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BIOMECHANICS

Osmotic Pressure Alters Time-dependent Recovery Behavior of the Intervertebral Disc

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Study Design. Disc recovery behavior under hypo- and hyperosmotic pressure.

Objective. To evaluate the effect of osmotic pressure on the unloaded recovery response of healthy discs.

Summary of Background Data. The intervertebral disc is a poroviscoelastic material that experiences large fluctuations in water composition throughout a diurnal loading cycle. Fluid flow out of the disc occurs through mechanical loading, whereas fluid flow into the disc occurs through passive diffusion because of an imbalance of ions between the disc and its surrounding environment. Osmotic pressure has been used to alter water uptake and tissue hydration.

Methods. Motion segments were prepared from the caudal spine sections of the skeletally mature bovines. A 300-N compressive load was applied for 2 hours before unloaded recovery for 12 hours. Hypo- and hyperosmotic pressure was used to alter the rate of water uptake and disc height recovery during unloaded recovery. A 5-parameter rheological model was used to describe the disc's time-dependent recovery behavior.

Results. The elastic response was not altered by changes in osmotic pressure; however, viscoelastic recovery was highly dependent on saline osmolarity and recovery time. The fast response of viscoelastic recovery was not dependent on osmotic pressure. The time constant for the slow response decreased whereas the slow response stiffness increased as osmotic pressure increased.

Conclusion. The fast response of viscoelastic recovery is governed by flow-independent recovery, whereas the slow response is related to flow-dependent recovery. The rate and magnitude of flow-dependent recovery are highly sensitive to changes in osmotic pressure of the saline bath. There is an osmotic pressure that reduces disc recovery behavior to an elastic response or flow-independent recovery.

Key words: disc biomechanics, disc recovery, flow-dependent recovery, healthy disc, intervertebral disc, nondegenerate disc, osmotic loading, osmotic pressure, poroelasticity, recovery mechanics, rheological models, time-dependent recovery, viscoelasticity.

Level of Evidence: N/A

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The intervertebral disc is subject to a wide range of compressive loads that cause fluctuations in mechanics, water content, and disc height.[1,2] The water contents of the nucleus pulposus and annulus fibrosus are altered by proteoglycan composition and osmotic pressure.[3–6] A decrease in proteoglycan content with injury or degeneration decreases the tissue's swelling capacity, altering fluid flow behavior into and out of the disc.[7–9] Fluid flow out of the disc occurs through mechanical loading, whereas fluid flow into the disc occurs through passive diffusion because of an imbalance of ions between the disc and its surrounding environment.[10–13] However, the role of osmotic pressure in fluid flow into the disc has not been well studied and is important for understanding disc recovery mechanisms.

The intervertebral disc is a poroviscoelastic material that experiences large fluctuations in water composition throughout a diurnal loading cycle. Mechanical loading on the disc results in an initial elastic response, followed by a time-dependent response. The viscoelastic response is influenced by intrinsic and extrinsic factors. Intrinsic factors include disc composition and geometry, whereas extrinsic factors include load magnitude and duration.[11,14–16] For example, the porosity of the solid matrix contributes to the time-dependent response because of fluid moving through pores with loading, providing the disc with its excellent resistance to compression.[17] Rheological models have been used to understand the role of these factors on the disc's time-dependent behavior, by monitoring disc height changes during creep, or unloaded recovery.[13,18] These models have been valuable in demonstrating significant differences in material properties with loading response, recovery behavior, injury, and degeneration.[11,19–22]

A diurnal loading cycle includes large compressive loads that are sustained throughout the day and reduced during bed-rest recovery. Previous studies demonstrated that the rate of disc height recovery during unloading is 3 to 4 times slower than the rate of disc height loss during loading, suggesting that passive diffusion of water molecules is not sufficient for full recovery within 8 hours. Incomplete disc height recovery also results in additional disc height loss during subsequent loading cycles.[23,24] More recent work has focused on the strong relationship between disc hydration and mechanical properties by using osmotic pressure to alter water absorption and intradiscal pressure during loading.[3,23,25–27] Stokes and coworkers observed a decrease in joint stiffness with an increase in water uptake during stress-relaxation (constant applied displacement).[27] Vergroesen *et al*[24] demonstrated that the effect of osmotic pressure on water absorption was highly dependent on magnitude of the applied load. However, it is not clear how osmotic pressure affects disc recovery behavior under low loading conditions that simulate bed-rest conditions.[2]

