



Intervertebral disc recovery after dynamic or static loading in vitro: Is there a role for the endplate?

A.J. van der Veen^{a,*}, J.H. van Dieën^b, A. Nadort^a, B. Stam^a, T.H. Smit^a

^a*VU University Medical Center, Department of Physics and Medical Technology, de Boelelaan 1117, 1081HV Amsterdam, The Netherlands*

^b*Faculty of Human Movement Sciences, VU University, Amsterdam, The Netherlands*

Accepted 19 October 2006

Abstract

In vivo studies on disc mechanics show loss of fluid from the intervertebral disc (IVD) during loading and full recovery during rest. Previous work indicated that in vitro recovery is hampered after static loading. The aim of the present study was to investigate the role of the endplate after dynamic and static loading on mechanical recovery in vitro.

Lumbar spines (caprine) were obtained from the local slaughterhouse and stored frozen. Twenty-four intervertebral discs were thawed and subjected to a compression test in a saline bath (37 °C).

The discs were pre-loaded at 20 N for 15 min. Three 15-min loading cycles (static: 2.0 MPa or dynamic: average load 2.0 MPa at 0.5 Hz) were applied, each followed by a 30-min period of unloading (20 N). After this protocol, the endplates of half of the discs were blocked with silicone paste and the long-term recovery protocol was applied; the discs were subjected to a single loading cycle (15 min of static or dynamic loading) followed by 10 h of unloading at 20 N.

All specimens showed a net loss of height and a gain in stiffness during the first part of the test. Eventually, height and stiffness were restored during a long-term recovery test. The difference in recovery between blocked and free endplates was marginal.

If fluid flow plays a role during recovery in vitro, the role of the endplate appears to be limited. Our findings show no influence of loading type on recovery in vitro.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Spine; Intervertebral disc; Fluid flow; Mechanical testing

1. Introduction

The primary function of the intervertebral disc (IVD) is to confer flexibility to the spine under intrinsic loads, due to gravity and muscular forces (Wilke et al., 1999; Nachemson and Morris, 1964). The mechanical properties of the IVD are non-linear (Panjabi et al., 1994; Kaigle et al., 1997). This is attributed to the complex structure of the IVD and to the material properties of its components, which are both visco-elastic and poro-elastic. The mechanical properties of the IVD are highly dependent on the water content of the disc (Gardner-Morse and Stokes, 2004; Kraemer et al., 1985; Iatridis et al., 2003; Huyghe et al., 2003; Perie et al., 2005). Consequently, fluid flow

plays an important role in the mechanical behavior of the IVD (Iatridis et al., 1997; White and Panjabi, 1990; Adams et al., 1996a, b; van Dieën et al., 2001; Koeller et al., 1984; McMillan et al., 1996).

The IVD continuously tends towards equilibrium between the external load on the spine and the swelling pressure of the disc (Urban and McMullin, 1988). The swelling pressure mainly depends on the proteoglycan concentration in the nucleus. The hydration of the IVD varies under the influence of loading. During daily activity, gravity and muscle forces will cause an increase in intradiscal pressure. As a result, the equilibrium is disturbed and the fluid flow is shifted towards outflow of fluid. This will increase the proteoglycan concentration and consequently the swelling pressure of the IVD until equilibrium is reached (Kraemer et al., 1985; Urban and McMullin, 1985). During rest, the flow direction is

*Corresponding author. Tel.: +31 204448304; fax: +31 204444147.

E-mail address: aj.veen@vumc.nl (A.J. van der Veen).

reversed. As a result, fluid flows back into the disc (Malko et al., 2002) and the IVD regains its mechanical properties. This cycle repeats itself in a stable daily pattern.

There are two possible pathways for fluid into the IVD. Fluid can travel through channels in the endplate and through the annulus fibrosus (Ogata and Whiteside, 1981). In vivo it has been shown that intravenously administered markers predominantly travel through the endplate (Rajasekaran et al., 2004). In addition, it has been suggested that the resistance of these channels depends on the flow direction (Ayotte et al., 2001): the resistance of inflow is thought to be lower than the resistance to outflow. This would explain why full recovery of water content could occur despite the fact that in vivo the available time for recovery is shorter than the loading time.

