

In Vivo and In Vitro Analysis of Rat Lumbar Spine Mechanics

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Abstract

Background Rodent lumbar and caudal (tail) spine segments provide useful in vivo and in vitro models for human disc research. In vivo caudal models allow characterization of the effect of static and dynamic loads on disc mechanics of individual animals with time, but the lumbar models have required sacrifice of the animals for in vitro mechanical testing.

Questions/purposes We therefore developed a novel displacement controlled in vivo lumbar spine noninvasive induced angular displacement (NIAD) test; data obtained with NIAD were used to compare angular displacement between segmental levels (L4/L5, L5/L6 and L6/S1), interobserver radiograph measurement agreement, and intraobserver radiograph measurement repeatability. Measurements from

NIAD were compared with angular displacement, bending stiffness, and moment to failure measured by an in vitro test. **Methods** Anesthetized Lewis rats were xrayed in a 90° angled fixture, and NIAD was measured at lumbar levels L4 to S1 by two independent and blinded observers. After euthanasia, in vitro angular displacement (IVAD), stiffness, and failure moment were measured for the combined L4-L6 segment in four-point bending.

Results NIAD was greater at L4/L5 and L5/L6 than at L6/S1. Combined coronal NIAD for L4-L6 was $42.8^\circ \pm 5.3^\circ$ and for IVAD was $61.5^\circ \pm 3.8^\circ$. Reliability assessed by intraclass correlation coefficient (ICC) was 0.905 and 0.937 for intraobserver radiograph measurements, and interobserver ICCs ranged from 0.387 to 0.653 for individual levels. The interobserver ICC was 0.911 for combined data from all levels. Reliability for test-retest NIAD measurements had an ICC of 0.932. In vitro failure moment correlated with NIAD left bending.

Conclusions The NIAD method yielded reproducible and reliable rat lumbar spine angular displacement measurements without required euthanasia, and allows repetitive monitoring of animals with time. For lumbar spine research studies performed during a course of time, the NIAD method may reduce animal numbers required by providing serial angular displacement measurements without euthanasia.

Clinical Relevance Improved methods to assess comparative models for disease or aging may permit enhanced clinical treatments and improved patient care.

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Each author certifies that his or her institution has approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

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Introduction

Intervertebral discs play a critical role in spine flexibility and ROM. Additional determinants of spine flexibility and ROM include spine ligaments, intrinsic spine muscles, and

trunk musculature and articulations with the axial and appendicular skeleton. Intervertebral disc disorders can alter ROM and cause back pain, as evidenced by multiple clinical etiologies including spondyloarthropathy [11, 15], disc infection [3, 16], and aging and disc degeneration [21, 35]. Comparative rodent models of these spinal disease states have offered mechanisms for better understanding of these conditions, and suggestions to improve clinical care [18, 33, 37, 38, 43]. Through enhanced overall understanding of the mechanics, physiology, and pathology of the intervertebral disc, additional preventative and curative clinical strategies may be developed [1].

Spine and intervertebral disc mechanics have been studied extensively in comparative rodent models, using the lumbar and caudal (tail) spine segments. Caudal models have used numerous mechanical, pharmacologic, and control treatments delivered *in vivo* and *in vitro* [13, 14, 22, 23, 27–29, 31, 32, 36, 42], and biomechanical testing *in vivo* [23, 28, 29, 42] and *in vitro* [13, 22, 27, 30, 36]. These various caudal *in vivo* models use invasive external fixators to deliver forces and assess force-displacement characteristics. Lumbar models have been used to deliver disc treatments *in vivo* [4] and *in vitro* [5, 6], but the biomechanical consequences were reported only for samples assessed *in vitro* after animal sacrifice [4–6]. Methods to noninvasively assess lumbar spine angular displacement or other biomechanical end points *in vivo* have not been described, possibly owing to technical difficulty for quantitative delivery of forces to the spine during load-controlled experiments.

