Intervertebral Disc Adaptation to Wedging Deformation

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Abstract: Although scoliosis includes wedge deformities of both vertebrae and discs, little is known about the causes of the discal changes, and whether they result from mechanical influences on growth and/or remodelling. Methods and Materials: An external apparatus attached to transvertebral pins applied compression and 15 degrees of angulation to each of two adjacent young rat caudal intervertebral discs for 5 weeks (four animals), or for 10 weeks (four animals). Each week, micro-CT scanning documented the *in vivo* discal wedging. After euthanasia, tail segments (three vertebrae and the 2 angulated discs) were excised and their flexibility was measured over a range of lateral bending. The angle of maximum flexibility was recorded. Then discs were fixed *in situ* (with the external apparatus in place) and sectioned for polarized light microscopy. Results: The disc-wedging deformity averaged 15 degrees initially, it averaged 20 degrees after 5 weeks, and then reduced to 10 degrees (in 10 week animals). The lateral bending flexibility showed a distinct maximum at an average of 1.1 degrees from the *in vivo* position in the 5-week animals, indicating structural remodeling of the discs almost to the deformed geometry. The 10-week animals had maximum flexibility at 1.4 degrees from the in vivo position (no significant difference between 5 and 10-week animals.) Collagen crimp angles [Cassidy *et al.,* Conn Tiss Res 1989, 23:75-88] were not significantly different between convex and concave sides, again suggesting that remodeling had occurred. Conclusions: In a mechanically induced scoliosis deformity in skeletally immature rats, the intervertebral discs underwent remodeling within 5 weeks. This indicates that this animal model is suitable for studying adaptive wedging changes in human scoliosis.

Keywords: Intervertebral_disc, wedging, animal_model, biomechanics

1. Introduction

Scoliosis involves wedging deformity of the discs as well as the vertebrae. Prior to skeletal maturity, the development of vertebral wedging is thought to involve asymmetrical growth in vertebral growth plates, modulated by asymmetrical loading. The cause of the wedging deformity in discs is not known, but may involve asymmetrical remodeling and/degeneration of the disc tissue. Lateral migration of the nucleus has been reported in human scoliosis [1]. During adolescent growth there is minimal vertical height increase in the discs [2]. The purpose of this study was to document morphological, mechanical and annulus structural changes in mechanicaly deformed intervertebral discs in a rapidly growing rat tail model.

2. Methods

2.1. In-vivo study - bent tail model

The tail discs of 8 rats were instrumented for either 5 or 10 weeks. The 8 animals were assigned to one of two groups of 4 animals each: 30° angulation of two disc levels for 5 or 10 weeks, (with the apparatus crossing two discs, each disc angulated nominally 15°, c.f. Mente et al. [3,4]). The apparatus was modified from that used previously [3,4] by use of radiolucent materials (fiberglass rings, nylon rods and nuts). The only radio-opaque materials were the bone-transecting pins and the loading springs, and these produced minimal artifact in the CT images. At weekly intervals (5-week animals) or 2-weekly intervals (10-week animals) *in vivo* micro-CT scans of the tail were performed to measure disc wedging and thickness. *Post-mortem* lateral bending stiffness was measured, followed by *in situ* fixation and histology to measure collagen crimp angles (by polarized light microscopy [5,6]). The objective was to determine whether the discs were structurally altered at 5 weeks, and whether the structural changes were greater after 10 weeks

2.2. Micro-CT of rat tail intervertebral disc

Micro-CT scanning provided a method to monitor precise geometrical measurement of the size and shape of the disc space between vertebrae *in vivo*. Animals were anesthetized at one or two-weekly intervals and imaged in the Explore Locus volumetric conebeam MicroCT scanner (GE Medical Systems, London, Ontario) set at 55 kVp, with 200 views (each taken at 1 degree increments of rotation). Four 150 ms exposures were averaged at each angle, for a total scan time of 9 minutes. Volumes were reconstructed from acquired scan data at a resolution of 94-micron per voxel side. These images (Figure 1) provided excellent resolution of the vertebral bony endplates, permitting precise disc-thickness and wedging measurements. The disc soft-tissue outline of the annulus periphery was visible but indistinct, precluding accurate non-invasive disc volumetric measurements. Disc dimensions (as evidenced by the space between vertebrae) were obtained from a mid-coronal plane section (See Figure 1). The vertebral lateral margins were identified manually, and the disc-bone interface was identified by a custom edge-detection algorithm. The average distance between edges (disc space) was calculated, and the wedge angle was found by fitting lines though the detected edges by linear regression. The angle between these lines provided a measure of disc wedging. Random errors in these measurements were associated with selection of the image plane to be evaluated, and manual identification of the vertebral lateral margins. In an empirical evaluation of measurement variability from these sources, the standard deviation of repeated measures was 8% (disc thickness) and 6% (wedge angle).

