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Presence of Intervertebral Discs Alters Observed Stiffness and Failure Mechanisms in the Vertebra

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Abstract

Ex vivo mechanical testing is an essential tool for study of vertebral mechanics. However, the common method of testing vertebral bodies in the absence of adjacent intervertebral discs (IVDs) may limit the physiological relevance of the results. The goal of this study was to determine the influence of IVDs on vertebral mechanical properties and failure mechanisms. Rabbit thoracic vertebral bodies were tested with and without IVDs in a stepwise fashion that incorporated a micro-computed tomography scan at each loading step. The image sequences were analyzed using digital volume correlation to quantify deformations throughout the vertebral body. The observed deformation patterns differed substantially between the groups. Specimens tested with IVDs exhibited a slow increase in strain in the inferior and posterior regions, followed by a sudden increase in strain in the anterior cortex right at the yield point. In contrast, the highest strains in the isolated vertebral bodies were in the posterior regions throughout the test. Specimens tested with IVDs had lower stiffness (507.49 ± 184.73 N/mm vs. 845.61 ± 296.09 N/mm; $p = 0.044$), higher ultimate displacement (2.00 ± 0.68 mm vs. 1.17 ± 0.54 mm; $p = 0.043$), and higher maximum shear strains (e.g. top 25th percentile: 0.19 ± 0.11 vs. 0.06 ± 0.07 mm/mm; $p < 0.0458$), and tended to have lower ultimate force (690.28 ± 160.25 N vs. 873.81 ± 131.48 N; $p = 0.056$). Similar work to failure (648.15 ± 317.86 N-mm vs. 603.49 ± 437.95 N-mm; $p = 0.844$) was observed between the two groups. These results indicate that testing vertebral bodies in the absence of IVDs can elicit artifactual failure mechanisms. These artifacts may be more prominent than the effects on vertebral strength and toughness.

Keywords

Vertebral body; mechanical testing; failure patterns; strength; load distribution

INTRODUCTION

Mechanical testing of vertebrae *ex vivo* is a widely used and essential method in research on the effects of aging, genetics, disease, and drug treatments and other interventions on the mechanical behavior of the vertebra. For these mechanical tests, the adjacent intervertebral discs (IVDs) are often removed prior to testing in order to obtain better control over the

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

loading conditions that are applied to the vertebra and to simplify calculations of vertebral stiffness. However, concerns have been raised that testing of isolated vertebrae produces highly idealized, non-physiological loading across the endplate (Yerby et al., 1998). Whether these loading conditions confound measurements of vertebral mechanical properties and failure mechanisms has not been established but is of central relevance to the design and interpretation of *ex vivo* mechanical tests.

The load distribution across the endplate depends on the anatomy and health of the adjacent IVDs. For healthy IVDs, the load distribution measured using stress profilometry is characterized by a high plateau of pressure in the nucleus pulposus, flanked by a steep drop in pressure over the approximate thickness of the annulus fibrosus (Adams et al., 2006; Pollintine et al., 2004). In degenerated IVDs, regions of high pressure occur instead in the annulus, and the percentage of the applied load distributed to the anterior half of the vertebra as compared to the neural arch increases dramatically from erect to flexed postures (Pollintine et al., 2004). In vertebrae with degenerated adjacent discs, trabecular bone is denser and stronger posteriorly than anteriorly (Hulme et al., 2007; Keller et al., 1989; Keller et al., 1993; Simpson et al., 2001), consistent with increased loading of the posterior region in erect postures. Moreover, the strength and fracture mode of vertebrae under axial compression can depend on IVD health (Hulme et al., 2007; Hansson and Roos, 1981). These studies highlight how variable the load distribution across the endplate can be and how these variations, by potentially altering the properties of the underlying bone tissue, might affect the mechanical response of the vertebral body.

This influence of loading conditions on vertebral mechanical behavior has been addressed using finite element (FE) models. By using different load distributions across the endplate to represent different severities of IVD degeneration, these studies have examined the effects of disc health on stress and strain distributions within the vertebra (Dai, 1998; Polikeit et al., 2004; Shirazi-Adl et al., 1984) and on the relative contributions of the cortical shell and trabecular centrum to vertebral strength and fracture risk (Buckley et al., 2006; Frei et al., 2002; Homminga et al., 2001; Silva et al., 1997). Interestingly, one of these studies predicted that the specific load distribution across the endplate had only a mild effect on estimates of vertebral strength, although the magnitude of the effect was largest for low-density vertebrae (Buckley et al., 2006). Other FE studies have examined the interplay between disc health and the spatial distribution of bone density in the vertebra, finding that as a result of bone adaptation in response to altered loading associated with IVD degeneration, bone density would be expected to decrease in the central transverse regions of the vertebra (Homminga et al., 2012). In contrast to the multitude of FE studies, the only experimental data available thus far comes from mechanical testing of slices of vertebrae with and without the disc tissues (Yerby et al., 1998) and from comparison of 2-D measurements of surface deformations of vertebrae to data on intra-discal pressure (Pollintine et al., 2010). True 3-D, experimental data are lacking.

