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

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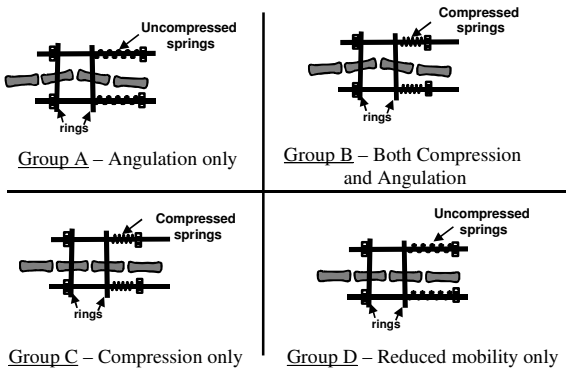
INTRODUCTION:

Scoliosis deformity consists of wedging asymmetry of the vertebrae and of the intervertebral discs in approximately equal proportions. The pathomechanism of progressive deformity of both the vertebrae and the discs is poorly understood. Wedging and narrowing of discs may be due in part to altered biomechanical environment. 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.

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 a published apparatus [1]. Four permutations of mechanical conditions (4 groups of animals, minimum 5 animals per group) were compared: 15 degrees Angulation (Group A), both Angulation and Compression (Group B), 0.1 MPa Compression (Group C), 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 0.1 MPa [2]. All live animal procedures were IACUC approved.

4 Groups of animals



In vivo micro-CT imaging (GE Medical Systems, London, ON) of the tails of anaesthetized animals was done at weeks 1 and 5. Each disc's mean disc space and wedge angle was determined from a straight line fit though points on the disc-vertebra margin.

Animals were euthanized after 5 weeks. Sections of the tail including 3 vertebrae and 2 discs (the intervention disc 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 (data not presented here) for gene expression and compositional studies. 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), and the 'finite-range' stiffness 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, quantified by crimp period [3] at three radial positions of the annulus – inner, middle and outer.

RESULTS:

Geometrical: Disc narrowing at the intervention levels was evident in micro-CT images. In the first micro-CT scan (2 days post-instrumentation), compressed discs (groups B and C) had lesser disc space (mean 0.60 mm) relative to the uncompressed groups A and D (mean 0.66 mm), representing 8.5% immediate narrowing. 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 15%, 36%, 32% and 16% in the four groups. The amount of disc narrowing did not differ significantly between groups ($p > 0.05$). Loss of disc space at control levels averaged 2% over the 5-week experiment.

Mechanical: Increased lateral bending stiffness relative to within-animal controls was observed at all groups. Stiffness averaged 55, 204, 51, 91 x 10^{-3} Nmm/deg in Groups A,B,C,D respectively. The mean stiffness at distal control levels was 50 x 10^{-3} Nmm/deg. The minimum stiffness was recorded at an angle close to the in vivo value (mean angle less than 1 degree in all groups), indicating that angulated discs had adapted to the imposed deformity.

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 (mean crimp periods 28.3 and 29.6 ± 6.8 µm. 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, where the crimp period averaged 38.7 µm. This is consistent with the fact that the intervention level discs were fixed with the apparatus in place, preventing swelling of the discs.

DISCUSSION:

All interventions (permutations of angulation, compression and reduced mobility) produced substantial changes in the intervertebral discs of these growing animals over the five-week experiment. 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.

'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.

It is concluded that the five-week growth period of these rats produces substantial disc tissue changes, probably comparable with secondary, biomechanically induced changes occurring during the adolescent growth phase in patients with scoliosis. Since the disc dimensions and properties were altered at the intervention levels of all four Groups, the reduced mobility that was present in all groups appears to be the major cause of the observed changes. The application of the rings and springs in Group D cannot be considered as a sham intervention, probably because it produces reduced mobility of the disc.

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