Contents lists available at ScienceDirect



Journal of the Mechanical Behavior of Biomedical Materials

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



# Elastic modulus varies along the bovine femur



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## ARTICLE INFO

Keywords: Cortical bone mechanics Micro-computed tomography Micro-CT gray-level to elastic modulus conversion

## ABSTRACT

Bone is a heterogeneous material and its mechanical properties vary within the body. Variations in the mechanical response of different bone samples taken from the body cannot be fully explained by only looking at local compositional information at the tissue level. Due to different states of the stress within bones, one might expect that mechanical properties change over the length of a bone; this has not been a matter of systematic research in previous studies. In this study, the distribution of the tissue elastic modulus along the bovine femur is investigated using three-point bending tests. Two bovine femora were split to seven and eight blocks from proximal to distal metaphysis, respectively and twenty beam shaped bone samples were extracted and tested from each block. Based on our findings, the longitudinal elastic modulus follows a gradient pattern along the bovine femur as it increases along the bone from the proximal metaphysis to mid-diaphysis and then decreases toward the distal metaphysis again. Considering long bones to be subjected to bending loads, this mechanism alters the bone structure to support load in the regions where it is needed; similar as outlined by Wolff's law. In another part of this study, microfocus X-ray computed tomography (µCT) was found unable to predict the same trend of changes for the elastic modulus via image-based or density-based elastic moduli calculations. This is insofar important as conventional finite element models of bone are often directly shaped from uCT data. Based on our findings, it seems that current computed tomography based finite element models generated in this manner may not adequately capture the local variation of material behavior of bone tissue, but this may be improved by considering the changes of the elastic modulus along the femur.

#### 1. Introduction

Bone is a smart structure/material, which is capable of adapting to applied forces (Wolff, 1892), while achieving an optimum and minimal-weighted structure with high load bearing capacity. In general, bone is not subjected to one loading case but to various loading conditions. Considering long bones for instance, human femur experiences complex bending, torsion and compression loading regimes during a gait cycle (Duda et al., 1998; Edwards et al., 2008). As bone adapts to mechanical loads throughout life (Wolff, 1892), it is expected that stronger or weaker regions form in mature skeleton due to habitual loading, and are optimized for the loads experienced. While several previous studies have reported differences in stiffness, ultimate strength and apparent density for various anatomical locations within the long bones (Atkinson and Weatherell, 1967; Les et al., 1997; Espinoza Orías et al., 2009; Rantalainen et al., 2011; Rohrbach et al., 2012), a systematic approach to characterize the degree of heterogeneity of micro-level mechanical properties along the bone seems to be missing in literature. From a structural point of view, bone is thicker where it has to withstand higher stresses. We hypothesize that the bone material also adapts to the condition of high stress in those regions and therefore, the elastic modulus is positively correlated to cortical bone thickness in bovine femur. As thickness varies in long bones, we hypothesize that there is a correlation between the elastic modulus and position along the bovine femur, with elevated moduli at mid-shaft.

The heterogeneity of the elastic modulus in bone is a key feature

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http://dx.doi.org/10.1016/j.jmbbm.2017.03.021 Received 17 August 2016; Received in revised form 5 March 2017; Accepted 25 March 2017 Available online 27 March 2017

1751-6161/ © 2017 Published by Elsevier Ltd.

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and many models try to take that into account in finite element (FE) analysis of bone tissue. FE models are extensively used in the study of the mechanics of bone and artificial implants e.g. (Duda et al., 1998; Polgar et al., 2003; Shahar et al., 2003; Poelert et al., 2012). In conventional FE modeling of the bone however, the regional dependency of mechanical properties is neglected and bone is assumed to be a homogeneous isotropic material (Polgar et al., 2003; Shahar et al., 2003). A more sophisticated approach in FE modelling is to extract the mechanical properties of bone from computed tomography (CT) data, converting local grey level numbers to bone mineral density (BMD) and subsequently to a local elastic modulus (Crawford et al., 2003a, 2003b; Taddei et al., 2006; Poelert et al., 2012). Whether the elastic modulus is positively related to bone mineral density in uCT measurements is still not clear. It has been previously shown in some studies that bone becomes stiffer with an increase in the degree of mineralization (Follet et al., 2004; Wagner et al., 2011). Some other studies, however, reported only a weak correlation between the bone mineral density and the elastic modulus (such as Rho et al., 1995). In this paper, we compared the bone mineral density to the elastic modulus to search for the validity of their relation in bovine bone. We hypothesize that the elastic modulus is not correlated to bone mineral density in bovine femur.

