Published in final edited form as:

J Mech Behav Biomed Mater. 2011 October ; 4(7): 933–942. doi:10.1016/j.jmbbm.2011.02.002.

Axial Creep Loading and Unloaded Recovery of the Human Intervertebral Disc and the Effect of Degeneration

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Abstract

The intervertebral disc maintains a balance between externally applied loads and internal osmotic pressure. Fluid flow plays a key role in this process, causing fluctuations in disc hydration and height. The objectives of this study were to quantify and model the axial creep and recovery responses of nondegenerate and degenerate human lumbar discs. Two experiments were performed. First, a slow compressive ramp was applied to 2000 N, unloaded to allow recovery for up to 24 hours, and re-applied. The linear-region stiffness and disc height were within 5% of the initial condition for recovery times greater than 8 hours. In the second experiment, a 1000 N creep load was applied for four hours, unloaded recovery monitored for 24 hours, and the creep load repeated. A viscoelastic model comprised of a “fast” and “slow” exponential response was used to describe the creep and recovery, where the fast response is associated with flow in the nucleus pulposus (NP) and endplate, while the slow response is associated with the annulus fibrosus (AF). The study demonstrated that recovery is 3-4X slower than loading. The fast response was correlated with degeneration, suggesting larger changes in the NP with degeneration compared to the AF. However, the fast response comprised only 10-15% of the total equilibrium displacement, with the AF-dominated slow response comprising 40-70%. Finally, the physiological loads and deformations and their associated long equilibrium times confirm that diurnal loading does not represent “equilibrium” in the disc, but that over time the disc is in steady-state.

Keywords

disc degeneration; viscoelasticity; axial compression; recovery; creep

1. Introduction

The intervertebral disc is a hydrated fibrocartilage comprised of the gelatinous nucleus pulposus which is surrounded by the structured fiber-reinforced annulus fibrosus. Over the course of daily activities, the disc maintains a balance between externally applied loads and internal osmotic pressure. Fluid flow plays a key role in this process, causing fluctuations in disc hydration and height. Disc hydration influences disc mechanics, particularly the

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The authors have no disclosures.

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stiffness and the creep properties in axial loading (Koeller, Funke et al. 1984; Race, Broom et al. 2000; Costi, Hearn et al. 2002; Johannessen, Vresilovic et al. 2004). Therefore, quantification of the disc's mechanical properties is highly dependent on loading history, which must be carefully controlled in *ex vivo* experiments. Viscoelastic mechanisms include three-dimensional fluid flow through the nucleus pulposus, annulus fibrosus, and endplate, as well as intrinsic solid phase viscoelasticity (Broberg 1993; Setton, Zhu et al. 1993; Argoubi and Shirazi-Adl 1996; Iatridis, Setton et al. 1998; Gu, Mao et al. 1999; Riches, Dhillon et al. 2002; Johannessen and Elliott 2005; Williams, Natarajan et al. 2007; Huang and Gu 2008; Schroeder, Elliott et al. 2008). However, the complex poroelastic flow is not yet well understood. Consequently, rheological models are used as a valuable tool to describe the time-dependent disc mechanics and to quantify disc viscoelasticity (Keller, Hansson et al. 1988; Cassidy, Silverstein et al. 1990; Li, Patwardhan et al. 1995; Johannessen, Vresilovic et al. 2004; Pollintine, van Tunen et al. 2010).

Rheological models mathematically describe viscoelastic behavior using a combination of springs and dashpots. They can be used to test for differences between study groups, to predict the time needed to reach a steady-state condition, predict the mechanical state of the disc after a pre-determined time, and to study the recovery response (Burns, Kaleps et al. 1984; Keller, Hansson et al. 1988; Li, Patwardhan et al. 1995; Johannessen, Cloyd et al. 2006; Pollintine, van Tunen et al. 2010). Studies have used combinations of viscoelastic solid Voigt models (i.e., spring and dashpot in parallel) to quantify the displacement-time behavior during creep, with the initial elastic response modeled as an additional spring in series (Burns, Kaleps et al. 1984; Keller, Hansson et al. 1988; Li, Patwardhan et al. 1995; Johannessen, Cloyd et al. 2006; Pollintine, van Tunen et al. 2010). Care must be taken when interpreting and extrapolating rheological models to ensure that sufficient experimental data is used to determine the model parameters, as a short experiment will give inaccurate predictions of the steady-state condition (Pollintine, van Tunen et al. 2010). The time-dependent behavior depends on many variables, including geometry, structure, and composition; therefore, previous models of creep in animal discs cannot be directly extrapolated to the human disc. Moreover, to our knowledge, no study has quantified and modeled the unloaded recovery response in human discs.

