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Original Research

A high-fat diet increases body weight and circulating estradiol concentrations but does not improve bone structural properties in ovariectomized mice

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ABSTRACT

Bone health is influenced by body mass and estrogen. The objective of the study was to determine whether high-fat diet-induced obesity affects bone structure and alters markers of bone turnover in ovariectomized (OVX) mice. We hypothesized that a high-fat diet would increase body weight gain and serum estradiol levels in OVX mice but would not improve bone structural parameter in OVX mice. Thirty-five C57BL/6 mice were either sham operated or OVX at the age of 4 months and then fed either a normal-fat diet (10% energy as fat) or a high-fat diet (45% energy as fat with extra fat from lard) ad libitum for 11 weeks. Ovariectomy increased body weight, serum tartrate–resistant acid phosphatase concentration, and expression of cathepsin K in bone; decreased serum estradiol concentration; and induced significant bone loss manifested by decreased bone volume/total volume (BV/TV), connectivity density (Conn.D), trabecular number, and trabecular thickness with increased trabecular separation and structural model index ($P < .01$). The high-fat diet increased body weight ($P < .01$) in OVX mice and nonsignificantly decreased BV/TV ($P = .08$) and Conn.D ($P = .10$). Despite having similar serum estradiol concentrations and higher body weight, OVX mice consuming the high-fat diet had lower BV/TV, Conn.D, trabecular number, trabecular thickness, and higher structural model index and trabecular separation than did sham mice fed the normal-fat diet. These findings indicate that increased body weight and elevated serum estradiol concentration induced by a high-fat diet do not mitigate ovariectomy-induced bone loss in mice.

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1. Introduction

Despite being a risk factor for many chronic diseases, obesity, a global public health problem affecting more than 300 million people, has long been considered to be beneficial to bone health in humans [1]. The increased body weight and the ability of estrogen synthesis by adipose tissue are 2 primary reasons for the alleged beneficial effects of obesity on bone [2,3].

Abbreviations: Conn.D, connectivity density; CTR, calcitonin receptor; CTSK, cathepsin K; Itgb3, integrin ß3; μCT, micro–computed tomography; RANKL, receptor activator nuclear factor-κB ligand; Sham, sham operated compared with OVX; SMI, structure model index; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TRAP, tartrate-resistant acid phosphatase; OVX, ovariectomy.

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In the United States, about 9% of adults older than 50 years have osteoporosis in either the femur neck or lumbar spine; an additional 49% have low bone mass and thus are at risk for developing the disease [4]. Women, accounting for 80% of individuals with osteoporosis, are especially at high risk mainly because of the sudden decrease in estrogen production associated with the loss of ovarian function at menopause [5].

Numerous studies have demonstrated that estrogen plays a critical role in bone metabolism. Estrogen deficiency after ovariectomy (OVX) causes a significant decrease in osteoclast apoptosis and increase in osteoclast formation that promotes bone resorption [6,7]. Estrogen treatment reverses bone loss in estrogen-deficient postmenopausal women and several OVX animal models [5,7,8]. The exact mechanisms through which estrogen deficiency and treatment exert these effects are still being determined. A number of signaling pathways have been implicated, including those involving oxidative stress and proinflammatory cytokines, such as tumor necrosis factor α, interleukin-1, macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating facto, and interleukin-6 [5,9–11].

Obesity induced by high-fat diets has been demonstrated to increase bone resorption and/or decrease bone formation resulting in reduced bone mass and strength in various animal models with normal ovary function. Also, bone mineral density and bone strength were not found to be affected by OVX-induced hyperphagia, which indicates that increased body weight in obesity is not advantageous to bone health [12–15]. Furthermore, evidence is accumulating indicating that obesity is associated with decreased bone mass and increased fracture risk in humans [16–19]. Recently, Feresin et al [20] reported that leptin receptor–deficient obese OVX Zucker rats, a genetic rat model for obesity, had lower trabecular thickness (Tb.Th) than did heterozygous lean OVX controls when animals were fed the same normal-fat diet (AIN-93G, Harlan-Teklad, Madison, WI). However, the extent to which diet-induced obesity affects bone structure and markers of bone formation and resorption, as well as circulating estrogen levels in an animal model of postmenopause (OVX), is unknown.

