

Mechanical Modulation of Intervertebral Disc Thickness in Growing Rat Tails

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Summary: Progression of scoliosis deformity during growth is thought to be caused by asymmetrical loading, resulting in asymmetrical growth with vertebral and disc wedging in a "vicious cycle." The purpose of this study was to quantify the changes in disc thickness during growth in rat tails subjected to compression or distraction loading for 6 or 9 weeks, to investigate the hypothesis that disc growth is mechanically modulated. Six-week-old Sprague-Dawley rats were studied with compression loading (13 animals) or distraction loading (15 animals) applied to their tails, and there were 8 sham animals. Loading was applied to tail segments by means of an external ring fixator. Radiographic measurements of disc thickness were made at biweekly intervals. From the initial to final radiograph, compressed discs had reduced thickness averaging (\pm SD) 0.50 ± 0.28 mm, distraction discs had average increased thickness of 0.20 ± 0.42 mm, and sham discs lost an average of 0.21 ± 0.18 mm of thickness (analysis of variance $p < 0.001$). There was an "initial change" in disc thickness averaging 0.18 ± 0.32 mm in nonloaded discs, which was similar in magnitude to the elastic deformation and was attributed to disc swelling under anesthesia. These results indicate that growth in disc thickness is mechanically modulated by axial loading in growing rats. **Key Words:** Intervertebral disc—Growth—Biomechanics—Scoliosis—Animal model.

Scoliosis deformities, independent of etiology, progress more during skeletal growth. It is thought that scoliosis produces asymmetrical loading on the spine, resulting in asymmetrical growth and vertebral and disc wedging in a "vicious cycle" (12,14,16). This mechanically modulated growth may be the cause of rapid progression of deformity during adolescent growth in both idiopathic (4,5,20) and neuromuscular scoliosis (1,10). Brace treatment is directed at controlling progression by minimizing the magnitude of asymmetrical loading of the spine.

If progression of spinal deformity is a result of mechanical modulation of growth, then it is important to know where and in which anatomical structure this occurs. The

scoliosis deformity involves wedging of both the discs and vertebrae (13,18,19). It previously has been shown that mechanical forces applied to the vertebrae of the rat tail modulate their growth; chronically applied compression inhibits the growth rate of vertebrae, whereas distraction enhances it (17). Furthermore, vertebral wedging resulted from asymmetrical compression of rat caudal vertebrae as a result of asymmetrical growth in the vertebral physes (7).

The contribution of the discs to the progression of scoliosis deformity is poorly understood. The influence of abnormal loads on intervertebral discs has been investigated in a number of animal studies. The majority evaluated the effects of loading on accelerated intervertebral disc degeneration rather than the influence of loading on the development of spinal deformity during growth (3).

This study was conducted to investigate the idea that disc wedging during growth in spinal deformities is mechanically modulated. The purpose of this study was

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to quantify the changes in disc thickness in growing rat caudal discs subjected to externally applied compression or distraction and to test the hypothesis that intervertebral disc thickness is mechanically modulated during growth.

METHODS

Loading apparatus was installed on the tail of 6-week-old Sprague-Dawley rats. General anesthesia was obtained with intraperitoneal ketamine 40–80 mg/kg and xylazine 5–10 mg/kg. A 1%-lidocaine block was administered at the base of the tail. Then, the external fixator was applied using two percutaneously inserted stainless steel pins (0.7-mm diameter) transecting each of two tail vertebrae (Fig. 1). There were initially 47 animals, but 11 were excluded because of technical problems (6 pinning errors, 2 glue failures, 1 vascular injury, 1 for health problems, and 1 for radiography problems), leaving 13 animals with compression loading, 15 animals with distraction loading, and 8 sham animals (three groups). The fixator was used to apply measured forces to the tail vertebrae (17). Calibrated springs on three threaded rods passing through the rings were compressed with nuts to apply compression (mean 54% body weight) or distraction forces (mean 45% body weight). The spring forces were adjusted weekly to maintain the total force applied as a proportion to the animals' body weight. In the sham group, the springs were omitted. The discs in each tail were divided into two groups—control discs (two caudal and two cephalad to the instrumentation) and loaded

discs (within the loading apparatus). The first instrumented vertebra was caudal 8, the second either caudal 10 (compression tails) or caudal 11 (distraction and sham tails). To maximize the number of discs studied per animal, there were three loaded discs for distraction and sham animals; for compression animals, only two loaded discs were used for reasons of stability (Fig. 2).