The majority of mechanical tests on IVDs have been performed in vitro. In vivo measurements are very complex, the applied loads on the disc are high and the accessibility is, obviously, low. We have shown earlier that the mechanical behavior of an IVD in vitro, submitted to static compression, does not resemble the in vivo behavior (van der Veen et al., 2005). Disc height was not regained after unloading, even if the duration of unloading was twice as long as the duration of loading. The fluid inflow, during recovery, was apparently hampered. We postulated that this is due to restricted flow through the endplate. This finding, however, appears to be in contrast with those of a study reporting full recovery of IVD mechanical properties (Johannessen et al., 2004). In this study, restoration of stiffness was found after a long recovery period following dynamic compression. Since full mechanical recovery was found, recovery of disc height was assumed, while in our study recovery was found to be incomplete. The two studies differed in loading type (static versus dynamic), parameter studied (disc height and mass versus stiffness) and length of the recovery period. In vivo loading contains both static and dynamic contributions and applying only static loading could be of influence on disc recovery. For example, the one-way valve, described by Ayotte et al. (2000), could be damaged by a constant high pressure. Alternatively, change of disc height during loading is not a direct measure for restoration of disc height. Therefore, stiffness might be restored even when disc height is not. Finally, recovery of both disc height and stiffness may require a much longer recovery period than we previously allowed for.

In the present study, we therefore directly compared the effect of loading type on restoration of disc height and stiffness of IVDs in vitro. Secondly, we investigated the effect of blocking the endplate route for fluid inflow on these properties of IVDs in vitro.

2. Materials and methods

Lumbar spine segments L1–L5 from 6 Dutch milk goats (± 4 years old, ± 60 kg) were obtained from the local slaughterhouse and frozen in their entirety for later usage. Before each test, a single disc was located by

radiographic examination and cut from the frozen spine with a band saw. In order to have maximal access for fluid to the endplate, the adjacent vertebral bodies were cut off as close to the endplate as possible. Subsequently, the cutting edge was brushed clean and rinsed out.

Mechanical tests were performed with a hydraulic testing device (Instron 8872, Canton, MA). During testing, the IVD specimens were placed between porous plates (pore size 40–100 μm), which allowed free passage of fluid to and from the endplates of the remaining IVDs (Maclean et al., 2006; van der Veen et al., 2005). All tests were performed in a saline bath (0.9% NaCl) at a temperature of 37°C (body temperature).

The outline of each IVD was transferred to graph paper before testing and the area of the endplate was calculated. This area was used to determine the compression load, required to obtain the test pressure of 2.0 MPa. The pressure corresponds to the nucleus pressure in humans during lifting a 20 kg mass (Wilke et al., 1999). It is expected that the compression load in quadrupeds is generally higher than in humans (Smit, 2002). The control of the testing device requires a small offset during the unloading in order to prevent backlash. This offset load during recovery was 20 N. The specimens were pre-loaded at a compression load of 20 N for 15 min to allow the disc to adjust to the test environment before testing started.

Segments were divided into two groups, the static group, of which the IVDs were subjected to static loading and the dynamic group; the discs in this group were subjected to dynamic (sinusoidal) loading. Each group consisted of twelve IVDs.

The test was divided into two subsequent protocols. The first protocol of the static group consisted of three loading cycles, each cycle consisted of a static loading phase of 2.0 MPa (15 min), followed by a recovery phase at 20 N (approximately 0.05 MPa). At the end of each cycle, a stiffness test was performed (ramp loading, 0.05–0.8 MPa in 2 s). The first protocol was immediately followed by the second protocol: the long-term recovery test. During the second protocol, the endplates of half of the samples of the static group (6 samples) were blocked with silicone paste. The long-term recovery test consisted of a single static loading phase at 2.0 MPa (15 min), followed by 10 h of recovery at 20 N. Blocking the endplates with silicone paste required a temporary removal from the saline bath. In order to stabilize the discs at the beginning of the second protocol, the long-term recovery test began with 15-min pre-loading at 0.05 MPa, to allow the IVD to regain equilibrium before testing.

The first protocol of the dynamic group consisted of three loading cycles. Each cycle in this group consisted of a dynamic loading phase with an average of 2.0 MPa (sinusoidal; 0.5 Hz, range: 0.05–3.95 MPa; 15 min), followed by a static recovery phase at 20 N (30 min) and a stiffness test (ramp loading, 0.05–0.8 MPa in 2 s). The first protocol was immediately followed by the second protocol: the long-term recovery test. During the second protocol, the endplates of half of the samples of the dynamic group were blocked. The long-term recovery test of the dynamic group consisted of a dynamic loading phase (average 2.0 MPa, 15 min), followed by 10 h of recovery at 20 N.