We therefore raised the following questions: (1) Could lumbar angular displacement be reproducibly noninvasively quantified *in vivo*? Reproducibility was assessed by interobserver and intraobserver tests and test-retest repeatability; and (2) would displacement controlled

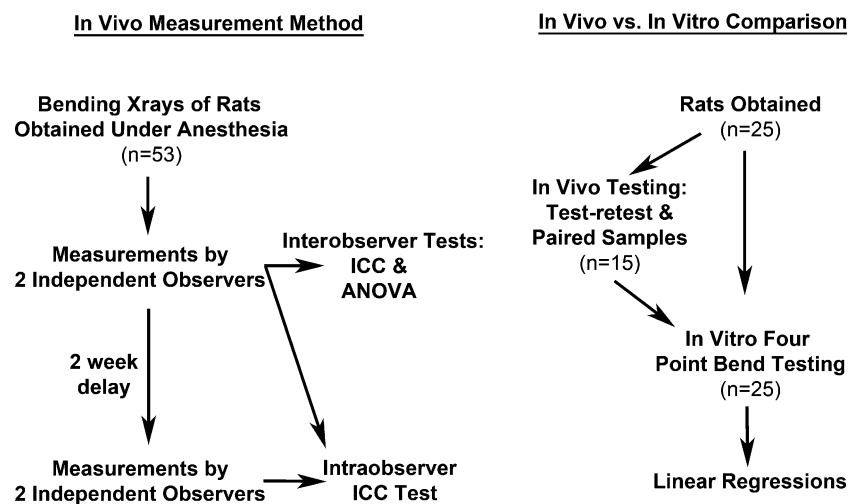
in vivo measurements correlate with *in vitro* load controlled tests?

Materials and Methods

In this observational study of rat lumbar spine angular displacement, we used bending radiographs of living animals under anesthesia to measure and compare segmental NIAD, and explanted lumbar specimens from euthanized animals to generate comparative IVAD measurements (Fig. 1). We used 78 male Lewis rats (275–300 gm) that had been obtained by other investigators for unrelated projects: (1) on arrival to the facility and before surgery ($n = 53$), or (2) postoperatively from limb surgery and immediately before euthanasia ($n = 25$). The first group of 53 animals was used to quantify segmental lumbar NIAD, and to determine intraobserver and interobserver agreement for measurement of the radiographs. Fifteen animals from the second group were used to measure NIAD test-retest repeatability, and for dual testing with NIAD and IVAD methods for paired sample induced angular displacement measurement comparisons; the remaining 10 animals from the second group were tested only for IVAD, and *in vitro* stiffness and failure moment. We had prior approval for our protocol from our Institutional Animal Care and Use Committee, and permission from the other investigators for use of their animals.

All animals were anesthetized with isoflurane (1.5% to 3%) anesthesia for NIAD testing. High-resolution standardized radiographs were obtained using a Faxitron model MX-20 with DC-4 digital camera (Faxitron X-Ray Corporation, Wheeling, IL, USA). Faxitron imaging was performed at 35 kV and 10-second maximal exposure. Animals were maintained in supine right and left bending

Fig. 1 A flowchart for the study design is depicted. The left column shows how interobserver and intraobserver radiographic measurements were assessed, and where the ANOVA assessments were made to compare segmental angular displacement for each observer and between observers. The right column shows how specimens were used for the test-retest, paired *in vivo/in vitro*, and *in vitro* biomechanics assessments.



positions in the AP plane with a radiolucent 90° bracket (Fig. 2A). Flexion and extension bending radiographs were not obtained. In vivo sagittal ROM was not measured owing to technical difficulties reproducibly immobilizing the pelvic girdle of the animals when in the lumbar flexed position, and inability to generate a three-point fulcrum by positioning in the 90° bracket. Digital xray images were repeated if pelvic rotation, assessed by symmetry of sacroiliac joints (posterior-inferior iliac spine) and pubic symphysis to the center of the sacrum, was not neutral and if three-point force was not in contact with the iliac wing and low flank (Fig. 2B). To assess NIAD test-retest reproducibility, animals were xrayed in the right-bending position, repositioned in the left-bending position, and xrayed, and then this process was repeated to obtain triplicate sets of radiographs ($n = 15$ animals) while being maintained under a single anesthesia.