The overall goal of this study was to determine the influence of the IVDs on vertebral mechanical properties and failure mechanisms. A method of digital volume correlation (DVC) (Hussein et al., 2012; Liu and Morgan, 2007) was used to track the deformation patterns that developed within the vertebral body prior to and at the onset of failure. The specific objectives were: 1) to quantify 3-D deformation and failure patterns in vertebral bodies tested with and without IVDs; and 2) to quantify the effect of the IVDs on vertebral mechanical properties.

METHODS

Specimen Preparation

Thoracic vertebrae (6 T9 and 6 T11) were dissected from fresh-frozen spines of female New Zealand White rabbits (18–28 weeks of age). All samples had partially open growth plates of similar thickness, as examined qualitatively using 3-D reconstructions of μ CT images. Six vertebrae were harvested with the adjacent intervertebral discs intact (“V+D specimens”) (Figure 1A). The remaining six specimens consisted of only the vertebrae (“V specimens”) (Figure 1A). Posterior elements were removed, and the cranial and caudal ends of the specimens were potted in polymethyl methacrylate (PMMA). Care was taken during the potting process to align the superior-inferior axis of the vertebral bodies with the axial loading direction.

Mechanical Testing and Micro-Computed Tomography (μ CT)

Each specimen was placed in a custom-designed, radiolucent, loading device. The device was filled with saline to hydrate the specimen throughout the test. The specimen was potted in a rectangular-shaped dish, which fit snugly within an inset region within the loading device. Hence, the specimen as a whole was constrained from rotating inside the loading device (Figure 1B), although the vertebral body in the V+D specimens was not itself constrained against rotation in any additional manner. After applying a pre-load of ~ 30 N for 20 minutes, the vertebral body was scanned via μ CT (μ CT 80, Scanco Medical, Brüttisellen, Switzerland; $36 \mu\text{m}/\text{voxel}$) (pre-load scan). The specimen was then compressed axially in a stepwise fashion. For the V specimens, each loading step consisted of applying a compressive displacement of 0.25 mm applied at a rate of 0.15 mm/sec. For the V+D specimens, the displacement increment was chosen such that the peak axial force that developed at the first loading step matched the average peak force observed at the first loading step in the V specimens. A μ CT scan was performed at each step, following a 20-minute relaxation period. The duration of each μ CT scan was approximately 90 minutes. The force was recorded right after load application, after the 20-minute relaxation period, and upon completion of the μ CT scan to assess the amount of relaxation taking place throughout a load increment. The overall amount of force relaxation was approximately 70% of the initial peak force, and the majority (87%) of the relaxation occurred during the initial 20-minute period. The combination of stepwise loading and μ CT imaging was continued until the ultimate force was reached (seven load increments on average). For both groups, the ultimate force was defined as the peak of the force-displacement curve constructed from the values of force recorded after the 20-minute relaxation periods.

3-D Measurement of Deformation and Failure

Digital volume correlation (DVC) was used to obtain direct, 3-D experimental measurements of the failure patterns in the vertebral body, as defined by the distributions of strains throughout the centrum (Hussein et al., 2012). DVC analyzes the images from the different loading steps in an automated fashion to track the movement and deformation of multiple, individual sub-regions throughout the entire centrum over the course of mechanical testing. In preparation for DVC, the images from the different loading steps were aligned to the pre-load scan using image registration (Scanco Medical, Brüttisellen, Switzerland). The sub-regions within the vertebral body were then defined by generating an irregular mesh, consisting of hexahedral sub-regions with side length ~ 1.44 mm, that conforms to the geometry of the vertebral body, using the IA-FEMesh software (The University of Iowa, Iowa City, IA) (Figure 2C). The sub-region size was chosen based on results from a previous study (Liu and Morgan, 2007) which found that sub-regions with side length ~ 1.44 mm provide the best combination of spatial resolution, accuracy, and precision in the strain measurements in the rabbit vertebral body. The images from a given

loading step were then analyzed together with the pre-load images using a custom, DVC algorithm (gradient-based, iterative, optimization technique) (Liu and Morgan, 2007) to determine the displacement and strain fields throughout the entire vertebral body (Figure 2D) at each load increment. The measured strain fields correspond to strain experienced as the vertebra is loaded to failure. For this DVC method, the errors in strain measurements were estimated to be within 345 $\mu\epsilon$ for the rabbit vertebra (Liu and Morgan, 2007).