In all tests, the applied force and the vertical displacement were recorded at a frequency of 2 Hz. Repeated measures analyses of variance were performed to test for effects of cycle (within factor, 3 levels) and loading type (between factor, dynamic versus static) on the following dependent variables: height loss over the cycle, change of stiffness over the cycle, and height recovery in the second part of the cycle. Pearson's coefficient of correlation was calculated between height loss and change of stiffness over the cycles. Student *t*-tests were used to compare height gain and stiffness after long-term recovery between samples with and without blocked endplates. Statistical analyses were performed using SPSS11.5.

3. Results

Each IVD lost height over the subsequent loading cycles in both the dynamic protocol and the static protocol. The loss of height per full cycle (comprising a loading and an unloading phase) shows that 30 min of unloading did not

compensate for 15 min of loading (Figs. 1 and 2). The average reduction in disc height per load cycle was compared between the static and dynamic loading protocol. No main effects or interactions of loading type and time were found (Table 1). Also the height recovery after each loading cycle was not significantly affected by loading type (Fig. 3; Table 1).

The change in IVD stiffness was opposite to the changes in disc height (Fig. 4). The stiffness increased after each

loading cycle and decreased back to baseline values after the long-term recovery. Again no main effects of loading type or interaction effects with loading type were found (Table 1). The coefficient of correlation between pooled disc height and of stiffness changes was -0.833 . The results of protocol 1 showed that loading type did not affect changes in disc height and stiffness.

In the long-term recovery phase, the discs gained an amount of height that was similar to the height loss over the preceding loading cycles (Figs. 1 and 2). Similarly, the decrease in stiffness after the long-term recovery was approximately equal but opposite to the loss of stiffness

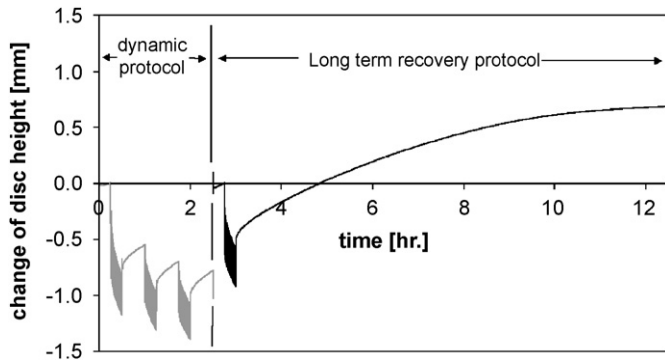


Fig. 1. Typical displacement curve for a disc in the dynamic group. The test is divided into two subsequent protocols. The first protocol (in gray) consisted of three loading cycles, each cycle comprising 15 min of loading (which due to the time scale appears as a shaded area), followed by 30 min of recovery. The second protocol (in black) consisted of one loading phase followed by 10 h of recovery. All samples were removed from the materials testing machine in between the first and second protocol.

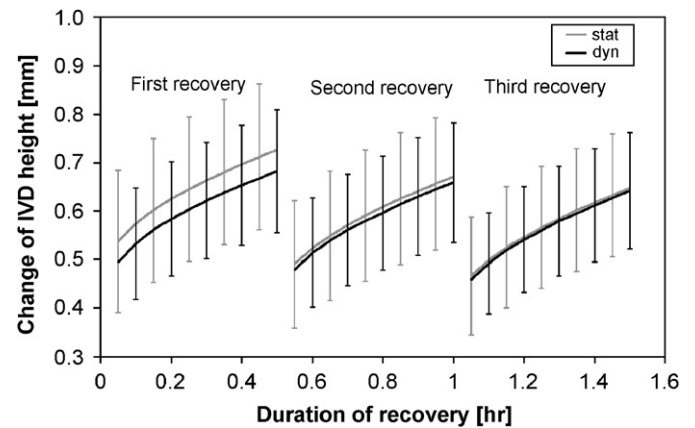


Fig. 3. The average gains of height (error bars sd) of the static and dynamic group during the subsequent unloading phases. The averages of the two groups were not significantly different.

