Mechanical behaviour of the intervertebral disc under sustained compressive loading

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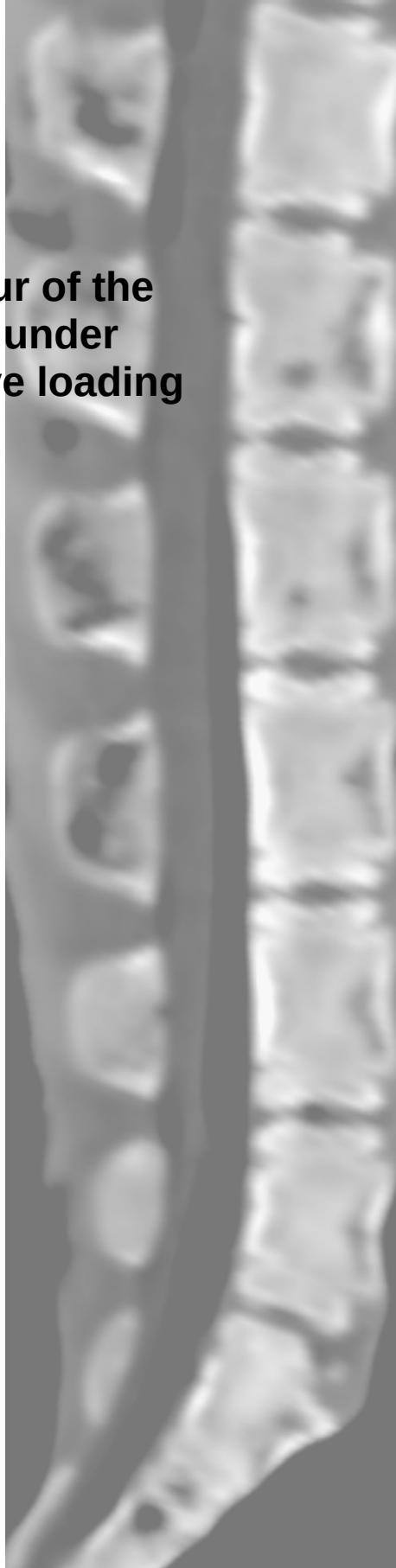

The studies described in this thesis were carried out at the Department of Physics and Medical Technology (FMT) of the VU University Medical Center Amsterdam.

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MECHANICAL BEHAVIOUR OF THE INTERVERTEBRAL DISC UNDER SUSTAINED COMPRESSIVE LOADING

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
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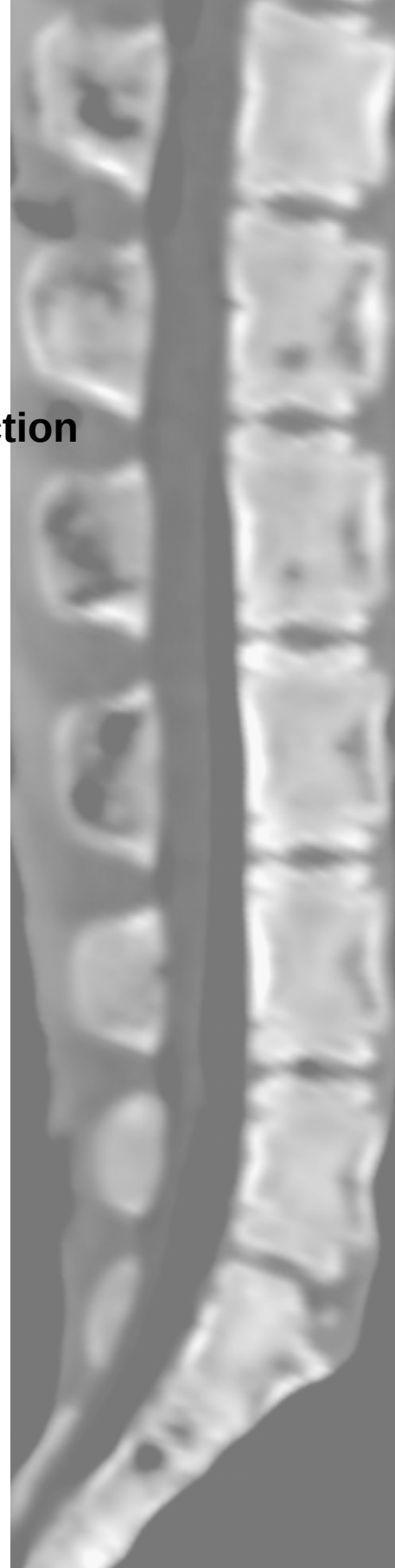
CHAPTER 1

General introduction



General introduction

Back pain is one of the most common musculo skeletal disorders in the Netherlands. It is a disorder with many possible etiologies. In general it is the patient's symptom that defines low back pain while the underlying pathology remains unclear. Many individuals experience low back pain during life[39;45], the lifetime prevalence in the US is 70% to 80%. In addition, back pain is the most common cause of disability in Americans younger than 45 years [45]. The annual incidence of back pain in the Netherlands is 17%[60]. The costs of back pain in the Netherlands in 1991 were 1.43 billion Euros. This can be divided in direct costs and indirect costs. Indirect costs are defined as the value of production losses of paid labour and related costs to society. Direct costs refer to those costs that involve monetary exchange[21] (e.g. physician services, hospital services, medical devices). Direct costs account for 20% of the total costs, which made it the fifth most expensive disease category regarding hospital care[31;77]. Low back pain is therefore a medical problem with major socio-economic consequences. A decrease in occupational back and neck pain has been reported in the Netherlands between 1980 and 2000 [60]. It has been suggested that GP's follow the guidelines for low back pain management. These guidelines, which advice to stay active instead of keeping bed rest, reduce the risk on chronic low back pain. However, although the duration of back pain is reduced due to these guidelines, it is unlikely that the number of new patients per year also decreased. There is a strong relationship between body mass and low back pain[37]. Obesity, which leads to an increased loading on the spine is a risk factor for back pain within the group of patients[80]. Since overweight is increasingly a problem in our society it is likely that the costs of back pain will rise.

Back pain is a multi-factorial disorder. One of the risk factors for low back pain is degeneration of the intervertebral disc[48]. The presence of disc space narrowing, osteophytes and sclerosis, is associated with non-specific low

back pain with odds ratios in literature ranging from 1.2 to 3.3[76]. The relation between low back pain and disc degeneration remains unclear. The nucleus itself is not innervated, while both the annulus and the endplates are only superficially innervated. Damage to these structures could lead to pain. Structurally disrupted discs show ingrow of nerves combined with reduction of disc height. This reduction in height might lead to entrapment of nerves branching off from the spinal cord. Low back pain, which is not caused by a muscular disorder, is always associated with structurally disrupted discs[4].

The view on risk factors for disc degeneration changed from one where age and mechanical factors were considered to be of importance to the current theory that genetic risk factors are predominant[10;39]. However, since the prevalence of degeneration shows a linear increase with age with 80% of all discs abnormal at 70 years of age[59], also other factors are involved.

Mechanical loading on the spine is also a risk factor for low back pain. Spinal injuries are very common among professional or amateur athletes[9] and occupational loads affects the risk of disc degeneration of the lumbar spine[49]. High mechanical loading among workers in the transport sector whose professional activity involves lifting heavy loads is indicated as a possible cause of the high frequency of low back pain[36]. Furthermore, it has been shown that sustained loading can cause damage to the intevertebral disc[15;25;86]. Mechanical loading also affects the cells of the disc. The response of the intervertebral disc cells subjected to sustained, static loading differs from discs subjected to dynamic loading. Static compression has a catabolic effect on the disc, whereas dynamic loads at an appropriate level may cause an anabolic response [79].

The Spine

The primary function of the spine to is give support to the upper body and to protect the spinal cord from impacts. The spine is a complex structure, which gives the body freedom of movement in three directions: flexion-extension, latero-flexion and axial rotation. In order to allow this movement

the spine consists of a chain of 24 vertebrae (7 cervical, 12 thoracic, 5 lumbar). The size and shape of the vertebral body and the orientation of the facet joints are determined by their location in the spine. The cervical spine is the most flexible part of the spine, while the thoracic spine (with the exception of the sacrum) is the least flexible part[81]. Soft tissue connects the adjacent vertebrae. The intervertebral disc and the facet joints form flexible connections between the rigid vertebrae and guides the mutual movements.

Gravity and, in particular, muscular forces cause loading on the spine. A motion segment is a statically undetermined structure. The load is divided by the nucleus, the annulus and the posterior elements. The load distribution depends on differences in stiffness between the load bearing parts. The distribution changes with ageing and degeneration. In older, degenerated discs load is shifted from the anterior elements to the posterior elements[3]. Fractures in the endplate are a common finding in the endplates of elderly people[53]. The load on a vertebral body is mainly an axial compression load which runs from one endplate to the next[30;72]. Resulting disc pressures of every-day activities have been measured in vivo[54;83]. The hydrostatic pressure in the nucleus varies from 0.1MPa during a nights rest to 2.5MPa during heavy lifting. Compression forces, such as during lifting, are large enough to cause damage to the spine, and are therefore thought to be an important cause of low back pain[75].

Motion segment

The smallest functional unit of the spine is a motion segment. A motion segment is made up of an intervertebral disc with its adjacent vertebrae. The intervertebral disc comprises of a layered structure, the annulus fibrosus, which encapsulates a gelatinous structure, the nucleus pulposus, in its centre and is bound by cartilage endplates at the top and bottom.

The endplate is a perforated plate of bone with on top a layer of hyaline cartilage[64]. The fibres of the annulus, which are under tension during axial

compression, are firmly rooted in the endplate. The thickness of the endplate below the annulus is therefore larger than the thickness in the centre. In young individuals, the boundary between the vertebral body and the endplate contains the epiphyseal growth plate[12;13]. This growth plate disappears during adolescence. The vertebral body supports the endplate in axial compression. The endplate is supported at the outer edge by a thin cortical shell and in the centre by trabecular bone. In other words, the structural stiffness of the support of the endplate also varies with its location under the endplate[1].

The annulus fibrosus is a composite structure, consisting of concentric bands of collagen fibres (mainly collagen type I). The annulus fibres run obliquely from the superior endplate of a vertebral body to the inferior endplate of the next vertebral body. All fibres within the same layer are oriented parallel, while fibres of adjacent layers lie in opposite direction. The angle of the fibres in relation to the endplate varies from 40 to 70 degrees. The angle with the endplate depends on the location radially in the annulus wall[57]. Each fibre extends for approximately half of the circumference of the disc. The radial thickness of the lamellae package varies between the posterior and anterior location. The alternate orientation of the annulus fibres creates a structure that allows bending but, at the same time, is ideal for withstanding compression and torsion loads on the disc. A compression load on the disc leads to an increased hydrostatic pressure in the nucleus which subsequently leads to a radial bulging of the annulus and tension on the annulus fibres[62].

The nucleus is a gelatinous structure that is enclosed by the annulus and both endplates. The main components of the nucleus are collagen (mainly type II), proteoglycans and water. Proteoglycans are very large molecules that are able to attract and bind water. Proteoglycans consist of a protein core with glycosaminoglycan side chains. The fixed negative charged chains give the disc a high osmotic pressure and its capability to maintain water

content under loading. The proteoglycan concentration of the disc changes with ageing and degeneration. The mechanical behavior of the gelatinous nucleus can be compared with that of a confined fluid, the nucleus being incompressible[40]. The fluid in the nucleus is confined, however, the encapsulating elements are porous. Consequently, fluid flow into or out of the disc plays a role in the mechanical behaviour of the intervertebral disc. When a spinal segment is compressed the hydrostatic pressure leads to outwards bulging of the annulus fibrosus [62] and to bulging of the endplate into the vertebral body[16;65].

The structural complexity of the intervertebral disc is combined with non-linear material properties of its components. Visco-elasticity indicates that the stress-strain relation of the solid material is time-dependent[46]. Poro-elasticity implies that fluid flow in an elastic matrix plays a role in the mechanical behavior of the disc[32;67]. Several studies have investigated the response of separate parts of a motion segment in sustained loading conditions[16;17;27;34;69]. Separate parts of the disc exhibit both visco-elastic and poro-elastic behavior [41;87]. Collagenous tissue, such as found in the annulus, show time-dependent deformation probably as a consequence of the release of hydrogen and salt-like bonds between fibrils and matrix[19]. In addition, bone shows non-linear and time-dependent material behavior [44;68;84;87] and it can thus be expected that the vertebral body will show time-dependent deformation when loaded. The mechanical properties of the disc are highly dependent on the water content of the nucleus[23;32;33;42;58]. This behavior is time-dependent as well. Daily activity after a night's rest will cause an increase in hydrostatic pressure of the nucleus. The intervertebral disc continuously tends towards equilibrium between the external load on the disc and the swelling pressure of the nucleus[74]. As a result, when the equilibrium is disturbed due to an increased external load, fluid flows out of the disc. The osmotic or swelling pressure depends on the proteoglycan concentration in the nucleus. Outflow of fluid will increase the proteoglycan concentration in the nucleus and consequently the osmotic pressure until

equilibrium is reached [42;73]. Hydration of the nucleus varies under the influence of loading. Parallel to the non-linear mechanical behavior of the nucleus, non-linear, visco-elastic, behavior of the annulus fibres also determines the overall mechanical response of the disc. This cycle of loading and recovery repeats itself in a stable, daily, pattern. During loading fluid flows out of the disc, during rest the flow direction is reversed[50]. As a result disc properties as disc height, stiffness and water content of the nucleus also change in a circadian rhythm.

There are two possible pathways for fluid flow into and out of the disc. Fluid can travel through channels in the endplate and through the annulus fibrosus[55]. *In vivo*, it has been shown that intravenously administered markers predominantly travel through the endplate[61]. In addition, it has been suggested that the resistance of endplate depends on the flow direction[8]: 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 after a night rest despite the fact that in vivo the available time for recovery is shorter than the loading time during the day.

Disc repair

Research effort is not only directed at understanding the etiology of degeneration and back pain. One of the goals is to find new, better, medical treatments and if possible, to obtain the knowledge to prevent to the development of low back pain. At the moment the treatment strategy consists of conservative treatment (as in the back pain management guidelines; pain control and mobilisation) and, if there is no progress, removal of the nucleus as starting point for fusion of adjacent vertebral bodies. With this method the function of the disc as flexible connection in the spine is lost. In a different approach an artificial disc replaces the degenerated disc. However, a less invasive method would be preferable in clinical use. Regenerative medicine might offer new strategies for repair of

degenerative discs, restoring function without removal of the degenerated discs. Different methods are under development [2;7;14;24;35;51;52;56;63;66;78]. These strategies vary from the application of growth factors to the use of stem cells techniques in tissue-engineering strategies. In order to be able to transform these concepts to a clinical application animal models and knowledge of the mechanics of tissue cultures are required[6;70].

Most disc related research is being conducted in small animal models[20;22;26;38;47]. The question remains whether results obtained in these models are valid for the human intervertebral discs. The cellular composition of discs of these animals differs essentially from human discs. Although cellular density is only 1% to 2% of the tissue volume, the cells are essential for maintenance of the disc. Notochordal cells are thought to play a role in the maintenance of extra cellular matrix in a healthy intervertebral disc[18]. In human discs notochordal cells disappear rapidly after birth whilst in some of the animal models the notochordal cells remain throughout life (e.g. pigs, rabbits and nonchondrodystrophoid dogs)[5;11;28;47]. Furthermore, handling, as part of the different treatment strategies for disc repair and regeneration, is difficult due to the small dimensions of the discs. Finally, the size of the disc determines its fluid flow. Therefore, the mechanical behaviour of a small disc e.g. the ratio between visco-elastic and poro-elastic behaviour will be different for small discs. Large animal models (pigs, dogs, goats and sheep) have been used because the morphology is more representative with the human spine[11;29;43;71;82;82;85].

Aim of the thesis

Mechanical loading plays a role in the life cycle of the intervertebral disc. On organ level daily loading can cause damage to the endplates, while at cellular level loading influences cell behaviour either positively or negatively. The aim of spinal research at the VU University Medical Center is to improve treatment strategies for low back pain, which requires a thorough insight in

the mechanics of the disc as an important structure in the healthy spine and in low back pain.

In literature mechanical behaviour of the disc is often described from short-term experiments, usually with as starting point the unloaded situation. The effect of loading history on disc mechanics is, in general, disregarded. This gap in knowledge on disc behavior under sustained loading is the starting point of the research into this field of biomechanics.

The question that is addressed in this thesis is how disc properties change over time due to sustained loading and to understand the mechanism behind this change in mechanical behaviour

Issues that are addressed in this thesis are the difference in time-dependent behaviour of the building blocks of a motion segment in chapter two, dissimilarity between time dependant behaviour during loading and unloading in chapter three, the role of the endplate in recovery in chapter four, the differences in fluid flow for fluid going into and out of the disc in chapter five and finally the influence of anticoagulation on the mechanical behaviour during sustained mechanical testing in chapter six.

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CHAPTER 2

Contribution of vertebral bodies, endplates, and intervertebral discs to the compression creep of spinal motion segments.

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Abstract

Spinal segments show non-linear behaviour under axial compression. It is unclear to what extent this behaviour is attributable to the different components of the segment. In this study, we quantified the separate contributions of vertebral bodies and intervertebral discs to creep of a segment. Secondly, we investigated the contribution of bone and osteochondral endplate (endplates including cartilage) to the deformation of the vertebral body.

From 8 porcine spines a motion segment, a disc and a vertebral body were dissected and subjected to mechanical testing. In an additional test, cylindrical samples, machined from the lowest thoracic vertebrae of 11 porcine spines, were used to compare the deformation of vertebral bone and endplate. All specimens were subjected to three loading cycles, each comprising a loading phase (2.0 MPa, 15minutes) and a recovery phase (0.001MPa, 30minutes).

All specimens displayed substantial time-dependent height changes. Average creep was the largest in motion segments and smallest in vertebral bodies. Bone samples with endplates displayed substantially more creep than samples without. In the early phase, behaviour of the vertebra was similar to that of the disc. Visco-elastic deformation of the endplate therefore appeared dominant. In the late creep phase, behaviour of the segment was similar to that of isolated discs, suggesting that in this phase the disc dominated creep behaviour, possibly by fluid flow from the nucleus.

We conclude that creep deformation of vertebral bodies contributes substantially to creep of motion segments and that within a vertebral body endplates play a major role.

Introduction

The main function of the intervertebral disc is to confer flexibility to the spine, while transferring the external load from one vertebral body to the next. Gravity and, in particular, muscle forces cause loading on the spine[26;38]. The load on a vertebral body is mainly axial compression, which runs from one endplate to the next[15;35]. Compression forces of every-day activities are large enough to cause damage to the spine, and are therefore thought to be an important cause of low back pain[37].

The response of a segment to compression loading is non-linear [18;27]. This is attributed to the non-linearity of the material properties and to the complex structure of the segment.

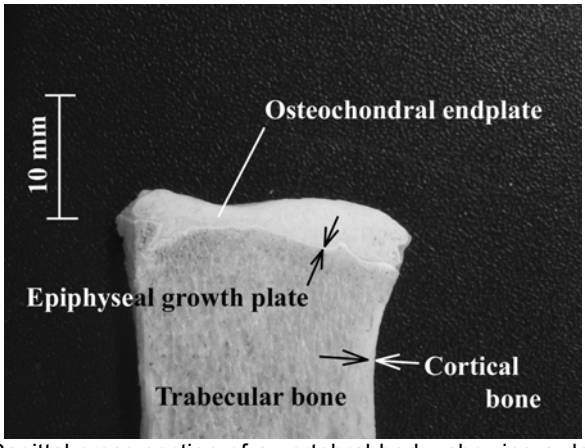

Figure 1. Sagittal cross-section of a vertebral body, showing a detail of the endplate region. (The tested sample contained both endplates.) The thickness of the endplate varies over the disc. Note that the epiphysial growth plate is hardly distinguishable due its limited height.

The smallest functional unit of a spine is a motion segment. A motion segment consists of an intervertebral disc with two adjacent vertebral bodies; the intervertebral disc is the most flexible part of a motion segment. The intervertebral disc comprises of a layered annulus with a gelatinous nucleus in its centre, bound by the endplates. The vertebral body supports the endplate (figure 1). The endplate consists of a perforated plate of bone and

a layer of hyaline cartilage[30]. Below the endplate an epiphyseal growth plate is present in young individuals[4;6;7]. In the present paper, we refer to this whole complex on top of the trabecular bone of the vertebral body, including epiphysial growth plate, cortical bone and the layer of hyaline cartilage, as the endplate.

The nucleus can be considered incompressible[19]. Consequently, when a segment is compressed, the annulus bulges outward[28], but also the endplate bulges into the vertebral body[8;29;31];. The endplate is supported by cortical bone at the edge and by trabecular bone in the center. The structural stiffness of this support, therefore, varies with the location on the endplate[1]. Due to the large differences in structural and material properties of the intervertebral disc, endplate and vertebral body it is to be expected that the role of the vertebral body in the deformation of a segment under compression is limited. However, vertebral bodies were shown not to be infinitively stiff[13;14]. Hence, the contribution of the vertebral bodies to the deformation of a motion segment cannot be neglected. If vertebral bodies contribute significantly to the deformation of the segment, this has to be taken into account in mechanical testing and computer simulations (e.g. finite element models) that describe the behaviour of the intervertebral disc.

This structural complexity is combined with non-linear material properties of the components. Several studies have investigated creep behaviour of the separate parts of a motion segment[8;9;13;17;34]. The materials of the disc exhibit both visco-elastic and poro-elastic behaviour[20;40]. Visco-elasticity means that the stress-strain relation of the solid material is time-dependent[22]. Collagenous tissue, such as found in the annulus, shows time-dependent deformation probably as a consequence of the release of hydrogen and salt-like bonds between fibrils and matrix[10]. Poro-elasticity implies that fluid flow, into or out of the disc, plays a role in the mechanical behaviour of the disc[16;20;32;36]. This behaviour is time-dependent as well. In addition, bone shows non-linear and time-dependent material behaviour[21;33;39;40] and it can

thus be expected that the vertebral body will show time-dependent deformation when compressed.

The influence of vertebral bodies on the deformation of motion segments of rodents was recently reported[23]. However, the separate contribution of the endplates to the deformation of a segment remains unclear, because in this study it was not possible to discriminate between the deformation of the endplate and the bone. The goal of the present study is to quantify the contribution of all the individual parts in a motion segment.

In the present study, porcine specimens were used. Compression tests were performed on single vertebral bodies, complete motion segments (including both outer endplates), isolated discs and the separate test on bone cylinders with and without endplates. This combination of results allows quantification of the effect of endplates on the creep of motion segments. We hypothesized that time-dependent deformation (creep) of the intervertebral disc, the endplates and bone all would contribute to the time-dependent deformation of the motion segment. Secondly, we hypothesized that the creep behaviour of the endplate has a strong influence on the early creep of the motion segment.

