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Obesity-related changes in bone structural and material properties in hyperphagic OLETF rats and protection by voluntary wheel running

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ABSTRACT

Purpose. To examine how the development of obesity and the associated insulin resistance affect bone structural and material properties, and bone formation and resorption markers in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat model.

Methods. This was a 36-week study of sedentary, hyperphagic, male OLETF rats (OLETF-SED), exercise-treated OLETF rats (OLETF-EX) and sedentary non-hyperphagic controls (LETO-SED) with data collection at 13, 20, and 40 weeks of age (n = 5–8 animals per group per timepoint).

Results. Body mass and fat (%) were significantly greater in OLETF-SED versus controls. OLETF-SED were insulin resistant at 13 and 20 weeks, with overt diabetes by 40 weeks. At 13 weeks, OLETF-SED had lower total body BMC and BMD and serum P1NP compared with LETO-SED. Differences in total body BMC and BMD between OLETF-SED and LETO-SED persisted at 20 weeks, with reductions in total and cortical BMD of the tibia. OLETF-SED also had lesser femur diameter, cross-sectional area, polar moment of area, and torque at fracture than LETO-SED. By 40 weeks, OLETF-SED had elevated bone resorption and reduced intrinsic bone strength. OLETF-EX did not show the excessive weight gain, obesity, insulin resistance or diabetes observed in OLETF-SED. OLETF-EX had greater BMD than OLETF-SED, and structural and material properties of the femur were significantly increased in OLETF-EX relative to OLETF-SED and LETO-SED.

Conclusions. The negative skeletal effects of excessive adiposity and insulin resistance were evident early in the progressive obesity with lasting negative impacts on intrinsic and extrinsic bone strength. Exercise protected against obesity-associated skeletal changes with marked benefits on the biomechanical properties of bone.

1. Introduction

Overweight and obesity are associated with increased risk of many chronic diseases, and recently it has been recognized that excess adiposity and associated metabolic dysregulation can negatively affect bone health [1–4]. Obese individuals have reduced bone mineral density (BMD) relative to body weight [3,5], and fracture risk at certain skeletal sites is increased in overweight or obese men and women who have normal BMD [6,7]. Moreover, insulin resistance (IR), as well as frank type 2

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diabetes (T2DM), also is associated with increased fracture risk [2].

The increased risk of fracture associated with obesity can be attributed, in part, to reduced bone quality, which is determined by both the structural and material properties of bone [8]. Numerous mechanisms have been hypothesized by which excess adiposity might influence bone quality, including altered hormone and cytokine release [9], metabolic dysregulation, changes in mechanical loading, increased adipogenesis at the expense of osteoblastogenesis [10,11], and alterations in insulin signaling [12]. Studies that examine changes in bone parameters during the development of obesity and metabolic dysregulation would provide valuable insight into the mechanisms by which obesity and diabetes reduce bone quality and increase fracture risk. However, these studies are not feasible in humans due to both the required duration and limitations in measurable bone outcomes. Consequently, various experimental animal models of genetic and diet-induced obesity and drug-induced diabetes have been used to study the effects of obesity and insulin resistance on bone.

Although useful, genetic and pharmacologic models, as well as models of diet-induced obesity have limitations that might compromise the translational relevance of the model to human disease. Drug-induced models of diabetes (e.g., streptozotocin) result in acute hypoinsulinemia and hyperglycemia, whereas humans develop hyperglycemia and hyperinsulinemia followed by pancreatic insufficiency, hypoinsulinemia and frank diabetes that occur in human T2DM. Pharmacologic animal models of insulin resistance or diabetes also adversely affect growth, including skeletal growth. To correspond to obesity and T2DM in humans, diabetes onset should not occur during rapid skeletal growth, but rather close to the time of skeletal maturation. In genetic models of obesity and diabetes that are due to structural or signaling defects in the leptin receptor (e.g., Zucker diabetic fatty), it is difficult to differentiate the effects of excess adiposity and insulin resistance from altered leptin signaling because leptin has direct effects on bone [13]. Likewise, diet-induced obesity (DIO), i.e., obesity that is induced by feeding rodents high-fat diets (e.g., 45% or 60% of kcal from fat as soybean oil and lard), also has limitations. Rat strains vary in their susceptibility to DIO and individual animals may be resistant to DIO. In addition, the amount, type, and source of fat added to the animal diet can affect the metabolic response, with high-saturated fat diets generally producing more severe metabolic dysfunction [14,15]. Especially important to the study of T2DM, some DIO models result in excess body fat and hyperinsulinemia, but normoglycemia [16].

