Biomechanical Evaluation of a Cervical Intervertebral Disc Degeneration Model

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved September 2015 by the
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ARIZONA STATE UNIVERSITY

December 2015

ABSTRACT

Introduction. Intervertebral disc degeneration (DD) is one of the most common diagnoses in patients with neck pain and contributes to worldwide disability. Despite the advances in diagnostic imaging today, little is known about functional status of cervical DD. The purpose of this research was to 1) develop and validate an ovine model of cervical spine DD, 2) to quantify and compare the effect of disc lesions on dynamic spinal stiffness, and 3) study the effect of disc lesions on spinal accelerations and displacements during two types of spinal manipulative therapy (SMT). Methods. Fifteen sheep received surgically induced disc injury to the mid-cervical spine via scalpel wound a minimum of five months earlier and 15 sheep served as controls. All animals were biomechanically assessed at the level of the lesion using swept-sine mechanical loads from 0-20 Hz under load control to quantify dynamic dorsoventral (DV) spine stiffness (load/deformation, N/mm). The effect of disc lesion on stiffness was assessed using a one-factor repeated measures ANOVA comparing 32 mechanical excitation frequencies. Tri-axial accelerometers rigidly attached to adjacent vertebrae across the target level further evaluated the effect of disc lesion on spinal motion response during two types of SMTs. A 2x6x2 repeated measures ANOVA examined the effect of disc lesion and SMT force-time profile on spine motion response. Postmortem histological analysis graded specimens at the target site and comparison was made with descriptive statistics. Results. Annular disc tears were only observed in the disc lesion group and the mild degeneration identified was localized to the injured annular tissue that did not progress to affect other areas of the disc. No difference in overall DD grading was found among the groups. DV stiffness was significantly increased in the disc lesion group by approximately 34% at 31 of 32 frequencies examined ($p<.05$). SMTs resulted in decreased displacements in the disc lesion group ($p<.05$), and SMT type significantly

influenced spinal accelerations for both the DV and axial planes. Conclusion. Disc lesions in the ovine cervical spine produce localized annular degenerative changes that increase the cervical spine dynamic stiffness and reduce its spinal motion response during manual examination and treatment that is further augmented by the force-time profile administered by the clinician.

DEDICATION

This work is dedicated to my close friends and family, those still with us and those who have passed, who have encouraged and inspired me in my career path. First, I thank my late parents, Joseph and Eris Colloca, for their love and reinforcement of the importance of education, while neither of them had even graduated from high school. I am very grateful to have known and been trained in research methods by the late Dr. Tony Keller. Tony's commitment to excellence and overwhelming work ethic set the bar for my research path while showing me what was possible. After Tony's death, Dr. Robert Gunzburg made absolutely sure that our research collaboration would continue through careful planning and collaboration. I'm thankful for Robert's shared vision in our projects, for his friendship, and for his tireless push for the next adventure. I was also greatly influenced by the late Dr. Donald D. Harrison, who taught me to think logically and vehemently pushed me to pursue an advanced degree beyond my Doctor of Chiropractic. I'm too very grateful for the opportunity to learn from the late Dr. William Harris. Dr. Harris' philanthropy has supported my research spanning two decades. I'm thankful for these individuals for their leadership, support, and belief in me and my goals.

Finally, and perhaps most importantly, I want to thank my wife, Mindy, and my three children, Melia Eris, Kai Christopher, and Nico Sebastiano, for affording me the freedom and time to pursue my dreams of furthering my education and participating in scientific research. Many of these instances took me away from them for data collection around the world for extended periods of time. I am so appreciative of the love, support, and patience of my family during these extended absences and apologize for the things that I missed at home. It is my hope and dream that my path will inspire you, Melia, Kai, and Nico, to follow your dreams and careers to the very peaks of the mountain tops.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Richard Hinrichs, for his guidance and direction over the many years of graduate study that have culminated in my PhD experience. Special thanks are also offered to my committee members, Dr. Shannon Ringenbach, Dr. James Abbas, and Dr. Steven Hooker for taking time out of their busy schedules and other University commitments to serve and help me in providing feedback, clarity and direction with my research. I thank Penny Pandelisev for helping me find my way through the administrative processes at ASU. I am also grateful for the many excellent professors at ASU who assisted in training me to think like a scientist rather than a clinician.

I acknowledge the team at the Adelaide Centre for Spinal Research under the direction of Professor Brian Freeman for their collaboration and shared excitement for this work. I'm grateful for the use of the South Australia Pathology and Institute for Medical and Veterinary Sciences staff and facilities. They always made us feel at home during our time in the laboratory and their efficient and friendly staff made data collection go smoothly. I thank Dr. Freeman, Dr. David Hall, and Dr. Robert Gunzburg for their spinal surgery expertise that made the model for this research possible. I'm also appreciative of the assistance of Dr. Robert Moore for organizing our research in Australia and assistance with histology and thank Dr. Julia Kuliwaba for her excellent work in the histological preparations and scoring necessary for evaluation of our model. I also want to thank and acknowledge Dr. Deed Harrison and Dr. Jeb McAviney for their assistance with our experimental set-up and data collection, and Dr. Mostafa Afifi Hegazy for his help with the arduous task of data processing and analysis.

I am also sincerely grateful to the International Spine Research Foundation, Chiropractic Biophysics Non-profit, and the William M. Harris Family Foundation for their financial support of this research.

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CHAPTER 1

GENERAL INTRODUCTION

Neck pain (NP) and related disorders arising from the cervical spine are common conditions that cause substantial disability. In fact, among 291 conditions identified in the *2010 Global Burden of Disease Study* (Hoy et al., 2014a), NP ranked 4th highest in terms of disability as measured by years lived with disability (YLD) and 21st in terms of overall burden (Hoy et al., 2014b). Over the past two decades, NP related disability has increased by approximately 30% (Hoy et al., 2014b). Neck pain has also been found to be negatively associated with physical health related quality of life (HRQoL) and a contributor of future poor physical HRQoL in the population (Nolet et al., 2014). Indeed, the overwhelming majority of low back and cervical pain is considered to be due to unspecified mechanical factors or disc degeneration, which is a common with aging and, hence, in people of working age (Williams & Sambrook, 2011). Cervical spine disc degeneration (DD) has been found prevalent in 26% of individuals under age 50 and nearly 50% in those 50-59 years with steadily increasing prevalence to over 85% in life's later decades (Teraguchi et al., 2014). Longitudinal studies have identified the progression of degeneration of the cervical intervertebral discs over ten years with development of neck related symptoms in 34% (Okada et al., 2009). This is of interest considering the estimated 1 year incidence of NP from available studies ranges between 10% and 21% (Hoy, Protani, De, & Buchbinder, 2010). Understanding how DD relates to cervical spine function and pain are natural first steps in responding to the economic and societal burden that neck related disorders presents.

The cervical spine is a multi-joint structure that simultaneously provides protection to its internal neural elements including the spinal cord and spinal nerve roots while providing both flexibility and stability through its dynamic motion ranges.

