ABSTRACT

Title of Thesis: EFFECTS OF LOAD HISTORY ON OVINE SPINAL RANGE OF MOTION

Giuliana Rotunno, Master of Science 2016

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Fischell Department of Bioengineering

Loading of spinal motion segment units alters biomechanical properties by modifying flexibility and range of motion. This study utilizes angular displacement due to an applied bending moment to assess biomechanical function during high-magnitude and prolonged compressive loading of ovine lumbar motion segments. High compressive loads, representative of physiological lifestyle and occupational behaviors, appear to limit fluid recovery of the intervertebral disc, thereby modifying spinal flexibility and increasing spinal instability. Intermittent extensions, or backwards bending movements, may provide a protective effect against the load-induced spinal instability. This study contributes a greater understanding of the effects of load history on the function and health of the lumbar spine. Findings may inform future efforts investigating adjustments in spinal posture to preserve or promote the recovery of lumbar spinal biomechanics.

EFFECTS OF LOAD HISTORY ON OVINE SPINAL RANGE OF MOTION

by

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Thesis submitted to the Faculty of the Graduate School of the
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Dedication

To my family.

Thank you for your love and constant support.

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Thank you to…

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Chapter 1: Introduction

1.1 Anatomy and Function of the Spine

The human spine is a mechanical structure consisting of five major regions: the cervical region (7 mobile vertebrae) which stabilizes the base of the skull to the spine, the relatively stiff thoracic region (12 mobile vertebrae), the strong and flexible lumbar region (5 mobile vertebrae), the sacral region (5 fused vertebrae) acting as the center of the pelvis, and the coccygeal region (4 fused vertebrae) or tailbone [Thompson and Netter 2010]. The neutral spine has a natural lordotic curvature in the cervical and lumbar regions and a natural kyphotic curvature in the thoracic and sacral regions (Figure 1). The functions of the spine include protecting the nerve roots and spinal cord; supporting body weight; providing attachment points for the ribs, shoulder girdle, and pelvic bones; and transmitting forces to allow postural stability and physical movement.

1.1.1 Vertebral Bodies

Vertebral bodies are the bony structures of the spinal column. A typical vertebral body consists of an anterior cylindrical body and posterior arch (Figure 2). The anterior body is the main axial load-bearing structure of the spine. It is composed primarily of cancellous bone encapsulated by an outer shell of cortical bone as well as superior and inferior end plates of compacted cancellous bone, which themselves are covered with thin layers of cartilage and act as the attachment sites to the intervertebral discs. The width and depth of vertebral bodies increase from the cervical to the lumbar regions due to increasing axial loads [Miele et al. 2012]. Similarly, compression strength

increases from the cervical to lumbar regions. However, the strength of vertebral bodies decrease with degenerative disease, injury, and age—especially beyond 40 years [Panjabi et al. 1980].

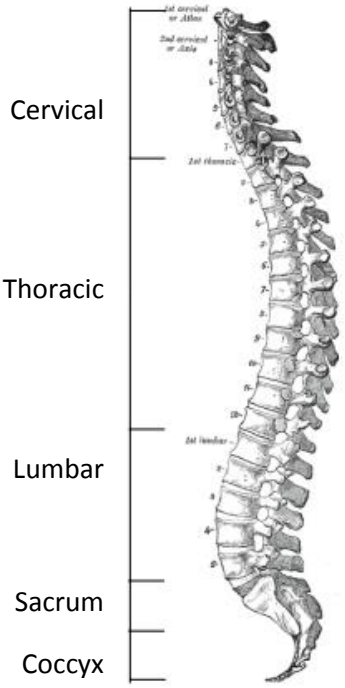

Figure 1: Lateral View Human Spinal Column. [adapted from Gray 1918]

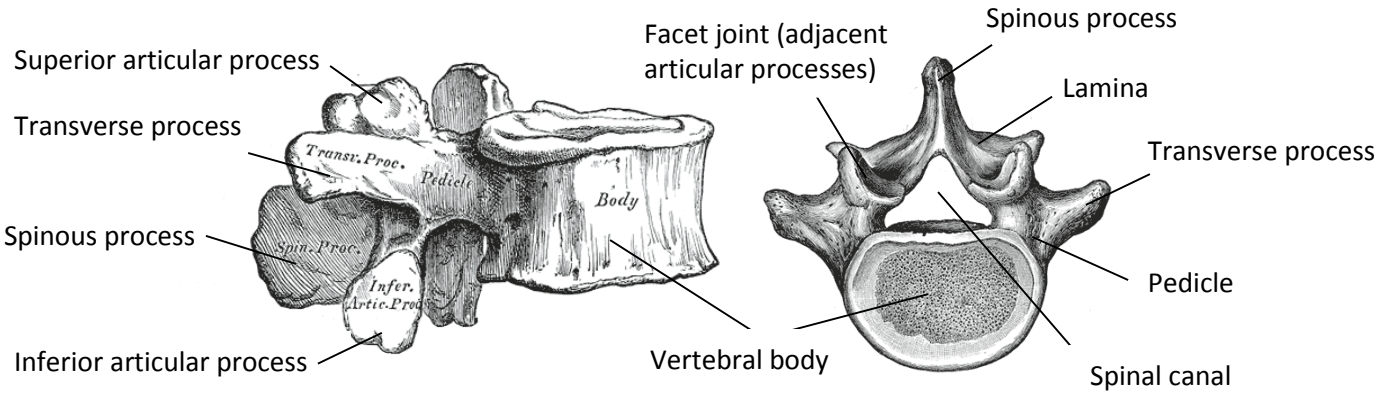

Figure 2: Vertebral Body and Nerve Structures.
Lateral View (left) and Superior View (right). [adapted from Gray 1918]

The vertebral arch begins bilaterally with pedicles which form junctions with: the laterally protruding transverse processes providing attachment sites to muscles and ligaments, the superior and inferior articular processes forming facet joints between neighboring vertebrae, the laminae extending around the spinal canal, and the posteriorly protruding spinous process providing additional attachment sites to muscles and ligaments [Miele et al. 2012]. The delicate spinal cord is enclosed within the rigid spinal canal, formed by the posterior face of the vertebral body and the vertebral arch, connected by facet joints end-to-end in space along the entire spinal column. Nerve roots above or below each vertebral level branch off from the spinal cord through spaces, formed by articulating facet joints, called neuroforamen [White and Panjabi 1990]. These spaces are clinically important because a reduction in diameter of the spinal canal and/or neuroforamen, most commonly due to injury or degenerative changes, is a direct source of pain [Moore et al. 2011].

1.1.2 Intervertebral Disc

The intervertebral disc sits between two vertebral bodies and is composed of an outer annulus fibrosus, which is continuous with the cartilaginous vertebral body endplates, and inner nucleus pulposus (Figure 3). Although these regions are strictly defined in the representative image, it is important to note that these strict boundaries do not exist anatomically [Humzah and Soames 1988]. The disc height increases from the cervical to the lumbar region from about 3mm to 9mm, again due to increasing axial loads [Zatsiorsky 1998].

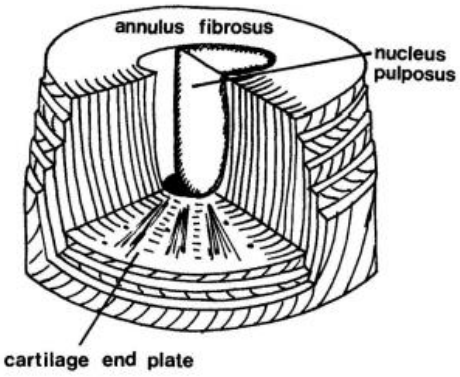

Figure 3: Three-dimensional Representation of Intervertebral Disc. [Humzah and Soames 1988] Reprinted with permission from John Wiley and Sons 2005.

The nucleus pulposus (NP) is located posterocentrally in the disc and may take up 30-50% of the disc's cross-sectional area. It is a soft, pressurized gelatinous region composed of poly-anionic proteoglycans, loose type II collagen fibrils, mineral salts, water, and cellular elements remaining from the primitive notochord [Martin et al. 2002]. The type II collagen fibers provide tensile strength to the NP [Cassinelli and Kang 2000]. The proteoglycans contain many glycosaminoglycan attachments which are highly hydrophilic, pulling water into the inner region of the intervertebral disc via osmosis. Water pressurizes the region by forming hydrogen bonds with the proteoglycans. This allows uniform force dispersion when the intervertebral disc transfers loads between vertebral bodies of the spinal column [Humzah and Soames 1988, Cassinelli and Kang 2000]. The water content in the NP may decrease due to short-term factors such as physical activity or long-term factors such as aging and disease. This leads to loss of spinal movement and therefore function [Cassinelli and Kang 2000].

The annulus fibrosus (AF) is designed for structural support with concentric layers of collagen fiber bundles. The orientation of the fibers alternates from layer to layer, with fibers oriented at an angle of approximately ± 30° with respect to the horizontal plane and 120° with respect to each other in adjacent layers. This arrangement results in equally distributed forces within the disc, which provides resistance to axial load, resistance to shearing and rotational forces, and tensile strength [Humzah and Soames 1988]. Fibers comprising the outer portion of the AF are highly organized and densely packed type I collagen fibers. Fibers comprising the inner portion of the AF are more loosely packed type I and type II collagen fibers with an increasing percentage of proteoglycans relative to the outer portion, giving way to a transition zone between the AF and NP [Whatley and Wen 2012].

All fibers of the AF except the outermost attach to the cartilaginous endplates of the vertebral bodies. The outermost layer, called Sharpey Fibers, attach directly to the vertebral bodies [Jones and Boyde 1974]. Because the intervertebral discs are avascular structures, the cartilage layers provide oxygen and nutrients for diffusion into the discs [Humzah and Soames 1988]. However, reduced porosity due to aging, degeneration, or injury, may lead to low permeability and reduced nutrient exchange [Wu et al. 2013].

1.1.3 Ligaments

Vertebral bodies and intervertebral discs are held together by groups of ligaments (Figure 4), including: the intertransverse ligaments (ITL) and interspinous ligaments (ISL), which attach to the transverse and spinous processes, respectively, or

adjacent vertebrae; the supraspinous ligament (SSL), which extends the length of the spinal column posterior to the ISL and attaches firmly to the tip of each spinous process; the capsular ligament (CL) surrounding each facet joint; the ligamentum flavum (LF), which originates bilaterally on the anteriorinferior laminar surface of each superior vertebral body and inserts on the posterosuperior laminar surface of each inferior vertebral body; the anterior longitudinal ligament (ALL), which extends the length of the spinal column anterior to the vertebral bodies; and the posterior longitudinal ligament (PLL), which extends the length of the spinal column posterior to the vertebral bodies. Ligaments are composed of unidirectional type I collagen fibers, providing strength and resistance, and elastin fibers, providing flexibility. Ligaments provide passive stabilization to the spinal column by both facilitating and limiting motion [Miele et al. 2012].

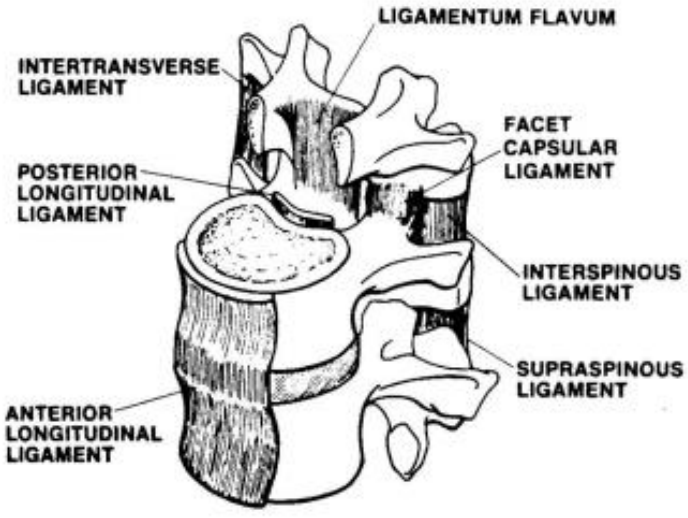

Figure 4: Ligaments of the Spine. [Panjabi et al. 1980]
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1.2 Spine Kinematics and Biomechanics

A motion segment unit (MSU) is the basic unit of study of the spine and consists of two adjacent vertebrae and their interposed intervertebral disc [Zatsiorsky 1998]. Each MSU has six degrees of freedom (Figure 5): translation and rotation along three orthogonal axes.

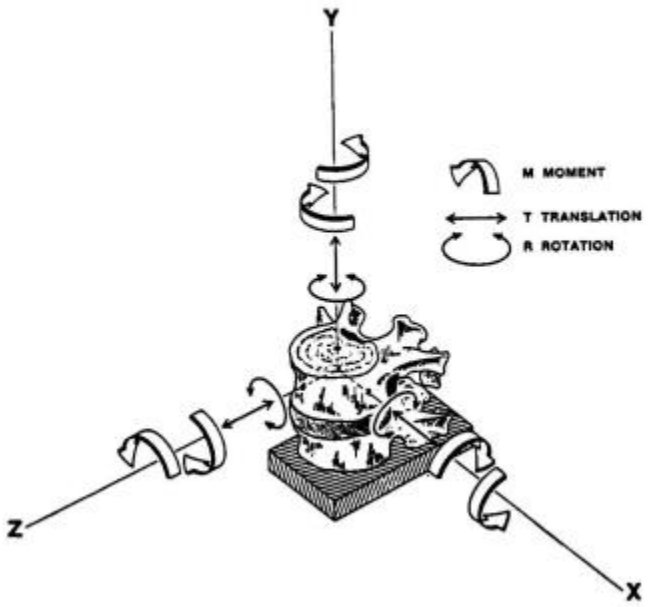

Figure 5: Three-dimensional MSU Coordinate System.
Kinematic range about three principal orthogonal axes. [Panjabi 1988]
Reprinted with permission from Wolters Kluwer Health, Inc. 1988.

1.2.1 Vertebral Range of Motion

There are two types of joints in the spinal column: intervertebral joints (synarthroses) between vertebral bodies and adjacent intervertebral discs and facet joints (synovial joints) between the articular processes of neighboring vertebrae. These joints allow the spine as a whole to produce three movements: flexion/extension, lateral

bending, and axial rotation [Zatsiorsky 1998]. Movement at the intervertebral and facet joints of the same MSU is coupled. The magnitude and direction of motion available at each MSU depends on the size of vertebral bodies and interposed discs, the orientation of facet joint surfaces, the tensile and elastic properties of spinal ligaments, and surrounding musculature [Zatsiorsky 1998, Miele et al. 2012].

Two parameters often used to discuss spinal kinematics and stability (or instability) are range of motion and neutral zone of motion. Range of Motion (ROM) is defined the angle through which a joint moves from anatomical position to the extreme limit of segment motion in a particular direction. ROM is used to diagnose spinal pathologies and is the most commonly reported kinematic characteristic of *in vitro* testing protocols [Panjabi et al. 1994, Crawford et al. 1995, Goel et al. 1995, Spenciner et al. 2006]. Vertebral bodies are considered rigid bodies, and the kinematic characteristics of MSU's are measured as the superior body with respect to the inferior body [Zatsiorsky 1998].

Stability of the spinal column is maintained by interdependent systems of vertebrae separated by intervertebral discs and articulating joints, joined together by passively restraining ligaments and controlled by neuromuscular activation. When a force is applied to an MSU, the unit will displace from a neutral position to a position where a significant resistance is encountered [Miele et al. 2012]. The neutral zone (NZ) is defined as this initial region of intervertebral motion around the neutral position where little resistance is given by the spinal column [Panjabi 1992]. After a maximum strain capacity of the NZ is reached, movement beyond that point causes the tissue deformity according to Hooke's law—a principle of physics stating that the force

required to extend or compress a spring by some distance is proportional to that distance—until any further movement results in permanent deformation or failure [Miele et al. 2012]. This region is called the elastic zone (EZ). Thus ROM involves the sum total displacement of the neutral and elastic zones (Figure 6).

The neutral zone appears to correlate with spinal stability—the interdependent spinal stability system adjusts to contain MSU movement within physiological thresholds of the neutral zone. NZ increases with spinal instability due to injury, muscle weakness, or degenerative changes and decreases with increasing muscle activation as well as the implementation of spinal fusion devices. Thus NZ calculations are used to measure clinical instability [Panjabi 1992, Wilke et al. 1998].

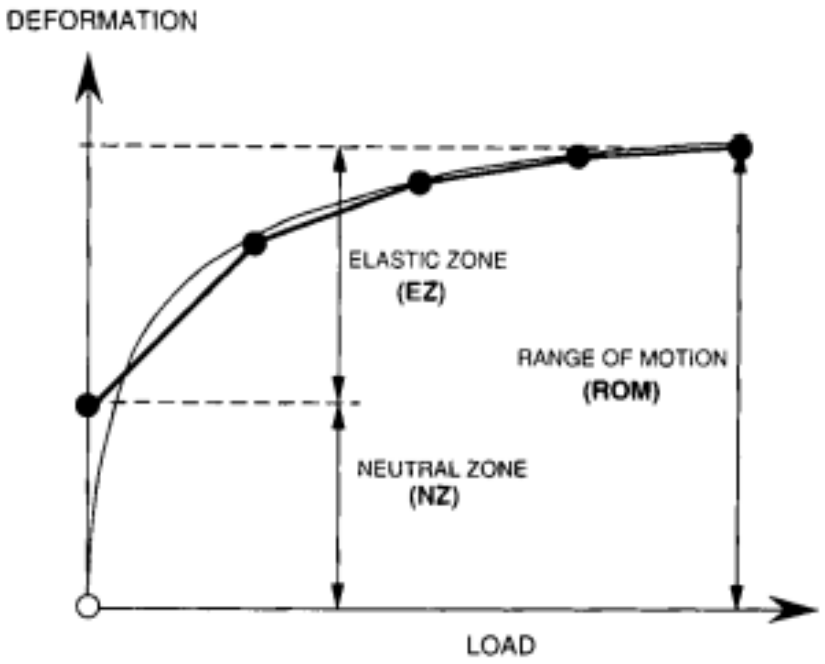

Figure 6: Load-Displacement Curve Illustrating Spinal Motion. The load-deformation curve of a soft tissue or a body joint is divided into two parts: neutral zone (NZ), the region of high flexibility, and the elastic zone (EZ), the region of high stiffness. The sum total of NZ and EZ is ROM. [Panjabi 1992] Reprinted with permission from Wolters Kluwer Health, Inc. 1992

1.2.2 Intervertebral Disc Mechanics

The intervertebral discs are essential to force transmission along the spinal column [Whatley and Wen 2012]. When a compressive force is applied to an MSU, the disc's NP pressurizes, expelling water as proteoglycan-induced osmotic pressure is overcome by the hydrostatic pressure created by disc deformation. The expelled water enters spaces between the fibrous lamellar layers of the AF and passes through the cartilaginous endplates of adjacent vertebral bodies. Conversely, when a compressive force is removed, the osmotic pressure of the NP is restored and the intervertebral disc returns to its original height [Johannessen et al. 2004, O'Connell et al. 2011].

Thus intradiscal NP pressures allows intervertebral discs to convert compressive axial loads into dispersed radial loads acting on the AF, allowing the discs to act as shock absorbers (Figure 7A). Because intervertebral discs contain both the pressurized NP's and tensile resistant AF's, they are able to maintain stability during normal flexion/extension, lateral bending, and torsional movements [Panjabi 1980, Humzah and Soames 1988]. Specifically, during eccentrically-placed loads, the AF fibers are compressed and bulge on the side of the applied force and contract in tension on the opposite side, and the NP is displaced to the opposite side of the applied force (Figure 7B-E). In these ways the viscoelastic properties of the intervertebral discs distribute stress along the spinal column to maintain stable posture and facilitate movement. Factors such as age, injury, nutritional imbalances, genetic conditions, and degenerative diseases compromise the spine's natural load-bearing mechanism and stability [Panjabi 1980, Adams and Roughley 2006].