Two of us (MEC, SB) used a modified Cobb technique to measure angles between the lumbar vertebrae ($n = 53$, Question 1; $n = 15$, Question 2). Digital measurements

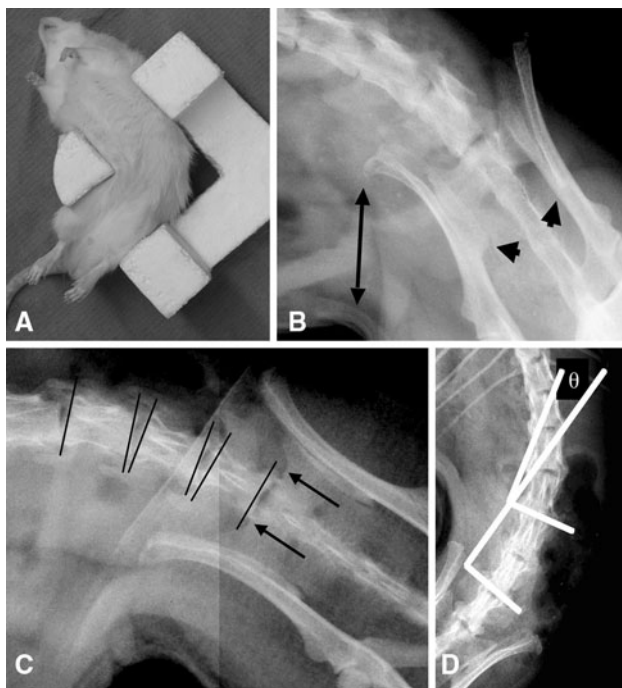


Fig. 2A–D (A) An anesthetized Lewis rat is shown in the bending bracket positioned for right-bending radiograph. (B) This high-definition digital radiograph shows inappropriate positioning of the animal in the bracket for right bend assessment with lack of correct force application on the iliac wing (double-ended arrow) and unacceptable rotation judged by the asymmetry of the posterior inferior iliac spines (arrowheads). (C) Bony landmarks for the pelvic measurements (arrows point to the bilateral sacral ala sulci, the most caudal location of the proximal sacral ridge) and vertebral end plate line placement (lines) are shown. The animal is well positioned with symmetric posterior inferior iliac spines and good contact of the iliac wing with the positioning bracket. (D) Cobb end plate measurement (θ) for the L4/L5 segment is shown.

were taken directly from the Faxitron images (Image-J Freeware, Version 1.37 h; National Institutes of Health, Bethesda, MD). Both observers were blinded and measured identical images. Measurements were performed by defining the proximal and distal end plates for L4, L5, L6, and a line connecting the sulci of the S1 sacral ala (Fig. 2C). To normalize between images, the end plates (referenced to the unique horizontal of each image by the software) were converted to reference from the sacrum that was defined as 0° based on methods reported in clinical studies [40, 41]. Sacrum-referenced end-plate positions were used to measure angulation differences between the proximal vertebra upper end plate and distal vertebra lower end plate (Fig. 2D), according to the Cobb method [10]. Angles between the L4/L5, L5/L6, L4-L6, and L6/S1 vertebrae were measured for each bending direction and summed for each animal to obtain segmental coronal NIAD (coronal NIAD = right bend NIAD + left bend NIAD). As a result of out-of-plane coupled movement mediated by the spine posterior elements, quantifications above L4 were not possible.