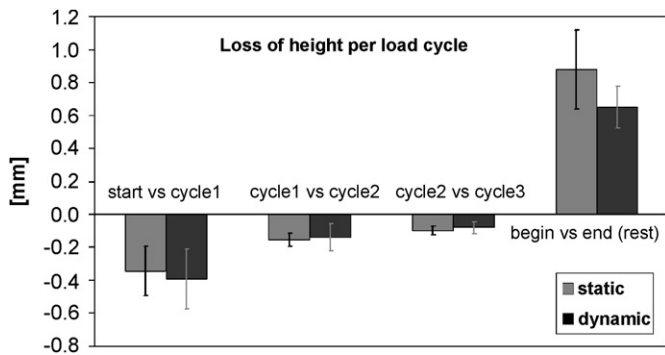


Fig. 2. Average changes of disc height (sd error bars) over the first, second, and third loading cycles. The change of disc height was measured at the end of each recovery phase. The last bar is the change during long-term recovery, measured between the beginning of the loading phase and the end of the recovery phase.

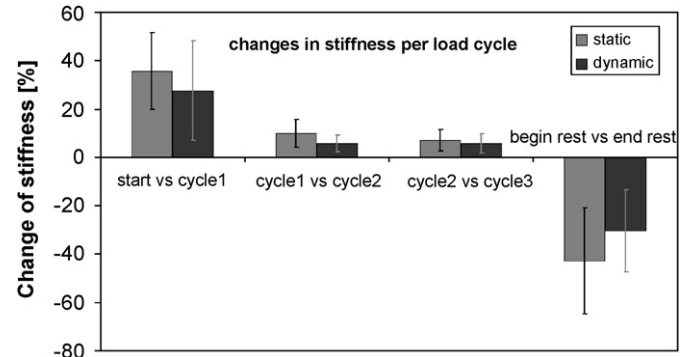


Fig. 4. Average changes in stiffness (error bars sd) during the first, second, and third full cycles and during long-term recovery. Stiffness test were performed at the end of each loading cycle.

Table 1

Repeated measures analyses of variance were performed to test for effects of cycle (within factor, 3 levels) and loading protocol (between factor: static versus dynamic)

	Height loss		Height recovery		Stiffness	
	F	p	F	p	F	p
Loading type	0.043	0.836	0.179	0.676	2.920	0.099
Cycle	78.805	<0.001	29.323	<0.001	50.102	<0.001
Loading type * cycle (interaction)	1.355	0.261	2.733	0.102	0.733	0.424

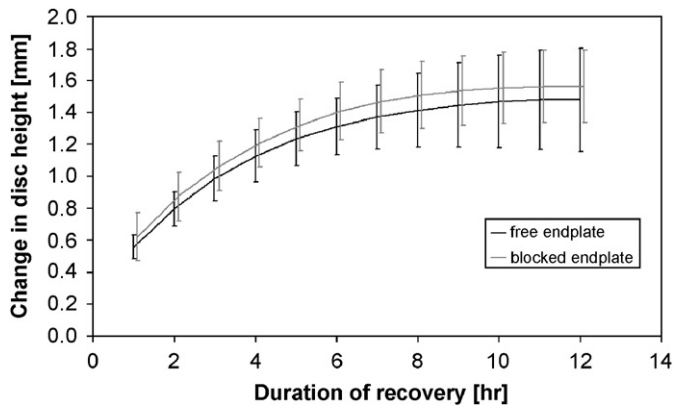


Fig. 5. Average gains in height (error bars sd) during the long-term recovery phase of discs with blocked and free endplates. Gains of disc height were measured from the beginning of the recovery phase and were not significantly different between the groups.

over protocol 1 (Fig. 4). These results indicate that long-term recovery allowed full mechanical recovery. However, the values after protocol 1 and 2 cannot be compared exactly, because the discs were taken from the materials testing machine in between the two protocols to seal the endplates of half of the samples. Comparing the samples with a blocked endplate to those with a free endplate during second protocol (Fig. 5), no significant differences in change of disc height or in stiffness of the specimen were found ($p = 0.725$ and 0.606 , respectively, Figs. 2 and 4).