Materials and methods

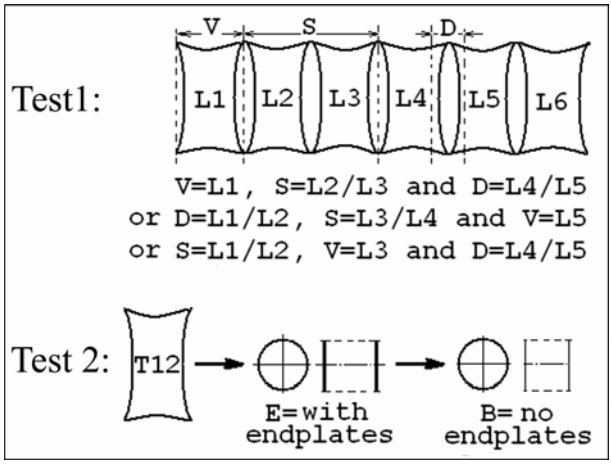

Figure 2. Location of the tested samples in the spine. In the first test motion segments (S), intervertebral discs (D) and vertebral bodies (V) were compared. Segment, disc and vertebral body were taken from alternate locations of the spines. For the second test a sample was machined from the lowest thoracic vertebral body of an additional spine. The cylindrical sample was tested with both endplates attached (E) and tested after removal of the endplates (B).

We performed two separate tests to quantify the creep behaviour of a complete motion segment and its components.

In the first test, time-dependent mechanical behaviour of motion segments (S), intervertebral discs (D) and vertebral bodies (V) was compared (figure 2). In the second test time dependent behaviour of cylindrical bone samples, before (E) and after (B) removal of the endplates, was compared (figure 2). The cylindrical samples were taken from the lowest thoracic vertebral bodies of 11 additional porcine spines,

Specimen

Lumbar spines (L1-L5) of eight, 10-month-old pigs were harvested and frozen for later usage. From each lumbar spine, one spinal motion segment (which was composed of a vertebra, a disc and a vertebra, including both outer endplates: S), one single vertebral body (including the endplates of the adjacent intervertebral discs: V) and one isolated intervertebral disc

(including its adjacent endplates: D) were taken. Segment, disc and vertebral body were taken from alternate locations of each spine (figure 2). The posterior part of the spine was removed at the pedicles, to avoid load bearing by the facet joints. A scalpel was used to remove all soft tissue from the outer endplates of segment and vertebral bodies. Since the outer endplates of motion segments and vertebral bodies do not have flat surfaces, the endplates were embedded in aluminum cups to ensure a good interface with the testing device. The free space between cup and specimen was filled with a metal alloy (Alloy: Cerrolow-136; melting point of 55°C). Finally, the cups were attached to the material testing machine.

The intervertebral discs were cut from the spine by two parallel cuts using a band saw. The remaining layers of trabecular bone on the inferior and superior sides of the disc were cleaned with gauze, which was soaked in saline, and rinsed. The intervertebral disc specimens were placed between two porous plates during testing (porosity code 1, 100-160 μm pore size). This allowed fluid flow from the bath to the endplates and vice versa.

The second test was performed on cylindrical samples taken from the last thoracic vertebral body of the thoracolumbar transition of 11 porcine spines. From the center of the vertebral body of each vertebra, a 10 mm cylindrical sample was machined, i.e. a trabecular bone plug with both endplates, including the hyaline cartilage, still attached. In order to have a good interface with the testing device, a thin layer of bone cement was used to level the outer surface of the samples. After testing of the cylindrical samples with the endplates attached (E), the endplates were removed by two parallel cuts (B), using a band saw. The remaining sample consisted only of trabecular bone. Again a thin layer of bone cement was used to level the outer surface of the samples.

Mechanical tests

All samples were thawed at room temperature prior to testing. The specimens were tested in a saline bath 37°C.

For the test on motion segments, intervertebral discs and vertebral bodies a trace of the shape of the intervertebral disc was made on graph paper; the total area of each intervertebral disc was measured and used to calculate the required force to obtain a disc pressure of 2.0 MPa. This calculated load was applied to all samples from the same animal.

For the test on cylindrical samples, the area of the cross-section of the cylindrical samples was calculated and used to determine the required test force to obtain a pressure of 2.0 MPa. The same loading pattern was applied in both tests. Specimens were preloaded at 0.001MPa for 15 min. Subsequently; they were loaded with 3 complete loading cycles with a loading phase of 15 min at 2.0 MPa and a recovery phase of 30 min at 0.001MPa. The repeated measurement allows assessment of non-recurrent deformation.

Compression tests were performed with a hydraulic mechanical testing device (Instron 8872, Canton, Massachusetts). Load and vertical displacement of the cross head of the Instron were recorded at a frequency of 2 Hz. The vertical displacement of the crosshead was equivalent to the height loss of the sample. The following dependent variables were calculated: height loss over each complete loading/unloading cycle, recovery of height during the unloading phases, the change of height during the interval from 2 – 60 seconds and during the final 10 minutes of each loading or unloading phase.

Statistics

A Student t-test for paired-samples was used to compare the means of two groups. Where multiple comparisons were made (e.g. comparing motion segments, discs and vertebral bodies and the three loading cycles), Bonferroni correction was applied resulting in a significance level of $\alpha=0.0167$.

Results

Test on motion segments, intervertebral discs and vertebral bodies

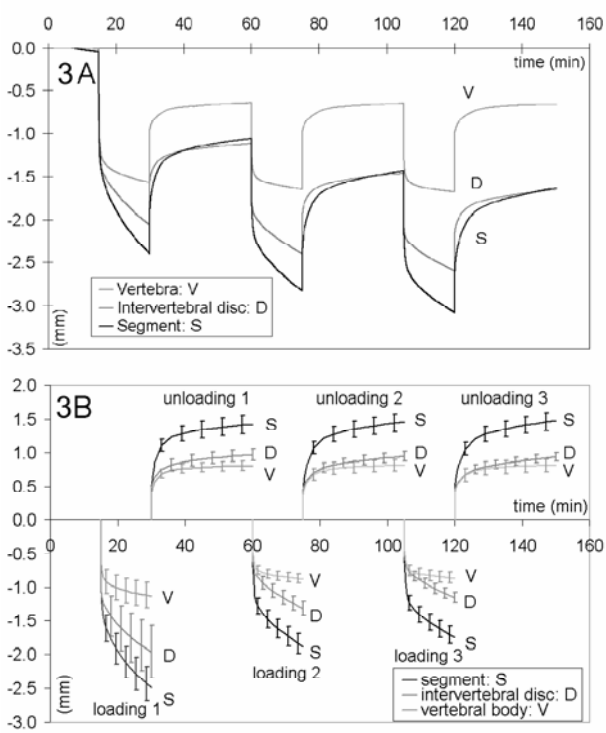

Figure 3a. Typical example of a compression load applied to a motion segment, an intervertebral disc, and a vertebral body showing the height change during three loading cycles. All samples in this figure were obtained from the same animal.
3b). Average changes in sample height of the motion segments, intervertebral discs and vertebral bodies in each loading and unloading phase. The change of height was calculated relative to the height at the end of the previous phase. Error bars indicate standard deviations.

The deformation under compression (figure 3a) was time-dependent in all three groups: motion segments (S), isolated discs (D) and vertebral bodies (V). The change of specimen height was calculated with respect to the height at the end of the previous loading phase (figure 3b). In all samples, the loss of height was smaller during the second and third loading cycle than

in the first cycle ($p<0.005$). The gain of specimen height during the unloading phases was almost invariant over the three cycles within each group.

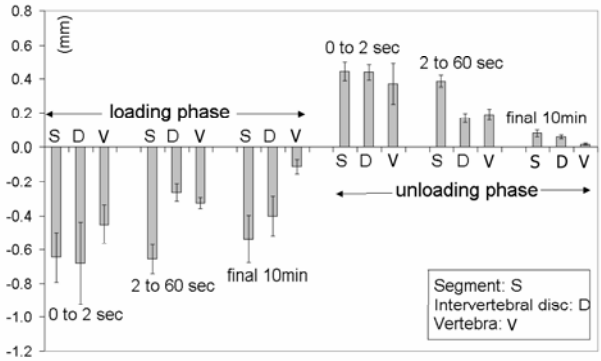

Figure 4. The average height loss and recovery of segments, discs and vertebral bodies over intervals from 0 to 2 seconds, from 2 to 60 seconds and during the final 10 minutes of the loading and unloading phases. Error bars indicate standard deviations.

The deformation rate decreased considerably over time during loading and unloading in all three groups (figure 4). During the loading phase of the test, the first two seconds of the test protocol, the compression load was increased from 20N to the required test load. After the loading phase the load was maintained at the maximum test level. The deformation of the motion segments was about twice that of both the single discs and vertebral bodies (figure 4, $p<0.001$). In the final 10 minutes of the loading phase the rate of deformation was clearly smaller in the vertebral bodies compared to motion segments and discs ($p<0.001$). A similar pattern was observed during the unloading phase. However, in the last 10 minutes of the unloading phase, height recovery was very small in all three groups.

Test on cylindrical samples

The change of height of the vertebral body contributes substantially to the mechanical behaviour of the motion segment, especially during the first minute of loading. To further differentiate between effects of the endplates

and the vertebral body, bone cylinders with endplates (E) and without endplates (B) were compared.

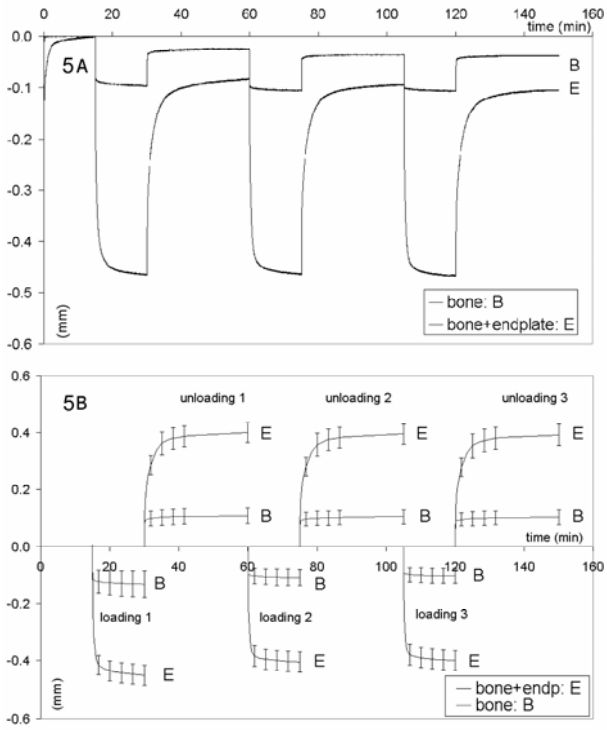

Figure 5a. Typical example of a compression load applied to a 10 mm diameter bone cylinder with and without endplates showing the height change during three loading cycles. The sample without endplates was obtained from the full sample. Note the different scale compared to figure 3.
5b). Average changes in specimen height of 10-mm bone cylinders with and without endplates (mean, sd) in each loading and unloading phase. Height was calculated relative to the height at the end of the previous (un)loading phase. The length of the sample with the endplates cut off was shorter than the sample with endplates still attached, the curve of the sample without endplates was corrected for this difference in height by multiplying the deformation with the ratio of the original sample heights and the sample height of with endplates cut off. Error bars indicate standard deviations.

The length of the sample with the endplates attached was 29.9 mm (SD = 2.0 mm). The length after removal of the endplates was 18.9 (SD = 2.2).

The thickness of the endplate of the bone plugs was measured; the average minimum value was 2.0mm (0.3mm SD). Figure 3a shows the deformation of such cylinders over three loading cycles. The deformation during compression and unloading was time-dependent in both groups (figure 5a).

The change of height of the sample with the endplates attached was, at the end of a loading cycle, about four times larger than without endplates (figure 5a, $p<0.001$). The loss of height per loading cycle was also larger for samples with the endplates attached ($p<0.004$). The loss of height per loading cycle decreased for all samples during the second and third loading cycle, in comparison to the first cycle ($p<0.008$).

The deformation was calculated relative to the displacements at the end of the previous phase (figure 5b), this value was corrected for differences in sample length. The average deformation of the samples with endplates was, at the end of the loading phase also four times larger than the average deformation of the bone samples (figure 5; $p<0.001$). Similarly to the tests on vertebral bodies, discs and motions segments, the recovery phases of all three cycles were virtually identical. The overall loss of height in the second and third loading cycle was almost zero. The overall loss of specimen height during the test can, therefore, be attributed to the first loading cycle.

The creep of both cylindrical samples decreased considerably over time (figure 6). The change of load was applied during the first two seconds of each loading phase. After this early phase, the creep of the samples with endplates was approximately five times larger than that of the samples without endplates ($p < 0.001$, figure 6).

During the final 10 minutes of each phase the deformation in both groups was small, but a significant difference between bone and bone + endplate persisted ($p < 0.001$, figure 6).

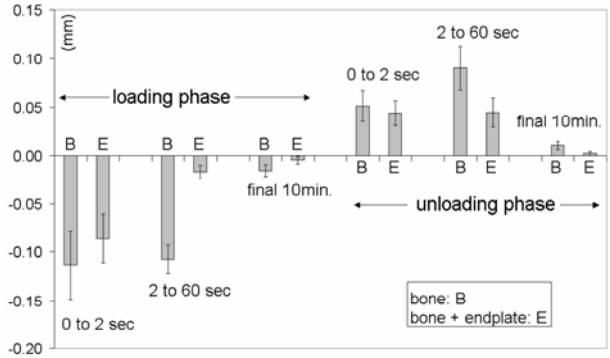

Figure 6. The average change of specimen height of the 10 mm cylindrical specimen of a vertebra and of the same specimen with the endplates removed. Height changes were calculated in the interval from 0 to 2 seconds, from 2 to 60 seconds and during the final 10 minutes. Error bars indicate standard deviation.

Figure 7 shows that creep of the vertebral bodies (V) from the first test was twice as large as creep of the cylindrical bone samples with the endplates attached (E, second test), which was in turn four times larger than creep of the sample without endplates ($p < 0.001$).

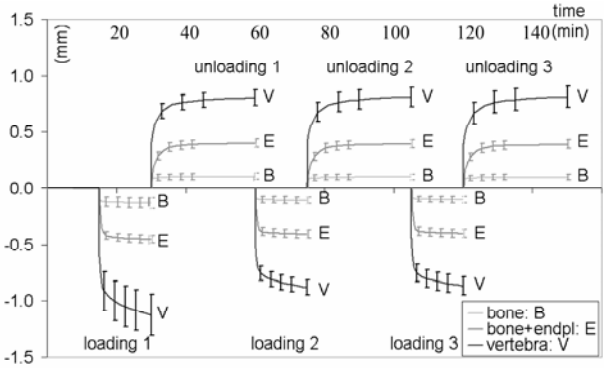

Figure 7. Average change in specimen height for bone, bone including adjacent endplates and single vertebra. Error bars indicate standard deviations.

Discussion

The mechanical behaviour of a motion segment during axial compression is complex. In the present study, we showed that time-dependent deformation of the disc, the endplates and the bone all contribute to the deformation of a motion segment in a prolonged and repeated compression test. However, in the present test it is not possible to discriminate between the creep of structures within the endplate. Most likely, the hyaline cartilage endplate, the bony endplate as well as the epiphyseal growth plate contribute to the creep.

Each loading or unloading phase can be divided into three distinct phases: The loading phase, the first two seconds in which the compression load increases or decreases to its desired test level (creep can not be determined whilst the load changes), the early creep phase (the interval from 2 to 60 seconds) and the secondary creep phase (the interval from 60 sec until the end of the loading or unloading phase).

During testing the endplate deforms and bulges into the vertebral body. The trabecular bone beneath the nucleus is compressed. However, the test on bone samples without endplates shows that creep of bone is already small during the early creep phase, suggesting that the bulging of the endplate into the vertebral body occurred mainly during the loading phase. This is in line with tests on human motion segments by Holmes and Hukins[13] who measured an instantaneous displacement of the endplate into the vertebral body of 0.14 mm during compression tests at 1600N, followed by a further creep of only 0.023 mm.

During the early creep phase, the deformation of the intervertebral disc was comparable to the creep of the vertebral body. The creep of the motion segment, however, was twice as large. This effect was seen both in loading

and unloading. An explanation for the difference in the early response of a segment and an intervertebral disc can be given by the composition of the tested specimens. The vertebral body and the intervertebral disc contained two endplates each, whilst the motion segment contained four endplates. The test on the cylindrical samples showed that creep in samples with endplates was much larger than creep of the identical samples with the endplates removed. Thus, creep of the bone was small as compared to that of the endplate; the endplate was more deformable than trabecular bone. In the test on motion segments, four endplates were present. The influence of these endplates in the deformation of the motion segment was therefore also large. Besides visco-elastic the disc is poro-elastic. The role of fluid flow in the mechanical behaviour of the disc is also time dependent. Rapid fluid flow, due to mechanical loading, affects the periphery of the disc whilst long term loading affects all regions of the disc[25]. Fluid flow from the nucleus is a slow process compared to visco-elastic deformation. Creep of a motion segment is by nature a combined visco and poro-elastic process. These processes work coincide from the beginning of the test. However, we assume that the duration of the early phase was too short for a large influence of fluid flow from the nucleus and that the endplates play a large role in the deformation during the early creep phase.

After the early creep phase, the behaviour of the intervertebral disc started to follow the pattern of the motion segment, whilst the creep of the vertebral body fell behind. Comparing tests of samples containing an intervertebral disc and tests on samples without, results show that creep of both bone and endplates was small compared to that of segment and disc. The effect of the endplate apparently decreased. This suggests that the intervertebral disc determines the late creep phase, probably through fluid loss from the nucleus and through visco-elastic creep of the annulus fibres.

Lumbar vertebral bodies and the cylindrical samples of the thoracolumbar region with the endplates still attached showed comparable mechanical behaviour under mechanical loading. However, the change of height of the vertebral body was approximately twice as large (figure 7). It is not to be expected that the properties of the lowest thoracic vertebral body differ substantially from those of lumbar vertebral bodies. The larger height loss of the vertebral bodies can probably be explained by the differences in the thickness of the endplate samples. The cylindrical sample was machined from the center of the vertebral body. The thickness of the endplate in the center of the disc is smaller than the thickness of the endplate under the annulus (figure 1). The endplate plays an important role in creep. Due to endplate thickness, the creep can be expected to be larger for a complete vertebral body.

Another difference between vertebral bodies and the bone plug is the presence or absence of a cortical shell. However, tests on cylindrical samples showed a very small deformation of vertebral bone during the creep phase. The effect of the presence of cortical bone, a changed stress level due to load bearing of the cortical shell, therefore will also be small.

In a segment, besides being compressed, the endplates bulge into the vertebral body. Pressure measurements in the intervertebral disc show that the compressive load in a healthy disc decreases towards the periphery [2]. This leads to higher loads in the centre of the vertebral body. The trabecular bone of the vertebral body carries this load, which locally leads to a higher deformation. In the present study, deformation of the endplate contributed substantially to the deformation of the segment. This may not be the same in degenerated discs. Degeneration is typically a problem of the mature human spine. In contrast to the animal model, the vertebral growth plates in adult human spines have closed in their early twenties[4]. Thinning of the endplate is observed with aging[12] and the loading profile within the intervertebral disc also changes with aging[3]. Bulging of the disc into the vertebral body may therefore be less pronounced in degenerated segments than in young

porcine segments. The present in vitro tests will, therefore, only reflect the mechanical behaviour of a disc at the equivalent age in human life. The effect of the above, changes in disc and endplate properties, on creep behaviour deserve a separate study.

The porcine disc is, both functionally and anatomically, an accepted model for mechanical testing of the spine. The heights of human and porcine vertebral bodies, measured at L4, are very similar[24]. The main difference between adult human and immature porcine vertebra, with respect to morphology, is the area of the vertebral body[24]. We therefore applied a load based on the area of the individual porcine disc to attain pressures comparable to those on human discs in daily life[38]. The average compression load during the loading phase in the present experiments corresponded to two times body weight of the animal; this is well below the compression strength of the spine.

Tests on immature porcine [5] and human intervertebral discs[11] showed that freezing influences creep behaviour of porcine discs, while creep behaviour of human discs was not altered. The change in porcine samples was largely attributed to changes in swelling pressure and permeability of the disc. Due to preparation of the samples, the number of samples to be tested and testing time, the use of fresh samples was undesirable. In the present study, all groups were frozen and thawed before testing. Therefore a comparison between groups is allowed.

In the present study, we have investigated how deformations of the disc, the endplates and the vertebral body contribute to the deformation of a motion segment in a compression test. We showed that the endplate contributes significantly to the creep of a single vertebra and that the vertebral body contributes to the creep of a segment. Creep deformation of a complete motion segment is thus determined by the behaviour of the bone, the endplates, the annulus and the nucleus. Each part has a separate time

scale. Creep of bone is present during the early creep phase, however, it is small compared to creep of the endplate. Creep of the endplate was substantial during the early creep phase and finally creep of soft tissue of nucleus and annulus dominates the late creep phase.

If the effect of remaining endplates in a compression test is not taken into account, the contribution of the segment to the deformation of the spine will be overestimated. Tests on motion segments should preferably be performed on a segment with the outer endplates cut-off.

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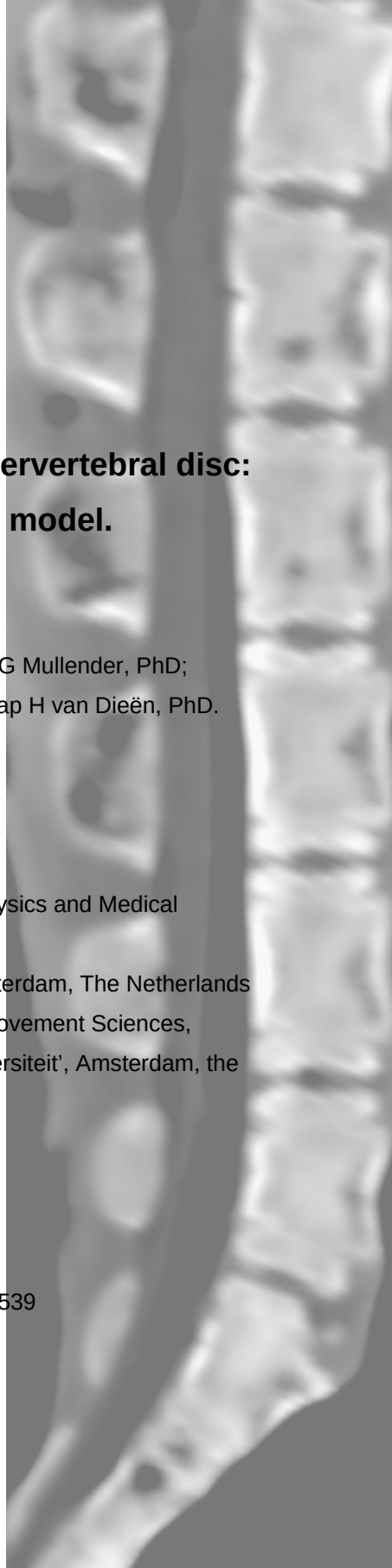

CHAPTER 3

Flow related mechanics of the intervertebral disc: the validity of an *in vitro* model.