The objective of the current study was to determine whether a high-fat diet-induced obesity mitigates or exacerbates bone structure deterioration and alters markers of bone resorption and formation in OVX mice. We hypothesized that a high-fat diet would induce significant increases in body weight gain and serum estradiol concentrations in OVX mice, but would not improve bone structural parameters. To test this hypothesis, bone structure and molecular characteristics of bone metabolism were determined in mice that were OVX or sham operated, and then fed a normal- or high-fat diet for 11 weeks.

2. Methods and materials

2.1. Animals and treatments

Thirty-six female C57BL/6 mice aged 4 months were either bilaterally OVX to mimic the postmenopausal osteoporosis or sham operated (Sham) by Charles River Laboratories (Wilmington, MA, USA). Upon arrival 1 week after the operation, mice were individually placed in Plexiglas ventilated cages located in an environmentally controlled pathogen-free facility with a 12-hour light/12-hour dark cycle. The animal protocol for the study was approved by the United States Department of Agriculture Agricultural Research Service Grand Forks Human Nutrition Research Center Animal Care and Use Committee. Animals were maintained and processed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were fed a Purina Rat Chow #5012 (Ralston-Purina, St Louis, MO, USA) while acclimating to our animal facility for 3 days. Then, sham or OVX mice were randomly assigned to 2 treatment groups (n = 9/group) and fed either a normal-fat purified diet (10% energy as fat, D12450B; Research Diets, New Brunswick, NJ, USA; Sham-10 or OVX-10) based on AIN-93G [21] or a high-fat diet (45% energy as fat) with extra fat from lard (Sham-45 or OVX-45) for 11 weeks. The composition of the experimental diets is described in Table 1. Mice had free access to diets and tap water throughout the study. Body weights were recorded.

2.2. Sample preparation

At the age of about 7 months, mice were euthanized with a ketamine cocktail (1.37:1 mixture of ketamine/xylazine [ketamine; Animal Health Co, St Joseph, MO, USA; xylazine: Phoenix Scientific, St Joseph, MO, USA]). Blood samples were collected and centrifuged at 1500*g* for 20 minutes at 4°C to obtain serum that was stored at –80°C until analyzed.

The right tibia of each mouse was removed, cleaned of adherent tissue, and stored at –20°C before being scanned by micro–computed tomography (μCT) as described below. The right femurs were flash-frozen in liquid nitrogen and stored at –80°C for later total RNA isolation.

2.3. Bone μCT evaluation

Either tibia or femur can be chosen for bone structure because structural parameters in proximal tibia and distal femur are highly correlated [12,22]. The tibia was placed in a holder of 10.2 mm in diameter and scanned using a Scanco μCT scanner 40 (Scanco Medical AG, Bassersdorf, Switzerland) at 12-μm isotropic voxel size with x-ray source power of 55 kV and 145 μA and integration time of 300 milliseconds. The gray-scale images were processed by using a low-pass Gaussian filter (sigma = 0.8, support = 1) to remove noise, and a fixed threshold of 275 was used to extract the mineralized bone from soft tissue and marrow phase. The reconstruction and 3-dimensional quantitative analyses were performed by using software provided by Scanco as previously described [12]. For tibial trabecular bone, 100 slices starting from about 0.1 mm distal to growth plate were chosen for analyses. The recommended guidelines for μCT scanning and bone histomorphometry nomenclature were followed. For tibial trabecular bone, bone volume (BV; mm^3), total volume (TV; mm^3), BV/TV (ratio of the segmented BV to the TV of the region evaluated; %), trabecular number (Tb.N; the average number of trabeculae per unit length; 1/mm), Tb.Th (mean thickness of trabeculae; mm), trabecular separation (Tb.Sp; mean distance between trabeculae; mm), connectivity density (Conn.D; a degree of connectivity of trabeculae normalized by TV;

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