Spine specimens were potted and tested in vitro in nondestructive and destructive bending ($n = 25$, Question 2). Animals were euthanized by carbon dioxide asphyxiation, spines explanted intact from L3 to S1, and muscle and ligaments were removed from the posterior elements preserving the discs, facets, and bony elements ($n = 25$). When necessary, samples were stored in saline-soaked gauze at -20°C . One sample was damaged during preparation, and was excluded. The proximal (L3 and half of L4) and distal (S1 and half of L6) ends of each segment were fixed with acrylic bone cement (COE Tray Plastic; GC America, Chicago, IL, USA) in custom rectangular aluminum fixtures, allowing free movement of the L4/L5 and L5/L6 levels. Four-point bending was performed with a 50-mm outer support span and inner support span of 22.5 mm with loads applied at 0.5 N/second (ELF 3200; EnduraTec, Eden Prairie, MN, USA) (Fig. 3A). Rotation of the rectangular fixtures allowed the samples to be positioned in four orthogonal anatomic directions. Hydration was maintained by wrapping the specimens in saline-soaked gauze. Samples were tested nondestructively in right and left lateral bending, extension, and flexion in random order and then tested to failure in extension over the combined L4-L6 segment. Flexion and extension testing were included in the in vitro testing to provide a more complete description of the mechanics in four-directions. The maximum load and number of cycles for the nondestructive testing were determined empirically from a series of practice samples and set at 4 N, corresponding to a bending moment of 55 N-mm. Each sample was loaded for five cycles (Fig. 3B). Stiffness was measured from the fifth cycle, after preconditioning of the sample had led to nearly

