical integrity of the bone and its resistance to fracture, *size-independent* mechanical properties

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[1] It should be noted that fracture risk in adults with type 2 diabetes does tend to increase in adults [12] (see Appendix); although fracture rates of diabetic children have not been reported, reduced bone mineral content and bone size have been observed in type 1 diabetic adolescents, which does imply an increased fracture risk [13].

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need to be measured [20], including yield and maximum stresses, stiffness, and fracture toughness. Strength, defined by the yield stress at the onset of permanent deformation or maximum strength at the peak load before fracture, is a measure of the force/unit area that the bone can withstand. Stiffness is related to the elastic modulus and defines the force required to give a specific elastic strain. The fracture toughness measures resistance to fracture. Additionally, although most prior studies have involved rats, the use of mice allows for greater genetic control which could be used in future studies to identify specific biological factors that are responsible for the high-fat diet-induced changes in bone.

Two possible ways by which obesity could affect bone properties are increased body mass and altered secretion of biological factors. Although the contribution of body mass to bone size and quality has been debated, it is well established that bone responds to external loads and that lean mass is more important than fat mass at predicting bone size and mineral density/content measures [21–27]. The latter observation is further supported by research showing that remodeling is influenced by dynamic rather than static loads [28,29].

The levels of several hormones are altered by obesity, many of which can impact bone. Adipocyte-derived hormones such as leptin, adiponectin, and resistin also play a role in bone's response to obesity by a variety of mechanisms [7]. As leptin influences osteoblast activity directly and indirectly through the central nervous system, it is typically considered in studies involving obesity and bone. Additionally, it has been suggested that insulin-like growth hormone I (IGF-I) acts to increase bone size [30] and is an interesting factor to consider in this study as increased IGF-I concentrations have been observed with increasing weight [31].

Despite the complex relationship between fat and bone, described succinctly by Reid [7], it is evident that both fat mass and lean mass affect bone health. The purpose of this study is to determine whether diet-induced obesity affects bone tissue quantity (bone size and mineral quantity measures), bone quality (defined by mechanical properties that affect fracture but are independent of bone size), or combinations of the two.

Materials and methods

Animals

All protocols were approved by the Institutional Animal Care and Use Committee and performed according to federal guidelines for the care and use of animals in research. Thirty 4-week-old C57BL/6 male mice were fed a high-fat diet (Research Diets High-Fat Diet 60 kcal% fat, 20 kcal% carbohydrate, 20 kcal% protein) ($n=15$ "HFD" group) or standard laboratory chow (PicoLab Mouse Diet 21.6 kcal% fat, 55.2 kcal% carbohydrate, 23.2 kcal% protein) ($n=15$, "Chow" group) for a diet duration of 19 weeks. All mice, grouped in cages of five animals each, were maintained under controlled temperature and photoperiod (12 hours light, 12 hours dark) with food and water provided *ad libitum*.

Body composition

Body weight was measured starting on postnatal day 37. At 1-week intervals throughout the study and once prior to sacrifice, all mice were weighed. Fat and lean body mass, percent fat, areal bone mineral density (aBMD), and bone mineral content (BMC) were determined at the completion of the study by dual-energy x-ray absorptiometry (DXA), as described by the manufacturer (Lunar PIXImus mouse densitometer).

Blood collection

Blood was collected at two intervals. In the first instance, mice were fasted for 4 hours before blood was collected through submandibular bleeding. For the second round of blood collection, mice were decapitated within 30 sec of mouse handling. Blood was collected in tubes containing ethylene-diaminetetraacetic acid (EDTA) and plasma was immediately separated by centrifugation and frozen at −80 °C.

Blood glucose and glucose tolerance test

Blood glucose levels were measured from the tail vein using an Ascensia ELITE XL Blood Glucose Meter. Fasting glucose measurements at 15 and 21 weeks of age as well as the glucose tolerance test at age 22 weeks were performed after 4 hours of fasting. For the glucose tolerance test, mice were injected IP with a 20% glucose solution at 2 g of glucose/kg of body weight and glucose levels were measured at 15, 30, 60, and 120 minutes.

Leptin level measurement

Serum leptin levels were measured using a Crystal Chem Inc. Mouse Leptin ELISA Kit.

IGF-I level measurement

Serum IGF-I levels were measured using an Immunodiagnostic Systems Inc. Mouse/Rat IGF-I ELISA Kit.

Mechanical testing

Following sacrifice, the whole mice were stored at −20 °C until dissection of the femora. At dissection, the right and left femora were isolated from any soft tissue with scissors and scalpel, after which bones were wrapped in gauze soaked with Hanks' balanced salt solution (HBSS) and stored at −20 °C. Prior to testing, the femora were thawed in room-temperature HBSS, and the size and geometry of all samples were measured with calipers. The left femora were tested in unnotched three-point bending to measure bending strength and stiffness. The right femora were tested in notched three-point bending to measure the fracture toughness. For notched testing, the femoral shaft was notched in the mid-diaphyseal region through the posterior wall using the method described by Ritchie et al. [32]. Notches were sharpened by polishing in 1 μm diamond paste with a razor blade to a root radius of ~5–10 μm. Notched and unnotched femora were placed in a three-point bending rig such that the posterior side was in tension and the anterior was in compression. Femora were submerged in HBSS at 37 °C for 1 minute to acclimate, then tested in the same environment at a displacement rate of 0.001 mm/s until fracture occurred. Broken halves were then dehydrated and the fracture surface was examined in an environmental SEM (Hitachi S-4300SE/N ESEM, Hitachi America). The femoral cross-sectional area and second moment of inertia were computed from fracture surface images. Notch half-crack angles were determined in the SEM from the fracture surface using techniques described in Ritchie et al. [32]. Stresses and strains were computed in accordance with the methods described by Akhter et al. [33]. The yield strength was determined as the stress at 0.2% plastic strain, and maximum strength as the stress at peak load. Bending stiffness was calculated as the slope of the linear region of the stress–strain curve. Fracture toughness values were defined at the onset of unstable fracture, *i.e.*, at the point of instability, using the procedures described in Ritchie et al. [32] for the toughness evaluation of small animal bone.

X-ray computed tomography and vBMD

Micro x-ray computed tomography was employed to measure the volumetric bone mineral density (vBMD) of all samples using synchrotron beamline 8.3.2 at the Advanced Light Source, Lawrence Berkeley National Laboratory. The three-dimensional resolution was 4.45 μm. The samples were imaged in absorption mode at 14 keV and