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Femoral osteocyte lacunar density, volume and morphology in women across the lifespan

Yasmin Carter [a], C. David L. Thomas [b], John G. Clement [b], David M.L. Cooper [a,*]

[a] Department of Anatomy & Cell Biology, University of Saskatchewan, Saskatoon, Canada
[b] Melbourne Dental School, University of Melbourne, Victoria, Australia

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ABSTRACT

Osteocytes are believed to be the primary agents of mechanosensing in bone. Due to this important role in the structure–function relationship of bone, osteocytes and the spaces they occupy (lacunae) are of increasing interest. Changes in lacunae with age are of particular interest in women since they are more susceptible to bone loss and fragility associated with senescent diseases including osteoporosis. This study's purpose was to test whether differences exist in lacunar density (lacunae/mm^3 of bone), orientation and morphology in the cortex of adult women spanning the human lifespan. Anterior blocks from the femoral shaft from 30 women aged 20–86 years were imaged by synchrotron-radiation micro-CT. No significant relation between lacunar density and age was detected. A significant reduction in lacunar volume with age ($p < 0.001$) was observed, alongside changes in lacunar morphology. When divided into two groups (<50 and >50 years) the younger group's lacunae were ~30% larger and were flatter ($p < 0.001$) and less equant (spherical) ($p < 0.001$). To our knowledge the observation that lacunar volume and morphology change over the human lifespan is novel, potentially resulting from preferential surface infilling within the extracellular space. The functional impact of this infilling is unclear but such a change in scale likely impacts the mechanosensing function of the osteocyte network. Limitations in resolution prevented us from assessing if this infilling is associated with disruption of the canaliculi. This hypothesis warrants further investigation as, if confirmed, it would represent a profound negative impact on the osteocyte network and may provide new insights into age-related bone loss.

1. Introduction

During life, human bone undergoes a constant process of remodeling, in which old bone is removed (resorption) and replaced with new bone (formation). In the young, these twinned processes are balanced; however, with advancing age there comes a net increase in bone resorption and a loss of bone architectural quality leading to fragility and fractures associated with diseases such as osteoporosis (Rodan and Martin, 2000). Studies examining the effect of aging at the cellular level have been restricted due to the encasement of the bone cells, osteocytes, deep within the bone matrix. Because the osteocytes themselves cannot be visualized using X-ray based techniques the spaces in which they reside, lacunae, are used as proxy.

Osteocytes are the most ubiquitous cells found in bone and are connected through an extensive network of cell processes which allow for communication and nutrient transportation (Busse et al., 2010). Together the lacunae and the spaces formed around the cell processes, the canaliculi, form a network considered vital to maintaining bone homeostasis. The exact role of the lacuno-canalicular network (LCN), however, is not yet fully understood. Several functions of the LCN have been proposed including initiation of bone remodeling via ion-sensation and regulation of osteoid matrix maturation and mineralization (Kamioka et al., 2001). Currently a general consensus exists that osteocytes play a role in translating mechanical stimuli into biochemical signals, forming the basis of the mechanosensation and transduction systems (Knothe Tate et al., 2004). The surface area of the LCN is immense and in the adult male has been calculated to be 400 times larger than that of the Haversian systems and 130 times greater than the surface area of the trabecular rods and plates (Johnson, 1966). The sheer scale of this network of connected spaces and cells suggests that differences in the scale or morphology of the LCN could affect mechanosensation/transduction (Schneider et al., 2010).

Changes in LCN with age are of particular interest, especially in women since they are more susceptible to the bone loss and

* Corresponding author. Address: Department of Anatomy & Cell Biology, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK, Canada S7 N 5E5. Fax: +1 (306) 966 4298.

E-mail addresses: yasmin.carter@usask.ca (Y. Carter), cdthomas@unimelb.edu.au (C. David L. Thomas), johngc@unimelb.edu.au (J.G. Clement), dml.cooper@usask.ca (D.M.L. Cooper).

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fragility associated with senescent diseases such as osteoporosis. Osteocyte density in humans correlates with the biomechanical quality of bone (Ma et al., 2008), with differing density and volume causing variations in the apparent stiffness of the bone matrix (Yeni et al., 2001). Several studies have reported that lacunar density (number of lacunae per mm^3 of bone), a reflection of the abundance of lacunae, declines with age in males and females (Mori et al., 1997; Mullender et al., 1996b; Qiu et al., 2002a; Torres-Lagares et al., 2010), the rate and causes of this decline, however, is debated. Mori and colleagues (1997) reported that age-related decline of lacunar density in trabeculae of the human femoral head did not begin until 70 years of age, which was then followed by a sharp decline. More recent studies by Mullender et al. (2005) reported the decline to be linear while Qiu et al. (2002b) determined the decrease to be exponential.

The state of lacunae populations in osteoporotic patients is an intriguingly complex research area which is currently hotly debated. A number of studies report lacunar density to be increased in women with osteoporosis (Mullender et al., 1996b; Soicher et al., 2011; Vashishth et al., 2005). In contrast to this, a more recent study by Mullender et al. (2005) found reduced lacunar density in osteoporotic patients. This is consistent with studies by Mori et al. (1997) and Qiu et al. (2003a) who report lower lacunar densities in patients with a history of osteoporotic fracture and Zarrinkalam et al. (2011), who found reduced lacunar density in the lumbar vertebrae of osteoporotic sheep. Previous research has demonstrated that in some individuals destined to experience an osteoporotic fracture, bone is made with fewer osteocytes than normal (Qiu et al., 2003a), suggesting that some individuals may be predisposed to developing an osteoporotic fracture.