Recovery of the disc height is dependent on the magnitude and duration of the applied load and unloaded recovery and on the surrounding environment. The time response in loading and unloading are expected to be different, as loading is driven by an active application of external pressure, which is balanced by osmotic pressure. In contrast, recovery is passive and relies solely on diffusion due to osmotic pressure. There is conflicting data about the disc's ability to recover following compression loading *in vitro*. In some studies full recovery is not achieved (e.g. (Kingma, van Dieen et al. 2000; Riches, Dhillon et al. 2002; van der Veen, Mullender et al. 2005; MacLean, Owen et al. 2007)), while in others full recovery is observed (e.g. (Koeller, Funke et al. 1984; Keller, Holm et al. 1990; Johannessen, Vresilovic et al. 2004; van der Veen, van Dieen et al. 2007)). Generally, full recovery is observed when the unloaded recovery time is several times longer than the loading time and the study is performed in a fluid environment. While a large portion of both creep and recovery are thought to occur within the first hour of loading or unloading (Koeller, Funke et al. 1984; Costi, Hearn et al. 2002), little is known about the recovery force-displacement behavior or the steady-state condition. Since both loading and unloading are key aspects of the *in vivo* diurnal cycle, a better understanding of the viscoelastic unloaded recovery response is essential. Moreover, because studies often use repeated experiments on the same sample, the appropriate recovery time to achieve repeatable mechanical behavior not affected by loading history is needed.

Disc degeneration is associated with an increased rate of creep or stress-relaxation (Kazarian 1975; Keller, Spengler et al. 1987; Li, Patwardhan et al. 1995; Pollintine, van Tunen et al. 2010). These changes are likely related to the decrease in both the water and glycoaminoglycan content with degeneration. A decrease in the glycoaminoglycan content will decrease the osmotic pressure, a key component in establishing the viscoelastic response and equilibrium. The role of degeneration on the creep and recovery response of human discs has not been fully established. Understanding the degenerative changes in creep and recovery is important for developing and testing potential biological treatments for the degenerated disc and to appropriately perform repeated testing of disc samples for *in vitro* experiments.

The objectives of this study were two-fold: 1) to determine the time required for unloaded recovery of disc height and stiffness following compressive loading in a human bone-disc-bone segment; 2) to measure the creep and recovery behavior of nondegenerate and moderately degenerate human lumbar discs and quantify the response using a rheological model. We hypothesize that the disc recovery time is longer than the loading time, repeated loading responses are equivalent when sufficient recovery time is used, and that the creep and recovery response are correlated with the disc's degenerative state.

2. Methods

Two load-recovery experiments were conducted on separate sets of samples to evaluate the disc recovery behavior: 1) a slow axial compressive ramp to 2000 N and, 2) an extended four hour creep experiment at 1000 N. The amount of time needed for recovery of the disc height and mechanics were evaluated in both experiments. Human lumbar spines were acquired from an approved source (NDRI, Philadelphia, Pennsylvania). T₂-weighted magnetic resonance (MR) images were obtained in order to determine the degenerative grade using the Pfirrmann scale, and values were averaged across three independent graders (Pfirrmann, Metzdorf et al. 2001). Briefly, the level of degeneration was determined by the MR signal intensity, the disc height, and structural features such as annular fissures and bulging. An integer scale from 1 to 5 was used to grade the samples, with nondegenerate discs having lower values. Bone-disc-bone samples were prepared by removing the muscles and facet joints from levels L1-L2 and L2-L3 and stored at -20°C until testing. Samples were potted in bone cement and allowed to hydrate overnight in a refrigerated phosphate-buffered saline (PBS) bath prior to mechanical testing. All mechanical tests were performed in a PBS bath at room temperature using an Instron 8874 testing system.

2.1 Slow Ramp Loading and Recovery

In the first experiment, the time required for the disc to recover from a ramp loading protocol was determined ($n = 8$ samples from the L1-L2 and/or L2-L3 levels of six lumbar spines, 22 - 77 years old (mean age = 42 years), degenerative grade = 1.0 - 4.0). A 20 N preload was applied to the sample to ensure contact with the loading platen. Axial compression was applied at a quasi-static rate of 1 N/s to 2000 N (loading time = 33 min) defining the "initial condition" of the disc (Johannessen, Vresilovic et al. 2004). Samples were allowed to recover unloaded in a PBS bath for 0, 1, 4, 8, 12, 16, and 24 hours and re-tested with the same loading protocol described above. The recovery times were tested in a random order to avoid bias in the viscoelastic recovery behavior due to loading history. Mechanical loading was repeated until all recovery times were tested on each sample. For recovery times greater than 4 hours, samples were placed in a refrigerated PBS bath and returned to room temperature one hour prior to re-testing. Force and displacement were measured during loading.

