Micron 71 (2015) 14–21

Contents lists available at ScienceDirect

Micron

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

X-ray imaging characterization of femoral bones in aging mice with osteopetrotic disorder

Shu-Ju Tu[a,*], Hong-Wen Huang[b], Wei-Jeng Chang[b]

[a] Department of Medical Imaging and Radiological Sciences, College of Medicine, Chang Gung University, Kwei-Shan, Tao-Yuan 333, Taiwan
[b] National Laboratory Animal Center, National Applied Research Laboratories, Nan-Kang, Taipei 115, Taiwan

ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 3 December 2014
Accepted 22 December 2014
Available online 3 January 2015

Keywords:
Micro CT
Trabecular bone
Cortical bone
Femur
Bone mineral density
Biomechanical strength

ABSTRACT

Aging mice with a rare osteopetrotic disorder in which the entire space of femoral bones are filled with trabecular bones are used as our research platform. A complete study is conducted with a micro computed tomography (CT) system to characterize the bone abnormality. Technical assessment of femoral bones includes geometric structure, biomechanical strength, bone mineral density (BMD), and bone mineral content (BMC). Normal aging mice of similar ages are included for comparisons. In our imaging work, we model the trabecular bone as a cylindrical rod and new quantitative which are not previously discussed are developed for advanced analysis, including trabecular segment length, trabecular segment radius, connecting node number, and distribution of trabecular segment radius. We then identified a geometric characteristic in which there are local maximums (0.0049, 0.0119, and 0.0147 mm) in the structure of trabecular segment radius. Our calculations show 343% higher in percent trabecular bone volume at distal-metaphysis; 38% higher in cortical thickness at mid-diaphysis; 11% higher in cortical cross-sectional moment of inertia at mid-diaphysis; 42% higher in cortical thickness at femur neck; 26% higher in cortical cross-sectional moment of inertia at femur neck; 31% and 395% higher in trabecular BMD and BMC at distal-metaphysis; 17% and 27% higher in cortical BMD and BMC at distal-metaphysis; 9% and 53% higher in cortical BMD and BMC at mid-diaphysis; 25% and 64% higher in cortical BMD and BMC at femur neck. Our new quantitative parameters and findings may be extended to evaluate the treatment response for other similar bone disorders.

1. Introduction

Bones are critical organs for protection and supporting of interior organs. Bones and muscles are integrated components of our musculoskeletal system for essential functions of biomechanical performance. Contrast to the commonly observed disorder of osteoporosis in the aging population or postmenopausal females, a rare condition of skeletal defect with deposition of excessive calcification to bones such as osteopetrosis may be developed (Bilezikian et al., 2008; Greenspan, 1991). As a consequence, bones may grow abnormal with exceptionally high density (Greenspan, 1991).

At our main facility of small animals, we observed incidents of massive calcification in femoral bones in the aging population of BALB/c mice in our quality control process. Among these rare and abnormal aging mice, we observed three mice with exceptionally high-density of trabecular bones in the proximal and distal metaphysis sites. Particularly, the entire space of diaphysis is filled with high-density of trabecular bones. Similar findings are not reported and discussed in the previous literature.

In clinical settings, dual-energy X-ray absorptiometry (DXA) is the major imaging examination of bone mineral density (BMD) and bone mineral content (BMC) (Blake et al., 1992; Laskey, 1996). However, the image acquired from DXA is a 2D X-ray radiograph. Essential characteristics of 3D geometric structure in trabecular and cortical bones are not able to be identified in DXA. In recent advancement of imaging technology, images of spatial resolution for X-ray based micro computed tomography (CT) are significantly improved to the scale of micrometers (Holdsworth and Thornton, 2002; Ritman, 2011). In addition, images of micro CT are truly volumetric and isotropic.

BMD and structure of trabecular bones are rapidly declined in aging mice. In general, trabecular bones in femoral bones are normally found at the proximal and distal metaphysis (Bilezikian et al., 2008). Typically, mid-diaphysis of a femur bone is filled entirely

* Corresponding author at: Department of Medical Imaging and Radiological Sciences, College of Medicine, Chang Gung University, Wen-Hua 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan. Tel.: +886 3 2118800; fax: +886 3 2118700.
E-mail address: sjtu@mail.cgu.edu.tw (S.-J. Tu).

http://dx.doi.org/10.1016/j.micron.2014.12.007
0968-4328/

with bone marrow and empty with trabecular bones (Bilezikian et al., 2008). To characterize the abnormality of femoral bones in these aging mice which are rarely observed, a complete study with a micro CT scanning system is performed to evaluate the associated geometric structure, biomechanical strength, BMD, and BMC for their femoral bones. Normal BLAB/c mice of similar ages are included for comparisons. In particular, we also developed quantitative parameters which are not discussed in previous literature such as trabecular segment number and connecting node number for advanced analysis.

2. Materials and methods

The ages of mice with an abnormal condition in femoral bones are 12, 17 and 20 months, while the corresponding normal aging BALB/c mice of similar ages are 12 months ($n = 3$). The strain type of our mice is BALB/c. Femoral bones were removed after euthanasia, freed from muscle and other soft tissues, and then fixed in the paraformaldehyde solution as the imaging specimen prior to the micro CT scanning. Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of Chang Gung University (ID: CGU12-147) and National Laboratory Animal Center (ID: IACUC2011001 and IACUC2013001).