Segmental instability and pathology including degeneration of the spine are believed to produce abnormal patterns of motion and forces which may play a significant role in the etiology of musculoskeletal conditions including neck pain. Figure 1 presents frontal, sagittal, and posterior views of general cervical spinal anatomy. Intervertebral discs are avascular pads of fibrocartilage that allow movement between vertebral bodies. Partly due to their avascular nature, discs degenerate far earlier than do other musculoskeletal tissues (Vernon-Roberts, Moore, & Fraser, 2007). Intervertebral disc degeneration is thought to be the first step in degenerative spinal changes and is typically followed by more severe indices involving the bony elements of the vertebral bodies including osteophytes and spinal stenosis (Boos et al., 2002). Figure 2 illustrates some of the anatomical changes that occur with disc degeneration.

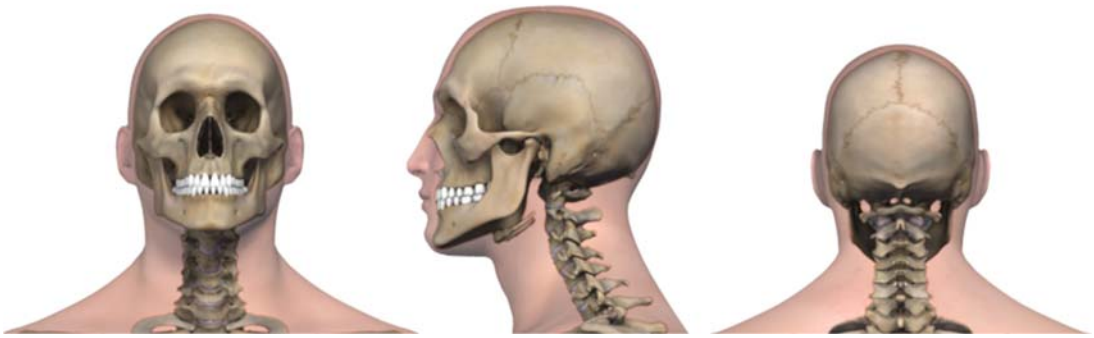

Figure 1. Cervical spinal anatomy showing frontal (left), sagittal (middle) and posterior views.

Progression of cervical intervertebral disc degeneration (DD) has been found to occur in 85% of individuals followed longitudinally for 10 years that had a positive correlation to clinical symptoms of neck pain and upper extremity symptoms (Okada et al., 2009). Disc degeneration alters disc height and the mechanics of the spinal column (Adams, Freeman, Morrison, Nelson, & Dolan, 2000) adversely affecting the behavior of

other spinal structures such as muscles and ligaments (Urban & Roberts, 2003) and the pain sensitive nerve endings inherent in these structures (McLain, 1994; Mendel, Wink, & Zimny, 1992). Degenerative cervical discs can also protrude causing radiculopathy producing numbness, paresthesia or pain into the upper extremity (Figure 3) (Nordin et al., 2009).

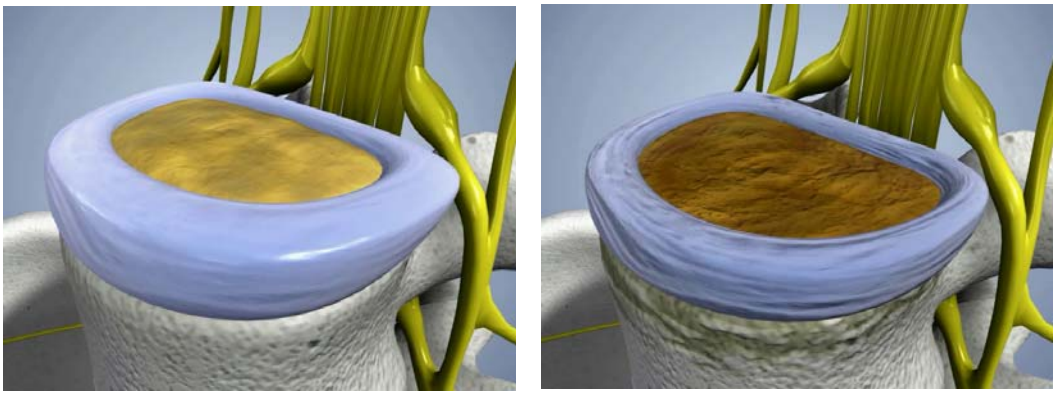

Figure 2. Intervertebral disc degeneration (right) is characterized by a loss of disc height, a loss of water content, and fibrotic change of the intervertebral disc tissue.

In the long term, DD can result in spinal stenosis, a major cause of pain and disability in the elderly; its incidence is rising exponentially with current demographic changes and an increased aged population (Urban & Roberts, 2003). While advances in diagnostic imaging technology have become a standard for evaluating the morphology of spinal structures, they do not provide any information about the functional status of the spine.

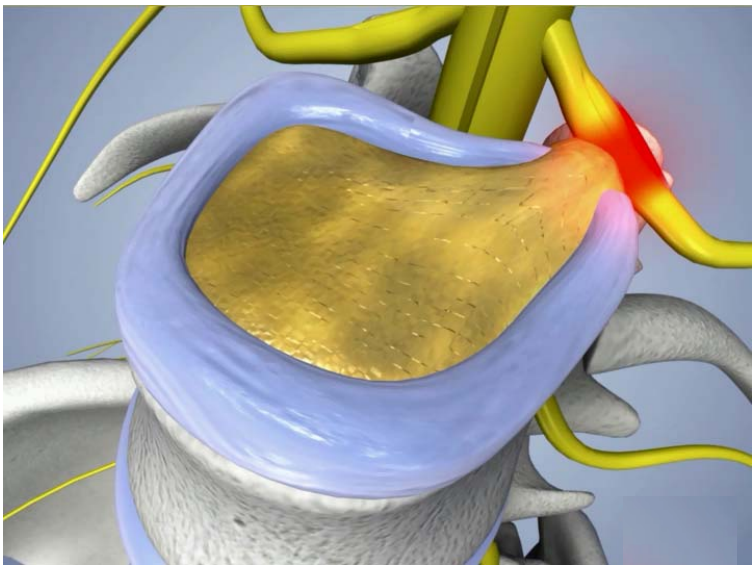

Figure 3. Intervertebral disc protrusion can be associated with DD and be the direct cause of irritation to the spinal nerve root resulting in upper extremity symptoms of pain, numbness, and weakness.

