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The poro-elastic behaviour of the intervertebral disc: A new perspective on diurnal fluid flow

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ABSTRACT

Diurnal disc height changes, due to fluid in- and outflow, are in equilibrium while daytime spinal loading is twice as long as night time rest. A direction-dependent permeability of the endplates, favouring inflow over outflow, reportedly explains this; however, fluid flow through the annulus fibrosus should be considered. This study investigates the fluid flow of entire intervertebral discs. Caprine discs were pre-loaded in saline for 24 h under four levels of static load. Under sustained load, we modulated the disc's swelling pressure by exchanging saline for demineralised water (inflow) and back to saline (outflow), both for 24 h. We measured disc height creep and used stretched exponential models to determine time-constants. During inflow disc height increased in relation to applied load, and during outflow disc height decreased to preload levels. When comparing in- and outflow phases, there was no difference in creep, and time-constants were similar indicating no direction-dependent resistance to fluid flow in the entire intervertebral disc. Results provoked a new hypothesis for diurnal fluid flow: *in vitro* time-constants for loading are shorter than for unloading and *in vivo* daytime loading is twice as long as night time unloading, *i.e.* in diurnal loading the intervertebral disc is closer to loading equilibrium than to unloading equilibrium. Per definition, fluid flow is slower close to equilibrium than far from equilibrium; therefore, as diurnal loading occurs closer to loading equilibrium, fluid inflow during night time unloading can balance fluid outflow during daytime loading, despite a longer time-constant.

1. Introduction

Diurnal loading of the human spine consists of ~16 h daytime loading, and ~8 h night time unloading, causing a 1.1% difference in body height between evening and morning (Reilly et al., 1984; Tyrrell et al., 1985). This is attributed to loss and gain of intervertebral disc height (McMillan et al., 1996; Reilly et al., 1984). Disc height changes due to axial compression and relaxation are the result of fluid flow (Adams et al., 1996; Iatridis et al., 1997; Koeller et al., 1984; McMillan et al., 1996; van Dieën et al., 2001; White and Panjabi, 1990), directed outward during loading, and inward during unloading (Urban and McMullin, 1988; Vergroesen et al., 2014). Although there is superimposed short-term loading and unloading with changes in activities during the day; overall, posture change and long-term fluid flow follows a diurnal rhythm, and is balanced over time *i.e.* the disc is in dynamic equilibrium.

Although daily activity varies in intensity, spinal loading during daytime is generally higher than during night time rest (Wilke et al., 1999). Additionally, the period for loading is twice as long as night time rest; therefore, inflow must, on average, be faster than outflow in order to maintain equilibrium. Currently, the predominant hypothesis explaining this equilibrium is a direction-dependent resistance to fluid flow through the vertebral endplate, favouring fluid inflow over outflow (Ayotte et al., 2001). However, this hypothesis may be only part of the explanation, as it is based on the assumption that fluid flow—only—occurs through the vertebral endplate. This assumption is based on *in vivo* studies which show the majority of gadolinium and ^{35}S-sulphate to be transported through the endplate into the nucleus (Arun et al., 2009; Rajasekaran et al., 2004; Roberts et al., 1996; Urban et al.,

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1982). However, nutrient diffusion from blood vessels is something other than interstitial fluid flow (Nachemson et al., 1970; Urban et al., 1982); therefore, fluid flow via the annulus fibrosus should not be discounted. In fact, the hydraulic permeability of the annulus fibrosus is 10 times larger than that of the cartilaginous endplates (Cortes et al., 2013; Wu et al., 2014). Additionally, the annulus fibrosus is permeable along the entire circumference, but the endplates are only permeable in their centre (Nachemson et al., 1970); consequently, the permeable area for fluid flow is smaller for the endplates. Since the hydraulic permeability and the permeable area are larger, the annulus is a likely pathway for interstitial fluid flow; furthermore, this increased permeability and permeable area might overcome the larger distance between nucleus and annulus than between nucleus and endplate. Accordingly, *in vitro* studies have shown that sealing of the vertebral endplates did not influence the response of the intervertebral disc to loading or unloading (Schmidt et al., 2014; Van der Veen et al., 2007), which strongly suggests that interstitial fluid flow due to diurnal loading and unloading occurs through the annulus fibrosus rather than the endplates.

Experimentally, the fluid flow in an entire intervertebral disc is difficult to monitor; however, disc height changes which are in part dependent on fluid flow can be monitored. With diurnal loading or unloading the height of the intervertebral disc shows an instant visco-elastic response due to tissue deformation, followed by non-linear and time-dependent creep, due to poro-elastic fluid flow (Emanuel et al., 2015). Disc height creep can be described using mathematical models (Van der Veen et al., 2013). Previously, these models have shown time-constants for unloading to be 2.5–27 times longer for unloading than for loading (MacLean et al., 2007; O'Connell et al., 2011b), indicating a slower fluid inflow than outflow, in contrast to *in vivo* loading (Reilly et al., 1984; Tyrrell et al., 1985). The paradox that—*in vitro*, unloading creep has a longer time-constant than loading creep, while *in vivo*, overnight fluid inflow is faster—has thus far not been resolved.