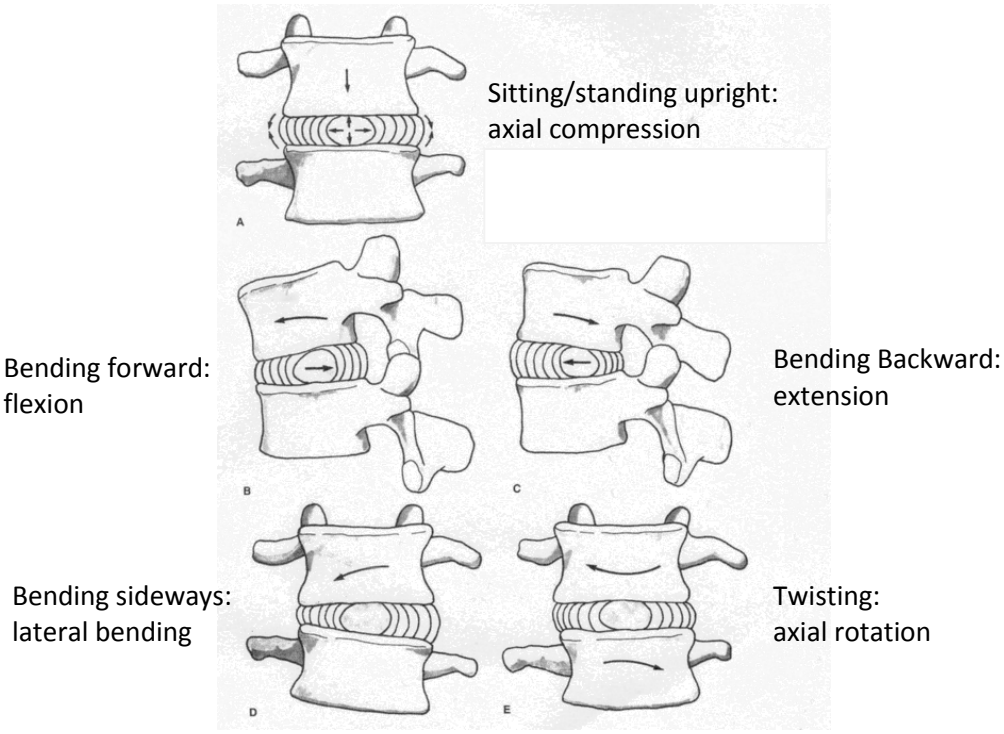

Figure 7: Pressure Dispersion and Movements of Intervertebral Disc.
(A) Axial Compression causes radial expansion of NP and AF. (B) Flexion causes posterior displacement of NP, anterior bulging of compressed AF fibers, and posterior tension of AF fibers. (C) Extension causes anterior displacement of NP, posterior bulging of compressed AF fibers, and anterior tension of AF fibers. (D) Lateral Bending causes ipsilateral displacement of NP, ipsilateral bulging of compressed AF fibers, and contralateral tension of AF fibers. (E) Axial Rotation causes strain on NP and AF fibers.
[adapted from Palastanga and Soames 2012]

1.3 Natural Loading Behavior of the Lumbar Spine

The lumbar spine naturally resists forces caused by body weight, torso and pelvic muscular activity, and additional external loads [Moore et al. 2011]. The average maximum flexion of a lumbar MSU *in vivo* is 15° [Adams and Hutton 1982], suggesting that at such large degrees of flexion, the lumbar spine provides substantial bending resistance. However, the average maximum extension and lateral bending of a lumbar MSU rarely exceeds 5° *in vivo* [Pearcy et al. 1984, Pearcy and Tibrewal 1984].

Similarly, the maximal axial rotation of a lumbar MSU has been reported to be 6° *in vivo* [Pearcy and Tibrewal 1984]. When bending or torsional moment loads on the lumbar spine do occur, they occur in conjunction with compressive loads, presumably due to body weight [Miller et al. 1986].

A simplified model of physiologic body loading on the lumbar spine is shown in Figure 8. The weight of the torso exhibits a force on the spine through the center of mass and induces a different bending moment at each individual vertebral level. The bending moment about a point is the product of the force and perpendicular distance to that point of rotation [Mow and Huiskes 2005]. The body weight force vector (F_{BW}), thought to lie along the line between the auricle of the ear to the center of the femoral head, is offset a distance (d) from the center of the MSU disc—this distance varies between MSU's due to the natural curvature of the spine. F_{BW} has two component forces: the shear force (F_S) acting along the plane of the disc and the axial load (F_A) acting along the plane perpendicular to that of the disc. The angle that the superior endplate of the MSU's upper vertebral body makes with the horizontal axis corresponds to the angle (θ) between the vertical F_{WB} and axial F_A. Thus the component forces can be calculated as:

$$F_A = F_{BW} \times \cos\theta$$

$$F_s = F_{BW} \times \sin\theta$$

As the angle θ increases, more of the body weight is transferred to the spine as shear force (F_S), thought to be resisted by the facet joint complexes and ligaments [Zatsiorsky 1998]. As the angle θ decreases, more body weight is transferred to the spine as axial force (F_A), absorbed and distributed by the intervertebral disc. The

bending moment (M) is resisted by the disc, ligaments, and facet joints. In fact, more resistance to the peak bending moment of extension is provided by the facet joints themselves than that of flexion [Dickey and Gillespie 2003], indicating that the posterior ligaments and intervertebral disc are heavily recruited for resistance in flexion.

The lumbar spine in particular must frequently resist especially large loads in axial compression—more than 1000 N may be imposed on the lumbar vertebrae by daily activities [Schultz 1987]. Although routine daily activities seldom impose large loads on the lumbar spine in bending or torsion movement, strenuous situations may occur, such as large trunk movements during traumatic events or when trunk muscle contractions are recruited inappropriately in unfamiliar and large weight bearing tasks [Miller et al. 1986]. Thus the loading scheme of the lumbar spine is further complicated by the location of the center of mass of an individual's upper torso, the anatomical curvature of an individual's spinal column, muscle activity, out-of-plane loads and moments, and the presence of disease or trauma [Zufelt 2008]. Thus clinical stability and load-sharing of the spine may be greatly affected by various factors.

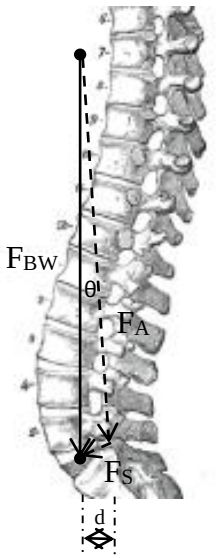

Figure 8: Simplified Lumbar Loading Mechanics. The action of the body weight force vector at the L5-S1 MSU induces a bending moment (M) and compressive force (F_{BW}), with two components: shear force (F_S) along the disc plane and axial force (F_A) perpendicular to the disc plane.
[adapted from Gray 1918]

1.3.1 Effects of Load History on Spinal Behavior

Loading within the physiological norms of the diurnal cycle, between 0.2 MPa at rest in a supine position and 0.6 MPa in an upright posture while performing a load bearing e.g. weight lifting activities of daily living, maintains the cellular and overall tissue health of the intervertebral disc while exchanging as much as 25% of the disc's water content within one 24-hour cycle [Sivan et al. 2006]. However, sustained compressive loads and/or repeated large compressive load cycling has been shown to generate increased AF stress concentrations and reduced NP pressures as well as reduced heights in lumbar intervertebral discs, which may lead to alterations in cellular metabolism, structural disruption, and therefore back pain [Adams et al. 1996].

Loading events are therefore known to influence the internal mechanics of intervertebral discs. Hydration plays a significant role of intervertebral disc mechanobiology with loading. Water distribution within the NP acts to resist compressive forces while the collagen fibers forming the AF acts to resist tensile forces from the NP's subsequent radial expansion thereby preventing NP bulging or herniation [Humzah and Soames 1988]. Multiple studies using human, ovine, and murine models have investigated the changes in intervertebral disc mechanobiology with varied axial compressive load histories and recovery periods. Higher loads cause greater water loss, leading to reduced disc height, reduced intradiscal pressure, and load transference from the NP to the AF creating shear stress peaks throughout the AF [Goodley 2014]. In fact, reduced intradiscal pressure under a constant load results in an imbalance between the transverse and axial stress components [Hwang et al. 2011]. After "safe load" recovery periods which promote maintenance of intervertebral tissue metabolism, discs exhibited full returns of intradiscal pressures, disc heights, and stress-relaxation properties [Adams and Hutton 1983, Adams et al. 1996, Argoubi and Shirazi-Adl 1996, Johannessen et al. 2004, Stokes and Iatridis 2004, Walsh and Lotz 2004, Sivan et al. 2006, van der Veen et al. 2006, Chan et al. 2011, Hwang et al. 2011, O'Connell et al. 2011, Walter et al. 2011].

During sustained loading of the spine, a progressive deformation of the spinal column, called "creep" occurs [Twomey and Taylor 1982]. The magnitude of loading forces as well as the loading direction of compressive or creep loading has been varied across all studies, making comparisons difficult. Compressive axial loads have been reported to increase simultaneously-measured stiffness and decrease ROM in

flexion/extension, lateral bending, and axial rotation [Panjabi et al. 1977, Adams and Dolan 1991, Janevic et al. 1991, Cripton et al. 2000, Gardner-Morse and Stokes 2003, Shirazi-Adl 2004]. These larger moment stiffnesses have been observed as generally more flat and linear load-displacement curves [Miller et al. 1986, Edwards et al. 1987, Janevic et al. 1991, Patwardhan et al. 2003]. On the other hand, prolonged compressive loads applied in the direction of moment testing have been reported to decease stiffness and increase ROM in flexion/extension, lateral bending, and axial rotation [Goel et al. 1988, Adams and Dolan 1991, Little and Khalsa 2005, Zhao et al. 2005, Busscher et al. 2011]. Siffnesses were shown to decrease particularly in the NZ, observed as more steeply linear slopes in the range of the NZ on the load-displacement curves [Busscher et al. 2011]. These results imply that stiffness of the MSU is not constant over the range of physiologic loads. This suggest that studies of load-sharing between active muscle and tendons and passive vertebral bodies, intervertebral discs, and ligaments during strenuous tasks of large compressive loads should take into consideration these changes in spinal flexibility and/or resistance characteristics.

1.3.2 Effects of Posture on Spinal Behavior

In similar ways, postural changes, especially during loading events, are known to influence the internal mechanics of intervertebral discs as well as spinal ROM. The effect of posture on spinal compressive strength and intradiscal pressure has been previously examined [Adams and Hutton 1983, Adams et al. 1994, Gooyers et al. 2012]. Results indicate that during compressive loading, intervertebral discs placed under flexion conditions, such as that of sitting positions, lose more fluid and therefore

lose height, especially from the NP, than do discs placed in neutral erect positions. This fluid flow during flexion is large enough to aid in the nutrition of lumbar discs during regular diurnal 24-hour cycles of daily living [Adams and Hutton 1983]. In full flexion, the anterior vertebral bodies become weight bearing and intradiscal pressure is high due to the tension response of the posterior intervertebral ligaments. In extension, i.e. lordotic curvature normally exhibited by the lumbar spine, the posterior vertebral arches and facet joints become weight bearing, allowing decreased intradiscal pressure and fluid re-distribution in the intervertebral disc [Adams and Hutton 1983]. However, the vertebral arches may be more easily damaged by smaller compressive forces.

The lumbar spine is thought to best able to resist high compressive forces when positioned at about 50% flexion [Adams et al. 1994], indicating that sitting or standing in a position of moderate flexion, i.e. flattening of the normal lordotic curvature or sitting/standing "up straight," is preferred when the lumbar spine is subjected to higher compressive forces. However, the lumbar spine does resist large compressive loads in its natural lordotic curvature, particularly when applied along a "follower" path that approximates the tangent to the natural curve of the lumbar spine [Patwardhan et al. 1999], suggesting that the lumbar spine allows physiological mobility under compressive "follower" loads and bending moments.

1.4 Clinical Relevance to Low Back Pain

Low back pain (LBP) is a multifactorial disorder affecting many individuals worldwide—approximately 15% of adults and 27% of the elderly. The prevalence of LBP continues to increase in the United States. The risk factors for LBP include: comorbidities, such as obesity, arthritis, anxiety, and depression; required occupational movements, such as heavy lifting, pushing, pulling, and prolonged walking, standing, or sitting; lifestyle behaviors, such as smoking, lack of exercise, and prolonged standing or sitting; increasing age; and degenerative diseases of the spinal vertebrae and/or intervertebral discs. Coupled with increasing health care costs, LBP causes significant impairments on physical and psychological health and well-being, work performance, and social responsibilities. LBP thus remains a difficult condition to manage [Manchikanti et al. 2012].

Recent studies have confirmed that mechanical stimulation of the lumbar intervertebral discs can reproduce the symptoms of severe and chronic back pain [Kuslich et a. 1991, Schwarzer et al. 1995]. Lumbar intervertebral discs can be sources of intrinsic pain with or without nerve root involvement, due to the fact that the outer third of the AF is innervated [Yoshizawa et al. 1980, Ashton et al. 1994]. However, the mechanism by which pain at the intervertebral disc is produced remains unclear. Several theories have been proposed: inflammatory disturbance [Crock 1986, Jaffray and O'Brien 1986], excessive mechanical deformation of the intervertebral disc tissues associated with abnormal loading of the posterolateral AF and depressurization of the NP [McNally et al. 1996, Adams et al. 2000], as well as internally displaced disc tissue

pressing directly on the pain receptors of innervated outer AF [Kramer 1990, Donelson et al. 1997].

Pain relief may be obtained through physical therapy exercises involving repeated full backward bending movements of the lumbar spine, although the mechanism through which pain is relieved has not been fully explained. Extension postures have been reported to improve and resolve symptoms of low back pain [Ponte et al. 1984, Nwuga and Nwuga 1985, Donelson et al. 1990, Donelson et al. 1991, Donelson and McKenzie 1992, Delitto et al. 1993]. Various theories have been investigated to explain pain reduction with backward bending. Extension movements cause anterior migration of the NP thereby preventing painful posterior protrusions of intervertebral discs [Schnebel et al. 1988, Schnebel et al. 1989, Beatie et al. 1994, Shepherd et al. 1995, Fennell et al. 1996]. Extension movements act to transfer compressive forces from the MSU to the posterior facet joints, effectively reducing NP compression and allowing rehydration, which can reduce forces acting on pain-sensitive tissues—these effects are magnified by continuous compressive "creep" loading [Adams and Hutton 1980, McNally and Adams 1992, Adams et al. 1996]. Extension movements also reduce stress concentration peaks in the posterior AF, which may reduce pain in patients whose painful discs are shielded by the vertebral arch in extension [Adams et al. 2000]. All theories of intervertebral disc mechanics may relate to spinal ROM and therefore overall function of the lumbar spine. Posture appears to be an important mechanical factor to consider when assessing ability of lumbar spine to resist injury.

1.5 Study Objectives

This study is a continuation of a previous study investigating the difference in biomechanical intervertebral disc properties between high compressive loading and low compressive loading during *ex vivo* ovine model experiments. High sustained compressive loading, called "adverse" loading, of 0.75 MPa with seven transient "challenge" 2 MPa loads applied every 15 minutes, caused increased strain, reduced endplate permeability, reduced disc heights, and reduced intradiscal pressure generation during challenge loading as well as and inhibited recovery of NP pressure generation post-challenge loading [Goodley 2014]. This study aims (1) to determine the effect(s) of "adverse" compressive loading on spinal ROM and (2) to determine whether or not postural interventions may provide protective effects on the spinal ROM with "adverse" loading. We hypothesize that (1) "adverse" compressive loading causes an increase in spinal ROM leading to instability and that (2) extension or backwards bending prior to "challenge" loads placed on spinal segments placed under "adverse" loading will have an interventional effect on those changes of spinal ROM.

To assess lumbar biomechanical function, force and displacement measurements were collected using a manufactured apparatus to apply pure moments in flexion/extension and axial rotation directions. To asses kinematic MSU rotation in each direction, local vertebral coordinate systems were constructed using optical markers to calculate Euler angles between each vertebral body.

Ex vivo mechanical tests of sheep lumbar motion segments were used for all data collection. The use of ovine models to investigate and extrapolate biomechanical behaviors of the human lumbar spine has been previously validated and is commonly

performed [Wilke et al. 1997, Smit 2002]. The enclosed findings provide additional understanding of the dynamics of loading and recovery of lumbar kinematics with and without postural intervention.

Applied loads used in this study were representative physiological values: high "adverse" compressive resting loads represent the effects of obesity, manual labor occupations, or other lifestyle factors, such as prolonged sitting or standing, which produce prolonged compressive stresses on the neutral spine; and "challenge" loads represent intermittent weight bearing activities of daily living or occupational labor causing short-term increased spinal compression. This continuous, or ramp loading, of the spine has been previously described and validated as a more physiological technique [Wilke et al. 1994, Crawford et al. 1995]. Increased and prolonged loading magnitudes limit hydration recovery of the intervertebral disc and may therefore induce laxity in the surrounding ligaments, resulting in accumulated destructive effects on spinal biomechanics.

Chapter 2: Spine Biomechanical Testing

2.1 Introduction

Methods of biomechanically testing the spine are generally categorized into two groups: flexibility protocols (load-controlled) or stiffness protocols (displacement-controlled). During flexibility testing, a linear and/or rotational load is applied to a specimen and the resulting translational and/or rotational displacement is measured. During stiffness testing, a translational and/or rotational displacement is applied to a specimen while the resulting load is measured [Panjabi 1988]. In order to achieve standardization of testing protocols, the following must be controlled: (1) the load experienced at various vertebral levels should remain constant, regardless of the stiffness of the intact spine, and (2) the loads or displacements applied should not inhibit or constrain the motion of the spinal segment [Goel et al. 1995].

The recommended testing method still remains controversial. Each method requires certain assumptions and offers different advantages. Although the stiffness protocol appears to better replicate *in vivo* conditions, which would allow for a better understanding of clinically valid responses to experimental interventions, the flexibility protocol offers better control over the complex variables involved in spinal biomechanics testing. Specifically, pure moment methods of flexibility testing protocols induce a similar loading profile at each vertebral level, allowing for comparisons between single as well as multi-levels [Goel et al. 1995].

2.1.1 Flexibility Protocol (Load-Control)

The flexibility method allows complete freedom of movement at all vertebral levels of the spine, achieving a more natural behavior of the spinal column. While the lowest vertebral body is fixed to a testing surface, many different types of translational and/or rotational loads may be applied to the highest free and unsupported end of the spinal segment [Panjabi 1988]. A typical and most common setup involves the superior surface of the free segment attached to a cable and pulley system, allowing load application in such a way to minimize shear stresses experienced by the spinal segment (Figure 9). This setup may also include the use of pneumatic actuators or gliding rails.

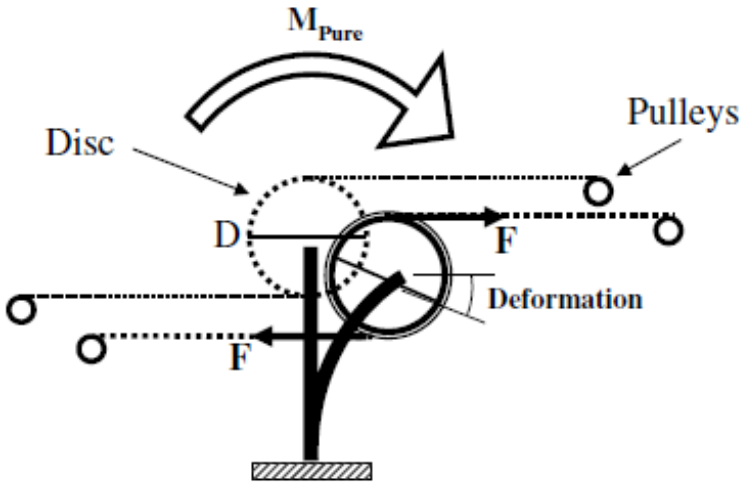

Figure 9: Illustration of Typical Flexibility Protocol Setup. Pure moment is applied using a parallel cable and pulleys system attached to the superior end of the free vertebral level. For the moment to remain pure (M_{pure}) and constant deformation, the two forces (F) tangential to the disc (D) must always remain parallel to each other. [Panjabi 2007] Reprinted with permission from Elsevier 2016

The pure moment applied to the free end of the spinal segment using this protocol is applied equally to all segments of the specimen and remains the same as the spine deforms during testing [Panjabi 1992]. This method is commonly used to investigate basic biomechanical characteristics of the spine and study clinically relevant problems, such as spinal injury or instability [Panjabi et al. 1984, Goel et al. 1986, Abumi et al. 1990], spinal trauma [Oxland et al. 1994, Panjabi et al. 1994], spinal fusion devices [Panjabi et al. 1988, Abumi et al. 1990, Wilke et al. 1998, Oda et al. 2001], and non-fusion devices [Hitchon et al. 2005, Kotani et al 2005]. Multidirectional mechanical properties of the spine, such as degrees of motion (flexion/extension, lateral bending, and axial rotation) may be obtained by applying moments along each of three rotational axes within the local vertebral coordinate system.

Studies involving applications of loads to induce rotations around more than one principle axis typically, however, involve removing the specimen or pieces of the testing apparatus from the testing frame between individual tests [Fraysur 2010]. Recently, testing machines have been developed to allow testing around multiple axes without specimen removal. These machines have the ability to drive one axis at a time, making them unable to induce combined loading scenarios which are normally exhibited physiologically [Wilke et al. 1994, Cunningham et al. 2003, Panjabi 2007].

One common modification to the flexibility protocol is the use of a follower load to simulate the stabilizing function of the surrounding muscles [Schultz et al. 1979, Panjabi et al. 1994, Patwardhan et al. 1999]. However, physiological stabilization is difficult to achieve may not show relevant spinal segment responses to a particular intervention technique or device [DiAngelo et al. 2002]. In fact, since physiological

spine loading cannot be measured non-invasively, assumptions must be made about loading limits during testing, leading to one important limitation to the flexibility protocol [Fraysur 2010]. Additionally, the scenario that similar magnitudes of moment are applied to each spinal level is not physiological, given the natural lordotic curvature of the spine [Zufelt 2008].

