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Compression-Induced Changes in Intervertebral Disc Properties in a Rat Tail Model

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Study Design. An Ilizarov-type apparatus was applied to the tails of rats to assess the influence of immobilization, chronically applied compression, and sham intervention on intervertebral discs of mature rats.

Objectives. To test the hypothesis that chronically applied compressive forces and immobilization cause changes in the biomechanical behavior and biochemical composition of rat tail intervertebral discs.

Summary of Background Data. Mechanical factors are associated with degenerative disc disease and low back pain, yet there have been few controlled studies in which the effects of compressive forces on the structure and function of the disc have been isolated.

Methods. The tails of 16 Sprague–Dawley rats were instrumented with an Ilizarov-type apparatus. Animals were separated into sham, immobilization, and compression groups based on the mechanical conditions imposed. *In vivo* biomechanical measurements of disc thickness, angular laxity, and axial and angular compliance were made at 14-day intervals during the course of the 56-day experiment, after which discs were harvested for measurement of water, proteoglycan, and collagen contents.

Results. Application of pins and rings alone (sham group) resulted in relatively small changes of *in vivo* biomechanical behavior. Immobilization resulted in decreased disc thickness, axial compliance, and angular laxity. Chronically applied compression had effects similar to those of immobilization alone but induced those changes earlier and in larger magnitudes. Application of external compressive forces also caused an increase in proteoglycan content of the intervertebral discs.

Conclusions. The well-controlled loading environment applied to the discs in this model provides a means of isolating the influence of joint-loading conditions on the response of the intervertebral disc. Results indicate that chronically applied compressive forces, in the absence of any disease process, caused changes in mechanical properties and composition of tail discs. These changes have similarities and differences in comparison with human spinal disc degeneration. [Key words: animal model, compression, immobilization, intervertebral disc degeneration, mechanical modulation] **Spine 1999;24:996–1002**

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Mechanical factors are often associated with disc degeneration and low back pain. The development of lumbar disc rupture is reported to be associated with activities that involve frequent bending and twisting and heavy physical work.[9] People who are physically fit have a decreased incidence of low back pain, whereas a sedentary lifestyle is associated with an increased risk for low back pain.[11,29] Results in epidemiologic studies have also shown that exposure to vibration increases risk of having low back pain.[11,36]

Excessive or abnormal compressive forces on human intervertebral discs are associated with accelerated degeneration and altered composition. The accelerated degeneration that occurs in discs adjacent to a spinal fusion is attributed to the altered mechanics in the spinal column caused by that fusion.[10] Decreased proteoglycan content in discs adjacent to the fusion was reported in an animal model using chondrodystrophoid dogs.[7] Altered mechanics in motion segments adjacent to a fusion include increased intradiscal pressure, mechanical stresses, intervertebral motion, and facet loads. All these changes are influenced by the length, location, and stiffness of the fusion.[13,16,26,30,37]

Increased intradiscal pressure may be partly attributed to increased compressive joint forces. The collagen content and ratio of Type I to Type II collagen in the anulus is affected by scoliosis, in which the total collagen concentration and ratio of Type I to Type II collagen are highest on the concave side of the curve.[4] The tissue remodeling and associated cellular activity in the anulus fibrosus were partly attributed to alterations in compression loading as an expression of Wolff's law. The changes seen in discs adjacent to a fusion and in scoliosis in humans result from a disease process and from altered mechanics. Significant insights can be gained by decoupling the effects of the disease process and the joint mechanics using animal models.

Most animal models of disc degeneration are naturally occurring or involve surgical intervention. However, several models of degeneration have been developed in which the animals' spines or tails are subjected to an extreme mechanical environment.[25] These mechanical interventions on the spine, which include forced bipedalism and bending of tails, cause morphologic changes in the disc and vertebrae similar to degenerative disc disease in humans.[6,12,14,19,25,27,31,38] Long distance running in dogs causes reductions in the proteoglycan content of cervical and thoracic intervertebral discs but