The intervertebral disc needs to maintain adequate hydration to absorb and transmit loads to surrounding tissues.[28,29] Our previous work used osmotic pressure to evaluate the effect of disc hydration on mechanical behavior during loading. We observed that hyperosmotic loading altered the time-dependent behavior by increasing the apparent compression stiffness.[3] Although previous studies have investigated the effect of osmotic swelling on water uptake during loading, it is still unclear how osmotic swelling, decoupled from mechanical loading, alters recovery response, and nutrient transport. Therefore, the objective of this study was to evaluate the effect of osmotic pressure on the unloaded recovery response of healthy discs.

MATERIALS AND METHODS

Specimen Preparation

Five caudal spine sections from skeletally mature bovines were acquired from the local abattoir (age ~18 months), and were selected based on their similarities to young and healthy human discs in disc height, matrix composition, and mechanical properties.[30–33] As mechanical and biochemical properties of the disc vary through the length of the spine, only the first three levels of the bovine caudal spine were used in this study.[31] Motion segments (n = 15) were prepared from the top three levels by removing the surrounding soft tissues and cutting through the mid-transverse plane of the superior and inferior vertebral bodies with a band saw. The inferior and superior vertebrae were embedded in polymetheylmethacrylate (PMMA) dental cement to ensure parallel loading surfaces and parallel alignment of the disc's mid-transverse plane with the loading platens. Potted motion segments were wrapped with saline-soaked gauze (0.15 M phosphate buffered saline, PBS) and frozen until testing.

Experiments were performed in a saline bath to provide continuous tissue hydration during testing. PBS solutions were prepared at a concentration of 3.0 M, and then diluted with de-ionized water to make 1.5, 0.75, 0.15, and 0.015 M PBS solutions (0.15 M PBS: 137 mM NaCl, 2.7 mM KCl, 5.4 mM Na_2HPO_4, and 0.6 mM KH_2PO_4). In addition, 1 N HCl or 2 N NaOH was used to adjust the solution pH to 7.4. The osmotic pressure of the saline solutions was calculated by the Van't Hoff equation, $\pi = MRT$, where M is the solution molarity (mol/L), R is the gas constant (8.3 J/mol-K), and T is the temperature (298 K).

Mechanical Testing

Samples were thawed in a refrigerated PBS bath for 24 hours to allow discs to reach steady-state hydration, and then allowed to equilibrate to room temperature before testing (Figure 1A). Motion segments were attached to custom grips in a water bath designed for an 858 Bionix hydraulic material testing machine (MTS Systems Corp., Eden Prairie, MN). A nominal preload (20 N) was applied and held for 10 minutes to ensure that the loading platen was engaged with the sample. Then, a 300 N axial compressive load (~0.5 MPa compressive stress)[32] was applied and maintained for 2 hours (Figure 1B). At the end of creep, the PBS solution was replaced with fresh PBS using a water pump

