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Preload substantially influences the intervertebral disc stiffness in loading–unloading cycles of compression

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ABSTRACT

Disc hydration is controlled by fluid imbibition and exudation and hence by applied load magnitude and history, internal osmotic pressure and disc conditions. It affects both the internal load distribution and external load-bearing of a disc while variations therein give rise to the disc time-dependent characteristics. This study aimed to evaluate the effect of changes in compression preload magnitude on the disc axial cyclic compression stiffness under physiological loading.

After 20 h of free hydration, effects of various preload magnitudes (no preload, 0.06 and 0.28 MPa, applied for eight hours) and disc-bone preparation conditions on disc height and axial stiffness were investigated using 36 disc-bone and 24 isolated disc (without bony endplates) bovine specimens. After preloading, specimens were subjected to ten loading/unloading cycles each of 7.5 min compression at 0.5 MPa followed by 7.5 min at 0.06 MPa.

Under 0.06 MPa preload, the specimen height losses during high loading periods of cyclic loading were greater than corresponding height recoveries during low loading phases. This resulted in a progressive reduction in the specimen height and increase in its stiffness. Differences between disc height losses in high cyclic loads and between stiffness in both load increase and release phases were significant for 0 and 0.06 MPa *vs.* 0.28 MPa preload.

Results highlight the significant role of disc preload magnitude/history and hence disc height and hydration on disc stiffness in loading/unloading and disc height loss in loading periods. Proper preconditioning and hence hydration level should be achieved if recovery in height loss similar to *in vivo* conditions is expected.

1. Introduction

The intervertebral disc, being made of viscoelastic tissues, exhibits a time-dependent response; for example, creep under constant compression force (Adams and Hutton, 1983; Kazarian, 1975; Keller et al., 1987; Koeller et al., 1984a,1984b; Markolf, 1972). The temporal response is believed to be due primarily to the fluid flow (i.e., loss–gain of water under loading–unloading, respectively) as well as the gradual rearrangement of collagen fibers and proteoglycans. The disc's water content can fluctuate by 15–20% during a diurnal cycle, resulting in an altered intradiscal pressure, magnetic resonance signal intensity, and internal load distribution (within the disc and adjacent vertebrae and between the disc, ligaments and facets) (Adams et al., 1987, 1990; Arun et al., 2009; Botsford et al., 1994; Hutton et al., 2003; Ludescher et al., 2008; Masuoka et al., 2007; O'Connell et al., 2011a, 2011b; Reitmaier et al., 2013; Urban and McMullin, 1988; Wilke et al., 1999).

An adequate understanding of the viscoelastic properties of the healthy non-degenerate intervertebral disc is important in the development of total and partial replacements by implants and tissue engineering with the aim to replicate as closely as possible the disc temporal response. The creep response of the intervertebral disc has previously been investigated in a number of *in vitro* studies (Adams and Hutton, 1983; Adams et al., 1996; Brown et al., 1957; Hirsch, 1955; Hirsch and Nachemson, 1994; Kazarian, 1975; Keller et al., 1987; Koeller et al., 1984a, 1984b; Kulak et al., 1975; Lin et al., 1978, 2009; Markolf, 1972; Virgin, 1951). These investigations highlighted the non-linear time-dependent behavior of the disc showing rapid decreases in the disc height and axial compliance early after loading that levels off with time till equilibrium. A recent *in vitro* study on porcine discs reported the effect of load magnitude and history on the disc angular stiffness and damping response (Zondervan et al., 2016).

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As in any other soft tissue such as the articular cartilage, the intervertebral disc hydration strongly influences its biomechanical behavior. Under load and in short term, the trapped fluid supports a major portion of the applied compression while being contained by stretched networks of collagen fibrils but later with time and drop in fluid content and pressure, the load is gradually transferred to the tissue non-fibrillar matrix and remaining structures (e.g., facets) (Argoubi and Shirazi-Adl, 1996; Shirazi-Adl, 1992). The disc hydration level in experimental studies could alter by evaporation when specimens are exposed to air, osmotic swelling when specimens are left unloaded while immersed in a solution bath and/or external loading/unloading that causes fluid inflow/outflow (Bezci et al., 2015; Costi et al., 2002; Ferguson et al., 2004; O'Connell et al., 2011a; Race et al., 2000; Vergroesen et al., 2014). The effects of preconditioning, preload or instantaneous fluid content on the disc axial stiffness under physiological cyclic loading regimes (high and low loading phases) either of the isolated intervertebral disc alone or combined with adjacent vertebrae have not comprehensively been investigated. This is important when attempting both to better understand the disc transient response as related to its water content and to adequately compare results of studies performed on discs at varying initial hydration levels.

Therefore, the purpose of this study was to evaluate the effect of changes in compression preload (disc hydration) on the axial compression stiffness in simulated physiological loading regimes. For this purpose, we reanalyzed the extensive data recorded (but not all reported) in a previous *in vitro* study on bovine lumbar motion segments (Schmidt et al., 2015) under cyclic axial compressive loads. We hypothesize that the axial stiffness of intervertebral discs in compression is substantially influenced by the preload magnitude and the number of loading–unloading cycles. Many *in vitro* studies reported an incomplete fluid and height recovery within an appropriate time-scale similar to *in vivo* (e.g., 8 h recovery for 16 h of loading) (Lee et al., 2006; Lin et al., 2009; O'Connell et al., 2011a; Reitmaier et al., 2012a, 2012b; van der Veen et al., 2005, 2007, 2009). Therefore due to low fluid imbibition, we further hypothesize that an incomplete recovery leads to a progressive increase in stiffness with each loading cycle.