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ABSTRACT

Study design

An *in vitro* mechanical study on porcine motion segments.

Objectives

To test the validity of *in vitro* studies on flow related mechanics of the intervertebral disc. In particular, to investigate whether fluid flows back into the disc during unloading after a loading cycle *in vitro*.

Summary of Background data

In vivo studies show both inflow and outflow of fluid in the intervertebral disc. The resistance to flow out of the disc is higher than to inflow. The fluid flow is regulated via unbalance between the external load and the osmotic pressure of the nucleus pulposus.

Materials

Eight porcine lumbar motion segments (without posterior elements) and 8 isolated discs were tested in a physiological saline bath (39 °C). The specimens were pre-loaded at 0.025 MPa for 15 minutes. Three 15-min loading periods at 2.0 MPa were applied each followed by an unloading period of 30 minutes.

Loads, axial displacements and nucleus pressure were recorded on line.

Results

Over three loading and unloading periods all specimens showed a net loss of height and mass. The time series of specimen height during the three unloading periods showed virtually identical responses. The pressure in the nucleus decreased in the subsequent loading periods and showed no increase during unloading.

Conclusion

The data show the limitations of an *in vitro* model for studying fluid flow related IVD mechanics. During loading outflow of fluid occurred, but inflow appears to be virtually absent during unloading. Poro-elastic behaviour cannot be reproduced in an *in vitro* model.

Introduction

The mechanical behaviour of a spinal motion segment is highly non-linear [13;23]. This is to a large extent attributable to the complex composition of the intervertebral disc, which exhibits both visco-elastic and poro-elastic behaviour [15] .

The intervertebral disc, and in particular the nucleus pulposus, has a high water content. The hydration of the disc varies under influence of loading. Consequently, fluid flow plays an important role in the mechanical behaviour of the intervertebral disc[2;3;10;15;20;32;33]. In a healthy disc there is a continuous tendency towards equilibrium between the external load and the swelling pressure of the disc [30]. The swelling pressure is dependent on the proteoglycan concentration in the nucleus. Thus the swelling pressure depends on the degree of hydration of the nucleus. When the external load changes, the proteoglycans will imbibe or express water until the swelling pressure balances the external load and a new equilibrium is reached.

After a night rest, daily activity causes an increase in intradiscal pressure due to gravity and, in particular, to muscle forces [21;35]. As a result fluid is expressed from the nucleus, which will increase the proteoglycan concentration and swelling pressure, until equilibrium is reached [16;29]. During resting periods the flow direction is reversed and fluid flows back into the intervertebral disc [17] and the disc regains its properties. The main path for fluid flow from the disc supposedly leads through the endplate into the vertebral body [25]. The vertebral body and the intervertebral disc are connected via channels through the bony endplate [22]. Recently, it has been suggested that the resistance of these channels to flow is direction-dependent, with the resistance to flow of fluid into the intervertebral disc being lower than the resistance to outflow [4]. This allows full recovery of fluid content during night rest.

Our knowledge of the mechanical behaviour of the intervertebral disc is largely based on *in vitro* testing of cadaveric material [1;24]. Yet, it is not clear

whether mechanical behaviour of the disc *in vitro*, due to fluid in- and outflow, resembles *in vivo* behaviour. Results in several studies raise doubts as to whether this is the case, especially for fluid inflow which is apparently reduced *in vitro*[12;14;31]. In this study, we investigated the mechanical behaviour of intervertebral discs *in vitro*. The mechanical behaviour of the intervertebral disc, under alternating axial compression and relaxation, was assessed in intervertebral discs taken from the lumbar spine of pigs. Intact motion segments as well as isolated intervertebral discs were tested.

Fluid flow, to and from the disc, plays an important role in the mechanical behaviour of the disc. Flow cannot be measured directly in intact motion segments. Changes of specimen height, changes of pressure in the nucleus and loss of mass of the intervertebral discs were used to monitor changes in mechanical behaviour under alternating loads.

Materials and methods

The lumbar spines (L1-L5) of eight, 10-month-old pigs were harvested and frozen for later usage. One spinal motion segment (comprising two vertebrae and an intervertebral disc) and one isolated intervertebral disc (including its adjacent endplates) were taken from each lumbar spine. The motion segment was L2-L3 in one half of the cases, and L3-L4 in the remaining cases. The associated isolated discs were L4-L5 and L1-L2, respectively. The posterior part of the motion segment was removed at the pedicles in order to exclude interference with the facet joints. To obtain isolated disc specimens, the adjacent vertebral bodies were cut off as close to the endplate as possible. Subsequently, the cutting edge was brushed clean. The specimens were thawed before testing. Each isolated intervertebral disc was weighed (Mettler-Toledo, Greifensee, Switzerland) before and after testing in order to estimate differences in fluid content. The change of mass of the complete motion segments could not be measured due to the method of embedding of the motion segments.

In the testing device, an Instron material testing machine (Instron 8872, Canton, Massachusetts), the isolated disc specimens were placed between two porous plates. During axial compression the porous plates allowed free passage of fluid to and from the endplates of the disc. The top plate was attached to the testing device via a ball-and-socket joint. The complete motion segments however, had to be embedded in aluminum cups for stability. The outer endplates of the specimen were embedded into the cups in bismuth. The cups were attached to the material testing machine. The posterior part of the motion segment was removed at the pedicles. Thus an open connection for fluid into and from the vertebral body was created.

All tests were performed in a bath with physiological saline at a temperature of 39°C (body temperature in pigs).

The total area of the intervertebral disc was measured. This area was then used to calculate the required force to obtain an overall pressure load

of 2.0 MPa. The forces corresponded to approximately 2 times the body weight of the animals. The specimens were pre-loaded at 0.025 MPa (20N) for 15 min. Subsequently they were loaded for 3 full loading cycles each consisting of a loading period of 15 min at 2.0 MPa and an unloading period of 30 min at 0.025MPa. The nucleus pressure was measured with a pressure needle (Gaeltec LTD, Dunvegan, Scotland), which was inserted into the nucleus and remained in position during the whole test. The load, displacement and pressure in the intervertebral disc were recorded at a frequency of 2 Hz.

Results

The average area of the specimens was 848 mm^2 thus the average external compression load was 1694N.

Over the three loading and unloading periods all specimens showed a net loss of height (Fig. 1). The total loss of height of the segments was (1.76mm±0.23 mm) and of the isolated discs (1.58±0.47 mm). The loss of height after the complete loading protocol shows that a total of 90 minutes unloading with virtually no compression load did not compensate the loss of height due to 45 minutes of loading. The loss of height in whole segments was larger than in isolated discs in each separate cycle of loading and unloading (Table 1).

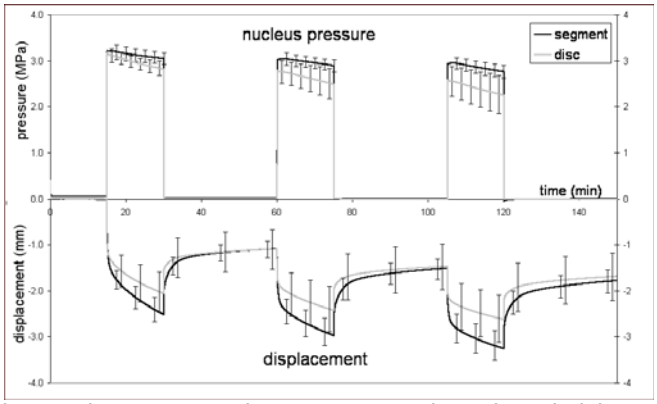

Figure1. Changes in average nucleus pressure and specimen height

	Segment	Intervertebral disc
cycle 1	-1.07±0.16	-1.01±0.38
cycle 2	-0.42±0.06	-0.36±0.08
cycle 3	-0.27±0.05	-0.21±0.04

Table 1. Change in specimen height (mm) after each complete cycle (loading plus unloading).

The reduction in height after each load change is compared between cycles in figures 2 and 3.

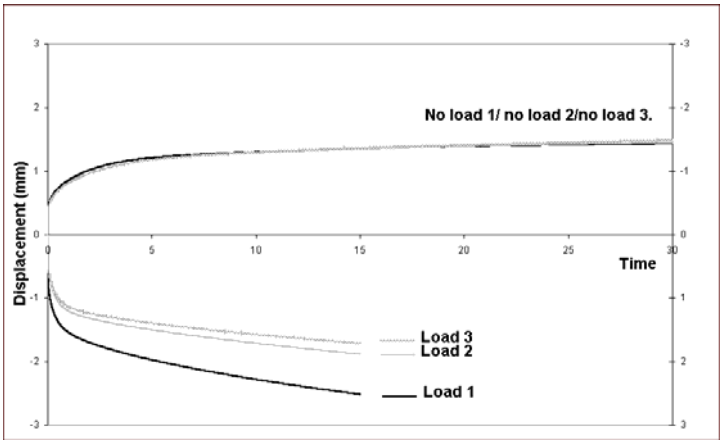

Figure 2. Overlaying height change data of 3 successive loading and unloading periods of the motion segments (time in minutes).

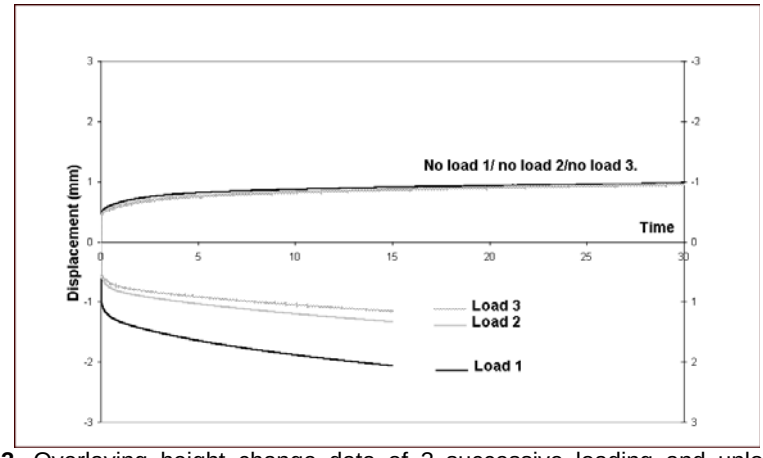

Figure 3. Overlaying height change data of 3 successive loading and unloading periods of the isolated discs (time in minutes).

Overlaying the time series of the specimen height during loading show that the loss of height decreased after each load cycle, whilst the responses in the unloading period were virtually identical. The slope of the displacement curves during loading of the successive load cycles (the slope is calculated in the interval from 5 to 15 minutes) decreased significantly ($p<0.01$) over the successive loading cycles. Although, the slope of the

displacement curve during unloading, calculated over the same interval as during the loading curve, increased in all specimens (fig. 4), this effect was marginal and the responses in the subsequent unloading periods have virtually identical appearances.

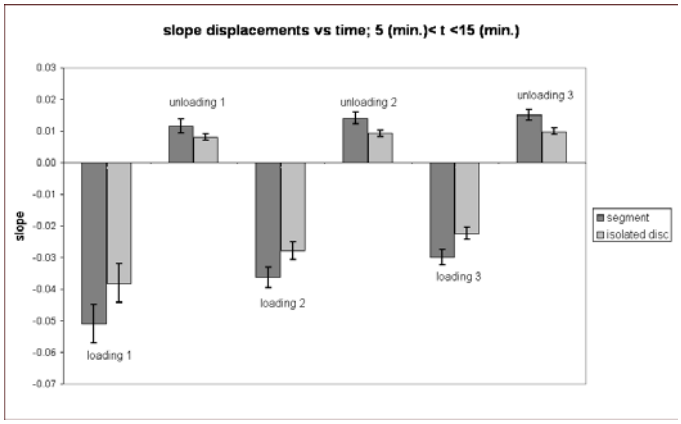

Figure 4. Average slope of the displacement curves in the interval from 5 to 15 minutes of the successive loading and unloading cycles.

The ratios of the slope during loading/unloading in the successive cycles were 8.6, 5.1 and 3.8 for the isolated discs and 8.9, 4.8 and 3.5 for the motion segments increased.

	Loading phase 1		Loading phase 2		Loading phase 3	
	slope	R^2	slope	R^2	slope	R^2
segment	-0.011±0.014	0.99	-0.011±0.012	0.98	-0.014±0.011	0.98
disc	-0.022±0.004	0.99	-0.020±0.004	1.00	-0.023±0.003	1.00

Table 2. The rate of reduction of the average nucleus pressure. (MPa/min).

In all three loading periods, the pressure of the nucleus decreased more or less linearly. This effect was more pronounced in the isolated discs than in the motion segments. The rate of nucleus pressure reduction in the isolated discs was about twice as high as the rate of pressure reduction in the motion segments (fig. 1, table 2). During the relaxation phase the nucleus pressure did not change significantly, the pressure showed no sign of an increase

during unloading. At the onset of the next loading cycle the nucleus pressure had not recovered.

All isolated intervertebral discs showed a significant reduction of mass after testing ($p < 0.03$). The average mass of the discs prior to the test was 14.72 ± 0.52 g, the average loss of mass after three complete load cycles was 0.65 ± 0.19 g.

Discussion

It is generally assumed that the external load and the osmotic pressure control the fluid content of the nucleus[20;24]. The intervertebral disc tends continuously, via attracting and expressing water from the nucleus, towards equilibrium between the external load and the swelling pressure. *In vivo* measurements of the intradiscal pressure in human discs have shown a gain in nucleus pressure during rest[35]. *In vivo*, an unloading period of approximately 7 hours out of 24 hours is sufficient for recovery of the disc's water content[17] and disc height[18;26]. In other words, the fluid balance in the intervertebral disc is shifted towards outflow of fluid during the day and reverts after removal of the external load in favor of fluid inflow during night. Fluid flow is therefore an important aspect in disc mechanics.

In the current research we have investigated whether the *in vivo* mechanical behaviour can be reproduced *in vitro*. After a repeated axial compression loading and unloading cycle, all specimens showed a net loss of height and mass in an *in vitro* test. Specimen height showed virtually identical responses during the three unloading periods. The pressure in the nucleus decreased in the subsequent loading periods and showed no increase during unloading.

The hydration of the disc depends on the loading history of the disc[30]. The swelling pressure is not a material property of the nucleus tissue but it changes with hydration of the disc. It is possible that the specimens at the time of testing were not equally hydrated. However, because test specimens have not been tested immediately post mortem, it is likely that, at the moment of testing, the specimens had sufficient time to adapt to the new, unloaded, situation. In the tests, this was confirmed in the results of the pre-loading phase when the load was close to zero (20 N compression load for 15 minutes). No major changes in specimen height or pressure were seen during this test phase.

The compression tests have been performed on porcine specimen. Although the biochemical composition of the porcine intervertebral disc differs from the human intervertebral disc, the porcine disc provides, with respect to functional characteristics and anatomical characteristics, an accepted model[7;19]. Furthermore, in pilot studies we have seen similar effects as described here in different species (goat, human). The porcine disc therefore appears to be a valid model for this aspect of disc mechanics[34].

The applied compression load corresponds to two times the body weight of the animal. In comparison with the static load in humans the test load can be considered high. However, based on the compression strength of the vertebral body of quadrupeds, the *in vivo* compression load in quadrupeds is expected to be higher than in humans[27]. The main reason is that the major part of the spinal load is related to muscle forces, which are likely to be higher in quadrupeds than in humans.

The test environment of the discs was as close as possible to the physiological, *in vivo*, environment. The temperature was raised to body temperature of the animal. This will influence decomposition of the disc; the decomposition rate will be increased compared to a test at room temperature. Since the temperature was kept constant during testing, the influence on the decomposition rate could be studied by comparing the successive loading and unloading phases. Overlaying the time series of the displacements of the successive unloading periods showed virtually identical responses (fig. 2 and 3). It is therefore unlikely that the changes in displacement curves between successive loading cycles can be attributed to decomposition, whilst nothing is seen in successive unloading curves. This is confirmed by tests on ovine intervertebral discs[8] and by pilot tests we performed for this research. These pilot tests were performed on porcine segments wrapped in saline soaked gazes at room temperature and showed similarly absence of recovery of disc height and intradiscal pressure.

For practical reasons we have chosen to use frozen materials. Although it has been argued in one study that this could influence fluid flow in the disc[6], another more recent study from the same group revealed no major effects of careful frozen storage over a time period comparable to the one used in the present study[9]. In addition, we have repeated the experiment on a single fresh specimen to see if the freezing and thawing of the discs had affected the results. The same absence of recovery was observed in this fresh specimen.

The test protocol in the current study was load controlled. During every loading phase the load was kept constant. Nevertheless, the measured nucleus pressure decreased in all specimens during the loading phase. Since the sum of all loads is constant by definition, this can only be explained if the load is internally redistributed from a load bearing nucleus to a load bearing annulus fibrosis. This result is in line with earlier findings in the literature[2;32] . During the unloading phase the pressure in the nucleus did not change significantly. The nucleus pressure showed no sign of an increase at the start of the next loading phase. The load did not shift back from the annulus to the nucleus. The load bearing capacity of the nucleus was therefore not restored.

The difference between the motion segments and the isolated discs was the presence of the vertebral body. In the isolated discs the rate of pressure reduction in the nucleus was twice as high as compared to the motion segments (fig. 1, table 1). Thus, the removal of the vertebral body resulted in a faster reduction of nucleus pressure. In comparison, the reduction of specimen height of the motion segment was larger than the loss of height of the isolated disc. This appears to be in conflict with the larger pressure reduction of the isolated discs. However the change of height of the motion segment is a combined reduction of disc and vertebral body height. Taking into account the stiffness of the vertebral bodies, the data suggest that the removal of the vertebral bodies has reduced the resistance against fluid outflow.

Overlaying the time series of the displacements of the successive unloading periods, the displacements showed virtually identical responses. The loss of mass indicated that fluid was expressed from the nucleus. The specimen height gain did not increase after each loading cycle, while the pressure gradient increased due to fluid loss. This suggests that, during the unloading phase, the mechanical responses were not determined by the osmotic pressure gradient. Therefore we hypothesize that the height gain after loading was dominated by visco-elasticity.

Since fluid flow cannot be measured directly, we have to rely on indirect measurements for an explanation. Recovery time was twice as long as the loading time, thus it was expected that the disc would regain its original properties. However, the continuous loss of nucleus pressure, specimen height and especially loss of mass in the current study demonstrate the opposite. In this test, all indicators point towards absence of fluid flow into the disc. This leads to the conclusion that the disc and in particular the nucleus did not regain its fluid content during unloading. The discharged fluid remained outside of the disc. The permanent loss of fluid was confirmed by the statistically significant loss of mass in the isolated discs.

This raises the question why the nucleus did not regain its fluid content during the unloading phase. One reason could be that proteoglycans could be dissolved[11;28] in the expressed fluid. This would directly lead to lower proteoglycan content in the next load step and to a shift in the equilibrium towards a reduced drive to inflow of fluid into the disc.

Secondly, we hypothesize that the fluid flow is hampered, *in vitro*, by congestion of the small pores in the endplate that function as primary pathways for fluid flow. The pores in the endplate might be blocked from one side. The pressure of the fluid, due to the external load, could be able to push the clots, which block the pores, away from the discharge opening and transport the fluid beyond the endplate. In that case, the channels remain closed for a reversed flow direction along a much less steep pressure gradient. It is conceivable that during inflow the clots are pushed into the

endplate pores, thus creating effectively a one-way valve. This would explain why the pressure reduction in isolated discs was steeper. With the vertebral body removed the endplate is more accessible for fluid outflow, whilst the pores in the endplate remain closed for inflow.

The outcome of these experiments is consistent with the results of other studies in which no or limited recovery was found[14;31]. In one study it has been reported that disc mechanics are restored after recovery[12]. However, the unloading phase during this study was 6 times longer than the loading phase. The duration of unloading phase was 18 hours, suggesting that inflow was slow compared to the outflow [5].

The intervertebral disc is a very complex structure. Its mechanical behaviour comprises visco elastic, poro elastic and biochemical aspects. Testing of the disc is likewise complex. The findings of the present study point at limitations of mechanical testing *in vitro*, especially when fluid inflow is vital to the mechanical behaviour of the disc.

Apparently mechanical behaviour of the intervertebral disc under alternating loads cannot be validly studied in spinal motion segments or isolated intervertebral discs ex vivo.

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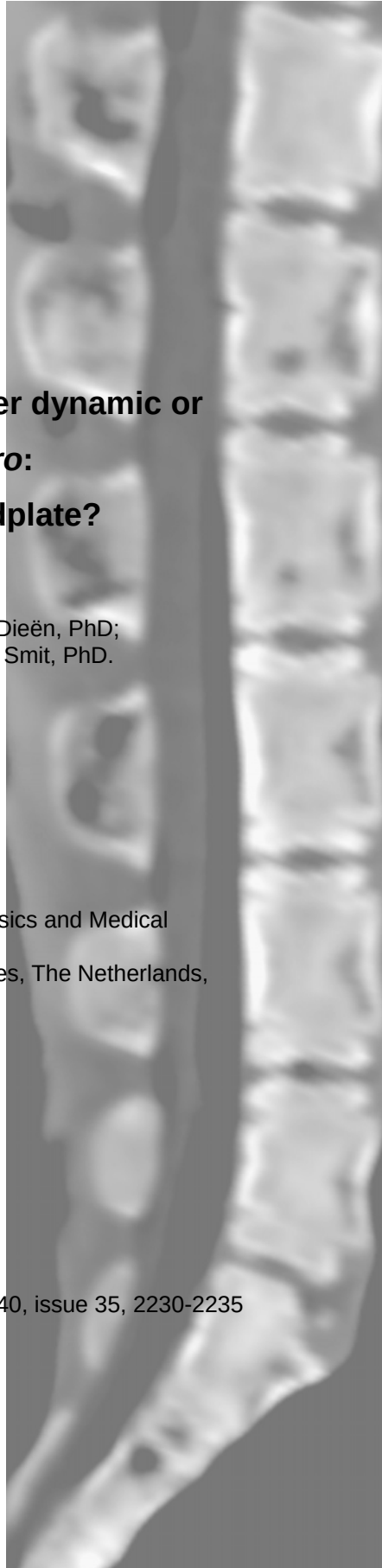

CHAPTER 4

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

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

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*.

