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Age-related changes in female mouse cortical bone microporosity

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ABSTRACT

Osteocyte lacunae are small cavities within the bone matrix. Their dimensions and spatial arrangement affect bone mechanical properties. Furthermore, their size and shape affect the strain in bone tissue close to the lacunae; hence, they are supposed to affect the mechanosensory function of the osteocytes residing in the lacunae. It was the purpose of this study to quantify the morphological features of osteocyte lacunae, whether these are affected by aging and whether these vary among different anatomical location. In addition, we aimed at quantifying the vascular canals as these affect bone's microporosity too. We quantified the microporosity in the fibular midshaft of young-adult and old female C57BL/6 mice. Using micro-computed tomography (μCT), we found that advanced age was associated with a significantly decreased vascular canal number and volume density. In aged mice, the mean volume of the lacuna was significantly smaller than in young animals and they were more round. Lacuna number density close to the neutral axis of the fibula was higher in older mice than in young ones. The characterization of bone microporosity presents a first step in further unraveling their potential role in age-related reductions in bone strength.

1. Introduction

Elderly people are vulnerable to low-trauma fractures; these fragility fractures are associated with substantial pain, morbidity, and even mortality and place a heavy burden on the individual. With an increasing population of elderly people, bone fragility has become a major public health problem, too. The high risk of sustaining a low-trauma fracture is related to reductions in bone mass and/or structural integrity [1,2], which are the main determinants of bone strength. With advancing age, an imbalance between bone formation and bone resorption results in thinning and complete loss of trabeculae and trabecularization of the cortex, which in turn weakens the bones, and increases fracture risk [3].

Bone fracture risk has mostly been associated with bone (micro-) structural parameters and resorption cavities [4–6]. Bone porosity also exists at a smaller length scale and includes vascular canals and the osteocyte lacuno-canalicular network (LCN). These cavities, their dimensions, and spatial arrangement can also directly affect bone strength and fracture risk, although their exact role is unclear. On the one hand, they can act as local stress concentrators to cause crack initiation [7,8]; on the other, they can act as barriers to slow down the propagation of microcracks [9]. In addition they can affect local bone mechanical properties [10] which again directly affects bone strength.

Alteration in bone microporosity may also affect bone strength in an indirect way. First, the vasculature plays a crucial role in nutrition of bone cells. Thus, any alterations in the vascular porosity could affect the supplement of minerals, oxygen, and nutrients for osteocytes, osteoclast and osteoblasts, hence, affect the integrity of bone [11]. Second, morphological alterations in the LCN could potentially affect the ability of osteocytes to sense and respond to mechanical stimuli as its morphology has been hypothesized to affect the transduction of strain at the level of the whole bone to the local osteocyte microenvironment [7].

Variations in bone microporosity may be related to the bone mechanical environment. A potential link between the morphology of osteocyte lacunae and local bone mechanical environment was first made by Vatsa et al. [12] who showed that osteocytes in the skull (small mechanical loads) were rounder and less longitudinally oriented than

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osteocytes in the load-bearing fibula; the latter being more elongated and directed parallel to the long axis of the bone.

The purpose of this study was to quantify potential age-related variations in the three-dimensional morphology of bone microporosity. Additionally, we aimed to investigate whether changes in the density and morphology of bone porosity are related to the local mechanical environment. We hypothesized that bone microporosity increases by aging. In addition to analyses of the whole midshaft, detailed site-dependencies of microstructural parameters were explored.

2. Materials and methods

2.1. Sample preparation and μCT imaging

One fibula per animal was extracted from six 5-month old female C57BL/6JRccHsd mice (22.35 ± 1.29 g, mean ± SD) as well as of six 23-month old female C57BL/6JRccHsd (30.12 ± 3.48 g, mean ± SD). Mice were purchased from Harlan (Horst, The Netherlands). Ethical approval for the animal procedures was given by the Animal Ethics Committee of KU Leuven (P075/2015). All mice were group housed in conventional conditions: 12-hour light, 12-hour dark cycle, standard diet (1% calcium, 0.76% phosphate), and water ad libitum in standard cages as reported previously [13]. Animals were bred and used in accordance with current Belgian national regulations for Animal Welfare and the 2010/63/EU directive. Mice were euthanized by cardiac puncture following deep anesthesia with i.p. pentobarbital (Nembutal, Ceva, Belgium; 100 mg/kg diluted 1:10 in PBS).

