Bone 57 (2013) 174–183

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Immature mice are more susceptible to the detrimental effects of high fat diet on cancellous bone in the distal femur☆

Jason A. Inzana [a,b,1], Ming Kung [a,1], Lei Shu [c], Daisuke Hamada [a,c], Lian Ping Xing [a,c], Michael J. Zuscik [a], Hani A. Awad [a,b], Robert A. Mooney [a,c,*]

[a] Center for Musculoskeletal Research, University of Rochester Medical Center, 601 Elmwood Avenue, Box 665, Rochester, NY 14642, USA
[b] Department of Biomedical Engineering, University of Rochester, 207 Robert B. Goergen Hall, Rochester, NY 14642, USA
[c] Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, Box 626, Rochester, NY 14642, USA

ARTICLE INFO

Article history:
Received 22 March 2013
Revised 1 August 2013
Accepted 8 August 2013
Available online 14 August 2013

Edited by: J. Aubin

Keywords:
High fat diet
Obesity
Trabecular bone
Age dependence
Diet correction

ABSTRACT

With the increasing prevalence of obesity among children and adolescents, it is imperative to understand the implications of early diet-induced obesity on bone health. We hypothesized that cancellous bone of skeletally immature mice is more susceptible to the detrimental effects of a high fat diet (HFD) than mature mice, and that removing excess dietary fat will reverse these adverse effects. Skeletally immature (5 weeks old) and mature (20 weeks old) male C57BL/6J mice were fed either a HFD (60% kcal fat) or low fat diet (LFD; 10% kcal fat) for 12 weeks, at which point, the trabecular bone structure in the distal femoral metaphysis and third lumbar vertebrae were evaluated by micro-computed tomography. The compressive strength of the vertebrae was also measured. In general, the HFD led to deteriorations in cancellous bone structure and compressive biomechanical properties in both age groups. The HFD-fed immature mice had a greater decrease in trabecular bone volume fraction (BVF) in the femoral metaphysis, compared to mature mice ($p = 0.017$ by 2-way ANOVA). In the vertebrae, however, the HFD led to similar reductions in BVF and compressive strength in the two age groups. When mice on the HFD were switched to a LFD (HFD:LFD) for an additional 12 weeks, the femoral metaphyseal BVF in immature mice showed no improvements, whereas the mature mice recovered their femoral metaphyseal BVF to that of age-matched lean controls. The vertebral BVF and compressive strength of HFD:LFD mouse bones, following diet correction, were equivalent to those of LFD:LFD mice in both age groups. These data suggest that femoral cancellous metaphyseal bone is more susceptible to the detrimental effects of HFD before skeletal maturity and is less able to recover after correcting the diet. Negative effects of HFD on vertebrae are less severe and can renormalize with LFD:LFD mice after diet correction, in both skeletally immature and mature animals.

Introduction

Obesity is reaching epidemic proportions in the United States and the developed world due, in part, to Western diets and decreased physical activity. In 2010, 33.8% of the U.S. adult population was obese. Childhood obesity is a particularly troubling public health concern. An estimated 16.9% of American adolescents are obese, with an alarming 9.7% of infants and toddlers also falling into this category [1]. Obesity is associated with an increased risk for several serious illnesses including type 2 diabetes, hypertension, and heart disease [2]. Associations between obesity and bone health, however, are still unclear. Evidence suggests that obese children are at risk of decreased bone mineral density (BMD) [3–5] and have increased fracture risk [3,6–8]. A recent study using peripheral quantitative computed tomography (pQCT) found reductions in volumetric BMD with increased fat mass in children, after correcting for lean mass, despite increased bone size [5]. The peak ages for increasing BMD and bone mineral content (BMC) are during adolescence, in the years 12–14 for girls and 13–15 for boys [9]. The lower BMD, BMC, and increased fracture risk in obese adolescents suggest that factors associated with obesity could be detrimental to the accrual of peak bone mass, a critical factor in the etiology of osteoporosis [10].

Many integral factors are associated with obesity, ranging from genetic to environmental. Excessive dietary fat, which is preventable, has become a particular concern in recent decades. It is recommended by the USDA that fats be reduced in the diet of Americans [11]. Several

* Corresponding author at: Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, Box 608, Rochester, NY 14642, USA.
E-mail addresses: jason_inzana@urmc.rochester.edu (J.A. Inzana), ming_kung@urmc.rochester.edu (M. Kung), lei_shu@urmc.rochester.edu (L. Shu), daisuke_hamada@urmc.rochester.edu (D. Hamada), lianping_xing@urmc.rochester.edu (L.P. Xing), michael_zuscik@urmc.rochester.edu (M.J. Zuscik), hani_awad@urmc.rochester.edu (H.A. Awad), robert_mooney@urmc.rochester.edu (R.A. Mooney).
[1] These authors contributed equally to this work.

8756-3282/$ – see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2013.08.003

studies have investigated the effects of a high fat diet (HFD) on bone in animal models, with the consensus that excessive dietary fat is detrimental to bone homeostasis and has a greater effect on trabecular than cortical bone [12–17]. These studies demonstrated adverse effects of the HFD on bone health in adult as well as adolescent mice or rats. Ionova-Martin et al. examined the effects of HFD on cortical bone from adolescence to adulthood in mice and observed similar trends in bone mineral and mechanical properties between the two age groups [18]. The possible differential impact between adolescents and adults of high dietary fat on cancellous bone, to the best of our knowledge, has not been reported. Studying this question may help determine if HFDs or the associated obesity and metabolic syndrome contribute to skeletal deficits in growing individuals; and whether this may lead to unrecoverable deficits later in life, even after potential interventions and life-style changes (e.g. diet). We hypothesized that 1) skeletally immature mice would be more susceptible to HFD-induced deterioration in cancellous bone structure, mineralization and strength compared to skeletally mature mice and 2) the HFD-associated deterioration in bone structure and strength would be alleviated after reducing dietary fat intake. These hypotheses were studied using skeletally immature (5 weeks old) and mature (20 weeks old) mice that were exposed to a HFD for 12 weeks and then transitioned to a low fat diet (LFD) for an additional 12 weeks. Mice that were maintained on the LFD throughout the experiment were used as controls.