4. Discussion

The disc has to perform its function in an environment with continuously changing loads. Mechanically this leads to a non-equilibrium state between the external load and the osmotic pressure in the disc, which results in changes in water content (McMillan et al., 1996; Pflaster et al., 1997). In vivo measurements of the signal intensity in MRI images of discs clearly show an increase in water content after a night's rest (Malko et al., 2002) and during the night disc height lost during the day is regained (McGill and Axler, 1996; Reilly et al., 1984). It has previously been found that recovery is hampered during in vitro testing of IVDs (van der Veen et al., 2005; Maclean et al., 2006). On the other hand, Johannessen et al. (2004) have reported full recovery of disc mechanical behavior in vitro. The purpose of this present study was to establish if this disparity can be attributed to loading type, duration of the unloading phase, or to the mechanical parameters used. In addition, we studied the importance of the endplate route versus the annulus route for fluid inflow for both protocols in vitro.

In the present study, all samples lost height after the three loading cycles (comprising a loading and a recovery phase), implying that 30 min of unloading did not compensate 15 min of loading. In both protocols, disc height was lost during the loading protocol, whilst no significant differences were found in the gains of disc height

during long-term unloading. Therefore, we conclude that the loading type does not affect the recovery of the IVD, with respect to disc height. However, the height gain in the 10-h recovery period appeared to allow a full recovery of disc height.

The instantaneous water content of the nucleus depends on the loading history of the disc (Urban and McMullin, 1988). The stiffness (or the initial height) of an IVD is therefore not a constant property of the disc but changes with the loading history. The axial stiffness of the IVD increased after each full cycle, in both loading protocols, and regained its original value after the long-term recovery. This implies that the axial flexibility of the spine, in vivo, declines during the day. In both dynamic and static loading, stiffness increased after loading and regained its original value during long-term unloading. Therefore, we conclude that the loading type does not influence the changes in stiffness of the disc.

In contrast with our previous findings, Johannessen et al. (2004) reported full recovery of disc mechanical properties after cyclic loading. The present study shows that this disparity was not due to loading type (static versus dynamic) or to differences in mechanical parameters studied (disc height versus stiffness). The disparity can be accounted for solely by differences in length of the recovery period. In the present study, full recovery was found of all parameters studied after long term recovery following both static and dynamic loading. However, the recovery phase required was, compared to the loading time, unphysiologically long, 18 h after 3 h of loading in the study of Johannessen et al. (2004) and 10 hours after 2.5 h of intermittent loading in the present study. This suggests that inflow of fluid into the disc is slow in vitro compared to the outflow. This is in contrast to in vivo and in vitro measurements that indicate that a short period of net inflow (8 h in humans) suffices to compensate a long period of net outflow (16 h in humans) (Ayotte et al., 2001; Tyrrell et al., 1985; Malko et al., 2002).

During the long-term recovery protocol, the endplates of half of the samples were blocked. One of the major routes for fluid into the disc was, therefore, no longer accessible. Surprisingly, this had hardly any effect on the recovery of the disc. Apparently, fluid inflow occurred solely via the annulus route. We have previously hypothesized that in the in vitro tested discs fluid flow through the endplate route is hampered in contrast with the in vivo situation possibly due to blood clots (van der Veen et al., 2005). The present results clearly support this hypothesis. In addition, Lee et al. (2006) found cell death in an IVD in culture, which they attributed to nutritional pathways through the endplate being blocked by blood clots. Alternatively, the endplate route could be less important for disc mechanics than commonly assumed in literature. This, however, needs further study.

The environment during testing was designed to mimic the in vivo conditions. The bath temperature was raised to body temperature, the endplates of the discs were accessible

for fluid from the bath and the average load was in a physiological range corresponding to two times body weight. The long duration of the test, combined with the high temperature of the bath (37 °C) could accelerate decomposition of the disc. But the virtually identical responses during successive recovery phases indicate that decomposition did not play any role during the first part of the test, in line with tests on ovine discs (Costi et al., 2002). However, an effect in the second part of the test, the long-term recovery phase, is conceivable, although complete recovery of height and stiffness argues against it. In addition, since the IVDs in both the dynamic and static protocol were subjected to the same conditions, any effect of decomposition on the IVDs will be approximately the same.

For practical reasons, we have chosen to use frozen materials. A recent study reveals no major effects of frozen storage over a time period comparable to the one used in the present study (Dhillon et al., 2001). Ongoing research in our lab supports these findings. Furthermore, the testing conditions are uniform in both loading protocols and cannot account for any differences.

The tests have been performed on IVDs from Dutch milk goats. This model has been used in other studies on the lumbar spine (van Dijk et al., 2002; Wuisman et al., 2002). The anatomy of the disc and the loading situation are comparable to a sheep model, which is an accepted model for spinal research (Wilke et al., 1997). The biochemical composition of a goat disc differs from other species. However, poro-elasticity in the IVD is a phenomenon common to many species (porcine, human, ovine and goat). The goat disc therefore appears to be a valid model for this aspect of disc mechanics.