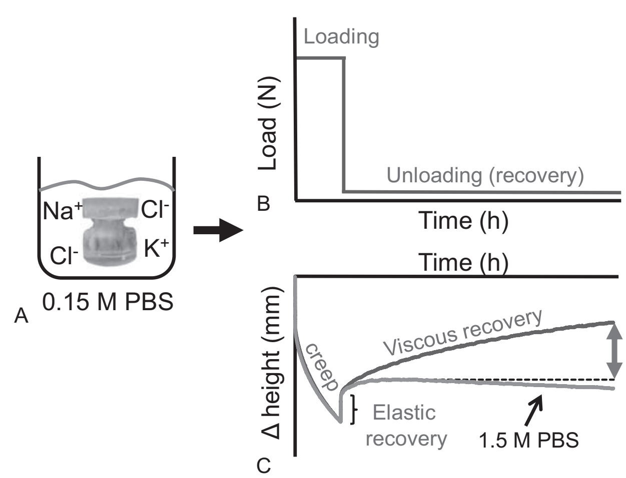

Figure 1. A, Samples were hydrated in a saline bath (0.15 M PBS) before testing. **B**, Samples were tested under axial compression for 2 hours, followed by unloaded recovery for 12 hours. **C**, Schematic of changes in disc height during testing. A reference disc height (dashed line) was defined as the maximum disc height recovered under hyperosmotic pressure (1.5 M PBS) and was used as a reference displacement. The difference between the reference displacement and final displacement after recovery was measured for the other experimental groups (blue line) (*i.e.*, 0.015 M, 0.15 M, and 0.75 M PBS). This difference in disc height recovery was used to estimate the osmotic condition that would result in elastic-only recovery behavior, and this osmotic condition was referred to as the balance concentration.

and samples were allowed to recover for 12 hours at 20 N. All samples were tested in 0.15 M PBS during creep; however, the PBS concentration was varied during recovery (0.015, 0.15, 0.75, or 1.5 M) (Figure 1C), with the 0.15 M PBS serving as the control. Force and displacement were recorded throughout the test.

Each sample was tested five times to perform a paired statistical analysis, and the testing order was randomized with a 24-hour recovery in 0.15 M PBS between tests. Upon completion of all saline recovery groups, the first test was repeated to confirm repeatability and to ensure that the disc was not damaged or altered during testing. A paired Student *t* test confirmed that there were no significant differences between the first and last tests ($P > 0.2$). After mechanical testing, discs were removed from the surrounding vertebrae and visually examined to confirm that they did not have any damage or degeneration.

Data Analysis

Experimental data were analyzed using a custom-written Matlab algorithm (Mathworks, Inc., Natick, MA). The time-dependent recovery response was curve-fit to a 5-parameter rheological model that included two Voigt solids with a spring in series (lsqcurvefit function, Equation 1). Each Voigt solid consists of a spring (S_i, N/mm) and a dashpot (η_i, N-s/mm) in parallel, and describes the slow and fast time-dependent responses under an applied stress (time constant $\tau_i = \eta_i/S_i$).[11,20,34] To simplify the model to four parameters, the elastic response parameter ($1/S_E$) was fixed based on the elastic displacement measured during recovery. Curve fits with a coefficient of determination (R^2) greater than 0.95 was considered a good fit.

$$d = L * \left[\left(\frac{1}{S_1} \left(1 - e^{\frac{-t}{\tau_1}} \right) \right) + \left(\frac{1}{S_2} \left(1 - e^{\frac{-t}{\tau_2}} \right) \right) + \frac{1}{S_E} \right] \quad (1)$$

As the equilibrium time is known to take longer than 12 hours,[11,35] five additional samples were used to evaluate long-duration recovery (48 hrs) in 0.15 M PBS. Data from the long-duration recovery tests were used to confirm model parameters determined from the 12-hour dataset. Once validated, the rheological model was used to predict equilibrium displacement (d_{eq}) and time (t_{eq}) for recovery. Equilibrium displacement was calculated at $t = \infty$, and equilibrium time was determined as the time where displacement was 99% of the equilibrium displacement. Total recovery at equilibrium was calculated as the displacement during recovery divided by the displacement during loading.

Finally, the saline concentration needed to yield a negligible viscous recovery response was estimated as a balance between external osmotic pressure and internal pressure during recovery. To calculate the "balance concentration", the disc height change at the end of recovery was measured with respect to a reference point (Figure 1C – red arrow), which was defined as the maximum disc height achieved during recovery in hyperosmotic loading (1.5 M PBS) (Figure 1C – dashed line). A linear regression was used to describe the data, and the x-intercept was defined as the balance concentration.

Statistical Analysis

A one-way repeated measures analysis of variance (ANOVA) was performed on recovery parameters, with a factor of osmotic loading condition. A Tukey *posthoc* analysis was performed to determine differences between groups. A two-way repeated measures ANOVA was performed on the percent recovery after 4, 8, and 12 hours of recovery (factors of time and osmolarity). All statistical analyses were performed using Matlab ($P \leq 0.05$ for significance). Values were reported as mean ± standard deviation, unless stated otherwise.

