Effect of sustained loading on the water content of intervertebral discs: implications for disc metabolism

D W McMillan, G Garbutt, M A Adams

Abstract

Objective—To examine regional changes in the fluid content of human intervertebral discs by comparing sagittal plane "profiles" of hydration before and after mechanical loading.

Methods—Cadaveric lumbar intervertebral discs were loaded to simulate a typical day's loading in vivo. Ten motion segments were subjected to a 1500 N compressive load for a period of 6 h with the superior vertebrae inclined by 4–8° to simulate a slightly flexed posture. Immediately after loading the discs were frozen at −80°C. Subsequently they were cut into slices perpendicular to the sagittal midline of the disc, and each slice was weighed before and after freeze drying. This enabled a profile of fluid content across the disc to be constructed. Fluid loss due to loading was estimated by comparing the water content of each loaded disc with that of an adjacent unloaded disc from the same spine.

Results—After 6 h of creep loading, disc height approached, but did not quite reach, an equilibrium. The mean fluid loss from all discs was 18%. All regions except the outer 2 mm experienced a significant loss of fluid (P < 0.01). The posterior mid-annulus showed the greatest fluid loss (30%), while the nucleus lost 15%.

Conclusions—A comparison with previously published work suggests that fluid exchange of this magnitude will have a considerable effect on disc cell metabolism and on metabolite transport.


The fluid content of the intervertebral disc is not constant but varies with external load and load history. When a load is applied to a disc, resulting in a stress that exceeds the osmotic pressure of the tissue, fluid is expelled. The proteoglycan concentration and hence osmotic pressure are thus increased as fluid is lost, until an equilibrium is reached and the osmotic pressure once again balances the applied stress. When the disc is unloaded, it imbibes fluid to achieve equilibrium. During daily activities, there is a net flow of fluid out of the disc, which is reversed at night when the disc is unloaded. In vivo magnetic resonance imaging (MRI) measurements indicate that the water content of lumbar discs falls by about 20% over the course of a day's activity.

Mechanically induced fluid loss from intervertebral discs may have a profound effect on disc metabolism. It is known that the cells of the disc respond to mechanical loading. Recent work suggests that the influence of load on the metabolism of articular cartilage cells is mediated through the effect on tissue fluid content and it is likely that the cells of the disc respond in a similar way. Whether the hydration of the intervertebral disc is changed osmotically or by mechanical loading, the observed effects on cell metabolism are similar. Thus the cells of the disc are sensitive to changes in hydration of the surrounding matrix.

Cellular activity is also dependent on the supply of nutrients and the dispersal of waste products such as lactic acid. Since the disc is avascular, nutrients and waste products must be transported by a combination of diffusion and fluid flow. Fluid flow is the more important mechanism for the movement of high molecular weight proteins which assist in regulating cell function, and large fluid shifts may also have a significant effect on small molecular weight solutes such as glucose. Rapid fluctuations in loading which occur during locomotion result in very little fluid flow and this is confined to the disc periphery. However, prolonged loading may result in much larger fluid exchanges throughout the entire disc.

In vitro experiments on cadaveric material are often used to clarify in vivo processes in biological tissues, but it is possible that postmortem changes may affect the viscoelastic properties of the disc and hence its response to long term mechanical loading. Keller et al have examined the creep response of pigs' spines before and after death. Differences were observed between the in vivo and postmortem response, but similar changes were seen in earlier work, even when the animal was not killed, and may be due to poor reproducibility of repeated creep tests. There is no reliable evidence that postmortem changes affect the in vitro response of the spine motion segment to sustained loading.

In previous experiments on cadaver spines, Adams and Hutton have shown that a compressive load equal to body weight can expel 10–15% of the fluid from lumbar discs in a four hour period, and Kraemer et al found that a slightly higher load can expel 8–11% over a 24 hour period. However, it is not clear just
Sustained loading and water content of intervertebral discs

Table 1  Specimen details of loaded discs and their corresponding control discs

<table>
<thead>
<tr>
<th>Sex</th>
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<th>Control</th>
<th>Loaded</th>
<th>Degeneration (scale 1-4)</th>
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<td>82</td>
<td>L3-L4</td>
<td>L4-L5</td>
<td>4, 3</td>
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Figure 1  Apparatus used to apply load to a motion segment, positioned at an angle \( \alpha \), to simulate a moderately flexed posture in life.

Figure 2  To measure a profile of hydration across the disc, 12 slices were cut from its midline. Six slices were cut from the anterior annulus, two from the nucleus, and four from the posterior annulus.