The IVD is a complex structure. Its mechanical behavior is equally complex. Fluid flow plays an important role in the mechanical behavior of the disc. The findings of the present study supports our earlier findings that the fluid flow through the endplate is hampered in vitro (van der Veen et al., 2005) but also shows that the applied loading protocol, dynamic or static, is of no influence on the mechanical behavior during loading or recovery.

References

- Adams, M.A., McMillan, D.W., Green, T.P., Dolan, P., 1996a. Sustained loading generates stress concentrations in lumbar intervertebral discs. *Spine* 21, 434–438.
- Adams, M.A., McNally, D.S., Dolan, P., 1996b. 'Stress' distributions inside intervertebral discs. The effects of age and degeneration. *Journal of Bone and Joint Surgery—British Volume* 78, 965–972.
- Ayotte, D.C., Ito, K., Perren, S.M., Tepic, S., 2000. Direction-dependent constriction flow in a poroelastic solid: the intervertebral disc valve. *Journal of Biomechanical Engineering* 122, 587–593.
- Ayotte, D.C., Ito, K., Tepic, S., 2001. Direction-dependent resistance to flow in the endplate of the intervertebral disc: an ex vivo study. *Journal of Orthopaedic Research* 19, 1073–1077.
- Costi, J.J., Hearn, T.C., Fazzalari, N.L., 2002. The effect of hydration on the stiffness of intervertebral discs in an ovine model. *Clinical Biomechanics* 17, 446–455.
- Dhillon, N., Bass, E.C., Lotz, J.C., 2001. Effect of frozen storage on the creep behavior of human intervertebral discs. *Spine* 26, 883–888.
- Gardner-Morse, M.G., Stokes, I.A., 2004. Structural behavior of human lumbar spinal motion segments. *Journal of Biomechanics* 37, 205–212.
- Huyghe, J.M., Houben, G.B., Drost, M.R., van Donkelaar, C.C., 2003. An ionised/non-ionised dual porosity model of intervertebral disc tissue. *Biomechanic and Modeling in Mechanobiology* 2, 3–19.
- Iatridis, J.C., Setton, L.A., Weidenbaum, M., Mow, V.C., 1997. Alterations in the mechanical behavior of the human lumbar nucleus pulposus with degeneration and aging. *Journal of Orthopaedic Research* 15, 318–322.
- Iatridis, J.C., Laible, J.P., Krag, M.H., 2003. Influence of fixed charge density magnitude and distribution on the intervertebral disc: applications of a poroelastic and chemical electric (PEACE) model. *Journal of Biomechanical Engineering* 125, 12–24.
- Johannessen, W., Vresilovic, E.J., Wright, A.C., Elliott, D.M., 2004. Intervertebral disc mechanics are restored following cyclic loading and unloaded recovery. *Annals of Biomedical Engineering* 32, 70–76.
- Kaigle, A.M., Holm, S.H., Hansson, T.H., 1997. 1997 Volvo Award winner in biomechanical studies. Kinematic behavior of the porcine lumbar spine: a chronic lesion model. *Spine* 22, 2796–2806.
- Koeller, W., Funke, F., Hartmann, F., 1984. Biomechanical behavior of human intervertebral discs subjected to long lasting axial loading. *Biorheology* 21, 675–686.
- Kraemer, J., Kolditz, D., Gowin, R., 1985. Water and electrolyte content of human intervertebral discs under variable load. *Spine* 10, 69–71.
- Lee, C.R., Iatridis, J.C., Poveda, L., Alini, M., 2006. In vitro organ culture of the bovine intervertebral disc: effects of vertebral endplate and potential for mechanobiology studies. *Spine* 31, 515–522.
- Maclelean, J.J., Owen, J.P., Iatridis, J.C., 2006. Role of endplates in contributing to compression behaviors of motion segments and intervertebral discs. *Journal of Biomechanics*, Epub ahead of print.
- Malko, J.A., Hutton, W.C., Fajman, W.A., 2002. An in vivo MRI study of the changes in volume (and fluid content) of the lumbar intervertebral disc after overnight bed rest and during an 8-hour walking protocol. *Journal of Spinal Disorders & Techniques* 15, 157–163.
- McGill, S.M., Axler, C.T., 1996. Changes in spine height throughout 32 hours of bedrest. *Archives of Physical Medicine and Rehabilitation* 77, 1071–1073.
- McMillan, D.W., Garbutt, G., Adams, M.A., 1996. Effect of sustained loading on the water content of intervertebral discs: implications for disc metabolism. *Annals of Rheumatic Diseases* 55, 880–887.
- Nachemson, A., Morris, J.M., 1964. In vivo measurements of intradiscal pressure. Discometry, a method for the determination of pressure in the lower lumbar discs. *Journal of Bone and Joint Surgery—American Volume* 46, 1077–1092.
- Ogata, K., Whiteside, L.A., 1981. 1980 Volvo award winner in basic science. Nutritional pathways of the intervertebral disc. An experimental study using hydrogen washout technique. *Spine* 6, 211–216.
- Panjabi, M.M., Oxland, T.R., Yamamoto, I., Crisco, J.J., 1994. Mechanical behavior of the human lumbar and lumbosacral spine as shown by three-dimensional load–displacement curves. *Journal of Bone and Joint Surgery—American Volume* 76, 413–424.
- Perie, D., Korda, D., Iatridis, J.C., 2005. Confined compression experiments on bovine nucleus pulposus and annulus fibrosus: sensitivity of the experiment in the determination of compressive modulus and hydraulic permeability. *Journal of Biomechanics* 38, 2164–2171.
- Pflaster, D.S., Krag, M.H., Johnson, C.C., Haugh, L.D., Pope, M.H., 1997. Effect of test environment on intervertebral disc hydration. *Spine* 22, 133–139.
- Rajasekaran, S., Babu, J.N., Arun, R., Armstrong, B.R., Shetty, A.P., Murugan, S., 2004. ISSLS prize winner: a study of diffusion in human lumbar discs: a serial magnetic resonance imaging study documenting the influence of the endplate on diffusion in normal and degenerate discs. *Spine* 29, 2654–2667.