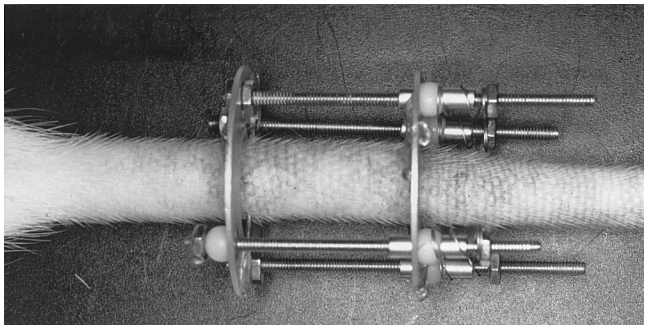

Figure 1. Rat tail instrumented with Ilizarov-type apparatus configured for axial compression loading of the tail. The apparatus consists of aluminum rings, stainless steel threaded rods, calibrated springs, and locking nuts. At 14-day intervals, the springs were unloaded and the apparatus modified with ball joints to allow for *in vivo* compression and bending tests.

increases proteoglycan content in lumbar discs.[33] The differences depending on spinal region were attributed to different biomechanical demands, showing that the characteristics of mechanical loading (type of forces and frequency and duration of loading) may influence disc composition. Changes in disc composition and morphology are expected to impact the mechanical properties of the disc.

Findings in these studies have shown that accelerated degeneration of the intervertebral disc results from altered mechanical loading conditions, but the type and magnitude of the loading that causes this acceleration remain unknown. In the current study, the hypothesis that chronically applied compressive forces cause changes in mechanical properties and composition of rat tail intervertebral discs was tested. Specifically, it was hypothesized that discs loaded chronically with compression, superimposed on the normal *in vivo* loading state exhibit decreased disc thickness, increased axial and angular stiffness, and decreased proteoglycan content.

Methods

Rat tail intervertebral discs were immobilized and loaded in compression using an Ilizarov-type apparatus. The effect of these loading conditions on caudal disc properties was assessed with *in vivo* biomechanical tests and *in vitro* biochemical analyses. The apparatus was applied to the 8th and 10th caudal vertebrae of 16 mature (4–5-month-old retired breeders) female Sprague–Dawley rats (Figure 1). The experimental procedure provided two instrumented discs and two control discs per animal.

The loading apparatus and surgical procedure used to install it were similar to that described by Stokes et al.[35] Animals were anesthetized with intraperitoneal injections of 10 mg/kg xylazine and 50 mg/kg ketamine, and anesthesia was maintained with supplementary injections of ketamine as needed throughout the procedure. The middle of the 8th and 10th caudal vertebrae were located by palpation and tagged with metallic markers, and preoperative radiographs were obtained to confirm the location of the tags. A variable-speed drill was used to insert two crossed 0.7-mm stainless steel Kirschner wires percutaneously through the tagged locations on each of the two vertebrae. The Kirschner wires were then glued (Loctite type 447 cyanoacrylate and Locquic accelerator; Loctite, Newington, CT) to pretreated (sandblasted and surface-primed) 35-mm diameter aluminum rings. Four stainless steel threaded rods were passed longitudinally through four holes distributed around the rings creating an Ilizarov-type apparatus. Ball joints, created using delrin balls (3/16 in. diameter; Small Parts, Inc., Miami Lakes, FL), linked the rods and rings and allowed application of compressive loads or bending moments to the vertebrae and two discs within the apparatus. Loads were applied using calibrated springs (stiffness, 0.30 N/mm) installed over each threaded rod and tightened against the distal ring.

Rats were separated into sham, immobilization, or compression groups, depending on the chronic loading state of the tail (Table 1). The animals in the sham group (n = 4) had tails instrumented with rings only. The immobilization group (n = 4) was instrumented with rings and rods but without springs, reducing motion of the two discs between the rings. The compression group (n = 8) was instrumented with rings, rods, and springs that served to immobilize and apply axial compressive forces to the two discs between the rings. The compressive forces were set and maintained at one of three load levels expressed as a percentage of the animal's body weight: 33% (n = 2), 67% (n = 2), or 100% (n = 4). *In vivo* biomechanical tests were performed on each animal at 14-day intervals to determine the disc thickness, angular laxity, and axial and angular compliance of the discs. To perform these tests, rats were anesthetized, and their chronic loads removed by loosening the springs. The calibrated springs were adjusted to set lengths using a micrometer and wrench to apply a range of compressive and bending test loads to the tail. Four compressive test loads (0%, 33%, 67%, and 100% body weight [BW]) and three test bending moments (13%, 25%, and 38% BW-cm) were applied, where 100% BW corresponds to 4 N, on average. Radiographs were obtained at each test configuration (Figure 2A) and labeled with a numeric code so that future measurements were made without knowledge of the loading group. Successive tests were performed at roughly 3-minute intervals to reduce viscoelastic effects.