The magnitudes of the principal strains and maximum shear strain were computed. These strains were examined throughout the vertebra as a whole. In addition, the strain magnitudes were examined in three separate, contiguous regions of the vertebra: superior, central and inferior. The superior and inferior regions were ~2.44 mm in height and included the growth plate and adjacent bone. These two regions could not be restricted only to the growth plate; due to the irregular topography of the growth plate, this anatomic feature was not contained solely within one transverse layer of DVC sub-regions. The average minimum principal strain and average maximum shear strain sustained at the yield point were calculated for each region.

Statistical Analyses

To compare the observed mechanical properties of the vertebral body between V+D and V groups, *t*-tests were used to compare the stiffness of the vertebral body (secant of the linear region of the load-displacement curve), ultimate force, ultimate displacement (the displacement that corresponded to the ultimate force), and work to failure between the V and V+D groups. For stiffness and work to failure, the displacement was computed using the displacement in the vertebral body only (*i.e.*, excluding displacement in the PMMA and the IVDs) and the force values recorded after the 20-minute relaxation period. The displacement in the vertebral body was computed using the axial strain along the superior-inferior direction multiplied by the vertebral height. This calculation was more accurate than simply examining the change in vertebral height, measured in number of μ CT slices, because the DVC calculations are accurate to within a fraction of the slice thickness.

To compare the observed deformation and failure patterns between V+D and V groups, the strains distributions just before yield and right at yield were considered for statistical analysis. For each of these two points during the compression test, *t*-tests were used on the distributions of minimum principal and maximum shear strains (inter-quartile range (IQR) and the top 1st, 2nd, 5th, 10th, 25th and 50th percentiles). Repeated-measures analyses of variance with Wilcoxon signed-rank *post-hoc* tests were performed to examine differences in average minimum principal and maximum shear strains among the three regions for the V +D and V groups, and also to compare the strain magnitudes between groups in each region.

RESULTS

Deformation and failure patterns were different in the presence of the IVDs. Vertebral bodies tested with IVDs ("V+D") exhibited a slow increase in both minimum principal and maximum shear strains in the inferior and posterior regions of the vertebral body prior to the yield point on the force-displacement curve (Figure 3B, D), followed by a sudden increase in both minimum principal and maximum shear strain in the anterior cortex right at the yield point (Figure 3C, E). In contrast, the isolated vertebral bodies ("V") consistently exhibited the highest minimum principal and maximum shear strains in the posterior regions, from the initial portion of the test (Figure 3G, I) through the yield point (Figure 3H, J). If large minimum principal and maximum shear strains were observed in the anterior regions of the cortex in the V specimens, these large strains developed only after the yield point had been exceeded. Overt cortical fractures were observed in the V+D specimens near the inferior end (Figure 4A–C). Cortical fracture occurred on the anterior and the posterior sides, and large

deformations occurred in the trabecular bone adjacent to the growth plate. The V specimens did not have clear cortical break; however, the trabecular centrum near the inferior third of the vertebra suffered large deformations, and trabecular connections were severed (Figure 4D–F).

Presence of the IVDs reduced the vertebral stiffness ($p = 0.044$), increased the ultimate displacement ($p = 0.043$), produced higher magnitudes of maximum shear strain at yield — as quantified by the top 25th percentile of maximum shear strain—and higher intra-vertebral variation (IQR) in maximum shear strain at yield ($p < 0.048$). These differences in maximum shear strain were most pronounced in the inferior region of the vertebral body ($p = 0.045$; Table 1). A trend towards lower ultimate force in the V+D group was found ($p = 0.056$) (Figure 5). No differences between groups were observed in work to failure ($p = 0.844$) or in any measure of the statistical distribution of minimum principal strain, whether right before or right at the yield point ($p > 0.208$). Similarly, no differences between groups were found for the maximum shear strain just prior to yield ($p > 0.096$), or for the top 1st ($p = 0.116$), 2nd ($p = 0.102$), 5th ($p = 0.057$), 10th ($p = 0.067$), and 50th ($p = 0.111$) percentiles of the maximum shear strain right at the yield point. For both minimum principal strain and maximum shear strain, no differences were found among the superior, central and inferior regions in either group ($p > 0.482$).