From in situ cadaveric models today we better understand the decreased load sharing capabilities of degenerated discs. Indeed, DD has been found to result in decreased hydrostatic pressure, strength, and elastic modulus (flexibility) that causes increased stress concentrations in the outer annulus fibrosus of the disc.(Hutton & Adams, 1987; Adams, 1995; Skrzypiec, Pollintine, Przybyla, Dolan, & Adams, 2007) Accordingly, numerous investigators have attempted to better the relationships between DD, spinal function, and pain (Kawchuk et al., 2001). Unfortunately, most clinical assessments to this extent are invasive or have the potential for harm, such as ionizing radiation, thus precluding their use in large populations. Furthermore, while diagnostic imaging techniques including X-ray and MRI (Figure 4) are valuable in providing visual images of degenerative lesions, they do not provide information about the functional status of the spine's degenerative state. The extent to which disc degeneration produces abnormal spinal motion patterns and forces in the cervical spine *in vivo* are yet to be

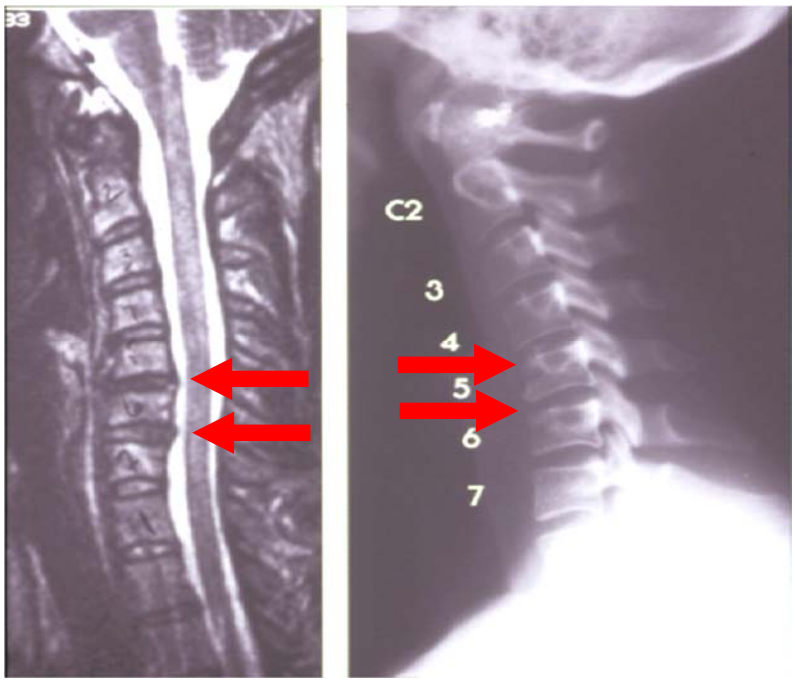

Figure 4. Sagittal plane T2 weighted magnetic resonance imaging (MRI) (Left) and plain film radiography (x-ray) (right) of the cervical spine. Noteworthy is the loss of signal intensity of the intervertebral discs at the C5-C6, and C6-C7 levels (arrows) on MRI and visualization of loss of disc height on corresponding x-ray. Adapted from Ruch WJ. Atlas of common subluxations of the human spine and pelvis. Boca Raton, FL, CRC Press, 1997.

determined. The ability to quantify *in vivo* spine segment motion (displacement) and stiffness (force/deformation) in response to forces is thus considered to be of clinical significance in terms of both diagnosis and treatment of spinal disorders including neck pain.

Indeed, a large proportion of the population who receive manual therapies have some degree of disc disease (Lisi, Holmes, & Ammendolia, 2005). To influence the peripheral pain generator, patients with discogenic disease commonly undergo spinal manipulative therapy (SMT) with the primary goals of reducing pain through normalizing loads and improving spinal mobility (Burton, Tillotson, & Cleary, 2000). A wide range of

manual techniques have been developed providing clinicians with choices of force amplitude, speed, and vector among other variables of SMT delivery in patient care. Force-time characteristics, including the applied force magnitude, speed, and/or frequency, have therefore been attributed to the underlying mechanisms of SMT (Keller, Colloca, & Beliveau, 2002). Both *in vitro* (Gal, Herzog, Kawchuk, Conway, & Zhang, 1997; Maigne & Guillon, 2000) and *in vivo* (Nathan & Keller, 1994; Keller, Colloca, & Gunzburg, 2003) biomechanical studies have examined segmental and intersegmental displacements and vibration responses during SMT, but few (if any) studies have quantified SMT-induced spinal kinematics in the degenerated IVD. Previous research in the lumbar spine by our group using a similar ovine degeneration model determined that vertebral kinematics were dependent on mechanical excitation pulse duration, and were significantly reduced in animals with degenerated discs (Colloca, Keller, Moore, Gunzburg, & Harrison, 2007). No study to our knowledge has quantified the motion response of the cervical spine during SMT comparing normal subjects to those with DD.

To answer this need, noninvasive biomechanical assessments have been developed and validated to evaluate the spine (Colloca, Keller, Moore, Harrison, & Gunzburg, 2009). Over the past 20 years, our research group has utilized a validated ovine model (Moore, Crotti, Osti, Fraser, & Vernon-Roberts, 1999; Moore, Vernon-Roberts, Osti, & Fraser, 1996; Moore, Osti, Vernon-Roberts, & Fraser, 1992; Osti, Vernon-Roberts, Moore, & Fraser, 1992) to investigate the *in vivo* biomechanics of normal and pathological spines focusing on the lumbar spinal region (Keller & Colloca, 2007; Keller, Colloca, Harrison, Moore, & Gunzburg, 2007; Colloca et al., 2007; Colloca, Keller, Moore, Gunzburg, & Harrison, 2008; Colloca et al., 2012). No research to date of our knowledge has developed an ovine model of the cervical spine to evaluate DD, its effect on biomechanical function of the spine, or the spine's biomechanical behavior

during clinical interventions such as during SMT. Thus, the purpose of the current project was broken down into three primary aims:

Part 1 was to develop a model of cervical spine DD using ovine intervertebral disc injury quantified histologically. Considering the past success in developing a lumbar spine DD model, we hypothesized that injury to the IVD would likewise result in marked histological changes in the intervertebral disc resulting in cervical DD at five month follow-up. Part 2 incorporated in vivo biomechanics testing to quantify and compare animals with disc lesions and normal control animals using a validated dynamic spinal stiffness assessment technique and examined differences in intersegmental mobility. Just as our previous work had demonstrated significant increases in dynamic spinal stiffness in animals with lumbar intervertebral disc degeneration, we hypothesized that DD in the cervical spine would be found to cause increased dynamic spinal stiffness compared with the control group in the neck. Likewise, our previous research has demonstrated decreased intersegmental motion responses during lumbar spinal manipulation in association with lumbar DD as quantified by accelerometers rigidly fixed to pins placed into the spinous processes. Thus, in Part 3, using similar methodology of mounting accelerometers to adjacent cervical spine vertebrae, we examined the acceleration response and calculated and compared the mobility of the normal and animals with disc lesions during spinal manipulative thrusts. We hypothesized that there would be decreases in intersegmental motion during manipulation in the cervical disc lesion group compared to the control group consistent with the biomechanical changes of DD.

To our knowledge, no such cervical spine DD model or in vivo cervical spine biomechanical examination has been conducted to date. The results of this dissertation will be helpful to clinicians and researchers alike in better understanding the

pathogenesis of cervical DD, and how the mechanical properties are altered in the presence of disc lesions or DD. These results are important to contribute to assisting with the global burden that neck pain and spinal disorders including DD present to society.

CHAPTER 3

GENERAL METHODS

Sample and Setting

Thirty adolescent Merino wethers (18-24 months old, approximately 50 kg) were used in this study conducted at the Institute of Veterinary and Medical Science (IMVS) Surgical Research Facility (Gillis Plains), a division of South Australia (SA) Pathology in Adelaide, Australia. Prior to commencement of the research, the study protocol was approved by the Animal Ethics Committee of the IMVS, SA Pathology. Prior to inclusion in the study all animals were subjected to a comprehensive physical assessment by a veterinary surgeon. The examination was conducted by a veterinary physician employed at the research facility and incorporated attitude, general physical appearance, hydration, appearance of mucous membranes and coat, and an assessment of respiratory, musculoskeletal, lymphatic, cardiovascular, gastrointestinal and urogenital systems. Standard forms were used to record these observations. Animals recruited to the study acclimatized for 1 week in open pasture before being transferred for approximately three days prior to surgery. Animal ethics approval and animal user permits are found in Appendix A.