2.2.2 Stiffness Protocol (Displacement-Control)

The stiffness protocol theoretically allows investigators to mimic *in vivo* behavior of the spine and can be run by a constrained rotational device or a commercial testing frames with a single degree of freedom (Figure 10). In this setup, the horizontal lever arm is attached to the superior surface of the specimen by a constrained fixture as well as a slide bearing of the vertical actuator, allowing the application of a compressive force [DiAngelo et al. 2003].

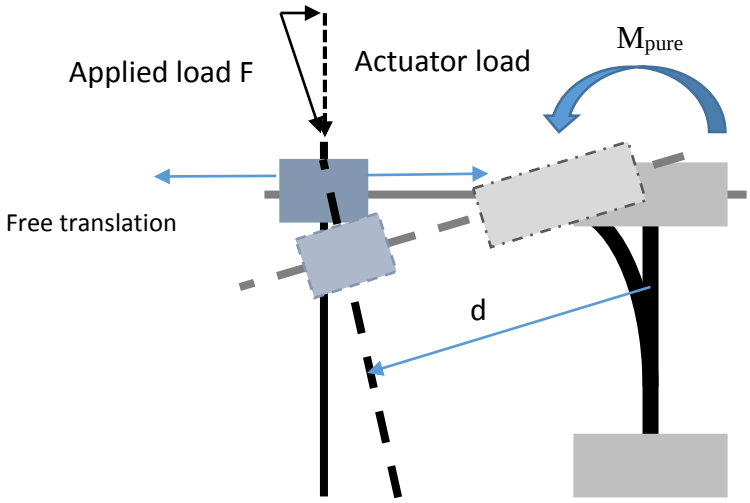

Figure 10: Illustration of Eccentric Stiffness Protocol Setup. A horizontal moment arm unconstrained along a slide bearing applies load at a distance normal to the specimen. [adapted from Zufelt 2008]

The displacement-input method of the stiffness protocol often causes practical difficulties. The location of displacement applied is of crucial importance—it is known that different input locations cause different rotation axes, which result in uneven distribution of loading and ambiguous load-displacement curves [Panjabi et al. 2000]. A location which produces natural physiological spinal movements is ideal but cannot be known unless a preliminary test is performed and may furthermore move during testing as the spine deforms [Panjabi 2007]. Additionally, if the rotation axis is not congruent with a natural axis of rotation, the resulting spinal movements may be constrained and may cause injury to the specimen [Grassmann et al. 1998].

2.2 Pure Moment Testing

A popular method of flexibility (load-controlled) mechanical testing is the pure moment protocol. This is a technique in which pure, relatively non-constraining moments are used to induce flexion/extension, lateral bending, and axial rotation in spine specimens of two or more vertebral levels. A pure rotational load is applied to a free and unsupported superior end of the spinal segment, while the opposite inferior end of the spinal segment is either fixed to the base of the testing frame or placed on a slide bearing mechanism, allowing for more physiological translational movements during bending motions. Since the top of the spinal segment is supported by the testing apparatus, shear deformation is minimized [Panjabi 1988, Goel et al. 1995]. The rotational moment may be applied via several constructs: deadweights on a rail or pulley system [Goel et al. 1995, Esses et al. 1996, Lysack et al. 2000], pneumatic actuators on a sliding rail system [Panjabi 1988, Panjabi 2007], or a multi-axial

hydraulic testing frame in conjunction with a cable and pulley system [Crawford et al. 1995, Esses et al. 1996, Eguizabal et al. 2010, Crawford 2011, Tang et al. 2012].

The most common testing frame used for pure moment protocols is hydraulic, attached to a cable and pulley system [Crawford et al. 1995]. Methods for improving the purity of pure moment loading in experiments where a servo hydraulic test frame is used to control the tension of a pulley-formed loop of cable have been described—a "sliding ring" mechanism attached to the superior end of the specimen for applying moment [Eguizabal et al. 2010] and most recently a "floating ring" mechanism with linear sliders and vertical bearings also attached to the superior end of the specimen for applying moment [Tang et al. 2012] (Figure 11). The modifications address specific aspects: (1) the maintenance of the parallelism of the cables, and (2) the minimization of friction due to cables traveling across the frictionless pulley system. Thus two parallel forces equal in magnitude and separated by some distance are applied, resulting in a cancellation of the opposing forces and application of a net moment in one direction (Figure 12).

Specimens are testing in sagittal plane bending (flexion/extension), coronal plane bending (lateral bending), and transverse plane rotation (axial rotation). Pure moment methods should ideally induce the same loading conditions for every test, allowing easy comparison between ROM values.

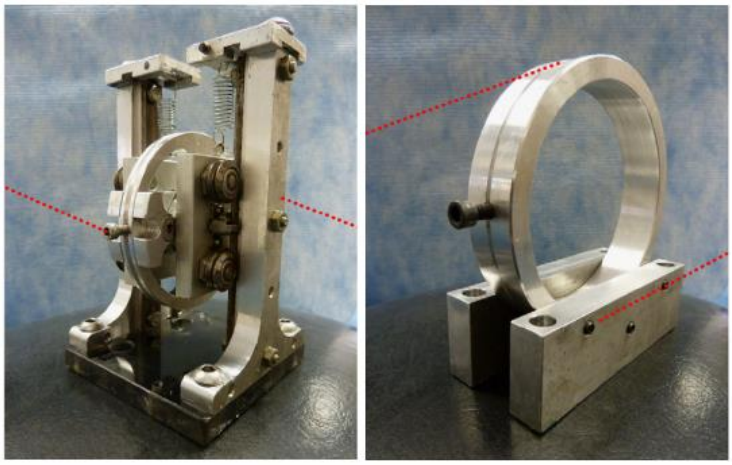

Figure 11: Close-Up of 3D "Floating Ring" (left) and "Fixed Ring" (right). Dotted red lines indicate the loading path for axial rotation in the floating ring setup. Dotted red lines indicate the loading path for flexion/extension and bending motion (after the ring or specimen is rotated 90° about its vertical axis) in the fixed ring setup. [Tang et al. 2012]
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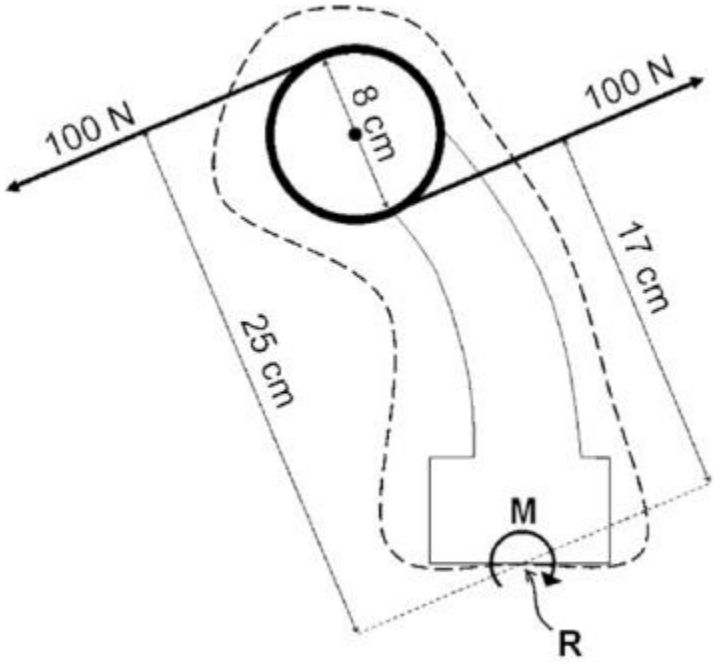

Figure 12: Example of Static Free-Body Diagram in Pure Moment Loading. Two 100 N forces applied by opposite ends of a cable loop to a spine specimen are balanced by a single reaction force (with x- and y-components) and a moment at the point R, representing the sensing origin of a multi-axial load cell below the specimen. When the two ends of the cable are parallel and separated by a diameter of the ring on the specimen (8 cm), although the forces cancel, the applied moment at the center of the ring (4 cm x 100 N + 4 cm x 100 N = 8 Nm) is the same as the reaction moment M of the specimen at the point R (25 cm x 100 N – 17 cm x 100 N = 8 Nm). [Crawford 2011]
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2.3 Construction of an Apparatus to Apply Pure Moments

The flexibility protocol via pure moment testing has become the most common and accepted method to investigate spinal segment ROM in each direction (flexion/extension, lateral bending, and axial rotation). The 3D "floating ring" mechanism to apply rotational moments has been shown to apply consistent moments despite varying specimen rigidity and length [Tang et al. 2012]. Since it has the advantage to allow additional directions of pure unconstrained motion with linear sliders and vertical bearings, a similar "ring" system, also with a linear slider and vertical bearings to counter-balance the mass of the "ring," was developed and produced (Figure 13) with assistance from Howard Grossenbacher (Department of Aerospace Engineering Machine Shop, University of Maryland). This system was used to apply bending moments to the specimens via a cable (braided Spectra cable, 200 lb. capacity) and low-friction pulley loop. Another "hex joint" system, thought to be more representative of a physiological torsional joint within the spine, was used to apply rotational moments to the specimens (Figure 14).

Both systems were attached to the superior ends of the specimens as well as an 858 Mini Bionix II material testing system (MTS Systems Corporation, Eden Prarie, MN). The multi-axial hydraulic actuator of the MTS system was programmed to apply either an upward force corresponding to a specific moment (as described previously), for bending movements of the spine, or a specific torsional moment for axial rotation of the spine. The inferior ends of the specimens were attached to a linear sliding mechanism fixed on the baseplate of the testing frame, ensuring that the specimens

were free in the plane of the MTS base and allowing for further unconstrained movements, more natural and physiologically present *in vivo*.

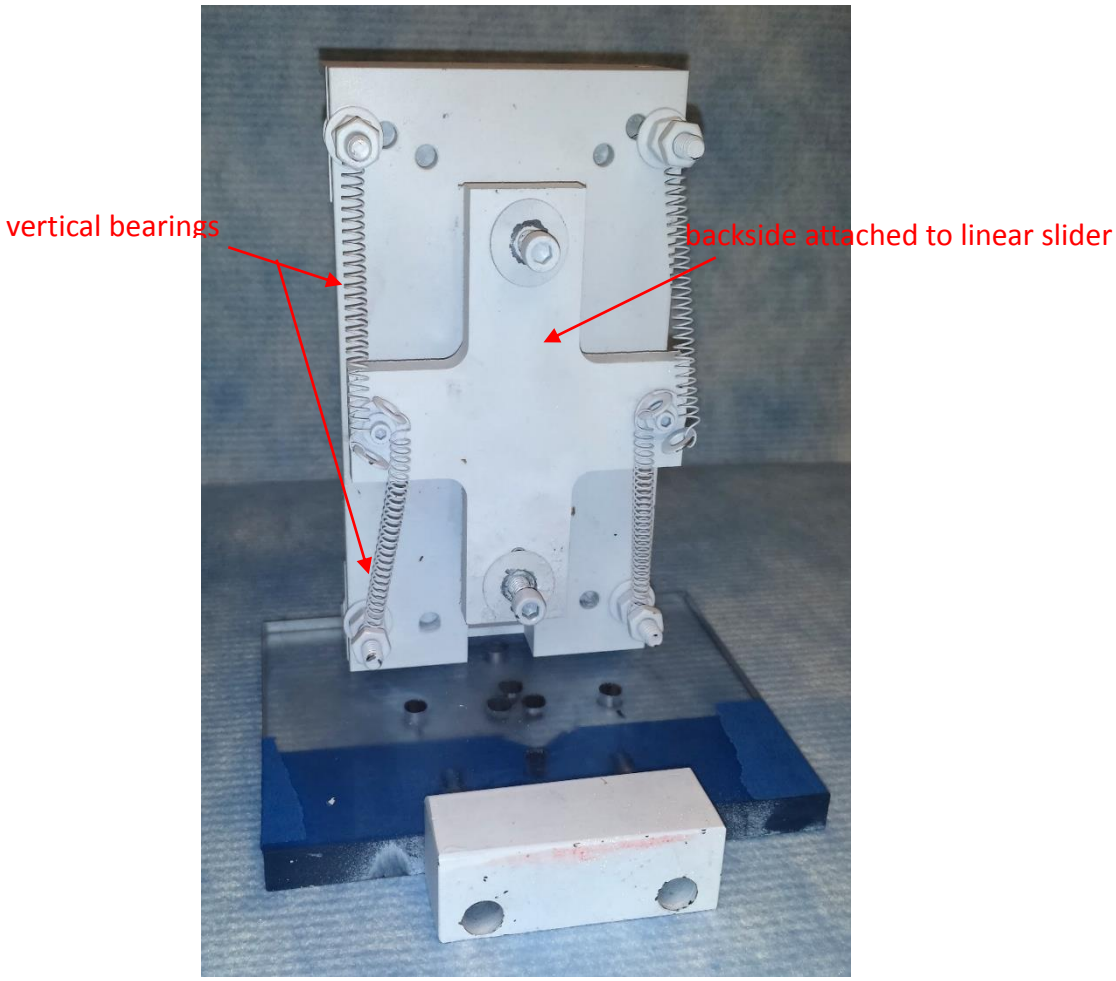


Figure 13: Pure Unconstrained Loading "Ring" Mechanism. Manufactured with linear slider and vertical bearings to apply pure moments in flexion/extension and lateral bending.

Figure 14: Hex Joint. Used to apply pure moments in axial rotation.

2.3.1 Flexion/Extension

Pure bending moments were induced via a single cable attached to the loading "ring" mechanism and routed to the actuator of the MTS system via low-friction pulleys attached to a static frame (Figure 15). The pulley positions were adjusted at the beginning of each test to achieve co-linearity of the cables extending from the loading "ring." Changing positions of the pulleys and directions of the cable loop allowed for loading in flexion and extension.

2.3.2 Lateral Bending

After rotating the spine 90° about its vertical axis and re-attaching the loading mechanism, pure bending moments were again induced via a single cable attached to the loading "ring" mechanism and routed to the actuator of the MTS system via low-friction pulleys attached to a static frame (Figure 15). Cable direction and pulley positions were adjusted for left and right lateral bending, and co-linearity of the cables extending from the loading "ring" was maintained prior to the start of each test.

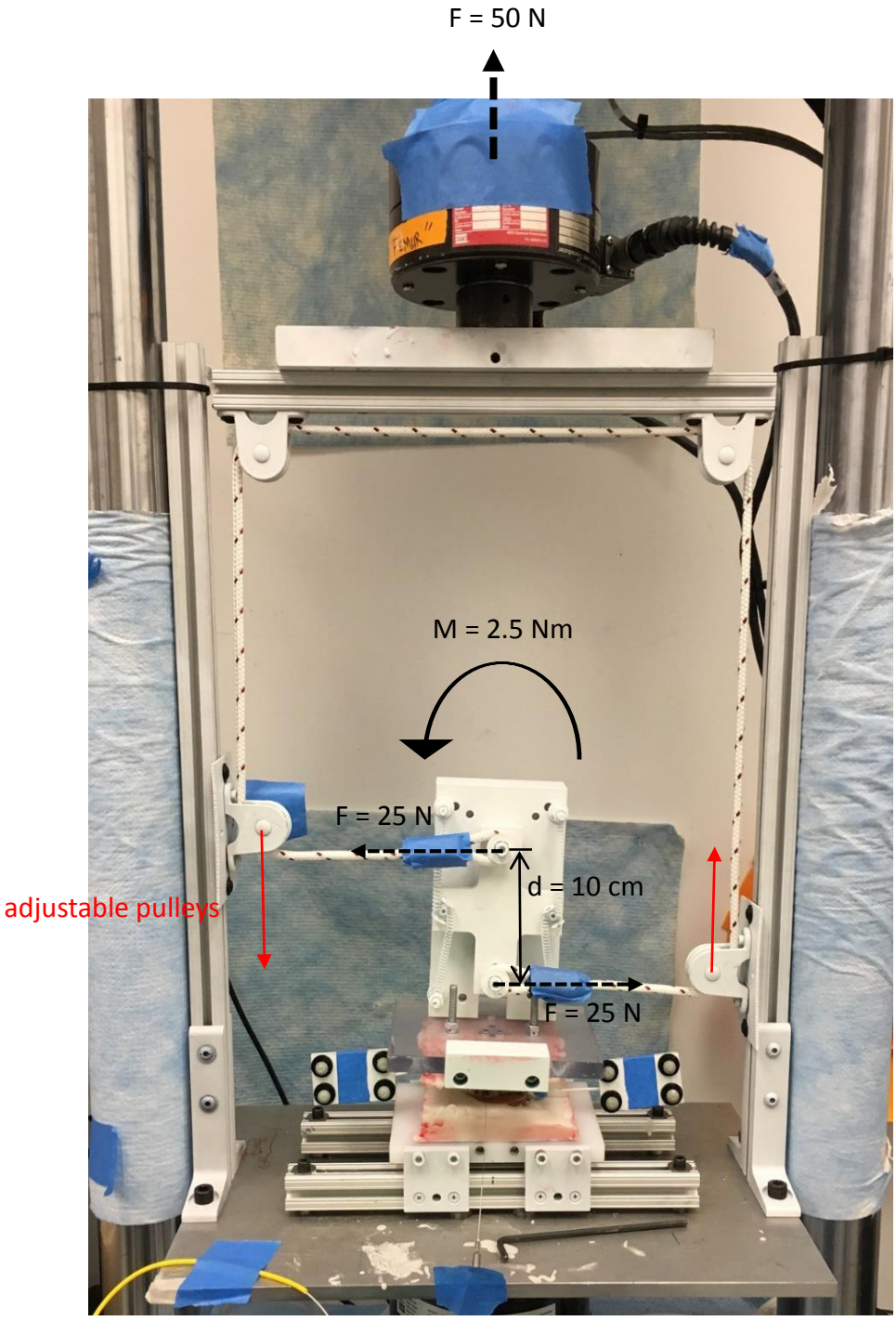


Figure 15: MTS System Setup for Flexion/Extension and Lateral Bending.
For example, one 50 N force applied by the MTS machine separates into two 25 N forces are applied by opposite ends of the cable loop and are parallel, separated by a diameter of the "ring" on the specimen (10 cm = 0.1 m). A resulting 2.5 Nm moment is applied at the center of the ring and along the length of the specimen
(0.05 m x 25 N + 0.05 m x 25 N = 0.05 m x 50 N = 2.5 Nm).

2.3.3 Axial Rotation

Torsional moments were applied via the "hex joint" and programmed axial rotation of the MTS system (Figure 16).

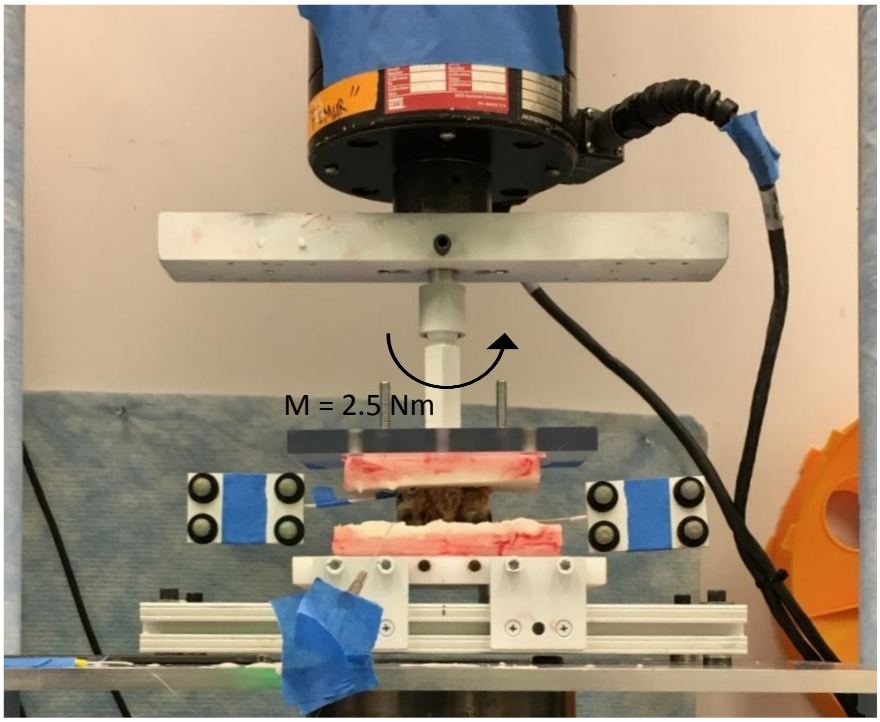


Figure 16: MTS System Setup for Axial Rotation.
For example, a 2.5 Nm moment is applied at the hex joint and along the length of the specimen by MTS machine.