Stiffness was calculated as the slope of the linear-region of the force-displacement curve. The initial disc height was calculated from sagittal MR images, as previously described (O'Connell, Vresilovic et al. 2007). Briefly, a custom algorithm (Matlab Inc.) was used to select the boundary along the superior and inferior vertebrae and connecting the points at the anterior and posterior height to create the disc space area. The average initial disc height was calculated by dividing the total disc space area by the anterior-posterior width. The unrecovered disc height was calculated as the change in position of the loading platen at the 20 N preload and was normalized to the initial disc height.

The data did not represent a normal Gaussian distribution and therefore the median and interquartile ranges are reported. A Wilcoxon test was used to compare stiffness and the unrecovered disc height to the initial values. The effect of degeneration was not evaluated due to the small sample size.

2.2 Extended Creep Loading and Recovery

In the second experiment, the time-response during creep, unloaded recovery, and a second creep was quantified (n=12 level L2-L3, 25 - 76 years old (mean age = 55 years), degenerative grade = 1.8 - 3.4). A 20 N preload was applied. A step load to 1000 N was rapidly applied in 1.5 s and held for 4 hours. The sample was then unloaded to 20 N and allowed to recover for up to 24 hours. The 4-hour 1000 N creep load was then repeated (total test time = 32 hours). Force and displacement were recorded during creep and recovery. The percent of recovery was calculated as the total displacement during recovery divided by the total displacement during the first creep test.

The displacement (d, mm) as a function of time (t, sec) and applied load (L, N) were fit to a 5-parameter rheological model (Equation 1) composed of two Voigt solids and a spring in series (lsqcurvefit function, Matlab Inc.) (Keller, Hansson et al. 1988; Johannessen, Cloyd et al. 2006),

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

which provides parameters for the elastic response (S_E), the fast response (τ_1 and S_1), and the slow response (τ_2 and S_2), where i to i+1 corresponds to the start and end times for the 3 stages of the test (i.e. first creep, recovery, and second creep). The elastic stiffness parameters S (N/mm) and time constants τ (sec) were calculated for each stage. The elastic response included the 1.5 s loading and the first 30 s after loading.

The Boltzmann superposition principle was applied to incorporate the loading history when fitting the model parameters to the recovery and second creep test response (Lakes 1999). Therefore, the experimentally measured displacement during recovery was fit to the model using a summation of the displacement during the first creep response and the recovery behavior ($d_{total}(t) = d(t=t_0 \rightarrow t_1) + d(t=t_1 \rightarrow t_2)$, Figure 1). Similarly, the model parameters for the second creep test were determined by incorporating the loading history from the first creep test and the recovery ($d_{total}(t) = d(t=t_0 \rightarrow t_1) + d(t=t_1 \rightarrow t_2) + d(t=t_2 \rightarrow t_3)$).

The displacement amplitude for the Voigt solid was calculated as L/S ; therefore, the displacement amplitude for the elastic response was L/S_E . The equilibrium displacement, where the viscous effects have completely dissipated, was calculated at $t = \infty$. The time to reach equilibrium was calculated at 99% of the equilibrium displacement. The percent of equilibrium displacement at the end of each creep and recovery test was determined as a

ratio of the measured displacement and the calculated equilibrium displacement. The relative contribution of each component (elastic, fast, and slow response) was calculated by dividing the displacement amplitude (L/S) by the total equilibrium displacement.

The median and interquartile range is reported. A Friedman test was used to compare the model parameters (elastic response (S_E), fast response (τ_1 and S_1), slow response (τ_2 and S_2)), equilibrium time, and equilibrium displacement across the two creep tests and recovery. A Dunns post-hoc test was performed when significance was found. To determine the effect of degeneration, a Spearman's correlation was performed to compare the average T_2 grade with the model parameters and the directly measured percent recovery. Significance was set at $p \leq 0.05$.