Design

Fifteen animals underwent cervical spine surgery to induce IVD degeneration at a single level from C2-C5 (Disc Lesion Group) and fifteen animals served as controls receiving a sham neck surgery at the same region (Control Group). Targeted spinal levels were determined by the surgeon based upon ease of access upon surgical approach. Animal demographics are presented in Table 1. Post-surgical monitoring and

maturation of the degenerative lesions were subsequently monitored at the research facility as well as the biomechanical testing that followed. An overview of the study design is depicted in Figure 5.

Table 1. Animal demographics, intervention received, and group assignment are presented for the 30 participants in this study. Animal weights and mean and standard deviation (S.D.) for the group and including spinal level targeted.

Subject	Intervention	Group	Weight (kg)	Level
1	Annulotomy	Disc Lesion	51.00	C:3/4
2	Annulotomy	Disc Lesion	46.00	C:3/4
3	Annulotomy	Disc Lesion	46.00	C:3/4
4	Annulotomy	Disc Lesion	39.50	C:3/4
5	Annulotomy	Disc Lesion	42.50	C:4/5
6	Annulotomy	Disc Lesion	39.50	C:3/4
7	Annulotomy	Disc Lesion	44.50	C:4/5
8	Annulotomy	Disc Lesion	56.00	C:3/4
9	Annulotomy	Disc Lesion	48.70	C:3/4
10	Annulotomy	Disc Lesion	48.60	C:3/4
11	Annulotomy	Disc Lesion	53.00	C:2/3
12	Annulotomy	Disc Lesion	54.00	C:3/4
13	Annulotomy	Disc Lesion	48.50	C:4/5
14	Annulotomy	Disc Lesion	47.50	C:2/3
15	Annulotomy	Disc Lesion	39.50	C:2/3
16	Exposure	Control	56.00	C:2/3
17	Exposure	Control	52.50	C:3/4
18	Exposure	Control	45.50	C:4/5
19	Exposure	Control	55.00	C:2/3
20	Exposure	Control	51.00	C:2/3
21	Exposure	Control	54.50	C:4/5
22	Exposure	Control	48.00	C:3/4
23	Exposure	Control	42.50	C:4/5
24	Exposure	Control	45.50	C:4/5
25	Exposure	Control	44.50	C:2/3
26	Exposure	Control	46.00	C:2/3
27	Exposure	Control	42.50	C:4/5
28	Exposure	Control	46.50	C:3/4
29	Exposure	Control	46.50	C:3/4
30	Exposure	Control	44.50	C:3/4
		Mean	**47.53**	
		S.D.	**4.87**	

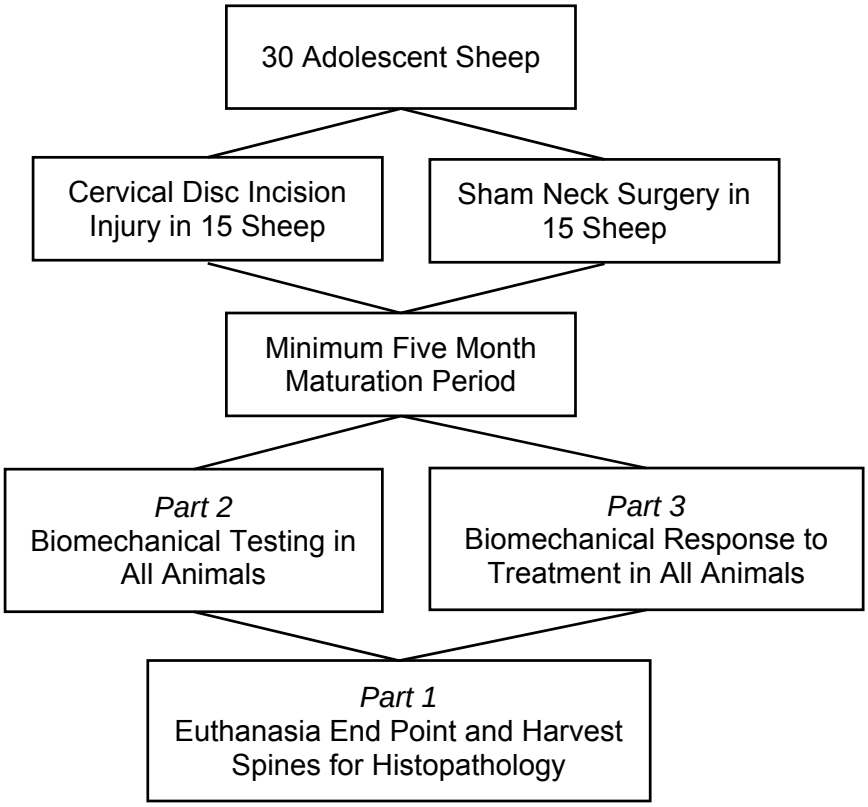

Figure 5. Study design for biomechanical evaluation of a cervical disc degeneration model.

Following a minimum five month period for healing to occur and maturation of the induced cervical IVD lesion to ensue, biomechanical testing consisting of in vivo dynamic spinal stiffness assessment was conducted at the target spinal level as presented in Part 2 of this study. Following biomechanical testing, spinal manipulative thrusts were randomly administered to the target spinal levels and the motion response of the spine recorded as presented in Part 3 of this study. At the conclusion of biomechanical assessment and treatment protocols, animals were euthanized and their cervical spines were harvested for histological processing and analysis of the IVDs at the target levels as per the methodology presented in Part 1 of this study.

CHAPTER 3

PART 1 – CERVICAL INTERVERTEBRAL DISC DEGENERATION MODEL

Introduction

The intervertebral discs are cartilaginous, articulating structures located between the vertebral bodies that allow movement (flexion, extension, and rotation) spinal column. The discs form a complex system, with an outer annulus fibrosus surrounding a central nucleus pulposus for shock absorption and tensile loading. Collagen fibers continue from the annulus into the adjacent tissues, tying this fibrocartilaginous structure to the vertebral bodies at its rim, to the longitudinal ligaments anteriorly and posteriorly, and to the hyaline cartilage end plates superiorly and inferiorly. The cartilage end plates in turn lock into the osseous vertebral end plates via the calcified cartilage (Roberts, Gratin, & Whitehouse, 1997).

Intervertebral disc degeneration is a multifaceted, chronic process involving progressive changes in disc composition, structure, and function occurring more quickly and typically with greater severity than those associated with normal aging. Uncertainties of the precise pathogenesis DD have brought about research agendas to investigate the number of disabling conditions arising from DD , including disc herniation, radiculopathy, myelopathy, and spinal stenosis. To date, few clinical options are available to directly manage the underlying problem DD highlighting the need for a greater understanding of this condition (Sobajima et al., 2005).