2.4 Performance of the Testing Apparatus

The test setup has its limitations in that there will always be an inherent difference in the input force command and the applied moment measured by the multi-axial load cell. This is due in part to an additional bending moment caused by the shifted center of mass when the specimen deforms under bending loads. However, this artifact is assumed to be present in the current "hex joint" mechanism used for axial rotation and has been documented to be present in typical "ring" mechanisms used for flexion/extension and lateral bending [Tang et al. 2012]. Additionally, since the ovine specimens were so small and often difficult to secure in bone cement, the slight variability in potting of each specimen, which may have caused off-center or misaligned attachment of both the "ring" mechanism and "hex joint," most likely contributed to artifact moments and additional shear stresses placed on the specimens.

The addition of the linear sliders and vertical bearings in the loading ring setup seems to alleviate some of this differential in moment. However, the main obstacle in achieving ideal pure moment loading in the flexion/extension and lateral bending directions is maintaining parallelism of the loading cable ends through applying enough tension prior to each test—the often required cable and/or pulley adjustments. Since the position of the cable loop and pulleys were secured at the beginning of each test and were subsequently controlled by the MTS system, the cables may not have remained co-linear throughout an entire test.

The main obstacle in achieving ideal pure moment loading in the axial rotation direction is steady and repeatable control of the multi-axial load cell by the MTS system. This additional challenge in applying pure moment loading via the MTS system

is due to the proportional-integral-derivative (PID) mechanism of the MTS system itself [Model 793.00 System Software 2005]. The proportional (P) gain exerts effects on present values of error, producing an output value proportional to the current error value in order to improve MTS system response. A high P gain results in a large change in the output for a given change in the error, causing the system to increase the speed of its response but may therefore become unstable, whereas a low P gain results in a small output response to a large input error, causing the system to become less responsive or sluggish. The integral (I) gain exerts effects on the past values of error, contributing to both the magnitude of the current error and the duration of the current error in order to minimize the amount of time it takes to improve the MTS system accuracy. The I gain therefore reduces the residual error which occurs with a pure proportional controller, but may cause the present output value to overshoot or undershoot its input command. The derivative (D) gain exerts effects on possible future values of error, contributing to the stability of the MTS system.

Pilot tests were performed to optimize the PID gains in both bending and rotational ROM testing; however, the use of the PID algorithm does not guarantee perfect control or stability of the MTS system, as seen by real-time multi-axial load cell discrepancies between input forces or moment commands and actual forces or moments applied (Figure 17). The reflective nature of the motion tracking markers also led to additional challenges when collecting data, since any and all reflective surfaces of the testing apparatus not carefully covered with spray paint and/or tape caused one or more signals to fluctuate or drop out during ROM trials. Unfortunately, ROM results from the reflective markers did not show noticeable movement in axial rotation. Thus

angular rotation in the clockwise "right" and counter-clockwise" left directions were measured by the MTS system itself—an important limitation in the use of reflective markers for full ROM analysis.

After pilot testing, there was a noticeable and consistent difference between angular displacements in both rotational directions—rotation in the counter-clockwise or "left" direction was observed in real-time to be quite unstable, resulting in consistently varied angular deformation than that of the clockwise or "right" direction. This was assumed to be due to the setup of the specimens within the MTS system. The multi-axial load cell would reach its limit of axial rotation in the counter-clockwise or "left" direction and over-compensate by applying a large moment in a short amount of time, causing the system to become unstable. Thus subsequent angular deformation analysis should only be carried out in one direction of axial rotation—clockwise or the "right" direction.

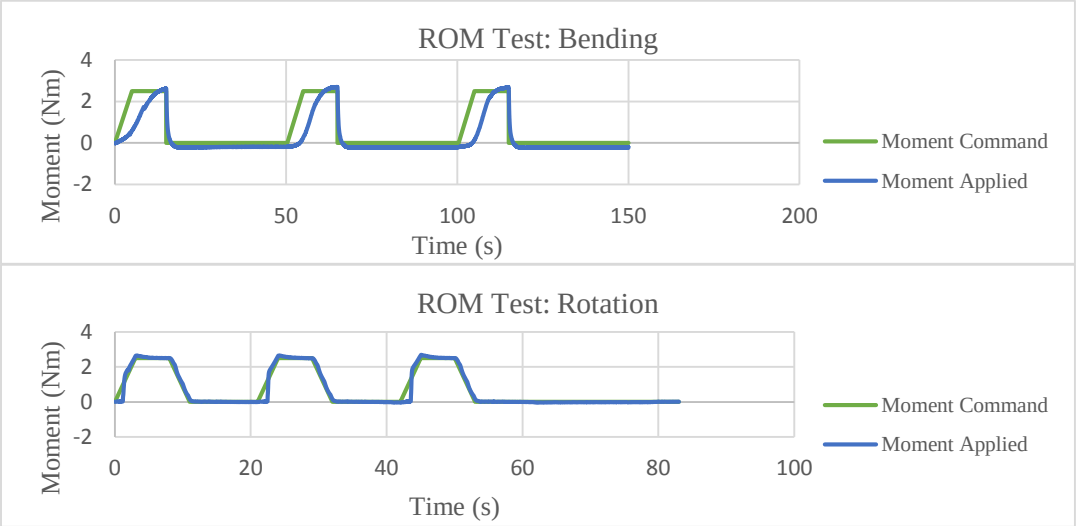


Figure 17: Real-Time ROM Tests. Over the course of one test with three cycles of loading, differences can be seen between the MTS input or moment command and the actual recorded moment applied for (top) one bending direction: flexion and (bottom) one rotation direction: clockwise or "right."

Repeatability analysis was performed in the ROM testing directions used in the following study: flexion, extension, and axial rotation. Over the course of one day, a control specimen was tested three times in each loading direction. ROM angular displacement was calculated—the method used for displacement calculations is described in the following chapter. Variations between tests and within tests were calculated from Analysis of Variance (ANOVA) and can be seen in Table 1. Variations both between and within tests were low for each direction of ROM testing. P-values greater than 0.05 indicate no statistical differences and therefore good repeatability of quantitative ROM measurements. This control specimen was not used in any other tests and is not a part of any subsequent data.

Table 1: ROM Measurement Repeatability. Variance and p-values of calculated degrees of angular displacement between and within ROM tests in flexion, extension, and axial rotation.

	Variation (degrees^2) between Tests	**Variation (degrees^2) within Tests**	**P-value**
Extension	0.020	0.043	0.65
Flexion	0.047	0.057	0.16
Axial Rotation	0.00053	0.000067	0.23

Chapter 3: Effects of Load History on Ovine Spinal ROM

3.1 Introduction

Load history alters intervertebral disc mechanical properties by modifying water distribution in the NP region, changing hydrostatic pressure and therefore tissue ROM response, when force is transmitted along the spine. The effects that "adverse" loading profiles have on ovine lumbar MSU flexibility and ROM were measured to investigate the effects of load history on spinal stability. Spinal segments subjected to "adverse" loading profiles were expected to, as a result of limited intervertebral disc fluid recovery, lose their stabilizing ability thereby generating increased ROM angular displacement profiles demonstrating increased flexibility. Additionally, interventional backward bending (extension) movements were expected to limit the loss of intervertebral disc fluid and prevent the loss of spinal stability. This study contributes to a greater understanding of load effects on lumbar flexibility and/or stiffness and overall health. Findings may also inform interventional efforts to reverse probable loss of spinal stability and function.

3.2 Materials and Methods

3.2.1 Specimen Preparation

Ovine lumbar motion segments (L2L3, L4L5) were previously harvested with surrounding muscular and ligamentous tissues as well as bony processes, such as transverse processes, removed. The harvested MSU's were wrapped in saline-soaked

gauze, and frozen (-20°C) until testing. The cross-sectional areas of the intervertebral discs were previously estimated by measuring disc long and short axes dimensions in order to define an applied force required to generate target loading pressures (Appendix A). Prior to testing, specimens were allowed to thaw overnight. Once thawed, superior and inferior vertebral bodies were potted in a custom-made fixture using Boswell Fastray Dental Cement (Bosworth Company, Skokie, IL) so that the potted specimen could easily be attached to the various components of the pure moment testing apparatus. The entire fixture was positioned in an 858 Mini Bionix II material testing system (MTS Systems Corporation, Eden Prairie, MN).

Ten ovine MSU's were used for testing with "adverse" loading conditions, five with backward bending extensional interventions and five without. The L2L3 and L4L5 segments exposed to "adverse" loading with or without backward bending motions were intermixed so that differences between those with or without interventional extensions could be studied independent of vertebral level.

3.2.2 Mechanical Testing

Prior to testing, each MSU underwent cyclic compression loading (0.05-0.25 MPa, 50 cycles at 1 Hz) to resolve any postmortem super-hydration effects [McMillan et al. 1996]. All ovine lumbar MSU's received: a relatively high "adverse" compressive loading regimen of 0.75 MPa (between 200 N and 400 N applied force), elevated from physiological resting levels; and multiple short-term, high-load exertion "challenge" loads of 2.0 MPa (between 500 N and 1000 N applied force) every 30 minutes. As previously described, the 0.75 MPa creep loads were intended to replicate *in vivo* forces during every day loading activities [Sato et al. 1999, Wilke et al. 1999, Claus et al.

2008], and transient 2 MPa load exertions were intended to replicate high force challenges such as heavy lifting, pushing or pulling, or other strenuous activities of daily life. The challenge loads provided an opportunity to conduct a parallel investigation, not reported in this present study, measuring intradiscal pressure and axial strain. The MSU's were divided into two groups to either receive or not receive: a backwards extension "intervention" of a non-damaging 4 Nm moment (80 N applied force) for 30 seconds prior to each "challenge" load. To minimize dehydration, the MSU's were sprayed with phosphate-buffered saline (Mediatech, Manassas, VA) during the entire loading protocol.

Prior to the initiation of the first 30 minute "adverse" loading period, and after each "challenge" load, the spinal ROM was measured by applying a ±2.5 Nm moment (50 N applied force) over 10 seconds in flexion/extension and axial rotation directions with no preload. Unfortunately, due to the parallel investigation of intradiscal pressure with a delicate pressure sensor placed laterally in each intervertebral disc, lateral bending was neither applied nor investigated. Data was gathered on the third cycle of testing in each direction to reduce the effects of the viscoelastic response.

During flexion/extension ROM testing (Figure 15), the midline that divides the specimen into equal medial-lateral halves was aligned to the custom-built loading "ring," the cable and pulley system, and the hydraulic multi-axial load cell actuator arm, using the spinous processes as a guide. The line that divides the vertebral body from the posterior vertebral arch of the inferior vertebrae of the specimen was aligned to the hydraulic actuator arm. The pulley positions were adjusted at the beginning of each ROM test to achieve co-linearity of the cables extending from the loading "ring."

Changing positions of the pulleys and directions of the cable loop allowed for loading in flexion and extension. The custom design of the loading "ring" mechanism attached to the superior vertebra of the specimen ensured that the applied torque would transmit to the specimen, negating the effects of minor misalignments. The baseplate sliding mechanism attached to the inferior vertebra of the specimen ensured that the specimen was free to move in a more natural path while bending.

During axial rotation ROM testing (Figure 16), both midlines that divide the specimen into equal medial-lateral halves and divide the vertebral body from the posterior vertebral arch of the inferior vertebra of the specimen were aligned to the hydraulic actuator arm. The superior vertebra of the specimen was attached to the "hex joint," and the inferior vertebra of the specimen was again attached to the baseplate sliding mechanism, ensuring more nature physiological movement while rotating.

Axial force, torsional moment, and torsional displacement were recorded by the MTS system at a frequency of 50 Hz. 3D kinematics of L2 and L3 or L4 and L5 were collected at 50 Hz for the full duration of each ROM test using an optical camera system (Vicon MXF40, Vicon Motion Systems Ltd., Oxford, UK). Arrays of four reflective markers mounted on lexan plates were affixed to each vertebra (Figure 18) using k-wires. The Vicon camera system utilizes four viewing angles (four separate cameras surrounding the testing area) to determine global positions of the reflective markers, and those positions were used to calculate relative 3D angular rotational displacements between vertebral by a custom MATLAB code (Appendix B).

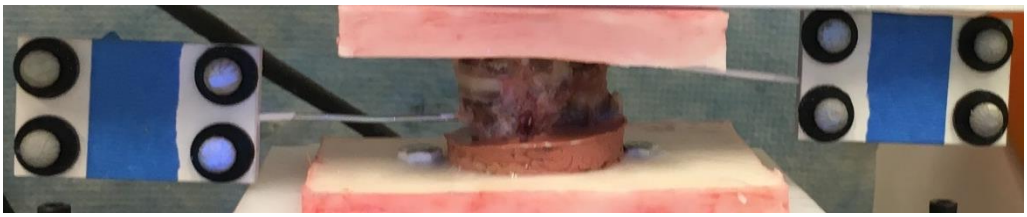


Figure 18: Reflective Marker Orientation and Attachment to Each Vertebra.

3.2.3 Three-Dimensional Motion Capture

To understand how the ROM data was acquired, the method with which 3D-coordinate reflective marker data is transformed into relative rigid body rotations must be explained. Six independent measurements—translations, rotations, or a combination of both—are required to fully describe the 3D motion of a rigid body.

The position of a body in space is defined by the position of three non-collinear points of that body, or its rigid extension [Panjabi et al. 1981]. Real-time measurement systems may be used to record the positional movements of a rigid body in space. The MATLAB code uses the global coordinate positions of at least three reflective markers for the rigid extensions of each vertebral body to determine a local coordinate system. Although only three markers are required to define the position of a rigid body, four were used to maintain a rigid body coordinate signal if any one of the four marker signals is interrupted.

The most common technique for calculating ROM rotation angles from marker coordinate data is the Euler method, in which any change in rigid body orientation (neglecting overall translation) is characterized by an equivalent sequence of three rotations, one about each fixed axis of the global coordinate system [Crawford et al. 1996]. That is, the reorientation of a rigid body, such as a vertebral body, from its initial

position described by i, j, k to its final position described by i', j', k', may be determined by calculating first the rotation Rx about the fixed x-axis followed by the rotation Ry about the fixed y-axis followed by the rotation Rz about the fixed z-axis (Rx → Ry → Rz). These angles Rx, Ry, and Rz are often called Euler angles [Panjabi et al. 1981, Panjabi et al. 1993, Oxland et al. 1992]. The rotation matrix R to transform any points from initial to final orientation is defined as:

$$R = Rot(x, Rx) \cdot Rot(y, Ry) \cdot Rot(z, Rx)$$

In this equation, *Rot (axis, angle)* represents the rotation matrix which is applied to rotate points by an *angle* about its corresponding *axis*. The columns of the matrix R are the vectors i', j', and k', respectively, as indicated in the following set of three Euler rotation matrices:

$$Rot(x, Rx) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & cos\, Rx & -sin\, Rx \\ 0 & sin\, Rx & cos\, Rx \end{bmatrix}$$

$$Rot(y, Ry) = \begin{bmatrix} cos\, Ry & 0 & sin\, Ry \\ 0 & 1 & 0 \\ -sin\, Ry & 0 & cos\, Ry \end{bmatrix}$$

$$Rot(z, Rz) = \begin{bmatrix} cos\, Rz & -sin\, Rz & 0 \\ sin\, Rz & cos\, Rz & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

When studying rotational motions of the spine limited to a single plane, as in this study, the selection of a particular rotation sequence is of little significance. Thus the sequence used matched the arbitrary sequence (Rx → Ry → Rz) commonly used in spinal biomechanics research [Crawford et al. 1996].

3.2.4 Data Analysis

Flexibility in each direction, flexion, extension, and axial rotation, was demonstrated by the load-displacement diagrams, and ROM was quantified by the magnitude of angular displacement [Panjabi et al. 1976]. Stiffness of the NZ was approximated as the inverse of the slope in the range of the NZ—the zone between the points of the largest changes in flexibility in the moment-angular displacement curve [Smit et al. 2009].

Post-challenge loading ROM measurements were normalized against respective baseline ROM values. Each specimen acted as its own control in order to reduce the effects of inter-specimen variability. Statistical differences between and within groups with and without the interventional backwards bending (extension) motion were assessed using single factor ANOVA, for which p-values less than 0.05 were accepted as significant.

3.3 Results

Results were obtained from the *ex vivo* biomechanical flexibility testing performed in repeated-cycle fashion, in each direction, on each of the ten MSU's throughout the entire "adverse" loading protocol at the following time points: (1) baseline, (2) post-challenge #1, (3) post-challenge #2, and (4) post-challenge #3. Load-displacement data collected during the third and last loading cycle were analyzed to determine the ROM for each specimen in each condition. This method provides sufficient time for the influence of viscoelasticity in the spinal tissues to wear off,

allowing the spine to reach near its final angular displacement during the last loading cycle [Wilke et al. 1998, Lee 2006]. The results quantified the effects of prolonged "adverse" loading on the ROM of ovine lumbar specimens.

3.3.1 ROM Flexibility in Extension

Flexion/extension (FE) flexibility curves for each specimen are included in Appendix C (Figures 32-33). The stiffness of the NZ can be generally described as high, as shown by the small slopes of these flexibility curves. This indicates apparent stability of the NZ in the extension direction. ROM results for each specimen in extension are included in Appendix D (Tables 3-4). Each specimen contains a ROM value in degrees, measured at baseline and after each challenge load. Percentages of change in ROM after each challenge load, as compared to baseline ROM, were calculated. Although exact values for NZ stiffness were particularly difficult to calculate given the FE flexibility curves, estimated NZ stiffness results for each specimen in extension are included in Appendix F (Tables 12-13). Each specimen contains an NZ stiffness value in Nm/degree, measured at baseline and after each challenge load. Percentages of change in NZ stiffness after each challenge load, as compared to baseline NZ stiffness, were calculated.

As shown in Figure 19, the mean L2-L5 ROM in the extension direction for ±2.5 Nm of applied moments within specimens without interventional extension movements were: 2.13° ± 0.95° at baseline, 2.17° ± 0.68° after the first challenge load, 1.91° ± 1.13° after the second challenge load, and 1.73° ± 0.71° after the third challenge load. No statistically significant differences between ROM after any post-challenge

load and baseline ROM were found in specimens without interventional extension movements (Appendix E Table 9). As shown in Figure 20, the mean percentages of change in ROM from baseline were: 15.09% after the first challenge load (S.D. ±49.28%), -0.66% after the second challenge load (S.D. ±55.35%), and -13.83% after the third challenge load (S.D. ±31.91%). As shown in Figure 19, mean L2-L5 ROM in the extension direction for ±2.5 Nm of applied moments within specimens with interventional extension movements were: 2.09° ± 0.82° at baseline, 1.90° ± 1.05° after the first challenge load, 1.70° ± 0.94° after the second challenge load, and 1.64° ± 0.62° after the third challenge load. Again, no statistically significant differences between ROM after any post-challenge load and baseline ROM were found in specimens with interventional extension movements (Appendix E Table 10). As shown in Figure 20, the mean percentages of change in ROM from baseline were: -9.88% after the first challenge load (S.D. ±24.52%), -22.71% after the second challenge load (S.D. ±19.50%), and -18.78% after the third challenge load (S.D. ±31.28%). The percentages of change in ROM results yielded no statistically significant differences between specimens with and without interventional movements (Appendix E Table 11).

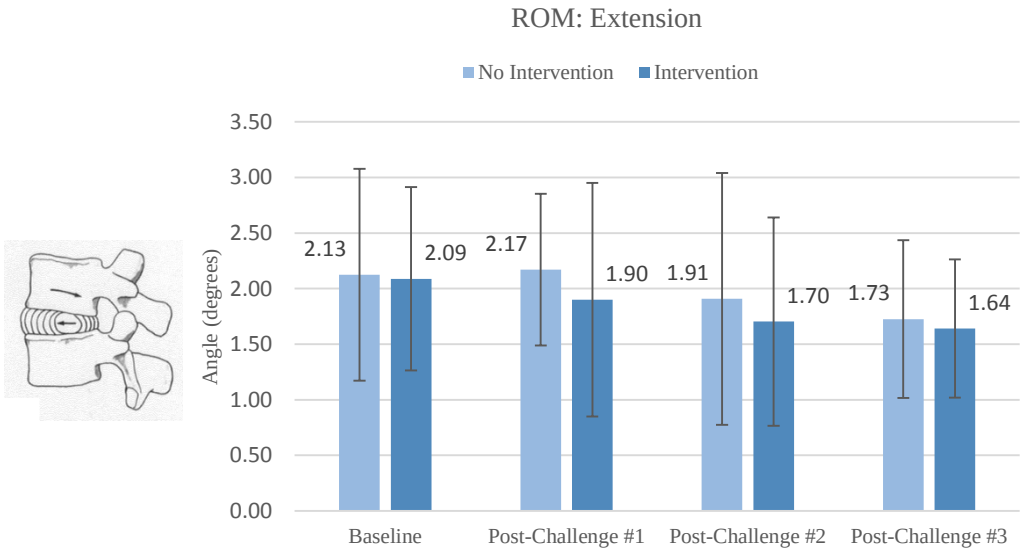


Figure 19: Extension ROM at Baseline and after each Challenge Load.
Mean angular displacement ROM values labeled, standard deviations marked with lines.