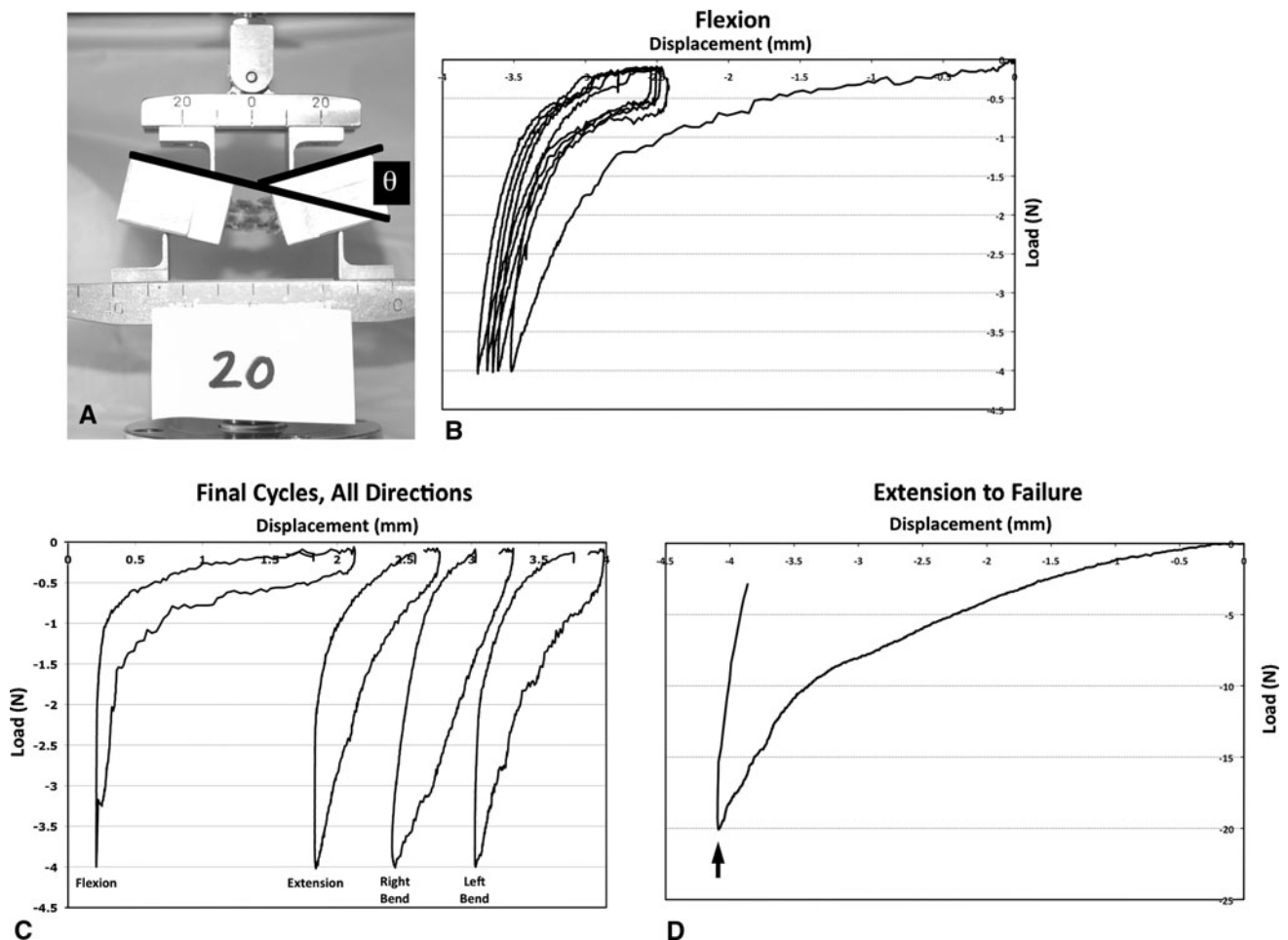


Fig. 3A–D (A) This photograph of the four-point bending fixture shows the specimen in maximal deflection in left lateral bending with angular deformation (θ) indicated. Displacement is in the downgoing vertical direction and the load cell is beneath the bottom fixture. (B) Continuous cycling data for a sample tested in flexion are shown. Displacement is graphed as decreasing from right to left during cycling reflecting the apparatus construction and applied force is

negative by convention. (C) Load-displacement traces for a representative sample in the fifth loading cycle for each of the specified directions are shown. Stiffness is the slope of curve between 2 and 4 N in the loading phase. The plots for each of the directions were superposed and are illustrated after translation to avoid overlap. (D) A representative bending to failure curve is shown; the arrow marks the failure load (20 N).

identical load-displacement traces for cycles 2 through 5 (Fig. 3C). At maximal deflection during the final nondestructive testing cycle, the sample angular deformation also was recorded photographically (Nikon D70, Melville, NY, USA). Composite L4-L6 segment IVAD was measured relative to the fixture edges in each loading direction from the photographs (Image-J Freeware) (Fig. 3A). Coronal IVAD was defined as the sum of the right and left IVAD measurements (as described previously) and sagittal IVAD as the sum of the flexion and extension IVADs. For the destructive tests (Fig. 3D), the failure bending moment and failure location (L4/L5 or L5/L6 disc) were recorded.

Bending stiffness (EI) for each of the four directions was calculated using the formula for four-point bending mechanics [7]. Variables are as follows: ΔF = force applied, y = $1/2$ inner support separation distance, x = $1/2$

(outer support separation – inner support separation), d = displacement of sample during test, and EI is the four-point bending flexural stiffness. The complete equation is: $d = (1/2 \Delta F x^2 * y)/EI_{\text{sample}} + (1/2 \Delta F x^3)/3EI_{\text{supports}}$. As reported previously [12], if the latter term in this formula is insignificant as a result of a large value of the EI supports term, then the formula simplifies to the following: stiffness (EI_{sample} , N-mm²) = $(1/16 * (\Delta F/d) * (\text{outer support separation} - \text{inner support separation})^2 * (\text{inner support length}))$. Testing in bending, rather than torsion or axial loading, was chosen as most comparable to the loading delivered in the NIAD testing method. Four-point bending was chosen over three-point assessment because a constant moment is applied to samples during measurement, and loads are not applied directly to the sample, each factor avoiding potential sources of error.

The potential effect that different observers impart to NIAD measurements, as this relates to application of the modified Cobb measurement technique described above, was quantified through independent blinded observers (MEC, SB) making measurements on identical digital radiographs ($n = 53$, Question 1). Segmental radiographic measurements were assessed for agreement between observers (interobserver measurement reliability) using the ICC for two independent observers taking single measures. After a delay to prevent measurement bias, the two observers performed repeat measurements of the same digital radiographs and intraobserver measurement agreement was assessed by ICC as above. ICC calculations were performed using the two-way mixed single-measure formula for consistency (SPSS Version 16; SPSS Inc., Chicago, IL, USA).

Reproducibility for application of the NIAD measurement method was quantified using ICC testing of NIAD measurements obtained from serial (triplicate) test applications during a single anesthesia, with radiographs obtained and digital measurements performed by one observer (MEC). Triplicate NIAD data sets were compared for measurement agreement using ICC as described. Correlations between the NIAD, IVAD, and in vitro biomechanics measurements were assessed by linear regression to estimate correlation (Pearson's r). One-factor ANOVA with Bonferroni post hoc testing was used to compare NIAD measurement means between levels for each rater, and one-factor ANOVA was used to compare segmental radiographic measurements between raters for each level.

