ISSLS Prize Winner: Dynamic Loading–Induced Convective Transport Enhances Intervertebral Disc Nutrition

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Study Design. Experimental animal study of convective transport in the intervertebral disc.

Objective. To quantify the effects of mechanical loading rate on net transport into the healthy and degenerative intervertebral disc in vivo.

Summary of Background Data. Intervertebral disc degeneration is linked with a reduction in transport to the avascular disc. Enhancing disc nutrition is, therefore, a potential strategy to slow or reverse the degenerative cascade. Convection induced by mechanical loading is a potential mechanism to augment diffusion of small molecules into the disc.

Methods. Skeletally mature New Zealand white rabbits with healthy discs and discs degenerated via needle puncture were subjected to low rate axial compression and distraction loading for 2.5, 5, 10, 15, or 20 minutes after a bolus administration of gadodiamide. Additional animals with healthy discs were subjected to high-rate loading for 10 minutes or no loading for 10 minutes. Transport into the disc for each loading regimen was quantified using post–contrast-enhanced magnetic resonance imaging.

Results. Low-rate loading resulted in the rapid uptake and clearance of gadodiamide in the disc. Low-rate loading increased net transport into the nucleus by a mean 16.8% and 12.6% in healthy and degenerative discs, respectively. The kinetics of small molecule uptake and clearance were accelerated in both healthy and degenerative discs with low-rate loading. In contrast, high-rate loading reduced transport into nucleus by a mean 16.8%.

Conclusion. These results illustrate that trans-endplate diffusion can be enhanced by forced convection in both healthy and degenerative discs in vivo. Mechanical loading–induced convection could offer therapeutic benefit for degenerated discs by enhancing uptake of nutrients and clearance of by-products.

Key words: intervertebral disc; nucleus pulposus; nutrition; mechanical loading; diffusion; forced convection; animal model; intervertebral disc degeneration; viscoelasticity.

Level of Evidence: 4
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The intervertebral disc is avascular and relies on transport from the microvessels in the adjacent subchondral bone to receive nutrients and expel waste products via diffusion. For this reason, trans-endplate transport plays a key role in disc homeostasis. For decades, a compromise in transport of small molecules has been thought to be a primary contributor to, or initiator of, disc degeneration.1–5

If disc degeneration is linked with reduced small molecule transport, strategies for enhancing transport into the disc have the potential to mitigate or even reverse the degenerative cascade. Diffusion is widely considered to be the primary mechanism for transport into the disc.6 However, diffusion in degenerative discs can be limited by increased calcification of the cartilage endplate, occlusion of the vertebral endplate marrow channels, or reduced proteoglycan content.6 Forced convection is a potential mechanism by which small molecule transport to and from the disc can be enhanced.

Convective transport occurs when the intervertebral disc is deformed via mechanical loading.7 Deformation of the disc induces bulk fluid flow, resulting in the clearance of fluid when the disc is compressed and uptake of fluid when the disc is distracted. Because the disc is viscoelastic and deforms in a rate-dependent manner, the rate of applied mechanical loading governs the amount of disc deformation and the extent to which convection contributes to net transport.8 The role of convection in disc transport is controversial. The predominant opinion in the literature is that convection does not contribute to the transport of small molecules in the
intervertebral disc.\textsuperscript{9,11} This viewpoint was pioneered by the landmark studies by Urban \textit{et al.},\textsuperscript{11} in which transport into the disc was quantified in moving versus nonmoving dogs after the bolus administration of radioactive tracer. At the single late time point (2 hr after tracer administration) at which transport was quantified, no differences between moving and nonmoving dogs were found.