3. Results

3.1 Slow Ramp Loading and Recovery

The first experiment included a slow ramp to 2000 N to determine the “initial condition”, followed by unloaded recovery times ranging from 0 to 24 hours and reloading. The average disc height was 8.86 (6.94 – 11.00) mm. The average displacement at 2000 N was 2.08 (1.78 – 2.51) mm, resulting in approximately 23% axial compression. The force-displacement response was nonlinear (Figure 2). Short recovery times (i.e., 0 and 1 hour) led to an elongated toe-region upon repeated testing, due to the unrecovered disc height. The force-displacement response following the remaining recovery times (greater than 4-8 hours) was similar to the initial condition (Figure 2).

The initial stiffness was 1498 (1294 – 1668) N/mm. The linear-region stiffness following the 0 and 1 hour recovery was 20 - 25% higher than the initial stiffness (373 & 291 N/mm higher, respectively; $p < 0.001$; Figure 3A). For recovery times of 4 hours or longer, the stiffness was not significantly different and was within 5% of the initial condition ($p > 0.3$).

The location of the loading platen at 20 N was used to calculate the unrecovered disc height. The unrecovered disc height following 0 to 4 hours recovery was significantly different from the initial condition. For example, the unrecovered height was 0.64 mm following the 0 hour recovery, or 7% of the platen location at 20 N ($p < 0.01$; Figure 3B). For recovery times of 8 hours or longer, the unrecovered disc height was within 1% of the loading platen location at 20 N ($p > 0.2$; Figure 3B).

3.2 Extended Creep Loading and Recovery

The second experiment consisted of a 4 hour creep test at 1000 N followed by a 24 hour recovery at 20 N and a second 4 hour creep test at 1000 N (Figure 1). The displacement at the end of the first creep test was 2.85 (2.14 - 3.53) mm, resulting in approximately 30% axial compression. At the end of recovery, 2.39 (2.21 – 2.64) mm of the creep displacement was recovered. The percent of recovered disc height ranged from 44 to 98% and was negatively correlated with degenerative grade ($r = -0.72$, $p < 0.01$; Figure 4). The displacement at the end of the second creep test was 2.09 (1.94 – 2.25) mm.

The displacement response was fit to a rheological model. The model fit very well to the experimental data for both creep tests and the recovery test as determined by a high R^2 and a low bias from the Bland-Altman analysis ($R^2 = 0.998$, bias = 0.0000 ± 0.0115 ; Figure 5). The model included an elastic response (S_E), a fast response (τ_1 and S_1), and a slow response (τ_2 and S_2) for each stage of the test (Table 1). The fast time constant, τ_1 , was on the order of minutes, and the slow time constant, τ_2 , was on the order of hours. The τ_1 for the first creep was not significantly different from the second creep ($p > 0.05$; Table 1, Figure 6A). In contrast, τ_1 for recovery was 5X longer than the creep tests ($p < 0.01$; Figure

6A). Similarly, τ_2 for the first and second creep tests were not different from each other ($p > 0.05$); however, τ_2 for recovery was 2.5X longer than the creep tests ($p < 0.001$; Figure 6B). There was a strong positive correlation of τ_1 with degeneration for creep ($r > 0.65$, $p \leq 0.02$; Figure 7A), but not for recovery (Table 2). The τ_2 did not correlate with degeneration for creep or recovery ($p > 0.1$; Table 2).

The displacement amplitude of the elastic response (L/S_E) for the first creep test was 50% larger than the second creep test ($p < 0.01$; Table 1, Figure 8A). The L/S_E during recovery was 30-60% lower than both creep tests ($p < 0.001$; Figure 8A). The displacement amplitude of the fast response (L/S_1) for the first creep test was significantly higher than for the second creep and was not significantly different for recovery ($p < 0.01$; Figure 8B). The displacement amplitude related to the slow response (L/S_2) was approximately 1.6 mm and was not significantly different across the creep or recovery tests ($p > 0.2$; Figure 8C). The elastic response L/S_E was correlated with degeneration for the first creep test ($r = 0.67$, $p = 0.02$), and was not correlated with degeneration for the second creep test or recovery ($p > 0.9$; Table 2). There was a strong positive correlation with degeneration for the fast response L/S_1 for creep and recovery ($r > 0.65$, $p \leq 0.02$; Figure 7B & C). The slow response L/S_2 was correlated with degeneration for the first creep test ($r = 0.66$, $p = 0.02$) and was not correlated with degeneration for the second creep test or recovery ($p > 0.2$; Table 2). The displacement amplitude of the elastic response contributed to 30-45% of the equilibrium displacement for creep and only 20% of the response for recovery (Figure 9). The displacement amplitude related to the fast response contributed 10-15% of the equilibrium displacement for creep and recovery. The displacement related to the slow response was 50% of the equilibrium displacement for creep and 70% of the displacement for recovery.

