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Organ and tissue level properties are more sensitive to age than osteocyte lacunar characteristics in rat cortical bone

Nina Kølln Wittig [a,1], Fiona Linnea Bach-Gansmo [a,1], Mie Elholm Birkbak [a], Malene Laugesen [a], Annemarie Brüel [b], Jesper Skovhus Thomsen [b], Henrik Birkedal [a,*]

[a] iNANO and Department of Chemistry, Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark
[b] Department of Biomedicine, Aarhus University, Aarhus, Denmark

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ABSTRACT

Modeling and remodeling induce significant changes of bone structure and mechanical properties with age. Therefore, it is important to gain knowledge of the processes taking place in bone over time. The rat is a widely used animal model, where much data has been accumulated on age-related changes of bone on the organ and tissue level, whereas features on the nano- and micrometer scale are much less explored. We investigated the age-related development of organ and tissue level bone properties such as bone volume, bone mineral density, and load to fracture and correlated these with osteocyte lacunar properties in rat cortical bone. Femora of 14 to 42-week-old female Wistar rats were investigated using multiple complementary techniques including X-ray micro-computed tomography and biomechanical testing. The body weight, femoral length, aBMD, load to fracture, tissue volume, bone volume, and tissue density were found to increase rapidly with age at 14–30 weeks. At the age of 30–42 weeks, the growth rate appeared to decrease. However, no accompanying changes were found in osteocyte lacunar properties such as lacunar volume, ellipsoidal radii, lacunar stretch, lacunar oblateness, or lacunar orientation with animal age. Hence, the evolution of organ and tissue level properties with age in rat cortical bone is not accompanied by related changes in osteocyte lacunar properties. This suggests that bone microstructure and bone matrix material properties and not the geometric properties of the osteocyte lacunar network are main determinants of the properties of the bone on larger length scales.

1. Introduction

The skeleton serves vital functions such as support for movement, organ protection, and as a calcium- and phosphate reservoir (Copp and Shim, 1963). Due to modeling and remodeling (Seeman, 2009), the structure and mechanical properties of bone change substantially with age (Mosekilde, 2000). For instance, a common problem for senescent individuals is reduced bone mass, bone mineral density (BMD) and increased risk of osteoporotic fractures and mortality (Johnell and Kanis, 2006). Hence, it is important to study how bone changes as a function of age.

The rat is a widely used animal model in studies of bone, and different aspects of how aging affects structural and mechanical properties of rat cortical bone on the organ (whole/significant part of bone, Fig. 1A) and tissue (Fig. 1B) level have been studied (Akkus et al., 2004; Bak and Andreassen, 1989; Danielsen et al., 1992, 1993; Fukada and Iida, 2004; Hoyer and Lippert, 1982; Iida and Fukuda, 2002; Jast and Jasiuk, 2013; Kiebzak et al., 1988; Nnakwe, 1995; Sontag, 1992; Takee et al., 2002; Vogel, 1979; Wronski et al., 1989; Zhang et al., 2015). Generally, the body weight (Danielsen et al., 1992, 1993; Fukada and Iida, 2004; Hoyer and Lippert, 1982; Iida and Fukuda, 2002; Jast and Jasiuk, 2013; Kiebzak et al., 1988; Nnakwe, 1995; Sontag, 1992), BMD (Danielsen et al., 1993; Fukada and Iida, 2004; Iida and Fukuda, 2002; Zhang et al., 2015), and load to fracture (Akkus et al., 2004; Bak and Andreassen, 1989; Danielsen et al., 1993; Hoyer and Lippert, 1982; Kiebzak et al., 1988; Takee et al., 2002; Vogel, 1979; Zhang et al., 2015; Bone Health and Osteoporosis, 2004) increase with age in the rat. However, discrepencies exist in the literature. For instance, Zhang et al. (2015) and Iida and Fukuda (2002) observed a decrease in BMD in male rat femora initiated at the age of 9 months and 18 months, respectively, and Akkus et al. (2004) observed a decrease in load to fracture in female rat femora from the age of 12 to 24 months.