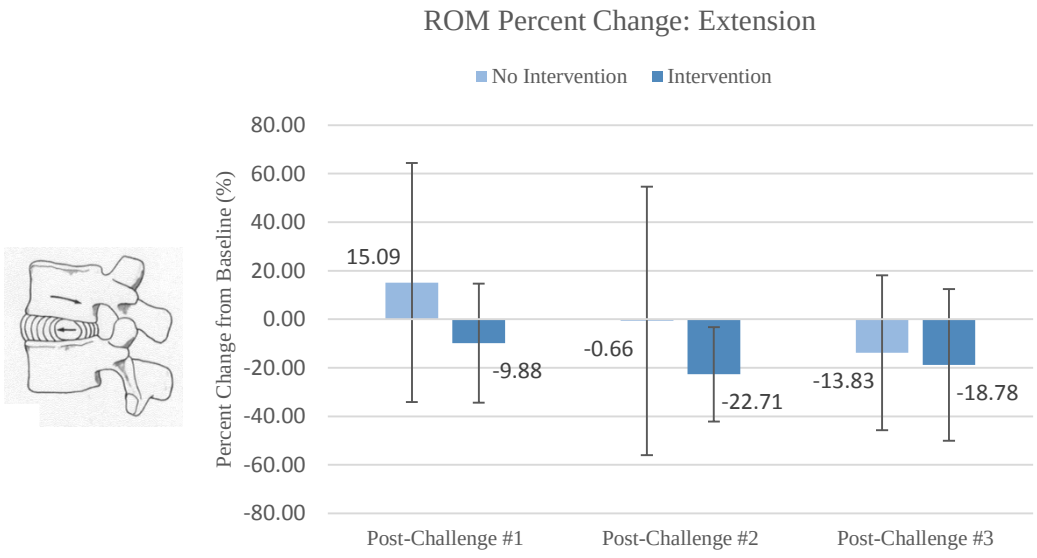
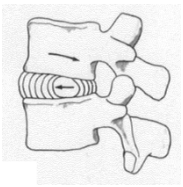


Figure 20: Percent Change (%) between Baseline ROM and each Post-Challenge ROM for Extension. Mean percent change in ROM values labeled, standard deviations marked with lines.

As shown in Figure 21, the mean L2-L5 NZ stiffness in the extension direction for ±2.5 Nm of applied moments within specimens without interventional extension movements were: 1.52 Nm/° ± 0.56 Nm/° at baseline, 1.48 Nm/° ± 0.54 Nm/° after the first challenge load, 1.91 Nm/° ± 1.03 Nm/° after the second challenge load, and 2.10 Nm/° ± 1.03 Nm/° after the third challenge load. No statistically significant differences between stiffness after any post-challenge load and baseline stiffness were found in specimens without interventional extension movements (Appendix G Table 18). As shown in Figure 22, the mean percentages of change in NZ stiffness from baseline were: 8.56% after the first challenge load (S.D. ±61.89%), 37.38% after the second challenge load (S.D. ±103.38%), and 40.87% after the third challenge load (S.D. ±56.37%). As shown in Figure 21, mean L2-L5 NZ stiffness in the extension direction for ±2.5 Nm of applied moments within specimens with interventional extension movements were: 1.58 Nm/° ± 0.73 Nm/° at baseline, 2.02 Nm/° ± 1.08 Nm/° after the first challenge load, 2.31 Nm/° ± 1.45 Nm/° after the second challenge load, and 2.12 Nm/° ± 1.35 Nm/° after the third challenge load. Again, no statistically significant differences between stiffness after any post-challenge load and baseline stiffness were found in specimens with interventional extension movements (Appendix G Table 19). As shown in Figure 22, the mean percentages of change in NZ stiffness from baseline were: 27.80% after the first challenge load (S.D. ±53.62%), 38.56% after the second challenge load (S.D. ±39.91%), and 38.79% after the third challenge load (S.D. ±57.48%). The percentages of change in NZ stiffness results yielded no statistically significant differences between specimens with and without interventional movements (Appendix G Table 20).



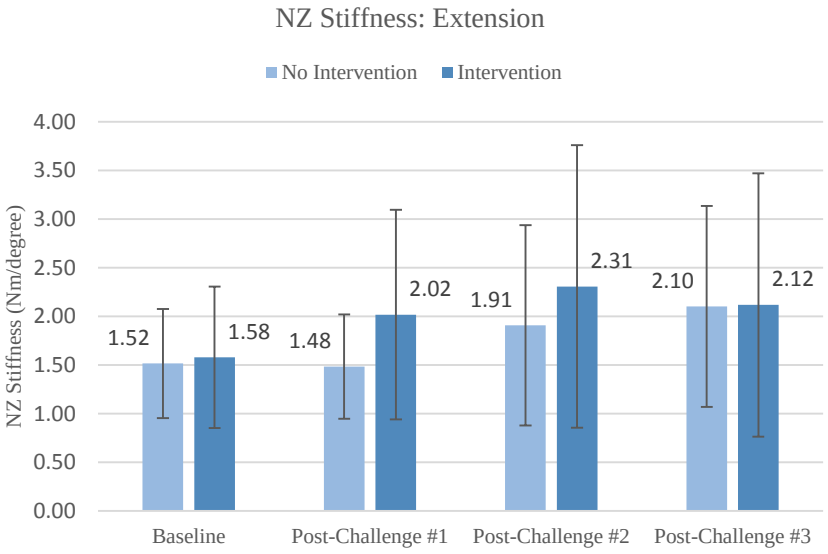
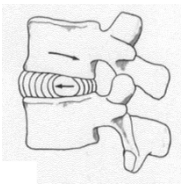


Figure 21: Extension NZ Stiffness at Baseline and after each Challenge Load. Mean stiffness values labeled, standard deviations marked with lines.



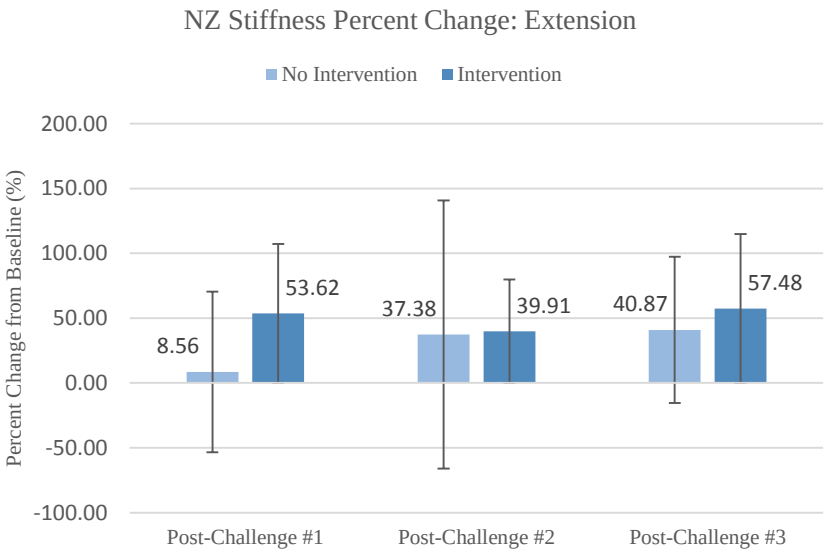


Figure 22: Percent Change (%) between Baseline NZ Stiffness and each Post-Challenge NZ Stiffness for Extension. Mean percent change in stiffness values labeled, standard deviations marked with lines.

3.3.2 ROM Flexibility in Flexion

Flexion/extension (FE) flexibility curves for each specimen are included in Appendix C (Figures 32-33). The stiffness of the NZ can be generally described as high, as shown by the small slopes of these flexibility curves. This indicates apparent stability of the NZ in the flexion direction. ROM results for each specimen in flexion are included in Appendix D (Tables 5-6). Each specimen contains a ROM value in degrees, measured at baseline and after each challenge load. Percentages of change in ROM after each challenge load, as compared to baseline ROM, were calculated. Although exact values for NZ stiffness were particularly difficult to calculate given the FE flexibility curves, estimated NZ stiffness results for each specimen in flexion are included in Appendix F (Tables 14-15). Each specimen contains an NZ stiffness value in Nm/degree, measured at baseline and after each challenge load. Percentages of change in NZ stiffness after each challenge load, as compared to baseline NZ stiffness, were calculated.

As shown in Figure 23, mean L2-L5 ROM in the flexion direction for ±2.5 Nm of applied moments within specimens without interventional extension movements were: 2.56° ± 0.55° at baseline, 3.83° ± 1.57° after the first challenge load, 3.80° ± 1.10° after the second challenge load, and 4.86 ° ± 1.94° after the third challenge load. The only statistically significant difference between ROM after any post-challenge load and baseline ROM was found after the final challenge (#3) in specimens without interventional extension movements (Appendix E Table 9). As shown in Figure 24, the mean percentages of change in ROM from baseline were: 45.49% after the first challenge load (S.D. ±27.81%), 47.43% after the second challenge load (S.D.

±20.41%), and 85.59% after the third challenge load (S.D. ±46.70%). As shown in Figure 23, mean L2-L5 ROM in the flexion direction for ±2.5 Nm of applied moments within specimens with interventional extension movements were: 2.86° ± 0.63° at baseline, 3.83° ± 1.42° after the first challenge load, 4.30° ± 1.75° after the second challenge load, and 4.44° ± 1.06° after the third challenge load. Again, the only statistically significant difference between ROM after any post-challenge load and baseline ROM was found after the final challenge (#3) in specimens without interventional extension movements (Appendix E Table 10). As shown in Figure 24, the mean percentages of change in ROM from baseline were: 30.81% after the first challenge load (S.D. ±20.78%), 46.21% after the second challenge load (S.D. ±32.26%), and 55.90% after the third challenge load (S.D. ±20.46%). The percentages of change in ROM results yielded no statistically significant differences between specimens with and without interventional movements (Appendix E Table 11).

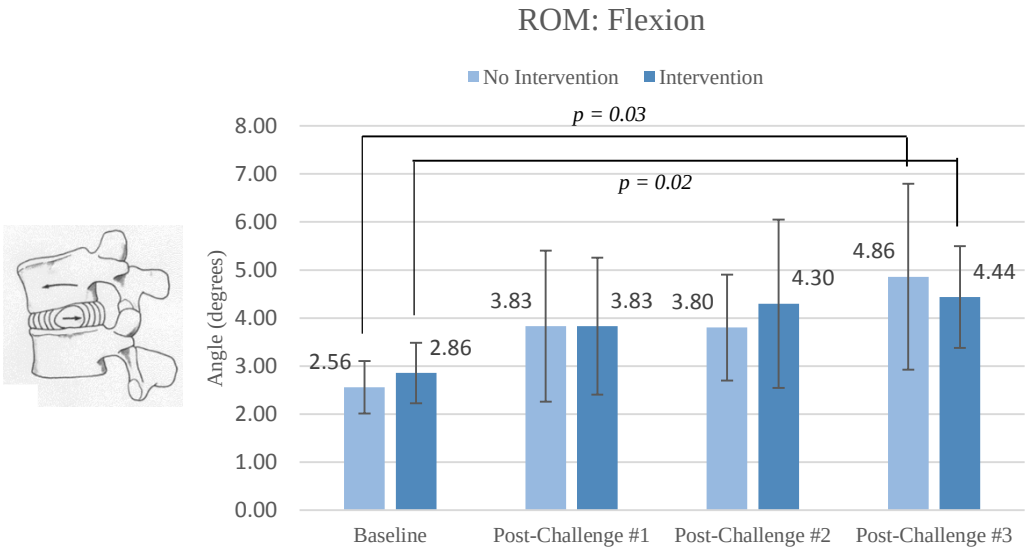
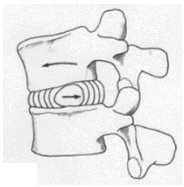


Figure 23: Flexion ROM at Baseline and after each Challenge Load.
Mean angular displacement ROM values labeled, standard deviations marked with lines.
Statistically significant p-values shown.



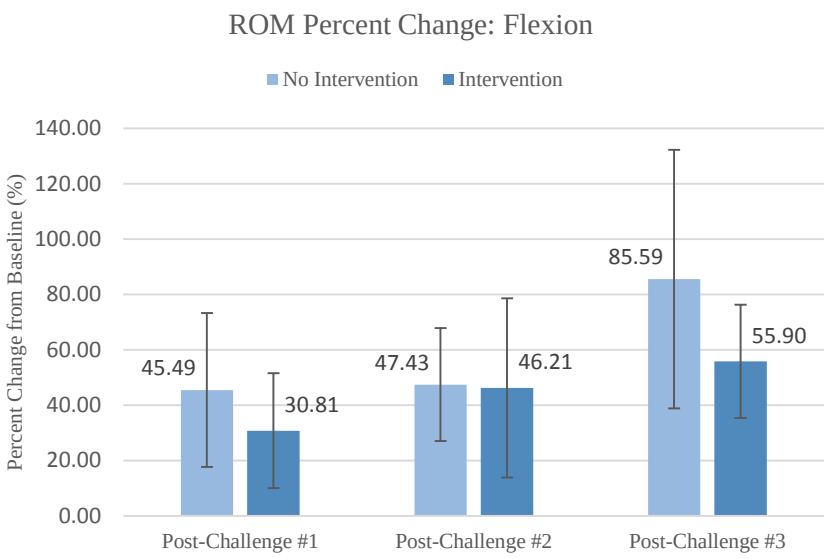


Figure 24: Percent Change (%) between Baseline ROM and each Post-Challenge ROM for Flexion. Mean percent change in ROM values labeled, standard deviations marked with lines.

As shown in Figure 25, the mean L2-L5 NZ stiffness in the flexion direction for ±2.5 Nm of applied moments within specimens without interventional extension movements were: 1.20 Nm/° ± 0.27 Nm/° at baseline, 0.80 Nm/° ± 0.34 Nm/° after the first challenge load, 0.79 Nm/° ± 0.26 Nm/° after the second challenge load, and 0.64 Nm/° ± 0.34 Nm/° after the third challenge load. Statistically significant differences between NZ stiffness after the second and third post-challenge loads (#2 and #3) and baseline NZ stiffness was found in specimens without interventional extension movements (Appendix G Table 18). As shown in Figure 26, the mean percentages of change in NZ stiffness from baseline were: -34.23% after the first challenge load (S.D. ±24.28%), -34.52% after the second challenge load (S.D. ±18.74%), and -46.88% after the third challenge load (S.D. ±27.79%). As shown in Figure 25, mean L2-L5 NZ stiffness in the flexion direction for ±2.5 Nm of applied moments within specimens

with interventional extension movements were: 0.93 Nm/° ± 0.21 Nm/° at baseline, 0.75 Nm/° ± 0.30 Nm/° after the first challenge load, 0.68 Nm/° ± 0.24 Nm/° after the second challenge load, and 0.58 Nm/° ± 0.12 Nm/° after the third challenge load. The only statistically significant difference between NZ stiffness after any post-challenge load and baseline NZ stiffness was found after the final challenge (#3) in specimens with interventional extension movements (Appendix G Table 19). As shown in Figure 26, the mean percentages of change in NZ stiffness from baseline were: -20.93% after the first challenge load (S.D. ±17.93%), -28.49% after the second challenge load (S.D. ±12.88%), and -36.60% after the third challenge load (S.D. ±8.34%). The percentages of change in NZ stiffness results yielded no statistically significant differences between specimens with and without interventional movements (Appendix G Table 20).

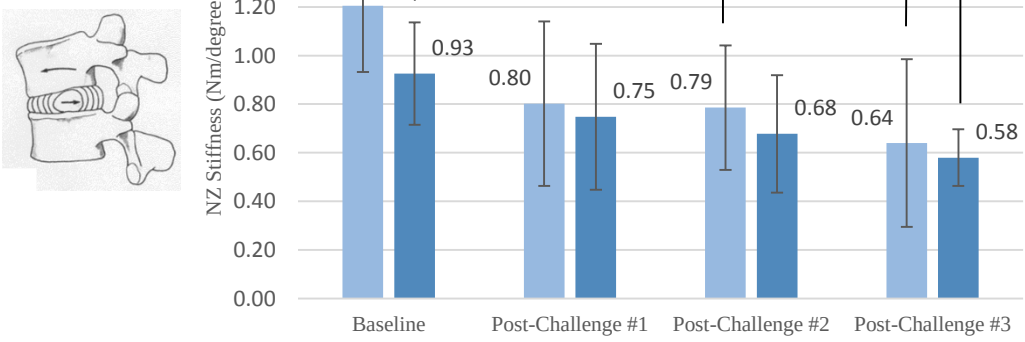


Figure 25: Flexion NZ Stiffness at Baseline and after each Challenge Load.
Mean stiffness values labeled, standard deviations marked with lines.
Statistically significant p-values shown.

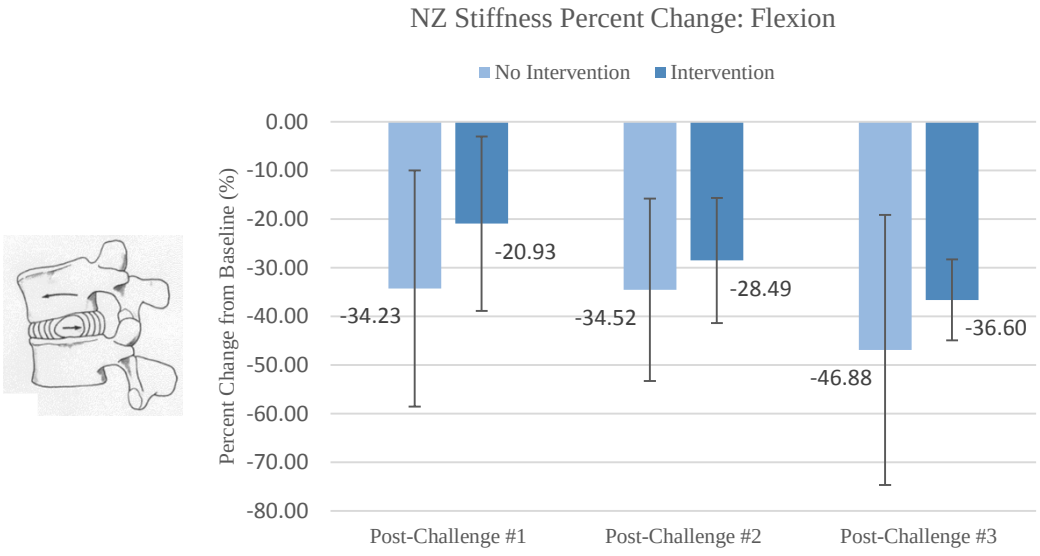


Figure 26: Percent Change (%) between Baseline NZ Stiffness and each Post-Challenge NZ Stiffness for Flexion. Mean percent change in stiffness values labeled, standard deviations marked with lines.

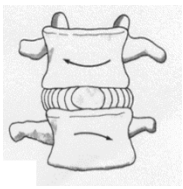
3.3.3 ROM Flexibility in Axial Rotation

Axial rotation (AR) flexibility curves for each specimen are included in Appendix C (Figures 34-35). The stiffness of the NZ can be generally described as low, as shown by the large slopes of these flexibility curves. This indicates apparent instability of the NZ in the axial rotation direction. ROM results for each specimen in axial rotation are included in Appendix D (Tables 7-8). Each specimen contains a ROM value in degrees, measured at baseline and after each challenge load. Percentages of change in ROM after each challenge load, as compared to baseline ROM, were calculated. Estimated NZ stiffness results for each specimen in axial rotation are included in Appendix F (Tables 16-17). Each specimen contains an NZ stiffness value in Nm/degree, measured at baseline and after each challenge load. Percentages of

change in NZ stiffness after each challenge load, as compared to baseline NZ stiffness, were calculated.

As shown in Figure 27, mean L2-L5 ROM in the axial rotation direction for ±2.5 Nm of applied moments within specimens without interventional extension movements were: 2.14° ± 0.65° at baseline, 2.12° ± 0.42° after the first challenge load, 2.91° ± 0.70° after the second challenge load, and 2.90° ± 0.39° after the third challenge load. No statistically significant differences between ROM after any post-challenge load and baseline ROM were found in specimens without interventional extension movements (Appendix E Table 9). As shown in Figure 28, the mean percentages of change in ROM from baseline were: 7.50% after the first challenge load (S.D. ±44.77%), 40.96% after the second challenge load (S.D. ±32.00%), and 45.34% after the third challenge load (S.D. ±47.06%). As shown in Figure 27, mean L2-L5 ROM in the axial rotation direction for ±2.5 Nm of applied moments within specimens with interventional extension movements were: 2.49° ± 1.19° at baseline, 2.66° ± 0.45° after the first challenge load, 2.93° ± 0.35° after the second challenge load, and 3.37 ° ± 0.90° after the third challenge load. Again, no statistically significant differences between ROM after any post-challenge load and baseline ROM were found in specimens with interventional extension movements (Appendix E Table 10). As shown in Figure 28, the mean percentages of change in ROM from baseline were: 43.00% after the first challenge load (S.D. ±105.01%), 69.50% after the second challenge load (S.D. ±146.15%), and 106.84% after the third challenge load (S.D. ±214.13%). The percentages of change in ROM results yielded no statistically significant differences

between specimens with and without interventional movements (Appendix E Table 11).



