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High bone mass in adult mice with diet-induced obesity results from a combination of initial increase in bone mass followed by attenuation in bone formation; implications for high bone mass and decreased bone quality in obesity

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ABSTRACT

Obesity is generally recognized as a condition which positively influences bone mass and bone mineral density (BMD). Positive effect of high body mass index (BMI) on bone has been recognized as a result of increased mechanical loading exerted on the skeleton. However, epidemiologic studies indicate that obesity is associated with increased incidence of fractures. The results presented here offer a new perspective regarding the mechanisms which may be responsible for the increase of bone mass and concurrent decrease in bone quality. Two groups of 12 week old C57BL/6 males were fed either high fat diet (HFD) or regular diet (RD) for 11 weeks. Metabolic profile, bone parameters and gene expression were assessed in these groups at the end of the experiment. Additionally, bone status was evaluated in a third group of 12 week old animals corresponding to animals at the start of the feeding period. Administration of HFD resulted in development of a diet-induced obesity (DIO), glucose intolerance, alteration in energy metabolism, and impairment in WAT function, as compared to the age-matched control animals fed RD. The expression of adiponectin, FABP4/aP2, DIO2 and FoxC2 were decreased in WAT of DIO animals, as well as transcript levels for IGFBP2, the cytokine regulating both energy metabolism and bone mass. At the end of experiment, DIO mice had higher bone mass than both control groups on RD, however they had decreased bone formation, as assessed by calcein labeling, and increased marrow adipocyte content. This study suggests that the bone mass acquired in obesity is a result of a two-phase process. First phase would consist of either beneficial effect of fat expansion to increase bone mass by increased mechanical loading and/or increased production of bone anabolic adipokines and/or nutritional effect of fatty acids. This is followed by a second phase characterized by decreased bone formation and bone turnover resulting from development of metabolic impairment.

1. Introduction

Obesity associates with a number of comorbidities among them increased fracture risk and poor fracture healing. The pathophysiology of obesity-related bone impairment is unclear and there are conflicting results from clinical and basic research trying to explain this phenomenon. Although obesity usually correlates with higher BMD, which in some individuals may have a protective effect against fractures (Reid, 2010), but it is also evident that in other individuals, e.g. postmenopausal women, obesity may increase risk of fractures (Compston et al., 2011; Premaor et al., 2011). Moreover, some studies point to the negative effect of obesity on BMD and fracture risk (Aguirre et al., 2014; Cawsey et al., 2014).

It is well recognized that a BMI value, which is used to diagnose obesity, is not reflecting impairment in fat metabolism. Growing evidence indicates that fat metabolism is an important determinant of bone and fat relationship (reviewed in Lecka-Czernik and Stechschulte, 2014). Indeed, individuals with type 2 diabetes, a disease of impaired fat function, have 2-fold higher risk of fractures despite higher BMD than non-diabetic control (Melton et al., 2008). The correlation between impairment in fat metabolic function and increased risk of fractures is also noticed with aging and in wasting diseases including anorexia nervosa (Cartwright et al.,

Abbreviations: BMD, bone mineral density; TMD, total mineral density; BMI, body mass index; HFD, high fat diet; RD, regular diet; DIO, diet-induced obesity; WAT, white adipose tissue; BAT, brown adipose tissue; MSCs, mesenchymal stem cells; mCT, micro-computed tomography.

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2007; Misra and Klibanski, 2014). In contrast, increase in fat metabolic function correlates with increased bone mass and bone quality (Lee et al., 2013; Rahman et al., 2013; Srikanthan et al., 2014).

Adipose tissue produces a number of adipokines, which either systemically or locally affect bone turnover. Among them, leptin and adiponectin regulate bone turnover through a combination of direct effects on bone cells, with leptin favoring bone formation while adiponectin having a negative effect on osteoblasts, and indirect mechanisms mediated by the central nervous system which often opposes their direct effects on bone (Kajimura et al., 2013; Naot and Cornish, 2014). This interplay between adipokines and bone is exacerbated in obese older adults and correlates with increased risk of fractures (Aguirre et al., 2014; Cawsey et al., 2014).

Animal studies highlight the complexity of fat and bone cross-talk and point to the effect of gene by nutrition interactions which may explain many conflicting findings (Bonnet et al., 2014). Thus, genetic background, age, sex, and rodent model determine bone response to obesity and/or dietary fatty acids either with decreased bone mass and quality (Cao et al., 2009; Woo et al., 2009), or with no effect (Styner et al., 2014), or with a positive effect on bone (Wei et al., 2014). Recently, it has been shown that mice which developed obesity as a result of prolonged feeding with HFD have higher bone mass than control animals fed RD (Wei et al., 2014). This study demonstrated that higher bone mass is a result of decreased bone resorption and low bone turnover due to decreased insulin signaling in osteoblasts. In contrast, HFD negatively affects new bone formation during fracture healing by increasing PPARγ activity in MSCs which results in development of callus adiposity (Brown et al., 2014). These examples indicate that both systemic and direct effects of obesity and dietary fatty acids on bone need to be considered simultaneously.