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[20;36]. The mechanical properties of the IVD are non-linear[12;22]. This is attributed to the complex structure of the IVD and the material properties of its components, which are both visco-elastic and poro-elastic. The mechanical properties of the IVD are highly dependent on its water content of the disc[7-9;14;23]. Consequently, fluid flow plays an important role in the mechanical behavior of the IVD[1;2;10;13;19;32;34].

The IVD continuously tends towards equilibrium between the external load on the spine and the swelling pressure of the disc [30]. 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 flow direction is shifted towards outflow of fluid. This will increase the proteoglycan concentration and consequently the swelling pressure of the IVD until equilibrium is reached[14;29]. During rest, the flow direction is reversed. As a result, fluid flows back into the disc[17] 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[21]. *In vivo*. it has been shown that intravenously administered markers predominantly travel through the endplate[25]. In addition, it has been suggested that the resistance of these channels depends on the flow direction[4]: 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 [31]. 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[11]. 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[3], 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*.

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, Massachusetts). 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 IVD's [16;31]. 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 [36]. It is expected that the compression load in quadrupeds is generally higher than in humans [27]. The control of the testing device requires a small offset during the unloading in order to prevent backlash. This offset load during recovery was 20N. The specimens were pre-loaded at a compression load of 20N for 15 minutes 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 IVD's.

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 minutes), followed by a recovery phase at 20N (approximately 0.05 MPa). At the end of each cycle, a stiffness test was performed (ramp loading, 0.05 MPa to 0.8 MPa in 2 seconds). 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 minutes), followed by 10 hours 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-minutes pre-loading at 0.05MPa, 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 MPa to 3.95 MPa; 15 minutes), followed by a static recovery phase at 20 N (30 minutes) and a stiffness test (ramp loading, 0.05 MPa to 0.8 MPa in 2 seconds). 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 minutes), followed by 10 hours of recovery at 20N.

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.

Results

Each IVD lost height over the subsequent loading cycles in both the dynamic protocol and the static protocol.

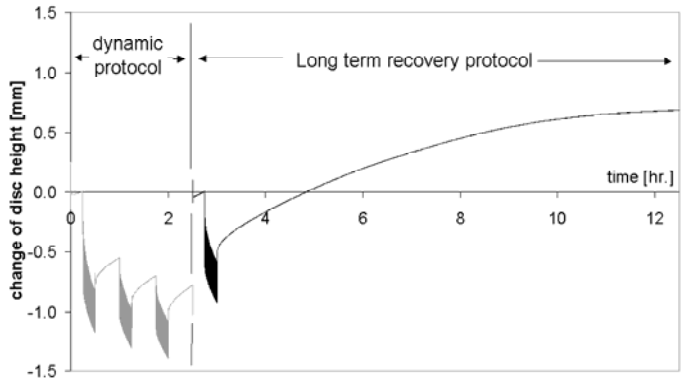

Fig. 1. Typical displacement curve for a disc in the dynamic group. The test is divided into two subsequent protocols. The first protocol (in grey) consisted of three loading cycles, each cycle comprising 15 minutes of loading (which due to the time scale appears as a shaded area), followed by 30 minutes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

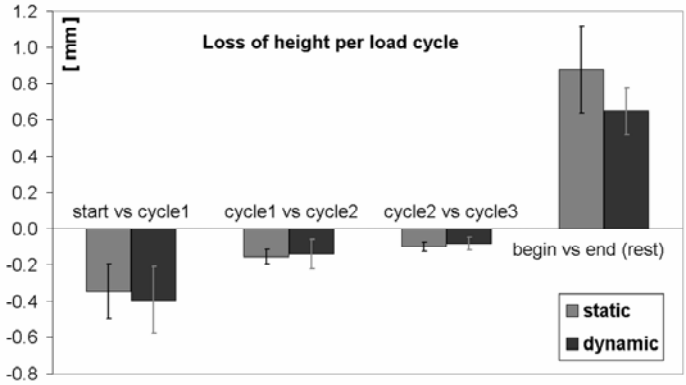

Fig.2. Averages changes of the disc height (sd error bars) over the first, second, and third loading cycles. The change of disc height was measured at the end of each recovery phase. The last bar is the change during long-term recovery, measured between the beginning of the loading phase and the end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

The loss of height per full cycle (comprising a loading and an unloading phase) shows that 30 minutes of unloading did not compensate for 15 minutes of loading. (Figures 1 and 2).

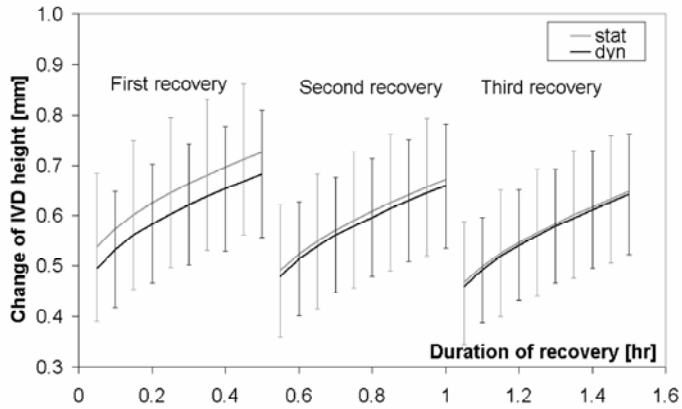

Fig.3. The average gains of height (error bars sd) of the static and dynamic group during the subsequent unloading phases. The averages of the two groups were not significantly different.

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 (Figure 3; Table 1).

	Height loss		Height recovery		Stiffness	
	F	p	F	p	F	p
Loading type	0.04	0.836	0.18	0.676	2.92	0.099
Cycle	78.80	<0.001	29.32	< 0.001	50.10	<0.001
Loading type * cycle	1.35	0.261	2.73	0.102	0.73	0.424

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).

The change in IVD stiffness was opposite to the changes in disc height (Figure 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.

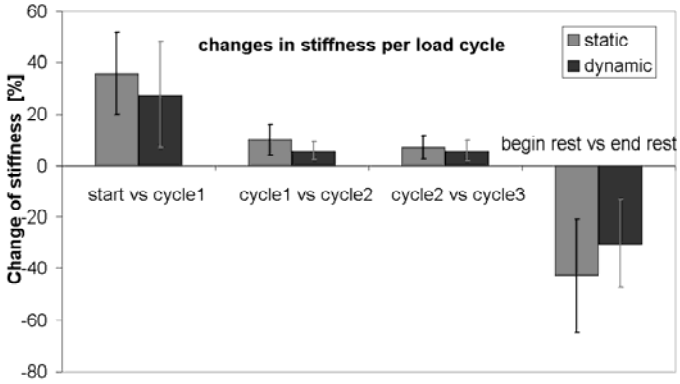

Fig.4. Average changes in stiffness (error bars sd) during the first, second, and third full cycles and during long-term recovery. Stiffness test were performed at the end of each loading cycle.

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 (Figures 1 and 2). Similarly, the decrease in stiffness after the long-term recovery was approximately equal but opposite to the loss of stiffness over protocol 1 (figure 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, no significant differences in change of disc height or in stiffness of the specimen were found ($p = 0.725$ and 0.606 respectively, Figures 2 and 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[19;24]. *In vivo* measurements of the signal intensity in MRI images of discs clearly show an increase in water content after a night's rest[17] and during the night disc height lost during the day is regained[18;26]. It has previously been found that recovery is hampered during *in vitro* testing of IVD's[16;31]. On the other hand, Johannessen et al.[11] 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 minutes of unloading did not compensate 15 minutes 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-hour 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[30]. 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[11]. 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 hours after 3 hours of loading in the study of Johannessen et al. (2004) and 10 hours after 2.5 hours 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 hours in humans) suffices to compensate a long period of net outflow (16 hours in humans)[4;17;28].

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 trough the endplate route is hampered in contrast with the *in vivo* situation possibly due to blood clots[31]. The present results clearly support this hypothesis. In addition, Lee et al.[15] found cell death in an IVD in culture, which they attributed to nutritional pathways through the endplate being blocked by blood clots.

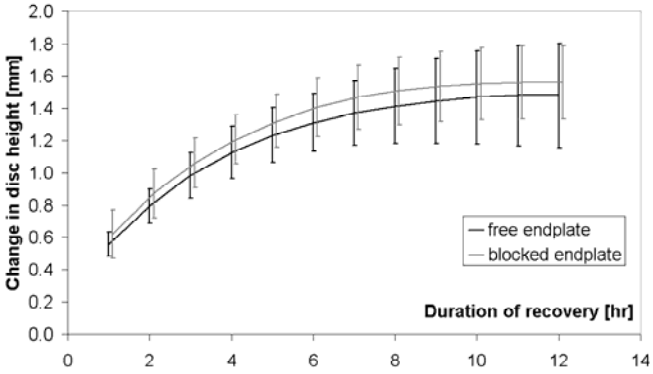

Fig.5. Average gains in height (error bars sd) during the long-term recovery phase of discs with blocked and free endplates. Gains of disc height were measured from the beginning of the recovery phase and were not significantly different between the groups.

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 ºC) 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 [5]. 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 IVD's in both the dynamic and static protocol were subjected to the same conditions, any effect of decomposition on the IVD's 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[6]. 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 IVD's from Dutch milk goats. This model has been used in other studies on the lumbar spine [33;37]. The anatomy of the disc and the loading situation are comparable to a sheep model, which is an accepted model for spinal research[35]. 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*[31] but also shows that the applied loading protocol, dynamic or static, is of no influence on the mechanical behavior during loading or recovery.

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CHAPTER 5

The influence of osmolarity in intervertebral disc loading: flow rate for fluid flow out of the disc exceeds flow rate into the disc.

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Submitted

Abstract

Fluid is squeezed out of the disc during compression and flows back during rest. As the daily period of activity is about twice the period of rest, it has been suggested that outflow must be slower than inflow. However, earlier *in vitro* experiments indicate the opposite. In order to address this paradox, we separated the effects of mechanical loading and osmotic pressure, and determined the respective time constants of fluid flow.

Axial compression (150, 300, 600 or 850N) was applied to twenty caprine lumbar discs for a period of 72 hours. The discs were immersed in saline during day one. The osmolarity of the fluid was lowered during day two and restored on day three. A stretched exponential function was used to quantify the creep.

The discs quickly lost height during day one, partly regained height on day two (inflow) and again lost height during day three (outflow). Time constants for changes in disc height due mechanical loading were much shorter than due to osmolarity changes. Time constants for outflow due to osmolarity changes were slightly lower than for inflow.

The difference in time constants of adaptation to mechanical loading and of adaptation to osmotic changes can explain the difference in mechanical behaviour between loading and rest. Outflow occurs due to high disc pressures under external mechanical loading and inflow due to osmolarity differences during rest This suggest that during the *in vivo* loading cycle fluid is lost shortly after rising and regained slowly during sleep.

Introduction

During daily activities, the intervertebral disc is subjected to varying mechanical loads. In response to such loads, it exhibits behaviour which is non-linear and time-dependent (visco- and poro-elastic mechanical behaviour) [14;28]. The intervertebral disc has high water content. Fluid flow in and out of the disc, therefore, plays an important role in the mechanical behaviour [1;2;11;14;16;25;27]. Disc height is governed by both the mechanical and osmotic pressures. The osmotic pressure of the nucleus depends on the difference between the osmolarity inside and outside the disc. Charged molecules in the nucleus attract and bind water and as such maintain disc height under loading. The unbalance between mechanical and osmotic pressures determines the direction of fluid flow. Whenever the disc pressure, due to the external load, exceeds the osmotic pressure fluid will flow out of the disc and when the unbalance is reversed fluid flows back [21]. It is clear that the hydration of the disc varies under influence of loading, but since these variations in water content also change the concentration of the negatively charged molecules of the disc, change in water content also changes the osmolarity of the disc. The osmolarity of a loaded disc is therefore higher than that of the unloaded disc.

The fluid content of the centre of the disc changes after sustained mechanical loading, while at the periphery, the hydration of the disc is more instantaneously affected by mechanical loading [16]. Due to the variation in loading 10-20% of the watery volume in the disc flows in and out of the disc in a daily cycle [15].

In general, there are two pathways for fluid into and out of the disc; fluid can flow through the endplate into the vertebral body or through the annulus fibrosis. The endplate route is thought to be the main route for solutes into the disc [18], the question remains whether fluid flow follows the same path. *In vivo* mechanical disc properties like water content, disc height and disc

stiffness restore in a daily cycle. We have shown previously in an *in vitro* study that the change of height of the loading phase is not equal to the unloading phase. The time required for recovery of disc height after mechanical loading exceeded the loading time [24], presumably because *in-vitro* outflow occurred faster than inflow. This contrasted with the *in-vivo* diurnal net change in fluid content, which typically is zero over a 24-hours cycle, while the duration of mechanical loading during daytime is longer than the recovery period during the night.

Using an isolated disc in a culture system both mechanical and osmotic pressures can be varied independently by varying the external mechanical load and the osmolarity of the external fluid. This allows estimation of time constants of the change in disc height due to mechanical loading and due to changes in osmolarity.

The aim of the present study therefore was to verify our hypothesis that fluid flow into the disc is slower than outflow. In addition, it is to be expected that disc properties depend on the loading history of the disc. Sustained loading leads to a change in water content of the disc. We hypothesized that higher water content is associated with an increased stiffness. We aimed to assess the relationship between the water content and the stiffness of the disc.

Materials and methods

Three-stages mechanical tests were performed on isolated intervertebral discs. We varied the mechanical load on the disc and the osmolarity of the fluid surrounding the disc independently to study their separate effects.

Five lumbar spines were harvested from approximately four-years old goats. Each spine was frozen in its entirety for later usage. When frozen, the discs were located using radiography. Each spine was divided into four separate intervertebral discs by use of a band saw. To obtain isolated disc specimens, the adjacent vertebral bodies were removed as close to the endplate as possible. Subsequently, the cutting edge was brushed clean. The frozen disc was thawed before testing. During testing, the disc was immersed in fluid.

Total testing time per intervertebral disc was 66 hours. This period was divided into one test phase of 18 hours and two phases of 24 hours. In total, four different loading levels were compared (150,300,600 and 850N). Five discs were tested per loading level. The compression load was kept constant throughout the test. The disc was immersed in saline (~295 mOsmol) during the first 18 and last 24 hours of the test. The osmolarity of the fluid was lowered during the second 24 hours (Figure 1).

The intervertebral discs were tested in a disc culture system. The culture system was fully closed. The compression load was applied at the top of the culture chamber. In order to allow fluid exchange via the endplate, the intervertebral discs were placed between porous glass plates (porosity code 2). The surrounding fluid (saline or demineralised water) was pumped through the system continuously. When changing the fluid, the system was flushed in order to remove all the fluid of the previous phase. Tests were performed at 37˚C.

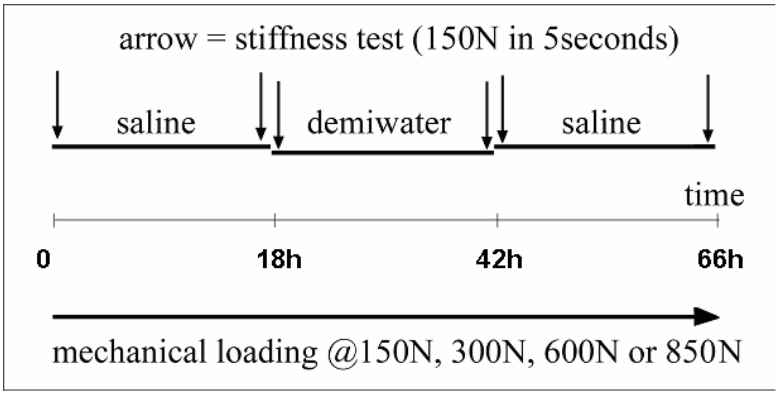

Figure 1: Test set up. The total testing time per specimen was 66 hours. In the first 10 seconds of the test the required axial compression load was applied, this load was maintained during the entire test. The invertebral disc was immersed in fluid of normal osmolarity (saline) during the first 18 hours of the test. At the onset of the second phase (24 hours) the fluid was exchanged with a fluid of low osmolarity (demineralised water; milli-Q). At the start of the final phase (24 hours) the surrounding fluid was exchanged for fluid with normal osmolarity (saline). Stiffness tests were performed at the beginning and end of each test phase.

Load was applied with a hydraulic mechanical testing device (Instron 8872 Canton, Massachusetts). Load and displacement were measured during testing at a sample rate of 2 samples/second. Change of disc height was calculated over each test phase. Stiffness measurements were performed at the beginning and the end of each test stage. For this the disc was loaded at a constant rate of 30 N/s for 5 seconds. The stiffness was calculated from the slope of the load-deformation curve.

During each test phase, a creep curve was established. In the study of relaxation of complex systems the stretched exponential function, or Kohlraush decay function, is frequently used as empirical decay law [5]. The advantage is that the curve is fitted with a minimum number of parameters. In literature this function was used to determine the indicators of the creep of the intervertebral disc[12].

The stretched exponential is of the form:

$$x(t) = d_\infty + (d_0 - d_\infty)(e^{(-(t/\tau)^\beta)})$$

Where *t* is the elapsed time, τ the time constant, β the stretch parameter, d_0 is the creep at t=0 and d_∞ is the creep at equilibrium (when t is infinite).

A stretched exponential function describes in general a decay function with two regimes. The first regime, for t until t = τ_0, a fast decay regime. The second regime, for t beyond t = τ_0, a slow decay regime[5]. This applies for a stretched exponential function with beta in the interval $0<\beta\leq1$. The response of an intervertebral disc to mechanical loading is typically a process with a beta in this interval [12]. The initial, fast response to a mechanical load is described by the faster-than-exponential-decay regime, followed by the creep behaviour, a decay regime that is slower-than-exponential. Time constant (τ) represents the time that a first order system requires to reach 63% of the asymptotic value after a step response.

The parameters τ, β and d_∞ were determined for each individual test phase (1,2 and 3). The fitted curve was optimised for the least summed square of the difference between the fitted curve and the measured data. The variance in data accounted for by the fitted curve (R squared) was used to describe the quality of the fit.

Statistics

Repeated measures analyses of variance were performed on the dependent variables: tau, beta and d_∞ to test the effects of osmolarity (within factor, 2 levels of osmolarity: low osmolarity and normal osmolarity) and to test the effect of loading level (between factor, applied mechanical load of 150N, 300N, 600N or 850N).

Repeated measures analyses of variance was performed on the dependent variable stiffness to test the effect of time on intervertebral disc stiffness, (within factor, stiffness at the begin and the end of the test phase)

and to test the effect of loading type on the stiffness (between factor, mechanical load, low osmolarity and normal osmolarity).

Results

During the first phase of the test, the height of the intervertebral disc decreased in response to the applied mechanical load (figure 2).

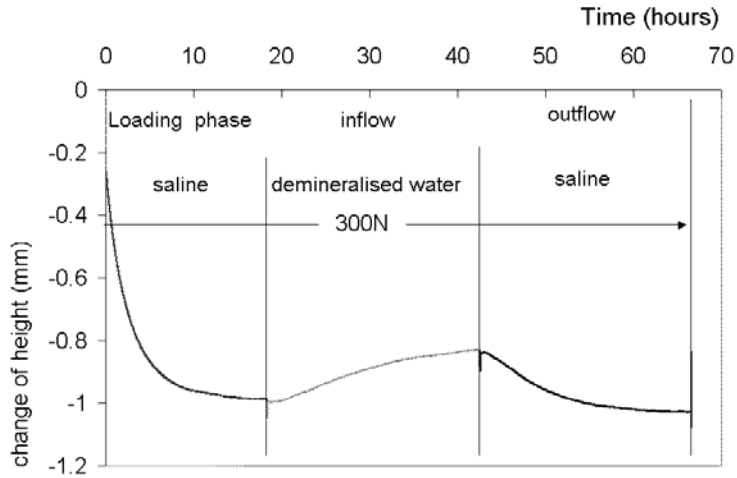

Figure 2: Typical test of 300N loading level. The total testing time was 66 hours. In the first 10 seconds a 300N compression load was applied, this load was maintained during the entire test. The disc lost height during the first 18 hours of mechanical loading, regained height after exchanging saline with demineralised water and again lost height during the final 24 hours of the test. The surrounding fluid was exchanged for fluid with normal osmolarity again (saline). Stiffness tests were performed at the beginning and end of each test phase.

After 18 hours the deformation rate of the disc approached zero, indicating that the disc approached equilibrium between the external mechanical load and the osmotic load. At the start of day two, saline was exchanged for demineralised water. The disc partly regained height during this phase. The degree of increase in height was related to the magnitude of the applied load (figures 3, 5). At the start of day three, demineralised water was replaced by saline again. This led to loss of the previous gained disc height. Again, the change of disc height was related to magnitude of the applied mechanical load (figure 3).

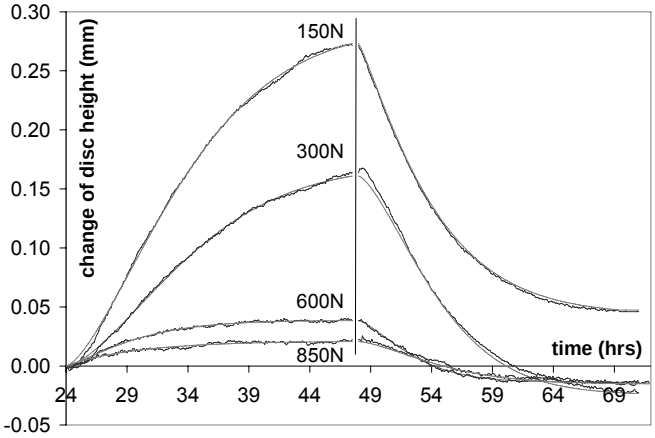

Figure. 3: Example of a stretched exponential curve and the corresponding measured data of a 150N, 300N, 600N and 850N test. Disc height increased after a drop in osmolarity of the surrounding fluid in the second phase (24hrs) and lost height again after restoring the original osmolarity level (24 hrs).

A stretched exponential function was fitted to the measured creep curves. Three parameters τ, β and d_∞ determine the shape of the stretched exponential as a function of the elapsed time. One sample of the 850N group showed large fluctuations (-0.03mm to 0.005mm) in the creep curve during the second phase. For this sample, during this phase, it was not possible to fit the stretched exponential function to the test data.

The time constant τ of the first phase was significantly smaller than time constant of phases 2 and 3 (figure 4, $p<0.003$). There was no main effect of force ($p=0.310$), but there was significant interaction between force and phase ($p<0.001$). In the second phase (inflow), the time constant decreased with increasing force ($p=0.013$), while in the third phase (outflow) there was no effect of force ($p=0.565$).

