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ORIGINAL ARTICLE

Intervertebral disc changes with angulation, compression and reduced mobility simulating altered mechanical environment in scoliosis

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Abstract
Purpose The intervertebral discs become wedged and narrowed in scoliosis, and this may result from altered biomechanical environment. The effects of four permutations of disc compression, angulation and reduced mobility were studied to identify possible causes of progressive disc deformity in scoliosis. The purpose of this study was to document morphological and biomechanical changes in four different models of altered mechanical environment in intervertebral discs of growing rats and in a sham and control groups.
Methods External rings were attached by percutaneous pins transfixing adjacent caudal vertebrae of 5-week-old Sprague–Dawley rats. Four experimental Groups of animals underwent permutations of the imposed mechanical conditions (A) 15° disc angulation, (B) angulation with 0.1 MPa compression, (C) 0.1 MPa compression and (R) reduced mobility ($N = 20$ per group), and they were compared with a sham group ($N = 12$) and control group ($N = 8$) (total of 6 groups of animals). The altered mechanical conditions were applied for 5 weeks. Intervertebral disc space was measured from micro-CT images at weeks 1 and 5. Post euthanasia, lateral bending stiffness of experimental and within-animal control discs was measured in a mechanical testing jig and collagen crimp was measured from histological sections.
Results After 5 weeks, micro-CT images showed disc space loss averaging 35, 53, 56 and 35% of the adjacent disc values in the four intervention groups. Lateral bending stiffness was 4.2 times that of within-animal controls in Group B and 2.3 times in Group R. The minimum stiffness occurred at an angle close to the in vivo value, indicating that angulated discs had adapted to the imposed deformity, this is also supported by measurements of collagen crimping at concave and convex sides of the disc annuli.
Conclusion Loss of disc space was present in all of the instrumented discs. Thus, reduced mobility, that was common to all interventions, may be a major source of the observed disc changes and may be a factor in disc deformity in scoliosis. Clinically, it is possible that rigid bracing for control of scoliosis progression may have secondary harmful effects by reducing spinal mobility.

Keywords Intervertebral disc · In vivo · Growth, deformity · Rat model, biomechanics

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Introduction

A scoliosis deformity, as measured by the Cobb angle, consists of wedging asymmetry of the vertebrae and of the intervertebral discs in approximately equal proportions [20, 30]. The pathomechanism of the progressive deformity of the vertebrae is thought to involve mechanical modulation of growth (Hueter-Volkmann principle). However, the mechanism by which the discs become wedged is poorly understood, but may also be due in part to altered biomechanical environment. Also, it is not known quantitatively how the biomechanical conditions of the discs are altered in a scoliosis deformity, but there is probably asymmetrical loading in addition to the angulation imposed by the lateral curvature. There is also evidence [13, 16, 24] of reduced and asymmetrical mobility in the spine, but it is

not clear whether this is due to changes in the tissue properties or shape of the discs, or to altered tissue properties, or size or shape of other surrounding structures (ligaments, muscles and the rib cage). Alterations on the mechanical environment might include alterations and asymmetry of loading and motion, as well as asymmetrical deformation (wedging).

In this study the mechanical environment of skeletally immature rat caudal intervertebral discs was altered to represent these putative alterations in the biomechanical environment of discs in progressive scoliosis. The intention was to distinguish the relative effects of imposed angulation, compressive force and reduced mobility to the progressive wedging of intervertebral discs by imposing permutations of alterations in biomechanical conditions in different groups of animals. Rat tail discs were selected because the rat tail is easily accessible for controlled application of compression forces [3, 10, 17, 26, 31] and because of the rapid growth of these animals [26].

The overall aim of this study was to determine how immature intervertebral discs respond morphologically and mechanically to different components of the altered mechanical environment that occur in scoliosis. The purpose was to document morphological and biomechanical changes in four different models of altered mechanical environment in intervertebral discs of growing rats and in a sham and control groups.

Methods

Animal groups and procedures

External rings were attached to adjacent caudal levels Cd7 and Cd8 vertebrae of 5-week-old (mean 140 g body mass) Sprague–Dawley rats by two percutaneous 0.5 mm diameter pins transfixing the vertebral bodies. Pins were installed under general anesthesia (Ketamine 80 mg/kg and Xylazine 10 mg/kg) with postoperative pain control (Buprenorphine 0.05 mg/kg) and with fluoroscopic (X-ray) visualization of the vertebrae and apparatus to ensure correct placement. The method and apparatus described by MacLean et al. [18] were employed, with a modification by which the rings could be installed either parallel or with an initial 15°- relative angulation (by angulation the rings each by 7.5° to the transverse plane of the vertebrae). Then threaded rods linking the rings were used to control the angulation between the rings, along with springs that could be mounted on the rods to apply a sustained compressive force (Fig. 1). The connecting rods and springs were adjusted to align the rings provisionally parallel to each other or at a small angle to compensate for misalignment measured radiographically (see below). Compression was imposed on the instrumented discs by compressing the calibrated springs to produce a force corresponding to the desired amount of stress [27]. The lengths of the springs were measured and adjusted weekly to maintain the desired force corresponding to the desired stress, using vertebral metaphyseal cross sectional areas as reported in Stokes et al. [27]. Animals were then followed for 5 weeks. All live animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