Results

Displacement controlled NIAD measurements showed different segmental motion by level for each observer,

reliable intraobserver radiographic measurements, an observer effect on digital radiograph measurements, and reproducible NIAD measurements with serial test application. Average coronal lumbar spine NIAD was similar between the L4/L5 bodies and the L5/L6 bodies for each observer (Table 1) ($p > 0.05$). Average NIAD was less at the L6/S1 junction as compared with the L4/L5 or L5/L6 levels ($p < 0.001$ for both raters for each comparison). Radiographic measurements performed on identical digital radiographs were different between raters ($p < 0.001$ at each segment), suggesting an observer effect when applying the modified Cobb measurement technique. The ICC intraobserver radiograph measurement reliability was 0.905 and 0.937 for each observer, and the interobserver ICCs ranged from 0.387 to 0.653 for measurements from individual segments (Table 1) and overall was 0.911 for measurements pooled and assessed as a single data set. NIAD test-retest reliability ICC was 0.932 for all segments assessed as a single data set, and average segmental ROM did not show progression during serial NIAD test applications (data not shown).

Comparison between NIAD and in vitro testing methods showed correlation between NIAD left bending and in vitro extension moment to failure. Average IVAD in four directions and in vitro bending stiffness in four directions were quantified (Table 2). In comparison to NIAD over the combined L4-L6 segment, IVAD measurements were greater in each specimen assessed by both methods (compared with Observer 1, measurement differences averaged $13.7^\circ \pm 6.9^\circ$; range 2.2° – 24.5° ; $n = 14$), and this also was shown by comparison of group averages for the techniques that had 12.8° more motion in the in vitro samples (Table 2). For the samples tested with in vivo and in vitro methods, we observed no correlation between NIAD and IVAD, and no correlation between NIAD or IVAD with in vitro stiffness (Table 2). Specimens loaded to failure in extension failed at the discs, with 14 of 25 (56%) occurring

Table 1. Lumbar in vivo noninvasive induced angular displacement

Spine level	NIAD		Interobserver	
	Observer 1 (degrees)	Observer 2 (degrees)	ICC	ANOVA
L4/L5	24.6 \pm 4.6	19.7 \pm 4.6	0.387	$p < 0.001$
L5/L6	22.3 \pm 4.2	18.6 \pm 4	0.517	$p < 0.001$
L6/S1	17.4 \pm 4.8	13.7 \pm 3.6	0.552	$p < 0.001$
L4-L6	42.8 \pm 5.3	37.9 \pm 5.6	0.653	$p < 0.001$
Intersegmental ANOVA				
L4/L5 vs L5/L6	$p = 0.28$	$p = 0.452$		
L4/L5 vs L6/S1	$p < 0.001$	$p < 0.001$		
L5/L6 vs L6/S1	$p < 0.001$	$p < 0.001$		

NIAD = noninvasive induced angular displacement; ICC = intraclass correlation coefficient; ANOVA – analysis of variance.

Table 2. Lumbar in vitro biomechanics assessments and correlations with angular displacement

Testing direction	L4-L6 segment in vitro measurements		NIAD vs IVAD angular displacement [§]	Failure moment correlations [§]		In vitro stiffness correlations [§]	
	IVAD (degrees)	Bending stiffness (N·mm ²)		vs NIAD	vs IVAD	vs NIAD	vs IVAD
Right bending	27.8 ± 3.8	11,036 ± 3,474	p = 0.34 (r = -0.27)	p = 0.83 (r = 0.049)	p = 0.86 (r = 0.05)	p = 0.71 (r = 0.11)	p = 0.23 (r = 0.35)
Left bending	27.8 ± 3.8	11,746 ± 3,220	p = 0.79 (r = 0.08)	p = 0.027* (r = 0.59)	p = 0.62 (r = 0.15)	p = 0.69 (r = -0.12)	p = 0.24 (r = 0.33)
Total coronal	55.6 ± 5.8	NM	p = 0.84 (r = -0.06)	p = 0.101 (r = 0.46)	p = 0.67 (r = 0.13)	NM	NM
Extension	22.4 ± 3.6	10,464 ± 3,125	NM	NM	p = 0.07 (r = 0.5)	NM	p = 0.42 (r = -0.24)
Flexion	39.1 ± 1.7	20,498 ± 11,189	NM	NM	p = 0.76 (r = -0.09)	NM	p = 0.61 (r = -0.15)
Total sagittal	61.5 ± 3.8	NM	NM	NM	p = 0.14 (r = 0.41)	NM	NM