DISCUSSION

This study assessed the impact of a commonly adopted simplification in *ex vivo* mechanical tests, which is removing the intervertebral discs prior to mechanical testing of vertebral specimens. Thoracic vertebral bodies were tested in compression, with and without the intervertebral discs to assess differences in failure mechanisms and measured mechanical properties. The results demonstrated a marked effect of the presence of the IVDs on vertebral deformation and failure patterns, even under simple axial loading. The spatial distributions of the strains differed substantially between V+D and V groups, both before and right at the yield point, and maximum shear strains were higher in the V+D than V group. There was also a trend towards lower ultimate force in the V+D than V group, while, no difference was found in work to failure. These results indicate that testing vertebral bodies in the absence of IVDs may not affect measurements of vertebral toughness, or possibly strength, but does affect the transmission of load throughout the vertebra and concomitantly, vertebral failure mechanisms.

The primary strength of this study is the use of direct, 3-D, experimental measurements to compare deformation patterns that develop in vertebral bodies, as they are loaded to failure. These DVC measurements provided detailed descriptions of the deformations throughout the entire vertebral body at each loading step, enabling identification of where high strain regions initiated within the bone and how the strain propagated with further loading. This allowed us to identify differences in failure behavior between the V and the V+D groups.

There are also limitations to this study. First, the posterior elements were removed prior to testing, rendering the loading somewhat non-physiologic. It is possible that load transmission through the facet joints mitigates the effects of changes in load distribution across the endplate. However, given that in the mechanical tests performed on vertebrae in the absence of IVDs, only the vertebral body is loaded, the interpretation of the present results remains unchanged. Removal of the posterior elements is a common procedure for biomechanical testing of vertebrae, at times because of limitations on the size of test samples (Buckley et al, 2007; Hussein et al., 2011; Kim et al., 2007; Kopperdahl et al., 2000; Mosekilde et al., 1989; Perilli et al., 2012), as was the case herein due to the size constraints imposed by the μ CT scanner. Second, given the quasi-static nature of the loading protocol,

the results of this study relate more directly to vertebral compression fractures caused by prolonged loading and may not apply to fractures due to short-term or dynamic loading conditions. Third, only twelve samples were included in this study. The statistical power for the comparison of ultimate force and work to failure was only 0.50 and 0.05, respectively, indicating that results for these parameters are inconclusive.

Some of the present results may be specific to the animal species and age. Although the age of the rabbits corresponded to skeletal maturity in this species, some portions of the physes in the vertebral bodies were not closed and may have experienced higher strains (Hardisty et al., 2010) than would the corresponding regions of adult human vertebrae, in which the physes are fully closed. Species and age differences in endplate structure, endplate topography, IVD degeneration, and vertebral microstructure must also be considered. The cartilage endplate in rabbits at skeletal maturity is thick, shows zonal arrangement similar to (though more disorganized than) that of articular cartilage (Kim et al., 2003; Scott et al., 1980), and is almost entirely separated from the growth plate via secondary ossification (Kim et al., 2003). In contrast, the cartilage endplate in adult humans is thin and undergoes secondary ossification only at the periphery (Kim et al., 2003). Rabbit endplates have higher permeability and lower porosity compared to values reported for human samples (Brown and T., 1976; Hee et al., 2011; Rodriguez et al., 2011). Rabbit vertebrae used in the present study were relatively uniform in endplate shape and curvature, whereas the topology of human vertebral endplates depends greatly on the extent of IVD degeneration (van der Veen et al., 2008). The adjacent IVDs in our rabbit spine segments were also all healthy (as judged from radiographic examination and gross visual examination) and thus would be expected to have many structural similarities to healthy IVDs in the human population (Kim et al., 2003). However, we cannot extrapolate the present inter-group differences to the case of degenerated IVDs. Finally, human vertebrae exhibit a thin cortex composed of very dense trabecular bone surrounding a large trabecular centrum, while rabbit vertebrae have a thicker, even denser cortex and comparatively fewer (and thicker) trabeculae in the centrum. All of these comparisons suggest that the deformation patterns seen in this study may not resemble those in adult human vertebrae and that further study is required to determine the extent to which the present results apply to the human population. Notably, the fracture patterns seen in the present study did not correspond precisely to the most common types of vertebral fractures or deformities in elderly or osteoporotic humans: wedge and biconcave fractures.