Subjects were aged between 27 and 82 years and all grades of disc degeneration were present. Degeneration was gauged at initial dissection on a scale of 1-4 using previously published criteria. Specimens were thawed at 3\(^\circ\)C for 12 h before being dissected into motion segments consisting of two adjacent vertebrae, the intervertebral disc, and ligaments. In all, 10 motion segments were used in the experiment. The discs adjacent to motion segments were carefully excised and immediately frozen at -80\(^\circ\)C to be used as controls for the loaded discs. Motion segments were usually dissected from L2-L3 and L4-L5 to make maximum use of cadaver spines, which were normally supplied from L1-L5, though some spines included S1. Occasionally, choice of specimen was influenced by evidence of postmortem damage to the apophyseal joints or vertebral bodies. Control discs were adjacent to loaded discs, and they were chosen so as to randomise any differences in hydration due to degeneration or lumbar level (table 1).

Motion segments were secured in two cups containing dental plaster (fig 1). Tissue dehydration was minimised by wrapping the motion segment in thin polyethylene film before setting in plaster. This ensured a humid environment around the disc, and during creep testing the disc surface appeared wet, suggesting that surface dehydration was not a problem. Water content of intervertebral discs may change slightly after death, and so a preliminary creep test of 300 N was applied to each specimen for 15 min using a Dartec hydraulic materials testing machine (Stourbridge, UK). The rationale behind this preliminary test is discussed below. Specimen height loss was monitored continuously using a linear variable displacement transducer (LVDT) mounted on the moveable ram of the Dartec.

After preliminary loading, each motion segment was positioned in 4-8\(^\circ\) of flexion, depending on its flexibility, and loaded at 1500 N for 6 h (fig 1). This amount of flexion simulates the “flat back” associated with lifting objects from the floor and with sitting, and was sufficient to unload the apophyseal joints and ensured they did not stress-shield the disc as creep progressed. The 1500 N load is appropriate to simulate the time averaged compressive force on the lumbar spine during light manual labour, or during car driving, where muscle tension, and hence spinal loading, is increased by the need to maintain stability in a vibrating environment. Load was not normalised for disc cross sectional area because there is no evidence that disc area is proportional to spinal load in life.

The distribution of compressive stress inside the intervertebral disc was measured by pulling a miniature pressure transducer along the mid-sagittal diameter of the disc while the motion segment was subjected to a compressive load of 2000 N. Measurements were performed before creep loading, and after 2 h and 6 h of creep loading, in order to show how the stress distribution changed following fluid loss from the tissue. Full details are reported elsewhere.

Methods

Five human lumbar spines were collected at necropsy examination and stored in sealed plastic bags at -17\(^\circ\)C for up to three months.

Figure 1  Apparatus used to apply load to a motion segment, positioned at an angle \( \alpha \), to simulate a moderately flexed posture in life.

Figure 2  To measure a profile of hydration across the disc, 12 slices were cut from its midline. Six slices were cut from the anterior annulus, two from the nucleus, and four from the posterior annulus.
Stress measurements after 2 h of loading necessitated a 10–15 min interruption of the creep test, during which time the average compressive force acting on the disc was approximately 1000 N. It is unlikely that this would allow any significant recovery of disc height lost during the preceding 2 h of creep, but this could not be confirmed because it was necessary to re-zero the LVDT when the stress measurements were made. Immediately after testing, the disc was excised from the motion segment, wrapped in Clingfilm, and frozen at −80°C. This took approximately 3 min. Preliminary tests showed that isolated discs take approximately 15 min to freeze hard at −80°C.

Subsequently, a sagittal strip 15 mm wide was cut from the midline of the frozen disc. The longitudinal ligaments were trimmed from each end of the strip until the characteristic oblique fibres of the annulus were visible. A cutting tool incorporating four razor blades was used to cut the frozen strip transversely into 2 mm wide slices as shown in fig 2. Six slices were cut from the anterior edge, four from the posterior edge, and two from the nucleus. The 12 samples were immediately placed in preweighed plastic vials and weighed before and after freeze drying. The hydration, $h_i$ of each sample was calculated as the weight of water in the sample divided by its dry weight. To quantify the fluid loss due to loading, each loaded disc was matched with a control disc from an adjacent level in the same spine that had not been loaded but was frozen, sectioned, and weighed in exactly the same manner. Matched pair $t$ tests compared the hydration of slices from loaded and control discs to determine which parts of the disc lost a significant amount of fluid, and a one way ANOVA was used to compare fluid loss from different parts of the disc.

**Results**

The preliminary creep test reduced specimen height by 0.13 mm on average. In the main

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**Figure 3** Creep curves for a 27 year old L1-L2 disc loaded to 1500 N for 6 h while positioned in 6° of flexion. Note that the test was interrupted after 2 h of loading, possibly allowing some recovery of the disc.