2. Methods

The specimen preparation, testing apparatus and loading protocol were described in detail previously (Schmidt et al., 2015) and are only briefly summarized here for completion. Sixty C1–C2 and C2–C3 specimens (fifty four from the previous experiment) from skeletally mature bovine tails were used. After the removal of all surrounding muscles, soft tissues, facets and transverse processes, each vertebra was sawn approximately 5 mm away from the disc yielding disc-body units consisting of an intervertebral disc with parts of the upper and lower vertebral bodies.

Twenty hours prior to testing, specimens were thawed initially for 18 h at 4 °C in phosphate-buffered saline (PBS, B. Braun Melsungen AG, Melsungen, Germany) to ensure uniform hydrated conditions (Fig. 1a). Specimens were then placed in a testing chamber filled with 39 °C (cow body temperature) PBS for additional two hours for temperature adjustment before tests start and during the entire tests allowing thus for a physiologically controlled environment.

The loading protocol started with 8 h preload at 0.06 MPa (corresponding to 27.7–45.8 N compressive force depending on the measured disc cross-sectional areas) (Fig. 1a). Subsequently, specimens were subjected to 10 high/low loading cycles, followed finally by 2 h of low loading at 0.06 MPa. Each loading cycle consisted of 7.5 min axial compression at 0.5 MPa (corresponding to 211.6–373.4 N) and a low loading period of 7.5 min at 0.06 MPa (Fig. 1b). Load applications and releases were performed at 100 N/s. The compression tests were carried out with a servohydraulic material testing machine (858 Mini Bionix II, MTS, MN, USA). To investigate the influence of the preloading, two additional loading regimes were considered. In the first one, the preload magnitude was increased from 0.06 MPa to 0.28 MPa as the average of subsequent cyclic low and peak values of 0.06 MPa (low loading) and 0.5 MPa (high loading), respectively. Conversely in the second regime, the 8 h preload period at 0.06 MPa was removed altogether from the protocol thus proceeding directly to the 10 loading cycles, which circumvents the free fluid loss after prior 20 h of free swelling.

The displacement was recorded at a frequency of 50 Hz. Each signal was dual-pass filtered with a 2nd order low-pass digital Butterworth filter at 10 Hz cut-off frequency. A schematic of the displacement response with time is shown in Fig. 1c. The compressive stiffness ($\Delta N/\Delta mm$) was calculated from the load–displacement curve in every phase of load increase (between 0.06 and 0.5 MPa) and release (between 0.5 and 0.06 MPa) as illustrated in Fig. 1d.

The study, from which main part of the current data were extracted, aimed to examine the recovery capacity of the disc post mortem (Schmidt et al., 2015). For this purpose and with the intention to impede or enhance fluid flow into and out of the discs, various endplate preparation conditions were considered (i.e., rinsing with PBS, irrigation with an orthopedic debridement system or injection of PMMA at the exposed endplate surface). In addition, isolated discs were considered with

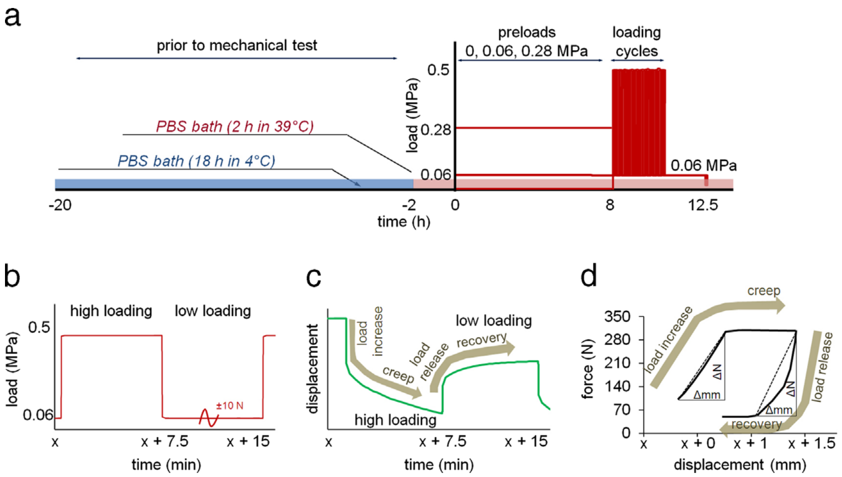

Fig. 1. (a) Time history of the compression test. Before testing, the specimens were immersed in a bath with phosphate buffered saline solution (PBS) for 18 h at 4 °C (blue bar). Subsequently, the specimens were placed for another 2 h in the testing chamber with PBS at 39 °C. The loading protocol consisted of 8 h preload at 0.06 MPa followed by 10 high loading (0.5 MPa) and low loading (0.06 MPa) cycles, each lasting 7.5 min (b). The effect of preload level was investigated by increasing the preload magnitude from 0.06 MPa to 0.28 MPa or by completely skipping the preload period going directly over to the 10 loading cycles. (c) Schematic of the temporal changes in the axial displacement as well as (d) the force–displacement curve of the first loading cycle. The compressive stiffness ($\Delta N/\Delta mm$) is calculated for every loading (between 0.06 MPa and 0.5 MPa) and load release (between 0.5 MPa and 0.06 MPa) phase. (For interpretation of the references to color in this figure legends, the reader is referred to the web version of this article.)

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