After the tests were completed, chronic loading was reapplied to the tail. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Vermont. At 56 days, animals were killed by CO_2 asphyxiation and thoracotomy, and the tails were harvested (Figure 3) and frozen at −20 C until use for *in vitro* biochemical analysis.

Table 1. Summary of Study Design

Group	Loading Conditions	No. of Animals
Sham	Instrumented with rings only	4
Immobilization	Instrumented with rings and rods to reduce motion of the two instrumented discs	4
Compression	Instrumented with rings, rods, and calibrated springs to immobilize and apply axial compressive forces to the two instrumented discs; spring forces were set and maintained to apply 33%, 67%, or 100% of the animal's body weight	8

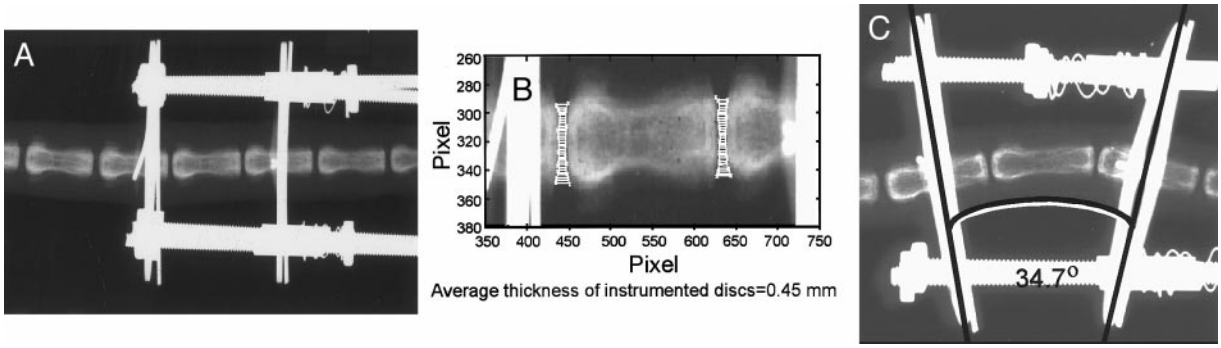

Figure 2. Radiographs of instrumented rat tails used for *in vivo* flexibility and thickness measurements. **A,** Tail subjected to axial compression test load of 100% body weight (BW). **B,** Example of disc space measurement in the same test. Average disc thickness of the two instrumented discs at 100% BW in this sample was 0.45 mm. **C,** Instrumented tail subjected to bending moment test load of 38% BW-cm, corresponding to approximately 15 N-mm.

Radiographs were scanned using a flatbed scanner with backlighting (Scanmaker III; Microtek Lab, Redondo Beach, CA) and passed to a computer on which images were magnified and digitized using commercial software (MATLAB; The Mathworks, Inc., Natick, MA). Disc thickness (*i.e.,* disc space) and relative ring angulation were marked and measured (Figure 2, B and C) for the different loading conditions. Measurement uncertainty for disc thickness and ring angle were calculated based on bias and precision error calculations.[1] The bias error was calculated using the x-ray measurement technique to make 12 linear and 12 angular measurements of calibration standards. The precision error was calculated using radiographs of an animal whose apparatus had been dismantled and reassembled four times at a given axial force and four times at a given bending moment. Based on this analysis technique, the total measurement uncertainty was ± 0.06 mm for disc thickness and $\pm 0.3°$ for ring angle measurements.