RESULTS

The instantaneous elastic displacement in creep was 1.06 ± 0.28 mm and accounted for 40% of the total disc height loss during loading (2.74 ± 0.69 mm; $\Delta d/\Delta t$ at 2-hours $= 0.41 \pm 0.10$ mm/h; pooled averages). Elastic displacement during recovery was not altered with osmotic pressure (0.53 ± 0.12 mm; $P > 0.7$) and accounted for approximately 30% of the total displacement during recovery in the control group (0.15 M PBS).

Viscoelastic recovery was highly dependent on saline bath concentration and recovery time ($P < 0.0001$) (Figure 2). Full disc height recovery was not observed within 12 hours for any test group (Figure 2A). Approximately 36% to 50% of disc height recovery occurred within the first 4 hours for all test groups. Recovery displacements for the 0.015, 0.15, and 0.75 M PBS groups were significantly different at 4, 8, and 12 hours of recovery ($P < 0.05$; Figure 2B). For the 0.015 and 0.15 M PBS groups, the disc height recovery increased over time (Figure 2B – white and black bars). However, recovery in a hyperosmotic solution

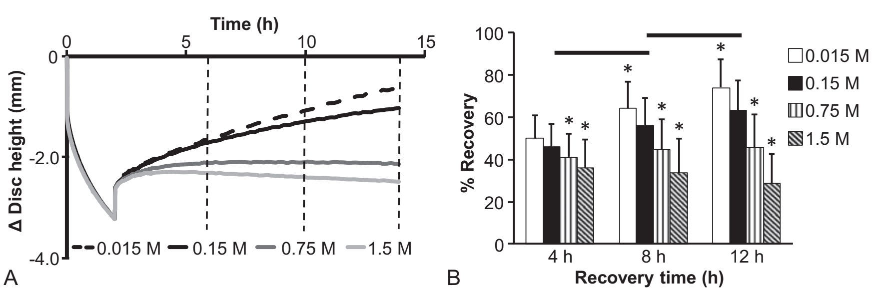

Figure 2. A, Disc height change during recovery for a representative sample. Dashed vertical lines represent time points used to calculate "percent-recovery", which was defined as the displacement during recovery divided by the displacement during loading. **B**, Percent-recovery after 4, 8, and 12 hours of recovery. The percent-recovery increased over time for the control group (0.15 M PBS; $P < 0.05$, black lines). *Represents differences between the osmotic loading group and the control.

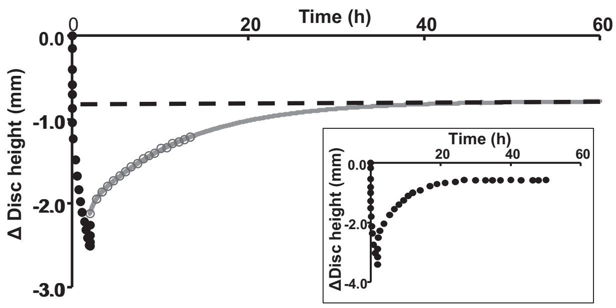

Figure 3. Disc height change from a representative sample. Circles represent experimental data, the grey line represents the model fit, and the dashed black line indicates equilibrium disc height. Inset – A small subset of specimens was allowed to recover for 48 hours, rather than 12 hours, to validate the predicted equilibrium time.

(*e.g.*, 1.5 M PBS) demonstrated a reverse trend in disc height recovery after ~4 hours of recovery, such that the disc exhibited features of loading rather than recovery (Figure 2B – diagonal stripped bar).

The rheological model described experimental data well ($R^2 = 0.99$; Figure 3 – circles versus solid line), except for the 1.5 M PBS group, because of the reverse trend during recovery (Figure 2A – light grey line). Therefore, model parameters were only reported for 0.015, 0.15, and 0.75 M PBS groups. The fast time constant, τ_1, was on the order of minutes, whereas the slow time constant, τ_2, was on the order of hours. The time constant and stiffness for the fast response were not dependent on osmolarity ($P > 0.3$) (Figure 4A and B). In contrast, the slow time constant decreased as bath osmolarity increased ($P < 0.01$; Figure 4C), whereas the stiffness for the slow response increased with osmolarity ($P < 0.0001$) (Figure 4D).