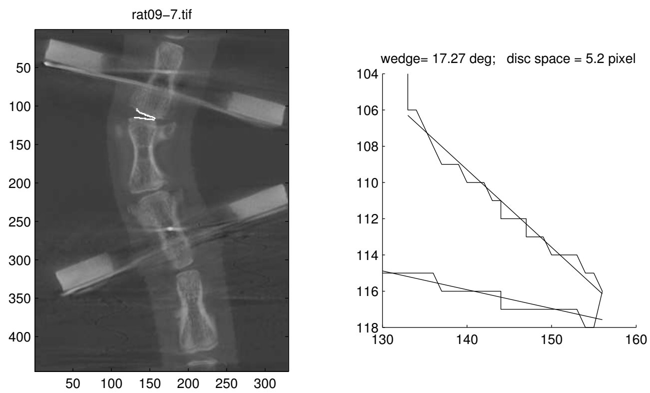

Figure 1. Left: mid-coronal section of rat tail Micro-CT scan, with edge detection of the upper instrumented disc bone-disc interface. Right: disc shape parameters derived from linear regression fit through the interface. (1 pixel=94 μm)

2.3. *Mechanical tests of rat tail intervertebral disc stiffnessl*

Specimens consisting of three vertebrae with the loaded two intervening discs were dissected from the tails (with loading rings still attached) and embedded in square-section end-fittings for mounting in a four-point bending jig. Two important elements of the design of this jig: were: (1) flexible pieces of shim stock engaged in grooves cut into the end fittings to create the four loading points (Figure 2, left). A fifth piece of flexible shim stock provided a 'hinge' permitting rotation of the upper loading yoke; (2) the end fittings were unbalanced relative to the outer supports, so that they provided an initial 'preload' moment that angulated the specimen in its initial position prior to the start of the test.

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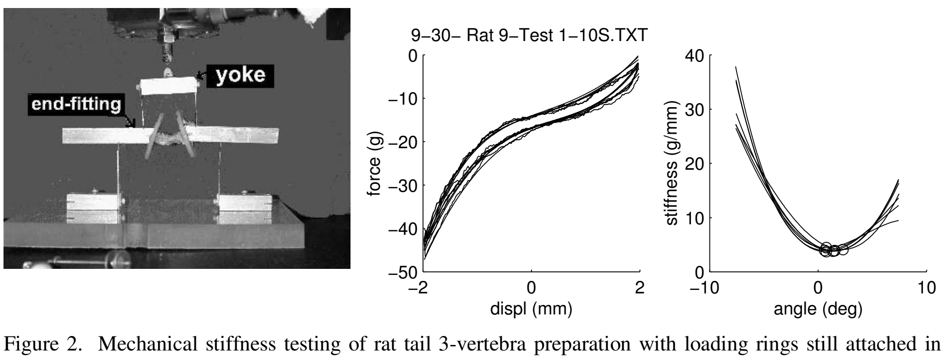

Figure 2. Mechanical stiffness testing of rat tail 3-vertebra preparation with loading rings still attached in testing jig (left), sample load-displacement data (center) and derived stiffness (right).

For testing, the jig was placed in a custom uniaxial micro-mechanical testing machine [7], operated under displacement control with five cycles of the actuator vertical displacement (saw-tooth waveform) that produced angulation or the specimen with a constant moment. Force and displacement were continuously recorded. Each of the ten loading or unloading segments of the load displacement data were fitted by a 4th-order polynomial. Each polynomial was differentiated to obtain a stiffness-angle relationship (Figure 2, right panel) which was corrected for the slightly non-linear geometrical relationship between displacement and angle, and between force and torque.

3. Results

3.1. Micro-CT

Disc wedge angle measurements (two discs per tail) were averaged by Group and Time post-instrumentation. These measurements show (1) that the disc wedge angle varied over time from the nominal 15° per disc intended value, (2) there was a steady loss of disc wedge angle after five weeks, presumably because of steadily increasing vertebral wedging (see Figure 3, left panel). Measurements of disc space (Figure 3, right panel) indicated a loss of disc space over time in these chronically compressed discs.