The study of cortical microstructure has gained momentum recently due to the increasing availability of high-resolution micro-CT, both desktop and synchrotron radiation-based (SR micro-CT). This technology provides highly accurate 3D images for analysis at micrometer or better resolutions and with much larger volumes of interest. SR micro-CT can be considered the gold standard in 3D bone imaging (Muller, 2009), producing more accurate and faster quantitative measurements than a similar desktop-CT setup (Bernhardt et al., 2004). SR micro-CT has been utilized to examine osteocyte lacunar density in 3D in rodents (Britz et al., 2012; Schneider et al., 2007) and humans (Carter et al., 2013; Hannah et al., 2010; Pacureanu et al., 2012). Quantification of human osteocyte lacunar morphology in 3D beyond simple measures of orientation has been limited (Carter et al., 2013), and mostly restricted to nano-scale studies of a few lacunae (van Hove et al., 2009; Vatsa et al., 2008). The purpose of this study was to test whether differences exist in the osteocyte lacunar density, orientation and morphology in the femoral cortex of adult women spanning the human lifespan using synchrotron-based micro-CT.

2. Materials and methods

2.1. Specimens

Cortical bone samples were obtained from the femora of 30 deceased women between the ages of 20 and 86. These femora form part of the Melbourne Femur Collection held at the University of Melbourne, Melbourne, Australia and were collected at autopsy with the informed consent of the donor's next-of-kin. The study was conducted with ethical approval from the Victorian Institute of Forensic Medicine (EC26/2000), the University of Melbourne (HREC 980139) and the University of Saskatchewan (Bio # 08–46). Individual samples with dimensions of approximately 2 × 2 × 5 mm were cut from the anterior mid-cortical region of the proximal femoral shaft. All samples were removed from the same region of the bone to minimize the effect of intra-individual variation in lacunar properties associated with the biomechanical axes (Carter et al., 2013). The proximal femoral shaft represents an ideal site of analysis as it has been the subject of recent investigation including regional variation in porosity (Thomas et al., 2005) and material density (Goldman et al., 2005). This region is subjected most strongly to compression forces with some incidental tensile forces dependant on bending associated with movement such as walking (Koch, 1917).

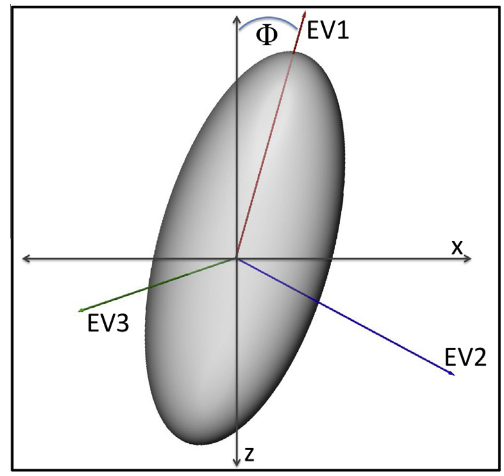

Fig. 1. Model demonstrating the coordinate system used for morphological analysis.

2.2. Synchrotron radiation micro-computed tomographic imaging (SR Micro-CT)

Synchrotron radiation micro-CT scanning was conducted at the Advanced Photon Source (APS), Argonne National Laboratory on beamline 2BM. Images were obtained using monochromatic X-rays with a photon energy of 27.9 keV and an effective pixel size of 1.47 μm. An exposure time of 250 ms per frame was employed for each of the 1500 frames spanning 180 degrees of rotation resulting in a scan time of approximately 17 min. The projection images were reconstructed to create a dataset containing 1408 slices (2048 × 2048 pixels).

2.3. 3D Quantitative morphometry

A cylindrical region of interest (ROI) with a diameter and height of 1.5 mm and a volume of 2.65 mm^3 was defined within each specimen (Fig. 2). Lacunae and canals within these ROI's were identified using a standardized global threshold, which separated the higher density bone from the air-filled lacunar and canal spaces, the same threshold was used for all specimens. Following segmentation, 'despeckling' was conducted on elements less than 10 μm^3, which were assumed to be noise, and elements above 2000 μm^3, which were assumed to be canals, the rest were assumed to be lacunae. These volume limits were based on confocal microscopy measurements of osteocyte volumes which found the minimum and maximum to be 28–1713 μm^3 (McCreadie et al., 2004).

Image stacks were cropped and analysis of the cortical canals was conducted using CT Analyzer 1.10.9.0 (SkyScan, Kontich, Belgium). Morphological analysis of the lacunar network was conducted using AMIRA 5.4.1 (Visage Imaging, Fuerth, Germany). Standard nomenclatures for canal (Cooper et al., 2003) and lacunar (Schneider et al., 2007) indices were used, for morphological parameters describing lacunar shape descriptions have been created by the authors (Carter et al., 2013). The extracted parameters