Advances in diagnostic imaging technology have become the most important method for the clinical assessment of intervertebral disc pathology (Pfirrmann, Metzdorf, Zanetti, Hodler, & Boos, 2001). Changes in signal characteristics of the disc in T-2 weighted MRIs reflect age related changes or degeneration. Indeed a number of morphological grading systems for IVD degeneration observed on MRI have been

proposed, yet studies focusing on the MRI characteristics of the disc structure are rare (Raininko et al., 1995) and the imaging technology is expensive. In contrast, histological analysis of the IVD clearly demonstrates the IVD's highly specialized and organized structure and integration with its adjacent tissues (Roberts, Evans, Trivedi, & Menage, 1988). Gross matrix changes, including increased lamellar disorganization and fissures, are features of degenerative discs that cannot be observed on MRI and solely observed using histological analysis (Roberts et al., 1988). Because tissue samples used for macroscopic histology can only be obtained from tissues removed during surgical interventions or from post-mortem specimens, it is easy to understand how MRI is commonly used in clinical settings (in vivo), while histology can be used to verify and validate imaging findings ex-vivo.

Recent advancements in molecular biology and tissue engineering have made it possible to contemplate directly treating the intervertebral disc itself (at molecular, cellular, and tissue levels) to alter the course of DD. A number of novel approaches have been proposed for studying DD, the most common being animal modeling. Initial experimental results have been encouraging for developing a lumbar DD model that following validation (Osti, Vernon-Roberts, & Fraser, 1990) has been used in a number of studies aimed at better understanding lumbar DD (Osti et al., 1992; Osti & Fraser, 1992; Moore et al., 1996; Moore et al., 1992; Vernon-Roberts et al., 2007; Ahlgren, Lui, Herkowitz, Panjabi, & Guiboux, 2000; Costi, Hearn, & Fazzalari, 2002; Fazzalari et al., 2001; Ghosh et al., 2012; Reid, Meakin, Robins, Skakle, & Hukins, 2002). These studies have substantiated the feasibility of studying DD by incorporating suitable animal models that closely mimic the specific aspects of human DD being targeted and have effectively begun to improve the understanding of the pathogenesis and pathophysiology of DD. Numerous animal models of DD have been proposed in the literature (Natarajan,

Williams, & Andersson, 2006), each with attendant advantages and disadvantages for the purposes of studying pathogenesis and pathophysiology of DD and testing novel therapies or interventions.

The classic “stab model” of Lipson and Muir (Lipson & Muir, 1924; Lipson & Muir, 1981) are arguably the most widely known classical studies to characterize an animal model of DD available. To this extent, full-thickness, ventral stab of annulus fibrosus (AF) of lumbar discs by a number 11 scalpel blade is induced into the disc through surgical intervention. Animals are then sutured and monitored over the course of weeks wherein DD ensues as evaluated by biochemical and histologic outcome measures that are similar to changes seen in human DD. A similar DD model was validated for the lumbar spine in sheep (Osti et al., 1990) that has been extensively studied in the lumbar region. Using this model (Colloca, Keller, Moore, Gunzburg, & Harrison, 2007b) the degenerated model discs, having been incised in the anterolateral annulus fibrosus five months prior to data collection, were consistently at a stage of moderate to advanced degeneration compared to the normal discs. In the animals subjected to the chronic lesion, macroscopically there was unequivocal evidence of the annular incision in the incised disc with extension of the lesion to involve the central nucleus pulposus in all cases that resulted in substantial loss of height due to breakdown of disc matrix. Microscopically all discs showed advanced repair of the most peripheral annular fibers or in some cases, more organized collagenized scar tissue. In most cases, the nucleus showed substantial migration with early clefting of the matrix in some cases. Similar reports (Costi et al., 2002) have documented the chronic degeneration of the lumbar sheep model using a control group subjected to surgical exposure. These findings led to the question of whether similar degeneration would occur in the cervical spine in the current proposal. No research to date of our knowledge has developed an ovine model

of the cervical spine intervertebral disc. Thus, the objective of Part 1 of this study was to develop a model of cervical spine DD using ovine intervertebral disc injury quantified histologically. Considering the past success in developing a lumbar spine DD model, we hypothesized that injury to the IVD would likewise result in marked histological changes in the cervical IVD resulting in cervical DD at a minimum of five month follow-up.

Methods

Induction of Cervical Disc Lesion. Fifteen animals underwent cervical spine surgery to induce IVD lesions at a single cervical spine level (Disc Lesion Group) and fifteen animals served as controls receiving a sham neck surgery in the same region (Control Group). In the fifteen animals in the disc lesion group, the sheep were fasted for 24 hours prior to surgery and anesthesia was induced with an intravenous injection of 1 g thiopentone. Lateral plain X-ray films were taken to verify normal cervical spine anatomy and to establish baseline parameters for comparison of changes at later stages of the study (Figure 6). General anesthesia was maintained after endotracheal intubation by 2.5% halothane and monitored by pulse oximetry and end tidal CO_2 measurement. To induce disc degeneration, the cervical spine was targeted via a direct anterolateral left-sided approach. The skin was incised to the left of midline and the paraspinal muscles were separated in their normal anatomical planes by gentle retraction. In the disc lesion group, when the mid cervical spine was visualized a #22 scalpel blade was introduced into the most easily approached disc with a controlled stab

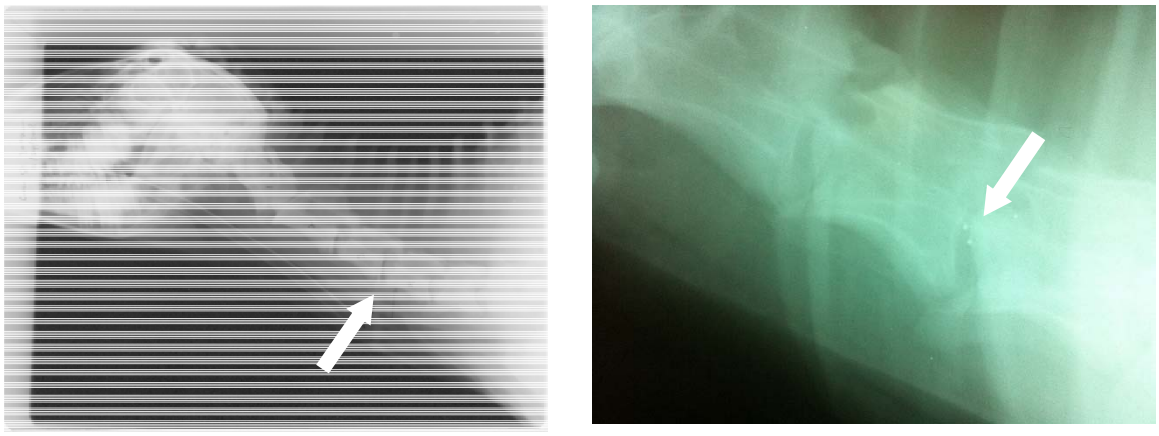

Figure 6. Lateral cervical spine radiograph of the ovine spine (Left) with an arrow demarcating the proposed level of experimental induction of intervertebral disc degeneration. (Right) Radiopaque tantalum beads are visualized identifying the surgical level targeted.

incision (annulotomy) to penetrate the full thickness of the firm outer layers through to the softer inner core using methodology previously described for the lumbar spine (Osti et al., 1990). Fluoroscopic control was used to check the posterior limit of the blade. Care was taken by the surgeon to protect both the spinal cord and the exiting nerve root during the stab incision procedure. Vascular and neural anatomy was resected and bleeding was controlled using direct pressure and electrocautery as required. In all animals, radiopaque tantalum beads were placed into the vertebral body bone on each side of the targeted disc level for future identification purposes (Figure 6).