The rheological model was used to predict equilibrium time and displacement. The predicted equilibrium time and percent recovery using model parameters determined from the 12-hour recovery data agreed well with experimental data from 48-hour recovery tests (unpaired Student *t* test, $P > 0.33$) (Figure 3 – dashed line *vs.* inset data). Equilibrium time was 33.0 ± 10.7 hours for the 0.15 M PBS group and was altered with osmotic loading ($P < 0.001$) (Figure 5A). The equilibrium time for the 0.015 M PBS was 51.2 ± 24.9 hours, which was ~1.5X greater than the control group equilibrium time. In contrast, the equilibrium time for the 0.75 M PBS group was 60% lower than the control group equilibrium time. Recovery at equilibrium was dependent on PBS osmolarity ($P < 0.001$). The predicted recovery was 74 ± 15% of the total disc height loss during loading for the 0.15 M PBS group, whereas full recovery was predicted for the 0.015 M PBS group

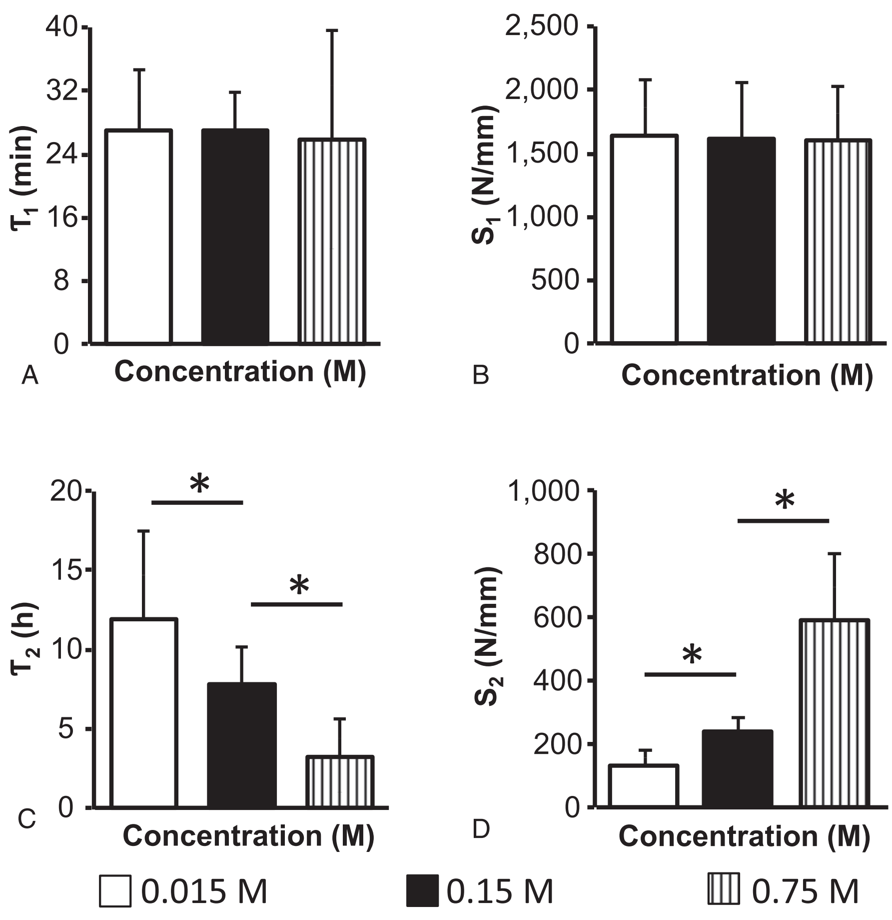

Figure 4. A & **B**, Fast response and (**C** & **D**) slow response model parameters. τ represents the time constant, which is a function of the dashpot and spring stiffness in the Voigt models, and S_i represents the spring stiffness. *Represents $P \leq 0.05$ with respect to 0.15 M PBS control group.