In understanding diurnal fluid flow it is important to consider that although they are balanced, the driving forces for out- and inflow of fluid are different in character: during loading, the mechanical compressive pressure increases intradiscal pressure, which expels water from the intervertebral disc (Ayotte et al., 2001; Vergroesen et al., 2014); whereas, upon unloading the swelling pressure of the nucleus pulposus attracts water into the disc (Urban and McMullin, 1988). This swelling pressure is generated by the proteoglycans' ability to attract and bind water, and is defined as: “equal to the compressive pressure that needs to be applied for the tissue to maintain its state of hydration” (Urban and McMullin, 1988). Swelling pressure (or potential) changes with nucleus hydration, and with alterations to the balance in the osmotic charge between the intervertebral disc and the surrounding tissues (Urban and McMullin, 1988, 1985; Urban and Maroudas, 1981). This results in the following process: during loading, intradiscal pressure increases, which expels water from the disc, and disc height is lost (Vergroesen et al., 2014). Simultaneously, with lower hydration swelling pressure increases exponentially (Urban and McMullin, 1988, 1985; Urban and Maroudas, 1981), the increase in swelling pressure opposes the effect of loading until both are balanced, and fluid flow stops (Fig. 1). Inversely, during unloading, the swelling pressure attracts fluid into the disc, increasing disc height; but as the disc rehydrates, the swelling pressure decreases and intradiscal pressure rises. Presumably, the pressure difference between intradiscal pressure and swelling pressure determines the rate of fluid in- or outflow. *In vivo*, this interplay between swelling pressure and intradiscal pressure does not reach equilibrium, because fluid flow is slow due to low permeability of the tissues (Cortes et al., 2013).

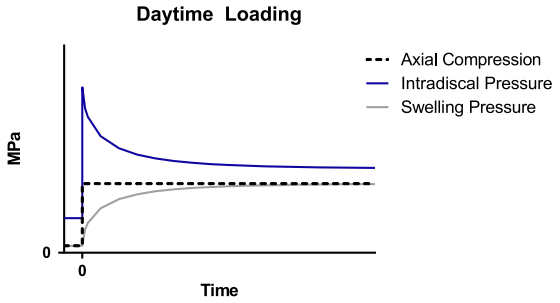

Fig. 1. The relation between mechanical loading of the spine and intradiscal and swelling pressures. Getting out of bed at $T=0$ instantly increases intradiscal pressure at a level of 1.5–2 times the axial pressure (dashed black line) (Nachemson, 1981). This increase in intradiscal pressure expels water from the intervertebral disc, but with this reduction of water content, the intradiscal pressure decreases (dark blue line) (Van der Veen et al., 2005; Vergroesen et al., 2014). The reduction in water content in turn increases the swelling pressure within the intervertebral disc (light grey line) (Urban and McMullin, 1988, 1985; Urban and Maroudas, 1981). The difference in stresses at equilibrium is due to a strain in the annulus fibrosus (intradiscal pressure is ~0.1 MPa in unloaded conditions). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

By investigating disc height creep, the fluid flow can be investigated *in vitro* (Van der Veen et al., 2005); however, there are some considerations that need to be accounted for if creep is to be translated to fluid flow alone. Firstly, a separation of visco-elasticity (tissue deformation) and poro-elasticity (fluid flow) should be made (Emanuel et al., 2015), which also separates the creep within the attached bone and endplates from the creep within the disc (Van der Veen et al., 2008). Secondly, loading magnitude is important as disc height and intradiscal pressure depend on loading history of the disc (Vergroesen et al., 2014; Wilke et al., 1999), and hydraulic permeability of tissues could be affected by the strain in the annulus (O'Connell et al., 2011a) or bulge of the annulus fibrosus (Brinckmann and Grootenboer, 1991). Thirdly, the duration of the measurement is important as modelling accuracy is increased with longer measurements (Van der Veen et al., 2013). Finally, the disc should be in equilibrium prior to the experiment to correct for hydration status (O'Connell et al., 2011b).

Here we investigate the fluid in- and outflow in an entire intervertebral disc by monitoring disc height changes *in vitro*. In order to separate the visco- and poro-elasic effects of different loads, we start by applying mechanical pressure to a disc submersed in saline until disc height creep stops, then, intradiscal pressure, swelling pressure, tissue strain and disc bulge are in equilibrium (phase 0: mechanical preloading). After reaching equilibrium, we change saline to demineralized water to increase the swelling pressure of the disc (phase 1; inflow). This should induce fluid inflow into the disc (*i.e.* disc height increase), by keeping the mechanical load constant we expect limited visco-elastic tissue deformation. Subsequently, changing back to saline should induce fluid outflow, restoring disc height (phase 2: outflow). We investigate the disc height creep curves with a stretched exponential function. Using the results of this study, we were able to formulate a new hypothesis on the balance of diurnal fluid flow.

2. Materials and methods

Five lumbar spines were harvested from 3 to 5 year old skeletally mature female Dutch milk goats, and stored at −20 °C. While frozen, the discs (L1–L2 to L4–L5) were located using fluoroscopy, and adjacent vertebral bodies were removed ~3.5 mm from the disc with a band saw. Subsequently, the cutting edge was brushed clean to remove sawing debris, and rinsed to remove blood cloths.