Control Group Exposure Sham Neck Surgery. In the control group the same surgical approach was conducted however once visualization of the spine and discs were accomplished from C2-C5, no scalpel incision was made to any disc at any level. Thus, we term the surgical intervention administered in the control animals as sham neck surgery in the control group. In all animals, the wound was closed in layers with continuous sutures. All animals received an intramuscular antibiotic injection 2 mL/50 kg (consisting of procaine penicillin 250 mg/mL, streptomycin 250 mg/mL, and procaine

HCL 20 mg/mL). A lateral radiograph was taken as a record of the spinal anatomy and to assist in the subsequent biomechanical testing. Progressive disc degeneration was also monitored in the DD group by lateral plain film X-ray under induction anesthesia prior to commencement of biomechanical testing.

Post-Operative Monitoring and Care. The sheep received intramuscular injections of 1 mg/kg Xylazine and 2 mg/kg Finadyne prophylactically for pain relief. As soon as each sheep was able to breathe spontaneously it was detubated and transferred to a holding pen until it was able to stand freely. A discharge report was subsequently made on a standardized form. All animals were observed closely for up to three days in indoor pens before being transferred to secure covered outdoor pens for four days further. Analgesia and anti-inflammatory treatment was continued for a further week as necessary, according to SA Pathology Animal Ethics Committee policy. Animals showing signs of full recovery from surgery were released one week after surgery and pen holding to open pasture where there was no restrictions on diet or physical activity. Animals were observed daily as a flock, but once a month the animal attendants made individual observations including an assessment of their physical appearance, body weight and gait. Where adverse were observations such as significant pain, bladder or bowel dysfunction or other adverse neurological symptoms a veterinary surgeon was consulted to decide on appropriate treatment. Where necessary the neurological examination included analysis of gait, bilateral patellar reflexes, foot sensitivity to cold temperature and sharp pinprick, and other reflexes. Animals that were judged as unfit to continue in the trial were sacrificed immediately and the appropriate tissues removed for analysis.

Animals were assessed via a clinical scoring system (Appendix B). Each observation was scored according to the Post Operative Pain Scoring System. The

criteria for continuing the study or euthanasia are: Level for continuing study: Score < 6; Euthanasia Endpoints: Score ≥12 for more than 24 hours; Score ≥ 9 for 48 hours. Animals were monitored as a flock. If an individual animal did not appear to be thriving and the flock scoring does not meet the level required for continuing the study an individual post-operative score monitoring form was completed (Appendix B). Analgesia (Ketaprofen, 2mg/kg, IM) was administered immediately post operatively and then as required (1.5 mg/kg, IM) at the discretion of the Veterinarian responsible for the animal. Following the in vivo biomechanical testing performed under induction anesthesia at the conclusion of the study as presented in Part 2 herein, animals were humanely euthanized by intravenous injection of 6.5 g sodium pentobarbitone.

Histological Preparation and Histopathological Grading. The cervical spines of each animal were immediately subsequently harvested and processed for histological analysis. Spinal motion segments were isolated post mortem by cutting through the cranial and caudal vertebral bodies close to the cartilaginous endplates using a bone saw. The left segment of each IVD was fixed in 10% neutral buffered formalin for 10 days. Decalcification was performed with fast decal solution (9.5% Nitric acid + 1% ethylenediaminetetracetic acid (EDTA)) until complete decalcification was confirmed by radiography. Neutralization was done with 6% Sodium Sulphate and storage in 70% Ethanol for processing was completed. Three 4 mm thick slices were cut immediately adjacent to the mid-sagittal plane and in each para-sagittal plane to the target level containing the annular lesion or control target (90 blocks, 3 per sheep) (Figure 7). These tissue slices were processed into paraffin; then five micron sections were cut and Haematoxylin & Eosin (H&E) stained. Two H&E sections from each of the mid-sagittal and para-sagittal slices were graded by an experienced researcher, blinded to section identity, using a published IVD degeneration grading system (Gries, Berlemann, Moore,

& Vernon-Roberts, 2000). This system, shown in Table 2, assigns a 4-point progressive degeneration grade (1 = normal, 2 = mildly degenerated, 3 = moderately degenerated, 4 = severely degenerated) to the nucleus pulposus, annulus fibrosus, cartilage end-plate and margins/subchondral bone, with the overall IVD grade calculated as the mean of these sub-compartment grades across the mid-sagittal and para-sagittal sections. Evaluation was conducted to examine histological differences between groups for overall disc scores and individual sub-region scores obtained from each anatomical IVD region evaluated using Mann-Whitney U Tests with alpha equal to 0.05.

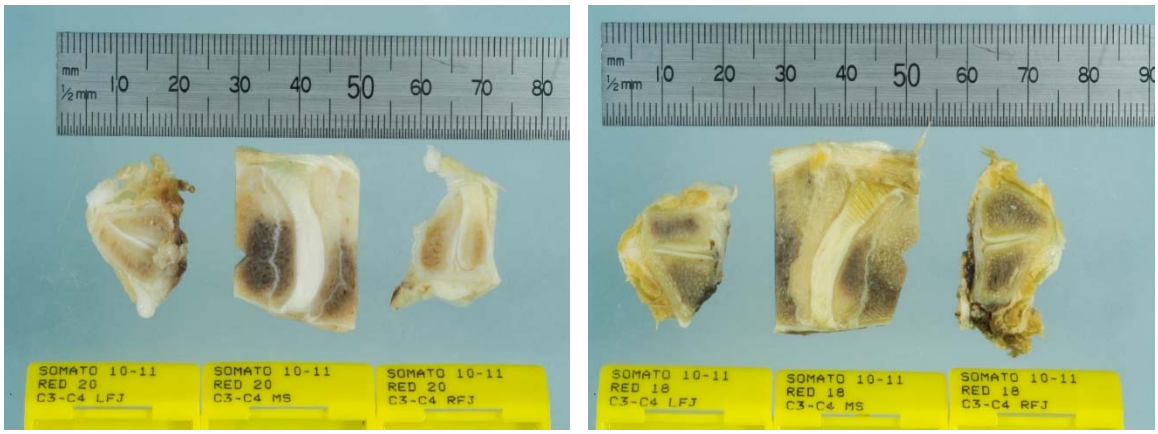

Figure 7. Mid-sagittal histological sections with scaling of the C3-C4 functional spinal unit demonstrating the left facet joint (LFS), intervertebral disc and adjacent vertebral bodies mid-section (MS), and right facet joint (RFJ) in a control animal subjected to sham neck surgery (left) and in the disc lesion group (right) that received scalpel injury to the intervertebral disc five months prior. Note the transverse fissure observed in the intervertebral disc on the specimen receiving previous disc injury (right) and hypertrophic changes of subchondral bone on the superior vertebral body endplate and the subsequent changes to the collagen lamellae accompanied by a decrease in intervertebral disc height, all indicative of DD captured by the grading system utilized herein.