For axial tests, loaded vertebral endplates were marked, and the average thickness of the two instrumented discs (*i.e.,* discs between the rings; Figure 3) was calculated. Axial compliance and disc thickness at zero load were calculated from the slope and *y* intercept of the thickness and load curves, respectively (Figure 4A). The compliance is a measure of the slope of the flexibility curve, and in a linear system, is the reciprocal of the stiffness. Compliance was calculated for axial compression and bending tests. In bending tests the angle between proximal and distal rings was used as a measure of disc angulation. The slope and *y* intercept of the angle and torque curves were used to obtain angular compliance and angular laxity of the two-disc tail segments, respectively (Figure 4B). Angular laxity was considered to be the angle that the tail segments bent because of a negligible (0 N-mm) bending moment and is similar to the concept of a neutral zone.[28]

To collect discs for biochemical analyses, the tails were thawed, soft tissues were removed with the aid of a dissecting microscope, and three intervertebral discs were dissected using a scalpel. The discs were grouped by level according to their locations in relation to the rings as instrumented (caudal disc 9–10, located inside the rings), adjacent (caudal disc 10–11, located just outside the rings), and control (caudal disc 11–12; Figure 3). Disc sections were refrozen to minimize loss of hydration or the gelatinous nucleus tissue during subsequent specimen handling.

In 10 of the 16 animals, the frozen tail discs were separated into nucleus pulposus and anulus fibrosus sections using biopsy punches (Miltex Instrument, Lake Success, NY). One punch was used to isolate the outer anulus from any remaining tendinous tissue (6 mm diameter), and a second smaller punch (3 mm diameter) was used to separate the nucleus from the inner anulus. The tissue sections, whether whole disc or separated by anulus and nucleus region, were immediately placed in preweighed vials, and their wet tissue weight was obtained. Vials were labeled using a numeric code so that analyses were performed without knowledge of the loading history of the specimens. Specimens were then lyophilized at −60 C for 48 hours to obtain a dry tissue weight. The dried tissue was digested with proteinase K, and aliquots analyzed for sulfated glycosaminoglycan and hydroxyproline content. Hydroxyproline, a measure of total collagen content, was obtained using a colorimetric method,[5] and glycosaminoglycan, a measure of proteoglycan content, was obtained using the dimethylmethylene blue dye binding assay.[9] Glycosaminoglycan and hydroxyproline measurements were normalized by dry tissue weight. Compositional measurements were reported as values for the whole disc, which required calculating the weighted average for the 10 discs, which were separated into nucleus and anulus sections (based on dry tissue weight of the anulus or nucleus section as a percentage of total tissue dry weight).

Two-way analysis of variance (ANOVA) was used to test for significant effects of group (sham, immobilization, compression) and time (0, 14, 28, 42, 56 days) on the *in vivo* biomechanical test variables (disc thickness under no load, axial compliance, angular laxity, angular compliance). Two-way

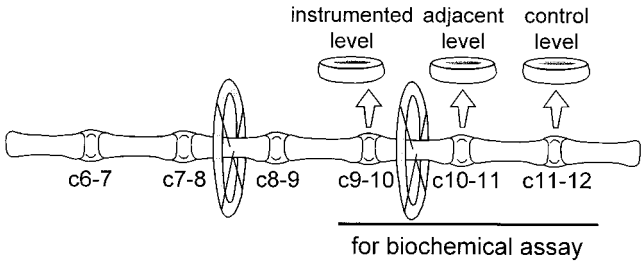

Figure 3. Schematic of Ilizarov-type apparatus applied to the rat tail and location of discs harvested for biochemical tests. The discs were grouped according to their locations in relation to the rings: instrumented (caudal disc 9–10, located inside the rings), adjacent (caudal disc 10–11, located just outside the rings), and control (caudal disc 11–12).