However, transport in the disc is a dynamic event—initially, the tracer is taken up into the disc but over time, it will be cleared from the disc.\textsuperscript{11} Quantifying transport at a single, late time point after a bolus of tracer administration cannot fully characterize the contribution of convection to net transport. The kinetics of disc transport has been illustrated in a recent finite element analysis of diurnal loading of the disc. Results from the model indicated no net accumulation of small molecules in the disc at the end of a diurnal period, but extensive exchange of solutes occurred with each loading and unloading cycle.\textsuperscript{10}

Cyclic exchange is important because disc homeostasis is dependent in part on the ability of the disc to not only receive nutrients but also expel waste products. The cyclic exchange of nutrients and waste products to and from the disc is crucial to disc nutrition, but it has not been previously investigated \textit{in vivo}. The purpose of this study was to investigate the role of cyclic loading on net small molecule transport to and from the disc. We hypothesized that cyclic loading-induced convection enhances small molecule trans-endplate transport in a rate-dependent manner. As forced convection could also be a potential therapeutic for disc degeneration, we also investigated the ability of forced convection to augment transport in a degenerative disc.

\section*{MATERIALS AND METHODS}
Twenty-four skeletally mature New Zealand white rabbits were used in this study. Animals were divided into treatment groups of healthy intervertebral discs, degenerative discs, or controls. Animals with healthy discs were subjected to either (1) low-rate, low-frequency loading, or (2) high-rate, low-frequency loading. Animals designated to the degenerative disc group were subjected to a disc puncture to induce degeneration. After degeneration was induced, discs were subjected to low-rate, low-frequency loading. Control animals were subjected to no loading (diffusion alone). Animals were randomly assigned to a treatment group (healthy \textit{vs.} punctured \textit{vs.} control, high \textit{vs.} low rate, etc.) before surgery. In all animals, small molecule transport into the intervertebral disc was quantified using post—contrast-enhanced magnetic resonance imaging (MRI).

\section*{Loading Interface}
A single lumbar motion segment interfaces with our custom external loading apparatus \textit{via} percutaneous posts implanted into the spine. After Institutional Animal Care and Use Committee approval, 21 New Zealand white rabbits underwent the surgical procedure to implant transfixing pins and percutaneous posts at a single lumbar motion segment (L45). Under general anesthesia and using standard aseptic techniques, a lateral approach was used to expose the lumbar spine. Two-millimeter diameter threaded stainless steel pins (IMEX Veterinary, Inc., Longview, TX) were placed transversely through the L4 and L5 vertebral bodies. To minimize any effects of pin placement on the target endplates, disc, or transport, we place the pins as far from the target disc as possible. In the L5 vertebra, the pins are placed just cranial to the caudal endplate. In the L4 vertebra, the pins are placed just caudal to the cranial endplate. After pin placement, 6-mm diameter stainless steel posts were seated on the ends of the pins through 4 small dorsal stab incisions. As shown in Figure 1, the posts extend dorsally percutaneously approximately 2 cm. In 16 of the 21 animals, the lumbar intervertebral discs were left intact. Animals were allowed a 3-week quiescent period to allow healing of the surgical site and bony ingrowth around the pins.

\subsection*{Loading Apparatus}
Controlled cyclic axial compression and distraction loading was applied to a single motion segment of the instrumented spines using a custom-designed loading apparatus shown in Figure 2. The \textit{in vivo} loading apparatus applied cyclic loading \textit{via} the percutaneous posts and transfixing pins implanted into the lumbar spine. A fully awake and alert rabbit was placed in a restrainer and the percutaneous posts were attached to the loading apparatus \textit{via} a pair of clamps. The clamps were connected, \textit{via} a series of bearings, to a computer-controlled linear actuator and a system of weights and pulleys, allowing for the application of displacement and load, respectively.

We have previously described the loading apparatus and spine interface used in this study.\textsuperscript{12} The animal model is based on the mouse tail model originally developed by Lotz \textit{et al.}\textsuperscript{13} In that model, transfixing pins were placed through adjacent caudal (tail) vertebrae and mechanical loads were applied externally. The model was subsequently adapted to the rat tail.\textsuperscript{14} However, there are limitations to the caudal disc model
and caudal discs respond differently to mechanical stimuli than lumbar discs. The transfixing pin technique was then adapted to the rabbit lumbar spine but only with application of static loads. We then developed our external dynamic loading apparatus for applying cyclic loads to the lumbar spine in vivo. We have previously validated our model using a spine phantom instrumented with a load cell. At all load magnitudes and frequencies, the error between target and applied load is less than 5%.

