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Can the contralateral limb be used as a control during the growing period in a rodent model?

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ABSTRACT

The contralateral limb is often used as a control in various clinical, forensic and anthropological studies. However, no studies have been performed to determine if the contra-lateral limb is a suitable control during the bone development period. The aim of this study was to determine the bilateral symmetry of growing rat tibiae in terms of geometric shape, mechanical strength and bone morphological parameters with developmental stages. Left and right tibias of 18 male Sprague–Dawley rats at 4, 8 and 12 weeks of age were scanned with micro-CT for bone-morphometric evaluation and for 3D deviation analysis to quantify the geometric shape variations between left and right tibiae. Overall tibial lengths and curvatures were also measured, and bone mechanical strength was investigated using three-point bending tests. Deviation distributions between bilateral tibiae remained below 0.5 mm for more than 80% of the geometry for all groups. Tibial lengths, longitudinal tibial curvatures, bone-morphometric parameters and mechanical strengths changed significantly during the growing period but kept a strong degree of symmetry between bilateral tibiae. These results suggest that bilateral tibiae can be considered symmetrical in nature and that contralateral limb can be used as a control during the growing period in different experimental scenarios.

1. Introduction

Bilateral symmetry of long bones is used by the researchers in musculoskeletal research and clinical practice. It is a commonly hypothesized concept used in geometric, biological and clinical studies. It is deemed as a reflection symmetry in which similar anatomical parts are arranged on opposite sides of a median axis so that only one plane of symmetry can divide the individual into substantially identical halves [1]. Usually, symmetries are perceived as extrinsic geometric and volumetric properties of shapes [2], implying that with a different orientation, the geometric shape of a structure is similar to another structure. The use of a contralateral limb as a reference is not new in animal studies. It has been used in various clinical, forensic and anthropological studies [3,4], with the implicit presumption of minimal or no significant differences between bilateral limb. Many rat bone modeling and remodeling studies have been conducted employing bilateral limbs with one of the limbs as control from experimental animals [5,6]. Rat tibial models have been used to investigate the effects of aging, mechanical loadings, surgical treatments, and genetic mutations on the changes in the microarchitecture and morphological parameters of the bones [7,8]. Also, they have been used for assessing the bone response under mechanical loading conditions [7]. Comparisons of the anatomical, morphological, and mechanical properties in one tibia are contrived respective to the bilateral limb based on the hypothesis that the observed changes under experimental conditions would be identical in both control and experimental tibiae [9]. Studies have been performed to establish physical and geometric similarities in animal models with paired bones [10]. However, there are no reports comparing three-dimensional (3D) symmetry for rat tibiae.

A comprehensive study of tibial morphology and anatomical features would be useful in understanding its adaptation to loading, medical treatment, and surgical intervention. Previous studies have assessed the asymmetry of long bones by evaluating cross-sectional, limited volumetric and mechanical properties [11,12], and implemented partial aspects to quantify left-right symmetry rather than using a full 3D geometric comparison. The most common methods implemented include measuring distances between anatomical landmarks [13], comparing mechanical strength and stiffness [14], as well as morphometric data [15], and

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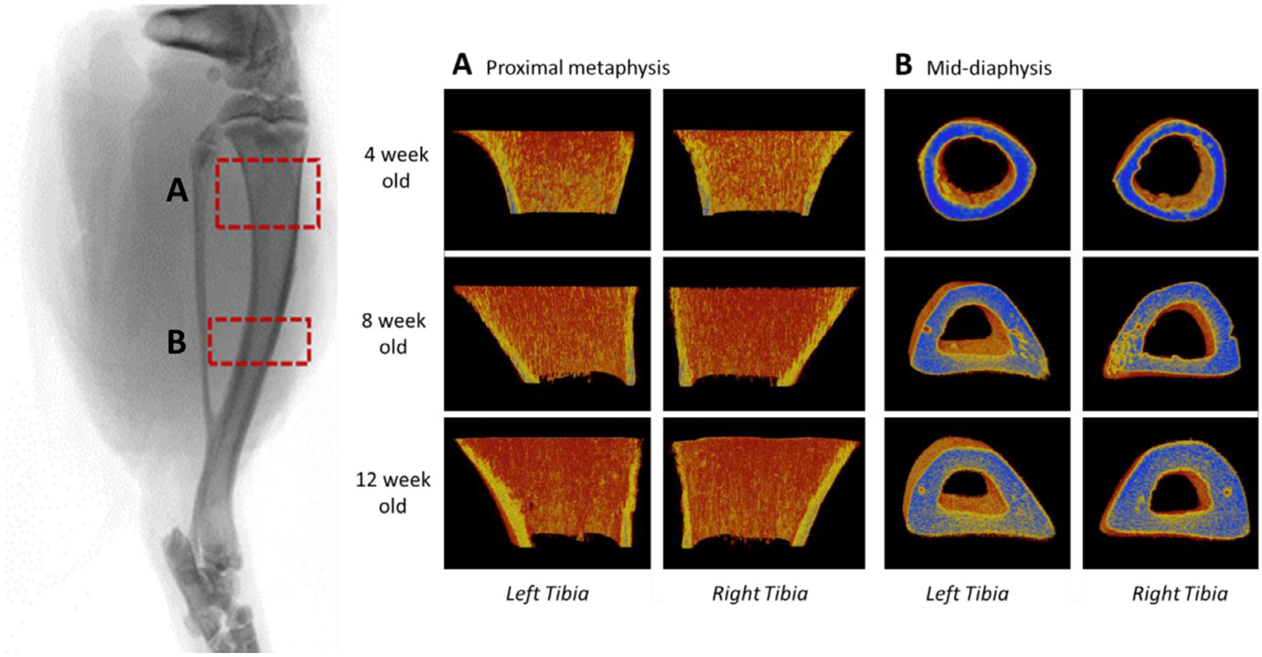