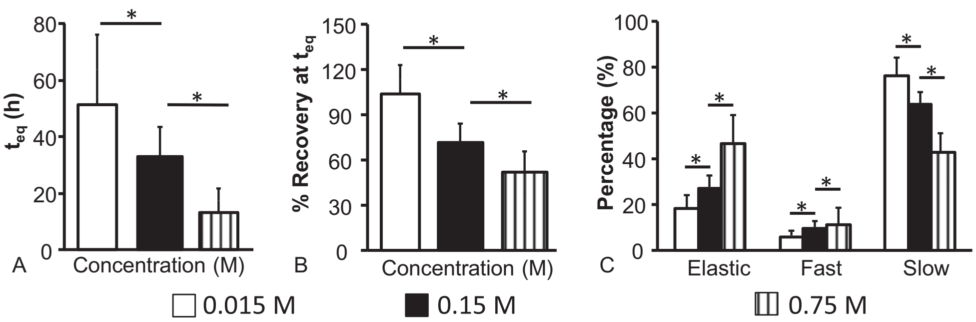

Figure 5. A, Equilibrium time (t_{eq}) for each osmotic group. **B**, Percent recovery at equilibrium (t_{eq}), (**C**) Contributions of elastic, fast viscous, and slow viscous responses shown as a percentage of the total recovery for each group. *Represents $P < 0.05$ with respect to 0.15 M PBS control group.

(104 ± 18%) (Figure 5B). The relative contributions of each model parameter were dependent on osmolarity ($P < 0.001$) (Figure 5C). Slow viscous recovery was the primary recovery mode for the hypoosmotic and control groups (>60%), whereas the elastic recovery was the primary mode for disc height recovery in the hyperosmotic group (~46%). The balance concentration between mechanical loading and osmotic pressure was 1.23 ± 0.16 M PBS (Figure 6).

DISCUSSION

During bed rest recovery, water is reimbibed into the disc. Full recovery of disc height and water content is important for preserving the mechanical function of healthy discs. In this study, we evaluated the influence of osmotic pressure on disc height recovery after creep to elucidate the effect of fluid-flow dependent recovery mechanisms. External osmotic pressure was created through ion imbalance between the saline solution and negatively charged proteoglycans. The data reported here decouple fluid-flow dependent behavior from fluid-flow independent behavior during recovery. That is, the elastic and short-term viscous recovery responses were independent of osmotic pressure, whereas passive diffusion of water was highly dependent on saline bath osmolarity and was the driving mechanism in long-term recovery.

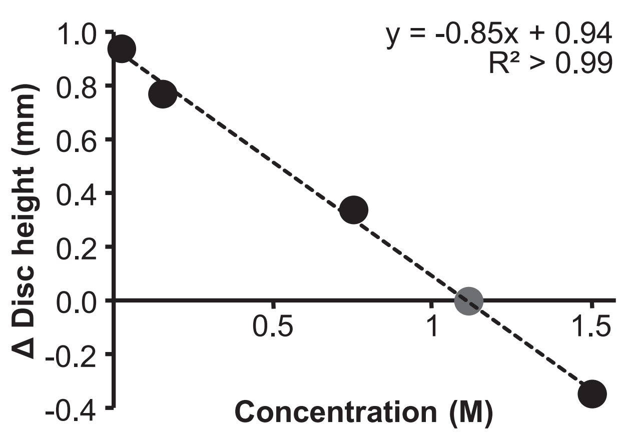

Figure 6. Balance concentration (red dot) for a representative sample. The balance concentration was defined as the x-intercept of a linear regression between the maximum disc height change during recovery and saline osmolarity (see Figure 2).

The rheological model provided a simplified model of the disc to differentiate between the short- and long-term viscous recovery. The physical meaning of short- and long-term recovery responses, which correspond to the fast and slow time constants, is not well understood. However, the model has been successful in noting differences in recovery mechanisms with degeneration and nucleotomy.[11,20] Previous studies observed significant changes in the fast response as a result of compositional changes in the nucleus pulposus, suggesting that the slow response may be related to fluid-flow through the annulus fibrosus. In the current study, osmotic pressure altered the slow response, but not the fast response. Taken together, our findings agree with the notion that the fast response is governed by flow-independent recovery (*e.g.*, immediate or recovery in air), which is related to the intrinsic properties of the disc. Therefore, discs subjected to severe morphological alterations with degeneration or nucleotomy would likely manifest as differences in the fast recovery behavior.

