Assessment of Axial Bone Rigidity in Rats with Metabolic Diseases using CT-based Structural Rigidity Analysis

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INTRODUCTION: Frailty fractures of the hip, spine or wrist result from osteoporosis and other bone diseases are common causes of disability, affecting up to 2 million Americans annually. A 50-year-old white woman has a 15-20% lifetime risk of sustaining a hip fracture, which is associated with long-term morbidity and a 20-33% mortality rate one year after fracture. Currently, the World Health Organization uses decreased bone mineral density (BMD), as measured by dual energy X-ray absorptiometry (DXA), to identify patients with osteoporosis ("2 SD below the mean for a young normal adult of the same sex") and osteopenia ("1 SD below the mean for a young normal adult of the same sex") and identify individuals at risk for fracture. For CTRA analysis, the axial (EA) rigidity for each transaxial section of mineral density, is not a true measure of bone density and has shown to be neither sensitive nor specific in its ability to predict future frailty fractures. In contrast, quantitative Computed Tomography (CT) based Structural Rigidity Analysis (CTRA), a 3D imaging modality, can provide information about specific changes in bone material and structure for both cortical and trabecular bone. While CTRA has been used extensively to assess fracture in studies of metastatic musculoskeletal lesions, efforts have not been made to assess the efficacy of this technique is assessing fracture risk in metabolic musculoskeletal diseases. Given the ability of CTRA to detect structural and material changes within trabecular and cortical bone, We hypothesize that CTRA can accurately assess the average and minimum axial rigidities of cortical and cancellous bones affected by metabolic diseases. To that end, we aim to use CTRA to assess the average and minimum axial rigidities of cortical and trabecular femur segments from normal (CON), ovariectomized (OVX), and partially nephrectomized (NFR) rats and compare the results to those obtained from mechanical testing as the gold standard measure.

MATERIALS AND METHODS: Thirty female Sprague Dawley rats (15 weeks old) were divided into 3 equally sized groups: 1) CON group: subjected to no surgical or dietary interventions. 2) OVX group: underwent ovariectomy to induce a state of low bone mass and micro-architectural deterioration. 3) NFR group: underwent 5/6 nephrectomy in addition to a modified diet containing 0.6% Ca and 1.2% P to induce renal osteodystrophy and severe secondary hyperparathyroidism. Both surgical procedures were conducted 1 week prior to the study. All animals were euthanized via CO2 inhalation at 4 months, and mid-diaphyseal (cortical bone only) and distal metaphyseal (trabecular + cortical bone) specimens were cut from each femur perpendicular to the anatomical axis. Sequential transaxial images through the entire cortical and trabecular specimens were obtained using micro-computed tomography (µCT40, Scanco Medical AG, Brüttisellen, Switzerland). Cortical and trabecular bone mineral densities (\(\rho_{\text{MIN}}\) g.cm\(^{-3}\)) were calculated using a hydroxyapatite phantom supplied by the manufacturer to convert X-ray attenuation coefficient to volumetric bone mineral density for CTRA analysis. For CTRA analysis, the axial (EA) rigidity for each transaxial section of bone cross-section through the bone was calculated by summing the density-weighted area of each isotropic voxel by its position relative to the density weighted centroid. Average (EA\(_{\text{AVG-CTRA}}\)) and minimum EA (EA\(_{\text{MIN-CTRA}}\)) axial rigidities were reported for each specimen. EA\(_{\text{AVG-CTRA}}\) represents the average axial rigidity of the entire segment, whereas EA\(_{\text{MIN-CTRA}}\) represents the axial rigidity of the entire segment at its weakest cross-section. For mechanical testing, specimens were preconditioned, using a triangular waveform to 0.33% strain for 7 cycles and a strain rate of 0.005 s\(^{-1}\), followed by uniaxial compression to failure at a strain rate of 0.01 s\(^{-1}\). Average axial rigidity (EA\(_{\text{AVG-mech}}\)) was calculated by multiplying with the average specimen cross-sectional area (\(A_{\text{AVG}}\) - assessed from transaxial µCT imaging, including bony sections only and reporting the cross-sectional area with the minimum area). A repeated-measures model was used to account for the within-animal correlation when comparing the slope and intercept parameters. Paired Student’s t-test was used to assess the correlation between EVB values obtained from mechanical testing versus CTRA based average and minimum EA values respectively.

RESULTS: CTRA-based EA\(_{\text{AVG}}\) and mechanical testing were well correlated with one another (\(R^2 = 0.74, P < 0.0001\)). This correlation improved significantly when the CTRA-based EA\(_{\text{MIN}}\) was correlated with the mechanical testing based minimum axial rigidity results (\(R^2 = 0.84, P < 0.0001\)) (Figure 1). The mixed model regression analysis indicated a significantly different slope and intercept for EA\(_{\text{AVG}}\) compared to EA\(_{\text{MIN}}\) (p = 0.028 and p = 0.022 respectively).

The CTRA-based average and minimum axial rigidities were correlated with the mechanical testing based average and minimum axial rigidities using a paired t test analysis (p = 0.37 and 0.18). Intra-group and intra-type t-test analysis of axial rigidity values between CON, OVX and NFR groups for both cortical and trabecular bone specimens showed correlation between the CTRA based and the mechanical testing based rigidity data (P > 0.13 for all cases).Significant differences in EA data between different bone types (cortical and trabecular - P < 0.0001) and groups (CON, OVX, NFR - P < 0.0001) were observed. Post hoc analysis of the intra group differences revealed that EA\(_{\text{AVG}}\) were not different between the OVX and NFR groups regardless of the CTRA and mechanical testing (P = 0.09 and 0.34 respectively). Cortical bone axial rigidity distribution occupied the upper right hand quadrant of both regression figures, whereas trabecular bone axial rigidity distribution filled the lower left quadrant of both regression figures with cortical and trabecular bones from control animals providing the highest rigidity values.

DISCUSSION: The results of this study support the hypothesis that axial rigidity of bones with metabolic pathologies can be accurately and quantitatively assessed in a rat model by conducting structural rigidity analysis on serial axial images of the affected bone. Axial rigidity measured non-invasively by µCT was well correlated with the results from mechanical testing as the gold standard measure. Minimum axial rigidity produced a stronger correlation with mechanical testing based minimum rigidity results (\(R^2 = 0.84\)) than their average counterparts (\(R^2 = 0.74\)). Furthermore, intra-group and intra-type paired Student’s t-test showed no significant difference in axial rigidity as determined by CTRA and mechanical testing (p > 0.13 for all cases).

SIGNIFICANCE: The structural rigidity analysis of µCT data can be used to accurately and quantitatively measure the axial rigidity of bones with metabolic pathologies in an experimental rat model. It appears that minimum axial rigidity is a better model for measuring bone rigidity than average axial rigidity.