**Figure 4** Hydration of a loaded 27 year old L4-L5 disc, compared to an L5-S1 control from the same spine. The greatest fluid loss of 1.43 g H$_2$O g$^{-1}$ dry weight was from the mid posterior annulus, while the nucleus lost 1.25 g H$_2$O g$^{-1}$ dry weight.
creep test, sustained compressive loading reduced disc height by 1.2-2.8 mm in 6 h, with an average of 2.1 mm (fig 3). This would overestimate the true height loss if any significant recovery occurred when testing was interrupted for 15 min. The rate of creep following the interruption took only a few minutes to regain its value just before the interruption (fig 3), suggesting that a few minutes was sufficient to reverse any fluid flow during the brief period of unloading immediately after stress profilometry. The rate of creep decreased from 1.5-2.5 mm h⁻¹ at the start of the creep test to 0.1-0.2 mm h⁻¹ towards the end of the 6 h loading period, but none of the specimens actually reached a creep limit.

All of the loaded discs, except the outer 2 mm, showed highly significant reductions in hydration when compared with unloaded controls (P < 0.01 or better). Values of hydration for a typical loaded disc and its control are shown in fig 4. Average values are summarised in table 2 and depicted in figure 5. The large standard deviations probably reflect variations in disc hydration between different spines. Variations in hydration between discs from the same spine were much smaller: when the hydration of control discs from the same spine were compared, the average difference in any one location was 10% or 0.34 g H₂O g⁻¹ dry weight. The maximum difference observed was 0.54 g H₂O g⁻¹ dry weight in slice 5 from the anterior inner annulus, and the minimum was 0.24 g H₂O g⁻¹ dry weight in slice 8 from the nucleus. For this reason, fluid loss from each loaded disc was estimated by comparing its hydration with that of its own control. Fluid loss was calculated as follows:

\[
\text{Relative fluid loss} = \frac{H(\text{loaded}) - H(\text{unloaded})}{H(\text{unloaded})} \times 100
\]

The mid-annulus experienced the greatest fluid loss of 27-30%, while the nucleus experienced a 15% fluid loss (fig 6). An ANOVA showed that significant differences existed between sites, and one tailed matched pair t tests confirmed that the middle annulus (slices
3 and 10) lost more fluid than the nucleus (slice 8) (P < 0.05). The variability present in the grouped results (figs 5 and 6) was partly due to differences in size of discs from different spines. Since each disc was cut into 12 slices, each 2 mm wide, a slice taken from the inner annulus of a small disc may correspond to a slice taken from the mid-annulus of a larger disc. As varying amounts of fluid are lost from the different regions, this affects the collated fluid loss for all the discs. No significant variations in fluid loss were seen with age or spinal level, but this may be attributable to the small number of discs tested. Also, the initial rate of fluid loss is greater from old degenerated discs, whereas young discs have more fluid to lose, and these effects may cancel to a certain extent after 6 h.

Figure 6 shows how the distribution of compressive stress varied across a typical intervertebral disc at various stages in the experiment. Further details of these measurements have been published elsewhere.

Discussion

Mechanically induced fluid loss varied across the disc (fig 6), being greatest in the mid-annulus, least in the nucleus, and least in the outer 2 mm of the disc. These changes can be compared with the stress distributions: in a loaded disc, the outer 2 mm of annulus shows very little compressive stress and the mid-annulus supports stress concentrations above the level experienced by the nucleus (fig 7). Fluid loss due to sustained loading increases this effect, leading to increased loading of the annulus. It appears that long term creep loading causes different regions of the disc to lose fluid according to the level of compressive stress in each region.

Most of the diurnal variation in a person’s height is due to variation in disc height. Individual motion segments in the present experiment lost between 1.7-2.2 mm. Initial disc height was not measured, but the height lost would be equivalent to about 17% of the height of a typical lumbar disc (12 mm). The length of the spine is approximately 33% of overall body height, and the discs provide approximately 25% of the spinal length. Thus a disc height loss of 17% corresponds approximately to an overall body height loss of 0.17 x 0.33 x 0.25 = 0.014 = 1.4%. This is comparable to the 1.1% diurnal in vivo change reported by Reilly et al and the 0.9% change reported by Krag et al. It might be expected that in vivo measurements of total height loss would be slightly lower than values calculated from the present experiment because the in vivo measurements are taken in an erect posture, which brings the apophyseal joints into contact and reduces disc height loss.

