Bone Reports 9 (2018) 74–83

Contents lists available at ScienceDirect

Bone Reports

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

Characteristics of bone strength and metabolism in type 2 diabetic model Tsumura, Suzuki, Obese Diabetes mice

Hiroaki Tanaka[a,*], Takenori Yamashita[b], Misao Yoneda[c], Satoshi Takagi[d], Toshihiro Miura[a]

[a] Graduate School of Health Science Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan
[b] Department of Radiological Technology, Faculty of Health Science, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan
[c] Department of Clinical Nutrition, Faculty of Health Science, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan
[d] Department of Physical Therapy, Faculty of Health and Medical Sciences, Tokoha University, 1230 Miyakoda, Kitaku, Hamamatsu, Shizuoka, 431-2102, Japan

ARTICLE INFO

Keywords:
Bone strength
Bone mass
Bone microstructure
Bone metabolism
Tsumura, Suzuki, Obese Diabetes mice
Type 2 diabetes mellitus

ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by hyperglycemia, hyperinsulinemia, and complications such as obesity and osteoporosis. The Tsumura, Suzuki, Obese Diabetes (TSOD) mouse is an animal model of spontaneous obese T2DM. However, bone metabolism in TSOD mice is yet to be investigated. The objective of the present study was to investigate the effects of T2DM on bone mass, metabolism, microstructure, and strength in TSOD mice.

Methods: We determined the following parameters in TSOD mice and Tsumura, Suzuki, Non-obesity (TSNO) mice (as controls): serum glucose levels; serum insulin levels; bone mass; bone microstructure; bone metabolic markers; and bone strength. We also performed the oral glucose tolerance test and examined histological sections of the femur. We compared these data between both groups at pre-diabetic (10 weeks) and established (20 weeks) diabetic conditions.

Results: Bone strength, such as extrinsic mechanical properties, increased with age in the TSOD mice and intrinsic material properties decreased at both 10 weeks and 20 weeks. Bone resorption marker levels in TSOD mice were significantly higher than those in the control mice at both ages, but there was no significant difference in bone formation markers between the groups. Bone mass in TSOD mice was lower than that in controls at both ages. The trabecular bone volume at the femoral greater trochanter increased with age in the TSOD mice. The femoral mid-diaphysis in TSOD mice was more slender and thicker than that in TSNO mice at both ages.

Conclusions: Bone mass of the femur was lower in TSOD mice than in TSNO mice because hyperinsulinemia during pre-diabetic and established diabetic conditions enhanced bone resorption due to high bone turnover. In addition, our data suggest that the bone mass of the femur was significantly reduced as a result of chronic hyperglycemia during established diabetic conditions in TSOD mice. We suggest that bone strength in the femur deteriorated due to the reduction of bone mass and because the femoral mid-diaphysis was more slender in TSOD mice.

1. Introduction

Diabetes is associated with an increased risk of fragility fractures (Janghorbani et al., 2007; Melton 3rd et al., 2008). Albright and Reifenstein (1948) were the first to report the presence of low bone mineral density (BMD) and a high incidence of fractures in diabetic patients. More recently, a meta-analysis showed that BMD is reduced in patients with type 1 diabetes mellitus (T1DM) but is increased in patients with type 2 diabetes mellitus (T2DM) (Vestergaard, 2007). T1DM has been shown to be associated with reduced BMD and insulin deficiency (Nyman et al., 2011; Silva et al., 2009). In contrast, studies were more inconsistent in patients with T2DM, who do not exhibit insulin deficiency, with some showing a similar BMD (Hampson et al., 1998; Tuominen et al., 1999), a higher BMD (Hanley et al., 2003; Strotmeyer et al., 2004), or even a lower BMD (Gregorio et al., 1994; Yaturu et al., 2009) compared to non-diabetic patients. There are

Abbreviations: T2DM, type 2 diabetes mellitus; TSOD, Tsumura, Suzuki, Obese Diabetes; OCN, osteocalcin; TRAcP5b, tartrate-resistant acid phosphatase 5b; TSNO, Tsumura, Suzuki, non-obesity; BMD, bone mineral density; T1DM, type 1 diabetes mellitus; CSMI, cross-sectional moment inertia; BMC, bone mineral content; PBS, phosphate-buffered saline; micro-CT, micro-computed tomography; OGTT, oral glucose tolerance test

* Corresponding author.
E-mail address: tanahiro@humanitec.ac.jp (H. Tanaka).

https://doi.org/10.1016/j.bonr.2018.07.004
Received 2 October 2017; Received in revised form 13 July 2018; Accepted 19 July 2018
Available online 21 July 2018

several factors which might explain such disparities, including differences in the severity and duration of the disease, along with the relative impairment of glucose metabolism (Vestergaard, 2007), or the fact that hyperinsulinemia can accelerate factors related to bone formation (Ashraf et al., 2013). These findings were reported in clinical investigations and studies involving animal models of T2DM (Fajardo et al., 2014). In T2DM, low bone mass may occur because of the impairment of glucose metabolism and high bone mass due to elevated insulin levels, as this can promote anabolic activity.

Several studies have used T2DM animal models to investigate the relationship between fragility fractures and diabetes, although a suitable animal model for T2DM is yet to be established. The Tsumura, Suzuki, Obese Diabetes (TSOD) mouse strain was first developed in 1992 through the selective inbreeding of obese ddY mice (Suzuki et al., 1999). Analysis showed that only male TSOD mice showed hyperglycemia, hyperinsulinemia, urinary glucose, and obesity (Suzuki et al., 1999). In contrast, the Tsumura, Suzuki, Non-obesity (TSNO) mouse strain was simultaneously established from ddY mice as a control, and exhibits neither obesity nor hyperglycemia (Hirayama et al., 1999). In TSOD mice, three quantitative trait loci were identified on the chromosome that determines genetic blood glucose levels (Nidd4 on chromosome 11), controls body weight (Nidd5 on chromosome 2 and Nidd6 on chromosome 1), and insulin levels (Nidd5 on chromosome 2) (Hirayama et al., 1999). Furthermore, it is evident that the existing literature does not address issues related to the skeletal phenotype in either TSOD or TSNO mice.