Fig. 1. Representative micro CT reconstructed image sets of the rat tibiae for the three age groups: (A) frontal sections through the proximal tibial metaphyses; (B) transverse sections through the tibial mid-diaphyses.

geometric parameters [12]. These analyses do not fully assess the degree of deviation between bilateral limbs in 3D geometric form. To our knowledge, there are no studies quantifying the symmetrical/asymmetrical nature of the rat tibial bones, a bone model commonly used by the scientific community. In some studies [16,17], bones were investigated during the rat growing period, where bones experienced developmental changes along with the effects of the experimental conditions. Comparisons were made either between experimented tibia with the contralateral controls or between run-trained, and jump-trained groups with paired experimented limbs comparing each other, assuming that there are no significant differences between the bilateral limbs. However, there is still a paucity of data on the investigation of mechanical, geometrical and morphological symmetry between bilateral tibiae to determine whether the contralateral tibia can be used as an equivalent control in experimental studies.

The goal of this study was two-fold. First, to determine the extent of overall 3D similarity between left and right growing rat tibiae. Secondly, the bone morphology, including cortical and trabecular bony segments, the whole bone geometry as well as the bone mechanical properties, were assessed and the degree of bilateral variability among the tibial pairs with developmental stages was investigated.

2. Materials and methods

2.1. Animals

Eighteen ($n = 18$) male Sprague–Dawley rats (Charles River Laboratories, Montreal) were divided into three groups ($n = 6$ per group): 4, 8 and 12-week old. Rats were housed at 25 °C with a 12-h light/dark cycle and provided with a standard laboratory diet and water ad libitum. On the experiment day, body weights were measured, rats were euthanized with carbon dioxide inhalation, and both tibiae were carefully dissected. The tibiae were imaged with micro-CT, and three-point bending tests were then performed. Animal care and use conformed to the guidelines of the Canadian Council on Animal Care (CCAC), and the experimental protocol was approved by the Institutional Animal Care Committee at the Sainte-Justine University Hospital, Montreal, Canada.

2.2. Microcomputed tomography (micro-CT) imaging and analyses

Micro-CT analyses of left and right tibiae were performed using a Skyscan 1176 *in-vivo* micro-CT (Skyscan, N.V., Belgium) scanner. The rat tibia was secured in a cylindrical Styrofoam holder and scans were performed with 18 µm voxel resolution, 65 kV, 384 µA, 350 ms exposure time, 0.65° rotation step, and a 1-mm Al filter. Images were reconstructed using NRecon software (v.1.6.10, Bruker-microCT, Belgium). For each scan, two regions covering 1328 cross sections were reconstructed corresponding to a total height of 10 mm each, one region starting from the knee joint and extending distally along the tibial diaphysis, and another region on the mid-tibial diaphysis (Fig. 1).

2.2.1. 3D analyses of whole tibiae

Reconstructed images of the left tibiae were imported into DataViewer v.1.5.2. Then, the images were flipped with respect to the sagittal plane and exported as new image sets. Next, an iterative optimization-based rigid 3D registration algorithm was employed using ImageJ (NIH, Bethesda, USA) to align the left (flipped) and right (real) tibiae on top of each other. Then both image sets were imported into the CTAn v.1.13 (Skyscan, Kontich, Belgium) and 3D meshed geometries were created. An in-house developed Matlab program was implemented to analyze the 3D deviations, where the left and right tibiae were used as the reference and target surface models, respectively. The resulted data encompassed signed deviations (orthogonal distance) using color maps, which illustrate the position of the target bone surface above or below the surface of the reference bone (Figs. 2 and 3). The light green/yellow colors illustrate a small deviation (<1.5 mm), whereas the dark red/blue colors refer to deviation >3.0 mm. Outward and inward deviations are represented by positive and negative values, respectively. Maximum and average positive and negative deviations were evaluated. The root mean square (RMS) values of deviations were also calculated, and correlation with the d_{avg} (+) and d_{avg} (−) values were quantified.