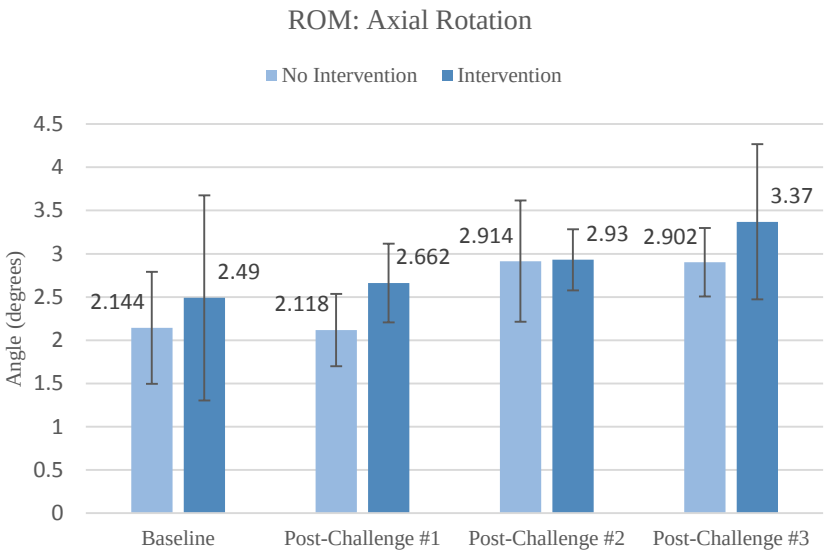
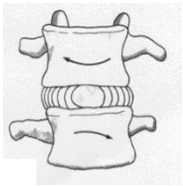


Figure 27: Axial Rotation ROM at Baseline and after each Challenge Load.
Mean angular displacement ROM values labeled, standard deviations marked with lines.



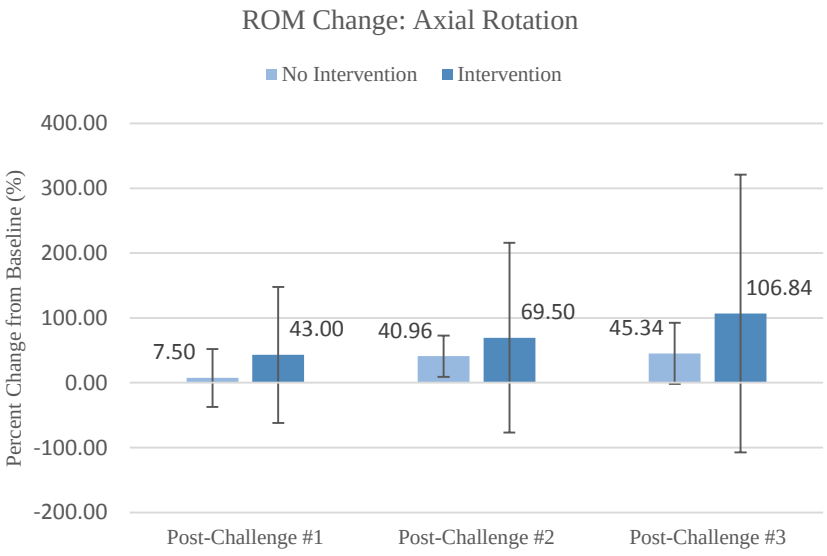
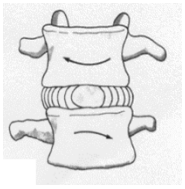


Figure 28: Percent Change (%) between Baseline ROM and each Post-Challenge ROM for Axial Rotation. Mean percent change in ROM values labeled, standard deviations marked with lines.

As shown in Figure 29, the mean L2-L5 NZ stiffness in the axial rotation direction for ±2.5 Nm of applied moments within specimens without interventional extension movements were: 0.06 Nm/° ± 0.04 Nm/° at baseline, 0.04 Nm/° ± 0.03 Nm/° after the first challenge load, 0.05 Nm/° ± 0.02 Nm/° after the second challenge load, and 0.08 Nm/° ± 0.06 Nm/° after the third challenge load. No statistically significant differences between stiffness after any post-challenge load and baseline stiffness were found in specimens without interventional extension movements (Appendix G Table 18). As shown in Figure 30, the mean percentages of change in NZ stiffness from baseline were: -19.72% after the first challenge load (S.D. ±63.07%), 1.77% after the second challenge load (S.D. ±57.31%), and 31.49% after the third challenge load (S.D. ±42.53%). As shown in Figure 29, mean L2-L5 NZ stiffness in the flexion direction for ±2.5 Nm of applied moments within specimens with interventional extension movements were: 0.06 Nm/° ± 0.05 Nm/° at baseline, 0.08 Nm/° ± 0.03 Nm/° after the first challenge load, 0.06 Nm/° ± 0.01 Nm/° after the second challenge load, and 0.04 Nm/° ± 0.01 Nm/° after the third challenge load. Again, no statistically significant differences between stiffness after any post-challenge load and baseline stiffness were found in specimens with interventional extension movements (Appendix G Table 19). As shown in Figure 30, the mean percentages of change in NZ stiffness from baseline were: -91.91% after the first challenge load (S.D. ±2.48%), -93.65% after the second challenge load (S.D. ±0.89%), and -94.94% after the third challenge load (S.D. ±1.96%). The percentages of change in NZ stiffness results yielded statistically significant differences between specimens with and without interventional extensional movements after all challenge loads (Appendix G Table 20).



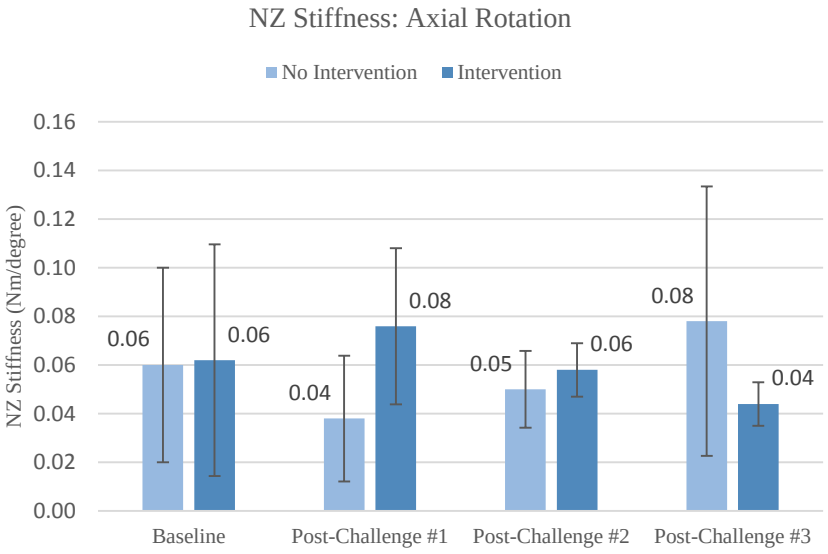
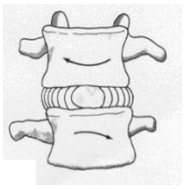


Figure 29: Axial Rotation NZ Stiffness at Baseline and after each Challenge Load. Mean stiffness values labeled, standard deviations marked with lines.



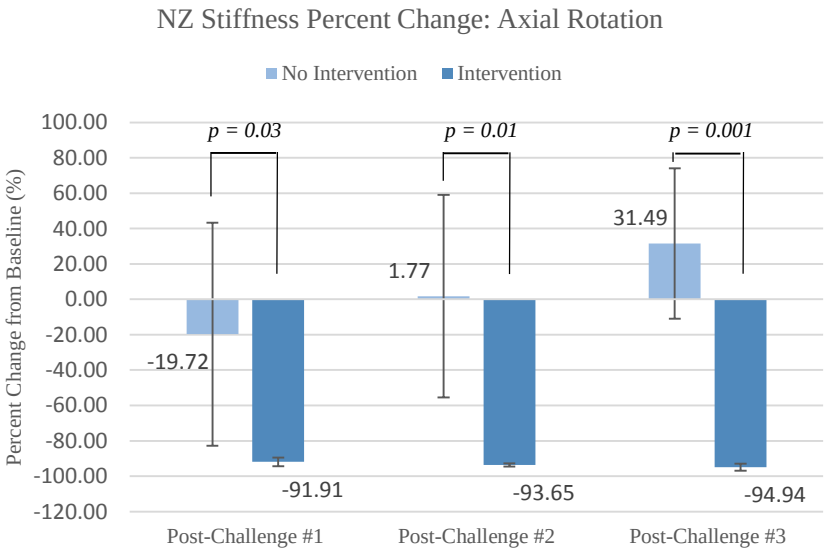


Figure 30: Percent Change (%) between Baseline NZ Stiffness and each Post-Challenge NZ Stiffness for Axial Rotation. Mean percent change in stiffness values labeled, standard deviations marked with lines. Statistically significant p-values shown.

3.4 Discussion

The objective of this *ex vivo* ovine model biomechanical study was to quantify and assess the ROM and NZ stiffness achieved after prolonged "adverse" compressive loading conditions, with and without intermittent extension or backwards bending movements. The current study found no significant differences between the change in ROM in any direction with and without interventional extension movement. The study found significant differences between the change in estimated NZ stiffness with and with and without interventional extension movement in only the axial rotation direction. However, this difference did not demonstrate protective effects of the interventional extension against spinal instability, i.e. decreased NZ stiffness. These variable results may be due to the variability in bone density and tissue parameters within different spinal MSU specimens—specifically the degree of spinal ligament stripping. This difficulty with a large variability over spinal specimens has previously been reported [Twomey and Taylor 1982, Wilke et al. 1998, Busscher et al. 2011]. Due to the limited availability of ovine specimens and timeframe of completing the present study, a small sample size was used.

Interesting trends over the course of the "adverse" loading protocol were observed. A slight decrease in ROM in the extension direction and complementary increase in NZ stiffness was observed over the course of the entire loading protocol in specimens with and without interventional extension movements. An apparent increase in ROM in the flexion direction and complementary decrease in NZ stiffness was observed over the course of the entire loading protocol in specimens with and without interventional extension movements. An apparent increase in ROM in the axial rotation

direction was observed over the course of the entire loading protocol in specimens with and without interventional extension movements. However, the NZ stiffness results in the axial rotation direction were not consistent with ROM data—a decrease in the NZ stiffness was observed over the course of the loading protocol in specimens with interventional extension movements, but an initial decrease and subsequent increase in NZ stiffness was observed over the course of the loading protocol in specimens without interventional extension movements.

The percentages of change in ROM and NZ stiffness may have a more significant impact on the larger relative effect of the "adverse" creep loading. However, these outcomes are difficult to compare to literature due to the fact that there have been few studies, most of which have used human cadaveric specimens, investigating ROM after prolonged creep loading—often applied in the direction of ROM testing, not in axial compression alone.

A larger ROM, and complementary reduced NZ stiffness, indicate that the spinal MSU's became more flexible and potentially more unstable after prolonged "adverse" loading, increasing the necessity for muscles to compensate [Panjabi 1992, Oxland and Panjabi 1992, Cholewicki et al. 1997]. Prolonged creep loading has been shown to induce fluid loss in the NP; slack collagenous fibers causing circumferential clefts within the AF; and laxity in the ligaments, facet joint capsules, and intervertebral discs—this laxity results in fewer reflexive stabilizing forces provided by surrounding musculature [Agroubni and Shirazi-Adl 1996, Solomonow et al. 1999]. It is obvious that in the present *ex vivo* study, surrounding spinal ligament and muscular stabilizing

forces are not present and therefore results should be carefully extrapolated and interpreted for the ovine model *in vivo* situation.

Results of the current study should be interpreted taking the study protocol into account. Different studies use highly different protocols, and recommendations for biomechanical testing of spinal segments are variable [Crawford 2010, Busscher et al. 2011]. The recommendations of previous studies were followed for preparation of the segments, test environment, and loading conditions [Crawford et al. 1995, Wilke et al. 1998, Lee 2006, Eguizabal et al. 2010, Tang et al. 2012]. Instantaneous loading with a non-damaging applied moment of ±2.5 Nm was recommended for ROM testing in ovine spinal segments [Lee 2006]. A pilot study in our test setup showed damage or potting slippage of ovine MSU's when loaded to more than 5 Nm in the cyclic ROM tests. Thus ±2.5 Nm appeared to be an appropriate magnitude of applied moment for ROM evaluation, and 4 Nm for 30 seconds appeared to be an appropriate applied moment for interventional extension testing without potting or specimen damage.

Axial compressive forces placing 0.75 MPa of "adverse" loading pressure and 2.0 MPa of "challenge" loading pressure on each MSU were chosen to replicate *in vivo* pressures placed on the lumbar spine during every day loading activities such as sitting or standing and transient exertional activities such as heavy lifting, pushing or pulling, etc. [Sato et al. 1999, Wilke et al. 1999, Claus et al. 2008, Goodley 2014]. Most studies describing biomechanical behavior apply a constant static or cyclic load between 15 and 20 minutes, or up to 30 minutes for older specimens [Kaigle et al. 1992, McGill and Brown 1992, Little and Khalsa 2005, Van der Veen et al. 2006]. As the present study uses older ovine MSU specimens stored for an extended amount of time,

"adverse" compressive loading was applied for 30 minutes prior to each "challenge" load and subsequent ROM testing.

Furthermore, compressive loads, in a pure axial direction or a more natural "follower" load path, as well as bending loads each have a different influence on the fluid loss of the NP, biomechanics of the intervertebral disc, and therefore motion behavior of spinal segments [Wilke et al. 1998, Patwardhan et al. 2003, Stanley et al. 2004, Tawackoli et al 2004]. Bending creep most likely results in little fluid loss but substantial viscoelastic strain of the fibers of the AF, but compression creep results in substantial fluid loss [Busscher et al. 2011]. In the present study, compression was applied in a purely axial direction due to the manufactured fixtures used for attachment of the MSU's to the ROM testing apparatus and MTS system. Thus load magnitude, creep type, and of loading time, and length of time during which specimens were placed under interventional extension backward bending conditions are important considerations when interpreting the results of the current study, especially in comparison to previous investigations.

3.4.1 Adverse Load History Decreases ROM and Increases NZ Stiffness in Extension

Although the current study found no significant differences between the change in ROM with and without interventional extension movement, interesting ROM trends in the extension direction over the course of the "adverse" loading protocol were observed. Without intervention, ROM in the extension direction initially increased after the first challenge load but then decreased after the second and third challenge loads, until the ROM decreased by approximately 13.8% compared to baseline ROM. With

intervention, ROM in the extension direction decreased after all challenge loads, until the ROM decreased by approximately 18.8% compared to baseline ROM. Although NZ stiffness was extremely difficult to quantitatively determine from the varying load-deformation flexibility curves, similar trends in NZ stiffness were observed. Without intervention, NZ in the extension direction increased over the course of the "adverse" loading protocol, until the NZ stiffness increased by approximately 41% after the third challenge load compared to baseline NZ stiffness. With intervention, NZ in the extension direction increased after all challenge loads, until the NZ stiffness increased by approximately 39% compared to baseline NZ stiffness. However, no statistically significant differences of ROM or NZ stiffness between baseline and challenge loads were observed, both between and within groups of specimens with and without interventional extension movements.

Contrary to our hypothesis, these results may indicate increasing spinal MSU stability in the extension direction after "adverse" compressive loading. The facet joints, which progressively absorb more loads during extension, may contribute to this stability. This effect may be compounded by reduced NP hydration and increased laxity of the AF ligaments, causing additional load sharing with the facet joints. The superior articular processes of the inferior vertebral body exert considerable frictional forces upon the superior vertebral body of the MSU, increasing resistance to motion in the extension direction, leading to reduced flexibility. Additionally, the interventional backwards extension movement does seem to provide a protective effect of increasing spinal stability in extension under "adverse" loading conditions.

3.4.2 Adverse Load History Increases ROM and Decreases NZ Stiffness in Flexion

Although the current study found no significant differences between the change in ROM with and without interventional extension movement, interesting ROM trends in the flexion direction over the course of the "adverse" loading protocol were observed. Without intervention, ROM in the flexion direction increased after all challenge loads, until the ROM increased by approximately 85.6% compared to baseline ROM. With intervention, ROM in the flexion direction increased after all challenge loads, until the ROM also increased by approximately 55.9% compared to baseline ROM. Statistically significant increases in ROM were observed between baseline and the third challenge load, in specimens with and without interventional extension movements. Although NZ stiffness was extremely difficult to quantitatively determine from the varying load-deformation flexibility curves, similar trends in NZ stiffness were observed. Without intervention, NZ in the flexion direction decreased over the course of the "adverse" loading protocol, until the NZ stiffness decreased by approximately 47% after the third challenge load compared to baseline NZ stiffness. With intervention, NZ in the flexion direction decreased after all challenge loads, until the NZ stiffness decreased by approximately 37% compared to baseline NZ stiffness. Statistically significant increases in NZ stiffness were observed between baseline and the second and third challenge loads, in specimens without interventional extension movements, and between baseline and the third challenge load, in specimens with interventional extension movements. However, no statistically significant differences

of change in ROM or NZ stiffness between groups of specimens with and without interventional extension movements were observed.

The fact that ROM is greater in flexion than extension is in agreement with previously-reported literature [Pearcy et al. 1984]. In agreement with our hypothesis, these results may indicate increasing spinal MSU instability in the flexion direction after "adverse" compressive loading. Reduced NP hydration as well as increased stress concentrations and laxity in the AF ligaments and any other remaining spinal ligaments may contribute to this instability during flexion. The previously removed musculature; ligaments, especially the ALL, PLL, and spinous ligaments; and bony processes during specimen harvesting, (all of which would which normally contribute to resisting excessive flexion movements) may have contributed to this instability. Additionally, the interventional backwards bending extension movement does seem to provide a protective effect against decreasing spinal stability in flexion under "adverse" loading conditions.

3.4.3 Adverse Load History Increases ROM and Decreases NZ Stiffness in Axial Rotation

Although the current study found no significant differences between the change in ROM with and without interventional extension movement, interesting ROM trends in the axial rotation directions over the course of the "adverse" loading protocol were observed. Without intervention, ROM in the axial rotation direction increased after all challenge loads, until the ROM increased by approximately 45.3% compared to baseline ROM. With intervention, ROM in the axial rotational direction also increased after all challenge loads, until the ROM increased by approximately 106.8% compared

to baseline ROM. The particularly large angular deformations in the NZ across most specimens and loading conditions indicate large spinal MSU instability in axial rotation. Without intervention, results with relatively large errors were found—NZ in the axial rotation direction initially decreased after the first challenge load but subsequently increased over the course of the "adverse" loading protocol, until the NZ stiffness increased by approximately 31% after the third challenge load compared to baseline NZ stiffness. With intervention, NZ in the axial rotation direction decreased after all challenge loads, until the NZ stiffness decreased by approximately 95% compared to baseline NZ stiffness. However, no statistically significant differences of ROM or NZ stiffness between baseline and challenge loads were observed within groups of specimens with and without interventional extension movements. Finally, although no statistically significant differences of change in ROM between groups of specimen with and without interventional extension movements were observed, statistically significant differences of change in NZ stiffness between groups were observed.

In agreement with our hypothesis, these results may indicate increasing spinal MSU instability in the axial direction after "adverse" compressive loading. The previously removed musculature, ligaments, and bony processes during specimen harvesting, as well as the dehydration and increased laxity of the AF fibers (all of which would which normally contribute to resisting excessive rotational movements) may have contributed to this high degree of instability. Additionally, the interventional backwards bending extension movement may not provide a protective effect against decreasing spinal stability in axial rotation under "adverse" loading conditions.

3.4.4 Limitations

The flexibility testing protocol was successfully implemented to quantitatively assess the motion of lumbar MSU's. The 3D motion capture system proved to be reliable in capturing and quantifying the kinematics of the spine in real-time. Although the flexibility method allowed each ROM test to be repeatable with consistency, variations in the features of each specimen such as age, bone density, and size of vertebral bodies required it to be handled individually and with care. In fact, the small size of ovine vertebral bodies necessitated the potting of specimen in a custom-made fixture with particular attention, making sure to keep the intervertebral disc level (parallel to the ground in its horizontal axis) and to cover enough surface area on each vertebral body with enough bone cement to maintain stability during ROM testing, reducing the possibility of specimen detaching from the bone cement.