Therefore, in the present study, we used TSOD mice to investigate the effect of T2DM progression on various key aspects of skeletal integrity, including bone mass, bone metabolism, bone microstructure, and bone strength. These parameters were characterized during prediabetic conditions (age: 10 weeks) and during established diabetic conditions (age: 20 weeks).

2. Material and methods

2.1. Animals

We studied male TSOD and TSNO mice (The Institute for Animal Reproduction, Ibaraki, Japan) (n = 6/group) from 6 weeks of age to 10 or 20 weeks of age. The mice were housed and had free access to food (CE-2; Clea Japan Inc., Tokyo, Japan) and water. The animal room was maintained at 22 ± 2 °C with a 12-h light (8:00–20:00) and dark (20:00–8:00) cycle. Before the mice were sacrificed by cervical dislocation at 10 and 20 weeks of age, we collected serum samples that were refrigerated until each analysis was carried out. A blood sample from each mouse was taken from the cavernous sinus with a capillary. Both femurs were removed from each mouse and cleaned of muscles and tendons. The right femur was wrapped in gauze soaked in phosphate-buffered saline (PBS) and stored at −40 °C until three-point bending test and micro-computed tomography (micro-CT) scanning were performed. The left femur was fixed in 10% neutral buffered formalin. Experimental protocols were approved by the Guidelines for the Care and Use of Laboratory Animals (Prime Minister's Office Directive No. 228, 2015).

2.2. Serum glucose level and oral glucose tolerance test

Serum glucose levels were measured using the Wako glucose CII-test (Wako Pure Chemical Industries, Osaka, Japan). An oral glucose tolerance test (OGTT) was performed on each mouse at 10 and 20 weeks of age. All mice were weighed and then deprived of food for the previous 20 h. A glucose (2 g/kg) solution was given by weight, and blood samples were taken from the cavernous sinus before and at 30, 60, and 120 min after administration of the glucose solution. In addition, the glucose area under the curve (AUC), an index of whole glucose excursion after glucose loading, was calculated in accordance with a previous study (Sakaguchi et al., 2016).

2.3. Biochemical analyses

Serum insulin levels were measured with the Mouse Insulin ELISA KIT (AKRIN-011T, Shibayagi, Gunma, Japan). Serum osteocalcin (OCN) levels were measured with Mouse Osteocalcin EIA Kit (BT-470, Biomedical Technologies Inc., Stoughton MA, USA). Serum tartrate-resistant acid phosphatase form 5b (TRAcP5b) levels were measured with Mouse TRAP™ Assay (Immunodiagnostic Systems Inc., Fountain Hills AZ, USA).

2.4. Determination of bone mineral content and bone mineral density of the femur

Bone mineral content (BMC) and BMD of the greater trochanter and the mid-diaphysis of the right femur in all mice were measured using dual X-ray absorptiometry with an apparatus for small animals (DICHROMA SCAN DCS-600; ALOKA, Tokyo, Japan) at both 10 and 20 weeks of age. The mice were anesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg) and the measurements were performed with extended hip and knee joints, i.e. flexion of each.

2.5. Micro-computed tomography measurements

Bone microstructure in the greater trochanter and the mid-diaphysis of the femur were assessed with micro-CT (SMX-90CT, SHIMADZU, Kyoto, Japan) at 23 μm × 23 μm × 23 μm voxel size with an X-ray power source of 90 kV and 110 μA. All bones were thawed to room temperature and placed in PBS during scanning. The greater trochanter of the femur was scanned at constant intervals of 23 μm in a region 460 μm in length from the inferior border of the femoral head, and the mid-diaphysis was scanned at the same intervals and length in a region of the central part of the femur. Then, the trabecular bone fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular spacing (Tb.Spac) were measured in the greater trochanter. The cortical bone volume (Ct.V), total bone volume (Tt.V), cortical volume fraction (Ct.V/Tt.V), cortical porosity (Ct.Po), cortical thickness (Ct.Th) and cross-sectional moment inertia (CSMI) were measured in the mid-diaphysis respectively. Three-dimensional measurements and structural analyses were performed with bone analysis software, TRI/3D-BON (RATOC System Engineering, Tokyo, Japan). The images were binarized with a threshold range between 1 and 255 (gray values), and then each parameter was measured automatically according to a software program. Grayscale images were segmented using a median filter to remove noise with a fixed threshold to extract bone components.

2.6. Biomechanical analyses

Extrinsic mechanical properties and intrinsic material properties of the femoral mid-diaphysis were determined using a three-point bending test. The site of testing was matched to micro-CT sampling sites for the femoral mid-diaphysis (centered at 50% of total bone length). Prior to the three-point bending test, anteroposterior surface diameters were measured at the femoral mid-diaphysis using calipers for the calculation of toughness. Bones were thawed at room temperature and placed in PBS until tested. The span between the lower supports was 10 mm for the femur, which was oriented posterior side down. Quasi-static, displacement-controlled loading (2 mm/min) was applied to the upper surface (anterior for femur) until a fracture was caused using a mechanical testing machine (EZtest; SHIMADZU, Kyoto, Japan). All bones were kept moist with PBS immediately prior to testing to maintain hydration. All data were analyzed with software (Factory SHiKiBU2000; SHIMADZU, Kyoto, Japan). Extrinsic mechanical properties included ultimate force (maximum load during the test), fracture