In the present study, we have investigated the effect of HFD on bone formation and correlated it with fat metabolic status. Our data demonstrate that initial response to HFD results in increased bone mass, however a prolonged exposure leads to attenuation of bone formation and correlates with changes in fat metabolic status causing a decreased efficiency in energy generation and decreased production of cytokines which possess endocrine/paracrine anabolic activity on bone.

2. Materials and methods

2.1. Animals

C57BL/6 mice, 12 wk old males, were purchased from the Jackson Laboratories (Bar Harbor, ME) and separated into two groups. Mice were fed *ad libitum* for 11 wks either regular diet (RD) providing 12 kcal% from fat (2016 Teklad Global, Harlan Laboratories, Indianapolis, IN) or high fat diet (HFD) providing 45 kcal% from fat (D12451; Research Diets, New Brunswick, NJ). The RD group consisted of 4 and HFD group consisted of 6 animals. Another group of mice (n = 4) represented a control for mice entering feeding experiment and were sacrificed at 12 wks of age. The animal treatment and care protocols conformed to NIH Guidelines and were performed using a University of Toledo Health Science Campus Institutional Animal Care and Utilization Committee protocol.

Fat and lean mass were evaluated at the beginning and at the end of experiment using a Minispec mq10 NMR analyzer (Bruker, Billerica, Massachusetts). Glucose disposal (glucose tolerance test, GTT) was measured after ip injection of 2 g/kg glucose to animals fasted for 4 hrs. Blood glucose was measured using a specific AlphaTRAK system appropriate for mice (Abbott Laboratories, North Chicago, Illinois). For indirect calorimetry, mice were placed in metabolic cages (CLAMS; Columbus Instruments, Columbus, Ohio) for 4 days and with a free access to food and water. Mice were housed individually at room temperature (22 °C) under an alternating 12-h light and 12-h dark cycle. After adaptation for 1 day, oxygen (VO2) consumption, carbon dioxide (VCO2) production, physical activity, and heat production were measured to determine energy expenditure. Respiratory exchange ratio was calculated as a ratio of the O_2 consumption and the CO_2 production.

2.2. mCT imaging of bone and marrow fat

mCT of the tibiae was performed using the μCT-35 system (Scanco Medical AG, Bruettisellen, Switzerland) as previously described (Liu et al., 2012). Briefly, scans were performed at 7 μm nominal resolution with the x-ray source operating at 70 kVp, and 113 μA settings. Scans consisted of 300 slices starting at the proximal end of tibias and images of trabecular bone were segmented at 220 threshold value using per mille scale following manual contouring starting 10 slices below the growth plate and extending to the end of the image stack. Scans of cortical bone at tibia midshaft consisting of 55 slices were obtained at 7 μm nominal resolution with the x-ray source operating at 70 kVp and 113 μA. Images of cortical bone were contoured in the entire image stack and segmented at 260 threshold using per mille scale. The analysis of the trabecular bone microstructure and the cortical bone parameters was conducted using Evaluation Program V6.5-1 (Scanco Medical AG, Bruettisellen, Switzerland) and conformed to recommended guidelines (Bouxsein et al., 2010). Total mineral density (TMD) was evaluated in cortical and trabecular tibia bone of 4 mice per group at the threshold 220.

For lipid evaluation, decalcified bone specimens were stained for 1 hr in solution containing 2% osmium tetroxide prepared in 0.1 M sodium cacodylate buffer pH 7.4, according to the protocol (Liu et al., 2012). Staining was carried-out in an exhaust hood and away from light due to osmium tetroxide toxicity and light sensitivity. Images of lipid depositions were acquired at 70 kVp and 113 μA settings and 12 μm nominal resolution. Image segmentation was done under global threshold condition by applying a gray scale threshold of 480–1000 using per mille scale with 3-dimensional noise filter set to sigma 1.2 and support 2.0. Lipid volumes were calculated directly from individual voxel volumes in 3-D reconstructions.

2.3. Bone histomorphometry

To permit static and dynamic bone histomorphometry animals were injected with 2.5 mg/ml calcein solution in 2% sodium bicarbonate at a dose 20 mg/kg body weight. First injection was performed 10 days and second 2 days before sacrifice and undecalcified tibiae were embedded in methyl methacrylate, sectioned and stained with either Golden Trichrome or Von Kossa/McNeal by Histology Core at the Department of Anatomy and Cell Biology, Indiana University (Indianapolis, IN). The histomorphometric examination was confined to the secondary spongiosa of proximal tibia and was performed using Nikon NIS-Elements BR3.1 system. The measurements were collected under 40× magnification from six representative fields per bone sample. The terminology and units used were those recommended by the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research (Parfitt et al., 1987).

2.4. Gene expression analysis

Total RNA was isolated from epididymal WAT using TRIzol (Sigma-Aldrich, St. Louis, MO). One microgram of RNA was converted to cDNA using the Verso cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA). PCR amplification of the cDNA was performed by quantitative real-time PCR using TrueAmp SYBR Green qPCR SuperMix (Smart Bioscience, Maumee, OH) and processed with