Radiographs were taken of each animal: (a) on the day of surgery immediately after anesthesia and again postoperatively after application of the fixator (with the animals still anesthetized), (b) at biweekly intervals for the duration of the experiment, and (c) at the end of the experiment before and after removal of the fixator. The rats were kept immobilized in a restraining tube for these subsequent radiographs without anesthesia. The duration of the experiment was 9 weeks for 28 animals and 6 weeks for 5 distraction and 2 compression animals.

On each radiograph, the disc-vertebra margins on each side of the discs were digitized using a flatbed digitizer (GTCO Corp., Rockville, MD, U.S.A.) interfaced to a personal computer to measure disc thickness. Three measures of thickness change were recorded for each disc (Fig. 3). First, the "initial change in disc thickness" was calculated as the difference in disc thickness measured from the preoperative (unloaded) and postoperative (loaded) radiographs. Second, the "unloaded change in disc thickness" was calculated based on the differences between the initial and final radiographs (both unloaded, without apparatus). Third, the "change in disc thickness under load" was calculated as the slope of the graph of disc thickness ver-

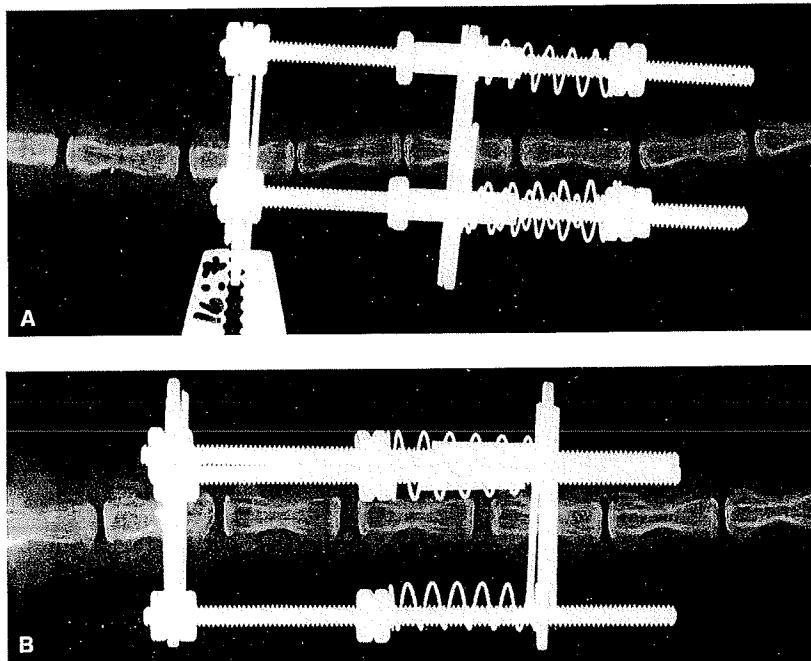


FIG. 1. Radiographs of rat tails, taken 9 weeks after application of the external fixator. **A:** An animal in the compression group. **B:** An animal in the distraction group.

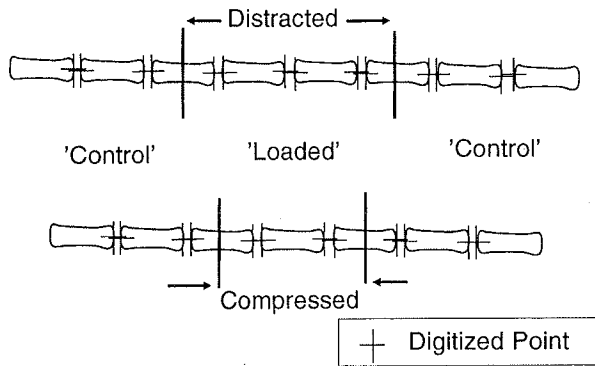


FIG. 2. A diagram showing the definition of "control" discs and "loaded" discs by reference to their position relative to the external fixator in compressed and distracted tails. Also shown are the points that were digitized for measurements of disc thickness.

time, multiplied by the duration of the experiment. All the measurements were in millimeters.