[§] Correlations for samples tested by in vivo and in vitro methods (n = 14, Pearson's r); * significant correlation (p < 0.05); NIAD = noninvasive induced angular displacement; IVAD = in vitro angular displacement; NM = not measured.

at the L5/L6 level, and an average failure moment of 138.2 ± 46.1 N·mm. For the samples tested with in vivo and in vitro methods, in vitro failure moment correlated with left bending NIAD only (Table 2). Comparing all available in vitro tested samples (n = 24), we observed a correlation between extension IVAD and failure moment (r = 0.44, p < 0.05) and sagittal IVAD and failure moment (r = 0.41, p < 0.05). No correlations were found for the (n = 24) in vitro sample comparisons for stiffness in any direction and failure moment (p = 0.35 to 0.82), or IVAD with stiffness in any direction (p = 0.26 to 0.55).

Discussion

In vitro biomechanical tests have been described in numerous studies using comparative rodent lumbar spine models [2, 6, 13, 14], but noninvasive in vivo lumbar mechanical testing methods for rodents have not been reported. We sought to develop a displacement controlled test to measure in vivo lumbar spine induced angular displacement (NIAD), and to assess the reliability of the measurements made by single observers, between observers, and by repeated application of the test. We also determined how the in vivo measurement method compared with standard in vitro biomechanics tests: angular displacement (IVAD), stiffness, and load to failure.

Our study has some limitations. First, it is limited by the use of displacement-controlled rather than load-controlled force application during the NIAD assessment. Displacement control applies a fixed displacement and delivers a variable force magnitude to the spines of individual animals, and would be expected to generate more data scatter and measurement error than load-controlled tests. Despite larger relative standard deviations for the NIAD measurements, test-retest measurement reliability was high, suggesting soft tissue displacement dissipation and variation of forces applied to the spine did not dramatically affect the measurements. Second, NIAD radiograph measurements for lumbar motion between observers were different, suggesting an observer effect. These differences were noted despite observers making blinded measurements from identical radiographic images; however, the measurement differences did not affect the conclusions drawn regarding relative segmental NIAD. Therefore, interpretation of measurement data using the NIAD method should be performed for each observer independently. Third, we had a relatively small number of animals used in the direct comparisons of in vivo and in vitro measurements (n = 14), which may have led to the limited correlations between the techniques in addition to the technical differences. Use of larger sample sizes, as was seen here in the comparisons of all in vitro specimens

($n = 24$), may improve correlations in future studies by providing a source for larger range in the data set. Several factors contributed to differences between the two methods: *in vivo* tests used the entire spine with soft tissues intact, whereas *in vitro* specimens used a two-disc segment without soft tissues; tests performed in different anatomic directions may limit comparisons; *in vivo* animal positioning was controlled but subject to movement error, whereas *in vitro* testing was rigorously maintained by the fixture; and different boundary conditions for *in vivo* displacement-controlled and *in vitro* load-controlled tests. Finally, although four-point bending is a strength of our approach, few published reports exist for comparison to our data. Despite these limitations, our findings suggest the *in vivo* lumbar NIAD measurement technique is a reliable method for comparative rat spine research.