We first acquire X-ray radiographic images for the entire femoral bone to show the global structures of cortical and trabecular bones at anatomical sites of proximal-metaphysis, diaphysis, distal-metaphysis, and femoral neck for the abnormal and normal aging mice. The radiography allows us to easily compare the bone structures between abnormal and normal aging mice. The images were acquired with both low X-ray energy of 30 kV and high energy of 100 kV. X-ray photons were transmitted through the specimen samples and received by a high-resolution detector made by a charge-coupled device (Bushberg et al., 2011; Laskey, 1996).

The micro CT imaging system (SkyScan 1076, Bruker micro CT, Belgium) is a commercial scanning instrument in which the X-ray tube and detector are housed in the same radiation-shield unit. X-ray beam is collimated as a cone beam system. The cone angle is less than 5 angular degrees. A charge-coupled device of high resolution with 11 million pixels and high quantum efficiency is installed as the detector. The distance between the X-ray tube and detector is 17.0 cm. Volumetric images with spatial resolution of 9.0 μm were obtained by a high-speed program based on the FDK reconstruction algorithm (Feldkamp et al., 1984; Tu et al., 2006). The animal couch is made of carbon fiber material. Fixed specimens of femoral bones were scanned with hardware settings of 50 kV and 360 projections. An aluminum filter of 0.5 mm thickness was placed at the exit window for optimal contrast. We used a standard phantom set (QRM-microCT-HA, QRM GmbH, Moehrendorf, Germany) for calculations of BMD in mg-HA/cm^3 and BMC in mg-HA (Barbour et al., 2010; Kalender, 2006).

In this work, we used anatomical sites of femoral bones for image assessment of trabecular and cortical bones. For the image analysis of distal-metaphysis, we first identified the growth plate as the geometric reference. A total length of 2.0 mm is then delineated as the region of interest (Bouxsein et al., 2010; Glatt et al., 2007). The distance between the region of interest and the growth plate is 0.5 mm (Bouxsein et al., 2010; Glatt et al., 2007). We delineated a length of 0.5 mm at the mid-point between the growth plate and trochanteric forssa of the femoral bone along the straight long-axis direction for analysis of mid-diaphysis (Barbour et al., 2010; Bouxsein et al., 2010). For biomechanical strength assessment of cortical bones in femur neck, images of micro CT are first rotated to the upright position. Then the central rotation axis was determined by the formula of center-of-mass. The region of interest with length of 0.1 mm along the straight long-axis was delineated (Hartog, 1987; Young et al., 2011b).

CTAn (CT-Analyzer, Bruker microCT, Belgium), Avizo (Visualization Sciences Group, Massachusetts, USA), and ImageJ (National Institute of Health, Maryland, USA) were used for the delineation of regions of interest and subsequent image analysis. The following parameters are obtained from CTAn: total tissue volume, bone volume, percent bone volume, trabecular separation, trabecular number, and fractal dimension. The following parameters are obtained by Avizo: trabecular segment number, trabecular segment number density, mean trabecular segment radius, mean trabecular segment length, connecting node number, and connecting number density (Bilezikian et al., 2008; Bouxsein et al., 2010; Glatt et al., 2007; Qiu et al., 2010). ImageJ was used for parameters of structural model index and surface area to volume ratio. In this work, trabecular bones are considered as a geometric structure which is interconnected by trabecular segments. A trabecular segment is modeled mathematically as a cylindrical rod. A connecting node is defined as the location in which different trabecular segments are connected.

For technical assessment of cortical bones at mid-diaphysis and femur neck, we used BMD, BMC, and the following parameters: total cross-sectional area, cortical cross-sectional area, cortical cross-sectional area fraction, cortical outer radius, cortical inner radius, cortical thickness, cross-sectional moment of inertia, rotational moment of inertia, sectional modulus, and buckling ratio (Hartog, 1987; Young et al., 2011b). These parameters are related to the biomechanical strength and may be used to evaluate the fracture risk of femoral bones.

Results are presented as the format of mean ± standard error. The *t*-test is performed with Excel. The *p*-value is used to determine whether a difference of two groups is statistically significant. We use the convention that a result is significantly different when the *p*-value is less than 0.05.

3. Results

To show the projection view of full-structure for the entire femoral bones in both groups of aging mice, we used the X-ray imaging technique of both 30 and 100 kV energies to build the two-dimensional digital radiographs as shown in Fig. 1A–D. Trabecular bones fill up the spaces of proximal-metaphysis, distal-metaphysis, and entire diaphysis in abnormal mice as shown in Fig. 1A and B, while trabecular bones are nearly invisible in normal aging mice as shown in Fig. 1C and D. In particular, the mid-diaphysis region is empty with trabecular bones in normal aging mice, while trabecular bones are densely populated in abnormal aging mice.

A series of volumetric images acquired from the micro CT projections is reconstructed for femoral bones of abnormal and normal aging mice as shown in Fig. 2A–C. Different viewing planes from the coronal, axial, and sagittal directions are respectively shown (Bushberg et al., 2011; Kalender, 2006). The interior space of femoral bone is filled up with the trabecular bones in the abnormal aging mice and empty in the normal aging mice.

3D images obtained by the computer processing technique of surface rendering for the abnormal and normal aging mice at the distal-metaphysis and mid-diaphysis are shown in Fig. 3A–D (Rubin et al., 1994; Vos et al., 2003). Trabecular bones of abnormal aging mice fill up the entire space of distal-metaphysis and mid-diaphysis as shown in Fig. 3A and B, while trabecular bones are empty in the normal aging mice as shown in Fig. 3C and D.

Quantitative assessment of micro-structure for trabecular bones at distal-metaphysis are listed in Table 1. The total tissue volume in mm^3 is reduced to 11.47% in abnormal aging mice with normal aging mice as the baseline reference; bone volume in mm^3 is