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Intervertebral disc changes in an animal model representing altered mechanics in scoliosis

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Abstract: The intervertebral discs become wedged and narrowed in a scoliosis curve, and this may be due in part to altered biomechanical environment. To study this, external rings were attached by percutaneous pins transfixing adjacent vertebrae in 5-week-old Sprague-Dawley rats and four permutations of mechanical conditions (4 groups of animals) were compared: (A) 15 degrees Angulation, (B) Angulation with 0.1 MPa Compression, (C) 0.1 MPa Compression, and (D) Reduced mobility. These altered mechanical conditions were applied for 5 weeks. After 5 weeks, disc narrowing at the intervention levels was evident in micro-CT images. Average disc space loss as a percent of the initial values over the 5 weeks was 19%, 28%, 22% and 20% four groups listed above. Increased lateral bending stiffness relative to within-animal controls was also observed at all groups. The minimum stiffness was recorded at an angle close to the *in vivo* value, indicating that angulated discs had adapted to the imposed deformity. In the angulated and compressed discs there was a small difference in the amount of collagen crimping in the disc annuli between concave and convex sides. All experimental interventions produced substantial changes in the intervertebral discs of these growing animals. 'Reduced mobility' was present in all interventions, and the changes in the discs with reduced mobility alone were comparable with those in loaded and angulated discs. This suggests that imposed reduced mobility is the major source of disc changes, and may be a factor in disc degeneration in scoliosis. Further studies are in progress to characterize gene expression, matrix protein synthesis and composition in these discs.

Keywords: Intervertebral disc; In vivo; Growth, Deformity; Rat model, Biomechanics

1. 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. The wedging and narrowing of the discs in a scoliosis curve may be due in part to altered biomechanical environment. The pathomechanism of the progressive deformity of both the vertebrae and the discs is poorly understood. The eventual aim of this study is to determine how immature intervertebral discs respond morphologically, mechanically and biologically to components of the altered mechanical environment that occurs in scoliosis. The purpose of the present study is to document morphological,and biomechanical changes in four different models of altered mechanical environment in intervertebral discs of growing rats.

2. Methods

External rings attached by percutaneous pins transfixing adjacent vertebrae in 5-week-old Sprague-Dawley rats were applied for 5 weeks, using a modification of the apparatus described by MacLean et al. [1] Four permutations of mechanical conditions (4 groups of animals) were compared: 15 degrees Angulation (Group A), 0.1 MPa Compression (Group C), both Angulation and Compression (Group B), and Reduced mobility (Group D). Angulation in Groups A and B was achieved by installing the rings each at 7.5 degrees to the transverse plane of adjacent tail vertebrae, then using connecting rods and springs to align the rings parallel to each other. Compression was achieved by compressing the springs to produce a force corresponding to the desired amount of stress [2].

The alignment of the rings and the lengths of springs were checked and adjusted if needed every week. In vivo micro-CT imaging (Explore Locus volumetric conebeam MicroCT scanner (GE Medical Systems, London, Ontario) of the tails of anaesthetized animals was done at week 1 and week 5. After semi-automated edge detection to identify the disc-vertebra margins, the mean disc space and the disc wedge angle of each disc was determined from a straight line fit though the points on the disc-vertebra margin.

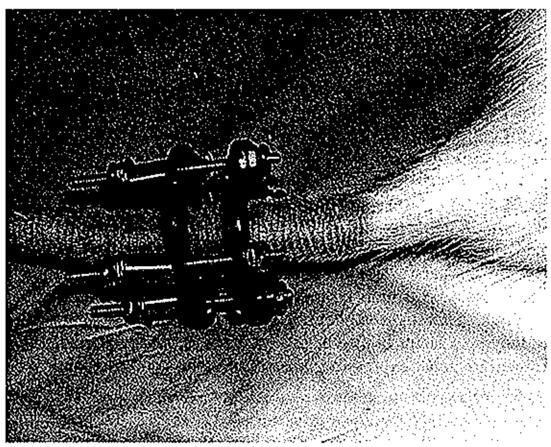

Figure 1: Photograph of rat tail with apparatus installed to produce both Angulation and Compression (Group B) at the instrumented intervertebral disc. Note: The rings were initially installed on a straight tail with a relative angulation of 15 degrees.

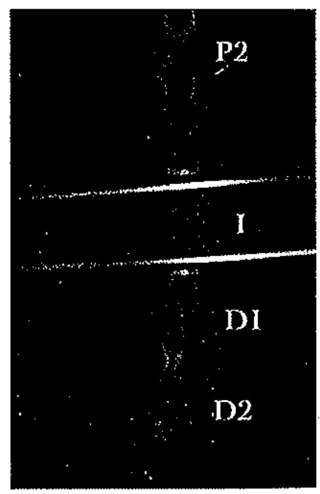

Figure 2: Coronal plane section through micro-CT image of rat tail in Group B, two days after installation of the apparatus. These images were used to measure the disc wedging and disc space. Labelled discs: I=Interventaion level, P2: proximal control; D1, D2 Distal controls

Animals were euthanized after 5 weeks. Sections of the tail including three vertebrae and 2 discs (the intervention level and the adjacent-distal control) were removed for mechanical testing,, and a disc two levels proximal to the intervention level, and the two discs distal to the intervention level were removed for histology and also (data not presented here) for gene expression and compositional studies, (Figure 2). The loaded and distal-adjacent intervertebral discs were tested mechanically to obtain curves for angle-rotation in lateral bending, using a custom four-point bending apparatus. The moment-rotation recordings were used to identify the angle at which stiffness was a minimum (degrees), the value of the minimum stiffness (mN/degree), and the 'finite-range' stiffness (measured over a range of +/- 5 degrees from the angle at which stiffness was a minimum.