Four Groups of animals ($N = 20$ per group) underwent permutations of the imposed mechanical conditions and were compared with a sham group ($N = 12$) and control group ($N = 8$) (total of 6 groups of animals, Fig. 1). In Group A (angulation) there was a 15°- angulation imposed by adding springs and locknuts to the rods but not compressing the springs so that motion or growth in the axial direction would not be affected. In Group B, a 0.1 MPa compression stress was imposed by compressing the calibrated springs, along with the 15°- angulation (Fig. 2). In Group C the 0.1 MPa compression stress was imposed, without angulation. In Group R, (Reduced Mobility Group) there was no imposed angulation and the springs were not compressed. This Group was termed the Reduced Mobility Group since the apparatus was observed to limit rotational tail motion in the instrumented region, although the absence of spring compression would not restrict motion in the axial direction. In a Sham Group (Group H) the rings were attached to the adjacent vertebrae without angulation, but no rods or springs were employed. In a Control group (Group NC) no apparatus was applied and the animals were simply housed and handled in the same manner as those in the experimental groups. The 15°- single-level angulation in groups A and B corresponded to the apical disc angulation at the apex of a moderately large human scoliosis deformity. Also, this was thought to be the largest angulation that could be tolerated by the rat caudal discs that are quite narrow relative to their diameter. In addition to the instrumented level, three additional discs were identified (Fig. 2), right panel) as the (P) proximal, (A) adjacent and (D) distal discs and provided measurements as within-animal controls.

Measurements of the disc space (separation on the vertebrae in the axial direction) and wedging of instrumented and adjacent control discs (Fig. 3) were made from micro-CT scans [Explore Locus volumetric conebeam MicroCT scanner (GE Medical Systems, London, ON, Canada)], 2 days after installation of the tail-loading apparatus (Week 1) and just prior to euthanasia (Week 5) under anesthesia and with tail-loading apparatus in place. Steel rod components of the apparatus were replaced with nylon rods for this imaging. The voxel dimension was 93 μm. Hence the typical disc space dimension was seven times the voxel size and typical disc diameter was 35 times the voxel size. The measured angulation of the rings relative to the