One particular limitation that is important to note is the challenge of attaching the lexan plates with reflective motion tracking markers to each vertebral body in such a way as to maintain constant signals to each camera. Because each specimen used in the current study had been used in a previous study, in which a hole was drilled horizontally into the superior vertebral body of each MSU, maintaining rigid attachment of the lexan plates containing motion tracking markers to that vertebral body via k-wire insertion was difficult—occasionally the real-time marker coordinate data collected from the motion tracking cameras showed unintentional movement after each loading cycle in one direction of spinal ROM. The reflective nature of the motion tracking markers also led to additional challenges when collecting data. Because the cameras are sensitive to any and all reflective surfaces within their capture volume,

particular care in spray painting components of the testing apparatus was taken. Additional surfaces found to be reflective during testing were covered with tape, if and when possible. When those additional reflections were unable to be covered, they often interfered with the motion tracking marker signals, causing one or more signals to fluctuate and/or drop out during part or all of a single trial in one ROM flexion/extension direction. As stated previously, due to the difficulty in measuring axial rotation with the reflective markers, the MTS system itself calculated degree of angular deformation in the ROM axial rotation direction. All limitations of the motion tracking system combined caused difficulty in generating the flexibility curves of each ROM test and therefore explicit calculation of MSU stiffness and complete determination of stability after prolonged "adverse" loading conditions.

The biomechanical ROM testing apparatus itself was shown to perform repeatable measurements but has many limitations. An inherent difference in the applied moment and the moment measured by the multi-axial load cell is always present, due to (1) the proportional-integral-derivative (PID) mechanism of the MTS system, and (2) an additional bending moment caused by the use of the "hex joint" in axial rotation or the shifted center of mass typical of pure moment "ring" testing mechanisms [Tang et al. 2012] in flexion/extension. Although pilot testing with ovine MSU's was conducted to optimize PID values for both axial force and torsional moment application, the use of the PID algorithm does not guarantee perfect control or stability of the MTS system.

Although the artifact moments caused by the shifting center of mass while testing flexion/extension ROM with the "ring" mechanism was partially alleviated

through the use of linear sliders and vertical bearings, the most important limitation in applying pure moments is maintaining parallelism of the loading cable ends by applying tension prior to each ROM test, as described previously. The loading cable may not have remained co-linear throughout the ROM test, as the MTS system was programmed to control vertical motion of the cable loop to apply and release bending moments. Artifact moments may also have been induced while testing axial rotation ROM with the use of the "hex joint," as it allows multidirectional movement as well as rotation, similar to a manufactured ball-and-socket joint. Additionally, the slight variability in potting of each specimen, which may have caused off-center or misaligned attachment of both the "ring" mechanism and "hex joint," most likely contributed to artifact, or non-pure, bending moments. Thus, pure ROM testing was not 100% successful throughout the entirety of this study.

Other limitations of the current study are related to the small sample size of ovine MSU specimens as well as the nature of the *ex vivo* ovine model testing. It is important to note that the 4 Nm backwards bending interventional extension moment was applied for just 30 seconds—this may not have been enough time to allow for rehydration of the inner NP and outer AF fibers, which may have prevented statistically significant differences between specimens with and without interventional extension movements from occurring. Additional influences that affect ROM and stiffness results include: age of the specimens; degree of stripping of surrounding motion-facilitating and motion-limiting musculature, tendons, ligaments, and bony structures; and residual hydration effects due to storing specimen in a freezer for an extended period of time. Furthermore, although the present study only tested single spinal units, multi-level

segments have been shown to be more representative of the normal physiological conditions [Adams 1995, Dickey and Kerr 2003, Goel et al. 2006].

As with all *ex vivo* studies, a final limitation of the current study is the non-physiological testing environment. Although the tested MSU's were repeatedly sprayed with saline throughout testing, the test environment and hydration status of the spinal segment is known to influence the biomechanical ROM characteristics [Pflaster et al. 1997, Race et al. 2000]. Thus the interpretation of the results should also carry the understanding that the current study was limited to using *ex vivo* specimens, specifically without surrounding tissues i.e. spinal-column stabilizing musculature, which have been stored for a long period of time and have been used in previous compressive loading experiments.

3.5 Significance

Load history influences the fluid-related biomechanics of the intervertebral disc and spinal ROM. High compressive "adverse" loads limit fluid recovery and pressure regeneration of the NP of the intervertebral disc, thereby limiting the ability of the AF and ligaments to provide MSU stabilization and prevent excessive motion. This higher ROM flexibility of the MSU's may suggest that creep deformation occurs during prolonged compressive loading. This indicates that surrounding musculature provides compensating stabilizing forces to reduce the risk of injury or low-back problems.

Chapter 4: Conclusions

4.1 Summary

Sheep lumbar motion segments are validated and often used to assess spinal biomechanical properties translatable to the human lumbar spine [Wilke et al. 1997, Smit et al. 2002]. The results of the current study provide an additional understanding of the dynamics of long-term compressive loading in ovine intervertebral disc mechanics and spinal ROM:

(1) High compressive "adverse" loads seem to cause an increase in ROM flexibility and complementary decrease in NZ stiffness and therefore increase in spinal instability in both flexion and axial rotation directions. This may be due to the limited fluid recovery of the intervertebral disc pressure regeneration of the NP, which induces laxity into the AF fibers and limits the surrounding spinal ligaments to prevent excessive bending and/or rotational motion. However, compensating forces of the facet joints seem to cause a decrease in ROM flexibility and complementary increase in NZ stiffness and therefore increase in spinal stability in the extension direction.

(2) Interventional backwards bending or extension movements may provide a protective but non-significant effect against the increased spinal instability in flexion/extension.

These results address the hypothesis that high "adverse" compressive loading conditions cause changes in spinal ROM and NZ stiffness, which may in turn be affected by interventional backwards bending motion.

These findings also indicate and suggest confirmation of the theory that surrounding musculature provides compensating stabilizing forces to reduce the risk of injury or low-back problems [Busscher et al. 2011] in everyday life. Body weight in a seated or standing position, occupational demands, household chores, and leisure-time physical activity behaviors often play roles in spinal instability and back pain. Further interpretation may therefore provide human physiological insight into load-induced biomechanical changes to guide and advance clinical investigations into the treatment of low back pain and loss of lumbar spinal function in patients.

4.2 Future Directions

The present investigation was performed as part of longitudinal study aiming to reveal the effects of load history on spinal ROM and intervertebral disc biomechanics and identify postural interventions effective at reversing spinal instability and critical loss of biomechanical function. The testing apparatus manufactured for use in the current study provides a method to apply physical therapy-type movements on MSU's loaded in the MTS system. Conclusions of the current study indicate that extension or backwards bending movements may act to mitigate the instability effects of prolonged static "adverse" loading on spinal biomechanics. Other physical therapy-type motions such as flexion, lateral bending, axial rotation, traction, or some combination may be investigated in future studies. A longer period of time to apply interventional postures is also encouraged. Regular completion of such interventional, non-invasive practices may improve long-term functional health by mitigating or reversing the damaging effects of "adverse" loading.

Additional manipulation of the testing fixtures used in conjunction with the MTS system may be performed to further elucidate the effects of various loading profiles, such as cyclic loading or creep loading in a specific bending directions, on specific tissue response. Different loading conditions may have different effects on spinal biomechanics, especially depending upon prior loading history.

The exact influence of differences in geometry of the vertebrae, facet joints, and intervertebral discs should be investigated further. The influence of spinal implants, fusions, and other interventional devices on spinal ROM and NZ stiffness may also be investigated with the use of the biomechanical testing apparatus. Although the *ex vivo* testing conditions of these types of biomechanical studies require stripping of the surrounding tendon and musculature tissues, the harvesting of surrounding spinal ligaments and bony structures should be carefully performed in future studies. Furthermore, although most studies only test single MSU's, multi-level segments may be more representative of normal physiological situations.

Finally, the goals of future investigations must simultaneously include the growth of spinal biomechanical knowledge and as well as its integration into patient care therapies through lifestyle behaviors and physical medicine and rehabilitation practices. Meaningful findings and theories must be translated from bench-top to clinical practice, in order to limit the loss of spinal function with diseases prevalent worldwide, such as low back pain. The overarching aim of scientific research is to improve upon and advance patient care.

Appendix A: Specimen Intervertebral Disc Geometries

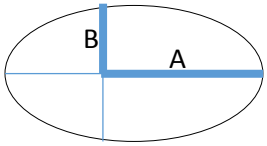


Figure 31: Two-Dimensional Representation of Intervertebral Disc. Segment A of long axis and segment B of short axis demonstrated by blue lines.

Table 2: Disc Geometry Calculations. To calculate the length of segment A of long axis and length of segment B of short axis, the long and short axes of each disc were measured 3 times, averaged, and divided in half. The area of each disc was calculated using the equation for the area of an ellipse: area = πAB. The axial compressive force to generate "adverse" and "challenge" loading conditions was calculated using the respective target pressures: F = P*area.

Specimen ID	Segment A of Long Axis (mm)	Segment B of Short Axis (mm)	Area of Disc (mm^2)	Force to Generate 0.75 MPa "Adverse" Load (N)	Force to Generate 2.0 MPa "Challenge" Load (N)
Sheep 5 L2L3	13.59	9.38	400.37	300.28	800.75
Sheep 5 L4L5	14.58	9.20	421.53	316.14	843.05
Sheep 6 L4L5	13.73	10.00	431.13	323.24	862.25
Sheep 8 L4L5	15.08	10.38	491.68	368.76	983.35
Sheep 7 L2L3	11.01	8.15	281.97	211.47	563.93
Sheep 7 L4L5	12.45	8.54	334.06	250.55	668.13
Sheep 9 L2L3	13.20	10.33	428.21	321.16	856.43
Sheep 10 L2L3	13.96	11.89	521.58	391.18	1043.16
Sheep 11 L2L3	13.33	10.55	441.64	331.23	883.28
Sheep 11 L4L5	14.16	10.40	462.63	346.97	925.26

Appendix B: MATLAB Code

The following MATLAB code and function returns relative rotational angles between vertebral bodies, used to calculate absolute angular displacement between the superior vertebral body relative to the inferior vertebral body:

```
 % load global positions of reflective markers
load data

 % calculate unit vectors of lower vertebral body to set up
orthogonal axes
l1x = data(:,1:3) - data(:,4:6);
l1y = data(:,7:9) - data(:,4:6);
l1x = l1x./(sqrt(sum(l1x.^2,2))*ones(1,3));
l1y = l1y./(sqrt(sum(l1y.^2,2))*ones(1,3));
l1z = cross(l1x,l1y);
l1z = l1z./(sqrt(sum(l1z.^2,2))*ones(1,3));
l1y = cross(l1z,l1x);
l1y = l1y./(sqrt(sum(l1y.^2,2))*ones(1,3));

% position matrix for lower vertebral body
RotGtol1(1,:,:) = l1x';
RotGtol1(2,:,:) = l1y';
RotGtol1(3,:,:) = l1z';

 % calculate unit vectors of upper vertebral body to set up
orthogonal axes
l2x = -data(:,10:12) + data(:,13:15);
l2y = data(:,16:18) - data(:,10:12);
l2x = l2x./(sqrt(sum(l2x.^2,2))*ones(1,3));
l2y = l2y./(sqrt(sum(l2y.^2,2))*ones(1,3));
l2z = cross(l2x,l2y);
l2z = l2z./(sqrt(sum(l2z.^2,2))*ones(1,3));
l2y = cross(l2z,l2x);
l2y = l2y./(sqrt(sum(l2y.^2,2))*ones(1,3));

% position matrix for upper vertebral body
RotGtol2(1,:,:) = l2x';
RotGtol2(2,:,:) = l2y';
RotGtol2(3,:,:) = l2z';

for i = 1:size(data,1)
    % calculate rotation matrix
    rot = RotGtol1(:,:,i)*RotGtol2(:,:,i)';
    % calculate rotation angles
    [rx ry rz]= GetEulerAngles(rot);
    ang(i,:) = [rx ry rz].*180./pi;
end
```

```
xlswrite('results.xls',ang);


function [rx ry rz]= GetEulerAngles(R)

% returns the rotation along x, y and z direction from a Rotation
Matrix

%Inputs:
    % R= 3x3 Rotation Matrix
%Outputs:
    % rx= Rotation along x direction in radians
    % ry= Rotation along y direction in radians
    % rz= Rotation along z direction in radians

%  R =
% [                            cos(ry)*cos(rz),
-cos(ry)*sin(rz),           sin(ry)]
% [ cos(rx)*sin(rz) + cos(rz)*sin(rx)*sin(ry), cos(rx)*cos(rz) -
sin(rx)*sin(ry)*sin(rz), -cos(ry)*sin(rx)]
% [ sin(rx)*sin(rz) - cos(rx)*cos(rz)*sin(ry), cos(rz)*sin(rx) +
cos(rx)*sin(ry)*sin(rz),  cos(rx)*cos(ry)]

% Author : Sandeep Sasidharan
% http://sandeepsasidharan.webs.com

ry=asin(R(1,3));
rz=acos(R(1,1)/cos(ry));
rx=acos(R(3,3)/cos(ry));
```

Appendix C: ROM Flexibility Curves

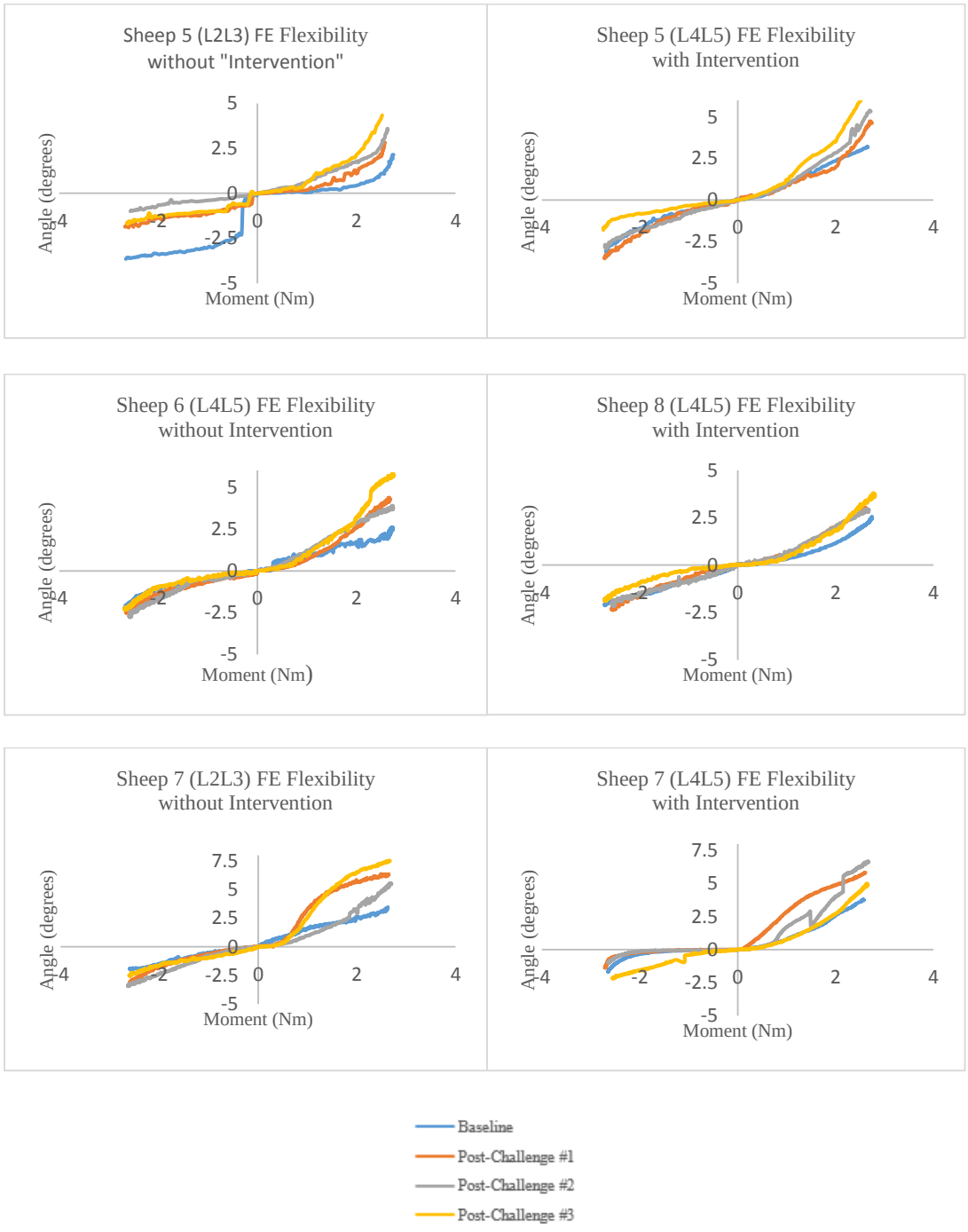


Figure 20: Flexibility curves during the last cycle of testing in flexion/extension (FE).

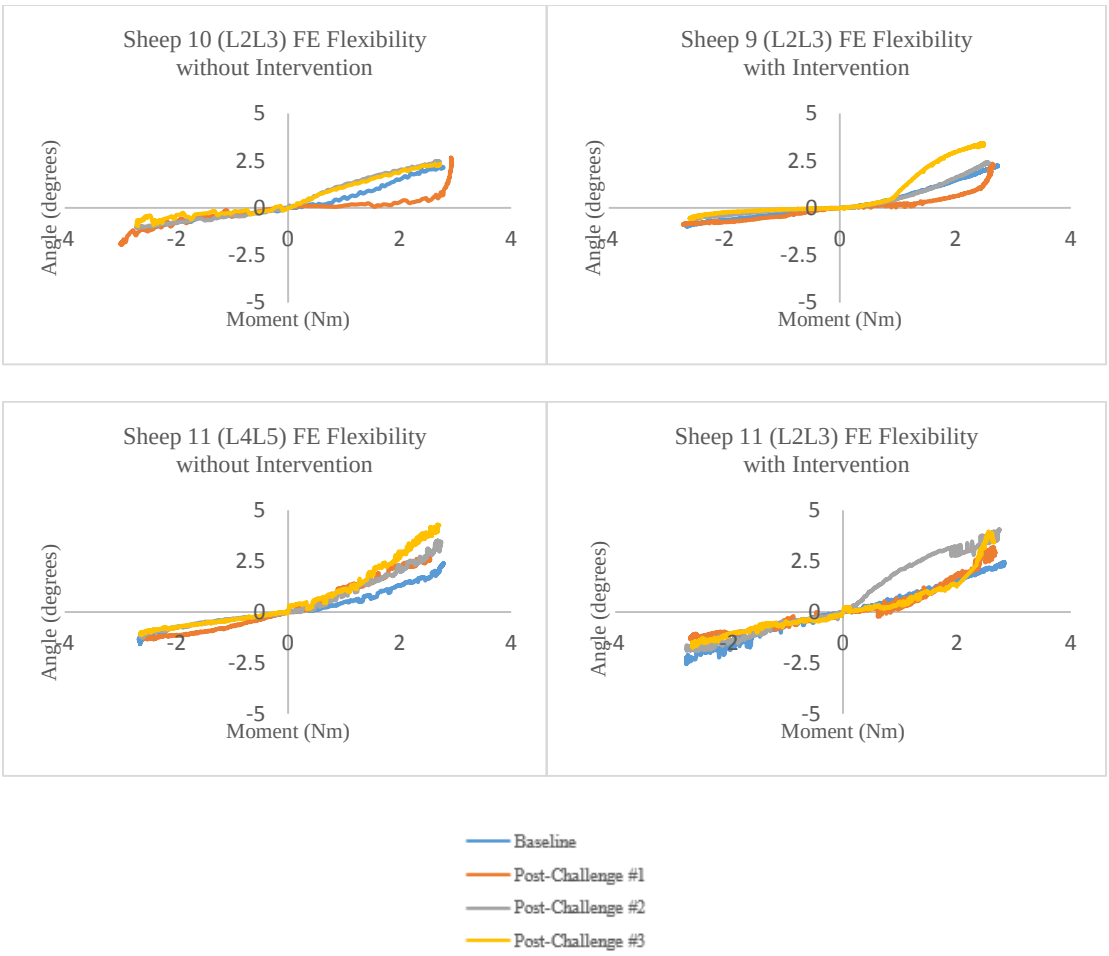


Figure 21: Flexibility curves during the last cycle of testing in flexion/extension (FE) contd.