Table 2. Grading system of histological changes of intervertebral discs (BEP – Bony End Plate; CEP – Cartilaginous End Plate) (Gries et al., 2000).

Grade	Annulus fibrosus	Nucleus pulposus	Cartilage end-plate	Margins/subchondral bone
1	Intact lamellae Narrow inter-lamellar matrix	Homogeneity Absence of clefting	Uniform thickness Intact attachment to bone	Even thickness of BEP Lamellar bone only
	Intact annulus attachment		Uniform calcification < 1/5 of depth	Distinct junction with CEP
	Vessels only in outer 1/3		Uniform cell distribution	Few vascular intrusions into CEP
2	Minor lamellar splitting and disorganization	Minor clefting	Minor cartilage thinning	Slightly uneven BEP
	Minor widening of matrix	Minor cell necrosis	Small transverse fissures	Schmorl's nodes
	Minor disorganization of attachment	Minor posterior displacement of annulus	Irregular thickening of calcified zone	Minimal remodelling of BEP
	Rim lesion without reparative reaction	Minor chondrone formation	Few invading vascular channels Small chondrones	Small marginal osteophytes
3	Moderate lamellar disorganization	Moderate clefting	Marked cartilage thinning	Moderately uneven BEP
	Moderate widening of matrix	Moderate cell necrosis	Marked thickening of calcified zone	Vascularized Schmorl's nodes
	Moderate fissuring of attachment	Cystic degeneration	Many transverse fissures	Moderate trabecular thickening

	Radiating tears, not involving outer 1/3 Minimal chondroid metaplasia Cystic degeneration Vessels in outer and middle 1/3 Rim lesion with minor reparative reaction	Posterior displacement within annulus Centripetal extension of collagen Moderate chondrone formation	Many vascular channels Many chondrones	Defect in bone lamellae Minimal fibrosis tissue in marrow spaces Medium-sized osteophytes
4	Extensive lamellar disorganization Radiating tears extending into outer 1/3	Complete loss of nucleus Loose body formation	Total loss of cartilage Calcification of residual cartilage	Marked uneven BEP Ossified Schmorl's nodes
	Extensive chondroid metaplasia Vessels in all zones Rim lesion with marked reparative reaction	Marked chondrone formation	Widespread fissuring	Large osteophytes Marked trabecular thickening Marked fibrosis of marrow spaces Cartilage formation

Results

Histopathological scores for the overall IVD grading of the mid-sagittal and para-sagittal sections for the target level and adjacent levels for all animals are shown in Table 3. Adjacent level specimens were not able to be graded in the in a minority of animals, however, this did not affect the grading scores of the target spinal levels. Mean overall histopathological IVD grading scores and sub-scores from individual IVD anatomical components for the targeted spinal level in all animals are provided in Table 4. Data for the mean overall histopathological grades and individual anatomical regions of the IVD evaluated (Annulus Fibrosis, Nucleus Pulposus, Cartilage Endplate, and Margins/Subchondral Bone) for the disc lesion and control groups are shown in Table 5 along with the results of the Mann-Whitney U Test analysis results. Figure 8 provides illustration of the group mean comparisons for the overall IVD histopathological grading analysis.

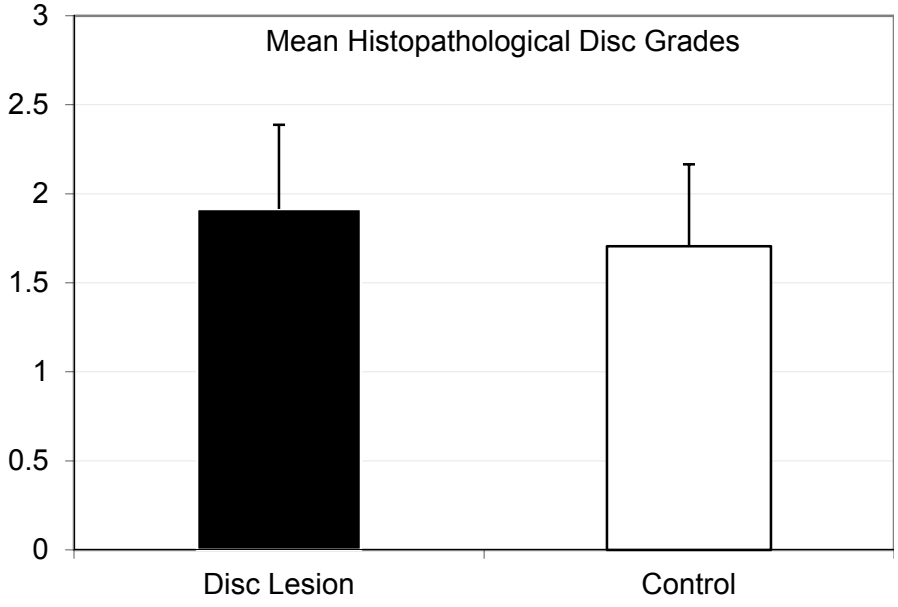

Figure 8. Mean overall histopathological disc grade scores for animals in the disc lesion and control groups. Standard deviations are indicated by error bars.

Table 3. Mean overall histopathological grading scores derived from the mid-sagittal and para-sagittal specimens for all levels examined including the target level and adjacent vertebral segments for all animals.

Subject	Group	Disc Level	Overall Disc Grades
1	Disc Lesion	C2/3	1.00
		C3/4	1.64
		C4/5	1.00
2	Disc Lesion	C2/3	1.28
		C3/4	1.58
		C4/5	1.36
3	Disc Lesion	C2/3	1.08
		C3/4	1.50
		C4/5	1.17
4	Disc Lesion	C2/3	1.00
		C3/4	1.28
		C4/5	1.17
5	Disc Lesion	C3/4	1.00
		C4/5	1.89
		C5/6	n/a
6	Disc Lesion	C2/3	1.36
		C3/4	1.47
		C4/5	1.08
7	Disc Lesion	C3/4	1.00
		C4/5	2.39
		C5/6	1.36
8	Disc Lesion	C2/3	1.36
		C3/4	2.47
		C4/5	1.69
9	Disc Lesion	C2/3	1.00
		C3/4	1.44
		C4/5	1.17
10	Disc Lesion	C2/3	1.33
		C3/4	1.89
		C4/5	1.33
11	Disc Lesion	C1/2	n/a
		C2/3	2.56
		C3/4	1.44
12	Disc Lesion	C2/3	1.67
		C3/4	1.89
		C4/5	1.31
13	Disc Lesion	C3/4	1.81
		C4/5	2.89
		C5/6	1.69
14	Disc Lesion	C1/2	n/a
		C2/3	1.75
		C3/4	1.50

