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Image Registration Demonstrates a Variable Effect of the Growth Plate on Vertebral Strain

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Abstract—Characterizing the biomechanical behavior of the vertebrae is important in understanding the impact of structural and material changes on spinal growth and fracture risk. The growth plate is critical for the normal development of the skeleton, with abnormalities leading to uneven maturation. Little is known about how growth plates affect the stress and strain experienced by the surrounding bone. Concentrated strain within the growth plate may influence mechanical cell signaling during development, lead to increased fracture risk at this site and may influence average bone strain measures. It is hypothesized that the growth plates and adjacent bony areas will take up a large amount of the strain within rat-tail vertebrae under axial compressive loading, leading to increased average bone strain measures. The sixth caudal vertebrae of 8 *rnu/rnu* rats were μ CT scanned in both loaded (20–32 N axial compression) and unloaded configurations. Image registration was used to calculate strain in the bone due to the applied load by finding a spatial mapping between the two scans. In seven of the eight rats, the majority of the strain measured within their vertebrae was concentrated in the growth plates. Five of the specimens had growth plates that demonstrated rigid behavior in contrast to compliant growth plate behavior seen in the other three rats. The presence of a compliant growth plate led to higher average (−0.03 vs. −0.01) and maximum (−0.13 vs. −0.02) strains. The strain within the growth plate is important to consider when interpreting apparent tissue level biomechanical data commonly reported in the literature [Ito, M., *et al.* Contribution of trabecular and cortical components to the mechanical properties of bone and their regulating parameters. *Bone* 31:351–358, 2002; Laing, K., *et al.* Binge alcohol treatment of adolescent rats followed by alcohol abstinence is associated with site-specific differences in bone loss and incomplete recovery of bone mass and strength. *Alcohol* 42:649–656, 2008; Sehmisch, S., *et al.* Short-term effects of parathyroid hormone on rat lumbar vertebrae. *Spine* (Phila Pa 1976), 34:2014–2021, 2009;

Shiraishi, A., *et al.* The combination therapy with alfacalcidol and risedronate improves the mechanical property in lumbar spine by affecting the material properties in an ovariectomized rat model of osteoporosis. *BMC Musculoskelet. Disord.* 10:66, 2009], as this study suggests strains are not uniformly distributed with high concentrations in and around the growth plate. This strain distribution may provide insight into the mechanical signals that cells experience during the formation of new bone, with the higher strains near the growth plate signaling cells to lay down more bone, while also leading to increased risk of fracture in this region.

Keywords—Growth plate, Biomechanics, Digital image correlation, Spine, Vertebrae.

INTRODUCTION

Characterizing the biomechanical behavior of the vertebrae is important in understanding the impact of structural and material changes on spinal growth and fracture risk. Vertebral trabecular bone at the apparent material level has been demonstrated to fail at a strain of 0.008 independent of density¹⁵; however, the measurements of apparent behavior used to identify this value may not necessarily be indicative of trabecular tissue level strain. Tissue level strains in trabecular bone have been shown to differ from the applied apparent level strains through the use of voxel-based finite element modeling of trabecular bone cores.^{6,7}

Human vertebrae contain both an inferior and superior growth plate which normally fuse following puberty.¹ The growth plate is critical for the normal development of the skeleton, with abnormalities leading to uneven maturation. The rate of bone lengthening is known to be affected by externally applied loading. In the spine, developmental pathologies of the growth plates can result in spondylolisthesis

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and scoliosis.²⁶ It would, therefore, be advantages to have a greater understanding of the growth plate's response to loading within a physiologic loading environment.

The growth plate is initially a dynamic structure that contains proliferating chondrocytes that create an extracellular matrix much like cartilaginous tissue and as such has a much lower modulus than bone.²⁹ As bone growth occurs, the growth plate tissue becomes mineralized. This rigid behavior may represent more mineralized growth plate tissue, a thinner growth plate, or regions of bony bridging, yielding a more rigid construct.

Rodents are often used as preclinical models for investigating the biomechanical consequences of spinal pathologies and therapeutic interventions.⁹ Growth plates are present within rat vertebrae throughout life¹³ (Fig. 1). Little is known about how growth plates affect the stress and strain experienced by the surrounding bone. As such it is important to consider the impact of growth plates in understanding the biomechanical behavior of whole vertebrae in rodent models.