- Reilly, T., Tyrrell, A., Troup, J.D., 1984. Circadian variation in human stature. *Chronobiology International* 1, 121–126.
- Smit, T.H., 2002. The use of a quadruped as an in vivo model for the study of the spine—biomechanical considerations. *European Spine Journal* 11, 137–144.
- Tyrrell, A.R., Reilly, T., Troup, J.D., 1985. Circadian variation in stature and the effects of spinal loading. *Spine* 10, 161–164.
- Urban, J.P., McMullin, J.F., 1985. Swelling pressure of the intervertebral disc: influence of proteoglycan and collagen contents. *Biorheology* 22, 145–157.
- Urban, J.P.G., McMullin, J.F., 1988. Swelling pressure of the lumbar intervertebral discs: influence of age, spinal level, composition, and degeneration. *Spine* 13, 179–187.
- van der Veen, A.J., Mullender, M., Smit, T.H., Kingma, I., van Dieen, J.H., 2005. Flow-related mechanics of the intervertebral disc: the validity of an in vitro model. *Spine* 30, E534–E539.
- van Dieen, J.H., Kingma, I., Meijer, R., Hansel, L., Huiskes, R., 2001. Stress distribution changes in bovine vertebrae just below the endplate after sustained loading. *Clinical Biomechanics* (Bristol, Avon) 16 (Suppl. 1), S135–S142.
- van Dijk, M., Smit, T.H., Burger, E.H., Wuisman, P.I., 2002. Bioabsorbable poly-L-lactic acid cages for lumbar interbody fusion: three-year follow-up radiographic, histologic, and histomorphometric analysis in goats. *Spine* 27, 2706–2714.
- White, A.A., Panjabi, M.M., 1990. *Clinical Biomechanics of the Spine*. J.B. Lippincott Company, Philadelphia, pp. 14–15.
- Wilke, H.J., Kettler, A., Claes, L.E., 1997. Are sheep spines a valid biomechanical model for human spines? *Spine* 22, 2365–2374.
- Wilke, H.J., Neef, P., Caimi, M., Hoogland, T., Claes, L.E., 1999. New in vivo measurements of pressures in the intervertebral disc in daily life. *Spine* 24, 755–762.
- Wuisman, P.I., van Dijk, M., Smit, T.H., 2002. Resorbable cages for spinal fusion: an experimental goat model. *Orthopedics* 25, s1141–s1148.