To test the hypothesis that intervertebral disc thickness growth is mechanically modulated, a two-way analysis of variance (ANOVA) was performed on each of the three disc thickness measurements. The two factors were *loading group* (three levels: sham, compression, distraction) and *disc level* (two levels: control discs, loaded discs). A repeated-measures analysis was used on the factor disc level, because these measurements came from the same animal. A significant interaction in this two-factor statistical design between the "loading group" and "disc level"

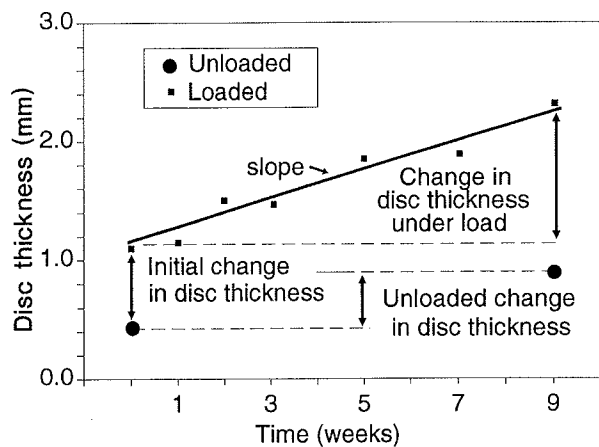


FIG. 3. Measurements of changes in disc thickness derived from a graph of disc thickness versus time (idealized graph for a distracted disc). The "initial change in disc thickness" was calculated as the difference in disc thickness measured from the pre- and postoperative radiographs. The "unloaded change in disc thickness" was calculated based on the differences between the initial and final radiographs (without apparatus). The "change in disc thickness under load" was calculated as the slope of the graph of disc thickness versus time, multiplied by the duration of the experiment. All these measurements were in units of millimeters.

factors indicated that growth of disc thickness is mechanically modulated. For post hoc comparisons, the Student-Newman-Keuls test was used. For all statistical analyses, $p < 0.05$ was considered significant.

RESULTS

Over the duration of the experiment, the compressed discs had a significant loss of thickness, and the distracted discs had a significant increase in thickness, compared with a loss of disc thickness in sham and control discs measured by "unloaded change of disc thickness" (Fig. 4). The average (\pm SD) change in thickness was -0.50 ± 0.28 mm for compression discs (i.e., reduced thickness), 0.20 ± 0.42 mm for distraction discs (i.e., increased thickness), and -0.21 ± 0.18 mm (i.e., reduced thickness) for sham discs (ANOVA $p < 0.0001$). No significant effect of loading group was detected for the "change in disc thickness under load" (ANOVA $p = 0.17$) (Table 1).

The "initial change in disc thickness" measurement (based on radiographs taken on the day of surgery before and after installation of the loading apparatus) was 0.17 ± 0.33 mm in nonloaded discs (Table 2). This initial increase in thickness was comparable in magnitude with the changes due to the imposed load in compressed and distracted discs (elastic effect). This widening of the nonloaded discs was attributed to disc swelling under anesthesia. Similar elastic effects were detected from the change in disc thickness in radiographs taken at the end of the experiment, before and after removal of the apparatus.

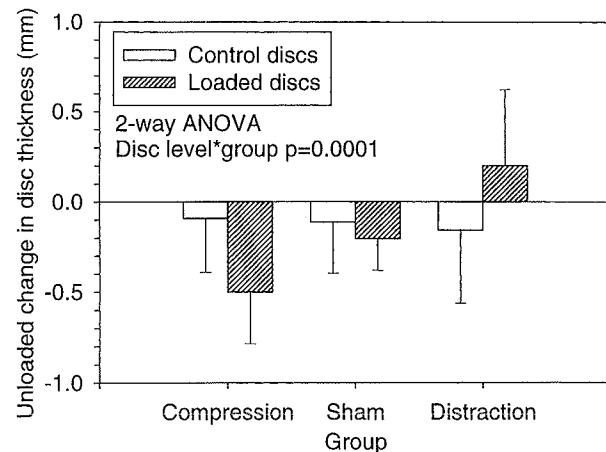


FIG. 4. Bar chart showing the breakdown of "unloaded change in disc thickness" by group (compression, sham, and distraction animals) and by disc level (loaded or control discs). Over the duration of the experiment, there was a loss of disc thickness in sham and control discs. In compressed discs, the loss was significantly greater, and in distracted discs there was a significant increase in thickness. ANOVA, analysis of variance.

TABLE 1. *Loaded change in disc thickness (means)*

Disc level/load type	Compressed tails	Distracted tails	Sham tails
Control discs	+0.14 ^a	-0.04 ^a	-0.02 ^a
Loaded discs	+0.01	+0.12	-0.08 ^a

No significant differences by two-way analysis of variance with repeated measures on disc level. Pooled SD = 0.39.

^aNonloaded discs.