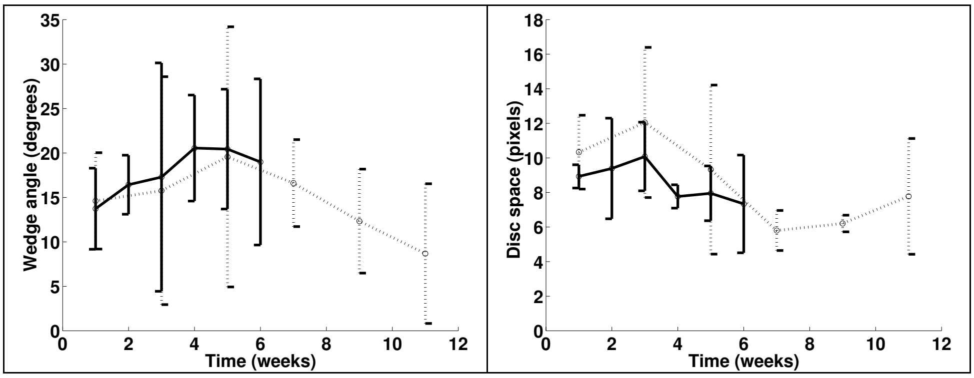

Figure 3. Mean (+/- SD) disc wedge angles (left), and disc space (right) measured from micro-CT scans, averaged for each group of 4 animals. 5-week animals (solid lines) were measured at weekly intervals; 10 week animals (broken lines) at two-weekly intervals.

3.2. Mechanical testing

The minimum stiffness was observed to occur at a distinct point in the angle-stiffness graphs, and occurred at a consistent angle over the multiple (five) loading cycles of each specimen. This minimum of stiffness (Figure 2, right panel) occurred in all tail specimens at a small positive angle, indicating that the discs were almost remodeled to their deformed state. (Acutely, the tails would have minimum stiffness when straight, *i.e.* at an angle of +30° in the stiffness-angle graphs.) There was no significant difference between the mean angle at which minimum stiffness occurred for the 2-disc animals (5-week animals 1.1° ± 0.4°; 10-week animals 1.4° ± 0.7°), indicating that the five week duration was adequate to achieve structural remodeling of the discs. In Figure 2, right panel, an angle of zero (abscissa) corresponds to the angulation of the tail during the *in vivo* experiment (nominally 30° angulation of the tail), as documented by the angle between the loading rings visible in the photograph (left panel, Figure 2).

3.3. Collagen crimp angles

Mean crimp angles were in the range 4.4 to 9.8 degrees. Paired differences averaged 1.1degrees (greater crimp angle on the concave side), but this difference was not statistically significant. Remodeling of the disc tissue was expected to equalize the concave and convex side crimp angles, with the disc fixed in the experimental state (loaded and angulated). The small, non-significant difference in this expected direction suggested that annulus tissue remodeling had occurred.

4. Discussion and conclusions

This study confirmed that the rat tail discs were substantially altered mechanically and structurally after 5 weeks. Mechanical changes were evidenced by the discs adopting a structure whose stiffness was minimum at close to the 15-degree angulated position (the minimum stiffness would normally occur at a straight tail position). Structural changes in the annulus apparently involved a remodeling of the crimped collagen fibers. These fibers have bi-refringent properties, such that alternating limbs of the zig-zag pattern appear bright under polarized light at differing angles when the microscope stage is rotated. This 'crimp angle' varies with the state of tension of the tissue [6]. The crimp angles measured here were lower than those reported for human discs [5], probably because in the present study the tissue was fixed in a loaded condition.

In this animal model, the changes were apparent after five weeks, during which time the recently weaned animals grew to close to their adult size. The changes in human adolescent discs probably occur much more slowly, and further studies are required to determine whether this rat model is an accurate representation of the mechanism of intervertebral disc wedging in the human spine with progressive scoliosis.

5. References

[1] Perie D, Curnier D, de Gauzy JS Correlation between nucleus zone migration within scoliotic intervertebral discs and mechanical properties distribution within scoliotic vertebrae. Magn Reson Imaging. 2003 Nov;21(9):949-53.

[2] Stokes IAF and Windisch L: Vertebral height growth predominates over intervertebral disc height growth in the adolescent spine. Spine: In press.

[3] Mente PL, Aronsson DD, Stokes IAF, Iatridis JC: Mechanical modulation of growth for the correction of vertebral wedge deformities. Journal of Orthopaedic Research, 1999; 17:518-524.

[4] Mente PL, Stokes IAF, Spence H, Aronsson DD: Progression of vertebral wedging in an asymmetrically loaded rat tail model. *Spine*, 22: 1292-1296, 1997

[7] Roemhildt ML, Coughlin KM, Peura GD, Fleming BC, Beynnon BD. Material properties of articular cartilage in the rabbit tibial plateau. J Biomech. 2005 Sep 13; [Epub ahead of print]

[5] Cassidy JJ, Hiltner A, Baer E: Hierarchical structure of the intervertebral disc. Connective Tissue Research 1989, 23: 75-88.

[6] Diamant J, Keller A, Baer E, Litt M, Arridge RGC: Collagen; ultrastructure and its relation to mechanical properties as a function of ageing. Proc Roy Soc Lond (B) 1972, 180:293-315.