Model of Intervertebral Disc Degeneration
In 5 of the 21 surgical animals, after pin and postplacement and before closing the incision, degeneration was induced at 2 levels of the lumbar spine via puncture with a 16G needle. Two discs in each animal were punctured—the disc between the pins (L45) and the disc 2 levels cranial (L23). In animals with punctured discs, degeneration was allowed to progress for 8 weeks with no additional manipulations during that time.

MRI Contrast Administration and Cyclic Loading
At the conclusion of the quiescent periods, post–contrast-enhanced MRI was used to quantify the contribution of cyclic loading to transport in the intervertebral disc. Animals in the loaded groups were attached to the loading apparatus. All animals were administered 0.3 mmol/kg of gadodiamide (Omniscan, GE Healthcare, United Kingdom, MW = 573) intravenously as a bolus. Thirteen animals in the intact (nonpunctured) group were subjected to low-rate, low-frequency axial compression and distraction loading (2.0 s/cycle, 0.5Hz, 0–200 N) for 2.5 minutes (n = 2), 5 minutes (n = 3), 10 minutes (n = 3), 15 minutes (n = 3), or 20 minutes (n = 2). Three animals in the nonpunctured group were subjected to high-rate, low-frequency loading (0.2 s/cycle, 0.5Hz, 0–200 N) for 10 minutes. Because the frequency of loading was the same for all animals, the total number of loading cycles was the same for the 10-minute low-rate group as it was for the 10-minute high-rate group. Three additional control (nonsurgical) animals were administered gadodiamide and allowed to sit without activity for 10 minutes.

Animals in the puncture (degenerated) group were subjected to 2.5 minutes (n = 3), 5 minutes (n = 1), or 10 minutes (n = 1) of low-rate axial compression and distraction loading (0.5 cycles/s, 200 N) after administration of gadodiamide.

Post–Contrast-Enhanced T1 MRI
Immediately at the conclusion of the designated loading or rest period, animals were euthanized and the lumbar spines were harvested. The spine was sectioned into motion segments and immersed in Fomblin Y04 (Solvay Solexis, Brussels), which facilitates high-quality magnetic resonance images by eliminating edge artifact. A Bruker 7T PharmaScan MRI (Bruker BioSpin, Billerica, MA) was used to obtain sagittal images for T1 relaxation time constant mapping using a RARE sequence (TR1–TR7 = 195–3000 ms, TE = 12.5 ms). Gadodiamide is a T1 shortening agent, thus the T1 constant provides a quantitative measure of gadodiamide transport into the disc. In animals with punctured discs, a series of images were acquired for T2 relaxation time constant mapping using an multi-slice multi-echo sequence (TR = 3000 ms, TE1–TE7 = 10.5–84.1 ms). T2 relaxation times correlate to water content and subsequently are an indirect assessment of disc health and an indicator of disc hydration.

T1 relaxation time constants were quantified in a circular region of interest in the nucleus pulposus (NP) at the loaded level. To normalize, T1 constants at the loaded levels were compared with the T1 constants at the adjacent, unloaded level of each animal. T2 constants were also quantified for a circular region of interest in the NP for punctured and intact discs. At the 10-minute time point, T1 constants at the experimental levels (L45; unloaded control, high-rate and low-rate loading) were normalized to their adjacent, unloaded levels (L23) as shown in Figure 3.

Histology
Degeneration of punctured discs was verified via histology. After the collection of MRI data, formalin fixed and decalcified lumbar motion segments from puncture-degenerated and intact groups were processed for histology. Forty-micrometer thick midsagittal cryosections of each motion segment were harvested. The spine was sectioned into motion segments and before closing the incision, degeneration was induced at 2 levels of the lumbar spine via puncture with a 16G needle.

Figure 2. A New Zealand white rabbit attached to the external loading apparatus for the application of dynamic in vivo mechanical loading.
cut at −20°C using a Micron HM505E cryostat (MICROM, Waldorf, Germany) and mounted on labeled microscope slides. Tissue sections were stained with safranin-O and fast green to assess disc proteoglycan content and overall disc structure. The intensity of safranin-O staining in the NP was quantified in ImageJ (NIH, Bethesda, MD), using the histogram tool.

