INTRODUCTION:
Fragility fractures of the hip, spine or wrist resultant from osteoporosis and other bone diseases are common causes of disability, affecting up to 2 million Americans. 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. While osteoporosis is assumed to be the cause of most fragility fractures, 25-OH-vitamin D deficiency is observed in 50% of postmenopausal women in the population who fracture their hip (not including those residing in retirement homes) and have no other cause for low bone mass. Vitamin D deficiency can result in osteomalacia, a bone material problem which has been diagnosed histologically (hypomineralized osteoid) in 13–33% of patients with hip fractures. Moreover, secondary hyperparathyroidism, as seen in patients with renal disease, can contribute to demineralization of both the cortical and trabecular bone and thereby increase the risk of fracture by compromising the material properties of bone. This compromise of bony material differs from osteoporosis; a disorder in which the micro-architectural deterioration of bone and subsequent loss of bone mass affects the structural integrity of bone, thereby increasing the risk of fracture.

Currently, the World Health Organization (WHO) uses decreased bone mineral density (BMD), as measured by dual energy X-ray absorptiometry (DXA), to identify patients with osteoporosis. The standard deviation 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 identifies individuals at risk for fracture. However, BMD, an areal projection of bone mineral density, is not a true measure of bone density and has been shown to be neither sensitive nor specific in its ability to predict future fragility 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. This technique is capable of non-invasive assessment of the axial, bending and torsional rigidities of bones from their trans-axial cross-sectional images. 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 in assessing fracture risk in metabolic musculoskeletal diseases.

Therefore, we hypothesize that CTRA can accurately assess the average and minimum axial rigidity of bones affected by metabolic diseases. To that end, we aim to use CT-based Structural Rigidity Analysis to assess the average and minimum axial rigidities of cortical and trabecular femur segments from normal, ovariectomized and partially nephrectomized rats and compare the results to those obtained from mechanical testing.

MATERIALS AND METHODS:
Thirty female Sprague Dawley (SD, mass: 250-275 g, ~15 weeks old) rats were obtained from Charles River Laboratories (Charles River, Charlestown, MA, USA) and were divided into three equally sized groups: the animals in the control group were not subjected to any surgical or dietary interventions; the OVX group underwent ovariectomy (a week prior to the start of the study) to induce a state of low bone mass and micro-architectural deterioration; and the NFR group underwent 5/6 nephrectomy in addition to being placed on a modified diet containing 0.6% Ca and 1.2% P to induce renal osteodystrophy and severe secondary hyperparathyroidism. The study protocol was approved by Beth Israel Deaconess Medical Center’s Institutional Animal Care and Use Committee (IACUC).

After dissection and cleaning of all adherent soft tissues , a mid-diaphyseal (cortical bone only) and a distal metaphyseal (trabecular + cortical bone) specimen was cut from each femur perpendicular to the anatomical axis using two parallel diamond wafering blades on a low-speed saw (Isomet, Buehler Corporation, Lake Bluff, IL, USA) under copious irrigation. The cortical midshaft specimens [H: 5.99 mm ± 0.28 mm (std. dev), Ø at mid-length: 3.64 mm ± 0.24 mm] were cut to maintain an approximate 2:1 height to diameter ratio, while the distal metaphyseal segments [H: 6.22 mm ± 0.73 mm, Ø at mid-length: 4.84 mm ± 0.41 mm] were cut from the growth plate, as identified from anterior-posterior contact radiographs to include the distal metaphyseal trabecular micro-structure. The metaphyseal cortex was shaved off at the laboratory using diamond wafering blades, ample lighting, and optical magnification to obtain trabecular only specimens. Sequential transaxial images through the entire cortical and trabecular bone sections were obtained using micro-computed tomography (µCT) (µCT40, Scanco Medical AG, Bruttisellen, Switzerland) at an isotropic voxel size of 30 μm per side, integration time of 250 ms and tube voltage and current of 55 kVp and 145 μA respectively, while applying a 1200 mg.cm−2 hydroxyapatite (HA) beam hardening correction curve. Average (EAavgCTRA) and minimum EA (EaminCTRA) axial rigidities were reported for each specimen. EAavgCTRA represents the average axial rigidity of the entire segment, whereas EaminCTRA represents the axial rigidity of the entire segment at its weakest cross-section. 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 (Instron 8511, Instron, Norwood, MA, USA).

RESULTS:
CTRA-based average axial rigidity was well correlated with mechanical testing based average axial rigidity results [EAavgCTRA = 1.23*EAavgHist - 3094, t = 12.88, P <0.0001, R2 = 0.74]. This correlation improved significantly when the CTRA-based EAavg was correlated to the mechanical testing based minimum axial rigidity results [EAminCTRA = 0.83*EAminHist + 1.1, t = 16.86, P < 0.0001, R2 = 0.84]. Tests of slopes in the mixed model regression analysis indicated a significantly steeper slope for EAavg compared to Eamin (p = 0.028) and a significant difference in the y-intercepts (p = 0.022).

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 the 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).

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. The distribution of the rigidity values along the regression lines followed the strength pattern observed in results from mechanical testing, where cortical and trabecular bone specimens from control animals were stronger than specimens from ovariectomized animals, which were in turn stronger than specimens obtained from animals undergoing partial nephrectomy.

DISCUSSION:
In summary, the results of this study suggest that 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. As expected by an analysis of the biophysics of bone subjected to mechanical load, minimum axial rigidity proved a better model for measuring bone rigidity than average axial rigidity. It remains to be seen whether analogous CT images in human patients could also be used to predict future fracture risk in patients with metabolic bone diseases. Future study across multiple disease models and imaging techniques involving larger sample sizes is warranted to evaluate the reproducibility and extensibility of these promising results. However, the results of this study suggest considerable potential in the use of µCT-based CTRA to quantitatively and non-invasively assess load bearing capacity of bones with metabolic diseases.