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Version Post-Print/Accepted Manuscript

Citation (published version) Hojjat SP, Beek M, Akens MK, Whyne CM. Can micro-imaging based analysis methods quantify structural integrity of rat vertebrae with and without metastatic involvement? J Biomech. 2012 Sep 21;45(14):2342-8. doi: 10.1016/j.jbiomech.2012.07.004. PMID: 22858318

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Publisher's Statement The final published version of this article is available at Elsevier via <https://dx.doi.org/10.1016/j.jbiomech.2012.07.004>.

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Can micro-imaging based analysis methods quantify structural integrity of rat vertebrae with and without metastatic involvement?

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ARTICLE INFO

Article history:
Accepted 8 July 2012

Keywords:
Spinal metastases
Structural integrity
Image-based quantification
Multi-modal image analyses
Finite element analyses

ABSTRACT

This study compares the ability of μ CT image-based registration, 2D structural rigidity analyses and multimodal continuum-level finite element (FE) modeling in evaluating the mechanical stability of healthy, osteolytic, and mixed osteolytic/osteoblastic metastatically involved rat vertebrae. μ MR and μ CT images (loaded and unloaded) were acquired of lumbar spinal motion segments from 15 rnu/rnu rats (five per group). Strains were calculated based on image registration of the loaded and unloaded μ CT images and via analysis of FE models created from the μ CT and μ MR data. Predicted yield load was also calculated through 2D structural rigidity analysis of the axial unloaded μ CT slices. Measures from the three techniques were compared to experimental yield loads. The ability of these methods to predict experimental yield loads were evaluated and image registration and FE calculated strains were directly compared. Quantitatively for all samples, only limited weak correlations were found between the image-based measures and experimental yield load. In comparison to the experimental yield load, we observed a trend toward a weak negative correlation with median strain calculated using the image-based strain measurement algorithm ($r = -0.405$, $p = 0.067$), weak significant correlations ($p < 0.05$) with FE based median and 10th percentile strain values ($r = -0.454$, -0.637 , respectively), and a trend toward a weak significant correlation with FE based mean strain ($r = -0.366$, $p = 0.09$). Individual group analyses, however, yielded more and stronger correlations with experimental results. Considering the image-based strain measurement algorithm we observed moderate significant correlations with experimental yield load ($p < 0.05$) in the osteolytic group for mean and median strain values ($r = -0.840$, -0.832 , respectively), and in the healthy group for median strain values ($r = -0.809$). Considering the rigidity-based predicted yield load, we observed a strong significant correlation with the experimental yield load in the mixed osteolytic/osteoblastic group ($r = 0.946$) and trend toward a moderate correlation with the experimental yield load in the osteolytic group ($r = 0.788$). Qualitatively, strain patterns in the vertebral bodies generated using image registration and FEA were well matched, yet quantitatively a significant correlation was found only between mean strains in the healthy group ($r = 0.934$). Large structural differences in metastatic vertebrae and the complexity of motion segment loading may have led to varied modes of failure. Improvements in load characterization, material properties assignments and resolution are necessary to yield a more generalized ability for image-based registration, structural rigidity and FE methods to accurately represent stability in healthy and pathologic scenarios.

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1. Introduction

Skeletal metastases most commonly affect the vertebral column (Wong et al., 1990). Spinal metastases can present as osteolytic, osteoblastic or with a mixed osteolytic/osteoblastic pattern of involvement. Non-invasive quantitative evaluation of the effect of osteolytic spinal metastases on biomechanical

stability has been conducted through image-based strain measurement (Hardisty and Whyne, 2009; Hardisty et al., 2010; Hojjat et al., 2011), structural rigidity analyses (Snyder et al., 2009; Entezari et al., 2011; Cory et al., 2010) and continuum-level finite element (FE) modeling (Hardisty and Whyne, 2009; Whyne et al., 2003; Eswaran et al., 2007; Ito et al., 2002; Kim et al., 2007; O'Reilly and Whyne, 2008). However, to date, limited work has been completed to directly compare these analytical tools or been applied to vertebrae with more complex mixed metastatic disease.