The time constant of the second phase was larger than the time constant of the third phase in the 150N, 300N and the 600N group. In contrast, in the 850N group the time constant was larger for phase 3 than for phase 2.

Repeated measures analyses of variance on all groups (150, 300, 600 and 850N) showed that the time constant for fluid inflow was larger than for out-flow (within subject factor) but appeared to be independent to the level of loading (between subject factor,($p<0.31$)).

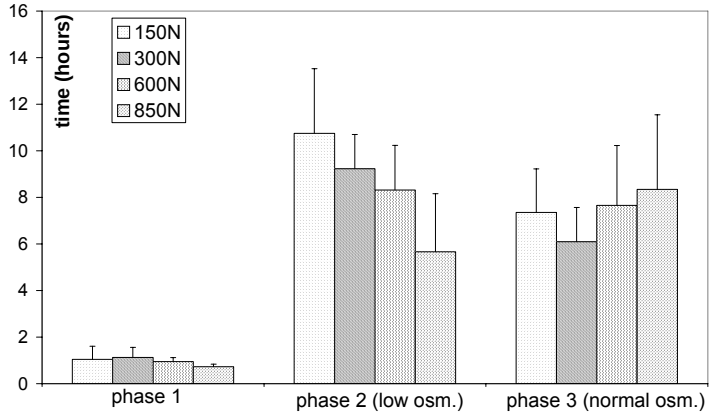

Figure 4.: The average times constant of successive test phases. The time constant of the mechanical loading phase is small compared to time constants of both osmolarity phases. The time constant of phase 3 (normal osmolarity) is lower than the time constant of the previous phase (except for 850N group). The time constant during inflow was significantly larger than during outflow ($p<0.002$). The difference between the loading levels of axial compression (the between subject factor) was not significant ($p<0.34$).

The parameter d_∞ in test phase 1 was on average -1.39 mm (SD 0.74), which was about ten times larger than the average parameter d_∞ in phase two and three (0.23 mm (SD 0.04) respectively –0.19mm (SD 0.05)). Comparison of phase two and three shows a clear dependency on the applied mechanical load (figures 3 and 5). There was no main effect of phase ($p=0.310$), but d_∞ became smaller with increasing force ($p<0.001$; figures 3 and 5). There was a significant interaction between force and phase ($p = 0.004$), with a slightly more pronounced decrease of d_∞ with increasing force for inflow than for outflow. The change in disc height ranged from 0.02-0.25 mm during inflow and from 0.02-0.20 mm during outflow and was not significant ($p=0.310$).

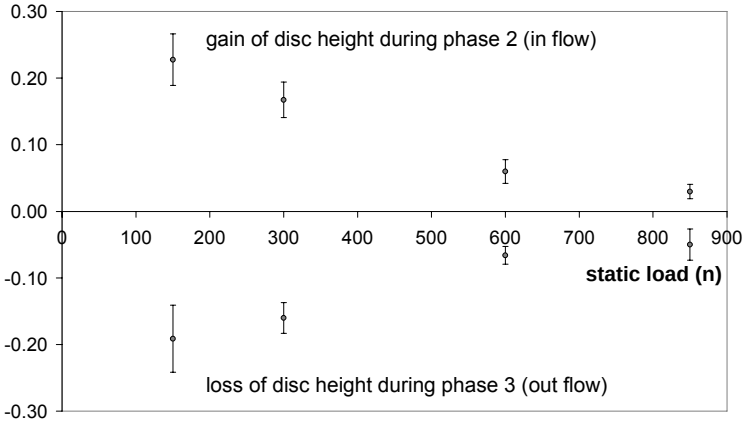

Figure 5.: Parameter d∞: Change in disc height due to changes in osmolarity for a disc at equilibrium between the external load and the osmotic pressure. The graph shows a relation between the external mechanical load (constant compression load of 150, 300 600 or 850 Newton) and changes due to osmolarity. The difference in d_∞ between the two levels of osmolarity (inflow and outflow) was not significant ($p<0.226$), while the difference due to the variation in axial compression load was ($p<0.001$).

The third coefficient of the stretched exponential function is parameter beta.

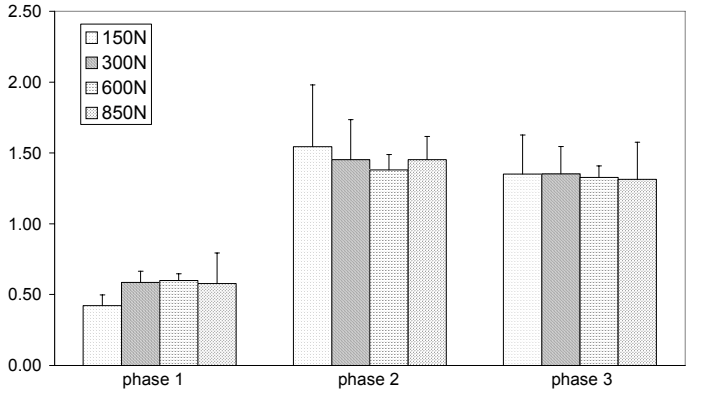

Figure 6.: Parameter Beta: average stretch coefficients of the tests with different external loads. Differences in beta due to mechanical loading and due to variation in osmolarity level were significant ($p<0.001$), while the difference between both osmolarity levels of the surrounding fluid was not. The beta's of the osmolarity phases were larger than 1.0 ($p<0.001$).

The average value of coefficient beta was 0.56 (SD=0.11) for the first phase. This is significantly lower than the values of beta for the second and the third phase ($p<0.001$). The value of beta for the second and third phase of the test was always larger than 1.0 with an average of 1.45 (SD=0.24) for the second phase and 1.34 (SD=0.20) for the third phase. The difference between the betas for inflow and outflow (c.q. the low osmolarity group and the normal osmolarity group) was not significant ($p = 0.223$), nor was there an effect of force ($p=0.820$) or an interaction effect ($p=0.925$).

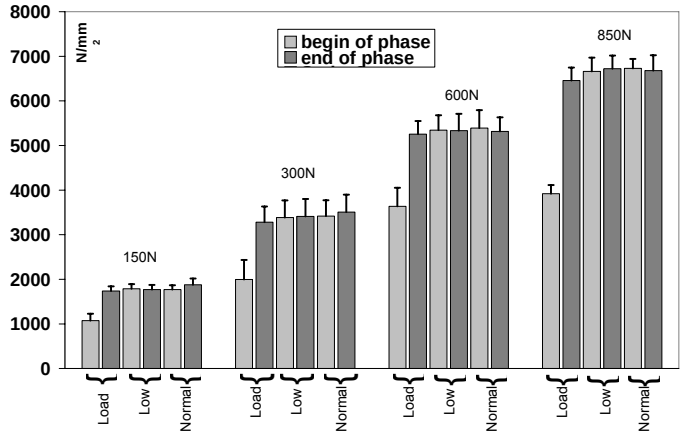

Figure 7: Change of stiffness due to mechanical loading and changes in osmolarity level. The change in stiffness due to load level was significant ($p<0.001$). The change of stiffness in time was significant for the mechanical phase and not for the osmolarity phases ($p<0.001$, $p<0.386$ and $p<0.462$).

Stiffness of the intervertebral disc was calculated at the start and end of each phase (figure 7). The stiffness of the intervertebral discs increased significantly after application of the mechanical loads at the onset of the test ($p<0.001$), with larger changes for higher forces ($p<0.001$). In contrast, varying the osmolarity of the fluid did not affect the disc stiffness (inflow and outflow, $p<0.386$ respectively $p<0.462$). The change in stiffness in time was significant during the first test phase (mechanical loading phase: $p<0.001$) but not significant for phases two and three

For one disc of the 850N group, the test was repeated after 48 hours at a load level of 150N. Disc behaviour was consistent with the other 150N tests, indicating that no damage had occurred as a result of the applied high mechanical load.

Discussion

In the present study, we measured the effects of changes in mechanical loading and changes in osmolarity on disc height and mechanical stiffness of the disc. To quantify the contribution of changes in osmolarity we separated the effects from the effect of mechanical loading in a three-stages mechanical test. The aim of the present study was to confirm our hypothesis that fluid flow into the disc is slower than outflow. Secondly, sustained loading leads to a change in water content of the disc. We hypothesized that higher water content is associated with an increased stiffness. We wanted to establish the influence of loading history on the disc properties.

Prolonged mechanical loading changes the osmolarity of the intervertebral disc. The production of extra-cellular matrix depends on the osmolarity of intervertebral disc [7]. Therefore, osmolarity affects maintenance of the disc *in-vivo*. An increase in loading level, both *in vitro* and *in vivo*, leads to an increased quantity of water being pressed out of the disc and a rise in osmolarity in the disc. The difference in osmolarity with respect to the surrounding fluid is, therefore, larger for a disc with an increased mechanical load. During the first test phase the disc was given the opportunity to reach equilibrium under mechanical loading. The osmolarity of surrounding fluid was iso-osmotic. When the fluid was exchanged for fluid of low osmolarity (demineralised water) an increase in difference of osmolarity between nucleus and test fluid was caused. This osmolarity step gave rise to an increase in disc height. Since the externally applied load was kept constant throughout the test, the increase in height was caused by inflow of fluid into the disc.

The osmolarity of the surrounding fluid was the same in all load cases (150N, 300N, 600N and 850N). Despite a larger osmotic pressure, the change in disc height appeared to stabilize at higher loads (figure 4). The difference in gain of disc height in the 600N test and 850N test was so small

(approximately 0.03 mm) that an increase above the 850N would probably not lead to an additional change in disc height and disc osmolarity.

Mechanical loading of the intervertebral disc leads to loss of disc height both *in vivo* and *in vitro*. The change of disc height was due to a combination of changes in water content of the nucleus and elongation of annulus fibres. This combined behaviour is typically a process with more than one time constant. This is confirmed by the parameters of the stretched exponential function of the first test phase. Parameter β was smaller 1 during the first test phase which describes a two-regime decay function. The initial, fast response to a mechanical load is described by a faster-than-exponential-decay, the creep behaviour by a slower-than-exponential decay. During the fast regime the annulus fibres are stretched and fluid of the periphery of the disc is directly secreted [16], while visco-elastic creep and change of fluid content in the centre of the disc are affected by long-term loading.

The beta of the phases two and three were significantly higher than 1.0 ($p<0.00$). This again describes a two-regime decay. The difference with $\beta < 1$ is that in the present case, the first regime is much slower than exponential instead of a faster-than-exponential. This effect may be explained by the composition of the intervertebral disc. Initially in test phases two and three, water could be attracted by osmolarity differences between the annulus and the environment while later on the fluid flow process could be accelerated when the water reaches the nucleus with its high proteoglycans content.

The time constants of the stretched exponential functions describing the different test phases also differ between the mechanical load step and both osmolarity steps. The time constant for adaptation to the mechanical load is small compared to the time constant changes due to osmolarity changes. In an earlier study[22;24], we have shown that, in repeated mechanical loading, the gain of height in the recovery phase is invariant, while the loss of height

in the loading phase decreases. The difference in disc behaviour between the loading phase and the osmolarity phase found in the present paper can provide an explanation for this. A different process possibly is responsible for fluid flow into or out of the disc. During loading fluid is mechanistically pressed out of the disc. Ideally, the over pressure in the nucleus would transform into an under pressure in the pores of the disc during unloading. This would enable a fast recovery of fluid content. However, due to the large compliance of the soft tissues (nucleus and annulus) this is not likely, therefore, fluid mainly flows back due to osmolarity differences. We presume therefore that the initial fast recovery, shown in our previous studies, was mainly visco-elastic.

Since the disc *in-vivo* is mechanically loaded in a daily cycle and, though never in equilibrium, is able to reach a stable diurnal cycle it is clear that this is only possible, when the behaviour during loading is compensated during unloading. Comparing the time constants of both osmolarity steps the time constant during inflow was larger than during outflow. In other words, it was easier for fluid to flow out of the disc than into the disc. This appears to be in contrast with a study of Ayotte [3;4]. This study focuses on endplate properties and shows that the flow rate for fluid travelling through the endplate into the disc is smaller than for fluid going out of the disc. In the present study, a complete intervertebral disc was tested. The disc is a complex structure of which the mechanical behaviour is a compound of the behaviour of the building blocks [23]. Conclusions on overall behaviour of the disc can therefore only be made when all elements of the disc are tested in their inter-dependence.

The last objective of the test was to measure the effect of fluid flow on intervertebral disc stiffness. This parameter was measured at the beginning and the end of each test phase. This gave us the opportunity to see the effect of creep on the compression stiffness of the disc. The stiffness

changed during the first phase but did not change during both osmolarity phases. Since the mechanical component of the creep already approached its equilibrium at the end of the first phase, we presume therefore that the change of stiffness is mainly related to visco-elastic strain of the fibres and not to the water content of the disc.

Disc nutrition has been implicated in the development of degeneration in the disc. It has been shown that impaired nutrition and accumulation of waste products are strongly related to disc degeneration [6;9;10;20]. In a healthy intervertebral disc the hydration, thus the osmolarity, changes due to changes in external loading. Osmotic pressures play a role in the intervertebral disc cell synthesis of matrix molecules at the transcriptional level. Extra-cellular matrix which is produced by disc cells depends on the osmolarity of intervertebral disc [7]. Although fluid flow plays a only a small role in nutrition of the intervertebral disc [19;20] mechanical loading does affect maintenance of the disc. Therefore, sustained loading without a sufficient unloading time might be detrimental for disc maintenance.

Changing the osmolarity of the surrounding fluid of a mechanically balanced intervertebral disc gave us the opportunity to investigate the effect of fluid flow in an intact disc. The osmotic disturbance, in a disc, which is mechanically in equilibrium, is un-physiologically large due to the use of demineralised water and saline. It is remarkable that the stiffness of the disc was not changed due to in- or outflow of fluid. Our hypothesis that in flow of fluid increases the stiffness of the disc, was not confirmed. We presume therefore that the increase in stiffness, in the first phase of the test, was mainly due to straining of annulus fibres and not to changed water content of the disc.

In conclusion, increase of disc height, due to fluid inflow based on an osmotic gradient, was slightly slower than loss of disc height due to fluid

outflow based on an osmotic gradient. The combined effect of fluid outflow and soft tissue deformation due to mechanical loading led to a much faster disc height loss. These results may suggest that *in-vivo* the water content changes rapidly in the beginning of the day and slowly recovers during the night.

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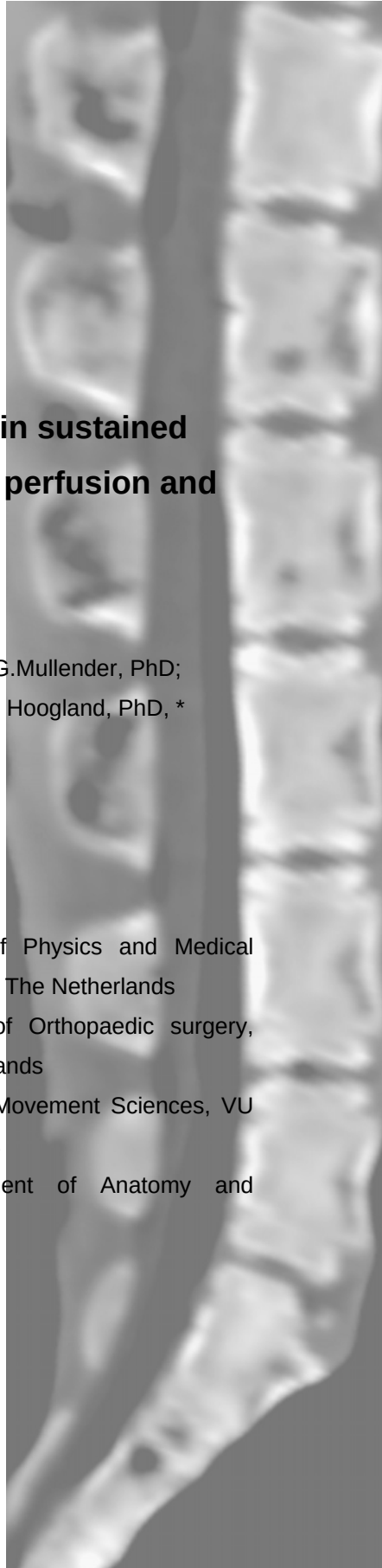

CHAPTER 6

Intervertebral disc mechanics in sustained compression: effects of heparin perfusion and frozen storage

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Submitted

Abstract

When an intervertebral disc is compressed fluid is expelled from the disc, while during rest fluid is absorbed. *In vivo* this leads to a stable diurnal cycle. *In vitro* studies, however, show no stabilization, even if the time available for inflow exceeds time for outflow. We hypothesized that blood clots obstruct mainly the inflow through the endplates. In the present study, we investigated whether *in vitro* mechanical behaviour of the disc recovers after perfusion with heparin to prevent blood clots.

Twelve lumbar spines (goat, approx. 4-year-old) were harvested; six goats were treated with heparin before euthanasia. Discs were tested within 3 hours after death or after 24 hours frozen storage.

Axial compression was applied in an organ culture system. Porous plates allowed fluid exchange via the endplates of the disc.

Discs were tested during eight cycles of three hours each. Each cycle comprised of two hours of dynamic loading (sine, 0.5Hz, 0.2-0.8MPa) and one hour of static loading (static, 0.2MPa). Each cycle was preceded by a stiffness test (compression; 150N=>300N in 1s.).

The mechanical behaviour of all discs stabilized in three cycles. After that, the loss of disc height due to loading was compensated during the recovery phase. The gain of disc height during the recovery phase was virtually constant over all cycles. Heparin perfusion had no effect on the results. Combined with previous results, this suggests that blood clots do not obstruct endplates *in vitro*, that inflow of fluid is slow and that the annulus plays a large role in the fluid flow process.

Introduction

The external load on the intervertebral disc changes constantly during daily activities. During the diurnal loading cycle, in which the load on the spine is low during night and is high during daytime, exceeds the loading time the duration of rest. As a consequence the pressure in the intervertebral disc changes. Pressures reported in the literature vary between 0.1 MPa for lying in prone position to more than 2 MPa for lifting activities[25].

The intervertebral disc is a complex structure with visco- and poro-elastic material properties. The response of the spine to mechanical loading is, therefore, non-linear and time dependent[13;18]. The water content of the disc and in particular of the nucleus varies under influence of external loading. As a consequence, fluid flow plays a large role in the mechanical behaviour of the intervertebral disc[10;11;24]. When the external load on the disc exceeds the osmotic pressure of the nucleus, fluid will flow out of the disc. This will lead to a change of disc height and water content of the nucleus. This in turn, will change the proteoglycan concentration of the disc. Fluid will be pressed out of the disc until equilibrium between the external load and osmotic pressure is reached[14;21]. When the imbalance between the external load and the osmotic pressure of the nucleus is reversed fluid flows back into the disc. The disc will regain its water content[16] and mechanical properties[23]. The regulating mechanism of fluid flow is the continuous tendency towards equilibrium between the external load on the intervertebral disc and the osmotic pressure in the nucleus.

There are two pathways for fluid flow into or out of the disc: through the endplate into the vertebral body and through the annulus fibrosus. It is generally assumed that the main flow path for nutrition of the nucleus cells leads through the endplate into the vertebral body [19]. The vertebral body and the intervertebral disc are connected via channels through the bony endplate[17].

It has been suggested that the resistance of these channels to flow is direction-dependent, with the resistance to fluid flow into the intervertebral disc being lower than the resistance to outflow[3]. This would allow full recovery of disc properties during eight hours of rest, after 16 hours of loading. However, there is no interconnection between the cartilage endplate and the lamellar subchondral collagen. The marrow contact channels end in capillary buds which connect the bone with the endplate, but do not penetrate into the cartilaginous endplate[7;12]. In an earlier study, we have shown that the mechanical behaviour of the disc *in vitro* is asymmetrical with respect to loading and unloading[22]. We found a net loss of height over repeated cycles. Although the recovery time was twice the loading time we could not show a stabilised mechanical pattern in three test cycles. A longer recovery time was needed to compensate the loss during loading [23]. Equilibrium was not reached within a single loading or unloading phase. This behaviour appears to be in contrast with *in vivo* behaviour where the duration of the loading phase exceeds the duration of rest (16 hours vs. 8 hours) but nevertheless a stable daily pattern occurs[15]. One explanation is that obstructed pores in the endplate, due to blood clots, could be responsible for this effect[22]. Alternatively, frozen storage has been shown to affect mechanical behaviour in sustained compression[5]. This may underlie this apparent difference between *in vivo* and *in vitro* behaviour.

Therefore we investigated in the present study the effect of the prevention of blood clotting by perfusion with heparin and the effect of frozen storage on disc mechanics. Furthermore, we have examined how fast mechanical behaviour stabilises over subsequent test cycles, with respect to disc height and compression stiffness, when loading time exceeds the unloading time.

Material and methods

The lumbar spines of twelve approximately 4-year-old goats were harvested. Six of the spines were taken from goats, which had been treated with heparin. The other six were collected from untreated goats. The spines of the untreated group, the control group, were obtained from a local slaughterhouse and stored at -20ºC.

This study was performed in compliance with the regulations of the Dutch legislation for animal research and Animal Ethics Committee of the VU University Medical Center approved the protocol.

The goats of the heparin treated group (six goats) received a large dose of heparin (20000 IU) 15minutes before death. The animals were euthanised with a lethal dose of pentobarbitalnatrium (0.75ml/kg). After death the complete spine was flushed with saline through an intravenous unit (iv). In the saline 50000 IU of heparin were solved. The iv unit was connected, immediately after death, to the azygos vein just proximal to its point of entry into the superior vena cava[8]. In order to accelerate the perfusion, the left and right arteria femoralis were closed with a ligature. Flushing of the spine was continued until clear fluid came out of the abdominal aorta. As a check on the effectiveness of the perfusion method, three neighbouring intervertebral discs of one of the spines of the heparin group were saved for histology. HE and DAP staining was performed on histological sections of the endplates obtained from these discs. The staining showed that in the endplate only occasionally minute traces of blood were found. Therefore, in intervertebral discs of the heparin group blood clots did not hamper fluid flow trough the endplate.

The procedure of flushing the spine with heparin took one hour; the intervertebral discs (L3/L4) of the heparin group were tested within three hours after death. The intervertebral discs (L3/L4) of the control group (fresh, not frozen) were tested within two hours after death at the slaughterhouse.

Discs (L2/L3) of the control group were frozen at -20ºC and tested 24 hours later.

The discs were located in the spine using radiography. The adjacent vertebral bodies were removed as close to the endplate as possible. The cutting edge was brushed and rinsed clean to remove loose particles.