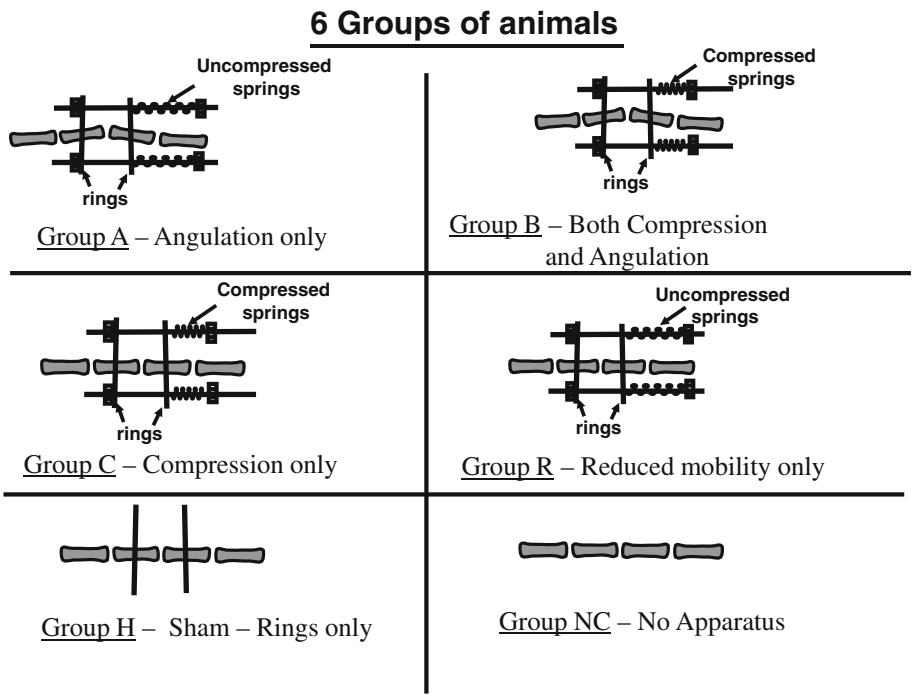

Fig. 1 Diagrammatic representation of the six groups of animals studied

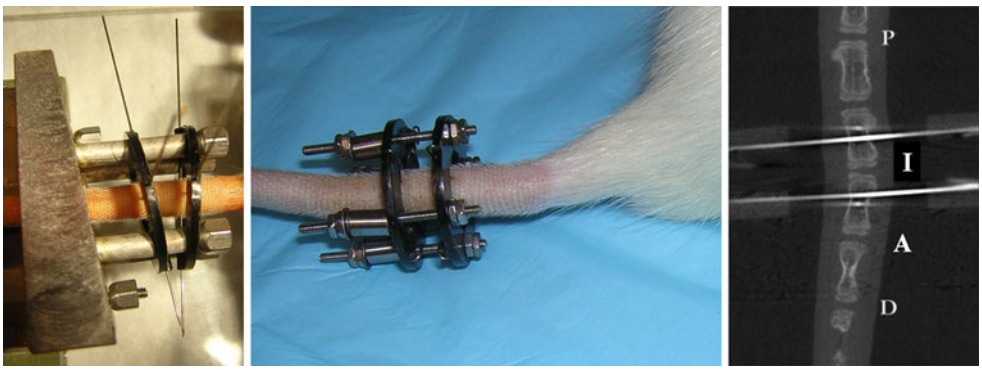

Fig. 2 Photograph and radiograph of rat tail with apparatus installed to produce both angulation and compression (Group B) at the instrumented intervertebral disc. *Note* the rings were initially installed on a straight tail with a relative angulation of 15°. *Left Panel* rings installed at a relative angle of 15°. *Centre Panel* rings made parallel by use of threaded rods and springs (hidden by steel tube protectors). *Right Panel* coronal plane section from Micro-CT of a Group B animal showing vertebrae angled at 15°, and identifying (*P*) proximal, (*I*) instrumented, (*A*) adjacent and (*D*) distal discs

vertebral axes was measured from Week 1 images and was used to provide an adjustment if needed to the alignment of the rings, as well as initial measurements of disc space and wedging for comparison with Week 5 measurements. The alignment of the rings and the lengths of springs were measured with a digital caliper (Mitutoyo, Japan) and were adjusted as needed every week.

Animals were euthanized after 5 weeks, when the average body mass was 440 g. After euthanasia, sections of the tails were cut off and skin and tendons were removed. The tails were then mounted in an apparatus for mechanical testing (see below). Sections of the tail including three vertebrae and two discs [the instrumented level (I-level) and the adjacent-distal control (A-level)] were removed for mechanical testing. After mechanical testing, the discs of a subset of nine animals in Groups A and B were fixed, sectioned and examined under polarized light.

Micro-CT measurements: disc thickness (width) and angle

Mid coronal plane sections of each disc were identified in the micro-CT volumetric reconstructions by means of MicroView (GE Healthcare) software. These planar images were saved and processed via custom software written in Matlab (Mathworks, Nattick, MA, USA). For each disc, four points were manually selected, two on each adjacent vertebra by means of a 'mouse' pointing device. These

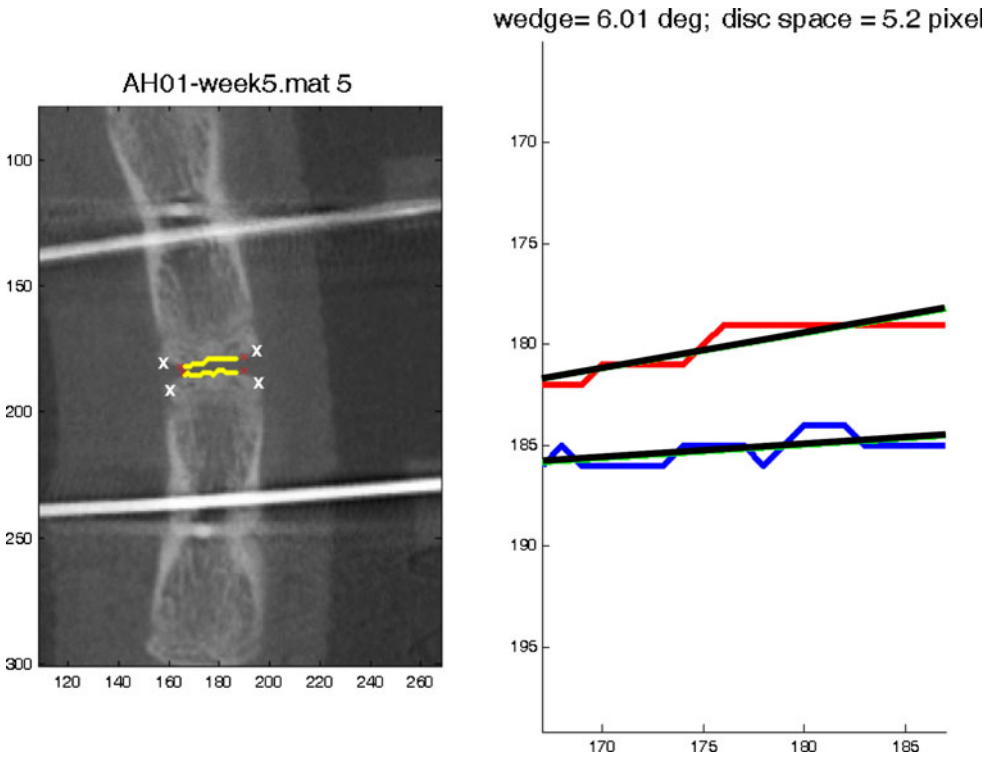

Fig. 3 *Left panel* Coronal plane section through micro-CT image of rat tail in Group A, 5 weeks after installation of the apparatus. The points marked *X* were manually selected and were used as 'seed' points for identifying the marked boundary between vertebra and disc in the central 50% of the endplate width by a method similar to that of Lai et al. [14]. The *right panel* shows an enlarged view of the boundaries, with a *straight line* fitted by linear regression. These *straight lines* were used to measure the disc wedging (angle between lines) and disc space (average separation)

points served as 'seed points' for semi-automated edge detection of the disc-vertebra margins, from which the mean disc space over mid 50% of the width of the endplate was calculated, based on the method of Lai et al. [14] (Fig. 3). The values for the two distal control levels A and D were averaged for comparison with the Instrumented (I-level) discs (Fig. 2).