The rate and magnitude of fluid-flow dependent recovery were highly sensitive to the saline osmolarity that, in turn, governed disc height recovery. The increase in water uptake and disc height recovery with hypoosmotic pressure agrees well with data in the literature.[13,27] Mechanical loading[13] and loading from osmotic pressure during recovery hinder fluid-flow into the disc and disc height recovery (Figure 2A). For example, disc recovery in the 1.5 M PBS group exhibited features of loading after 4 hours of recovery, suggesting a change in fluid-flow direction from inflow to outflow. During diurnal recovery, intradiscal pressure is ~80% lower than the stress measured during standing[2]; however, little is known about changes in the tissue environment osmolarity throughout a diurnal loading cycle.[9] These findings suggest that full disc height recovery can be achieved *in vitro* through a combination of low osmotic pressure and low mechanical loading.

The osmotic condition that limited disc height recovery to an elastic-only response after creep was approximately 1.2 M PBS. Similarly, data reported by Vergroesen *et al* demonstrated a balance between osmotic pressure and mechanical loading with respect to the disc's ability to increase water uptake during compression. The balance between mechanical loading and osmotic pressure was near 1.8 MPa (1200N for caprine discs),[32,36] which was half of the balance stress calculated for bovine discs in this study (~3.6MPa for 1.2 M PBS). Differences between the two studies are likely because of geometrical differences in disc height and volume (disc height: caprine = 3–4 mm, bovine = 7 mm),[32,36] as the disc area, glycosaminoglycan content, and water composition are comparable between the two species (~600–680 mm^2, ~600 μg/mg dry weight).[36–38] Establishing a relationship between disc behavior and external osmotic pressure will be helpful for measuring intradiscal pressure without the use of pressure sensors, which might alter disc mechanics because of annular injuries.[39]

The bovine caudal disc is an ideal animal model to study disc mechanics of the healthy human disc, based on similarities in biomechanical composition, disc height, swelling pressure, and mechanical properties.[30–33,40] However, the bovine discs do not exhibit signs of degeneration or aging like the human disc.[31] Negatively charged proteoglycans of the intervertebral disc play a crucial role in tissue swelling, by attracting water molecules into the disc. The proteoglycan content in the annulus fibrosus is lower in the bovine disc than the human disc,[31] suggesting that some differences may exist for mechanical properties measured under osmotic loading conditions.

There are some limitations that should be noted. Bone-disc-bone motion segments were used in this study to examine the individual response of a single intervertebral disc to osmotic pressure, which limits our ability to observe differences in stress distribution with surrounding tissues. During testing, creep was not long enough to reach equilibrium; however, loading was intentionally kept short to investigate multiple recovery conditions for each specimen. Each sample was tested multiple times to perform a paired statistical analysis. Although full recovery in disc height was not observed, we did observe complete recovery in disc joint mechanics, which is comparable to data in the literature.[11,21,41] Disc rehydration behavior depends on many factors.[3,11,13] Direct comparison with data reported in the literature is difficult because of variations with animal models, experimental protocols, including recovery with an applied load, removal of endplates and adjacent vertebrae, and level of disc degeneration.[13,22,25] Therefore, future work will investigate the effect of degeneration on time-dependent recovery mechanics with osmotic pressure.

There are conflicting data in the literature about achieving full disc height recovery *in vitro*.[35,42–45] Incomplete disc height recovery observed in this study for the control group agrees with the results of previous studies[11,35]; however, the findings reported here demonstrate that osmotic pressure can be used in combination with low mechanical loading to achieve full disc height recovery *in vitro*. In conclusion, osmotic pressure causes significant changes in time-dependent recovery response of healthy discs. This study provides a better understanding of disc rehydration mechanisms and will be important for understanding diurnal recovery of healthy, injured, and degenerated discs.

➢ Key Points

- The fast response of the disc in unloaded recovery is because of its intrinsic properties, such as the extracellular matrix.
- The slow response of the disc in recovery is because of its flow-dependent properties and is altered by osmotic loading.
- Osmotic pressure alters the rate and magnitude of flow-dependent recovery behavior.
- There exists a balance between osmotic pressure and mechanical loading (~3.6 MPa in this study).
- Full disc height recovery, after mechanical loading, can be achieved *in vitro* through a combination of low mechanical loading and hypoosmotic pressure.

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