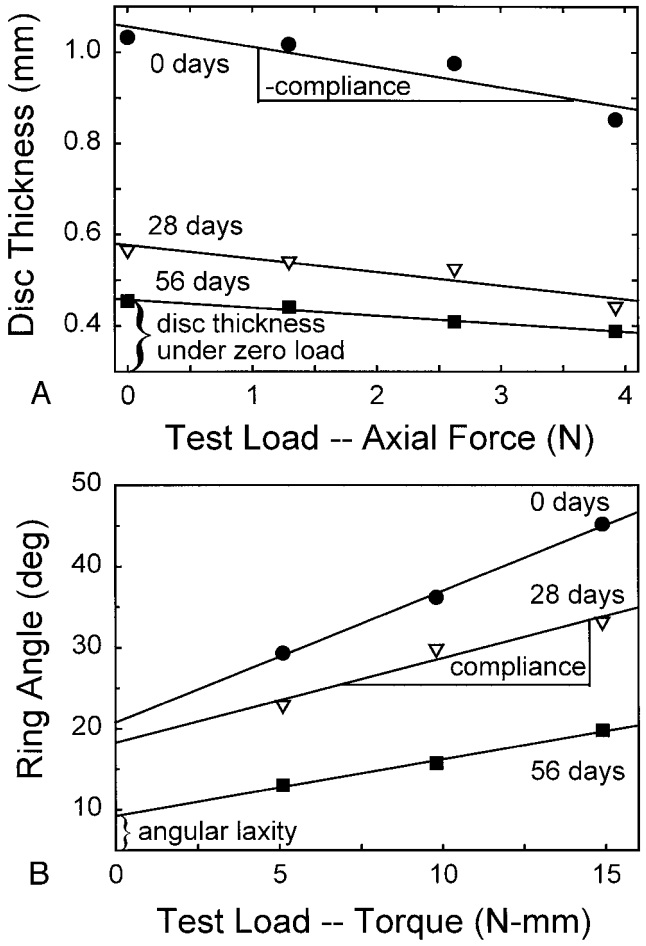

Figure 4. *In vivo* biomechanical measurement results exhibiting structural biomechanical parameters: disc thickness under zero load, axial compliance, angular laxity, and angular compliance. **A,** Disc thickness *versus* axial force curve in rat 12 (compression at 100% body weight [BW] applied chronically) at different times during the experiment. Axial compliance was calculated from the slope of the thickness–force curve. Disc thickness and axial compliance decreased with time of experiment. **B,** Ring angle *versus* torque curve for rat 12 at different times during the experiment. Angular laxity decreased in this specimen without a change in slope (*i.e.,* no change in angular compliance) of the angle–torque curve.

ANOVA was used to test for effects of group and disc level (control, adjacent, loaded; Figure 3) on the composition variables (water content, glycosaminoglycan, hydroxyproline). One-way ANOVA was also used to test for the effect of region (anulus fibrosus, nucleus pulposus) on the control levels of those samples that were separated into nucleus and anulus samples. For all ANOVAs, $P < 0.05$ was considered significant. When significant effects were detected, *post hoc* comparisons were made using a significance level adjusted according to the Bonferroni criterion for multiple comparisons. Values are expressed as mean ± standard deviation.

■ Results

All 16 animals successfully completed the 8-week study protocol. One animal had a pin-track infection that was treated topically with antiseptic cream (Panalog; Squibb and Sons, Princeton, NJ) and resolved. The average animal weight was 412 g at the beginning of the study, 397 g after 3 weeks, and 412 g again at the end of the protocol, indicating that animals returned to original weight after an initial adjustment period. The apparatus weighed roughly 12 g and was apparently tolerated well by the animals, evidenced by their ability to lift and easily move their tails with the apparatus attached. The animals were also able to move and control their tails both proximal and distal to the apparatus, although the segments within the apparatus were largely immobilized.

Axial compression tests indicated disc thickness was generally linearly related to axial force (Figure 4A; typical load–thickness curve for rat 12). The correlation between ring angle and test torque also appeared linear (Figure 4B; typical torque–angle curve for rat 12). Intervertebral disc thickness with no load averaged 0.93 ± 0.14 mm in all specimens at 0 days (Figure 5A). Subsequently, there was a significant decrease with time in disc thickness, which was detected at 14 days in the compression group, and at 42 days in the immobilization group, but not in the sham group (based on the significant