Mechanical testing: lateral bending stiffness

The loaded and distal-adjacent intervertebral discs were tested mechanically to obtain torque–angle relationships in lateral bending, using a custom four-point bending apparatus in a miniature single axis (compression) mechanical testing machine. Displacement and force were recorded during a series of four sawtooth displacements (i.e. displacement increasing and decreasing linearly with time) with 4-s cycle time. The tail sections consisting of three vertebrae and the intervening I-level and A-level discs with skin and tendons removed were mounted in end fittings and installed sideways in a jig (Fig. 4) that converted the linear motion to an angular motion. The springs and rods were removed from the tail apparatus, and the rings were left in place. Each disc was tested in isolation by immobilizing the other disc. To immobilize the I-level the two rings were connected by threaded rods and nuts. Alternatively, the A-level was similarly immobilized (Fig. 4). The sequence of testing these two levels was randomized. The angular rotation and torque applied were calculated from the force–displacement recordings, using the dimensions of the testing jig that converted linear to angular motion. Ascending and descending segments of the torque-rotation recordings were identified and a cubic polynomial was fitted to each segment (Fig. 4). The angle at which stiffness was a minimum was identified by differentiation of the cubic polynomial and the 'finite-range' stiffness (N mm/°) measured over a range of ±5° from the angle at which stiffness was a minimum (Fig. 4).

Collagen crimping

After mechanical testing, sections of the instrumented (I-level) and distal (D-level) discs of Groups A and B animals were fixed in 10% formalin with glutaraldehyde with the loading apparatus (rods and springs) in place and then decalcified [Decal (R) Decal Chemical Corp, Tallman, NY, USA] after removal of the loading springs. These discs were examined under polarized light for measurement of collagen crimp period (method of Cassidy et al. [6]). Crimp period is an indication of the state of stretch of collagenous tissue [8] and was used here to identify evidence of residual asymmetry between concave and convex sides of angulated (Groups A and B) discs. Also, discs of five additional euthanized animals (from an unrelated experiment) of an age corresponding to the age at surgery were examined as controls to determine acute effects. Sections of these acute control tails were set up with rings and angulated and compressed, then

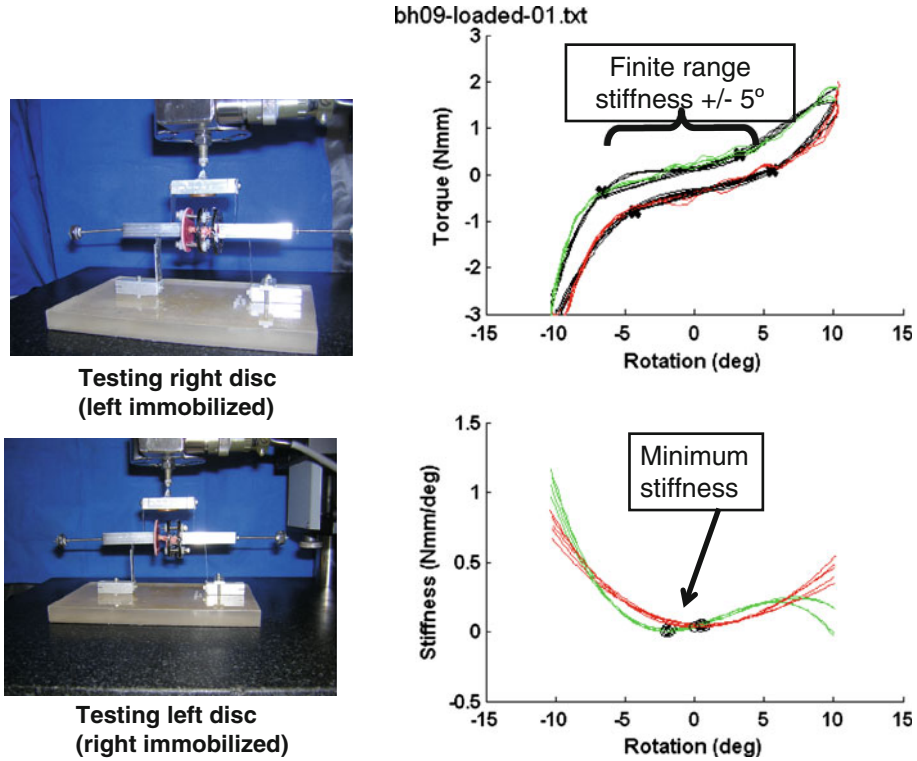

Fig. 4 Mechanical testing in 4-point lateral bending in an apparatus (*left panels*) that converted linear motion to angular motion via flexible knife edges. Tail segments consisting of three vertebrae (two discs) were mounted in end fittings. Each disc was immobilized in turn, to provide measurements of stiffness of the other disc. *Right panels* show an example of data from one disc tested cyclically. The *upper panel* includes ascending (*upper*) and descending (*lower*) portions of the recording with nonlinearity and hysteresis. The 'finite-range' stiffness was measured as the gradient of a line joining the marked points that were at ±5° from the angle at which stiffness was minimum. The *lower panel* shows the first derivative of a third-order polynomial fitted to individual torque-increasing and torque-decreasing segments of the recordings. It was used to identify the angle at which stiffness was a minimum