Tail sections were then fixed in 10% formalin with glutaraldehyde with the loading apparatus in place, and decalcified. Frozen sections were imaged under polarized light to demonstrate collagen crimping, which was quantified by crimp angle and crimp period [3]. These crimp measurements were made at three radial positions of the annulus – inner, middle and outer.

3. Results

3.1 *Geometrical:*

Disc narrowing at the intervention levels was evident in micro-CT images. Compressed discs (groups B and C) had slightly reduced disc space at the initial measurement, relative to other groups (Table 1), and there was subsequent loss of disc space in all groups over the 5-week duration of the loading. Average disc space loss as a percent of the initial values over the 5 weeks was 19%, 22%, 28% and 20% four groups (Table 1). The amount of disc narrowing did not differ significantly between groups.

Table 1: Disc space and loss of disc space (mm) at the intervention l intervertebral disc level in four groups of animals. Group A (Angulation), Group B (both Angulation and Compression, Group C (0.1 MPa Compression) and Group D (Reduced mobility).

	Initial (after 2 days)	Final (week 5)	Difference
Group A (N=9)	0.69 (0.12)	0.55 (0.13)	-0.13 (0.16)
Group B (N=31)	0.64 (0.14)	0.47 (0.14)	-0.18 (0.21)
Group C (N=8)	0.50 (0.16)	0.39 (0.14)	-0.11 (0.25)
Group D (N=11)	0.60 (0.15)	0.48 (0.08)	-0.12 (0.18)

3.2 *Mechanical*

Increased lateral bending stiffness relative to within-animal controls was observed at all groups. Trends were similar for both group-wise mean values of stiffness, as measured at minimum stiffness, and as measured over the finite range of displacement (+/- 5 degrees). (Table 2) The minimum stiffness was recorded at an angle close to the *in vivo* value, indicating that angulated discs had adapted to the imposed deformity.

Table 2: Average lateral bending stiffness (mN/degree) at intervention levels, and at distal control levels of four groups: Group A (Angulation), Group B (both Angulation and Compression), Group C (0.1 MPa Compression) and Group D (Reduced mobility).

	Group A	Group B	Group C	Group D
Minimum stiffness (intervention level)	12.6 (n=6)	60.0 (n=8)	15.2 (n=9)	61.7 (n=7)
Minimum stiffness (distal-adjacent level)	16.9 (n=4)	21.1 (n=6)	16.9 (n=3)	0.34 (n=6)
Finite-range stiffness (intervention level)	55.0 (n=6)	210 (n=8)	50.7 (n=9)	129 (n=7)
Finite-range stiffness (distal-adjacent level)	47.3 (n=4)	54.1 (n=6)	39.7 (n=3)	38.1 (n=6)

3.3 *Histology (Collagen crimping)*

At the intervention levels of the Group B (angulated and compressed) discs there was a small non-significant difference in the amount of collagen crimping in the disc annuli between concave and convex sides. This was consistent with the collagen having remodeled to a similar strain condition on each side of these angulated discs. The crimp periods at these levels was significantly less than at the within-animal control discs. This is consistent with the fact that the intervention level discs were fixed with the apparatus in place (angulation and compression present).

Table 3: Mean (and Standard Deviation) of collagen crimp length (arbitrary units) and crimp angle (degrees) in 4 groups of animals: Group A (Angulation), (Group B) both Angulation and Compression, Group C (0.1 MPa Compression) and Group D (Reduced mobility) at the intervention, proximal adjacent and two-distal discs. *Note:* more crimped collagen has shorter crimp period, and greater crimp angle.

	Proximal		Intervention level disc		Two-Distal	
	Convex	Concave	Convex	Concave	Convex	Concave
Crimp Length (arbitary)	93.4 (11.7)	87.2 (11.9)	69.6 (27.8)	66.5 (35.3)	93.8 (16.1)	93.5 (21.3)
Crimp Angle (degrees)	23.8 (8.5)	24.0 (8.7)	23.1 (16.4)	26.9 (7.2)	22.5 (8.17)	19.6 (4.9)

4. Discussion and Conclusion

All experimental interventions produced substantial changes in the intervertebral discs of these growing animals over the five-week period. This five-week period corresponded to a large proportion of the post-natal growth of the animals (bodyweight typically increased from 125 to 400 g). The changes included narrowed disc space and increased lateral bending stiffness, and there was evidence of collagen remodeling to accommodate the deformed position, also compatible with the observation that minimum lateral bending stiffness was measured close to the *in vivo* 'bent-tail' configuration in angulated discs.

The discs at the level immediately distal to the experimental intervention level, as well as discs two distal and proximal to the experimental intervention levels were used as within-animal controls. It should be noted that in the angulated tailed (Groups A and B) there were compensating curves (Figure 2) that could result in some wedging of these discs, especially at the adjacent-distal level.

'Reduced mobility' was present in all interventions, and the changes in the discs with reduced mobility alone were comparable with those in loaded and angulated discs. This suggests that reduced mobility is the major source of disc changes, and may be a factor in disc degeneration in scoliosis. Further studies are in progress to characterize gene expression, matrix protein synthesis and composition in these discs.

Acknowledgement

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