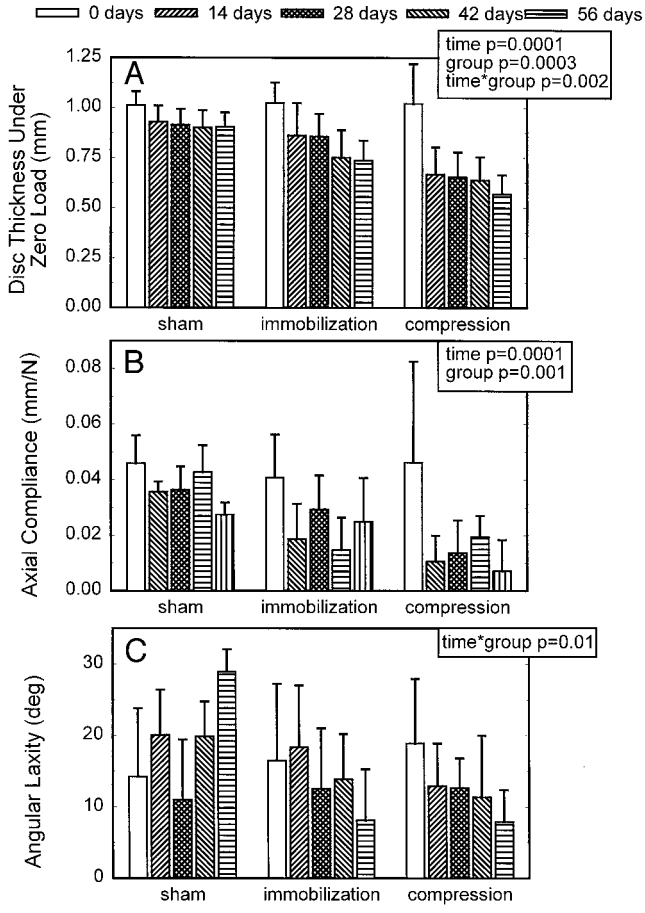

Figure 5. Mean ± standard deviation for (**A**) disc thickness, (**B**) axial compliance, and (**C**) angular laxity by group and time of experiment. The compression and immobilization groups exhibited similar changes in mechanical and structural properties, with compression accelerating the rate and increasing the magnitude of those changes. Small changes in these parameters were detected in the sham group.

group-time interaction and multiple comparison tests). The largest decrease in disc thickness was observed in the compression group, but at 56 days this value was not significantly different from the disc thickness of the immobilization group at 56 days.

Axial compliance for instrumented levels averaged 0.045 ± 0.015 mm/N in all samples at 0 days (Figure 5B). A significant decrease in axial compliance in the compression group was detected at 14 days and thereafter, but no significant effects were detected in the immobilization or sham groups. For *in vivo* bending tests, angular laxity of the instrumented sections initially averaged 18.1° ± 10.1° in all animals (Figure 5C). The sham group showed an increased angular laxity with time that was significantly different from the values in the compression and immobilization groups at 56 days. Angular laxity decreased with time in both the compression and immobilization groups. Angular compliance had an average of 1.33 ± 0.50°/N-mm in all animals at 0 days with no significant group or time effects detected for this variable.

Biochemical results indicated there were differences in glycosaminoglycan content of the intervertebral discs by disc level and by group (Figure 6). Glycosaminoglycan content in the instrumented level was significantly greater than in the adjacent and control levels in all three groups. There were no significant effects of disc level on water or hydroxyproline contents. The quantities of glycosaminoglycan and hydroxyproline in the discs in the compression group were significantly greater than in the discs in the sham and immobilization groups. Significant effects of disc regions (anulus *vs.* nucleus) were detected in all compositional measures. The water and glycosaminoglycan contents were highest in the nucleus, but the hydroxyproline was highest in the anulus. Water content, glycosaminoglycan, and hydroxyproline in control discs averaged 0.60 ± 0.08 g and 0.77 ± 0.08 g H_2O per gram wet tissue, 27 ± 12 mg and 152 ± 140 mg glycosaminoglycan per milligram dry tissue, and 67 ± 26 mg and 57 ± 22 mg hydroxyproline per milligram dry tissue in the anulus fibrosus and nucleus pulposus regions, respectively.