The frozen discs were thawed immediately before testing. The overall testing time was 25 hours and was performed in a disc culture system. Since the mechanical behaviour of the disc depends on the loading history the disc discs were preloaded for one hour at 0.2MPa. The remaining testing time was divided into eight loading cycles: each loading cycle consisted of a sinusoidal loading phase (0.2-0.8MPa, 0.5Hz, 2hours) and a static unloading phase (0.2MPa, 1hour). The loading protocol was based upon *in vivo* intradiscal pressure measurements in humans by Wilke et al [25]. The test system was filled with fluid (DMEM, +10%FBS and 1 % antibiotics) and was fully closed from the environment. In order to allow fluid flow via the endplates, the intervertebral disc was placed between two porous glass plates (porosity code 2). A peristaltic pump circulated the surrounding fluid continuously. Tests were performed at 37˚C.

A mechanical testing device (Instron 8872 Canton, Massachusetts) applied an axial compression load to the top endplate of the disc. Load and displacement were measured during static testing at a sample rate of 10 samples per second. Change of disc height was calculated over each test phase. Stiffness measurements were performed at the beginning of each loading and unloading phase. For the stiffness test, the external load on the disc increased from 150N to 300N and back at a constant rate of 30 N/s. The associated stiffness was calculated from the slope of the load-deformation curve.

Statistics

Mixed design measures analyses of variance were performed on the dependent variables height and stiffness during loading and recovery to test the effects of time (within factor: eight steps in time) and the effect of the treatment (between factor: control group (fresh), frozen and heparin).

Results

In figures 1, 2 and 3 the change of disc height is depicted. Figure 1 shows the raw data of the change of disc height (typical) of an intervertebral disc from the heparin group. This graph is also illustrative for the displacement curves in both other groups.

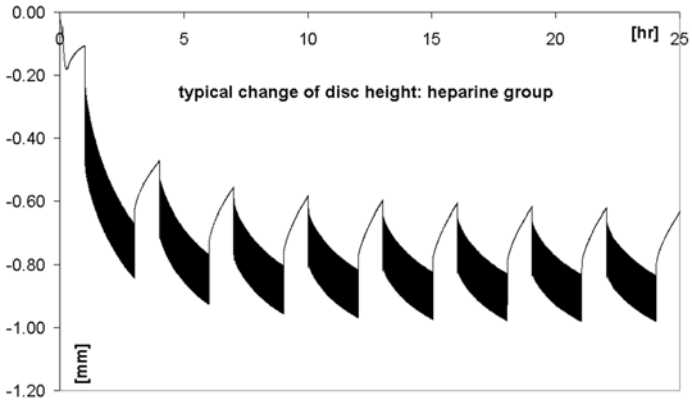

Figure 1. This figure depicts the change of the height of an intervertebral disc of the heparin group (typical result). The disc lost height during the sinusoidal loading phase while height increased during the static unloading phase. The change of height decreased in subsequent cycles.

Disc height was lost during the loading phase and partially regained during the unloading phase. The gain of disc height during recovery was virtually constant over the test cycles. The mechanical behaviour stabilized after the third test cycle (figure 2). The disc moved towards a stable cyclic behaviour within three test cycles. However, equilibrium was not reached at the end of either the loading or the unloading phase.

The loss of disc height per cycle was time dependent (Figure 2: $p<0.001$). Loss of disc height decreased during the first three loading cycles and remained stable from the fourth loading cycle. The difference in change of height was approximately 40% between first and second cycle, 10%

between second and third cycle, 5% between and third and fourth cycle and 1% between the following test cycles. Pairwise comparisons between test cycles showed that the change of disc height was significantly different between the first three cycles ($p<0.005$). Differences in loss of disc height between heparin treated, the frozen and the untreated disc were not significant ($p<0.514$).

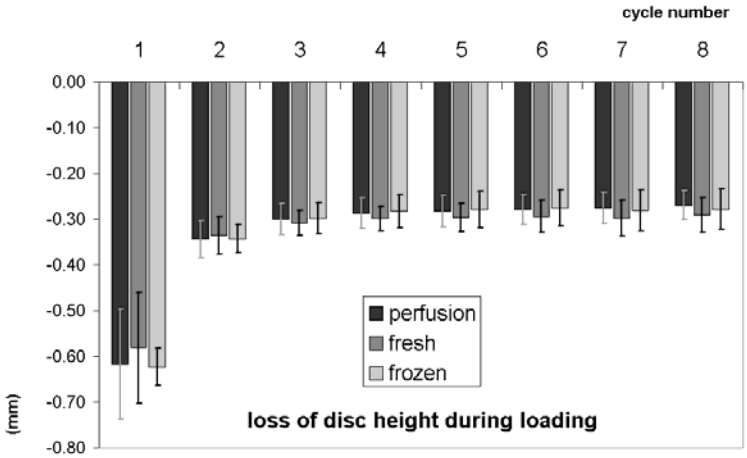

Figure 2. The average loss of disc height during the loading phase in subsequent cycles for all groups. The change in disc height during loading stabilized after three cycles. No significant differences were observed between the perfusion group, the fresh and the frozen group.

The gain of disc height in the subsequent cycles was virtually constant throughout the test (Figure 3: the disc height increased approximately 3% between first and second cycle, 1% or less between the subsequent cycles). Pairwise comparisons between the consecutive cycles showed that the increase during unloading was not significantly different in subsequent unloading cycles. The overall change of disc height per full cycle was therefore mainly determined by changes during the loading phase. Differences in gain of disc height between the untreated, heparin treated and frozen discs were not significant. The loss of height was compensated by the

gain of disc height after the third cycle. The net loss of height per cycle was not significantly different from the next cycles (figure 2 and 3). This applied to the heparin treated, untreated and frozen samples.

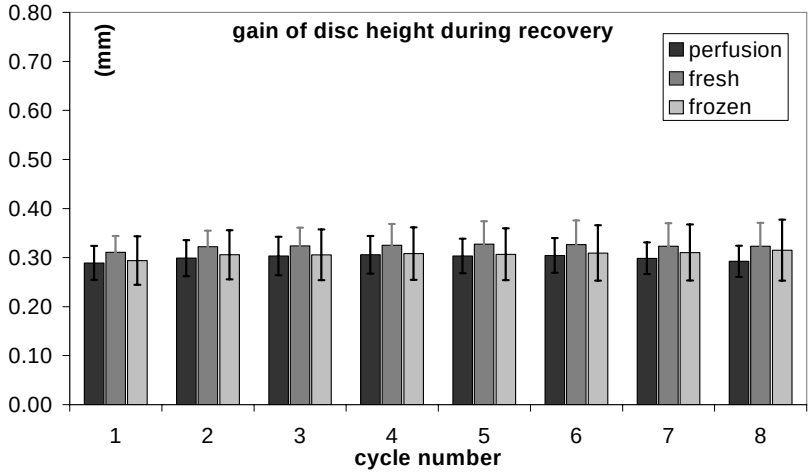

Figure 3. The average gain of disc height during the recovery phase in subsequent cycles for all groups. The increase in disc height during the recovery was in almost constant over all test cycles.

The intervertebral disc stiffness also stabilized after three cycles in all groups. Figure 4 shows the change of stiffness of a single intervertebral disc from the heparin group (typical result). The slope of the load-deflection curve of each single sinusoidal loading cycle was used as indicator of the instantaneous stiffness. The stiffness increased during all loading cycles and decreased during recovery but the increase was the largest during the first cycle (figure 4).

The disc stiffness increased mainly during the first test cycle (figure 5) and reached a stable pattern of increase and decrease during the next cycles. The change of stiffness during the second test cycle and further was less than 2 percent. Differences between the groups (perfusion, fresh and frozen) were not significant.

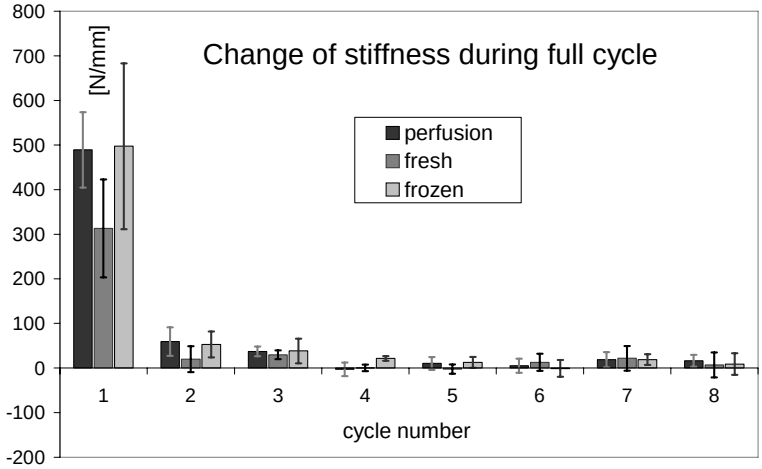

Figure 4. Increase of intervertebral disc stiffness in the heparin group (typical result). The change of stiffness decreases in subsequent cycles. Since the differences in other groups are comparable to the heparin group only the latter is shown.

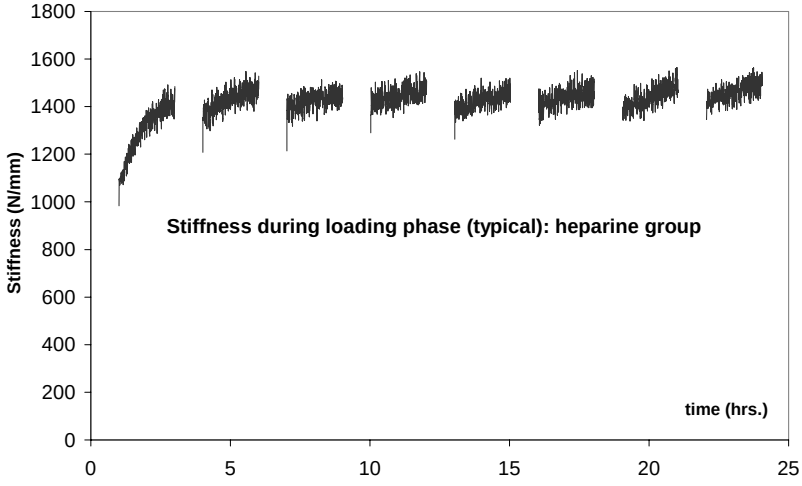

Figure 5. This graph shows the change of intervertebral disc stiffness in subsequent loading cycles (loading plus unloading). The mechanical behaviour stabilized after three cycles.

Discussion

In the present study, we investigated the effect of prevention blood clotting (heparin) and the effect of frozen storage on disc mechanics. We compared changes in disc height and stiffness and found no differences with control discs. Secondly, we investigated the cyclic mechanical behaviour of these discs between subsequent loading cycles (loading plus unloading) and found that stable behaviour occurred after the third loading cycle. Histological staining (DAP and HE) on endplates of spare discs confirmed the validity of our perfusion method.

In vivo the duration of external loading on the spine exceeds the duration of rest. The loading time is approximately 16 hours while the unloading time is 8 hours. This leads to a stable cyclic pattern in which the creep behaviour during loading is in balance with creep during recovery. Focussing on fluid flow, this implies that the fluid flow out of the disc during loading is compensated by fluid flow into the disc during rest. In an earlier study, we have shown that *in vitro*, even when the unloading time was twice the loading time, the loss of height was not compensated during unloading [22]. We presumed that the endplates of the disc were the limiting factor for fluid inflow. To overcome this barrier we now have flushed the spines of half of the goats with heparin. In contrast with our expectations, we did not find an improvement of the inflow of fluid during recovery. Nor did we find any difference in mechanical response due to freezing of the discs. It is therefore unlikely that obstruction of the endplate or frozen storage is responsible for the difference in mechanical behaviour between loading and unloading.

In our previous studies, the duration of the unloading phase was twice that of the loading phase, while the number of cycles (loading plus unloading) was limited to three. In the present study, we have chosen to mimic the *in vivo* loading pattern in which loading time is twice as long as unloading time. This study showed that, although the discs did not reach

equilibrium, the overall cyclic mechanical behaviour of the disc stabilized after three test cycles in all groups. The loss of disc height of the loading phase during the first three test cycles was not compensated during recovery. The gain of disc height of the recovery phases of the successive cycles was virtually identical. There was a very small increase in the gain of height, but this change was small in comparison to the changes of mechanical behaviour during loading. This is in line with the long duration required for full recovery found in our previous study [23]. The present study shows that disc properties (height and stiffness) changed mainly during the first loading phases. These changes decreased in subsequent loading phases until the invariant recovery phase could compensate the changes of the loading phase.

Initially we interpreted the dissimilarity in mechanical behaviour between loading and unloading phases [23] as indicating a limited validity of *in vitro* models of the motion segment and disc. The present results suggest that a stationary mechanical behaviour, similar to the *in vivo* situation, can be reached within three loading cycles. Indicating that *in vitro* behaviour, as found in the present study, can be compared to mechanical behaviour *in vivo*. A slow recovery of water content of the disc during night is followed by a short period of loss of water after rising in the morning[2]. Then, during daytime, a cyclic mechanical behaviour is found dependent on loading conditions and thus on daily activities. Fluid flow into the disc would then not play a substantial role during load changes during daytime since this process would be too slow.

In an earlier study, we have shown that fully blocking the endplate with a silicon paste had no effect on the recovery rate of the disc [23]. In that study the disc did fully recover, but the time to recovery was very long (12 hours). Heparin perfusion had no effect on the results of the present test. It has been reported that the permeability of the endplate for inflow and outflow is asymmetric. Ayotte et al [4] showed a direction dependent resistance for fluid

flow through the endplate with resistance for outflow being larger than for inflow. This is in contrast with the paper of Accadbled et al[1] who showed a 35% larger permeability for outflow than for inflow. However, the present study, which showed no differences between heparin treated and frozen discs, suggests that flow through the endplate was not hampered in the previous *in vitro* studies. Indicating that role of the endplate *in vitro* is limited. The annulus seems to play a major role in the fluid flow process during recovery. Suggesting that the major pathway of fluid flow is through the annulus, not through the endplates. This seems to be in contrast with the generally accepted route for intervertebral disc nutrients. Rajaskaran et al.[19] showed that these solutes diffuse mainly through the endplate. Indicating that the inflows of nutrition and fluid are different processes.

The present study shows that the mechanical behaviour of the intervertebral disc stabilized during a repeated loading and recovery cycle. The disc, however, is not in equilibrium at any stage. Loading history of the disc determines the mechanical behaviour of the disc. This has implications for testing of intervertebral discs in general. The mechanical properties of the disc differ, shortly after rest, from properties after prolonged loading. This can be compared to *in vivo* behaviour where mechanical properties during bed rest presumably differ from disc properties during daytime. The mechanical test should be designed for either situation. Measurements on disc height, creep rate and intervertebral disc stiffness can only be mutually compared if the tested discs have the same loading history. Preloading of the disc can bring a disc in the required stable state, daytime or rest.

Tests were performed on mature caprine specimens. Use of quadrupeds as a model for human discs is generally accepted for mechanical testing [6;20]. Although the size and biochemical composition of the caprine disc differs from the human disc, it is a common model for spinal research on the adult human spine [9].

The test environment was designed to mimic the *in vivo* environment of the intervertebral disc. The specimens were tested in an organ culture system. In the system the temperature was raised to body temperature of the animal. The total testing time was 25 hours, mechanical behaviour stabilized within 9 hours and remained at that level. We have, therefore, no indication that decomposition of the disc played a major role in the outcome of the study.

Our hypothesis was that the fluid flow into the disc was hampered due to blood clots or due to frozen storage. However, perfusion of the spine with heparin or freezing of the intervertebral disc had no effect compared to the response of an untreated (fresh) control disc. This suggests that the endplate was not obstructed with blood clots after all and that the *in vitro* testing, in contrast with our earlier finding, appears to be a valid model for *in vivo* behaviour.

The present results, combined with our previous studies, suggest that *in vitro* fluid flow occurs mainly through the annulus, with a fairly rapid outflow and a slow inflow. After the rapid outflow, a stationary cyclic pattern can occur apparently determined by visco-elastic creep of the annulus. This mechanical behaviour may be representative for *in vivo* disc mechanics.

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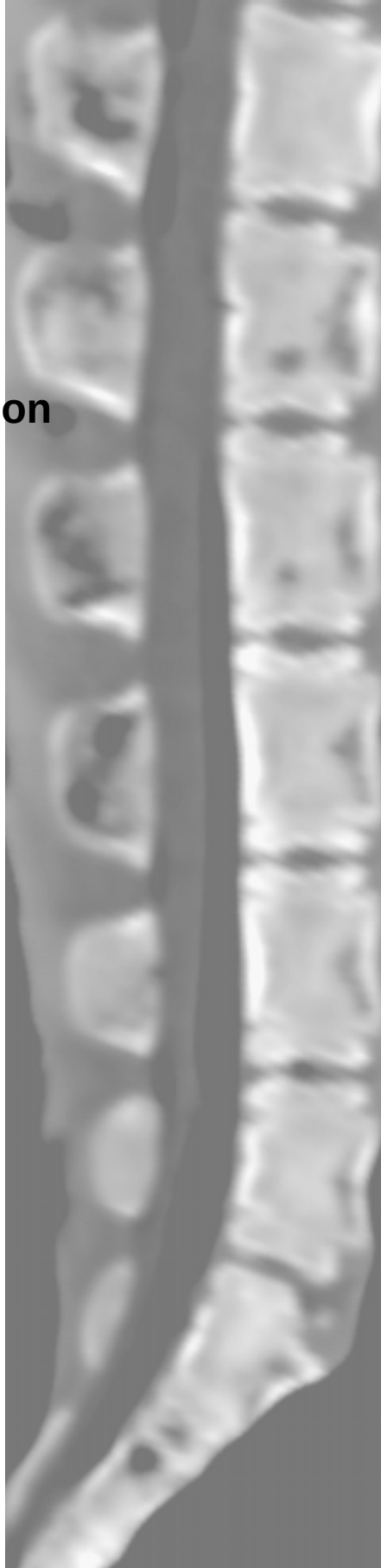
CHAPTER 7

General discussion



General discussion

The topic of this thesis is the response of the intervertebral disc to sustained axial mechanical loading.

Sustained loading may play an important role in disc degeneration. It has been shown that sustained static loading can cause damage to the intervertebral disc[2;3;13]. In addition, intervertebral disc cells respond differently to sustained static loading and to dynamic loading[10]. In literature, mechanical behaviour of the disc is often described from short-term experiments, usually with the unloaded situation as starting point. The effect of the loading history of the disc, e.g. the minimum load during rest, is in general discarded. This gap in knowledge on disc behaviour under sustained loading is the basis of the present research in this field of biomechanics. The aim of this thesis is to describe changes of disc properties in time due to sustained loading and to understand the mechanism behind these changes in mechanical behaviour.

The second chapter of this thesis addresses the mechanical behaviour of a motion segment and its separate components (intervertebral discs, vertebrae, motion segments and bone samples) during axial compression. Bone samples without endplates showed almost instant deformation followed by a marginal creep. Bone samples with the endplates attached showed much larger creep. Indicating that the cartilage endplate is more deformable than the supporting trabecular bone (visco-elastic behaviour). After the early creep phase fluid flow from the disc and visco-elastic stretching of the annulus come into play. Creep of a motion segment is by nature a combined visco and poro-elastic process. The time constant of this combined process is much larger than the creep of bone and endplate. Creep of a motion segment can be divided into an early and a late phase due to the different behaviour of the building blocks. Creep of the endplate is substantial during the early phase of the test, while creep of the soft tissues

dominates the late phase of the test. Due to the substantial influence of the endplates on the overall time-dependent behaviour of a segment a test specimen should consist of the intervertebral disc with only half of each vertebral bodies attached. In this set-up, creep deformation can be measured, while the stiffness of the supporting structure allows bulging of the endplate into the vertebral body.

Deformations of the disc, the endplates, and of the trabecular bone contribute to the deformation of a motion segment. In the third chapter of this thesis, it is shown that the mechanical response of a disc subjected to a repeated loading cycle is asymmetrical, i.e. the changes in disc properties occurring during the loading phase were not compensated during unloading. In spite of the recovery phase being twice as long as the loading phase. Both the change in disc height and the hydrostatic pressure in the nucleus were measured. The overall loss of disc height during axial loading was not fully restored during rest. Also the nucleus pressure, which decreased during subsequent loading phases, showed no full recovery during rest, indicating that the osmotic pressure of the nucleus did not restore during unloading. These results pointed the ongoing research into the direction of the endplate. The hypothesis was that the flow rate through the endplate was smaller for fluid going in to the disc than out of the disc. Obstruction of the endplate route by blood clots was thought to be responsible for this.

In vitro recovery of disc properties, however, is feasible. It has been reported that disc properties were restored after tong term recovery (18hours of rest)[2]. In chapter 4, fluid flow through the endplate was investigated as well as the effect of loading type on recovery (static versus dynamic loading). Samples were divided into two groups; one group was subjected to a static loading and the other group to dynamic loading. In half the samples of each group the endplates were blocked in the second part of the test, which consist of a long-term recovery test. In contrast to our expectations, no

difference was found in the response of discs with the endplates open and discs with the blocked endplates. In addition, it was shown that the disparity between loading and recovery behaviour was not due to the loading type. Full recovery was found for all parameters studied after long-term recovery. This contrasts with the observation that in vivo a relatively short period of 8 hours of rest, suffices to compensate a period of 16 hours of activity [1;4;5]. This suggests that inflow of fluid into the disc is slow compared to the outflow.

Initially we interpreted the findings in the preceding experiments as an indication of limited validity of *in vitro* models of the motion segment and disc. In order to further investigate this incongruity, we attempted to uncouple the fluid flow and visco-elastic behaviour. In chapter 5, we investigated the differences between inflow and outflow.

The protocol in chapter 5 was divided into three separate test phases. During the first phase, the nucleus pressure of the disc was enabled to reach equilibrium with the applied external load. At the start of the second phase, the surrounding fluid was exchanged for fluid of a lower osmolarity. At the start of the last phase the osmolarity was changed back to the initial level. The external load remained on the disc throughout the entire test.

During the first phase, fluid was pressed out of the disc while, at the same time, the annulus fibres were stretched. As a result the disc height decreased. During the second phase, disc height was partially regained. Since the applied load was constant, this can only be explained by inflow of fluid. During the last phase the change in osmolarity led to a decrease in disc height. When comparing the time constants of the last two test phases it was established that the time constant for fluid flow into the disc was larger than for the reversed flow direction. The ability to attract and retain water is dependent on the applied external load. During loading the hydrostatic pressure in the disc is increased. Therefore fluid is squeezed out of the disc, this will increase the proteoglycan concentration in the disc and

consequently the osmotic pressure. Fluid will flow out of the disc until the external load is balanced by the osmotic pressure[3;6]. The instantaneous water content of the disc depends thus on the loading history of the disc [7]. Therefore, properties (e.g. disc stiffness and height) of an intervertebral disc are not intrinsic properties, but change with the loading history.