Non-invasive image-based techniques that accurately reflect vertebral integrity are needed to evaluate fracture risk and

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treatment effects in both clinical and preclinical scenarios. Robust measures could ultimately improve the management and outcome of patients with established spinal metastases. The objective of this work was to compare the ability of μ CT image-based strain measurement, 2D structural rigidity analyses and multimodal continuum-level FE modeling in the evaluation of mechanical stability in healthy, osteolytic and mixed osteolytic/osteoblastic metastatically involved rat vertebrae. It is hypothesized that these methods could be used to accurately demonstrate the biomechanical stability of rat vertebrae despite variability in the presence and pattern of the metastatic involvement.

2. Materials and methods

2.1. Animal model

Fifteen 4-week-old rnu/rnu rats were divided into three groups based on pathologic involvement of the spine: healthy, osteolytic metastases, and mixed osteolytic/osteoblastic metastases. Osteolytic and mixed lesions were developed via intracardiac injection of MT1 human breast cancer cells, and Ace-1 canine prostate cancer cells, respectively (Burch et al., 2007; LeRoy et al., 2006). Three weeks post injection the presence of vertebral metastases was confirmed via bioluminescent imaging, prior to sacrifice. L1–L3 vertebral motion segments were then dissected for further analysis.

2.2. Image acquisition

2.2.1. Micro computed tomography

Each sample ($n=15$) was imaged in an unloaded configuration adjacent to bone calibration phantoms (Skyscan Corp) at a $(14\ \mu\text{m})^3$ isotropic voxel size (x-ray source: 90 mA and 80 kVp, 900 views covering 360° of rotation, GE Explore Locus, General Electric Co.). Repeat μ CT images of each sample were acquired within a calibrated customized axial loading device (Hojjat et al., 2011). Static axial loading was applied to achieve sufficient deformation to produce detectable strain in the L2 vertebra (based on the image registration algorithm (Hardisty and Whyne, 2009)) without causing fracture (45 N to 65 N based on qualitative assessment of the level of metastatic destruction visible within the unloaded scans).

2.2.2. Micro magnetic resonance imaging (μ MRI)

μ MR images were acquired of all specimens with metastatic involvement ($n=10$) using a custom-built quadrature birdcage coil (Stark Contrast MRI Research, Germany) tuned to the 1 H frequency (~ 300.3 MHz). T1 and T2 weighted μ MR scans were acquired at a $(60\ \mu\text{m})^3$ isotropic voxel size in a 7 T preclinical Biospec system (Bruker, Ettlingen, Germany). The T1-weighted μ MR images accentuate bone ultrastructure enabling accurate registration with μ CT data sets (Fig. 1b, echo time (TE)=7.6 ms, repetition time (TR)=500 ms, rapid

acquisition with refocused echoes (RARE) factor=2, and 3 signal averages). The T2-weighted μ MR images were acquired to accentuate soft tissue contrast and differentiate lower intensity bone marrow from higher intensity tumour tissue (Fig. 1c, TE=48 ms, TR=3000 ms, RARE factor=4 and 1 signal average).

All μ CT and μ MR scans were aligned to a global axis in Amira visualization software (AmiraDev, Visage Imaging, USA) prior to further analyses.

2.3. Image-based strain measurement

Due to computational limitations, image-based strain was only calculated within vertebral bodies. The cropped μ CT image of each unloaded L2 vertebral body was registered to the corresponding loaded image through a rigid registration using a quasi Newton optimizer (initial and final step sizes of $10^{-3} \times$ and $10^{-7} \times$ voxel size, respectively). A normalized mutual information metric was implemented to account for intensity differences between the scans. The initial rigid vertebral registration was then improved using a multi-resolution deformable registration algorithm, implemented as a plugin to Amira from the Insight ToolKit data base (Hardisty and Whyne, 2009). The deformable registration initially divided the unloaded scan into 8 regions, which were registered to corresponding regions in the loaded scan using an affine transform (allowing translation, rotation, scaling and shearing). Each subregion was similarly divided for two more iterations to yield the final level of analysis (Hojjat et al., 2011). The final registration yielded an overall deformation field, which was used to calculate the strain in each image block (Hardisty and Whyne, 2009; Hojjat et al., 2011). The axial strain values calculated from spatial displacement of the μ CT voxels were quantified through the calculation of mean, median, 10th percentile, and 90th percentile strains, with negative values corresponding to compression.