In contrast to physical strain gage measurements or global changes in displacement measured during biomechanical testing, image registration of μ CT scans can be used to spatially resolve full strain fields. Deformable registration techniques have been previously applied to quantify tissue strain.^{2,12,17,23,25,27,30} Patterns of intensity used to spatially align images provide a method of back calculating tissue strain in both bone and soft tissues. This investigation aims to characterize the biomechanical response of rat-tail vertebrae to axial compressive loading through image-based strain measurement. It is hypothesized that the growth plates and adjacent bony areas will be

responsible for a large amount of the strain generated within rat-tail vertebrae under axial compressive loading, leading to increased average bone strain measures.

METHODS

The sixth caudal vertebrae of 8 *rmu/rmu* rats (8–9 weeks of age) were μ CT scanned (GE Explore Locus, General Electric Company, Fairfield, CT, USA) first in an unloaded configuration and subsequently in a loaded configuration (20–32 N axial compression to an equivalent stress of ~ 10 MPa). The mechanical testing was designed to maximize the prefailure load to the specimens in order to generate a sufficiently high-strain magnitude. Larger strain magnitudes increase the certainty of the analytic results (increasing the signal-to-noise ratio), lessening the impact of scanner resolution and any motion that may have occurred as a result of creep. Further, in order to maximize the strain applied to the specimens, the amount of prefailure load that each specimen could safely bear was individually estimated. The estimates were based upon the stability of the pins within the adjacent vertebrae; in a few pilot specimens, fracture occurred during load application in the vertebrae through which the load was applied (adjacent to the vertebral body of interest). The pins holes in these vertebrae were always the site of fracture because they created stress risers within the bone that would not otherwise have been present.

Imaging was carried out over 907 views covering 360° of rotation (2.5 h scan time) with an X-ray source at 90 μ A and 80 kV. Reconstruction of volumes from

FIGURE 1. (a) MicroCT cross-section of the growth plate within the sixth caudal vertebrae of *rmu/rmu* rats. The growth plate appears as a dim, less dense line (white arrows) between two relatively much more dense regions of bone. The growth plate in these vertebrae has a characteristic undulated structure that cannot be entirely captured in a single cross-section. (b) A 3D reconstruction of the growth plate from a manual segmentation of the CT data, more clearly showing the undulated structure. (c) A histological nondecalcified section depicting the vertebral growth plate in *rmu/rmu* rats stained with Goldner-Masson that stains cell nucleuae dark brown to black, cytoplasm and muscle brick red, connective tissue, bone and acidic mucous substances stain green. The growth plate is well defined by the absence of calcified tissue, appearing as a white band within the structure. Left is distal.

the X-ray projections was carried out with the GE Explore Locus Recon utility to $17.5 \mu\text{m}/\text{voxel}$. The unloaded configuration of the specimen was imaged while immersed in agar. The load was applied to samples with custom designed μCT compatible spring loaded telescoping device (Fig. 2); loads applied to the samples were allowed to creep for 30 min. After 30 min, the viscoelastic response of the tissues loaded was found in calibration experiments to have sufficiently dissipated. The device was designed to transfer axial compressive loads to the vertebra of interest through the adjacent vertebrae and intervertebral discs of the excised spinal motion segment (fifth to seventh caudal vertebrae). Previous pilot work had indicated the potential of this configuration for applying axial compressive loading to vertebrae in intact rat-tails.³ The device was calibrated by loading select specimens inline with a ELFM-T2M, 500 N capacity subminiature tension/compression load cell (Entron Devices Inc., Fairfield, CT, USA) and by calibration against a materials testing machine 250 lbs load cell (MTS Bionix 858, Eden Prairie, MN, USA) in a specialized calibration set up.

A digital volume correlation method, previously demonstrated and validated, relying on image registration was used to calculate strain and displacement fields in the bone due to the applied load.¹⁰ This technique utilizes both loaded and unloaded images of the specimen. The registration was implanted in a multiresolution framework. The algorithm begins with an iterative optimization of affine mapping parameters to ideally match up the entire loaded and unloaded scans based upon a mutual information metric. The affine mapping parameters allow a transform with rotation, scaling, shearing, and translation (12 DOF). Once an acceptable fit was

obtained, the moving scan was partitioned into eight pieces by bisecting the scan along the three axes. The affine transform found for registering the whole moving scan was used as the initial guess in these eight subpieces. These eight pieces formed the second level of registration and were each individually registered. The subpieces then each spawned eight more individual pieces and so on until the maximum level of analysis desired was reached. Once the registration was completed, the displacement field and strain field were calculated:

$$e = \frac{1}{2} (\nabla \vec{A}^T + \nabla \vec{A})$$

$$\vec{A}(x, y, z) = \vec{T}(x, y, z)P(x, y, z) - P(x, y, z)$$

$$T = \begin{bmatrix} J_{11} & J_{12} & J_{13} & X_{\text{Trans}} \\ J_{21} & J_{22} & J_{23} & Y_{\text{Trans}} \\ J_{31} & J_{32} & J_{33} & Z_{\text{Trans}} \\ X_{\text{Center}} & Y_{\text{Center}} & Z_{\text{Center}} & 1 \end{bmatrix}$$

where e is the strain matrix, A is the displacement vector, T is the affine transform found from registration, and P is the voxel location. The gradient of the displacement field and strain were calculated at the center of registration regions with finite differencing; elsewhere the fields were interpolated using third order B splines (Insight Segmentation Toolkit, Bethesda, MD, USA).

