INTRODUCTION:
Animal studies using well-controlled, non-invasive exogenous loadings have been invaluable in broadening the understanding of bone mechanobiology. The axial loading protocol for the mouse ulna has become a particularly popular model (Lee et al., 2002; Robling et al., 2002). This loading protocol consists of placing the forelimb of an anesthetized mouse between two vertically oriented cups, which support the olecranon and the flexed wrist. With this model, the target axial force is determined using a calibration procedure that relates the applied force to the measured strain in several sacrificed animals.

Robling et al. (2002) reported large variances in the ulnar midshaft strain for identical axial loads and proposed a method to partially account for the variance based on differences in ulnar geometry among animals. However, the reported variation of the strains was only partially explained by the corresponding variation in geometric properties (e.g., \(I_{max}\)). Kotha et al. (2004), using a similar loading protocol, applied a 6.5 N peak force to five excised rat ulna specimens and reported an average compressive strain of approximately -2080 \(\mu\varepsilon\) and a range from -1580 to -2650 \(\mu\varepsilon\). The associated variance due to the sensitivity of the experimental setup (i.e. repeatability) has not been examined. The purpose of this study is to quantify the sensitivity of predicted midshaft strain to perturbations of the ulna bone position and orientation in the loading setup. A finite element (FE) simulation is conducted to ideally control the applied boundary conditions to the mouse ulna and to limit any confounding effects of operator involvement.

METHODS:
The left forelimb of a C57BL/6 strain mouse was scanned with a Scanco vivaCT 40 microCT scanner. The scan was performed using the following settings: 55 kVp, 145 \(\mu\)A, 400 ms integration time, 2x averaged data factor, 10.5 \(\mu\)m voxel size; a 1200 mg-HA/cm\(^2\) beam hardening correction algorithm provided by Scanco was also applied. A 3D grayscale representation of the ulna was created and the most proximal point of the olecranon was identified. The 3D volume was aligned such that the vector defined by the olecranon and the point on the surface of the ulna with the largest Euclidean distance from the olecranon were aligned with the z-axis (Fig. 1). The elastic modulus and Poisson’s ratio of the bone elements were 13.3 GPa and 0.3, respectively. The voxels in the model were downsampled by a factor of two, resulting in 21.0 \(\mu\)m voxels. A direct voxel to hex element conversion was performed to create the unperturbed FE model. All the FE models were solved using the Scanco FE software (v1.15b). The ulna model had 1,166,563 nodes and 1,063,357 elements.

RESULTS:
Uniaxial strain in the z-direction is presented for selected results from the first axial force perturbation sub-study. An equivalent ‘gauge’ strain was calculated for the second orientation perturbation sub-study using the strain tensor of each element.

The maximum strain at the medial aspect of the midshaft for the unperturbed loading (indicated by #5 in Fig. 1) was -3122 \(\mu\varepsilon\). For the perturbed loading cases, the greatest strain was -4795 for loading #1; the least strain was -1446 for loading #9 (Fig. 2). The strain values were most sensitive to changes in load position in the direction roughly corresponding to the \(I_{max}\) direction, i.e., in the primary plane of bending. For every 100 microns shift in the axis of applied axial loading in the plane of \(I_{max}\), the maximum compressive strain at the midshaft changes by 418 \(\mu\varepsilon\) or 13% of the unperturbed value.

The perturbation of the vertical orientation of the mouse ulna yielded similar results as the first sub-study. The largest increase in compressive strain at the midshaft was 1741 \(\mu\varepsilon\). Thus, for an orientation change of 1\(^\circ\), the expected change in strain would be 423 \(\mu\varepsilon\) or 13.6% of the unperturbed value.

Figure 2. Strain (uniaxial in the z direction) distributions for perturbed force/support application positions 1, 5 and 9 (first sub-study).

DISCUSSION:
The purpose of this study was to quantify the sensitivity of longitudinal strain to perturbations in the position and orientation of the applied axial force for the axial ulnar loading technique of the mouse forelimb. Changes of 100 micron in position or 1\(^\circ\) in orientation resulted in changes on the order of 13% in both cases. Robling et al. (2002) reported that a peak strain magnitude increase from 2000 \(\mu\varepsilon\) to 4000 \(\mu\varepsilon\) corresponded to an approximate increase in bone formation rate from 0 to 400 \(\mu\)g/cm\(^2\)/year per for C57BL/6 mice. Considering that the change in microstrain caused by small perturbations to the axial loading protocol boundary conditions presented here resulted in approximately an equivalent magnitude as the reported range of strain that was tested (which assumed no variation existed between loading cycles), additional consideration must be afforded how different specimens are loaded accurately and/or how the same specimens are loaded consistently.

The amount of perturbation to the orientation and axial force position used in this study were selected to approximate what the authors considered a reasonable variation that could occur during the physical testing protocol. A change in axial force location of 400 microns corresponds to approximately 11% of the 3.7 mm width of the spherical cup used to support the flexed carpus and olecranon. A change of vertical orientation of 4\(^\circ\) corresponds to a 1 mm displacement at the distal end of the ulna assuming the olecranon is fixed. Although the actual variability that might occur during a repeatability study is not currently known, the potential effect on imposed strain is significant and must be addressed for continued confidence in the results related to this protocol.

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