An alternative rodent model in which to study the skeletal effects of obesity and T2DM is the Otsuka Long-Evans Tokushima Fatty (OLETF) rat. Selectively bred for null expression of the cholecystokinin-1 receptor, OLETF rats exhibit a within-meal feedback defect for satiety, resulting in hyperphagia and obesity, and the spontaneous development of insulin resistance (IR) and type 2 diabetes [17,18]. Body mass and fat pad mass are significantly greater than non-hyperphagic lean Long-Evans Tokushima Otsuka (LETO) controls by 5 weeks of age, and IR is evident by age 13 weeks. Obesity and IR progressively worsen, resulting in frank T2DM by age 40 weeks. The OLETF rat model is well-suited for the study of the effects of obesity and insulin resistance on bone in humans because the onset of insulin resistance is progressive and coincides with near completion of skeletal growth, which occurs at ~16 weeks in the rat [19].

Currently, lifestyle modifications, such as exercise, are recommended for the treatment of obesity and T2DM. Exercise improves overall metabolic health by increasing insulin sensitivity [20], reducing chronic inflammation [21–23], and reducing circulating lipids and might, therefore, indirectly benefit bone in obese, diabetic individuals. In addition, in normal weight, healthy individuals, exercise has direct positive effects on bone strength due to increased mechanical loading on the skeleton. Mechanotransduction to the osteocytes occurs via cell deformation, changes in extracellular fluid shear stress, pressure gradients, and electrical fields [24,25], and the osteocytes modulate signaling to osteoblasts and osteoclast to increase bone formation [26]. A unique feature of the bone's adaptive response to exercise, which differs from cardiovascular or metabolic adaptations is that bone becomes refractory to loading relatively quickly. Animal data suggest that the maximal benefit is achieved after as few as 40–100 loading cycles [27], and mechanosensitivity is restored after 8–24 h of rest in experimental animals [28].

Voluntary wheeling running (VWR) and forced treadmill running are used to study the effects of exercise in rodents. VWR is spontaneous and allows intermittent exercise, and, unlike forced treadmill running, VWR does not induce systemic stress (i.e., elevated circulating corticosterone) [15,29]. Elevated corticosterone might negatively impact bone, as well as glucose homeostasis, and therefore confound the effects of exercise on bone. For these reasons, and because intermittent exercise is more osteogenic than continuous exercise [27], we used VWR in the present study.

The overall objective of this project was to improve our understanding of potential relationships between excessive adiposity, insulin resistance and bone health by using a rat model of hyperphagic obesity that results in progressive expansion of adipose tissue and loss of insulin sensitivity. The specific aims were: 1) to examine the aging-related temporal changes in skeletal outcomes (BMD, markers of bone formation and resorption, and bone structural and material properties) relative to the development of excess adiposity and insulin resistance in OLETF rats and lean LETO controls; and 2) to evaluate the potential of daily exercise via voluntary wheel running to mitigate detrimental effects of adiposity and T2DM on skeletal health in OLETF rats compared with LETO controls. We hypothesized that OLETF rats would have decreased BMD, structural and material properties, and increased bone resorption relative to formation compared with lean LETO-SED controls. We also hypothesized that voluntary wheel running would protect OLETF-EX against the negative skeletal effects of obesity and associated insulin resistance.

2. Methods

2.1. *Experimental design and animal protocol*

This was a 36-week longitudinal study of sedentary, hyperphagic, male OLETF rats (OLETF-SED), exercise-treated OLETF rats