The equilibrium displacement (calculated as the total displacement at $t = \infty$) was not significantly different between the creep tests ($p > 0.05$; Table 1, Figure 8D). For recovery the equilibrium displacement was 10-25% lower than the creep tests ($p < 0.01$; Figure 8D). There was a significant positive correlation of the equilibrium displacement of the first creep test with degeneration ($r = 0.75$, $p < 0.01$), but not for the second creep test or recovery ($p > 0.1$). At the end of the first and second creep test (4 hours of loading), the measured displacement was 86% and 81% of the equilibrium displacement, respectively; at the end of the recovery, the measured displacement was 98% of the equilibrium displacement (Figure 9). The equilibrium time (calculated as the time to reach 99% of the equilibrium displacement) was 14.1 (13.4 – 15.8) hours for the first creep test and was not significantly different for the second creep test ($p > 0.05$). The equilibrium time for recovery was 36.9 (33.4 – 47.5) hours and was 2.5X greater than the equilibrium time for creep ($p < 0.001$). The equilibrium time was not correlated with degeneration for creep or recovery ($p > 0.1$).

4. Discussion

The objective of this study was to measure the creep and recovery response of the human disc in axial compression using two experiments: a slow compressive ramp to 2000 N (applied over 30 min) and 1000 N creep (rapidly applied and held for 4 hours). The applied compressive loads were selected to represent moderate levels of physiological stress (0.58MPa and 1.16MPa) experienced during walking or while standing and carrying an object (Wilke, Neef et al. 1999). The measured linear-region stiffness (1500 N/mm) was within the range previously reported (Brinckmann and Grootenboer 1991; Shea, Takeuchi et al. 1994; Okawa, Shinomiya et al. 1996; Beckstein, Sen et al. 2008; Cannella, Arthur et al. 2008), suggesting that our protocol and population was representative of established standards. In addition, the correlations with degeneration in this study were consistent with established literature, which has demonstrated that the disc creep rate is faster in

degenerated discs (Kazarian 1975; Keller, Spengler et al. 1987; Li, Patwardhan et al. 1995; Pollintine, van Tunen et al. 2010).

The recovery time for both experiments was much longer than the loading duration. In the first experiment, where loading was applied gradually for a short duration (~30 min) to 2.1 mm axial displacement, the discs immediately recovered 70% of the displacement and achieved full recovery of stiffness and disc height within eight hours, or 16X longer than the loading time. In the second experiment, where a rapidly applied load was held for a longer duration (4 hrs and 2.9 mm displacement), the disc immediately recovered only 20% of the creep displacement. Furthermore, the recovery response was predicted to reach to equilibrium after 40 hours, or 10X longer than the loading time and ~3X longer than the predicted creep equilibrium time. The observation for full recovery *in vitro* and the longer recovery time required, in comparison to the loading time, is somewhat controversial (Kingma, van Dieen et al. 2000; van der Veen, Mullender et al. 2005; MacLean, Owen et al. 2007). Yet, this observation is consistent with previous studies where full recovery is observed, but only when the unloaded recovery times are much longer than loading times (Koeller, Funke et al. 1984; Keller, Holm et al. 1990; Solomonow, He Zhou et al. 2000; Johannessen, Vresilovic et al. 2004; van der Veen, van Dieen et al. 2007). These responses suggest that the mechanisms for axial disc mechanics are highly time-dependent, and the rate and duration of loading impacts the recovery response. Importantly, the repeatability between tests supports repeated *in vitro* mechanical testing if sufficient rehydration and recovery are used, but care must be taken to confirm recovery.