As an alternative to bone mineral density, we propose that complementary approaches to CT-based calculations can be developed based on location dependency of the elastic modulus in long bones, to precisely map the local elastic properties of the bone in finite element models. After proper calibration of the method for each case, an accurate estimation of the elastic modulus may be found for long bones. In this study, we report results from miniature three-point bending experiments on samples taken at regular intervals along the long axis of two bovine femora. Through comparison with  $\mu$ CT data, we can compare CT predicted longitudinal elastic modulus with the actual measurements. From the findings of our experiments, we propose that complementary empiric laws to the current CT-based approaches can more accurately predict the local longitudinal elastic modulus along the bovine femur.

#### 2. Materials and methods

Two bovine femora were tested in this study. Femur-1 was tested to investigate the correlation of the elastic modulus with position along the long axis of the bone, cortical bone thickness and for comparison with local elastic moduli predicted by  $\mu$ CT. Three-point bending experiments were repeated on femur-2 to check if the relationship found for femur-1 can predict the elastic modulus for other bones. Bovine femora were obtained from local meat wholesalers and stored at -20 °C until scanning and sample preparation.

#### 2.1. Microfocus X-ray computed tomography (µCT)

 $\mu$ CT imaging on femur-1 was performed at the University of Southampton  $\mu$ -VIS X-Ray imaging center using a Custom 225 kVp

Nikon/Metris HMX ST scanner (Nikon Metrology, Brighton, MI, US) equipped with a 2000×2000 pixel flat panel detector. To ensure sufficient flux a W target was selected, the voltage was set at 200 kV and no pre-filtration was used. The current was set at  $29 \,\mu A$  (5.8 W), and the source to detector distance was 672.56 mm, resulting in an isotropic pixel resolution of 59.1 µm in x, y and z directions for the scan. The femur was placed upright in a shielded Perspex tube and positioned onto the rotation stage, so that the long axis of the bone coincided with the stage's center of rotation (CoR). The position of the specimen and the distance from the X-Ray source were such that ensured the whole width and depth of the bone remained within the field of view during the full 360 degree rotation. To cover the whole specimen at the aforementioned resolution, we divided the imaging volume into three sub-volumes (i.e. bottom-, middle- and top-raster) and each raster was scanned separately keeping imaging conditions constant. In more detail: 3142 projections were taken over the 360degree rotation (Angular Step=0.1146 deg), with 4 frames per projection being averaged in order to improve the signal to noise ratio. Exposure time of each projection was 354 ms and the gain was set to 30 dB. Once the scans were complete, the reconstructions parameters were defined using CTPro software (Nikon Metrology, Brighton, MI, US) and reconstructed in CTAgent (Nikon Metrology, Brighton, MI, US) reconstruction software, which uses a filtered back-projection algorithm. The output volume data were scaled to Hounsfield units using a known density phantom (in our case water), following Nikon's recommendations. The three calibrated volumes were then concatenated into a single volume using Fiji/ImageJ (National Institute of Health, Bethesda, MD, US) and orthogonal central cross-sections along the XZ and YZ planes (Z being in line with bone's long axis) were exported for further analysis. Thickness of the bone cortex in each anatomical location within the blocks was measured in several sites from the uCT data and in MATLAB (The Math Works, Natick, MA, US). On each cross-section and at approximately the middle of the bone cortex, a line profile (width 10 pixels) was measured using Fiji's profile tool (Fig. 1). This allowed averaging the measured gray value, over a larger bone area, reducing possible bias due to local pixel variations and beam hardening effects.

Calibrated Hounsfield units (HU) along each profile were converted to the bone mineral density (BMD) using Eq. 1 (Schneider et al., 1996) and consequently to the elastic modulus (E) using Eq. 2, the empirical relationship proposed by Rho et al. (1995).

$$BMD (g/cm^3) = 0.0007 \times HU + 0.3489 \tag{1}$$

$$E (GPa) = 9.11 \times BMD^{1.326} (g/cm^3)$$
<sup>(2)</sup>

#### 2.2. Sample preparation

Femur-1 was cut along its longitudinal and perpendicular axes using a bandsaw (BG 200, Medoc, Logrono, Spain). The bone was then divided into 8 blocks along its longitudinal axis (block height= 2 cm), excluding the epiphyses. This configuration is shown in Fig. 2. Blocks 1



Fig. 1. - Line profile used to extract the CT graylevel number and mineral density from the anterior section of femur-1.

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