immediately fixed in 10% formalin. These controls served to identify immediate effects of angulation and compression on collagen crimp period. After fixation and decalcification the discs were cut into two halves ('convex' and 'concave' sides). Each half was then further cut into two parts using a blade angled at approximately 60° to the coronal plane (i.e. aligned with the plane of collagen fibres). These tissue samples were mounted frozen in Optimum Cutting Temperature compound (Tissue-Tek-OCT, Sakura Finetek, Torrance, CA, USA) and frozen sections (8 micron thickness) were made parallel to the cut surface.

Sections were imaged under polarized light to demonstrate collagen crimping, which was quantified by crimp period [6] (Fig. 5). These crimp measurements were made at three radial positions of the annulus—inner, middle and outer in each of five sections. Crimp period measurements were made by marking a series of 'peak' or 'valley' points on several fibres (Fig. 5). The crimp of each fibre was measured as the mean distance between adjacent marked points, and these values were then averaged to give a value for each radial position of each disc annulus.

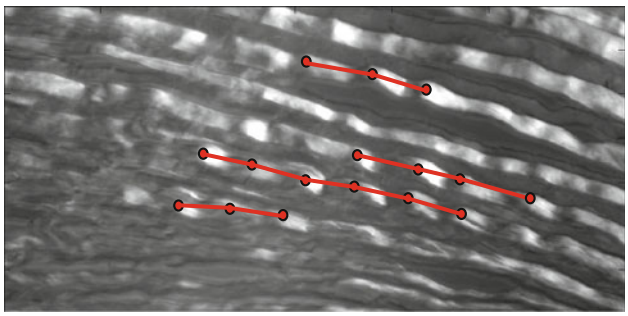

Fig. 5 Micrograph (frozen section, imaged under polarized light) showing collagen crimping. The four sets of points identified the crimp period (mean distance between adjacent points) of four fibres

Statistical analyses

Groupwise and level-wise comparison were made by analyses of variance of individual measurements of intervertebral discs, or of paired differences (between sides, or levels) with post hoc *t* tests (Bonferroni correction). Analyses were made with SPSS software (SPSS Inc., Chicago, IL, USA). The *p*-level 0.05 was considered significant.

Results

Disc space

Disc narrowing at the instrumented (I) levels was evident in micro-CT images (Fig. 6). Compressed discs (groups B and C) had slightly reduced disc space averaging 0.11 and 0.17 mm less than that of adjacent levels at the initial Week 1 measurement ($p = 0.06$ and $p = 0.01$, respectively) (Table 1), and there was subsequent loss of disc space in all 'intervention' groups over the 5-week duration of the experiments. Average disc space loss as a percent of the adjacent level values at 5 weeks was 35, 53, 56 and 35% in the four intervention groups (Fig. 7, Table 1) ($p < 0.001$ for all four groups A, B, C and R). The amount of disc narrowing did not differ significantly between the four groups A, B, C and R. There were missing observations (7, 2, 9, 5 and 1 animals in Group A, B, C, R, H, respectively) resulting from motion artifacts in CT images of these anesthetized animals.

The disc space of instrumented compared to adjacent control disc spaces was significantly different in Groups A, B and R (paired *t* tests). In Groups A and B this presumably resulted from the imposed disc compression, but the initial narrowing in the 'R' Group discs, with apparently no