The results show that the flow rate of fluid going into the disc is smaller than the flow rate for fluid going out of the disc. Again, this appears to be in contrast with *in vivo* observations. The disc *in vivo*, though never in equilibrium, is able to reach a stable diurnal cycle in disc properties. It is clear that *in vivo* changes due to loading are compensated during recovery. The previous experiments have shown that changes during recovery are slow compared to changes during external loading. This indicates that *in vitro*, in order to reach a stationary mechanical behaviour, the magnitude of changes in subsequent loading phases have to decrease, until these changes are in balance with the changes in the recovery phase.

The role of fluid flow trough the endplate and the disparity between loading time and recovery was further investigated in chapter 6. In the third chapter of this thesis, we hypothesized that the endplates of the disc were the limiting factor for fluid inflow. To this end changes in mechanical properties of intervertebral discs treated with heparin were compared with untreated discs. Heparin was used to prevent the clotting of blood in the endplate. In the experiments, it was attempted to achieve stationary mechanical behaviour in a protocol that mimics the ratio of loading time versus rest of the diurnal loading cycle. The intervertebral discs were tested for 24 hours divided in 8 loading cycles. Comparing the mechanical behaviour of both groups, there was no improvement in disc recovery of the treated discs. As such, results of chapter 6 were in line with the outcome of the experiments in chapter 4. The previously suggested explanation that obstruction of the endplate could be responsible for the asymmetric mechanical behaviour is therefore unlikely.

The results showed stabilization of mechanical behaviour after three loading cycles. In the first three loading phases subsidence decreased whereas height recovery in each rest phase remained constant. After these initial loading cycles, changes during loading were negligible compared to the changes in the recovery phase. From then on the recovery phase was able to keep pace with the loading phase. This implies that fluid flow out of the disc can be compensated during rest, even when inflow in general is slower than outflow. These results show that a dynamic equilibrium, similar to the in vivo situation, can be reached in vitro.

As shown in chapter 5, the time constant for changes due to an osmotic pressure, which is responsible for recovery, is much larger than for changes due to a mechanical load. Mechanical tests in which the diurnal cycle plays a role should therefore include at least an 8-hour rest period in order to incorporate the fluid inflow. Since the time constant of mechanical loading is shorter than for recovery visco-elastic deformation appears to be the dominant factor in disc mechanics. It is likely that the role of fluid flow on cell nutrition is limited as well. Observations on the *in vivo* behaviour of other species support this view. The ratio between the duration of loading and unloading varies between species. Large grazers have a short unloading phase compared to loading (e.g. horses are able to sleep upright). Degeneration, however, is not a common problem in these species. In the intervertebral disc nutrition is scarce and cell metabolism is slow. During growth of the spine cells are programmed to reproduce, while after reaching adulthood the decline in cell count in the disc might not fully be compensated by renewal. This will lead to a reduced number of cells being available for maintenance of the extra cellular matrix, which again will lead to an increased cell death. In this view, intervertebral disc degeneration is not a disease but an unavoidable aspect of ageing. This off course changes when degeneration is accompanied by pain. Since in the majority of elderly humans degeneration is found, future therapies should not focus on

degeneration in general, but on restoring pain free function of the disc for instance by restoring the available space for impinched nerves or restoring the mechanical function of the disc to an adequate level. Supporting the patient's body in restoration of disc function by injecting stem cells combined with extra cellular matrix might be a promising therapy in the future.

Mechanical loading on the intervertebral disc leads to loss of disc height both *in vivo* and *in vitro*. This change in disc height is caused by a combination of changes in water content and elongation of annulus fibres. The difference in behaviour during loading and unloading, the invariance of the recovery phase and the slow recovery suggests that cyclic behaviour is mainly visco-elastic. During compression the hydrostatic pressure of the disc increases. Due to this mechanical factor fluid flows out of the disc. During unloading the tension of the annulus fibres relaxes which leads to a reduction of the hydrostatic pressure in the disc. As a result of the elevated osmotic pressure fluid flows back into the disc. Differences in disc behaviour between loading and unloading probably find their origin in fluid mechanistically being squeezed out of the disc during loading while osmotic differences suck fluid in during rest.

The *in vivo* changes in human discs might be compared to the disc behaviour found in this *in vitro* study. A slow recovery of water content of the disc during night is followed by a short period of loss of water after rising in the morning. During daytime, when the disc is subjected to varying loads, further changes in disc properties are mainly visco-elastic, since fluid inflow is too slow to follow the load changes.

High compression loads of every-day activities *in vivo* are known to be responsible for damage to the endplates[9]. Several studies link endplate damage to degeneration. The *in vivo* loading history determines the fluid content of the disc and thus the instantaneous disc mechanics. Avoiding high loading shortly after rising in the morning, when the disc is super

hydrated, could reduce the risk of degeneration, endplate fractures[8] and herniated discs.

The results presented in this thesis have implications for testing of intervertebral discs in general. The disc is a complex system; during sustained loading each part plays a distinct role in time in the overall mechanical behaviour of the disc. Therefore, specimens for mechanical testing should be tested as complete as possible, with the supporting trabecular bone present, i.e. with only half of the vertebral body attached.

The loading history determines the mechanical behaviour of the disc. Disc behaviour shortly after rising presumably differs from disc behaviour during daytime. A mechanical test on an intervertebral disc should therefore be designed for the applicable situation. Preloading of the disc can bring a disc in the required stable state. Reports on disc properties like disc height, creep rate and stiffness can only be mutually compared if the tested discs have the same loading history. Again preloading is a prerequisite to fulfil the boundary conditions for mechanical testing. To enable comparison with literature preloading should be standardized and the magnitude and duration should be reported. In addition, differences in mechanical properties between for example degenerated and non-degenerated discs should be interpreted against the background of variation in properties over time due to loading history of the disc.

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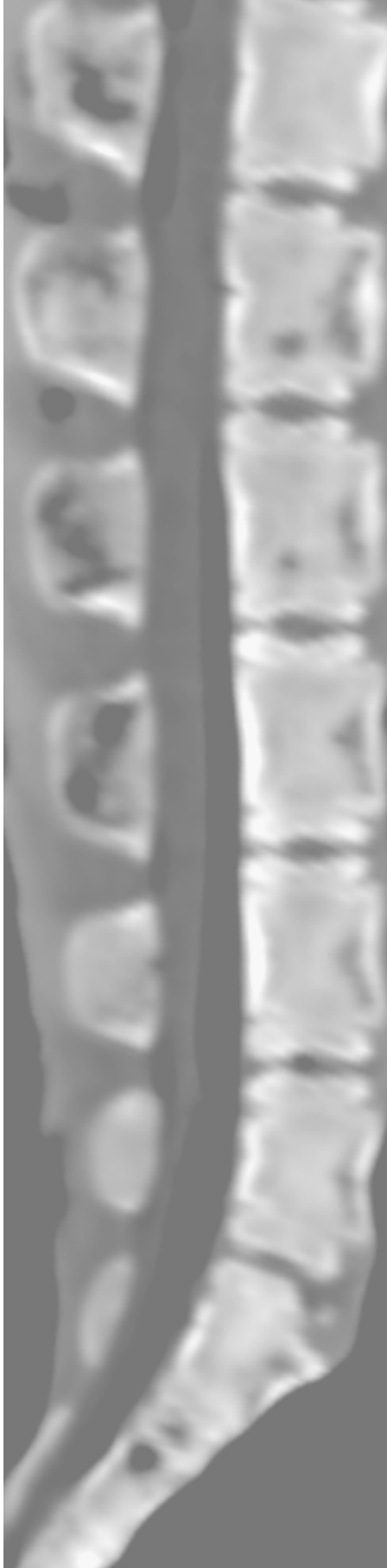
SUMMARY



The spine is a flexible structure that gives support to the upper body while providing freedom of movement to the trunk. The intervertebral disc serves as an elastic hinge between two vertebral bodies and provides flexibility to the spine. The disc comprises of the nucleus pulposus, the annulus fibrosus and the superior and inferior endplate.

The nucleus is a gelatinous structure, which consists of proteoglycans and collagen fibres, but mainly (up to 80%) of water. The chemical composition of the disc changes with age. The water content of the nucleus, however, also depends on the loading history of the disc.

The annulus fibrosus encapsulates the nucleus pulposus in its centre. It is a ring-shaped, layered structure of collagen fibres. All fibres in the same layer are parallel to each other. Each fibre runs at an angle of approximately 60° from endplate to endplate (the angle varies between 40° and 70° degrees). From one layer to the next the orientation of the fibres switches from approximately +60° to –60°.

Finally, the endplates enclose the nucleus at the top and bottom, separating the intervertebral disc from the vertebral bodies. The endplate is a perforated plate of bone with on top a layer of cartilage. The fibres of the annulus are anchored in the endplates.

The weight of head and trunk, but in particular the loads of back and abdominal muscles cause mechanical loading on the spine. The dominant loading type on the spine is axial compression. External loading on the spine leads to an increased hydrostatic pressure in the nucleus and to tension in the fibres of the annulus. The layered structure of the annulus with its alternating fibre direction is ideal for containing the hydrostatic pressure, while the flexibility in flexion/extension direction is maintained.

The osmotic pressure of the nucleus gives the disc the ability to withstand external loads. The negatively charged proteoglycans in the nucleus can attract and retain water. The percentage of water in the nucleus also

depends on the external load. An increased hydrostatic pressure leads to a decreased amount of water in the nucleus and consequently to an increased difference in osmolarity relative to the environment. Fluid continues to flow out of the disc until the external load is balanced by the osmotic pressure of the nucleus. Simultaneous with the fluid flow fibres of the annulus are stretched. As a result the intervertebral disc loses height. The mechanical behaviour of the disc is poro-elastic and visco-elastic. Thus mechanical properties of the disc are non-linear and strongly time dependent.

In literature, mechanical behaviour of the disc is often described from short-term experiments, usually with the unloaded situation as a starting point. The effect of loading history on disc mechanics is, in general, disregarded. This gap in knowledge on disc behaviour under sustained loading is the starting point of the research in this thesis.

The aim of this thesis is to elucidate the role of loading history on the mechanical behaviour of a spinal segment in general, and the intervertebral disc in particular.

After a general introduction, the second chapter of this thesis addresses the difference in mechanical behaviour of a motion segment and its separate components during axial compression. Intervertebral discs, vertebrae, motion segments without posterior parts and samples of the trabecular bone of the vertebral body (both with and without endplates) were subjected to a repeated compression test. Each of the three loading cycles consisted of a loading phase (2.0 MPa, 15 minutes) and a recovery phase (zero load for 30 minutes).

Bone samples without endplates showed almost instant deformation, followed by a marginal creep (time constant approximately 0.02 minutes). With a time constant of approximately 0.25 minutes samples with the endplates attached showed a much larger creep. The largest time constant

and change of specimen height were found in the creep of the intervertebral discs (time constant approximately 1 hour).

The marginal creep of bone samples shows that the role of bone in the creep behaviour of a disc is limited. However, bulging of the endplate into the vertebral body is a significant factor during the initial phase of the test.

Each component plays a distinct role in the time dependent behaviour of a motion segment. The optimal configuration for mechanical testing of motion segments is therefore the intervertebral disc with both half vertebral bodies attached, *i.e.* without the outer endplates.

In the third chapter of this thesis is shown that the mechanical response of a disc, subjected to a repeated loading cycle is asymmetric, *i.e.* changes in disc properties occurring during the loading phase are not fully restored during unloading. This seems to contradict the observations *in vivo* that loss of disc height is fully restored during recovery in spite of the fact that the duration of the loading time exceeds the duration of recovery (16 hours of daily activity vs. 8 hours of sleep). The recovery phase (zero load, 30 minutes) in the experiment of chapter 3 was -unlike the *in vivo* situation- twice as long as the loading phase (2.0 MPa, 15 minutes). Yet disc height did not recover during unloading. Also the nucleus pressure, which decreased during the subsequent loading phases, showed no full recovery during unloading, indicating that the osmotic pressure in the nucleus did not recover.

These results induced us to hypothesize that the flow rate through the endplate was smaller for fluid going into the disc than out of the disc. Obstruction of the endplate route by blood clots was thought to be responsible for this discrepancy.

All the applied loads in the previous chapters were static loads. *In vivo* loading, however, has a dynamic nature. Chapter 4 addresses fluid flow through the endplates and the effect of loading type on recovery of disc

properties. To this end, the samples were divided into four groups of intervertebral discs. Two groups were subjected to static loads (three loading cycles with a nucleus pressure of 2.0 MPa. each followed by a recovery phase). The other groups were subjected to dynamic loads (three loading cycles with an average nucleus pressure of 2.0 MPa, frequency of 0.5 Hz, each followed by a recovery phase). In the second part of the test, after the static or dynamic loading protocol, the endplates were blocked with silicon paste in half the number of discs of, while the endplate was left open in the other half. The test was restarted with an extra static or dynamic loading cycle, which was followed by a 10-hour long recovery period.

During the first part of the test, all samples showed a loss of disc height coinciding with an increase in disc stiffness. There was no difference in the change of disc properties between the statically and dynamically loaded groups. The expectation was that during the second part of the test, the recovery of the group with blocked endplates would be different from the group with open endplates. However, in spite of blocking one of the routes for fluid flow of the disc, no significant difference in recovery was found. Full recovery was measured for all parameters studied after long-term unloading. The required recovery time until full restoration was long compared to the loading time. This suggests that inflow of fluid into the disc is slow compared to outflow and contrasts with the *in vivo* observation that 8 hours of recovery suffice to compensate 16 hours of loading. Furthermore, the role of the endplate route for fluid flow seems to be limited. These findings were interpreted as an indication that the validity of *in vitro* models of the disc is limited.

In order to further investigate the differences between flow behaviour into and out of the disc, an experiment was designed in which the visco-elastic and poro-elastic behaviour were uncoupled. The experiment of chapter 5 was divided into three separate test phases. In the first phase, the disc was given the opportunity to reach equilibrium with an external load (static

compression: 150N, 300N, 600N or 850N). During this mechanical phase fluid was pressed out of the disc while at the same time the annulus fibres were stretched. At the start of the second phase of the test, the osmolarity of the environment was lowered (24-hours). The external load was maintained on the disc throughout the entire test. This change in osmolarity gave rise to an increase in disc height. Since the applied load was constant, this increase in disc height can only be explained by inflow of fluid. At the start of the final 24-hours of the test, the osmolarity of the environment was changed back to the initial value, again without changing the external load. Due to this increase in osmolarity, the change in height was reversed.

The time constant of the change in height was calculated for each test phase. The intervertebral disc lost height rapidly on the first day (time constant approximately 1 hour), slowly regained height on the second day (time constant approximately 8.6 hours) and finally lost height again on the third day (time constant approximately 7.2 hours). The time constant for fluid flow into the disc was larger than for the reversed flow direction. The changes in height on the second and third days were related to the applied load; the larger the applied load, the smaller the change in height.

The large difference in time constants between adaptation to mechanical loading and osmotic differences suggests that two different processes drive the mechanical behaviour of the disc. During mechanical loading fluid is actively pressed out of the nucleus, combined with a rapid stretching of the annulus fibres, while during recovery fluid flows back slowly due to osmotic differences.

Prolonged mechanical loading changes the osmolarity of the intervertebral disc. The instantaneous water content of the disc depends on the loading history of the disc. Therefore the mechanical properties of the intervertebral disc (e.g. disc stiffness and height) are not an intrinsic property, but change with the loading history.

In the third chapter, it was hypothesized that blood clots in the endplates were the limiting factor for inflow. In chapter 6 a study to further test this hypothesis is described. Mechanical properties of two groups of discs were compared. In one group heparin was used to prevent blood clotting in the endplates, while the endplates of the other group were untreated. It was also attempted to achieve a stationary mechanical behaviour in a protocol that mimics the ratio of loading time versus the unloading time of a diurnal loading cycle. Thus when the duration of the loading phase exceeded that of the recovery phase by a factor of two.

The intervertebral discs were tested for 24 hours, divided into 8 separate loading cycles. Each cycle consisted of a loading phase (2 hours of sinusoidal loading) and a recovery phase (1 hour, static loading). When comparing the mechanical behaviour of the treated discs with the control group no improvement in disc recovery was found. These results are in line with the outcome of the experiments in chapter 4, where no differences were found in a comparison between discs with open and closed endplates. The combined results make our previously postulated hypothesis that obstruction of the endplate is responsible for the difference between the mechanical behaviour of the loading and the unloading phase unlikely since recovery appeared to be slow in all cases.

The overall mechanical behaviour of both groups stabilized after three cycles. From then on the recovery phase compensated the changes of the loading phase. This implies that fluid flow out of the disc can be compensated during recovery, even when inflow in general is slower than outflow. However, since fluid flow is a slow process it is unlikely that a full recovery of water content in the nucleus was reached. Visco-elastic behaviour dominated the overall mechanical behaviour of the disc from the third cycle. The results show that a dynamic equilibrium, similar to the *in vivo* situation, can be reached *in vitro*. However, if a full exchange of fluid of the nucleus with the environment is required, a long recovery phase (minimum of eight hours) should be part of the test.

Mechanical loading on the intervertebral disc leads to loss of disc height both *in vivo* and *in vitro*. The *in vitro* results presented in this thesis can be translated to the in vivo behaviour of human discs. Change of disc height is caused by a combination of a change in water content and elongation of annulus fibres. The large difference in time constants between adaptation to an increased mechanical load and a decreased mechanical load suggests that two different processes are responsible. During axial compression the increased hydrostatic pressure squeezes fluid out of the disc, while during unloading the changes in osmotic pressure drive fluid back into the disc. The latter is a slow process. Therefore the cyclic behaviour during daytime is mainly visco-elastic; the nucleus fluid acquired during night is already lost during the first hour after rising.

In vivo the disc is never without loading. The loading history of the intervertebral disc determines the instantaneous mechanical properties of the disc. The disc height and the stiffness vary with the water content of the nucleus in a daily cycle. High compression loads of every-day activities *in vivo* are known to be responsible for damage to the endplates. Several studies link endplate damage to degeneration. Disc properties shortly after rising presumably differ from the properties during daytime. Avoiding high external loading shortly after rising in the morning when the disc is super-hydrated might reduce the risk of endplate fracture and herniated discs.

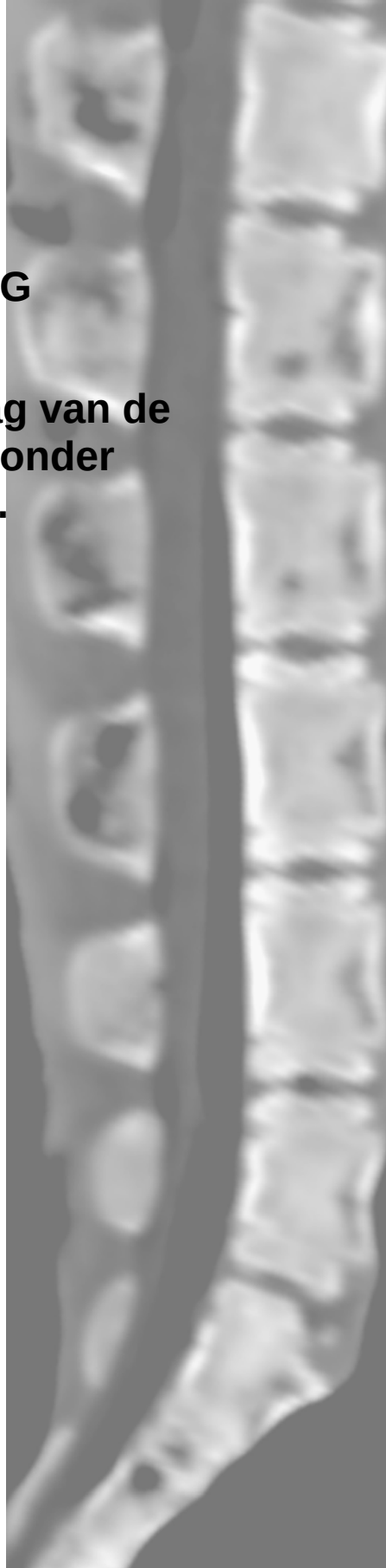
These results also have implications for testing of motion sections and intervertebral discs. The motion segment is a complex, time- and load-dependent system; during sustained loading each part plays a distinct role in the mechanical behaviour of a segment in time. Specimens for mechanical testing should therefore be tested as complete as possible: both endplates and only half of the supporting trabecular bone of vertebral body present, thus the outer endplates removed.

Since mechanical properties of motion segments and discs shortly after rising differ from the properties during daytime, a mechanical test should be designed for the desired time point in the daily cycle. Therefore, preloading of the specimen is required.

Finally, results of studies on disc properties like disc height, creep rate and stiffness can only be mutually compared with other studies when the tested discs have the same loading history. Again preloading of the disc is required to bring the disc in this required state.

SAMENVATTING

Het mechanisch gedrag van de tussenwervelschijf onder duurbelasting.



De wervelkolom geeft structuur aan de romp; het is een van de hoofdstructuren van het steun- en bewegingsapparaat. De wervelkolom is flexibel en geeft zo bewegingsvrijheid aan het lichaam. Om deze rol te kunnen vervullen moet de wervelkolom, en alle onderdelen waaruit zij is opgebouwd, in staat zijn om de optredende mechanische belasting te dragen. De belasting op de wervelkolom komt voornamelijk voort uit de werking van buik- en rugspieren en wordt in mindere mate veroorzaakt door het gewicht van hoofd en romp. De grootte van deze spierkracht wordt bepaald door voorkomende dagelijkse activiteiten. Zo is, tijdens een onderzoek van de groep van Wilke, de laagste belasting gemeten tijdens liggen en de hoogste belasting tijdens tillen. De belasting op de wervelkolom was tijdens het tillen 25 keer hoger dan tijdens liggen. Uit dit onderzoek blijkt tevens dat, *in vivo*, de belasting op de wervelkolom nooit nul is.

De wervelkolom is opgebouwd uit 24 wervels. De zeven cervicale wervels bevinden zich in het halsgebied, 12 thoracale wervels bevinden zich in het borstgebied en 5 lumbale wervel bevinden zich in het buikgebied. Het verschil tussen enerzijds thoracale wervels en anderzijds de lumbale en cervicale wervels is de aanwezigheid van gewrichtsvlakken voor de ribben op de thoracale wervels. Verder neemt de grootte van de wervellichamen toe naarmate ze lager in de wervelkolom zitten. Tenslotte is de stand van de gewrichtsvlakken aan de achterzijde, de facetgewrichten, afhankelijk van de positie in de wervelkolom. Deze gewrichtsvlakken bepalen in welke richtingen de wervelkolom kan bewegen.
Elk van de wervels is met de naastliggende wervels verbonden doormiddel van een tussenwervelschijf (in het vervolg afgekort tot tws). De tussenwervelschijven zijn de flexibele elementen van de wervelkolom en zorgen voor de beweeglijkheid van de romp. De wervels kunnen tijdens het voor- en achterwaarts buigen bewegen in de flexie/extensie richting, bij het zijdelings buigen bewegen in de latero-flexie richting en bij een draaibeweging van de romp draaien om haar eigen as. De mate van

bewegelijkheid, per richting, is afhankelijk van de plaats van de wervel in de wervelkolom.