2.4. 2D structural stability

The unloaded μ CT images of the whole L2 vertebrae were segmented using atlas-based demons deformable registration, level set curvature evolution and an intensity-based thresholding algorithm (Hojjat et al., 2010) (Fig. 2). The segmented whole vertebrae were converted to equivalent density (ρ_{EQ}) images using the data obtained from the calibration phantoms. The elastic modulus (E) was calculated for each voxel using the relationship derived by Cory et al. ($E=8362.8(\rho_{EQ})^{2.56}$) (Cory et al., 2010).

Axial rigidity ($EA = \int E(\rho_{EQ}) dA$) was calculated for each axial μ CT slice (Snyder et al., 2009). The design of the tubular loading device limited loading to the axial direction and, consequently, the yield load (F_z) was defined as a product of the yield strain (ϵ) and the axial rigidity ($F_z = \epsilon \times EA$). Yield strain for our samples was chosen to be 0.96% (McNamara et al., 2006). The lowest value of F_z was identified as the predicted yield load (Fig. 3).

2.5. Continuum-level finite element analyses

The unloaded μ CT scans and the T1 and T2 weighted μ MR images of the L2 vertebrae with metastatic involvement were resampled to $(35\ \mu\text{m})^3$ isotropic voxel size to facilitate multimodal registration. The μ CT scans and T2 weighted μ MR images were registered independently to the T1 weighted μ MR images using a rigid registration (quasi Newton optimizer, as above) to ensure accurate 3D overlap of all three image sets. A normalized mutual information metric was

Fig. 1. (a) μ CT image, (b) T1 weighted μ MR image, (c) T2 weighted μ MR image of the L1–L3 motion segments with mixed osteolytic/osteoblastic metastatic involvement following rigid registration.

Fig. 2. Representative axial slice of the L2 rat vertebra with the bony tissue segmented (cross hatched) using the automated segmentation algorithm (used in calculating structural rigidity).

utilized to account for intensity variations between the μ CT and μ MR scans (Fig. 1).

The unloaded μ CT images of the whole vertebrae (healthy and metastatically involved) were segmented using an atlas-based demons deformable registration and a level set curvature evolution algorithm (Hojjat et al., 2010). The cortical shell was segmented out using an additional iteration of level set curvature evolution to reduce edge effects in material property assignments required for the finite element analyses (Fig. 4a). In the metastatic vertebrae, outer segmentation boundaries from the μ CT data sets were wrapped around the T2 weighted μ MR image to segment out the vertebral ROI. An intensity-based threshold equal to the mean of the segmented region plus half of one standard deviation ($\mu + \sigma/2$) was chosen by observation to define the osteolytic tissue intensity (Fig. 4b).

A novel multi-step image-based FE approach was utilized to yield accurate representation of tumour involvement through μ MR based segmentation, heterogeneous distributions of bone density based on μ CT intensity (Cory et al., 2010), and boundary conditions representing spinal motion segment loading through

registration of loaded and unloaded μ CT images. Specifically, tetrahedral FE meshes were constructed based on refined and simplified triangular surfaces generated from the μ CT and μ MR segmentations of the trabecular centrum, cortical shell and tumour volumes, respectively (AmiraDev). The number of tetrahedral elements was optimized to $\sim 60,000$. This value was based on a mesh refinement study that was previously conducted for subject-specific FE models of rat vertebrae based on the median von Mises stress and the median axial strain values (Sigal et al., 2008). Average element size was further reduced by 30% in our models. The meshes were then imported into Abaqus 6.10 (Simula, Pawtucket, RI).

