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Research Paper

Orientation of whole bone samples of small rodents matters during bending tests

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ABSTRACT

The influence of the orientation of rat bones on their mechanical response is analyzed in this research. 28 femora obtained from 14 Sprague-Dawley rats were subjected to three-point bending tests, comparing the anteroposterior and posteroanterior orientations. The results show that the whole-bone loading capacity of the femora tested in the posteroanterior orientation clearly exceeds that of the anteroposterior oriented bones. Likewise, the intrinsic (tissue-level) loading capacity of the bones tested in the posteroanterior orientation is manifestly higher than that of the bones tested in the opposite direction. The analysis carried out shows that applying beam theory for symmetric cross-sections leads to underestimating the stress state in the cross-section. In this sense, it is generally recommendable to use the non-symmetric beam theory in order to obtain the normal stresses during bending tests. The geometric, intrinsic and global changes resulting from the orientation of the bones was assessed, finding out that it is the variation in the intrinsic properties which explains the change measured in the whole-bone properties. The experimental scope was increased, including 8 additional femora on which a series of Vickers tests were carried out in the anterior and posterior regions of the cross-section. In all cases the hardness obtained in the anterior region is larger than in the posterior region. This result confirms that the mechanical properties of the bone tissue depend on its position in the cross-section and provides a reliable explanation to understand the response of the bones when subjected to bending tests. These results stress the importance of reporting the orientation of the bones in any scientific paper because, otherwise, it would be impossible to properly assess its impact and relevance.

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1. Introduction

Numerous recent studies use techniques of mechanical characterization for determining the properties of bone tissue under different loading conditions and purposes. More than thirty years ago, Stein and Granik published a pioneering work where they proposed an in-vivo method for assessing the mechanical strength of human tibias through bending tests (Stein and Granik, 1982). In 1992 Turner and Burr (Turner and Burr, 1993) described the basics of biomechanics as well as various methods for characterizing the mechanical properties of bones in a tutorial article. Since then, and particularly in the last ten years, many contributions analyzing aspects of the mechanical behavior of bones have been published.

Bones can be tested by applying either constant loads until failure or cyclical loading, giving rise to fatigue (Beaupied et al., 2007). In both cases mechanical tests may involve torsional, compressive, tensile or bending loads, the latter being the most widely used method for testing long bones, particularly for rodents. Mechanical testing of bones has enabled the determination of important biological properties. To illustrate this point, and while not intending to be exhaustive, the following noteworthy list of findings can be mentioned. Several researches have correlated biomechanical and clinical parameters, particularly bone mineral density, to assess bone quality, by means of torsion (Ford and Keaveny, 1996; Garnier et al., 1999) compression (Myers and Wilson, 1997; Turner, 1989) or bending (Stenström et al., 2000; Tommasini et al., 2005). Studies carried out to determine the tensile strength of cortical bone revealed a correlation between mechanical properties and porosity (Dong and Guo, 2004; Wachter et al., 2002). As mentioned, long bones of rodents are mostly tested through bending tests (Goss et al., 2004; Li et al., 2005a, 2005b) for different purposes such as establishing correlations with clinical and genetic parameters (Akhter et al., 2000) or to assess the influence of disease and medical treatment.

Nevertheless, for the moment little attention has been paid to establish a rigorous methodology to define how to properly perform and interpret the tests commonly used to characterize the mechanical response of bone tissues. The review paper by Jepsen et al. (Jepsen et al., 2015), recently published, represents a remarkable exception as they "focus on whole-bone tests of long-bone diaphysis [...] to better define biomechanical mechanisms in the skeleton by recommending guidelines to systematically evaluate phenotypic changes in mouse long bones". Their paper aims at establishing common standards about how to perform the tests, as well as how to interpret and deliver the results in order to avoid wrong practices and misunderstandings.

In this research, an experimental scope was designed consisting in testing rat femora by means of three-point bending tests comparing the results obtained from the tests performed in the anteroposterior (AP) direction to those in the posteroanterior (PA) direction.

The aim of the paper is twofold. First, we focused on determining the influence of the orientation of the femora (AP and PA) during the bending tests on the experimental results. Not only several whole-bone mechanical properties were obtained but also a number of tissue-level properties. Particular attention was paid to determine the relationship between the geometry of the bones and the intrinsic properties on the whole-bone parameters. Obtaining these intrinsic properties implies firstly to determine the stress state in the cross-sections of the bones resulting from bending. Traditionally, this has been carried out by applying beam theory and considering, for the sake of simplicity, that the bone cross-section is symmetrical. In this paper an improved experimental and analytical methodology based on the beam theory for non-symmetric cross-sections was used to calculate the normal stresses in a bending test. This analysis constitutes the second line of research in this work.

2. Materials and methods

2.1. *Study design*

Fourteen female Sprague–Dawley rats (thirteen weeks old, weighing between 250 and 350 g) were originally available for the research. All the animals were treated in the Centre for Animal Experimentation of the University of Cantabria (UC); the experimental protocol was approved by the Animal's Research Ethics Committee of the UC. Their sacrifice was conducted by CO_2 inhalation, the rodents being previously subjected to subcutaneous anaesthesia with a mixture of Ketamine (75 mg/kg) and Medetomidine (0.5 mg/kg). The samples provided by the surgeons (28 femora) were taken from the operating room to the laboratory of mechanical characterization LADICIM (Laboratory of Science and Engineering of Materials) in a portable refrigerator. In order to preserve the integrity of the biological material, the bones were stored in a freezer at $-18\,^{\circ}C$ (Evans et al., 1990; Fischer-Cripps, 2011). The length of the femora was 37.1 ± 1.8 mm while their weight was 0.36 ± 0.07 g. The morphological properties of the bones (particularly, the dimensions of the cross-sections) were determined using a Skyscan 1172 micro-CT (μCT), the resolution being 20 μm. Fig. 1 shows an example of one of the femora tested in this work; the four representative views of the bone (lateral, medial, cranial and caudal) are included in the figure.

2.2. *Testing protocol*

The three-point bending tests were performed with a Servosis electromechanical testing machine, under quasi-static and displacement control conditions, applying a constant rate of 1mm/min. During the tests, the femurs were immersed in a bath of Hanks' solution at 37 °C to mimic the physiological environment and avoid dehydration. The distance between supports was fixed at 15 mm which is over the limit proposed by Turner (1989) to minimize shear deformation.

The fourteen rats were divided at random into two groups which will be subsequently referred to as groups A and B, respectively. Right (R) and left (L) femora of group A and right femora of group B were tested in the PA direction (from posterior to anterior), see Fig. 2(a), whereas left femora of group B were tested in the AP direction, represented in Fig. 2 (b). Rodents belonging to group A play the role of the control