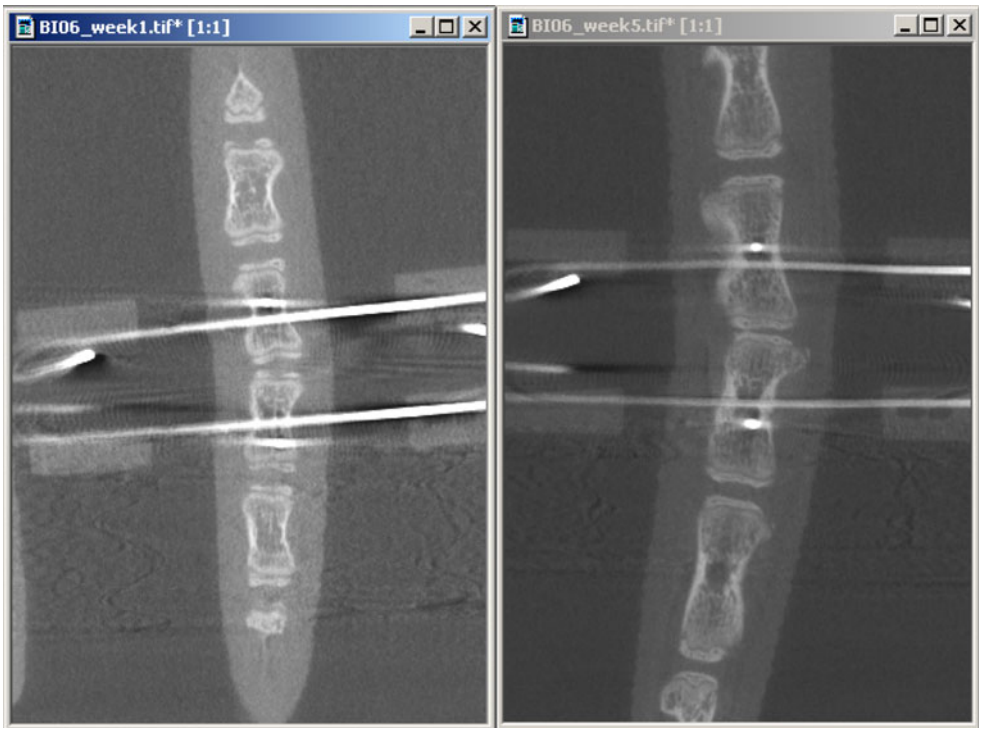

Fig. 6 Example of coronal plane sections from in vivo CT imaging of rat tail at Week 1 (*left*) and at Week 5 (*right*) of an animal in Group B (both compression and angulation of the disc). The in vivo images were made with the apparatus in place, including the springs compressing the disc

Table 1 Mean disc space (and SD) and loss of disc space (mm) at the intervertebral disc level and mean of two distal-adjacent levels in six groups of animals

	Initial (Week 1 -after 2 days)		Final (Week 5)		Week 5 difference (adjacent-instrumented)
	Instrumented	Adjacent	Instrumented	Adjacent	
Group A ($N = 13$)	0.63 (0.15)	0.63 (0.07)	0.45 (0.16)	0.70 (0.10)	0.24 [35%]**
Group B ($N = 18$)	0.59 (0.16)	0.70 (0.12)	0.31 (0.16)	0.65 (0.09)	0.35 [53%]**
Group C ($N = 11$)	0.48 (0.21)	0.65 (0.15)	0.30 (0.15)	0.69 (0.07)	0.39 [56%]**
Group R ($N = 15$)	0.46 (0.21)	0.64 (0.11)	0.41 (0.15)	0.64 (0.11)	0.22 [35%]**
Group H ($N = 11$)	0.75 (0.13)	0.75 (0.08)	0.62 (0.21)	0.64 (0.09)	0.02 [3.4%]
Group NC ($N = 8$)	0.64 (0.12)	0.56 (0.12)	0.63 (0.18)	0.62 (0.10)	−0.01 [−2.0%]

Group A (angulation), Group B (both angulation and compression, Group C (0.1 MPa compression), Group R (reduced mobility), Group H (sham), Group NC (control)

** Highly significant difference at Week 5 between instrumented and internal control adjacent discs ($p < 0.001$, paired *t* test)

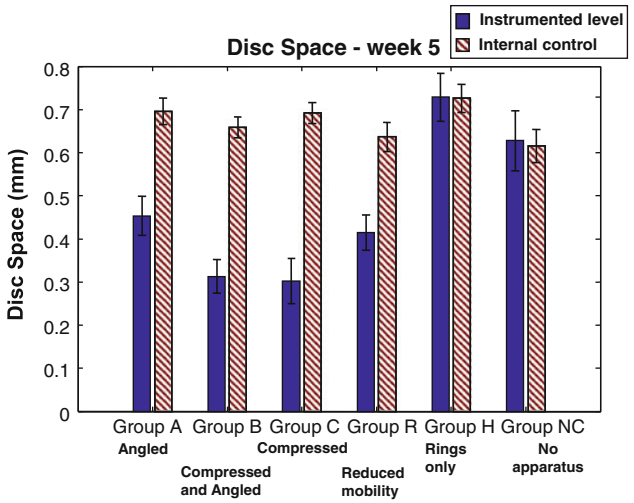

Fig. 7 Disc space measurements at Week 5 (Mean and SEM) (*solid fill* instrumented level; *shaded* average of two adjacent internal control levels)

further narrowing over the duration of the experiment, was unexpected.

There was also a loss of disc wedge angle over 5 weeks in Group A (mean 6.3° loss) and in Group B (mean 9.3° loss). This was attributed to asymmetrical growth of adjacent vertebra that consequently became wedged (c.f. Mente [19]).

Mechanical

Increased lateral bending stiffness relative to within-animal (distal) controls was observed in groups A, B, R, H and NC and the difference was significant in Groups B and R ($p = 0.001$ and $p = 0.005$, respectively) (Table 2). The minimum stiffness was recorded at an angle close to the in vivo (deformed) value, indicating that angulated discs had adapted (remodeled) to the imposed deformity (Table 2). All mean values of this angle were not significantly different from zero. Some stiffness data were lost because of technical failures in the testing apparatus or damage to the tail specimens during dissection. Measurements were lost for 6, 6, 6, 8, 1 and 3 animals in Groups A, B, C, R, H and NC at the instrumented level and for 6, 12, 7, 11, 2 and 3 animals at the distal adjacent (D) level, as indicated in Table 2.