DISCUSSION

This study was performed to test the hypothesis that disc thickness in growing rats is mechanically modulated. Using the measurements of the "unloaded change in disc thickness," there was a change in thickness over the duration of the experiment that depended on the loading state of the disc. These results were in agreement with our hypothesis that intervertebral disc thickness is mechanically modulated during growth. The fact that compression caused reduced thickness and distraction caused increased thickness is compatible with the idea that asymmetrical loading of both vertebrae and discs may contribute to scoliosis progression during growth.

The reason why the "change in disc thickness under load" did not exhibit the significant effect of loading on intervertebral disc thickness demonstrated by the "unloaded change in disc thickness" is not clear. The former (based on the slope of the disc thickness versus time graphs) were taken while the discs were loaded and were therefore subject to growth change as well as the elastic deformations resulting from the applied loads. These loads were not constant (they were increased in proportion to the animals' body mass), and the intervertebral disc stiffness may have changed during the course of the experiment. It was intended to have equal magnitude of load in the two loaded groups, but this was confounded by growth acting against the constraints of the external fixator. The growth tended to increase the compressive load over time while decreasing the distraction load over time, despite the weekly readjustments. The difference between the average distraction forces and the average compression forces is relatively small (54 versus 45% of body weight), so the effect on growth might be of a similar magnitude, which would not be detectable with the statistical power of this experiment.

The radiographic measurements of disc dimensions were technically difficult and initially confounded by the effects of anesthesia. The initial change in disc thickness of nonloaded discs was attributed to disc swelling under anesthesia. This effect of anesthesia on disc thickness has been reported previously (8).

It appears that the magnitude of the experimentally imposed loads (on the order of half body weight) was com-

TABLE 2. *Initial change in disc thickness (means)*

Disc level/load type	Compressed tails	Distracted tails	Sham tails
Control discs	0.16 ^a	0.14 ^a	0.25 ^a
Loaded discs	-0.02	0.65	0.26 ^a

Pooled SD = 0.37.

^aNonloaded discs.

parable with the *in vivo* loads, because the unloading with anesthesia produced a thickness change similar to that resulting from the experimentally imposed forces. In the bovine tail, Oshima et al. (11) found that the swelling pressure in caudal discs is similar to that in human lumbar discs, implying that the prevailing loads cause similar stresses. Little is known about how musculoskeletal connective tissue growth and development are influenced by mechanical environment. Intuitively, it appears unlikely that the level of physical activity, and hence magnitude of transient mechanical loading, influences growth of ligaments, tendons, etc., although apparently it does influence their tissue material properties and remodeling. However, the results of this study indicate that chronically applied forces modulate growth of intervertebral discs.

The tail pinning procedure was technically demanding, especially for animals in the compression group. Six animals with compression loading were eliminated from the study because they developed a buckled tail, apparently because of a loose or poorly placed pin, and some of these became infected. It appeared that the rigidity of the crossed-pin fixator design minimized infection problems, and that infections were secondary to loose pins and not vice versa. Among animals included in the study, there were some transient pin tract infections that were successfully treated topically, but none produced radiological evidence of either bone or disc infection, and we believe this did not interfere with the intervertebral disc growth.

Another potentially confounding factor in these experiments was that the external fixator reduced the motion of the affected discs. However, the disc thickness measurements, when compared between the instrumented levels of the sham animals and the "control" levels in the same animals, did not show any significant differences, indicating that the immobilization effects on disc thickness were not important. The measured reductions in disc thickness over time that occurred in all but the distracted discs was unexpected in these growing animals. It may be an artifact due to the advancing secondary ossification centers in the epiphyses, rather than a true narrowing of the intervertebral disc. Conversely, Hulse Neufeld (2) reported radiographic measurements showing that the intervertebral discs in the lumbar region of rats increased in thickness during growth.

In 9 weeks, the animals grew linearly ~25%, similar to adolescent growth of the human spine and thorax (6,9,15). Taylor (18) investigated growth of the human spine using radiographs to determine vertical and horizontal disc and vertebrae dimensions. The vertical increase in disc height was dependent on vertebral level, being greatest in the lumbar region (i.e., L4-L5 disc thickness ranging from roughly 4 mm at birth to ~10 mm at 14 years). Lumbar disc growth of nonambulating and non-weight-bearing patients with cerebral palsy was less than normal and less than that of ambulatory cerebral palsy adolescents, leading the author to conclude that vertical growth of intervertebral discs depends on the physical activity associated with weight bearing in the erect posture. Our results with this rat tail model are in agreement with Taylor's findings (18) in human spines, and provide further evidence that compression or distraction forces on the vertebral column can inhibit or enhance the growth of the disc.

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