A considerable proportion of the discs’ fluid was expelled during the six hour creep test. The sum of the fluid loss across the whole annulus and the nucleus of the two 27 year old specimens was 21.7% and 19.9% respectively. The annulus of a lumbar disc has three times the volume of the nucleus, so the overall fluid loss was 21.2%. Typically, a young (unloaded) disc has an 80% water content so a fluid loss of 21.2% will reduce disc volume by about 17.0%. This compares with in vivo MRI measurements of diurnal change in the lumbar disc volume of young people of 16.2%. Thus height loss and volume loss of the discs in the present experiment are equivalent to diurnal changes in vivo. Any redistribution of water during the short time it took to cut out and freeze the disc after testing would lead to an underestimation of fluid flow.

The closeness of this agreement may be fortuitous, because a wide variety of loads is...
applied to the spine in everyday life, and the duration of loading in a "long day" may be 12-16 hours rather than the six hours used in the present experiment. However, the creep curves shown in fig 3 indicate that the rate of creep decreased by a factor of 10 during the six hour loading period, so it appears unlikely that diurnal creep in life would be much greater — or much less — than in these experiments.

The good agreement between water loss in vivo and in vitro is at variance with the study by Johnstone et al,13 who concluded that disc hydration changes markedly postmortem. Their conclusion was based upon measured differences in hydration profiles between postmortem discs and discs removed from patients undergoing spinal surgery. However, these differences could be explained by the surgical discs being more degenerated than the cadaveric ones, because degenerated discs have a flatter hydration profile with less water in the nucleus,13 similar to the surgical discs. Johnstone et al reported that their surgical specimens were essentially non-degenerated ("grade 2"), but it is not clear how this was decided because the specimens consisted of pieces of anterior annulus only, whereas most of the structural changes which characterise disc degeneration are found in the nucleus and posterior annulus. Johnstone et al also reported differences in the swelling pressure profile between postmortem and surgical discs, but these could have occurred when the cadaveric discs, cut away from the adjacent vertebrae, were frozen slowly at —20°C, and subsequently thawed overnight at 4°C. When intervertebral discs are cut free from bone, they lose their inherent internal pressurisation and so water can be attracted from the annulus to the depresurised nucleus by the higher fixed charge density of the latter. This does not happen in intact spines, living or dead, because the nucleus is permanently pressurised by tension in the ligamentum flavum26 and outer annulus fibrosus.27 Thus the swelling pressure difference noted by Johnstone et al could be an artefact caused by cutting cadaver discs free from their adjacent vertebrae and then leaving them unloaded for several hours. In the present experiments, all discs were frozen at —80°C immediately after being cut away from the vertebrae. Preliminary tests showed that they were frozen solid after 15 minutes, and little fluid redistribution could occur during this period. One could argue that cadaveric spines spend rather longer in the relatively unloaded state in the necropsy room than do the spines of living people lying in bed, but this is unlikely to have much effect on disc hydration because these discs are still pressurised, and their rate of swelling falls greatly after the first few hours of low loading, just as the rate of creep falls greatly after a few hours of high loading. In the present experiments a 300 N preload was applied for 15 minutes in an attempt to guard against the possibility that extra swelling postmortem might have affected the initial stress distributions within the disc. The load and duration were somewhat arbitrary because of the lack of reliable information concerning such swelling. The average height lost during this preliminary creep test was 0.13 mm, which is approximately 6% of that lost during the main creep test, so its effect on stress distributions or water content could not have been large.

The precise amount of creep in life will depend on many factors, and one of these is posture. Prominent studies have shown that flexed postures expel more fluid from the nucleus pulposus than do erect postures,8 presumably by stretching and thinning the posterior annulus, and by increasing the pre-stress of the disc by stretching the intervertebral ligaments. The present results suggest a third important influence of posture: flexion unloads the apophyseal joints10 and prevents them from limiting creep height loss, even after six hours. At the end of the present experiments, intradiscal pressure was reduced by 36% (indicating substantial unloading of the apophyseal joints) if the motion segment was loaded in a simulated flexed posture (per motion segment) and not in flexed postures. These results are reported elsewhere.22 A gradual transfer of load from disc to apophyseal joints would cause the rate of creep to fall faster than in the present experiment, and a creep limit may possibly be reached in a few hours. By removing this natural restraint to progressive creep, sustained flexion may result in extremely dehydrated discs, especially if it is associated with high muscle activity. Some consequences of disc fluid loss will now be considered.