Bone elements were assigned transversely isotropic elastic material properties. The equivalent density values for each element were calculated based on the μ CT intensity values (using the calibration phantoms) and converted to elastic modulus based on the relationship derived by Cory et al. for rat bone (Cory et al., 2010; Sigal et al., 2008). The cortical shell was considered as a separate material, based on the work of Pahr et al., and assigned a constant elastic modulus to avoid partial volume edge effects (Pahr and Zysset, 2009). The osteolytic tumour was modeled as an elastic isotropic material (poroelastic material properties were not included in the model based on the quasi-static loading scenario) (Whyne et al., 2000) (Table 1). Displacement boundary conditions were applied to each node on the end plates and facets of the L2 vertebrae based on the displacement vectors calculated from the registration of the unloaded and loaded μ CT scans. The final mesh was analyzed in Abaqus 6.10 using a high performance Opteron **cluster supercomputer** (Centre For Computational Biology High Performance Facility, Hospital for Sick Children, Toronto) (Fig. 5).

The FE axial (ZZ) strain patterns were qualitatively analyzed and compared to the results obtained from image-based strain measurement method. Mean, median, 10th percentile and 90th percentile axial strain values were also quantified for the whole vertebrae as well as the vertebral bodies alone. Average values for the maximum principle strain and stress values and Von Mises stress values were also calculated for the whole vertebrae.

Fig. 3. Schematic of the structural rigidity based prediction of failure load. dA represents a single pixel in an axial slice with dimensions $14 \times 14 \mu\text{m}^2$. The density of each pixel is converted to elastic modulus (E) which in turn is used to calculate axial rigidity (EA) and predict failure load (F_z) (Snyder et al., 2009).

Fig. 4. Segmentation of a vertebra with mixed osteolytic/osteoblastic metastatic disease: (a) the trabecular centrum and cortical shell on an axial μ CT slice and (b) the osteolytic tumour based on an equivalent T2 weighted μ MR axial slice.

Table 1
Material property assignments utilized in the finite element models.

Material	Type	Elastic modulus (E) in MPa	Poisson's ratio (η)	Shear modulus (G) in MPa
Trabecular centrum ^a	Transverse isotropic	$E_1 = 2787.6 (\rho_{EQ})^{2.56}$ $E_2 = 2787.6 (\rho_{EQ})^{2.56}$ $E_3 = 8362.8 (\rho_{EQ})^{2.56}$	0.33	$G = E/2(1 + \eta)$
Cortical shell	Transverse isotropic	$E_1 = 5,000$ $E_2 = 5,000$ $E_3 = 15,000$	0.33	$G = E/2(1 + \eta)$
Osteolytic tumour	Isotropic	$E_1 = E_2 = E_3 = 0.0036$	0.4995	$G = E/2(1 + \eta)$

^a Trabecular centrum refers to the whole vertebrae excluding the cortical shell.

^b Average E_3 values were calculated to be 7406 MPa, 4550 MPa, 4550 MPa for the healthy, osteolytic, and mixed vertebrae, respectively.

Fig. 5. The automatically generated tetrahedral mesh, with the displacement boundary conditions applied to the endplates and facets of the finite element model.

Fig. 6. Representative axial μ CT slices of the three groups displaying the median micro-architecture for (a) healthy, (b) osteolytic, and (c) mixed osteolytic/osteoblastic samples.

Fig. 7. Strain patterns in a vertebral body with mixed osteolytic/osteoblastic metastatic involvement generated via (a) image-registration, (b) FEA and (c) FEA with osteolytic tumour elements removed (bone only). The strain patterns results based on the two techniques yield qualitatively similar patterns as shown in the 2D coronal slices.

2.6. Destructive mechanical testing

The first and third lumbar vertebrae were potted in bone cement and attached to a servo-hydraulic testing system (MTS Bionix 858, Eden Prairie, USA). The motion segments were loaded in axial compression to failure at a rate of 1 mm/min (Won et al., 2010). Yield loads were identified as the values at the end of the linear region of the generated load vs. deformation plots.