It is becoming increasingly clear, that the micro- and nanostructural properties of bone are central in determining the quality (Felsenberg and Boonen, 2005) and performance of bone on the macroscale (Dunlop and Fratzl, 2010; Zimmermann and Ritchie, 2015; Tai et al., 2007), and studies employing techniques with high spatial resolution of bone are becoming more frequent (Hesse et al., 2015; Langer et al.,

* Corresponding Author.
E-mail address: hbirkedal@chem.au.dk (H. Birkedal).
[1] These authors contributed equally to the present work.

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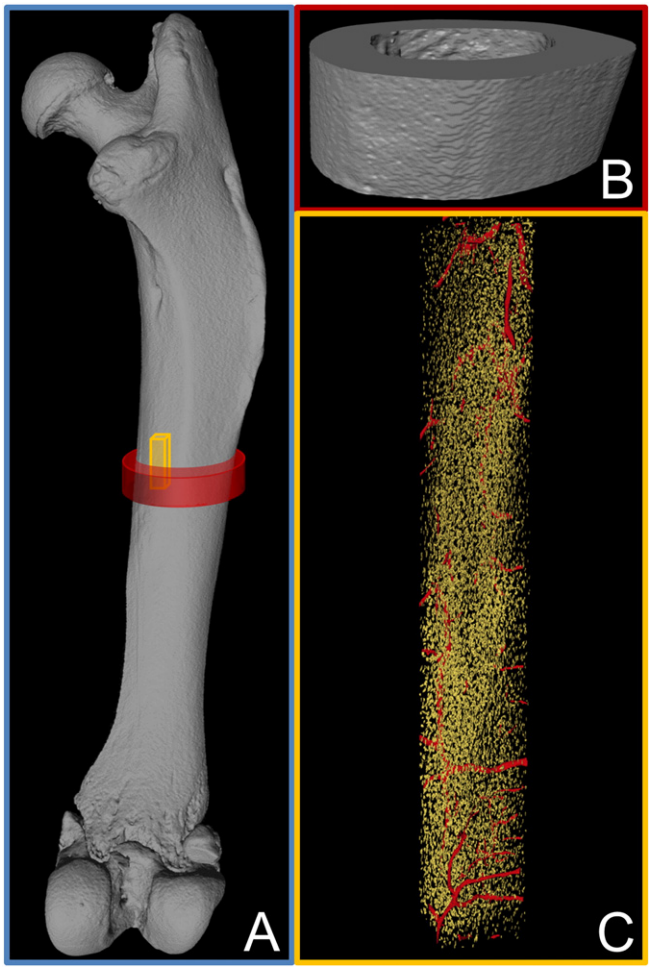

Fig. 1. Length scales investigated in this study range from A the whole bone (blue box) to B the tissue level (red box) and C the material level (yellow box). All images are renderings of μCT data, the whole bone and tissue level from desktop μCT and the material level from synchrotron μCT. In the latter, the internal void space is shown with blood vessel canals in red and osteocyte lacunae in yellow. In the blue box, red and yellow boxes indicate the measured volumes at the tissue and material level, respectively.

2012; Mader et al., 2013; Schneider et al., 2010). Hence, studies that correlate structural and mechanical properties of bone over several length scales are needed in order to better understand age-related changes.

Osteocytes orchestrate bone remodeling and act as mechanotransducers, but an increased understanding of how osteocytes and their activities influence the properties of bone at the tissue level is still needed (Bonewald, 2011; Klein-Nulend et al., 1995; Robling et al., 2008; Skerry et al., 1989; Tatsumi et al., 2007). Considering the large extent of the osteocyte lacunar–canalicular network (LCN) (Schneider et al., 2010), a change in osteocyte lacunar geometry or number could have a significant impact on e.g. the quality and mechanical properties of the whole bone.