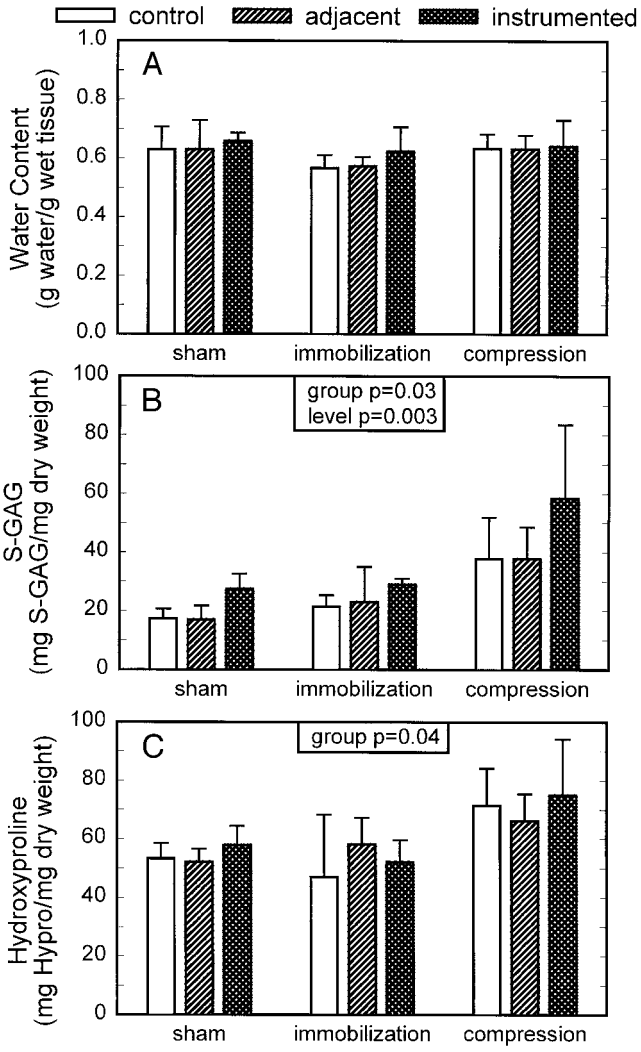

Figure 6. Mean ± standard deviation for (**A**) water content, (**B**) glycosaminoglycan per dry tissue weight, and (**C**) hydroxyproline per dry tissue weight by group and disc level. A significant increase in glycosaminoglycan with disc level shows that in all groups, instrumentation of the tail resulted in an increase in proteoglycan content in the instrumented disc.

■ Discussion

In the current study, the hypothesis that mechanical compression and immobilization cause changes in mechanical properties and composition of rat tail discs was examined. Specifically, the hypothesis was that discs loaded chronically in compression have decreased disc thickness, increased axial and angular stiffness, and decreased proteoglycan content. The results indicate that chronically applied compressive joint forces in the absence of a disease process can cause changes in mechanical properties and composition of tail discs.

Application of pins and rings alone (sham group) resulted in minimal changes in biomechanical behavior *in vivo*. Immobilization resulted in decreased disc thickness, axial compliance, and angular laxity. Chronically applied compression caused changes similar to those seen with immobilization alone, although axial compression induced those changes more quickly and with greater magnitude. Biochemically, an overall increase in proteoglycan content of the loaded intervertebral discs was observed. Although the increased compressive stiffness and disc thickness of the compressively loaded discs in this model indicate some similarities to the process of human disc degeneration, the decrease in angular laxity and increase in proteoglycan content are in contrast. This model does not simulate the loading conditions in human discs; however, the well-controlled loading environment on the discs provides a means of isolating the influence of specific joint-loading conditions on the response of the intervertebral disc.

Some limitations of this study are noteworthy. Chronically applied compressive loads of 33–100% BW were pooled into one group of "compression" animals, which

may mask more subtle differences in output variables with force magnitude. Because additional compressive stresses were all considered in a low range of physiologic stresses, pooling of these animals was not considered a problem. Specifically, the application of 100% BW of external compressive forces resulted in axial compressive stresses on the loaded discs of approximately 0.15 MPa above physiologic loading. The limited number of animals used in this study resulted in a power of approximately 50% for all factors (group, time, and disc level). The relatively low power did not affect our conclusions when the null hypothesis was rejected (*i.e.,* when $P < 0.05$) but prevented definitive conclusions when significant differences were not obtained (*i.e.,* when $P > 0.05$, there is a risk of having a Type II error or of accepting a false null hypothesis).

Results in previous animal studies showed changes in disc structure and composition under complex loading conditions. Forcing rats to walk bipedally resulted in increased kyphosis, osteophyte formation, and disc herniation.[6,12,14,19,38] Increasing the lordosis on rat spines, by forced ambulation along the inside surface of a small diameter cylinder, caused discs to narrow initially then widen toward normal size.[31] Lindblom[27] bent rat tails into a U shape and reported structural changes to the anulus on the compression but not the tension side of the anulus. The results of these studies all indicate that structural change in the disc results from mechanical loading, yet the complicated experimental designs of the studies leave unclear the mechanism or specific force components responsible. Mechanically, complex loading conditions in these experiments may be considered to be comprised of a combination of compression and shear forces and bending moments. The current results provide evidence that immobilization alone and static compressive forces not only reduce disc thickness, but also affect axial stiffness, angular laxity, and disc composition.