De tussenwervelschijf kan worden onderverdeeld in drie delen: de nucleus pulposus, de annulus fibrosus en de beide eindplaten.
De nucleus pulposus is de geleiachtige kern van de tussenwervelschijf. De nucleus is opgebouwd uit proteoglycanen (lange ketens van water aantrekkende moleculen), uit collageenvezels (vooral collageen type I), maar vooral uit water. Ongeveer 80% van het gewicht van de nucleus bestaat uit water. De onderlinge verhouding van de bestanddelen van de nucleus is leeftijdsafhankelijk. Echter, het watergehalte van de tussenwervelschijf is ook afhankelijk van de belasting op de tws. Het watergehalte daalt onder belasting maar is na een nacht rusten weer toegenomen tot het oorspronkelijke niveau.
De annulus fibrosus is een ringvormige structuur die de nucleus pulposus in radiale richting opsluit. De annulus is opgebouwd uit rondlopende lagen collageenvezels (vooral collageen type II). In elke laag lopen de collageen-vezels parallel aan elkaar. Deze vezels lopen van de onderste eindplaat van de bovenliggende wervel naar de bovenste eindplaat van de wervel eronder. De hoek die de vezels met de eindplaat maken is afhankelijk van de plaats in de tussenwervelschijf, deze hoek varieert tussen de 20 en 40 graden. De hoek die de vezels ten opzichte van de eindplaat maken draait in elke opeenvolgende laag om. Met andere woorden: vanuit de nucleus gezien wisselt de richting van de vezels van gemiddeld +30 graden naar –30 graden. De opbouw van de annulus, met zijn gelaagde structuur en met zijn afwisselende vezelrichtingen, zorgt ervoor dat de nucleus vloeistofdruk kan opbouwen en zo dat de externe belasting door de tws gedragen kan worden.
Het laatste onderdeel van de tws is de eindplaat. De eindplaten sluitende nucleus pulposus, aan boven- en onderzijde, af van het wervellichaam. Het wervellichaam bestaat uit sponsachtig bot gevuld met beenmerg. De eindplaat zelf bestaat uit vezelig kraakbeen, de vezels van de annulus zijn in

de beide eindplaten verankerd. Als de wervelkolom wordt belast, loopt de druk in de nucleus op. Hierdoor stulpt de nucleus in zowel de radiale als in verticale richting uit.

De voornaamste belasting op een wervelsegment, dat uit twee wervellichamen en de tussenwervelschijf bestaat, is een compressiekracht die van eindplaat naar eindplaat loopt. Ten gevolge van de externe belasting wordt in de nucleus pulposus een vloeistofdruk opgebouwd hierdoor worden de collageenvezels van de annulus fibrosus op trek belast. De proteoglycanen, de negatief geladen moleculen in de nucleus die het water vasthouden, kunnen onder belasting minder water binden en laten het overtollige water los. Dit water gaat via de annulus of de eindplaten terug in het lichaam. Doordat er water uit de nucleus verdwijnt, neemt de concentratie van proteoglycanen toe. Het verschil in osmotische waarde tussen de nucleus en zijn omgeving neemt dus toe en hiermee het vermogen van de nucleus om mechanische belastingen te dragen. De nucleus blijft water uitscheiden totdat de uitwendige compressie belasting in evenwicht is met het dragende vermogen van de nucleus. Met andere woorden, totdat evenwicht is bereikt met de osmotische druk van de nucleus. Tegelijkertijd met het uitstromen van vloeistof uit de nucleus worden de vezels van de annulus door de trekkracht opgerekt. De tws verliest hoogte en de hydrostatische druk in de tws neemt af. In deze processen speelt tijd een belangrijke rol. Het vervormen van de tws in de tijd, als gevolg van een constante duurbelasting, wordt kruip genoemd. Het gecombineerde proces van oprekken van de annulus en het in en uit stromen van vloeistof van de nucleus is een complex proces waaraan alle bouwstenen van de tussenwervelschijf hun bijdrage leveren.

De wervelkolom is één van de eerste structuren die aan het begin van de ontwikkeling wordt aangelegd. Reeds 14 dagen na conceptie is de tws in aanleg aanwezig. Dit is het begin van een lang ontwikkelingsproces dat pas

voltooid is als men lichamelijk volgroeid is. Gedurende deze groei veranderen zowel de grootte als de belasting van de tws. De tws is de grootste structuur in het lichaam dat niet door het vaatstelsel (bloedvaten) van voeding wordt voorzien. De tws is niet gevascularisseerd, dit houdt in dat het transport van voedingsstoffen van en naar de cellen van de tws via een diffusieproces verloopt. De cellen in de nucleus hebben derhalve een laag metabolisme. Hierdoor zijn de nucleuscellen niet goed in staat om optredende schade aan de structuur van de tws te herstellen. De kwaliteit en functionaliteit van de tws gaat in de loop van de jaren achteruit. Deze verminderde functie of degeneratie wordt bij de meerderheid van oudere mensen gevonden. De degeneratie wordt gezien als een mogelijke bron van pijnklachten. Het is daarmee niet verwonderlijk dat de meerderheid van de mensen gedurende een periode van hun leven last krijgen van (lage)rugpijn. Per jaar krijgt 17% van de Nederlanders last van lage rug pijn. Het is daarmee één van de grote sociaal-economische kostenposten in Nederland. In Nederland alleen leverde dit in 1991 een bedrag van 1.4 miljard euro aan kosten op.

Het doel van het wervelkolomonderzoek binnen het VU Medisch Centrum is het verbeteren van de behandeling van lage rugpijn en, zo mogelijk, het voorkomen er van. De tussenwervelschijf speelt een belangrijke rol in de mechanica van de wervelkolom en bij het ontstaan van lage rugpijn. Daarom is een grondige kennis van de mechanica van de tws noodzakelijk om het zojuist geschetste doel te bereiken. In de literatuur, echter, wordt het mechanische gedrag vooral beschreven vanuit kortdurende testen en veelal vanuit een onbelaste toestand. Hierdoor wordt er te weinig rekening gehouden met de belastingsgeschiedenis van de tws. Het doel van het onderzoek dat in dit proefschrift staat beschreven, is het opvullen van deze lacune in kennis. De titel en leidraad van dit proefschrift is de invloed van duurbelasting op het mechanisch gedrag van de lumbale tussenwervelschijf *in vitro*.

In het tweede hoofdstuk wordt getoond dat alle delen van een wervelsegment (dus bot, eindplaat en de verbindende zachte weefsels) een rol spelen in de totale vervorming van de tussenwervelschijf. De vervorming ten gevolge van een duurbelasting gaat in de loop van de tijd naar een constante eindwaarde. De tijdconstante is de tijd die het proces nodig heeft om twee-derde van deze eindwaarde te bereiken. De verwachting is dat elk van de afzonderlijke delen van een segment een andere tijdconstante heeft. In deze studie vergelijken we de bijdrage van de verschillende delen aan de totale vervorming van het segment. Hiervoor hebben we naast een wervelsegment en een tussenwervelschijf ook een losse wervel getest. Behalve naar het verschil in mechanisch gedrag van de afzonderlijke delen, wordt in deze studie ook gekeken naar het verschil in mechanisch gedrag tussen bot van het wervellichaam met en zonder eindplaten. Hiervoor zijn met een holle boor botpluggen uit het wervellichaam geboord. Alle groepen zijn op een vergelijkbaar belastingsniveau getest (drie cycli, elk bestaande uit een belastingfase; gedurende 15 minuten een statische druk van 2.0 MPa en een rustfase; gedurende 30 minuten onbelast). De verschillende delen, bot, eindplaat en tussenwervelschijf blijken een verschillend mechanisch gedrag te vertonen. De tijdconstante van de verschillende groepen varieert sterk. De botstructuur van het wervellichaam heeft een korte tijdsconstante (de tijdconstante van bot is ongeveer 0.02 minuut). Het kruipproces van het bot is relatief snel op zijn eindwaarde, de tijd speelt hierdoor een slechts beperkte rol bij het belasten van bot. Hierna volgt het kruipen van de eindplaat (de tijdconstante van bot + beide eindplaten is ongeveer 0.25 minuut) en tenslotte duurt het kruipen van de weke delen het langst (de tijdconstante van de gehele tussenwervelschijf is bijna een uur). Het gevolg is dat in een segment bot en eindplaat gedurende de vroege kruipfase een rol spelen, terwijl gedurende de late kruipfase vooral de zachte weefsels vervormen. Uit de resultaten blijkt verder dat de vervorming van de eindplaat groot genoeg is om een rol te spelen in de totale

vervorming van een segment. Het laatste heeft consequenties voor de test opzet voor mechanische testen van bewegingssegmenten in het algemeen, in geval van een mechanische test moet het bewegingssegment bestaan uit de tws en beide naastliggende, halve, wervellichamen en niet zoals vaak wordt gedaan een tws met de gehele naastliggende wervels met de buitenste eindplaten er nog aan vast.

In het derde hoofdstuk laten we zien dat het herstel van de hoogte van de tussenwervelschijf, na wegnemen van de belasting, niet even snel verloopt als het verlies van hoogte tijdens het belasten. Bij de mens is de lengte van de rustperiode is niet gelijk aan de belastingsduur. Mensen hebben een dagnachtritme dat ook in het krachtenspel in de wervelkolom doorwerkt. Van de 24 uur in een dag wordt ongeveer twee derde deel van de tijd actief gebruikt en slechts een derde gerust. Ondanks het verschil in rust en belastingsduur heeft het lichaam na 24 uur weer haar uitgangssituatie bereikt. Het verlies aan lichaamslengte, wat door de dagelijkse belasting is ontstaan, is na voldoende (nacht)rust weer hersteld. Met andere woorden, de vloeistof die ten gevolge van het belasten uit de tussenwervelschijf is weggestroomd, is na rust weer opgenomen.
Dit is in tegenspraak met de *in vitro* experimenten van de hoofdstukken twee en drie. Tijdens deze experimenten was de duur van de rust tweemaal zo lang als duur van de belasting. Ondanks de langere hersteltijd was het verlies aan hoogte na ontlasten niet gecompenseerd. Gedurende de drie belastingcycli (elke cyclus bestond uit 15 minuten belasten en 30 minuten rust) bleek de tussenwervelschijf hoogte te verliezen. De kuipkrommen gedurende de herstelfase waren echter vrijwel identiek. Tevens nam de hydrostatische druk in de nucleus af gedurende de belastingsfase en herstelde niet gedurende de rust. Dit is in tegenspraak met het dagelijkse belastingspatroon. In eerste instantie hebben wij de verklaring hiervoor gezocht in obstructie van de eindplaat. In de eindplaat lopen kanaaltjes van het wervellichaam naar de nucleus. Als deze kanaaltjes aan de buitenzijde

geblokkeerd zijn door bijvoorbeeld bloedstolsels zouden deze met name het instromen kunnen belemmeren en zo het herstel vertragen. Op deze manier zou er een verschil kunnen ontstaan tussen *in vivo* en *in vitro* mechanisch gedrag. De studie laat zien dat de eindplaat mogelijk een rol speelt in de verschillen tussen *in vivo* en *in vitro* mechanisch gedrag.

In de studie van het vierde hoofdstuk is gekeken naar de invloed van de eindplaat op het herstel. In hoofdstuk drie was de externe belasting een statische compressiekracht, de belasting varieerde niet in de tijd. Echter, de belasting in het dagelijks leven varieert continu. Gezien de tijdconstante van het mechanisch gedrag van een segment is de verwachting niet dat dit een wezenlijk verschil maakt op het herstel, desondanks is ook dit aspect in de studie van hoofdstuk vier meegenomen.
In deze studie zijn De geteste wervelkolommen zijn afkomstig uit geiten. De tussenwervelschijven zijn in vier groepen verdeeld. Twee groepen hebben een statisch belastingsprotocol ondergaan (2.0 MPa) en de andere twee groepen een dynamisch protocol (sinusvormig, gemiddelde druk 2.0 MPa, 0.5 Hz). Zowel de statische als de dynamische groep is onderverdeeld in twee subgroepen: een groep met een open eindplaat en een groep met een afgesloten eindplaat.
Het testprotocol bestaat uit twee delen: het eerste deel is een belastings-protocol wat vergelijkbaar is met het protocol uit het vorige hoofdstuk en het tweede deel bestaat uit een enkele belastingscyclus gevolgd door een lange rust periode van 10 uur. Voorafgaand aan het tweede deel van de test is elke tussenwervelschijf uit de opstelling genomen. Waarna de beide eindplaten van tussenwervelschijven uit de groep met afgesloten eindplaat zijn dicht gesmeerd met siliconenpasta, terwijl de eindplaten van de rest zijn vrij gebleven.
Gedurende het eerste deel van de test vertoonde alle groepen een verlies aan hoogte en een toename van compressiestijfheid. Er was in deze fase dus geen verschil met de bevindingen uit eerdere experimenten. De

resultaten van het experiment bevestigen dat *in vitro* dynamische belasting geen effect heeft op het herstel gedurende de rustfase. De verwachting was dat gedurende het tweede deel van de test, de 10 uur durende herstelfase, er een verschil zou ontstaan tussen de groepen met een afgesloten en de open eindplaat. Tenslotte, één van de routes voor de vloeistofstroom is afgesloten. Het verschil tussen de groep met afgesloten en open eindplaat bleek echter marginaal te zijn. De mechanische eigenschappen zijn na de lange rustperiode in beide groepen terug op de uitgangswaarde. Een tussenwervelschijf kan kennelijk wel herstellen, maar dat duurt erg.lang. Het verschil in mechanische eigenschappen, was statistisch gezien niet significant. Kennelijk is de rol van de eindplaat inzake het herstel van de tussenwervelschijf klein.

Deze resultaten laten zien dat de invloed van de eindplaat niet kan verklaren dat, *in vitro,* het herstel van de tussenwervelschijf achter blijft bij het inzakken ervan. Het volledig herstel *in vivo*, waarbij er 16 uur belast wordt tegenover 8 uur rust, suggereert dat de stroomsnelheid van vloeistof die de tussenwervelschijf ingaat hoger moet zijn dan van vloeistof die eruit stroomt. Dit is in tegenspraak met het gedrag dat in voorgaande experimenten wordt getoond: onvoldoende herstel zelfs als de rustfase tweemaal zo lang is als de belastingsfase.

Als door een externe belasting vloeistof uit de tussenwervelschijf wordt geperst verandert de concentratie aan proteoglycanen in de nucleus en daarmee de elektrische lading van de nucleus (dwz de osmolariteit). Om de verschillen in stroomsnelheid tussen de in- en uitstroom van vloeistof te onderzoeken, hebben wij in hoofdstuk vijf de effecten die ontstaan door verschil in osmotische waarden gescheiden van het effect dat ontstaat door het mechanisch belasten. Gedurende de eerste fase van de test is een compressiekracht van 150, 300, 600 of 850N op de tussenwervelschijf gezet. Deze kracht is gedurende de hele test van 66 uur op de tussenwervelschijf blijven staan. De test is onderverdeeld in drie fasen. De

eerste fase van 18 uur is gebruikt om een evenwicht tussen de osmotische druk van de nucleus en de uitwendige kracht te bereiken. Aan het begin van de tweede fase, die 24 uur duurt, is de osmolariteit van de vloeistof waarin de test wordt uitgevoerd verlaagd. De verwachting is dat gedurende deze fase de vloeistof weer de tussenwervelschijf instroomt. Aan het begin van de derde en laatste fase van 24 uur is de osmolariteit weer terug gebracht op het oorspronkelijke niveau. De vloeistofstroom zou hierdoor moeten omkeren, de tws moet de vloeistof weer uitscheiden.

De tussenwervelschijf verloor snel hoogte op de eerste dag, herwon traag een deel van de hoogte op de tweede dag en verloor deze hoogte weer tijdens de derde dag. Het gemeten hoogteverschil is afhankelijk van de grootte van de aangebrachte belasting: hoe hoger de belasting, hoe lager de hoogtewinst. Van de verschillende testfasen is de tijdconstante van de hoogteverandering bepaald. Het hoogteverlies tijdens het aanpassen aan de mechanische belastingen, gedurende de eerste dag, gaat het snelst (tijdconstante 1 uur). Ook in de volgende twee fasen, waarin vloeistof in en uitstroomt door osmose verschillen, blijkt hoogteverlies sneller te gaan dan hoogtewinst (tijdconstante van instroom 8.6 uur, van uitstroom 7.2 uur). Deze getallen laten duidelijk zien dat de aanpassingen aan de osmoseverschillen gedurende de laatste twee testfasen veel trager zijn dan het aanpassen aan de mechanische belasting.

Deze uitkomst suggereert dat het hoogteverlies zoals is beschreven in eerdere hoofdstukken vooral wordt veroorzaakt door het snel oprekken van de annulus vezels gecombineerd met een snel vloeistofverlies door het actief uitpersen van vloeistof door de belasting. Het herstel daarentegen kan worden opgedeeld in een snel, elastisch, herstel van de opgerekte annulus vezels en een passief, traag instromen van vloeistof.

Tijdens eerdere experimenten werd gedurende een cyclisch belastings-protocol, waarbij de rustperiode langer duurde dan de belastingsperiode, geen evenwicht bereikt. De hypothese was dat dit werd veroorzaakt doordat

dat de eindplaat verstopt was door bloedstolsels. De veronderstelling was dat de eindplaat hierdoor vloeistof wel naar buiten zou doorlaten maar niet naar binnen. Dit model wordt echter niet ondersteund door de resultaten uit hoofdstuk 4. Een verstopte eindplaat lijkt niet de oorzaak te zijn van het verschil in het mechanisch gedrag tussen belasten en rust. Om de invloed van de eindplaat nader te onderzoeken zijn de tussenwervelschijven voor het experiment van hoofdstuk 6 in twee groepen verdeeld. De eerste groep is behandeld met heparine, dit middel zorgt ervoor er geen bloedstolsel gevormd worden, de tweede groep was niet behandeld.

Tijdens deze studie wordt de tws belast volgens een belastingsregime waarbij de belastingsfase, net als in het dagnachtritme van de mens, tweemaal zo lang is als de rustfase. De totale testduur is 24 uur. De totale testduur is opgedeeld in acht belastingscycli. Elke cyclus bestaat uit een belastingsfase (2 uur, sinusvormig 0.5Hz en een gemiddelde druk: 0.5MPa) gevolgd door een rustfase (1 uur, statische belasting, druk: 0.2MPa).

Het mechanische gedrag van de tussenwervelschijf stabiliseerde zowel in de heparine groep als in de controle groep na drie cycli. In de daarop volgende cycli werd het hoogteverlies van de belastingsfase gecompenseerd in de herstelfase. Deze bevinding ondersteunt de hypothese dat het hoogteverlies vooral komt door een snel vloeistofverlies gecombineerd met het snel oprekken van de annulus vezels terwijl de rustfase wordt gekenmerkt door een snel herstel van de opgerekte annulus vezels en een traag instromen van vloeistof in de nucleus.

Er is geen verschil gevonden tussen de resultaten van de groep behandeld met heparine en de groep zonder heparine, derhalve wordt de hypothese dat de eindplaatroute voor vloeistofstroom geblokkeerd was door bloedstolsels niet door de resultaten ondersteund.

De resultaten, die in dit proefschrift zijn gepresenteerd, kunnen vertaald worden naar het gedrag van de tussenwervelschijf in het dagelijkse leven.

De respons van een tussenwervelschijf op een mechanische belasting wordt mede bepaald door zijn belastingsgeschiedenis. De dikte en de stijfheid van de tussenwervelschijf variëren in dezelfde dagelijkse cyclus als de waterinhoud van de nucleus. De interne verdeling van de belasting in de tussenwervelschijf is ook hiervan afhankelijk. Het is denkbaar dat schade aan de eindplaat te vermijden is door 's ochtends, op het moment dat de waterinhoud van de nucleus het hoogst is, piek belastingen te vermijden.

De resultaten uit dit onderzoek hebben ook gevolgen voor het *in vitro* onderzoek naar de mechanica van de tussenwervelschijf in het algemeen. *In vivo* zijn de mechanische eigenschappen van de tussenwervelschijf afhankelijk van het meetmoment in de dag-nacht cyclus, dus van de belastingsgeschiedenis voor dat moment. Evenzo zijn de resultaten van *in vitro* onderzoek afhankelijk van de belastingsvoorgeschiedenis. Het voorbelasten, voorafgaande aan het eigenlijke onderzoek, bepaalt de uitkomst ervan. Daarom moet het onderzoek, via deze voorbelasting, gericht zijn op een gekozen moment in de dag-nacht cyclus van de mens.

Bij een meerderheid van de ouderen wordt in een of meer tussenwervelschijven degeneratie gevonden. Degeneratie is een risicofactor voor rugpijn, Dit houdt in dat de kans op rugpijn in een groep met degeneratie groter is dan in een groep zonder degeneratie. Degeneratie wordt mogelijk veroorzaakt doordat de nucleuscellen, na het bereiken van volwassenheid, niet in staat zijn om afbraak van en schade aan de tws te compenseren. De dagelijkse belastingen kunnen leiden tot schade aan de tussenwervelschijf en zo degeneratie te bespoedigen. Echter, het niet belasten van de wervelkolom is geen optie omdat de wervelkolomcellen belasting nodig hebben om goed te functioneren. Het lijkt er dus op dat ouder worden gepaard zal gaan met degeneratie van de tws. In deze visie is degeneratie van de tussenwervelschijf geen ziekteverschijnsel maar een natuurlijk onderdeel van het ouder worden. Dit verandert natuurlijk als de degeneratie gepaard gaat met pijnklachten. Gegeven het algemeen

voorkomen van degeneratie en het ontbreken van een één-op-één relatie tussen degeneratie en pijnklachten zal er niet gezocht moeten worden naar methodes om degeneratie in het algemeen te voorkomen, maar naar methodes om de klachten te verhelpen bij de groep die er nadelige gevolgen van ondervindt. Het ondersteunen van de lichaamseigen reparatiefunctie, bijvoorbeeld door het injecteren van eigen vetstamcellen,om zo de tussenwervelschijfstructuur te reconstrueren, is een mogelijk een veelbelovende therapie voor deze groep.

De kennis opgedaan in dit promotieonderzoek, is nodig voor het ontwikkelen van deze nieuwe behandelwijzen.

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