The displacement controlled NIAD method we described for the lumbar spine has precedent in techniques reported in the literature for clinical spine deformity. In either normal volunteers [20], or patients with scoliosis [19, 39], induced translation and bending of the spine can be generated with displacements of the trunk made with reference to the pelvic girdle. Analogous, although load-controlled, experiments delivering unilateral static bending in a rodent caudal model have been done to study experimental scoliosis [23, 31, 32, 36], with unidirectional initial angles averaging 18.1° to 26° over a two-disc segment [23, 32]. Although these caudal deformation assessments would compare favorably with our lumbar NIAD observations, differences between lumbar and caudal biomechanics have been reported for axial [13] and torsional [14] testing, thereby limiting validity for direct comparison of the caudal data to our lumbar measurements. Direct comparisons for our findings do not appear in the clinical or experimental literature. Regarding the accuracy agreement of the *in vivo* measurement method, angular deformation quantifications were generated using a modified Cobb technique. The Cobb method is the gold standard for angular measurement between vertebrae in clinical scoliosis and spine care, with 95% of interrater measurements for idiopathic scoliosis within 5° to 8° of one another when measured graphically [8, 17], or within 2.6° to 4.8° when measured digitally [9, 24]. The *in vivo* segmental NIAD averages had interobserver agreement within 4.9° for each measured segment. Digital Cobb interobserver measurement reliability as reported in clinical applications, evaluated by ICC, varies widely by the radiographic parameter assessed, with low values (0.25 to 0.33) associated with small measurement magnitudes and not easily identified landmarks, and high values (0.93 to 1) associated with larger measurement magnitude and more easily identified landmarks [25]. The relatively small magnitude of segmental measurements made by the *in vivo* method

(13.7° to 24.6°) suggests that the low to moderate ICC values reflect what would have been expected, as was the higher ICC obtained for the larger magnitude combined L4-L6 segment measurement. These comparisons suggest the *in vivo* quantification technique is comparable in accuracy and precision to the Cobb method of measuring spinal deformity clinically.

Comparison of *in vivo* NIAD to *in vitro* end points revealed correlation only for *in vivo* left bending and *in vitro* extension moment to failure. The lack of correlation between NIAD and IVAD, and either of these with other parameters measured by the *in vitro* testing method was thought to represent the relatively small sample used for the investigation, and lack of sufficient data nonhomogeneity to strengthen observed associations. Measurement data for the two methods were each normally distributed (data not shown), and when orthogonally graphed formed rounded scatterplots. Further study to test correlation between the measurement techniques is warranted with larger study populations, and populations containing subjects with altered lumbar spine mobility to provide larger data ranges.

Regarding comparison of the *in vitro* lumbar spine testing data to that contained in the literature, no rodent lumbar spine four-point bending data currently exist. Four-point bending has been reported for a murine caudal model [27], but with the established differences between lumbar and caudal mechanics in axial and torsion testing [13, 14], direct comparison to the murine data is difficult to make even when corrected for relative size [1, 2]. Four-point bending measurements were reported from rabbit and canine cadaver spines using a similar method to assess *in vitro* stiffness [12]. The stiffness of rabbit and dog spines was in the range of 4 to 6 N-m² with little difference seen between the species. Direct comparison of our current and prior data yields approximately 250-fold greater stiffness in the larger animals. Approximating the disc and vertebral body geometry of the spine as a rigid rod, spine stiffness as a function of specimen size would be expected to relate to the spine radius squared. If relative animal mass is assumed to be proportional to vertebral body size, then a typical 4-kg New Zealand White rabbit is approximately 14-fold larger than the rats (0.275–0.3 kg) used in this study and would be expected to have average spine stiffness approximately 200-fold larger than the rodents. Using this rationale, the data between the species are comparable.

The *in vivo* and *in vitro* lumbar spine tests described in this study can be used to objectively quantify spine mechanics. The noninvasive *in vivo* method can be used to measure spine angular displacement longitudinally at multiple times on one animal and thus may be useful for testing experimental animals with time without requirement for euthanasia if absolute biomechanical stiffness and

ultimate load to failure data are not required to answer the research question. After euthanasia, the in vitro method to test bending angular displacement and stiffness in four anatomic directions and failure moment in extension provides additional objective means to measure spine end points. Used in this way, the methods are complementary and may limit or prevent the need for euthanasia at multiple times to assess mechanics, as has been described [26, 34], thereby limiting the number of animals required in complex study designs.

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