The rheological model, while not specifically tied to disc structure, provides insights into mechanisms of disc viscoelasticity and particularly to differences between loading and recovery responses. The largest differences between creep and recovery were in the time constants, which were 2.5 and 5X longer for recovery; however, the displacement amplitudes of the responses were of similar magnitude. Previously we proposed that change in the time constants is related to alteration in the fluid flow pathway, while change in the displacement amplitude is related to a difference in the quantity of fluid being acted upon by that pathway (Vresilovic, Johannessen et al. 2006). The results here support this concept, where the mechanisms for fluid inflow and outflow (e.g., the time constants) are different for loading and recovery, while the volume and distance of fluid being exchanged (e.g., the displacement amplitude, L/S) should be the same. Some of the differences between the creep and recovery time constants may be related to the directional dependence of flow (and strain-dependent permeability) through the endplate and annulus fibrosus (Gu, Mao et al. 1999; Ayotte, Ito et al. 2001; van der Veen, Mullender et al. 2008). However, it is likely that the major reason for the differences observed between the creep and recovery time constants are the large differences in the driving forces of mechanical load and osmotic pressure (Riches, Dhillon et al. 2002; Ferguson, Ito et al. 2004; Schroeder, Sivan et al. 2007). That is, during creep loading, the externally applied forces overwhelm the osmotic pressure and rapidly drive fluid out of the system, while during unloaded recovery, only the osmotic pressure is acting and fluid flows into the disc at a slower rate.

Disc fluid flow under load is a complex three-dimensional problem with anisotropic, inhomogeneous, strain-dependent permeability and solid matrix modulus through the NP, AF, endplate, and vertebral body and with inhomogeneous fixed charge density and osmotic pressure (Urban and Maroudas 1979; Ohshima, Tsuji et al. 1989; Gu, Mao et al. 1999; Iatridis, MacLean et al. 2007; Heneghan and Riches 2008; Jackson, Yuan et al. 2008). The mechanisms of disc flow and transport under load remain largely unknown and will require continued development of sophisticated constitutive models and experiments (Iatridis, Laible et al. 2003; Ferguson, Ito et al. 2004; Yao and Gu 2007; Mokhbi Soukane, Shirazi-Adl et al. 2009). Yet, the simple rheological model used in this study can be interpreted

toward an understanding of flow in the various disc compartments. We assume that the fast response (t_1 , L/S_1) is primarily related to fluid flow through the NP and/or endplate, and that the slow response (t_2 , L/S_2) is primarily related to fluid flow through the annulus fibrosus. Previous trans-endplate nucleotomy studies with partial NP removal through the central endplate (fully opening the endplate to flow) with partial NP removal only altered the fast response and not the slow response (Johannessen, Cloyd et al. 2006; Vresilovic, Johannessen et al. 2006). This supports that the fast response is in the EP and NP (which was altered) and the slow response is in the AF (which was not altered) (Vresilovic, Johannessen et al. 2006).

The correlation with degeneration of specific model parameters (fast response), and the lack of correlation of other parameters (the slow response) provide additional insights to this general observation. The model parameters of the **fast response** significantly correlated with degeneration. The longer t_1 with degeneration (Figure 7A) is likely related to reduced permeability, presumably within the NP and EP, as the tissue becomes denser and more compacted. Consistent with this observation are changes with degeneration that include: reduced NP apparent diffusion coefficient reduction measured using magnetic resonance imaging (Antoniou, Demers et al. 2004) and the decreased NP swelling pressure measured in confined compression (Johannessen and Elliott 2005). Although the NP has an increased permeability (faster and easier flow) with degeneration (Johannessen and Elliott 2005), the other changes, such as decreased osmotic pressure, may overwhelm this effect at the whole disc level. This is supported by an in vivo MR study where the flow of gadolinium contrast from the vertebral body across the EP was faster with EP disruption and in degeneration, and the flow slowed as the contrast moved through the NP (Rajasekaran, Babu et al. 2004). It would appear that presumed “blockage” to flow through the EP may not be a relevant barrier in the degenerated disc, rather, reduced permeability of the NP, particularly during compression loading, seems to slow diffusion.

While the correlations of the fast response model parameters with degeneration are interesting, it is notable that the fast response represents a relatively small fraction of the total creep or recovery displacement (10-15%, Figure 9). This is consistent with a previous study of cyclic compressive loading where t_1 (the fast time constant) increased with nucleotomy, however, this response was quite small compared to the large changes observed with prolonged compressive cyclic loading (Vresilovic, Johannessen et al. 2006). Similarly, some in vitro studies observe by blocking the flow pathways that the fluid flow in the AF is a larger contributor than the EP pathway (Ohshima, Tsuji et al. 1989; van der Veen, van Dieen et al. 2007). Thus, while the fast response's fraction of the total response is small, this study cannot address whether this reflects its relative role and importance for large and small solute transport and disc nutrition. Sophisticated analytical and experimental models of disc transport flow would be needed to address this question (Nachemson, Lewin et al. 1970; Urban, Holm et al. 1978; Ferguson, Ito et al. 2004; Yao and Gu 2007; Mokhbi Soukane, Shirazi-Adl et al. 2009). Moreover, it is important to keep in mind that while the AF may be the major contributor to the mechanical equilibrium between axial loading and osmotic pressure, this mechanism may be partially uncoupled in the case of nutrition, where the delivery of nutrients must occur via the surrounding blood supply and transported through the disc. Indeed, in vivo MRI studies suggest that the EP is the primary contributor over the AF in the movement of contrast agent (and presumably nutrients) from the blood supply into the disc (Rajasekaran, Babu et al. 2004) and that this transport is load-dependent (Arun, Freeman et al. 2009).