2.7. Statistical analyses

Normality of the distribution of the quantitative measures was confirmed using one sample Kolmogorov-Smirnov (KS) tests (SPSS statistical analysis software). Pearson correlation coefficients were used to quantitatively compare the mean, median, 10th percentile and 90th percentile vertebral body strain values obtained from the image-based strain measurement with equivalent measures obtained from the continuum finite element analyses. Pearson correlations were also used to evaluate the relationship between the image-based measures (the rigidity-based predicted yield load, the quantitative strain values obtained from the image-based strain measurement algorithm and the quantitative strain and stress values obtained from continuum finite element analyses of the whole vertebrae) and the experimentally measured yield load. Results were obtained grouping all 15 samples and subsequently considering each group separately (healthy, osteolytic and mixed osteolytic/osteoblastic) to account for the micro-structural differences between the samples (Fig. 6).

3. Results

Qualitatively, the image-based strain measurement method was able to resolve the strain patterns throughout the healthy

and metastatically involved vertebral bodies. As expected, the results showed high strains around areas of osteolytic destruction in the metastatic samples as well as the growth plates of all vertebrae (Fig. 7a). Quantitatively, the strain values for both the osteolytic and mixed metastatic samples were higher than those found in the healthy vertebral bodies suggesting a reduction in mechanical stability.

Quantitatively, in considering all samples, the only relationship found between strain calculated using the image-based registration approach and experimentally measured yield load was a trend toward a weak negative correlation with mean strain values ($r = -0.405$, $p = 0.067$). A significant negative correlation between the experimental yield load and the median strain was found separately in the healthy and osteolytic groups ($r = -0.809$, -0.832 , respectively). A significant negative correlation was also observed between the mean strain in the healthy group and the experimental yield load ($r = -0.840$). The relationship between 10th percentile strain and experimental yield load in the osteolytic group also demonstrated a trend toward a negative correlation ($r = -0.749$, $p = 0.072$) (Table 2). No other significant correlations were detected.

Considering the combined group that includes all samples, the rigidity-based predicted yield load did not demonstrate any relationship with measured yield loads. Separately, a significant ($p < 0.01$) positive correlation was found between the predicted and the experimental yield load in the mixed metastatic group

Table 2
Pearson correlation coefficients (R) and the respective p -values of the bivariate correlations between quantitative strain measures obtained using image registration and the experimental yield load (significant relationships, $p < 0.05$, are highlighted in bold text).

Analysis group	Combined		Healthy		Osteolytic		Mixed osteolytic/osteoblastic	
	R	p	R	p	R	p	R	p
Mean	−0.349	0.101	−0.270	0.33	−0.840	0.038	−0.138	0.413
Median	−0.405	0.067	−0.809	0.049	−0.832	0.04	−0.596	0.145
10th percentile	−0.005	0.493	−0.104	0.434	−0.749	0.072	−0.09	0.443
90th percentile	−0.340	0.108	−0.363	0.274	−0.614	0.136	−0.221	0.361

Table 3
Pearson correlation coefficients (R) and the p -value of the bivariate correlations between rigidity based predicted yield load and the corresponding experimental yield load (significant relationships, $p < 0.05$, are highlighted in bold text).

Combined		Healthy		Osteolytic		Mixed osteolytic/osteoblastic	
R	p	R	p	R	p	R	p
0.195	0.243	0.593	0.146	0.788	0.057	0.946	0.007

($r=0.946$) and a similar positive trend in the osteolytic group ($r=0.788$, $p=0.057$). The absolute value of the predicted yield load was, however, observed to be 1.5 to 2 times higher than the experimental yield load. No other significant correlations were found (Table 3).

Qualitatively, the strain patterns obtained from the FE analyses matched well with the strain patterns generated by the image-based strain measurement algorithm (Fig. 6). Quantitatively, a significant positive correlation was found in comparing results for mean vertebral body strain generated based on the image-based strain measurement and the FE method in the healthy group ($r=0.934$). Statistical trends were observed between image-based strain measurement and the FE method results with respect to mean strain values in the combined group ($r=0.377$, $p=0.083$) and the 90th percentile strain values in the healthy group ($r=0.760$, $p=0.068$). No other significant correlations were observed with respect to any of the quantitative strain values in any of the study groups (Table 4).