Materials and methods

Animals and tissue processing

Animal studies were performed in accordance with protocols approved by the University of Rochester's Committee on Animal Resources. Male C57BL/6J mice were purchased from Jackson Research Labs (Bar Harbor, ME) at 5 and 20 weeks of age to represent skeletally immature and mature mice, respectively. These ages were chosen based on studies of bone density as well as bone tissue and mechanical properties in C57BL/6J mice peaking in the age range of 16–24 weeks [19–21]. After a brief acclimation period, mice from each age group were placed either on a high fat diet (HFD; 60% kcal fat; Research Diets, Inc., New Brunswick, NJ) or low fat diet (LFD; 10% kcal fat; Research Diets, Inc., New Brunswick, NJ) for 12 weeks. After those 12 weeks, half of the mice from each group were switched to or continued on the lean diet (HFD:LFD or LFD:LFD, respectively) for an additional 12 weeks, while the other half were sacrificed for tissue collection ($n = 7–8$ per age group, diet, and time point). Immediately after isolation and removal of soft tissue, the right femurs were used for micro-computed tomography (micro-CT) imaging, while the third lumbar (L3) vertebrae were wrapped in saline-soaked gauze and frozen at −80 °C until the day of micro-CT and biomechanical testing.

Glucose measurements

Sixteen hours prior to sacrifice, food was removed from mouse cages to allow measurement of fasting blood glucose. Immediately before sacrifice, the mice were anesthetized under isoflurane gas, the distal tip of the tail was excised, and blood samples were collected to measure blood glucose levels using One Touch glucose meters (Lifescan, Inc.; Milpitas, CA).

Serum leptin measurements

At the time of sacrifice, blood samples were collected via heart puncture. Sera were frozen at −80 °C until analysis. Serum leptin levels were quantitated using a mouse leptin ELISA kit (EMD Millipore, St. Charles, MO). Sera were diluted 1:4 before analysis. All procedures were according to the manufacturer's instructions.

Micro-computed tomography

Femurs and L3 vertebrae were scanned by micro-CT (VivaCT 40; Scanco Medical; Bassersdorf, Switzerland), at a 10.5-micron isotropic resolution using an integration time of 300 ms, energy of 55 kVp and intensity of 145 μA. For trabecular analysis in the distal femoral metaphysis, a 200 μm region proximal to the growth plate was used for quantification. Femoral cortical bone was measured at the mid-diaphysis by averaging over a 200 μm region (19 slices). For vertebral measurements, the volume within the endosteal margin of each vertebral body was used to assess trabecular bone. Cortical thickness was measured at the mid-level of each vertebral body by averaging over a 200 μm thick region. Total cross-sectional bone area was similarly measured from the region between the caudal endplate and transverse processes. The trabecular bone morphology of the femoral metaphysis and vertebral bodies, including the bone volume fraction (BVF), connective density (Conn.D), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and structural model index (SMI) was determined using Scanco's 3D analysis tools (direct model).

Biomechanical testing

The whole bone strength of L3 vertebral bodies was tested under compressive loading through a modified, published method [22,23]. Briefly, the L3 vertebrae were dissected of all soft tissue including the

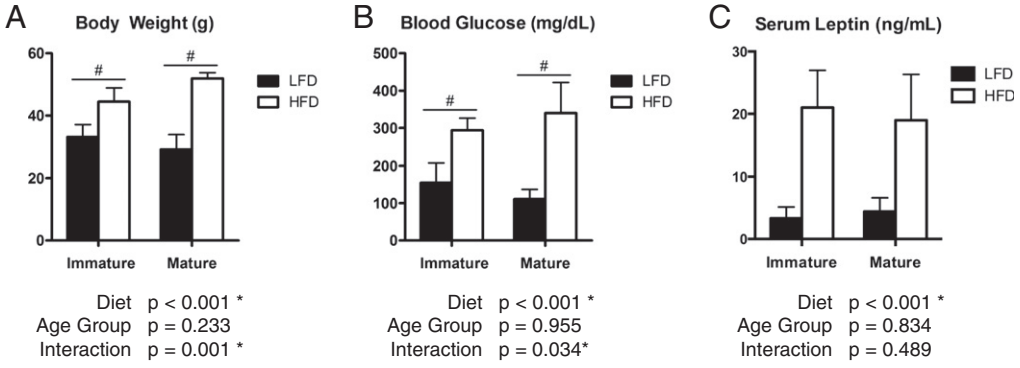

Fig. 1. High fat diet increases body weight, blood glucose and serum leptin levels significantly. Mice on the high fat diet (HFD) were significantly heavier (A) and had significantly higher fasting blood glucose levels (B) compared to low fat diet (LFD) controls. The interactive effect (diet × age group) was also significant in both body weight and blood glucose, which indicates that a synergistic effect existed between diet and age groups. Serum leptin levels (C) were also significantly elevated in the HFD-fed mice. Bars represent means and error bars represent SD. * indicates $p < 0.05$ by two-way ANOVA. # indicates $p < 0.05$ for intra-age group comparisons made with Bonferroni's post-hoc test when the interaction is significant.