Regions that were individually registered and used to calculate the strain varied from $260 \times 240 \times 170 \mu\text{m}^3$ to $750 \times 840 \times 580 \mu\text{m}^3$. The zz strain distributions in the rat-tail were characterized by plotting strain contours, calculating the mean strain, median strain and strain in the 10th and 90th percentile of the strain distribution.

FIGURE 2. Custom designed μCT compatible spring loaded telescoping device for application of axial compressive loading to rat-tail spinal motion segments.

Axial strain was successfully calculated in whole rat vertebrae by image registration, ranging from 0.008 to 0.04 in compression with an average axial strain of 0.016 (Table 1). None of the vertebrae showed evidence of damage due to the applied loading. The nature of the growth plate largely determined the strain magnitude and distribution, as evident in the contour plots of the strain field (Fig. 3). In seven of the eight rats, the majority of the strain measured within their vertebrae was concentrated in the growth plates and surrounding bone.

Interestingly, the structural behavior of the rat-tail growth plates was not consistent across specimens. The specimens yielded two distinct strain patterns based on the rigidity of their growth plates [rigid growth plates ($n = 5$) and compliant growth plates ($n = 3$)] (Table 2). The presence of a compliant growth plate led to maximum axial strains exceeding 0.1 (0.1–0.16 in compression) compared to maximum axial strains in the more rigid growth plate group remaining below 0.04 (0.022–0.032 in compression) (Fig. 4). The rigid growth plates were thinner and deformed much less, consistent with the strain measurements.

Centrally located strain concentrations of low magnitude and more limited spatial extent were observed in the vertebral trabecular bone (Fig. 3). All rats showed elevated strains in trabecular regions of the bone: four in the more caudal regions, three in the more cranial regions and one evenly distributed between the two regions. In five of the specimens, the strain distributions were consistent with primarily axial compressive loading. The other specimens had strain distributions that indicated loading was slightly asymmetric, causing limited bending and shearing behavior.

MicroCT-based image registration techniques were successful in determining the full strain field present in rat-tail vertebrae under axial compressive loading. The growth plates were found to absorb the majority of the strain within the rat vertebrae; however, the behavior of the growth plates was not consistent across all specimens. The results confirm the hypothesis that the growth plates increase the average strains within the vertebrae. However, the results do not demonstrate a uniform effect. Although 8- to 9-week-old *rnu/rnu* rats were used in this analysis, the rodent spine may have some inconsistency in its level of skeletal maturity, leading to altered mechanical behavior. Villemure and Stokes have shown that rat tibial growth plate's effective modulus increases with the age of the animal.²⁹

The variability of compliance in the growth plate is important to consider when interpreting biomechanical data generated from axial loading of rat vertebrae. The standard protocol for measuring strength of bony specimens of applying an increasing compressive load will give a false picture of the strain during the elastic phase of loading. Increasing loading will first compress the growth plate and only following compression of this structure will significant displacement and strain occur in the trabecular bone and vertebral shell. This observation of how a growth plate structure will effect the strain experienced by the surrounding bone tissue is supported by investigation of growth plate tissue material properties within rodents,²⁸ rabbits^{8,18} and bovine specimens,^{4,5} both in tension,^{4,8,28} and compression.⁵ Growth plate material properties have been found to vary in the lateral plane, with the interior⁵ and the anterior⁴ parts of the growth plate found to be the stiffest and strongest. The aggregate modulus of the growth plate has been found to be lower in

TABLE 1. Summary of axial strain measurements for rat-tail vertebrae loaded in axial compression, calculated by image registration.