The rat tail model with Ilizarov-type apparatus effectively applied controlled loading to the tail intervertebral discs *in vivo* with little interference in the animal's routine activities. Immobilization and compression are expected to result in different physiologic responses, yet these effects could not be uncoupled with the apparatus used in the study. The use of sham, immobilization, and compression groups was intended to provide insight into these different loading conditions. A decrease in disc thickness and axial compliance (although not significant) was observed in the sham group, yet it was small compared with that in the immobilization and compression groups. The angular laxity of tails of the sham group increased over time, whereas angular laxity of immobilization and compression groups decreased. The increase in angular laxity of the sham group may be related to mechanics altered by the presence of an apparatus on the tail or may be an effect of pinning the vertebrae. Of note, immobilization resulted in decreased disc thickness, axial compliance, and angular laxity. Chronically applied compression caused changes similar to those caused by immobilization alone, although axial compression induced larger changes that appeared more quickly.

The most significant biochemical finding was the increase in glycosaminoglycan content in discs at the instrumented levels compared with content of those at the control levels. Because the adjacent level did not have an associated increase in glycosaminoglycan content relative to the control, we concluded that the increase in glycosaminoglycan of the instrumented level was related to the mechanical environment of the instrumented discs and not to the instrumentation procedure or disc level along the tail. This result was not anticipated in light of studies on the spine and articular cartilage, in which results showed decreased proteoglycan synthesis or content with increased static compression,[20] hydrostatic pressure,[17] or immobilization.[24,34]

However, results in studies *in vitro* using human and bovine tail discs indicate that the response to load is not monotonic.[18,22,32] Although application of relatively low loads caused an initial increase in synthesis rates, increased loading caused a decrease in the biosynthetic response. This bimodal response was also found for articular cartilage and was partly attributed to decreased interstitial pH or transient changes in the mechanical environment of the cells with compressive deformations.[3,15]

Results in animal modeling studies also indicate that compressive force magnitude is an important determinant of biosynthetic response of the intervertebral disc. The current results indicate that relatively small compressive stresses (up to approximately 0.15 MPa above physiologic) produced an overall increase in proteoglycan content. Experiments using animal models in which larger compressive forces were applied to the intervertebral discs resulted in altered composition and cellular apoptosis. Hutton et al[20] used springs to apply high compressive forces to the lumbar spines of mature dogs for up to 27 weeks and found a decrease in total proteoglycans and chondroitin sulfate in experimental discs, particularly in the nucleus. Colliou et al[8] used an external apparatus to apply compressive forces to the tail of a mouse. They found that high external forces increased the number of apoptotic cells, but the compressive stresses on the discs were in the range of 0.4–1.3 MPa, up to 10 times greater than those applied in this study.

Taken together with the previous findings, the current results support the concept that, in intervertebral disc tissues, there appears to be a threshold at which low forces stimulate glycosaminoglycan synthesis, its accumulation (and in general the anabolism), whereas larger forces reduce the amount of glycosaminoglycan. Further work is necessary to isolate those physical signals on the cells that cause this biosynthetic response. It should also be noted that the presence of notochordal cells in the nucleus of the rat, mouse, and other animals may enable those intervertebral discs to respond to their mechanical environment in a different manner, or at different stress magnitudes than human discs do.[2]

The biomechanical results were similar to human disc degeneration in some respects and different in others. The average axial compliance at 0 days was 0.045 mm/N and corresponded to a compressive modulus for the whole disc of 0.73 MPa (based on assumptions of linearity, disc diameter of 6 mm, and average disc thickness of 0.93 mm). This modulus falls within the range of values reported in human anulus fibrosus and pig lumbar spine.[21,23] The decreased axial compliance was similar in magnitude to the twofold increase in compressive modulus reported for human anulus fibrosus with degeneration.[21] The decreased angular laxity in this study is in contrast to the slight increase in joint laxity reported in human motion segments with increasing degenerative grade (laxity in this article is similar to the concept of neutral zone in Mimura et al[28]).

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