The time constant of the **slow response**, which contributes approximately 50% of the equilibrium displacement (Figure 9), is not affected by degeneration. While degeneration changes the transient disc response over the time scale of minutes (t_1), it has less of an effect

on the slower rate effects over the scale of 4 to 10 hours (t_2). This may explain why quasi-static AF tensile tests (Acaroglu, Iatridis et al. 1995; Guerin and Elliott 2007; O'Connell, Guerin et al. 2009) and AF permeability measured in compression (Iatridis, Setton et al. 1998) show few changes with degeneration. Several displacement parameters correlated with degeneration only for the first creep test (equilibrium displacement, elastic displacement L/S_E , slow displacement L/S_2), but not during the recovery or second creep test (Table 2). It is not clear why these displacements did not correlate with degeneration for the later tests, but may be related not yet reaching equilibrium before beginning these portions of the test. Nonetheless, the effect of degeneration to increase the total creep displacement is consistent with the literature (Kazarian 1975; Keller, Spengler et al. 1987; Li, Patwardhan et al. 1995; Pollintine, van Tunen et al. 2010) and with the trans-endplate partial nucleotomy (Johannessen, Cloyd et al. 2006), and is likely due to loss of NP proteoglycan and associated fixed charge density and osmotic pressure (Urban and McMullin 1988).

There are important distinctions between these *in vitro* creep-recovery experiments and diurnal loading. The samples in this study were equilibrated in an unloaded state prior to testing, in contrast to *in vivo* diurnal loading where the disc is always subject to some amount of compressive load. Therefore, it is likely that the samples tested had a higher initial fluid content versus discs at the beginning of the diurnal cycle. This is supported by the larger change in lumbar disc height in this study (2-3 mm) compared to diurnal studies where approximately 1.5 mm (Adams, Dolan et al. 1987) is observed. The diurnal cycle exhibits restoration of the disc height over the 24-hour period (Adams, Dolan et al. 1987; Botsford, Esses et al. 1994; Paajanen, Lehto et al. 1994; Park 1997; Roberts, Hogg et al. 1998; Karakida, Ueda et al. 2003); however, this restoration is not recovery to the equilibrium state. Indeed, diurnal loading represents a “steady-state” condition governed by an always present compressive load; not an unloaded equilibrium condition between fluid flow into and out of the disc. This notion is supported by *in vivo* unloaded conditions such as microgravity and extended periods of bed rest where increased disc height and spine length are observed (Wing, Tsang et al. 1991; Cao, Kimura et al. 2005).

It would be impractical to attempt to replicate the diurnal cycle for *in vitro* experiments of creep and recovery due to the lack of equilibrium state and the complexity of the loading cycle. Thus, this experiment was based on an initial condition of unloaded equilibrium; however, this introduced limitations to the study. First, the displacement during the first creep test was 20% greater than the displacement during the second creep test. This is likely due to the initial equilibrium at zero load followed by a 20 N preload that was not held to equilibrium, and to not reaching unloaded equilibrium by the start of the second creep test. These effects resulted in the first creep test having a 0.7 mm higher elastic displacement compared to the second creep test. This effect between 0 and 20 N may also explain why some of the creep parameters correlated with degeneration for creep 1 but not creep 2, and suggests that these are related to low load neutral zone effects. Another potential limitation is that the creep and recovery tests did not run until equilibrium (predicted to be 14 and 40 hours, respectively). While this may have affected the model parameters, there was sufficient data to perform the model fit with confidence to equilibrium since the change in displacement at the end of the tests was less than 0.02 mm/hour. In spite of these limitations, the protocols used here and the model parameters calculated represent the physiological range of axial displacements and loads experienced during daily activity.