Rat #	Average strain	Strain in 10th percentile	Strain in 90th percentile	Median strain	Standard deviation	Load applied (N)
1	-0.0437	0.0141	-0.1600	-0.0124	0.0811	27
2	-0.0080	0.0074	-0.0222	-0.0068	0.0216	30
3	-0.0160	0.0137	-0.0323	-0.0026	0.054	30
4	-0.0043	0.0042	-0.0139	-0.0014	0.014	30
5	-0.0100	0.0023	-0.0258	-0.0039	0.027	30
6	-0.0089	0.0058	-0.0228	-0.0034	0.029	30
7	-0.0083	0.0962	-0.1195	-0.0102	0.097	20
8	-0.0282	0.0033	-0.1003	-0.0117	0.048	32
Average	-0.0159	0.0184	-0.0621	-0.0065	0.0556	29

FIGURE 3. Coronal slices of rat vertebrae | CT scans. A representative specimen from rigid growth plate group in the (a1) unloaded configuration with the growth plates *encircled* in ovals, (a2) with axial strain contours plotted on top of the unloaded configuration, and (a3) in the loaded configuration. (b1–3) The same configurations for a representative specimen from the compliant growth plate group, yielding greater displacements and strains in the growth plate regions.

TABLE 2. Strain distribution comparison calculated from image registrations between specimens groups based on growth plate compliance.

Mean + STD	Compliant growth plate, $n = 3$	Rigid growth plate, $n = 5$	Overall, $n = 8$
Minimum strain (10th percentile)	$0.038 + 0.042$	$0.007 + 0.004$	$0.018 + 0.030$
Average axial strain	$-0.027 + 0.015$	$-0.0094 + 0.004$	$-0.016 + 0.013$
Maximum strain (90th percentile)	$-0.127 + 0.025$	$-0.023 + 0.006$	$-0.062 + 0.052$
Load applied (N)	$26 + 5$	$30 + 0$	$29 + 4$

compression ($0.67\text{--}1.29\text{ MPa}$,⁵ $0.57\text{--}1.41\text{ MPa}$ ⁸) than tension ($23\text{--}48.6\text{ MPa}$,⁴ $17.7\text{--}27\text{ MPa}$ ⁸), both of which are much lower than the apparent elastic modulus of trabecular bone ($67\text{--}450\text{ MPa}$ ¹⁵).

The large difference in elastic modulus between the growth plate and the trabecular bone is consistent with the results of this investigation, with much of the strain being taken up by the growth plate. The high

strains within the growth plate may well be within the physiologic realm corresponding with findings that high-compressive loads can decrease the rate of lengthening, which are thought to occur because of a decrease of size of hypertrophic cells.²⁹ Stresses up to 0.2 MPa (corresponding to approximately 0.33 strain) in compression have been shown to decrease the rate of lengthening of bone.²⁴

FIGURE 4. Comparison of the axial strain distributions of rigid and compliant growth plate groups. The compliant growth plate group has a much smaller peak.

Image registration yields a spatial strain distribution without having to make assumptions about the magnitude or distribution of applied loading or inherent material properties of the bony or soft tissues (as is required in finite element modeling). However, the method does assume that the structure that is being scanned remains intact and undergoes elastic deformation. The method will not properly measure strains in regions that have been damaged or undergone plastic-like deformations. The complexity and variability in material properties within the growth plate make it difficult to draw inferences about how μ CT-based measurements may predict its material behavior. Uncertainty in the material properties of the growth plate create a challenge in utilizing the finite element method to model the behavior of whole vertebrae and calculate similar spatial distributions of strain.

Bulk biomechanical testing is unable to determine the distribution of strain within specimens, and thus would not differentiate between trabecular and growth plate strains. The importance of the growth plate in the biomechanical response of rat vertebrae is more easily discernable through the application of image registration to calculate strain fields.

However, it should be noted that all specimens in the study contained growth plates and therefore definitive conclusions as to the effect of the presence of growth plates cannot be made. However, the more rigid growth plate group does provide a clue as to the behavior of vertebrae without growth plates, as the growth plate during maturation will fuse (in most species) and be remodeled to resemble the adjacent trabecular bone. Using image registration-based

methods to investigate growth plate mechanics is limited by the ability of the technique to only capture the deformation between two distinct time points, therefore not allowing for the study of the possible path between the two points. This is particularly relevant in the growth plate as the viscoelastic properties²¹ are undoubtedly important to the function of the structure and they cannot be appreciated with the current method given μ CT scanning times.