Each fibula was positioned in a custom fixture filled with PBS and scanned using a SkyScan 1172 (Bruker, Kontich, Belgium) μCT scanner. A 6 mm stack of the middle part of each fibula was scanned at 5 μm resolution. The samples were rotated over 180° at a rotation step of 0.4°. The X-ray settings were standardized to 49 kV and 200 μA with an exposure time of 590 ms. Two times frame averaging was used. The total scan time for one sample was about 1 h. Following scanning at macro level, 1.4 mm of the middle part of the same fibula was scanned at a nominal resolution of 0.7 μm (Fig. 1) to acquire microstructural cortical bone properties. The samples were rotated over 180° at a rotation step of 0.2°. The X-ray settings were standardized to 80 kV and 124 μA with an exposure time of 4123 ms. Four times frame averaging was used. The total scan time for one sample was approximately 6 h. One scan produced 2000 contiguous slices with a nominal resolution of 0.70 μm. Each slice contained 4000 × 4000 pixels. The scanning parameters at this resolution were selected to enhance the signal-to-noise ratio and the image contrast. The X-ray projections were reconstructed using a modified back-projection [14] reconstruction algorithm (NRecon 1.6.4.6 software-SkyScan) to create cross-sectional images. Reconstruction parameters included ring artifact reduction (RAR = 15), beam hardening correction (BHC = 20%), and misalignment correction. Additionally, a smoothing filter with a Gaussian window kernel (2 pixels) was applied.

2.2. Image processing and quantitative morphometry

Segmentation of cortical bone porosity was performed using our previously reported technique [15]. Briefly, a histogram-based global thresholding method was applied to the reconstructed bone to segment the mineralized tissue and nonmineralized structures. Small elements (noise) outside the cortical bone were removed using the sweep operation in CTAn software (v.1.14.4.1, SkyScan) which removed all but the largest object. Then, intracortical porosity comprising lacunae and canals was segmented by inverting the image and using the 3D despeckled filter in CTAn. The objects < 100 μm^3 were considered to be noise, elements with a volume ranging between 100 and 2000 μm^3 were assumed to be osteocyte lacunae, and the objects > 2000 μm^3 were considered to be canals. These volume limits were used in previous synchrotron-based studies [16–18] and were based on the confocal microscopy measurements indicating a size between 28 and 1713 μm^3 for each osteocyte [19]. Note that canaliculi were not segmented since their size was below the scan resolution used in this study. 3D renderings of the lacunae and canal network were created using Mimics

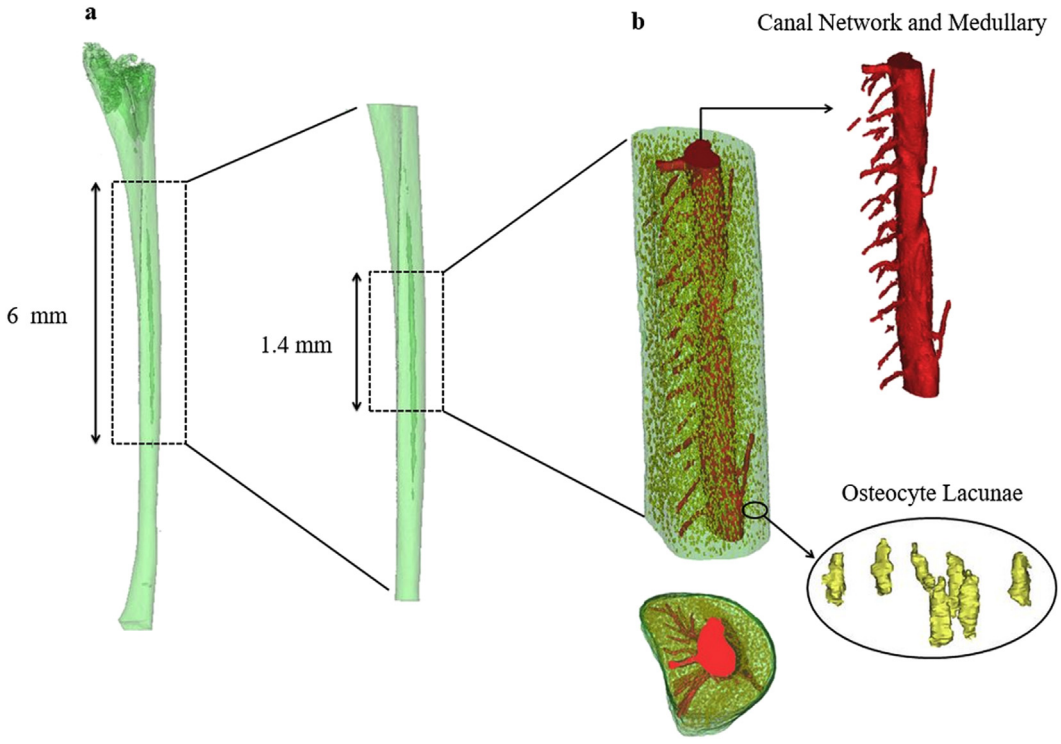

Fig. 1. Representation of volumes of interest at macro- and micro-level. (a) 3D rendering of a whole mouse fibula at 5 μm resolution illustrating the volume of interest for macro-analyses; (b) 3D rendering of the reconstructed images at 0.70 μm resolution demonstrating the volume of interest for micro-analyses including canal network, medullary canal and osteocyte lacunae.