In conclusion, the current study demonstrated that recovery is much slower than creep loading. The creep and recovery response were well described by a model comprised of a constant and two exponentials representing the elastic, fast, and slow response. While these may not directly correspond to unique mechanisms, it was possible to infer that the NP and EP drive the fast response and the AF drives the slow response. The fast response was

correlated with degeneration, suggesting larger changes in the NP with degeneration compared to the AF. Finally, the physiological loads and deformations and their associated long equilibrium times confirm that diurnal loading does not represent “equilibrium” in the disc, but that over time the disc is in steady-state. In addition to contributing toward understanding mechanisms of disc function, these results demonstrate that in vitro studies can be performed with repeated mechanical testing if sufficient rehydration and recovery are used between tests.

Acknowledgments

This study was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIH AR50052) and by the Penn Center for Musculoskeletal Disorders. Human tissue provided by National Disease Research Interchange (NDRI).

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Figure 1. Schematic of the applied load (top) and the displacement response (bottom) with time. A 1000N creep load was applied between during $t_0 - t_1$ (creep 1) and $t_2 - t_3$ (creep 2). The first creep test was followed by a 24-hour recovery at 20N ($t_1 - t_2$).

Figure 2. Force-displacement response curve for a representative sample, for the initial condition and recovery groups of 0, 1, 4 and 24 hours. The curves for recovery groups of 8, 12 and 16 hours were similar in behavior to the initial, 4 and 24 hour recovery groups (data not shown).

Figure 3. Median and interquartile range for recovery times between 0 and 24 hours of A) the change in the linear-region stiffness and B) unrecovered disc height. * denotes a significant difference from the initial condition, $p \leq 0.05$.

Figure 4.
The percent of recovery was significantly correlated with degeneration.

Figure 5. Representative sample showing the first creep and recovery experimental data (circles) with model fit (solid line) and equilibrium displacement predicted by the model (dashed line).

Figure 6.

Median and interquartile range for the short and long time constants for creep and recovery.

* denotes differences between groups.

Figure 7.

The short time constant (τ_1) for creep (A), the displacement (L/S_1) related to the fast response for creep (B) and recovery (C) were significantly correlated with degeneration ($p \leq 0.02$).

Figure 8.

Median and interquartile range for displacement amplitude, calculated as L/S , of the A) elastic response, B) short time constant, C) long time constants for creep and recovery. D) Equilibrium displacement calculated at t_{∞} . * denotes significant difference.

Figure 9.

Displacement of the elastic spring response and the two Voigt models (fast and slow response) for A) the first creep test, B) the second creep test, and C) recovery normalized to the equilibrium displacement (i.e. the displacement at 16hrs and 40hrs for creep and recovery, respectively). The displacements are shown for the duration of the experiment and at the equilibrium time determined (shown by the black circle) by the model.

Table 1

Model parameters for each stage of experiment (e.g., Creep 1, Recovery, and Creep 2). Variables defined by Equation 1 in Methods section.

	Creep 1	Recovery	Creep 2
τ_1 (min)	8.09 (6.12, 11.75)	35.8 (28.9, 57.7)	5.85 (4.26, 8.10)
L/S_1 (mm)	0.29 (0.21, 0.44)	0.28 (0.24, 0.38)	0.20 (0.16, 0.30)
τ_2 (hrs)	3.07 (2.96, 3.43)	8.00 (7.47, 9.26)	3.40 (3.19, 3.73)
L/S_2 (mm)	1.51 (1.31, 1.72)	1.64 (1.48, 1.83)	1.70 (1.59, 1.81)
L/S_E (mm)	1.44 (1.08, 1.71)	0.53 (0.49, 0.59)	0.76 (0.65, 0.79)
Equilibrium disp. (mm)	3.29 (2.55, 3.99)	2.49 (2.25, 2.71)	2.66 (2.42, 2.83)
Equilibrium time (hrs)	14.1 (13.6, 15.8)	36.9 (34.4, 42.7)	15.6 (14.7, 17.2)

Table 2

Model parameters that correlate with degeneration (denoted by Y) for each stage of experiment (e.g., Creep 1, Recovery, and Creep 2). Variables defined by Equation 1 in Methods section.

	Creep 1	Recovery	Creep 2
τ_1 (fast response)	Y, see Fig 7a	no	Y, see Fig 7a
L/S_1 (fast response)	Y, see Fig 7b	Y, see Fig 7c	Y, see Fig 7b
τ_2 (slow response)	no	no	no
L/S_2 (slow response)	Y	no	no
L/S_E (elastic response)	Y	no	no
Equilibrium displacement	Y	no	no
Equilibrium time	no	no	no