Nevertheless, comparison of the present results to prior biomechanical studies on human vertebrae reveals some correspondence. In the present study, the differences between the V and V+D groups can be attributed to artifacts caused by the absence of the IVDs. Removing the IVDs and potting the endplates of the V specimens in PMMA (Figure 1A) may “strain-shield” the epiphysis and physis and transfer a more uniformly distributed load to the mid-transverse segment of the vertebral body. Examination of the strain distributions throughout the vertebral body (Figure 3, Figure 4, Figure 5E–H, Table 1) suggests that this shielding effect manifests principally in the maximum shear strain, rather than in the minimum principal strain, shifts the location of largest shear strains from the anterior to posterior aspects of the vertebral body, and moves the location of highly deformed trabecular bone from points adjacent to the endplate to points slightly closer to the mid-transverse plane. Our strain data are in agreement with prior human studies that reported a more homogeneous strain distribution in the absence of IVDs (Eswaran et al., 2007) and larger strains at the anterior cortex in the presence of IVDs (Yerby et al., 1998). Moreover, our finding that ultimate strength is one of the least sensitive outcome measures to removal of the IVDs is also consistent with a prior finite-element study on human vertebrae, which estimated the sensitivity of vertebral strength to the load distribution across the endplate (Buckley et al., 2006).

The present results also have bearing on studies that investigate biomechanical consequences of the interplay between vertebral bone properties and disc quality. Bone loss in the anterior and central regions (Homminga et al., 2012; Keller et al., 1989; Keller et al., 1993; Simpson et al., 2001) of the vertebra in response to IVD degeneration may make these regions susceptible to failure when testing is performed in the absence of the IVDs, because such testing likely creates a more uniform load distribution across the endplate than exists via the degenerated IVDs. Similarly, increased density in the vertebral cortex and periphery with IVD degeneration (Homminga et al., 2012) may shift the regions of failure to the inner centrum when testing is performed without IVDs. These scenarios illustrate how removal of the IVDs prior to testing may confound conclusions regarding the potential effect of bone remodeling in response to IVD degeneration on vertebral failure.

Taken together, these findings indicate that testing vertebral bodies in the absence of IVDs can elicit artifactual failure mechanisms. These artifacts may lead to false conclusions in studies of the effects of IVD degeneration and other diseases, bone remodeling, and drug treatments. Thus testing with IVDs, though more complicated in experimental procedures, may be necessary for maximizing the clinical relevance of the test results.

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Figure 1.
(A) Schematic for “V+D specimens” and “V specimens”; (B) components of the loading device

Figure 2.
Schematic for generating the 3D, irregularly shaped mesh to be used for DVC analyses of deformations in whole vertebral bodies

Figure 3.

Force-displacement curve and distributions of minimum principal strain (B, C, G, H) and maximum shear strain (D, E, I, J) (posterior right quadrant removed from the rendering for viewing purposes) right before (*) and right at (**) yield for: (A–E) V+D and (F–J) V specimens

Figure 4.

3-D renderings of vertebrae from (A–C) the V+D group and (D–F) the V group for the scans performed at the preload and final load increments: Renderings in C and F show the vertebral surface, while renderings A, B, D, and E show frontal half-sections. Brackets indicate regions of large deformations; circles indicate regions of overt, cortical fracture. For the V specimen, the portions of the cranial and caudal endplate that are embedded in PMMA are not shown

Figure 5.

Comparison of mechanical properties between V+D and V groups: the height of each bar indicates the group mean, and the error bar ± 1 standard deviation. # and ## denote $p < 0.05$ and $p < 0.01$, respectively. The bar graphs of the IQR in strains are presented for the load increment just before yield (indicated by the single asterisk in Figures 3A and 3F) and for the load increment right at yield (indicated by the double asterisks in Figures 3A and 3F)

Table 1

Average $\pm$ standard deviation of strain in the superior, central and inferior regions of the vertebra for the V+D and V groups. Values marked with different superscript letters are different between groups, within a given region ($p = 0.045$)

Group	Superior region	Central region	Inferior region
<i>Minimum principal strain [mm/mm]</i>			
V+D	-0.049 ± 0.125	-0.062 ± 0.110	-0.060 ± 0.097
V	-0.044 ± 0.053	-0.049 ± 0.076	-0.050 ± 0.065
<i>Maximum shear strain [mm/mm]</i>			
V+D	0.124 ± 0.096	0.158 ± 0.118	0.130 ± 0.046^a
V	0.051 ± 0.053	0.043 ± 0.066	0.056 ± 0.064^b