AN IMPROVED METHOD TO ASSESS TORSIONAL PROPERTIES OF RODENT LONG BONES
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INTRODUCTION: Torsion is an important testing modality for rodent bones to calculate the resistance of the bone structure to twisting moments and to derive the shear properties of the bone tissue as a means for quantifying the effect of species, age, disease or drug affect on bone structural and material properties. Traditionally, the torsional properties for rodent long bones have been calculated using equations derived for isotropic, homogeneous, beams with symmetric cross-sectional geometry. However rodent long bones are anisotropic, inhomogeneous and have variable, asymmetric, cross-sectional geometry along the length of the bone. When a uni-axial torque is applied to a rodent long bone, it bends and warps out of plane. The resulting stress and strain profiles are complex. In order to derive the shear properties of the bone tissue, the effects of bone size and geometry must be excluded from the overall structural response. Levenston et al.† has proposed modeling the whole bone structure as a multi-segmented prismatic beam with variable cross-sectional geometry along the bone. They also proposed a multi-segment, prismatic beam model they predicted the shear modulus for a corresponding finite element model of a rat femur to be ~3% of the value assigned in the FEM. With the wide-spread use of micro-computed tomography (μCT) it is now possible to generate serial trans-axial images of the variable cross-sectional geometry and bone micro-structure for rodent long bones. We hypothesize that a multi-segmented, transversely isotropic, composite, elliptical beam model derived from serial trans-axial μCT images that accounts for the width-wise variation in bone cross sectional geometry and density will improve the accuracy of the torsional properties predicted for rodent long bones. Therefore, the aim of this study were: 1) validate this hypothesis using reverse engineered rat femurs manufactured from a material with known properties based on μCT images of actual rat femurs; 2) derive constitutive relationships expressing the torsional properties of rat bone as a function of bone volume fraction or bone mineral density for normal, osteoporotic and dystrophic rodent rats.

METHODS: After IACUC approval 21 female Sprague Dawley rats were divided into three equal groups: control (CON) underwent no surgical or dietary interventions; ovariectomized (OVX) underwent ovariectomy 1 wk prior to the study; nephrectomized (NFR) underwent 5/6 nephrectomy 1 wk prior to the study and fed a modified diet (0.6% Ca and 1.2% P04). The CON, NFR and OVX animals were euthanized at 2, 3, and 4 months, respectively; both femurs from each animal were used. Both ends of the femurs were embedded in PMMA and the unsupported length of the femur diaphysis measured with a caliper.

Serial transaxial μCT images were obtained through the non-embedded segment of each femur (isotropic voxel size 20 μm; interrogation time 250 ms; tube voltage 70 keV; current 0.114 mA). Mineralized bone was segmented using an adaptive thresholding procedure and the cortical bone volume fraction (Ct.BV/TV), average cortical thickness (Ct.Th), cross sectional area (A) were calculated for each transaxial image using standard quantitative morphology algorithms.

Serial transaxial μCT images from three control rat femurs were exported as a rapid prototyping system (3D Systems, 138 μm voxel size) and five segment prismatic replicas of each femur were manufactured from InVision m100 resin (E = 775 MPa, v = 0.34, G = 290 MPa).

All femurs (including the rapid prototype resin femurs) were subjected to uniaxial torque until failure using a custom apparatus of our own design that accommodates out of plane bending and warping while applying uniaxial torque along the neutral axis. The applied torque and angle of twist along the unsupported length of the femur were measured.

The effective torsional material properties for each cross section were derived from an equivalent ellipse for the bone profile on that cross section that assumes the periosteal (a0) and endosteal (a1) boundaries to be parallel and the cortical thickness of the femoral diaphysis to be uniform. The torsional constant, k, for a tubular beam with elliptical cross section is calculated: 
\[ k = \frac{\pi}{4} \left( a_0^2 - a_1^2 \right) \]
where \( \xi \) is the ratio of the minor axis length to the major axis length, \( a_0 \), the endosteal diameter is a function of the periosteal diameter, \( a_o \), and the average cortical thickness of the specimen: 
\[ a_o = a_p - Ct.Th \]

The shear modulus for that cross-section is: 
\[ G = \frac{1 + \frac{2\alpha}{\pi}}{\frac{\pi^2}{2}a_p^4} \cdot \frac{T}{\xi \left( a_0^2 - a_1^2 \right)} \]
where \( T \) is the applied torque and \( \alpha \) is a constant representing the twist per unit length of the diaphysis. The effective torsional modulus for the entire femoral diaphysis is derived from the relative contribution of each cross-section: 
\[ G = \left( \frac{1}{k} \right) \sum_{i=1}^{n} \frac{1}{\xi_i} \left( \frac{1}{a_0^2 - a_1^2} \right) \]
Note that the cross-section with the minimal shear modulus will dominate the torsional behavior of the entire femur.

RESULTS: The torsional properties derived from serial transaxial μCT images for the InVision m100 resin based prototype rat femur assuming the diaphysis to be a multi-segmented tubular beam comprised of variable elliptic cross-sections overestimated the actual material properties of the resin by 6%. Applying the multi-prismatic method presented by Levenston et al. underestimated the actual shear modulus by 7%. Linear regression models expressing the shear modulus and shear strength for normal and pathologic rat bone femurs as a function of either bone volume fraction or apparent bone mineral density accounted for 81% of the variation in these properties (Table 1).

<table>
<thead>
<tr>
<th>Equation</th>
<th>R² Value</th>
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<tbody>
<tr>
<td>G = 4.13(Ct.BV/TV)^1.49</td>
<td>0.81</td>
</tr>
<tr>
<td>τ = 30.44(Ct.BV/TV)^1.46</td>
<td>0.83</td>
</tr>
<tr>
<td>G = 3.16(ρAPP)^1.24</td>
<td>0.81</td>
</tr>
<tr>
<td>τ = 23.30(ρAPP)^1.20</td>
<td>0.81</td>
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DISCUSSION: Geometric modeling of the whole bone has evolved considerably in recent decades. Early models considered bone as a solid cylinder with constant circular cross-section throughout the length of the bone. Improvements in the modeling of whole bones have included modeling the cross-section as a solid ellipse, hollow cylinder and elliptical tube with an area and torsional constant equivalent to either the midshaft or the minimal cross-section of the bone. Levenston et al. approached this problem using a multi-prismatic model in which each prismatic segment represented a corresponding cross-section through the bone with similar area and torsional constant. Using the multi prismatic approach without addressing cross-sectional geometry reduced the calculated modulus error by half, compared to the alternate methods thereby emphasizing the importance of this concept. Since non-invasive high resolution imaging modalities were not widely available at that time, Levenston et al. proposed a compromise solution to use a multi-prismatic model with five true bone cross-sections capable of reducing the modulus errors to 3%

While the resin used to model the rat femur consisted of an isotropic material, the overriding goal was to manufacture exact replicas of rat femur geometry using a material with known mechanical properties and then subject them to actual torsion using testing procedures identical to those used for the normal and pathological rat femur subsequently tested. In that regard, the method established here was validated with actual torsion data as opposed to finite element estimations. Using this analysis technique, a regression model with either shear modulus or shear strength as the dependent variable and bone volume fraction or apparent density as the independent variable described at least 81% of the variation in torsional properties of normal and pathologic rat cortical bone. The relationships generated from this study describe the mechanical properties of cortical bone over a wide range of bone density and common skeletal pathologies. Coupled with the structural rigidity technique introduced by the authors, the relationships reported here provide a non-invasive method to assess fracture risk in bones affected by pathology and/or treatment options.