The effect of age on osteocyte lacunar characteristics has predominantly been studied in humans and results have been contradictory. Carter et al. reported a decrease in osteocyte lacunar volume (Lc.V) and radii along with a change in lacunar shape, but found no change in osteocyte lacunar orientations or densities with age in women (Carter et al., 2013). However, other studies found the osteocyte lacunar density (Lc.D) to decrease with age (Busse et al., 2010; Mullender et al., 1996). Busse et al. reported an increased amount of hypermineralized calcium phosphate occlusions in osteocyte lacunae with age (Busse et al., 2010). This finding combined with the decrease in Lc.D with age was suggested to be a consequence of osteocytic apoptosis, possibly contributing to failure or delayed bone repair in aged bone. In contrast, Mullender et al. did not find an increased occurrence of osteocytic apoptosis with age (Mullender et al., 1996).

Despite the well established nature and widespread use of rat bone as a model for macrostructural bone studies, few studies have examined rat bone at the sub-100 μm scale. Rat cortical bone is not subjected to Haversian remodeling under normal conditions (Bentolila et al., 1998; Currey, 2002). Thus, the rat provides an excellent opportunity to study the effect of age on bone properties without the interference of Haversian remodeling. Rat femoral cortical bone consists of a central bone zone surrounded by circumferential lamellar bone on the endosteal and periosteal surfaces (Danielsen et al., 1993; Sontag, 1986). With age, the proportion of the two bone zones changes, with central bone being replaced by lamellar bone through combined periosteal apposition and endosteal resorption (Sontag, 1986, 1992). We have previously showed that there are marked differences between central and lamellar bone in rat cortical bone with regard to bone ultrastructure and osteocyte lacunar properties (Bach-Gansmo et al., 2013, 2015). The degree of bone mineralization has also been reported to differ between the two bone zones (Shipov et al., 2013).

The aim of this study is to link the evolution of bone with age across length scales ranging from the material to the organ level (Fig. 1) to test whether there is a coupled age-dependence of the osteocyte lacunar geometry or number and e.g. the quality and mechanical properties of the whole bone. We herein investigate the age development of rat cortical bone using multiple complementary techniques including X-ray micro computed tomography (μCT) at two distinct length scales and biomechanical testing. At the organ level (Fig. 1A) we determine the body weight, femoral length, and areal bone mineral density (aBMD). At the tissue level (Fig. 1B) we determine the mid-diaphyseal load to fracture (F_{max}), tissue volume (TV), bone volume (BV), marrow volume (MV), and tissue density (ρ_{tiss}). At the material level (Fig. 1C) high resolution synchrotron radiation μCT (SR μCT) data is used to determine different osteocyte lacunar properties as well as the local degree of mineralization, the material density (ρ_{mat}). The difference between ρ_{mat} and ρ_{tiss} stems from the length scale over which they are measured: ρ_{mat} will be a measure of the material density excluding osteocyte lacunae and blood vessels while ρ_{tiss} is a value obtained at a larger length scale thus averaging over lacunae and the smallest vessels. To the best of our knowledge, it is the first study to correlate this many parameters covering such a large span in length scales and animal ages.

2. Materials and methods

2.1. Animals and sample preparation

Forty-two 13-week-old female Wistar rats were randomized according to weight into 6 groups ($N = 7$). The rats received an injection of saline into the investigated right hind limb at 14 weeks, as the animals were used as control animals in a different study. The animals were weighed weekly. The animals were sacrificed 0, 4, 8, 12, 16, or 28 weeks after study start, i.e. at ages from 14 to 42 weeks. The right femora were dissected free, cleaned, and the length of the femora was measured using a digital caliper. The femora were frozen in Ringer's solution at −20 °C until further used. The experiment complied with the EU Directive 2010/63/EU for animal experiments, and was approved by the Danish Animal Experiments Inspectorate.

2.2. Dual-energy X-ray absorptiometry (DEXA)

The femora were DEXA scanned (Sabre XL, Norland Stratec, Pforzheim, Germany) using a pixel size of 0.5 mm × 0.5 mm. The areal Bone Mineral Density (aBMD) of the entire femora was determined using the scanner software. Quality assurance was performed by scans of the two solid-state phantoms provided with the scanner. The