Axial Ulnar Loading in the C57BL/6 Mouse: Contribution of Inter-Animal Geometric Variation to Midshaft Periosteal Strain

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Introduction

Non-invasive, axial loading of the mouse ulna has become a popular model to study bone adaptation [1,2]. A load-strain calibration experiment with a uniaxial strain gauge attached to the ulnar diaphysis of a small number of sacrificed animals is typically used to determine the relationship between applied exogenous load and periosteal strain. Large variations in calibration study results suggest that animal-specific calibrations may be necessary. However, it is unclear if the observed variations result primarily from inter-animal differences or from experimental complexities associated with gauge placement and limb alignment during loading [3,4]. Additionally, the optimal set of geometric variables for performing an animal-specific calibration are not known as the contribution of the various geometric parameters to periosteal strain has not been well studied. Our hypothesis is that inter-animal geometric variation within the C57BL/6 strain accounts for the variation in microstrain previously reported during load-calibration studies. A theoretical model is used to compute simulated gauge strains from mice scanned with microCT. The relationship between individual geometric parameters and simulated gauge strain is also investigated.

Methods

The data were collected as part of a larger study and the VA Palo Alto Institutional Animal Care and Use Committee approved all procedures.

Three sections of the right forelimb of C57BL/6 mice (N=39, female, age: 16-18wk), positioned in a custom acrylic (below) were scanned with microCT.

Custom non-metallic fixture used to replicate mouse forelimb posture during the ulnar axial loading protocol

Mouse forelimb positioned in fixture

Statistics

Linear statistical models (JMP, SAS Institute Inc.) were used to investigate the predicted variance associated with individual variables for the simulated gauge strain.

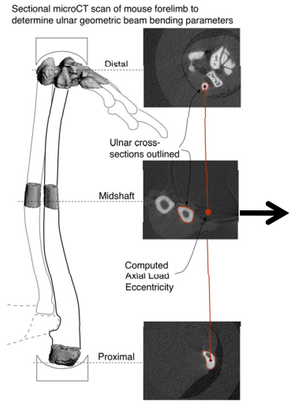
MicroCT Scan Protocol

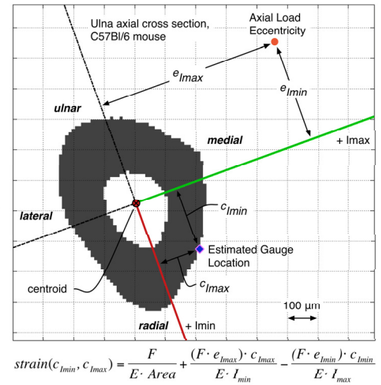
- *vivaCT* 40 microCT (SCANCO Medical, Switzerland)
  55 kVp, 145 mA, 347 ms integration time,10.5 micron isotropic voxel
- Beam hardening correction algorithm specified by Scanco (1200 mg-HA/cm^3)

Theoretical Model of Gauge Strain

- Homogenous beam theory
  - Elastic modulus, E: 20 Gpa
  - Simulated in-vivo compressive load of 1 N, assumed 65% of load supported by the ulna [5]

Sectional microCT scan of mouse forelimb to determine ulnar geometric beam bending parameters



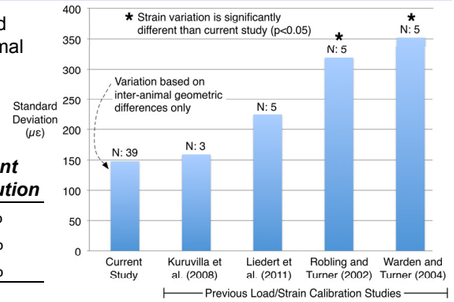


Results

The predicted mean and variation in simulated midshaft gauge strain resulting from inter-animal geometric differences was -985 ± 148 με/N.

Distribution of loading components contributing to predicted mean strain

	Strain Magnitude	*Percent Contribution*
Axial Compression	134 □□	11 %
Bending, I_{min} axis	975 □□	79 %
Bending, I_{max} axis	124 □□	10 %



Comparison of strain variation Data normalized to 1N loading [1,2,6,7].

Linear Statistical Models

Simulated gauge strain was best predicted by the single parameter I_{min}, which accounted for 54% of the residual variance. The combined terms of eccentricity and second moment of inertia (e_{Imax}/I_{min}) accounted for 78% of the variance and the full gauge strain due to bending about the I_{min} axis term ($e_{Imax}*c_{Imax}/I_{min}$) accounted for 89% of the variance and reduced the residual RMSE to 50.4 με. In contrast, the section modulus term associated with bending about the I_{min} axis (c_{Imax}/I_{min}) only accounted for 63% of the variance with no additional reduction in variance when the section modulus term associated with bending about the I_{max} axis (c_{Imin}/I_{max}) or the area term were included in the model, a potential result associated with the high correlation of c_{Imax} and I_{min} (correlation coefficient of 0.90). The combined beam bending terms ($e_{Imax}*c_{Imax}/I_{min}$) and ($e_{Imin}*c_{Imin}/I_{max}$) accounted for 99% of the variance of the simulated gauge strain.

Significance: Understanding the contribution of observed gauge strain variation attributed to the natural geometric variation among animals is critical for indentifying the optimal set of animal-specific normalization parameters. The presented theoretical model and scan protocol provides a potential method for estimating in-vivo animal-specific periosteal strain with less experimental variation than previous load/calibration gauging studies.

Discussion

The theoretical model resulted in mean strains similar to those previously reported in the literature (data not shown). However, the variation of the simulated gauge strain was less than that of all the compared studies, suggesting that the inter-animal geometric variation may be only one component contributing to the variation observed in the load/calibration gauging studies. Variation in gauge strain can be caused by variations in gauge position, gauge orientation, gauge fixation, glue line thickness, etc., and these can substantially add to the strain variability resulting from bone geometry alone. When compared to other studies, the data suggest experimental complexities not associated with inter-animal differences may contribute to increases in observed standard deviation of inter-animal strain by over 130% (approximately 200με). Previous simulation studies have quantified a similar change in measured strain magnitude could be produced with a 190 micron circumferential shift in gauge placement [3] or a 1.5° change in bone alignment [4].

The majority of the geometric parameters investigated were correlated (results not presented), confounding the direct relationships between variances associated with individual geometric parameters and the computed strain variability. Interestingly, the parameters defining eccentricity (e_{Imax} and e_{Imin}) were not well correlated with other geometric parameters suggesting that measurement of the two eccentricities (to be used for animal-specific normalizations) may be necessary to fully account for inter-animal differences in periosteal strain.

References

[1] Liedert et al., Bone, 2011. [2] Robling & Turner, Bone, 2002. [3] Wagner & Beaupre, Amer Soc Biomech. Conf, 2011. [4] Wagner & Beaupre, ORS, 2249, 2011. [5] Kotha et al., J Biomech, 2004. [6] Kuruvilla et al., J Musculoskelet Neuronal Interact, 2008. [7] Warden & Turner, Bone, 2004.

Acknowledgements

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