Collagen crimping

Acutely, asymmetrical crimp period (10% difference) between convex and concave sides of the disc annuli was measured with larger period on the convex side ($p = 0.05$). This asymmetry was compatible with convex side tissue being under relatively more tension (Table 3). However,

Table 2 Mean (and standard error of the mean) lateral bending stiffness (mN mm/°) and angle (relative to in vivo alignment) where stiffness was minimum

	Group A	Group B	Group C	Group R	Group H	Group NC
Finite-range stiffness (instrumented level)	60.2 (10.5) ($N = 14$)	193.8 (32.2) ($N = 14$)	36.8 (4.5) ($N = 14$)	91.2 (22.7) ($N = 12$)	46.8 (6.1) ($N = 11$)	35.1 (9.1) ($N = 5$)
Finite-range stiffness (distal-adjacent level)	47.9 (6. 6) ($N = 14$)	46.0 (6.5) ($N = 6$)	39.5 (5.7) ($N = 13$)	36.5 (5.1) ($N = 9$)	35.7 (5.5) ($N = 10$)	24.0 (5.7) ($N = 5$)
Angle at min. stiffness (instrumented level)	0.05 (0.26) ($N = 14$)	−0.53 (0.19) ($N = 14$)	−0.32 (0.16) ($N = 14$)	−0.59 (0.17) ($N = 12$)	0.36 (0.20) ($N = 11$)	−2.03 (0.65) ($N = 5$)

Group A (angulation), Group B (both angulation and compression), Group C (0.1 MPa compression) and Group R (reduced mobility), Group H (sham), Group NC (control). Differences between mean values at instrumented levels relative to distal adjacent levels were significant in Groups B and R ($p = 0.001$ and $p = 0.005$, respectively)

Table 3 Mean (and SD) of collagen crimp period (μm) in three groups of animals: Group A (angulation), (Group B) both angulation and compression and acute group (5 additional discs from euthanized animals fixed immediately after application of angulation and compression)

	Convex	Concave
Group A	36.0 (7.4)	38.0 (8.1)
Group B	35.7 (10.2)	35.1 (11.9)
Acute	46.1 (9.5)	41.2 (6.8)

More crimped collagen has shorter crimp period

this asymmetry was not evident (no significant concave–convex difference) in the instrumented level discs of Groups A (angulated) and B (angulated and compressed) after they were euthanized after 5 weeks. The crimp period at the instrumented levels of Groups A and B animals was significantly less than in the Acute Group ($p < 0.01$). Also the crimp period in these instrumented discs (mean 37.0 and 35.4 μm, respectively, in Groups A and B) was significantly less and at the distal within-animal control discs ($p = 0.03$ and $p = 0.01$ for Groups A and B, respectively), where the mean crimp period was 43.5 and 42.2 μm, respectively, for Groups A and B. The greater values at the distal adjacent control levels were consistent with the swelling that likely occurred during fixation. (The instrumented level discs of all three groups were fixed under compression with rings and springs in place).

Discussion

All experimental interventions produced substantial narrowing of the intervertebral disc space of these growing animals over the 5-week period. This 5-week period corresponded to a large proportion of the post-natal growth of the animals (bodyweight increased from mean 140 to 440 g). The changes also included increased lateral bending stiffness, and there was evidence of collagen remodeling to accommodate the deformed position from the crimp measurements (symmetry of crimp period after 5 weeks in Groups A and B) as well as the observation that minimum lateral bending stiffness was measured close to the in vivo (deformed) configuration in angulated discs. The small non-significant convex–concave side differences in crimp period at the instrumented levels were consistent with the collagen having remodeled in these angulated discs. The initial loss of disc space at 1 week in discs with imposed compression (Groups B and C) was presumably a consequence of disc bulging together with fluid loss.

This study was designed to distinguish between the effects of different components of the mechanical interventions that included compression, angulation and reduced mobility, and the four experimental groups all demonstrated loss of disc space. Reduced mobility was present in all interventions, and the disc narrowing in the discs with reduced mobility alone (Group R) was comparable with that in loaded and angulated discs. This suggests that reduced mobility was an important contributor to loss of disc space, since the restricted mobility was common to the instrumented levels of all experimental groups. The stiffness of the springs in the apparatus was expected to reduce angular motion. This effect would be present even when the springs were not compressed, although in this case there would be minimal if any constraint in the axial direction. In the Groups A and R animals the springs were kept uncompressed so only the springs on the concave side of any bending motion would contribute to this angular stiffening. The effect of the springs in the axial direction was considered to be negligible in these Group A and R animals.

The very early differences between instrumented and adjacent disc space (at Day 2) were expected for the compressed discs (Groups B and C), but not for the reduced mobility discs (Group R). The initial narrowing in the 'R' Group discs, with apparently no further narrowing over the duration of the experiment, was unexpected.

