HOSTED BY

ELSEVIER

CrossMark

Available online at www.sciencedirect.com

ScienceDirect

Osteoporosis and Sarcopenia 2 (2016) 25–29

http://www.elsevier.com/locate/afos

Original article

Femoral bone structure in Otsuka Long-Evans Tokushima Fatty rats

Akira Minematsu [a,*], Tomoko Hanaoka [b], Yoshihiro Takada [b,c], Shunji Okuda [d], Hidetaka Imagita [a], Susumu Sakata [e]

[a] Department of Physical Therapy, Faculty of Health Science, Kio University, 4-2-2 Umaminaka, Koryo-cho, Kitakatsuragi-gun, Nara 635-0832, Japan
[b] Division of Health Science, Graduate School of Health Science, Kio University, 4-2-2 Umaminaka, Koryo-cho, Kitakatsuragi-gun, Nara 635-0832, Japan
[c] Department of Human Behavior, Faculty of Human Development, Kobe University, 3-11 Tsurukabuto, Nada-ku, Kobe, Hyogo 657-8501, Japan
[d] Department of Modern Education, Faculty of Education, Kio University, 4-2-2 Umaminaka, Koryo-cho, Kitakatsuragi-gun, Nara 635-0832, Japan
[e] Department of Physiology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8521, Japan

Received 17 August 2015; revised 16 November 2015; accepted 31 December 2015
Available online 14 March 2016

Abstract

Objectives: Type 2 diabetes mellitus (T2DM) increases fracture risk despite normal to high levels of bone mineral density. Bone quality is known to affect bone fragility in T2DM. The aim of this study was to clarify the trabecular bone microstructure and cortical bone geometry of the femur in T2DM model rats.
Methods: Five-week-old Otsuka Long-Evans Tokushima Fatty (OLETF; n = 5) and Long-Evans Tokushima Otsuka (LETO; n = 5) rats were used. At the age of 18 months, femurs were scanned with micro-computed tomography, and trabecular bone microstructure and cortical bone geometry were analyzed.
Results: Trabecular bone microstructure and cortical bone geometry deteriorated in the femur in OLETF rats. Compared with in LETO rats, in OLETF rats, bone volume fraction, trabecular number and connectivity density decreased, and trabecular space significantly increased. Moreover, in OLETF rats, cortical bone volume and section area decreased, and medullary volume significantly increased.
Conclusions: Long-term T2DM leaded to deterioration in trabecular and cortical bone structure. Therefore, OLETF rats may serve as a useful animal model for investigating the relationship between T2DM and bone quality.

Keywords: Cortical bone geometry; Micro-CT; Otsuka Long-Evans Tokushima Fatty (OLETF) rats; Trabecular bone microstructure

1. Introduction

Type 2 diabetes mellitus (T2DM) increases the risk of fracture despite normal to high levels of bone mineral density (BMD) [1–3]. Vestergaard reported a 1.4-fold increase in hip fracture risk in patients with T2DM, as compared with in healthy people without T2DM, though BMD Z-score of the spine and hip in T2DM patients was high [4]. Thus, it seems likely that bone fragility in T2DM is affected by bone quality rather than bone mass. Bone quality, that reflects bone strength, encompasses bone architecture, turnover, damage accumulation and mineralization [5]. Low bone turnover in T2DM was evident by decreased bone formation/resorption markers, though both the markers were reported to increase in several studies [1–3,6,7]. Moreover, T2DM was associated with decreased total bone area, decreased trabecular bone score (TBS) [2,6,8], decreased cortical bone area and increased cortical porosity [2,3,6,9]. Accumulation of advanced glycation end products impaired enzymatic cross-links and resulted in excessive formation of non-enzymatic cross-links, leading to a decline in bone properties and detrimental effects on bone cells [1–3,6,10]. Patients with inadequately controlled T2DM showed about 1.5-fold increase in fracture risk despite having a higher BMD, as compared with healthy people without

* Corresponding author.
E-mail address: a.minematsu@kio.ac.jp (A. Minematsu).
Peer review under responsibility of The Korean Society of Osteoporosis.

http://dx.doi.org/10.1016/j.afos.2015.12.001

diabetes. When T2DM was adequately controlled, there was no difference in fracture risk between T2DM patients and healthy people [11]. Low trabecular bone score was associated with worse glycemic control in T2DM [8], suggesting that hyperglycemia may affect factors that influence bone metabolism. However, there has been no consensus regarding the effects of T2DM on bone metabolism and properties as yet.