In comparing the results of the FEA to the experimental yield load, significant negative correlations were found with median and 10th percentile strains in the combined group ($r=-0.454$, and -0.637 , respectively). Similar trends were also found in comparing the mean strain in the healthy group ($r=-0.366$, $p=0.09$) and 10th percentile strain in the mixed group ($r=-0.726$, $p=0.083$) to the experimental yield load. No other significant correlations were observed between FE based measures and experimental yield load (Table 5).

4. Discussion

Robust non-invasive measures that are able to quantify structural stability of bones with varying degrees of pathologic involvement can provide an important preclinical/clinical tool to direct and evaluate treatment. The objective of this work was to compare the ability of three image-based approaches to represent structural integrity in healthy and metastatically involved rat vertebrae. It was found that the ability of each of the three image-based methods was limited, in that they were unable to consistently represent the experimental mechanical behavior of both healthy and metastatically involved vertebrae.

As expected, the image-based strain measurement algorithm yielded higher compressive strain values in the metastatic cases, with high strains demonstrated at the growth plates in all

vertebrae. Quantitative strain values did not correlate significantly with experimental yield load in the combined group, but significant correlations with the experimental yield load were observed in the healthy (median strain) and osteolytic (mean and median strain) groups. Mechanical testing of spinal motion segments, while more physiologic, results in higher variability in applied loading (i.e., due to shear of the intervertebral discs). Incremental loading applied within a μ CT scanner may better characterize loading immediately prior to failure and improve correlations of image-based strain with failure load. Image registration provides full field deformation and strain information in the vertebrae without any assumptions as to force models or applied loading. However, the image-based strain measures are limited with respect to spatial resolution (deformation and strain) based on the multi-resolution deformable registration algorithm, which requires sufficient pattern for image matching (i.e., trabecular network). The limited strain resolution tends to smooth the strain field, moving maximums and minimums towards the centre of the range (Hardisty and Whyne, 2009).

Previously, structural rigidity analyses have demonstrated moderate correlations in predicting failure and assessing post fracture stability ($r=0.74$ and $r=0.55-0.77$, respectively), of single vertebrae with simulated and naturally occurring osteolytic metastases (Whealan et al., 2000; Windhagen et al., 2000), with excellent sensitivity but limited specificity (44% to 70%) in clinical prediction of fracture risk (Snyder et al., 2009). Surprisingly, in contrast, structural rigidity was not able to predict experimental yield loads in the combined data set. Strong correlations were found in evaluation of individual groups, suggesting that a single structural rigidity–yield load relationship may not be able to represent failure risk in healthy and metastatic vertebrae. Additional factors related to the 3D trabecular structure (i.e., the relationship between trabecular length and propensity to bending or buckling failure) and specific pathologic density-modulus relationships may be required to yield a more general predictive relationship. As well, the absolute value of the structural rigidity predicted yield load was considerably higher than the experimental yield load. Improvement of the density-modulus relationship specific to anatomical site or the type of bone disease present may be required to improve the accuracy of the predicted yield load values.

Previous FE modeling has demonstrated the importance of tumour size, bone density and the magnitude of load applied to the spine in predicting fracture in metastatic vertebrae (Hardisty and Whyne, 2009; Whyne et al., 2003; Eswaran et al., 2007; Ito et al., 2002). The multimodal imaging, segmentation and registration algorithms allowed for construction of vertebral models representative of the complex distribution of metastatic tumour within bone. Utilization of the deformation field obtained from the loaded-unloaded μ CT-based image registration as the displacement boundary condition for the FEA, ensured consistency between the FE and image-based strain measurement techniques. It further allowed simulation of non-uniform loading that occurs *in vivo* via the intervertebral disc and facets without the

Table 4

Pearson correlation coefficient (R) and the p -value of the bivariate correlation between quantitative strain measures obtained using FEA and image-based strain measurement (significant relationships, $p < 0.05$, are highlighted in bold text).