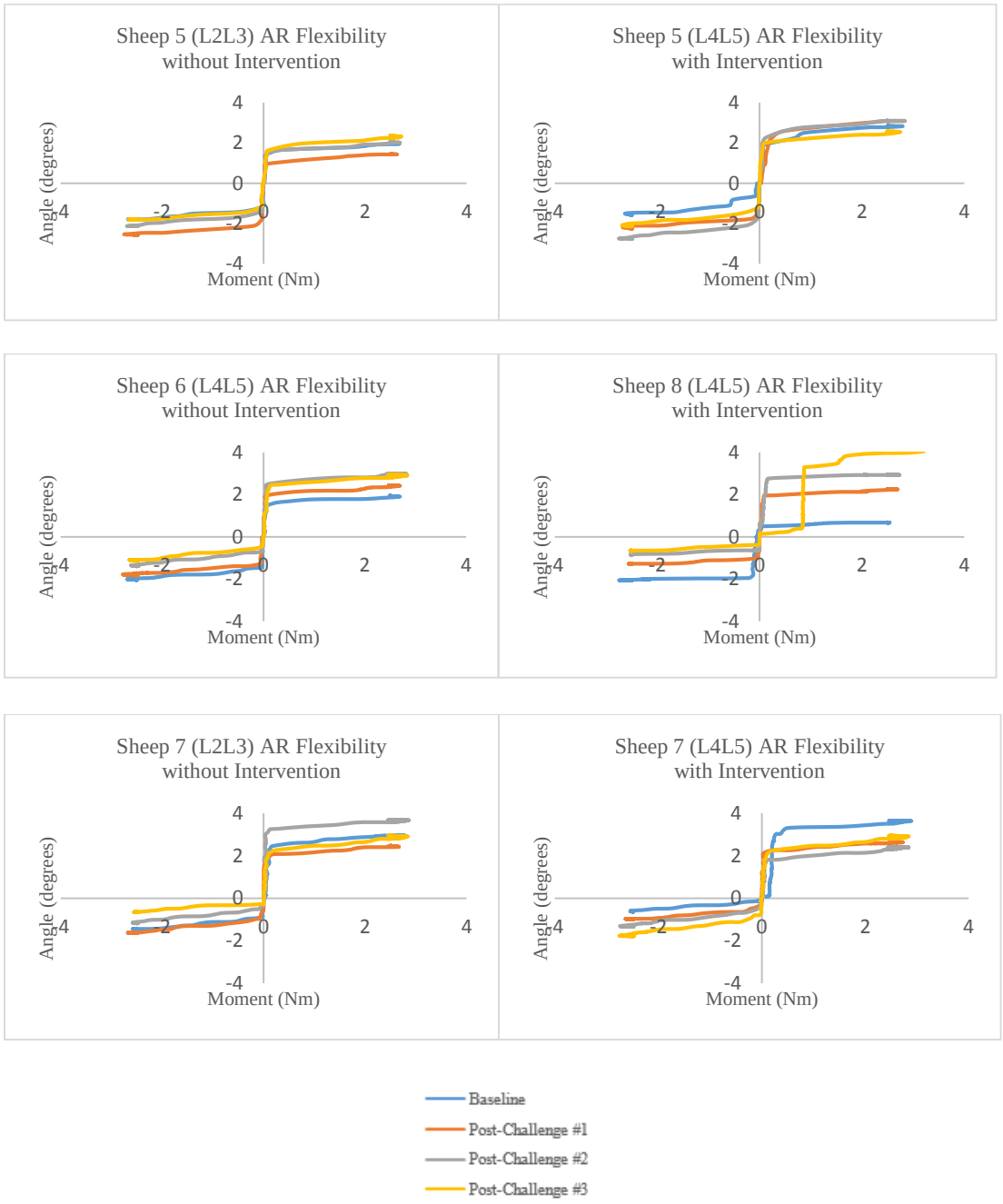


Figure 22: Flexibility curves during the last cycle of testing in axial rotation (AR).

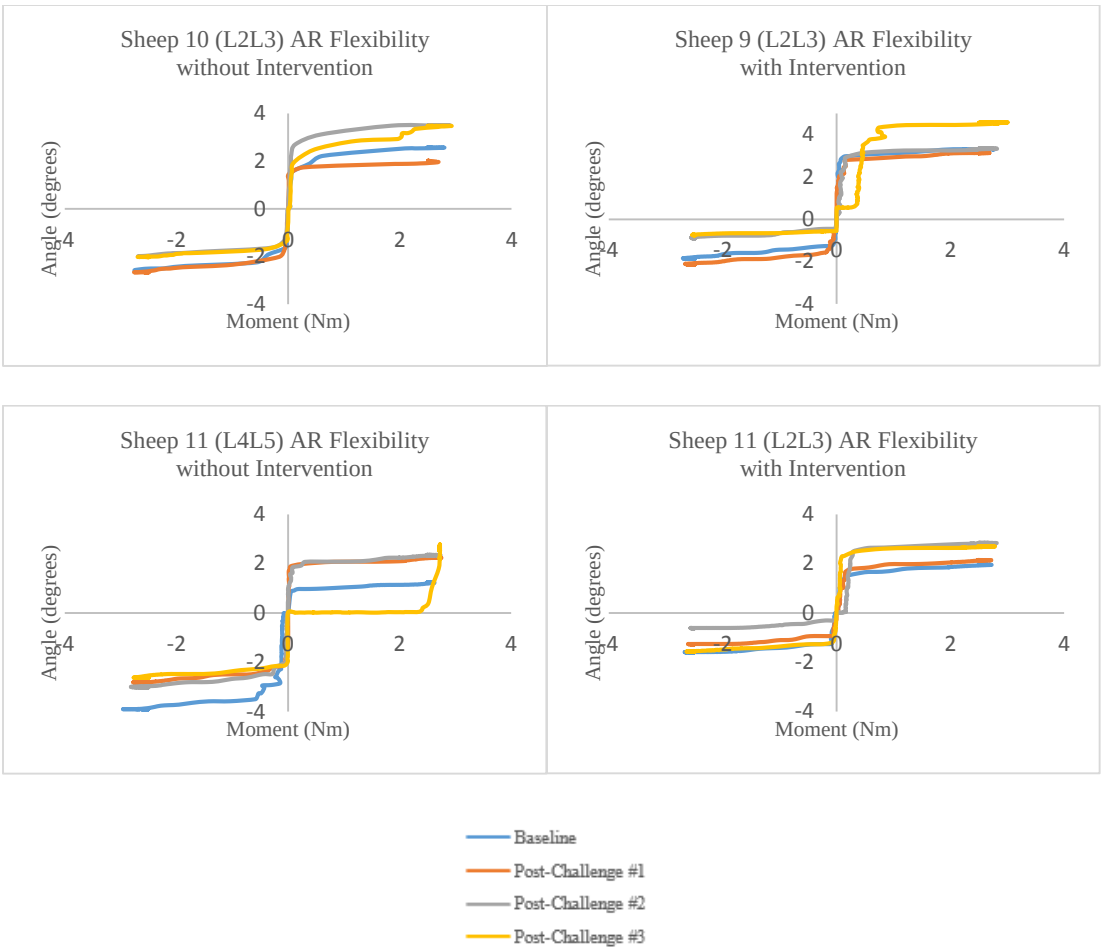


Figure 35: Flexibility curves during the last cycle of testing in axial rotation (AR) contd.

Appendix D: ROM Data

Table 3: ROM for each specimen in extension, without intervention. Absolute degree of angular deformation (ROM), percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (degrees)	Post-Challenge #1 (degrees)	Post-Challenge #2 (degrees)	Post-Challenge #3 (degrees)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L2L3	3.66	1.91	1.00	1.70	-47.81	-72.68	-53.55
Sheep 6 L4L5	2.28	2.52	2.77	2.32	10.53	21.49	1.75
Sheep 7 L2L3	1.95	3.13	3.46	2.55	60.51	77.44	30.77
Sheep 10 L2L3	1.17	1.96	1.07	0.95	67.52	-8.55	-18.80
Sheep 11 L4L5	1.57	1.33	1.24	1.11	-15.29	-21.02	-29.30
Mean	2.13	2.17	1.91	1.73	15.09	-0.66	-13.83
Std. Dev.	0.95	0.68	1.13	0.71	49.28	55.35	31.91

Table 4: ROM for each specimen in extension, with intervention. Absolute degree of angular deformation (ROM), percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (degrees)	Post-Challenge #1 (degrees)	Post-Challenge #2 (degrees)	Post-Challenge #3 (degrees)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L4L5	3.13	3.50	2.91	1.80	11.82	-7.03	-42.49
Sheep 8 L4L5	2.13	2.39	2.16	1.91	12.21	1.41	-10.33
Sheep 7 L4L5	1.65	1.39	0.98	2.18	-15.76	-40.61	32.12
Sheep 9 L2L3	0.98	0.88	0.57	0.57	-10.20	-41.84	-41.84
Sheep 11 L2L3	2.55	1.34	1.90	1.75	-47.45	-25.49	-31.37
Mean	2.09	1.90	1.70	1.64	-9.88	-22.71	-18.78
Std. Dev.	0.82	1.05	0.94	0.62	24.52	19.50	31.28

Table 5: ROM for each specimen in flexion, without intervention. Absolute degree of angular deformation (ROM), percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (degrees)	Post-Challenge #1 (degrees)	Post-Challenge #2 (degrees)	Post-Challenge #3 (degrees)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L2L3	2.14	2.82	3.60	4.35	31.78	68.22	103.27
Sheep 6 L4L5	2.61	4.37	3.89	5.78	67.43	49.04	121.46
Sheep 7 L2L3	3.48	6.36	5.53	7.54	82.76	58.91	116.67
Sheep 10 L2L3	2.17	2.66	2.48	2.33	22.58	14.29	7.37
Sheep 11 L4L5	2.40	2.95	3.52	4.30	22.92	46.67	79.17
Mean	2.56	3.83	3.80	4.86	45.49	47.43	85.59
Std. Dev.	0.55	1.57	1.10	1.94	27.81	20.41	46.70

Table 6: ROM for each specimen in flexion, with intervention. Absolute degree of angular deformation (ROM), percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (degrees)	Post-Challenge #1 (degrees)	Post-Challenge #2 (degrees)	Post-Challenge #3 (degrees)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L4L5	3.21	4.75	5.39	6.02	47.98	67.91	87.54
Sheep 8 L4L5	2.55	3.04	2.94	3.79	19.22	15.29	48.63
Sheep 7 L4L5	3.79	5.83	6.67	4.99	53.83	75.99	31.66
Sheep 9 L2L3	2.26	2.33	2.42	3.43	3.10	7.08	51.77
Sheep 11 L2L3	2.47	3.21	4.07	3.95	29.96	64.78	59.92
Mean	2.86	3.83	4.30	4.44	30.81	46.21	55.90
Std. Dev.	0.63	1.42	1.75	1.06	20.78	32.36	20.46

Table 7: ROM for each specimen in axial rotation, without intervention. Absolute degree of angular deformation (ROM), percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (degrees)	Post-Challenge #1 (degrees)	Post-Challenge #2 (degrees)	Post-Challenge #3 (degrees)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L2L3	2.00	1.44	2.06	2.38	-28.00	3.00	19.00
Sheep 6 L4L5	1.95	2.44	2.99	2.94	25.13	53.33	50.77
Sheep 7 L2L3	2.94	2.46	3.67	2.93	-16.33	24.83	-0.34
Sheep 10 L2L3	2.58	2.02	3.50	3.48	-21.71	35.66	34.88
Sheep 11 L4L5	1.25	2.23	2.35	2.78	78.40	88.00	122.40
Mean	2.14	2.12	2.91	2.90	7.50	40.96	45.34
Std. Dev.	0.65	0.42	0.70	0.39	44.77	32.00	47.06

Table 8: ROM for each specimen in axial rotation, with intervention. Absolute degree of angular deformation (ROM), percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (degrees)	Post-Challenge #1 (degrees)	Post-Challenge #2 (degrees)	Post-Challenge #3 (degrees)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L4L5	2.85	3.10	3.12	2.58	8.77	9.47	-9.47
Sheep 8 L4L5	0.69	2.27	2.94	4.05	228.99	326.09	486.96
Sheep 7 L4L5	3.63	2.66	2.40	2.93	-26.72	-33.88	-19.28
Sheep 9 L2L3	3.32	3.13	3.35	4.59	-5.72	0.90	38.25
Sheep 11 L2L3	1.96	2.15	2.84	2.70	9.69	44.90	37.76
Mean	2.49	2.66	2.93	3.37	43.00	69.50	106.84
Std. Dev.	1.19	0.45	0.35	0.90	105.01	146.15	214.13

Appendix E: ROM Statistical Analysis

Table 9: ANOVA Statistical Results Comparing each Post-Challenge ROM with Baseline ROM in Specimens without Intervention. P-values of mean post-challenge ROM compared to mean baseline ROM within group of specimens without interventional extension, in each biomechanical testing direction.

Testing Direction	Post-Challenge #1	Post-Challenge #2	Post-Challenge #3
Extension	0.94	0.75	0.47
Flexion	0.13	0.05	0.03
Axial Rotation	0.94	0.11	0.06

Table 10: ANOVA Statistical Results Comparing each Post-Challenge ROM with Baseline ROM in Specimens with Intervention. P-values of mean post-challenge ROM compared to mean baseline ROM within group of specimens with interventional extension, in each biomechanical testing direction.

Testing Direction	Post-Challenge #1	Post-Challenge #2	Post-Challenge #3
Extension	0.76	0.51	0.36
Flexion	0.20	0.12	0.02
Axial Rotation	0.77	0.45	0.22

Table 11: ANOVA Statistical Results Comparing Specimens with and without Intervention. P-values of percent change of post-challenge ROM compared to baseline ROM between group of specimens without interventional extension movement and group of specimens with interventional extension movement, in each biomechanical testing direction.

Testing Direction	Post-Challenge #1	Post-Challenge #2	Post-Challenge #3
Extension	0.34	0.43	0.81
Flexion	0.37	0.95	0.23
Axial Rotation	0.51	0.68	0.55

Appendix F: NZ Stiffness Data

Table 12: NZ Stiffness for each specimen in extension, without intervention. Estimated stiffness, percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (Nm/degree)	Post-Challenge #1 (Nm/degree)	Post-Challenge #2 (Nm/degree)	Post-Challenge #3 (Nm/degree)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L2L3	0.91	1.95	2.87	2.08	114.29	215.38	128.57
Sheep 6 L4L5	1.24	1.12	0.90	1.15	-9.68	-27.42	-7.26
Sheep 7 L2L3	1.24	0.81	0.72	1.15	-34.68	-41.94	-7.26
Sheep 10 L2L3	2.28	1.47	2.77	3.61	-35.53	21.49	58.33
Sheep 11 L4L5	1.91	2.07	2.28	2.52	8.38	19.37	31.94
Mean	1.52	1.48	1.91	2.10	8.56	37.38	40.87
Std. Dev.	0.56	0.54	1.03	1.03	61.89	103.38	56.37

Table 13: NZ Stiffness for each specimen in extension, with intervention. Estimated stiffness, percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (Nm/degree)	Post-Challenge #1 (Nm/degree)	Post-Challenge #2 (Nm/degree)	Post-Challenge #3 (Nm/degree)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L4L5	0.88	0.75	1.00	1.83	-14.77	13.64	107.95
Sheep 8 L4L5	1.36	1.05	1.32	1.40	-22.79	-2.94	2.94
Sheep 7 L4L5	1.80	2.64	3.60	1.09	46.67	100.00	-39.44
Sheep 9 L2L3	2.73	3.28	4.15	4.48	20.15	52.01	64.10
Sheep 11 L2L3	1.13	2.37	1.47	1.79	109.73	30.09	58.41
Mean	1.58	2.02	2.31	2.12	27.80	38.56	38.79
Std. Dev.	0.73	1.08	1.45	1.35	53.62	39.91	57.48

Table 14: NZ Stiffness for each specimen in flexion, without intervention. Estimated stiffness, percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (Nm/degree)	Post-Challenge #1 (Nm/degree)	Post-Challenge #2 (Nm/degree)	Post-Challenge #3 (Nm/degree)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L2L3	1.64	0.98	0.86	0.65	-40.24	-47.56	-60.37
Sheep 6 L4L5	1.14	0.55	0.68	0.41	-51.75	-40.35	-64.04
Sheep 7 L2L3	0.89	0.37	0.45	0.31	-58.43	-49.44	-65.17
Sheep 10 L2L3	1.19	1.21	1.15	1.20	1.68	-3.36	0.84
Sheep 11 L4L5	1.16	0.90	0.79	0.63	-22.41	-31.90	-45.69
Mean	1.20	0.80	0.79	0.64	-34.23	-34.52	-46.88
Std. Dev.	0.27	0.34	0.26	0.34	24.28	18.74	27.79

Table 15: NZ Stiffness for each specimen in flexion, with intervention. Estimated stiffness, percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (Nm/degree)	Post-Challenge #1 (Nm/degree)	Post-Challenge #2 (Nm/degree)	Post-Challenge #3 (Nm/degree)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L4L5	0.77	0.58	0.50	0.43	-24.68	-35.06	-44.16
Sheep 8 L4L5	0.97	0.78	0.82	0.65	-19.59	-15.46	-32.99
Sheep 7 L4L5	0.65	0.42	0.36	0.49	-35.38	-44.62	-24.62
Sheep 9 L2L3	1.12	1.22	0.95	0.62	8.93	-15.18	-44.64
Sheep 11 L2L3	1.12	0.74	0.76	0.71	-33.93	-32.14	-36.61
Mean	0.93	0.75	0.68	0.58	-20.93	-28.49	-36.60
Std. Dev.	0.21	0.30	0.24	0.12	17.93	12.88	8.34

Table 16: NZ Stiffness for each specimen in axial rotation, without intervention. Estimated stiffness, percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (Nm/degree)	Post-Challenge #1 (Nm/degree)	Post-Challenge #2 (Nm/degree)	Post-Challenge #3 (Nm/degree)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L2L3	0.03	0.04	0.06	0.05	33.33	100.00	66.67
Sheep 6 L4L5	0.05	0.03	0.04	0.09	-40.00	-20.00	80.00
Sheep 7 L2L3	0.05	0.08	0.05	0.04	60.00	0.00	-20.00
Sheep 10 L2L3	0.04	0.01	0.03	0.04	-75.00	-25.00	0.00
Sheep 11 L4L5	0.13	0.03	0.07	0.17	-76.92	-46.15	30.77
Mean	0.06	0.04	0.05	0.08	-19.72	1.77	31.49
Std. Dev.	0.04	0.03	0.02	0.06	63.07	57.31	42.53

Table 17: NZ Stiffness for each specimen in axial rotation, with intervention. Estimated stiffness, percent change between each post-challenge load and baseline ROM.

Specimen ID	Baseline (Nm/degree)	Post-Challenge #1 (Nm/degree)	Post-Challenge #2 (Nm/degree)	Post-Challenge #3 (Nm/degree)	Post-Challenge #1 vs. Baseline (% change)	Post-Challenge #2 vs. Baseline (% change)	Post-Challenge #3 vs. Baseline (% change)
Sheep 5 L4L5	0.05	0.08	0.06	0.05	-89.61	-92.21	-93.51
Sheep 8 L4L5	0.02	0.05	0.06	0.03	-94.85	-93.81	-96.91
Sheep 7 L4L5	0.14	0.04	0.04	0.05	-93.85	-93.85	-92.31
Sheep 9 L2L3	0.03	0.12	0.06	0.04	-89.29	-94.64	-96.43
Sheep 11 L2L3	0.07	0.09	0.07	0.05	-91.96	-93.75	-95.54
Mean	0.06	0.08	0.06	0.04	-91.91	-93.65	-94.94
Std. Dev.	0.05	0.03	0.01	0.01	2.48	0.89	1.96

Appendix G: NZ Stiffness Statistical Analysis

Table 18: ANOVA Statistical Results Comparing each Post-Challenge NZ Stiffness with Baseline NZ Stiffness in Specimens without Intervention. P-values of mean post-challenge NZ stiffness compared to mean baseline NZ stiffness within group of specimens without interventional extension, in each biomechanical testing direction.

Testing Direction	Post-Challenge #1	Post-Challenge #2	Post-Challenge #3
Extension	0.93	0.48	0.30
Flexion	0.07	0.04	0.02
Axial Rotation	0.33	0.62	0.57

Table 19: ANOVA Statistical Results Comparing each Post-Challenge NZ Stiffness with Baseline NZ Stiffness in Specimens with Intervention. P-values of mean post-challenge NZ stiffness compared to mean baseline NZ stiffness within group of specimens with interventional extension, in each biomechanical testing direction.

Testing Direction	Post-Challenge #1	Post-Challenge #2	Post-Challenge #3
Extension	0.47	0.36	0.46
Flexion	0.31	0.12	0.01
Axial Rotation	0.60	0.86	0.43

Table 20: ANOVA Statistical Results Comparing Specimens with and without Intervention. P-values of percent change of post-challenge NZ stiffness compared to baseline NZ stiffness between group of specimens without interventional extension movement and group of specimens with interventional extension movement, in each biomechanical testing direction.

Testing Direction	Post-Challenge #1	Post-Challenge #2	Post-Challenge #3
Extension	0.61	0.98	0.96
Flexion	0.35	0.57	0.45
Axial Rotation	0.03	0.01	0.001

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