Animal models were widely used to examine not only T2DM pathophysiology but also the effects of T2DM on bone quality factors. Since each DM model strain had unique characteristics, the most appropriate DM model was chosen for the study's purpose [12]. Otsuka Long-Evans Tokushima Fatty (OLETF) rats displayed the clinical and pathological features of human non-insulin-dependent diabetes mellitus (NIDDM), as well as the characteristics of late-onset hyperglycemia, chronic DM, mild obesity, male inheritance, hyperplastic foci of pancreatic islets and renal complications [13]. Omi et al. investigated bone metabolism in OLETF rats, and found that OLETF rats can serve as a useful model for NIDDM with osteopenia [14]. However, they did not investigate trabecular bone microstructure (TBM) or cortical bone geometry (CBG). Only a few studies have reported bone-related parameters in OLETF rats so far, though bone structure has been investigated in other rat strain models [15–22]. Thus, TBM and CBG remained unclear in OLETF rats, of which symptoms were similar to those of human T2DM.

The aims of this study were to clarify TBM and CBG in OLETF rats in order to gain further insight on bones in long-term T2DM patients and also to examine whether OLETF rats can serve as a useful animal model for investigating the relationship between T2DM and bone quality.

2. Methods

2.1. Animals

This study was performed in accordance with the Animal Experimentation Guidelines set forth by the Committee for Research Facilities of Laboratory Animal Science at Kio University. Five-week-old male OLETF (n = 5) rats were used as a model for T2DM, and LETO (n = 5) rats were used as controls (Japan SLC Inc., Hamamatsu, Japan). All rats were housed in cages at 23 ± 2 °C under a 12-h day–night cycle for 17 months. They were fed a standard rat chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* throughout the experiment. At the age of 18 months, femurs were harvested from all rats under anesthesia.

At the point of analysis (at the age of 18 months), the onset of T2DM in OLETF rats was demonstrated by increased levels of blood glucose concentration (229.3 ± 104.5 mg/dL) and of HbA1c (8.3 ± 1.3%), as compared with those of LETO rats (blood glucose concentration, 70.8 ± 11.5 mg/dL; HbA1c, 5.0 ± 0.1%).

2.2. Bone structure analysis

Left femurs were dissected out, and soft tissues were removed. Using an X-ray micro-computed tomography (micro-CT; Hitachi Medical Corp., Tokyo, Japan), distal femurs were scanned at 65 kV, 90 μA, and with a voxel size of 21.3 μm, in the high-definition mode for analysis of TBM. The region of interest (ROI) for TBM was a 2 mm-length portion of the metaphysis, and therefore the first slice was scanned 1 mm distal from the physeal-metaphyseal demarcation. Likewise, the central portion of femurs were simultaneously scanned by micro-CT at 65 kV, 90 μA, and with a voxel size of 18.1 μm using the high-definition mode for analysis of CBG. The ROI for CBG was a 2 mm-length portion distal from the center of the femur. Scanned data were transmitted to a personal computer, in which TBM and CBG of the ROI were analyzed using the bone analysis software (TRI BON 3D, RATOC System Engineering Co. Ltd., Tokyo, Japan). Individual TBM and CBG parameters were obtained by analyzing 104 slice data from one ROI per animal.

Parameters of TBM were tissue volume (TV), bone volume (BV), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), connectivity density (Conn.D), trabecular bone pattern factor (TBPf), structure model index (SMI) and degree of anisotropy (DOA). Parameters of CBG were cortical bone volume (CV), all bone volume (AV), medullary volume (MV), cortical bone ratio (CV/AV), cortical bone thickness (Ct) and cortical bone section area (CSa).

2.3. Statistical data analysis

All values are expressed as mean ± standard deviation. Differences between OLETF and LETO rats were examined with the Mann–Whitney U test. All statistical analyses were performed using the Excel Statistics software (Excel 2012 version 1.15 for Windows; Social Survey Research Information Co. Ltd., Tokyo, Japan). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Body weight and femur length

There was no significant difference in final body weight between LETO rats (575.7 ± 18.3 g) and OLETF rats (496.1 ± 87.6 g). However, femur length was significantly shorter in OLETF rats (39.4 ± 1.2 mm) than in LETO rats (41.8 ± 0.3 mm).

3.2. TBM and CBG parameters

OLETF rats at the age of 18 months showed the deterioration in TBM and CBG parameters compared with LETO rats at the same age (Table 1). In TBM parameters, BV, BV/TV, Tb.N and Conn.D were significantly lower, and Tb.Th, Tb.Sp, TBPf and SMI were significantly higher, in OLETF rats than in LETO rats. With respect to CBG parameters, CV, CV/AV and CSa were significantly lower in OLETF rats than in LETO rats. To the contrary, MV was significantly higher in OLETF rats than in LETO rats (Table 1).