The mechanical role of the growth plate has previously been investigated by Sairyo *et al.* who modeled the pediatric spine using finite element analysis. In their work, the growth plates were modeled as significantly more compliant than the adjacent trabecular bone (10 MPa vs. 100 MPa).¹⁹ In agreement with our results, they showed that strains were more varied and greater with the presence of growth plates in whole pediatric vertebrae, compared to adult controls, with increased strains near the growth plate.¹⁹

Other work has further focused on strain patterns within the growth plate tissue. These investigations dealt with growth plates that were removed from bones prior to testing, thus did not include information on the surrounding bone. Villemure *et al.* investigating the mechanics of growth plates in vitro using confocal microscopy and texture correlation found large variability in strain distribution throughout the growth plate layers, with the greatest strains occurring in the reserve and hypertrophic zones.²⁸ Villemure's results are consistent with the findings in this paper, in which growth plate strain distributions were also found to be highly variable within and between specimens [with large strains (up to 0.4) resulting from an applied 0.05 strain]. Similarly, Fujii *et al.* found that tensile failure predominantly occurred in the hypertrophic zone of the growth plate.⁸ Radhakrishnan *et al.* using atomic force microscopy also found differences in growth plate stiffness with depth, finding exponentially increasing stiffness from the reserve zone to the mineral zone.¹⁸

The maximum strains measured within this study (0.1–0.16) appear very high for bony structures prefailure. However, it is important to consider two important points when interpreting these high-strain values: first, they occur near and around the growth plate and second, the length scale on which they are measured lies between apparent and tissue level behavior. The apparent axial strains measured within this study (apparent level or average strains of 0.009–0.02) were similar to previously found apparent level strains in trabecular bone on the order of 0.01.¹⁵ These large strains measured are in line with the strains that have been reported in the growth plate and for individual trabecular failure. Villemure *et al.* reported strains from 0.05 to 0.40 in the growth plate under

compressive loading²⁸ and Hernandez *et al.* measured failure strains in individual trabeculae that varied from 0.018 to 0.20.¹¹

The growth plate's extracellular matrix contains proteoglycans, collagen, and water, and is modeled as a biphasic material.^{5,8} The growth plate in long bones⁴ and in vertebrae has an undulated structure (Fig. 1), however regardless of the orientation of the specific undulation of the growth plate, the cells within it grow in lines that are aligned with the long axis of the bone.^{4,5} In our study, a similar undulated structure of the growth plates within the vertebrae was found. Interestingly, Cohen *et al.* found denser bone on the proximal side, while in this study denser bone was observed on the distal side of the growth plate.⁴ This structural difference may have an effect on the permeability of the two sides, affecting the viscoelastic response of the growth plate.⁵

Image registration was able to successfully quantify the biomechanical response within the growth plate due to the large contrast difference between the growth plate and adjacent bone. However, the presence of the growth plate limits the ability of the image registration method to accurately spatially resolve strain in the trabecular bone of whole rat vertebrae. Large strains in the growth plate lead to adjacent bone appearing highly strained due to the limited spatial resolution possible with this image registration technique. The growth plate hinders the image registration methods from completely spatially resolving where the strain occurs because there is no contrast within the structure when it is imaged with CT. Further, the ability to calculate accurate strain is dependent upon the ability to accurately register subregions. These subregions must have sufficient contrast texture to accurately find the displacement. The growth plates have no internal contrast on CT, thus the only features that are used to accurately measure displacement in the region around the growth plate is the surrounding trabecular bone and the interface of the growth plate with the bone. The limited spatial resolution possible with this technique due to the underlying structure indicates a potential benefit of the utilization of other computational analysis approaches; whereas image registration does not require as much *a priori* knowledge it nevertheless may result in lower resolution results than is possible with the finite element method.

The ease of interpretation of the strain fields was also limited by the loading technique. The load was applied through the adjacent vertebral bodies and intervertebral discs in order to represent a more physiologic scenario. However, this resulted in load application that was not entirely axial compressive, as evidenced by the presence of limited tensile strains, suggesting small amounts of bending. The presence of

wide distribution of strains (Fig. 4) strongly suggests some asymmetry in the load applied; however, this may accurately represent the physiological loading scenario. This aberration of the loading is noted as a limitation of the loading protocol; however, load was predominantly applied in the axial direction.

CONCLUSIONS

Compressive loading of the rat-tail vertebrae resulted in strain concentrations within the growth plates. The amount of strain experienced by the growth plates was highly variable, yielding two distinct patterns demonstrating rigid and compliant growth plate behavior. The specimens with compliant growth plates showed greater strain concentrations within and around the growth plates with greater average whole specimen strain experienced than specimens possessing a more rigid growth plate. The observed strain concentrations within and adjacent to the growth plate may lead to increased fracture risk near the growth plate. This strain distribution may provide insight into the mechanical signals that cells experience during the formation of new bone with higher strains near the growth plate signaling cells to lay down more bone. Finally, the amount of strain within the growth plate is important to consider when interpreting mechanical testing data obtained from specimens that contain growth plates, which is commonly reported in the literature,^{14,16,20,22} as this study suggests strains are not uniformly distributed with high concentrations in and around the growth plate.

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