Statistical Analyses
Statistical analyses were conducted using Minitab (Minitab Inc., State College, PA). Statistical differences in NP T1 constants between loaded and unloaded levels of animals subjected to low-rate loading were assessed via paired t tests at the 5, 10, and 15-minute time points for healthy discs and at the 2.5-minute time point for degenerative discs. To compare the effects of loading rate on transport, statistical differences between control, high-rate and low-rate loaded groups were assessed at 10 minutes using an analysis of variance with Fisher post hoc test. Statistical differences in NP T2 constants and safranin-O staining intensity between punctured and intact discs were assessed using independent means t tests.

RESULTS
The 21 surgical animals tolerated the surgery well. There were no complications related to dynamic in vivo loading.

Degeneration Induced by Needle Puncture
At 8 weeks after needle puncture, histology showed substantial alterations to overall disc structure, which were indicative of degeneration. As shown in Figure 4, reductions in disc height, disorganization of the annulus fibers, and fibrosis of the NP were observed on histology of punctured discs (Figure 4B) relative to intact discs (Figure 4A). T2 constant mapping illustrated a significant mean 25.5% ($P < 0.001$) reduction in nucleus T2 constants in punctured discs compared with intact discs. This corresponded with a significant mean 22.5% ($P = 0.004$) reduction in safranin-O staining intensity in the nucleus of punctured versus intact discs. These results indicate a loss of nucleus proteoglycans in the punctured discs compared with intact controls.

Convection in Healthy and Degenerative Discs
In intervertebral discs subjected to low-rate loading, transport into the disc is governed by a combination of diffusion...
plus loading-induced convection. At adjacent, unloaded discs, transport is governed by diffusion alone. Low-rate axial compression and distraction loading simulated the rapid uptake and clearance of gadodiamide in the NP, as shown in Figure 5. In healthy discs, transport into the nucleus was enhanced at 5 minutes ($P < 0.001$) and 10 minutes ($P = 0.03$) of low-rate loading by a mean $16.8\%$ and $7.59\%$, respectively, compared with unloaded control discs.

In degenerative discs, low-rate axial compression and distraction loading applied for 2.5 minutes significantly enhanced net transport of gadodiamide into the NP of the loaded disc compared with the unloaded degenerative disc by a mean $12.6\%$ ($P = 0.008$). As shown in Figure 6, the transport kinetics of gadodiamide uptake and clearance were accelerated in degenerated discs, with maximal enhancement occurring at 2.5 minutes of loading in degenerative discs compared with 5 minutes of loading in healthy discs.

**Effects of Loading Rate in Healthy Discs**

In intact healthy discs, low-rate axial compression and distraction loading significantly enhanced gadodiamide concentration in the NP by a mean $6.5\%$ compared with the unloaded control animals ($P < 0.001$). After the same number of cycles of high-rate loading, gadodiamide concentration in the nucleus was decreased by a mean $16.8\%$ compared with the unloaded control discs ($P < 0.001$), as shown in Figure 7.

**DISCUSSION**

Our results illustrate for the first time that loading-induced convection can augment the transport of small molecules to and from the intervertebral disc *in vivo*. In discs subjected to cyclic loading, transport into the disc is governed by a combination of diffusion plus loading-induced convection. At the adjacent, unloaded levels of the lumbar spine, transport is governed by diffusion alone. Our results demonstrate the dynamic nature of convective transport in the disc. At early time points, the net concentration of gadodiamide in the disc is enhanced compared with diffusion only controls, whereas at later time points, net gadodiamide concentration is reduced as it is cleared from the disc via convective fluid flow. In a healthy disc, net transport in the nucleus was maximally enhanced by a mean $16.8\%$ with the application of low-rate loading compared with unloaded discs.