Although the stiffness of the apparatus appeared on examination of animals in vivo to eliminate any angular motion, it would not completely immobilize the tail segment. Rigid immobilization has been associated with loss of caudal disc space in older animals [10, 18]. This suggests that reduced mobility was a major source of disc changes and may be a factor in disc degeneration in scoliosis. Immobilization effects have also been reported in surgically fused canine discs [2, 4, 5] and immobilization has been implicated as producing degeneration of articular cartilage [29].

It was expected that the loss of disc space and increase in stiffness would be greatest in Group B animals in which compression, angulation and reduced mobility were all present. However, the disc space loss was equally large in the Group C (compressed) discs. In Group B the combined angulation and compression of the disc was observed in CT images to produce near impingement on the concave side. This may have produced an effective limit on the loss of disc space and also was probably responsible for the very large increase in stiffness in the Group B discs. Increased stiffness could result from a combination of disc space narrowing (geometrical effect) and altered tissue properties. Comparing the findings between groups, the increased stiffness was greatest (and significant) in the groups R and B while the disc narrowing was greatest in Groups B and C, so there was no direct correlation between disc narrowing and increased stiffness. The possible contribution of altered tissue properties is not known from this study.

The adjacent control disc space values were unexpectedly variable between the sham and control groups, and ANOVA and post hoc tests showed that the control (Group NC) adjacent discs had significantly smaller disc space (averaging 0.56 mm) than the Sham (Group H) adjacent discs (averaging 0.75 mm).

The physiological level of compressive stress experienced by the rat tail is not known precisely. The 0.1 MPa sustained compression applied in Groups B and C corresponded to 1 N at Week 1, increasing to 2.2 N at Week 5 (to maintain the same stress on the disc). Thus, it was just less than bodyweight (125 g) at Week 1 and about half bodyweight (400 g) at Week 5. In vivo studies of compression of mature rodent tails designed to produce degenerative changes [10, 15, 17, 18] have generally used larger stresses.

Disc narrowing has been reported in axially loaded (compressed) rodent discs [10, 15, 17, 18] and rabbit spine discs [11] and in 'immobilized' discs [10] and mechanically stressed (over-active) rats [22], and there was reduced growth of axially compressed discs of young rats [26]. Apoptosis and disc narrowing have been reported in compressed skeletally mature mouse tail discs [17] and imposed bending produced cellular changes preferentially on the concave side [7]. It was expected that the younger growing animals in the present study would have more metabolically active tissue with a higher rate of tissue synthesis and remodeling and consequently more sensitive to the effects of mechanical stress than in the older (typically 12-week-old) animals reported previously. However, there was little axial direction growth observed in any of the rat discs, similar to the relatively small amount of axial growth of spinal discs relative to growth of the vertebrae previously reported in both adolescent humans [28] and in rats [9].

Ching [3] reported greater reduction of disc space in rat tails subjected to static than cyclic compression, and there was a reduction in angular compliance at the loaded levels. Stiffness changes in discs subjected to compressive loading in vivo were thought to be due to stiffening of tissues surrounding the disc (longitudinal ligaments, tendons, muscle, skin), rather than disc tissue [21], suggesting that the inconsistent findings (increased stiffness only in Groups B and R, and large variability between animals in each group) may be related to variability in the amount of tissue surrounding the discs that was removed. It was reported that there was no change in mechanical lateral bending stiffness in the segment of the mouse tail subjected to static in vivo bending [7].

Within-animal control discs at the level immediately distal to the experimental instrumented level (level A), as well as discs two distal and proximal to the instrumented levels (levels P and D) provided the basis for comparison with the instrumented discs. It should be noted that in the angulated tails (Groups A and B) there were compensating curves (Fig. 2) that could result in some wedging of these discs, especially at the adjacent-distal level. In mechanical testing, the minimum stiffness of distal adjacent discs was found to be asymmetrical and this was attributed to remodeling of these discs in 'secondary' curves.

The compressed vertebrae of animals in Groups A and B became wedged during the 5-week experiments as a result of asymmetrical growth [19], with consequential reduction in the disc wedge angles. In human scoliosis both the vertebrae and discs develop wedging of approximately equal magnitude during growth. It is not known whether there is any paracrinal or other interaction between vertebral growth plates and adjacent discs that might produce this complementary wedging.

In humans there was altered disc composition in patients with idiopathic scoliosis as well as those having scoliosis associated with cerebral palsy, so the changes were thought to be secondary to the spinal curvature, rather than causal [23]. Differences in hydration and in biosynthetic activity in discs of humans with scoliosis were attributed to ineffective response to a pathological mechanical environment by Antoniou et al. [1]. In the rat tail model there is an imposed disc deformity, so this is considered to be a model of secondary changes. Endplate calcification has been observed in discs of humans with scoliosis [25] and in a porcine model of induced scoliosis [12] and is considered as a possible cause of nutritional compromise and consequent disc degeneration and wedging in scoliosis. The resolution of the micro CT scans used in the present study was insufficient to quantify possibly similar effects in the rat tails.

The loss of disc space was present in all experimental interventions and 'reduced mobility' associated with the stiffness of the apparatus was present in all interventions. This suggests that reduced mobility was a major source of disc changes and may be a factor in disc deformity in human scoliosis. This finding suggests that rigid bracing for control of scoliosis progression may have secondary harmful effects.

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Conflict of interest None.

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