Analysis group	Combined		Healthy		Osteolytic		Mixed osteolytic/osteoblastic	
Strain quantity	R	p	R	p	R	p	R	p
Mean	0.377	0.083	0.934	0.010	0.304	0.309	0.561	0.162
Median	0.296	0.142	−0.263	0.335	0.026	0.484	0.526	0.181
10th percentile	−0.082	0.385	0.676	0.105	0.612	0.136	−0.112	0.429
90th percentile	0.329	0.115	0.760	0.068	0.179	0.387	0.603	0.141

Table 5

Pearson correlation (R) coefficient and the p -value of the bivariate correlation between quantitative strain measures obtained using FEA and the experimental yield load (significant relationships, $p < 0.05$, are highlighted in bold text).

Analysis group	Combined		Healthy		Osteolytic		Mixed osteolytic/osteoblastic	
Strain quantity	R	p	R	p	R	p	R	p
Mean	−0.366	0.09	0.244	0.346	−0.651	0.117	−0.361	0.275
Median	−0.454	0.045	0.162	0.397	−0.570	0.158	−0.427	0.236
10th percentile	−0.637	0.005	0.119	0.424	−0.248	0.344	−0.726	0.083
90th percentile	−0.181	0.260	0.203	0.372	−0.605	0.140	−0.005	0.497
Max prin. strain	−0.003	0.496	−0.187	0.387	0.458	0.219	−0.273	0.329
Max prin. stress	−0.156	0.290	−0.485	0.204	−0.148	0.406	0.186	0.383
Von Mises stress	0.067	0.407	−0.228	0.356	0.207	0.369	0.002	0.499

computational time, expense and assumptions required to model complex soft tissue structures and contact. However, the loaded-unloaded μ CT-based image registration limits the analysis to a quasi-static loading scheme due to the time required to acquire the high resolution images, despite the reported poroelastic behavior of the metastatic spine (Whyne et al., 2000, 2001).

Qualitatively, there was good agreement between the strain patterns obtained from FEA and the image-based strain measurement algorithm in all 15 samples. Quantitatively, only the mean strain values in the healthy vertebral bodies from the two analysis techniques demonstrated a significant correlation. The resolution differences between the two methods, the presence of high strains in the osteolytic tumour elements, limitations in the density-modulus relationship (particularly for osteoblastic tissue) and accurate FE representation of the growth plates may have limited the direct comparison of strain values. Similarly, the inability of the FEA to yield strong correlations with experimental yield loads may be due to limitations in the material property and loading assignments.

Post hoc power analyses demonstrated sufficient power for detection of moderate correlations between methods in the combined group ($n=15$) with a 94% probability of observing a significant correlation with a Pearson coefficient of 0.7. The post hoc power for the individual groups ($n=5$, 33%) increases the possibility of type II errors in these analyses.

Overall, the conclusion of this work is that in their present form none of the three image-based methods are able to represent the complex structural behavior of both healthy and metastatic rat vertebrae. This study demonstrated limited ability of frequently studied and applied computational methods to predict failure loads in healthy and pathologic rat vertebrae which is important as measures that work for single cases (i.e., healthy vertebrae) may be applied erroneously to pathologic cases. Image-based measures to be used in the metastatic spine need to be robust enough to represent the physiologic variation that exists in the pathology (differing amounts and patterns of tumour volume, osteolysis and osteoblastic involvement). Despite qualitative agreement found between the 3D image-based registration and the FE method, quantitative agreement was limited, which

may be explained in part by resolution mismatch and FE material property assignments. Future work focused on determining differences in the mechanisms of failure may direct improvements to computational image-based approaches. Ultimately, the availability of image-based non-destructive techniques that can quantify structural stability may improve the efficiency (reduce sample sizes) of preclinical studies and allow temporal mechanical evaluation of tumour growth and treatment effects over time.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

Acknowledgements

This work has been supported by Canadian Institutes of Health Research (CIHR) grant number 724180902. We would also like to thank the Centre for Computational Biology High Performance Facility, Hospital for Sick Children, Toronto, for supporting our FE analyses.

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