To our knowledge, this is the first study to illustrate this phenomenon *in vivo*. Results from this study contrast with the work by Urban et al., which reported no contribution of convection to net transport in the discs of moving versus nonmoving dogs. In this previous work, however, net transport into the disc was quantified at only a single time point 2 hours after bolus tracer administration. It is possible that the dynamics of tracer uptake and clearance were affected at early time points in that study, but at the time point measured, the tracer had cleared and concentration had reached a steady state. Our data indicate that convection affects both small molecule uptake and clearance, and thus quantifying transport at multiple, early time points is necessary to demonstrate the contribution of convection to net transport.

Our results also illustrate that net transport into the disc is significantly affected by the rate of mechanical loading. Ten minutes of low-rate loading significantly enhanced transport into the nucleus by a mean $6.5\%$, whereas the same number of cycles of high-rate loading significantly reduced net transport into the disc by a mean $16.8\%$. The dependence of transport on loading rate is likely due to the viscoelastic nature of the intervertebral disc. At low rates of loading, substantial deformation of the disc occurs, stimulating bulk fluid flow and convective transport. At high rates of loading, transport into the disc is restricted, likely caused by loading-induced increases in nucleus fixed charge density and hydrostatic pressure.

As the mechanical and biochemical properties of degenerative discs differ greatly from healthy discs, we also investigated the effects of low-rate loading on net transport in a degenerative model. Disc degeneration was induced in the New Zealand white rabbit lumbar spine via puncture of the disc with a 16G needle. This puncture model reproduces many of the hallmark characteristics of degeneration...
commonly observed in humans, including reductions in disc height, structural changes to the nucleus and annulus, and loss of proteoglycans.\textsuperscript{21,22}

The magnitude of the therapeutic effects from loading was comparable for healthy and degenerated discs. In degenerated discs, low-rate loading maximally enhanced transport into the nucleus by a mean 12.6%. However, the transport kinetics of small molecule uptake and clearance were shifted in the degenerated discs compared with the healthy discs. This is likely due to the loss of proteoglycans in the degenerated discs, which reduces the osmotic pressure, reduces the stiffness of the disc, and increases deformation in response to a compressive load.\textsuperscript{21,24} Increased deformation of the disc with loading could stimulate greater bulk fluid flow than in the healthy disc, leading to the acceleration of transport kinetics in the disc.

Although the results for this study are promising, there are limitations. In this exploratory study, the sample sizes of each treatment at each time point are small. In addition, there are only 5 time points from 2.5 to 20 minutes. We were also able to compare only the effects of loading rate at a single 10-minute time point. Ideally, the kinetics of uptake and clearance should be characterized at additional time intervals for a longer period of time with larger sample sizes at each time point. The effects of cyclic loading rate should be evaluated at each time point to quantify differences in uptake and clearance and additional loading rates should be explored. In spite of the small sample size, there were statistically significant differences between treated discs and control discs at almost all time points and treatments in this study. This suggests that cyclic loading can have a substantial treatment effect on trans-endplate transport. Further research with larger sample sizes and additional treatment groups and time points is necessary to fully characterize the potential enhancement of transport via cyclic loading.

Overall, results from this research may have significant implications for the clinical treatment of low back pain and disc degeneration. Clinically, degenerative discs demonstrate a reduction in diffusion of 11.5% to 15%,\textsuperscript{21,24} In our animal model, we were able to enhance transport by 16.8% in a healthy disc and by 12.6% in a degenerative disc. This suggests that in a degenerative disc, forced convection is a potential strategy to restore transport to the levels of normal healthy discs. Enhanced uptake of nutrients and clearance of byproducts could enhance the regenerative potential of the disc. Clinically, enhanced transport could be achieved via physical therapy regimens that cause cyclic compression and distraction of the lumbar discs.\textsuperscript{27} Enhancing transport into the discs of patients with early-stage disc degeneration via forced convection could stimulate a regenerative response, potentially culminating in the slowing or reversal of the degenerative cascade.

### Key Points

- Low-rate cyclic loading enhances small molecule transport via forced convection.
- High-rate cyclic loading diminishes small molecule transport.
- Net transport was maximally enhanced by a mean 16.8% and 12.6% in healthy and degenerative discs, respectively, subjected to low-rate loading.
- The transport kinetics of small molecule uptake and clearance is different for healthy versus degenerative discs.

### References