Disc cells in the middle of the annulus, which is the region most affected by fluid loss, are most active in synthesising proteoglycans. Bayliss et al18 showed that the cells of the disc respond directly to changes in hydration: synthesis rates in the anterior annulus fell as hydration was increased or decreased from the in vivo resting value. In the present experiment, most regions experienced too large a fluid loss to directly compare to the experimental results of Bayliss et al. However, the mean hydration of slice 6 from the inner annulus changed from 3.1 to 2.5 g H2O g−1 dry weight following creep (fig 5). Relating this experimental result to those of Bayliss et al19 for the inner annulus, such a change would have halved the rate of proteoglycan synthesis in this region from 4 to 2 × 10−4 mmol h−1 g−1 dry weight. Larger changes in hydration experienced by the mid-annulus would be expected to reduce synthesis rates even more. Evidently sustained periods spent in any one posture would have a detrimental effect on disc cell synthesis.

Although load induced tissue dehydration inhibits disc cell synthesis2 and generates stress concentrations within the posterior annulus,20 the overall effect of fluid loss may not be entirely detrimental. This is because fluid expelled at 300 N preload was returned when loading is reduced, during periods of rest or sleep. This fluid exchange has the potential to enhance disc nutrition by boosting the transport of metabolites between disc cells and the nearest blood vessels around the disc periphery. Rapid fluid "pumping", associated
with locomotion, affects only the disc periphery, but the results of the present experiment show that longer term fluid flow, associated with long term changes in activity or posture, affects all regions of the disc by a considerable amount. (Even the outermost annulus, which does not appear to be dehydrated by loading, will experience a large fluid flow if fluid flows horizontally through it.) This conclusion regarding the beneficial effects of fluid flow may appear at variance with those of Urban et al, who showed that the penetration of small solutes into dog discs could be predicted by diffusion theory alone, even when the dogs were exercised. However, the duration of exercise in their experiment was only two hours for most of the dogs, and the horizontal spines of dogs probably experience smaller fluctuations in load than does the human spine. Also, their theoretical predictions were based upon two constants which had to be evaluated by fitting theoretical curves to the experimental data, so it is not surprising that theoretical and experimental results agreed. The supply of nutrients by diffusion has consistently been shown to be barely sufficient to meet the nutritional requirements of human disc cells. The available evidence suggests that long term fluid exchange of the sort demonstrated in the present experiment should have a significant effect on the transport of small solutes within the human intervertebral disc.

For large molecular weight solutes the situation is much clearer: these molecules diffuse very slowly, and for them fluid flow is an important transport mechanism. Some of the molecules which control and coordinate cell metabolism are high molecular weight proteins, and large molecules such as collagen and proteoglycans represent the main structural components of the disc matrix that are manufactured by disc cells. Therefore, increases in fluid flow to and from the disc would be expected to have a large effect on the disc's ability to repair itself, or to remodel in response to changing mechanical demands.

This conclusion is supported by the results of an experiment monitoring the effects of spinal fusion on the metabolism of dog discs. Fusion shields discs from some of the loading that produces fluid flow, while not affecting the diffusion of nutrients. A large build up of lactic acid was found in fused discs, resulting in cell death and indicating poor transport of metabolites out of the disc. In contrast, the discs adjacent to the fusion, which would be subjected to a greater range of movement and loading than normal, showed increased metabolic activity indicating improved transport of nutrients. All results were consistent with a decreased nutritional supply in the fused discs and an increased supply in the adjacent discs. Therefore, large fluid exchanges in the disc resulting from prolonged changes in loading may be sufficient to make the difference between adequate metabolite transport, which would reduce the ability of disc cells to repair a defective matrix, and a good supply of nutrients which would ensure a healthy metabolism.

CONCLUSIONS

Sustained loading reduces the fluid content in all regions of the intervertebral disc except the outermost 2 mm. Since metabolism of disc tissue is directly related to fluid content, it will be suppressed as the day progresses and the disc loses fluid. However, diurnal fluid exchange is sufficiently large to contribute significantly to overall fluid transport in those regions of the disc where the diffusion of nutrients is deficient. Thus intervertebral disc metabolism depends on mechanical loading, and also on loading history.

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2. Ishihara H, Urban JPG, Hall AC. A 20 s application of physiological levels of hydrostatic pressure can simulate matrix synthesis in some regions of the bovine intervertebral disc in vitro. J Physiol (Lond) 1993;467:939-43.
Sustained loading and water content of intervertebral discs


Vesalius 1543: The first plate of the muscles. "Anterior view of the body from which I have cut away the skin together with the fat, and all the sinews, veins and arteries existing on the surface. No portion of the membrane which we call fleshy [deep fascia] has been left, even in those regions in which this becomes muscle-like"... "These plates display a total view of the scheme of muscles such as only painters and sculptors are wont to consider..."
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