15	Disc Lesion	C1/2	n/a
		C2/3	2.08
		C3/4	1.67
16	Control	C1/2	n/a
		C2/3	2.61
		C3/4	1.67
17	Control	C2/3	1.97
		C3/4	2.00
		C4/5	1.78
18	Control	C3/4	1.25
		C4/5	1.83
		C5/6	1.00
19	Control	C1/2	n/a
		C2/3	1.00
		C3/4	1.44
20	Control	C1/2	n/a
		C2/3	1.97
		C3/4	1.17
21	Control	C3/4	1.00
		C4/5	1.00
		C5/6	1.00
22	Control	C2/3	1.67
		C3/4	2.00
		C4/5	1.67
23	Control	C3/4	1.00
		C4/5	1.25
		C5/6	2.00
24	Control	C3/4	1.78
		C4/5	2.39
		C5/6	2.00
25	Control	C1/2	n/a
		C2/3	1.67
		C3/4	1.56
26	Control	C1/2	n/a
		C2/3	1.33
		C3/4	1.08
27	Control	C3/4	1.44
		C4/5	1.58
		C5/6	1.78
28	Control	C2/3	1.00
		C3/4	1.47
		C4/5	1.08
29	Control	C2/3	2.00
		C3/4	1.67
		C4/5	2.00
30	Control	C2/3	1.33
		C3/4	1.81
		C4/5	1.63

Table 4. Overall IVD Histopathological grading scores and their individual anatomical sub-region anatomical site scores for the targeted spinal levels in all animals.

Subject	Disc Level	Group	Overall Disc Grade	Annulus Fibrosis Grade	Nucleus Pulposus Grade	Cartilage Endplate Grade	Margins Subchondral Bone Grade
1	C3/4	Disc lesion	1.64	2.00	1.00	2.00	1.33
2	C3/4	Disc lesion	1.58	2.00	2.00	1.67	1.00
3	C3/4	Disc lesion	1.50	1.50	2.00	1.50	1.50
4	C3/4	Disc lesion	1.28	1.67	2.00	1.00	1.00
5	C4/5	Disc lesion	1.89	2.00	2.00	2.00	1.50
6	C3/4	Disc lesion	1.47	2.00	2.00	1.33	1.00
7	C4/5	Disc lesion	2.39	2.33	3.00	2.00	2.67
8	C3/4	Disc lesion	2.47	2.67	3.00	2.67	2.00
9	C3/4	Disc lesion	1.44	1.67	2.00	1.33	1.33
10	C3/4	Disc lesion	1.89	2.00	2.00	2.00	1.67
11	C2/3	Disc lesion	2.56	2.33	3.00	2.67	2.67
12	C3/4	Disc lesion	1.89	2.00	2.00	1.67	2.00
13	C4/5	Disc lesion	2.89	3.00	3.00	3.00	2.67
14	C2/3	Disc lesion	1.75	2.00	2.00	2.00	2.00
15	C2/3	Disc lesion	2.08	2.00	3.00	2.00	2.00
16	C2/3	Control	2.61	2.00	3.00	3.00	2.67
17	C3/4	Control	2.00	2.00	2.00	2.00	2.00
18	C4/5	Control	1.83	1.67	2.00	2.00	2.00
19	C2/3	Control	1.00	1.00	1.00	1.00	1.00
20	C2/3	Control	1.97	2.00	2.00	2.00	2.00
21	C4/5	Control	1.00	1.00	1.00	1.00	1.00
22	C3/4	Control	2.00	2.00	2.00	2.00	2.00
23	C4/5	Control	1.25	1.33	2.00	1.33	1.00
24	C4/5	Control	2.39	2.00	3.00	2.67	2.33
25	C2/3	Control	1.67	1.67	2.00	1.67	1.67
26	C2/3	Control	1.33	1.33	2.00	1.33	1.33
27	C4/5	Control	1.58	1.67	3.00	1.33	1.33
28	C3/4	Control	1.47	1.67	2.00	1.33	1.33
29	C3/4	Control	1.67	1.67	2.00	1.67	1.67
30	C3/4	Control	1.81	2.00	3.00	1.33	2.00

In the disc lesion group, annular damage (tears) were observed as well as annual damage visualized in the form of annular tears on the anterolateral aspect of the disc that were not similarly appreciated in the control group (Figure 9). While large tears were not observed in the annulus, other appreciable mild degenerative changes were also identified in the control group including lamellar splitting and widening. When

comparing the IVD scores obtained from the individual anatomical regions that compose the overall disc grading score, significantly greater degeneration was observed in the Annulus Fibrosis in the disc lesion group compared to the control group, U=52, p=0.011 (Table 5). No significant difference was observed when analyzing the nucleus pulposus, cartilage endplate, or margins of subchondral bone individually between groups. Similarly, with respect to overall histopathological scoring, the mean disc degeneration grade for the disc lesion group was not found to be significantly different than that for the control group, U=89.5, p=0.35 (Figure 8, Table 5).

Table 5. Group mean histopathological disc grades and standard deviations (S.D.) at the target spinal level for the overall and sub-region (annulus fibrosis, AF; nucleus pulposus, NP; cartilage endplate, CE; and margins/subchondral bone, SB) grading schemes. The results of the Mann-Whitney U Test and p-values are also shown.

Group	Overall Mean Disc Grade	S.D.	AF Grade	S.D.	NP Grade	S.D.	CE Grade	S.D.	SB Grade	S.D.
Disc Lesion	1.91	0.47	2.08	0.38	2.27	0.59	1.92	0.55	1.76	0.59
Control	1.71	0.46	1.69	0.36	2.14	0.64	1.72	0.58	1.69	0.51
U	89.5		52		100.5		84.5		107	
p value	0.35		0.01*		0.64		0.25		0.84	

Figure 9. Mid-sagittal histopathological section of C3-C4, the site of previous annulotomy in a disc lesion animal. A large annual tear is visualized, a characteristic not observed in the control group.

Discussion

Unlike the stark differences of progressive disc degeneration observed between normal animals and those undergoing annulotomy in the ovine lumbar spine (Colloca et al., 2007; Osti et al., 1990), the ovine cervical spine model presented in the current study only revealed localized mild degenerative changes to the annulus fibrosis that did not progress to degeneration of other regions of the IVD. The overall disc degeneration grading system by Gries et al (Gries et al., 2000) did not yield differences between the disc lesion and control groups in this ovine cervical spine degeneration model. The extent of degeneration in the present study was also different than that of the ovine lumbar spine, an important consideration for those attempting to generalize DD

progression in the ovine lumbar spine to the cervical spine. When evaluating the tissue specimens at the target cervical IVD (intersegmental level of the annulotomy or sham neck surgery focal point) degenerative changes collectively in the annulus fibrosus, nucleus pulposus, cartilage end plate, and vertebral margins and subchondral bone, did not advance to grade 3 and grade 4 levels of DD as has been commonly seen in the lumbar DD model previously (Gries et al., 2000; Osti et al., 1990; Moore et al., 1996; Moore et al., 1999; Fazzalari et al., 2001; Vernon-Roberts et al., 2007). Further, the amount of pathology identified in the control animals similarly resembled the changes observed in those with disc lesions, a finding that we did not anticipate.

There are a number of considerations that may help to explain these findings. First, the annulotomy induced in the disc lesion group were all administered via a left sided anterolateral approach. In reviewing the raw histopathological data it is evident that in the animals receiving annulotomy (disc lesion group) large annular tears are clearly visualized in the corresponding para-sagittal section. In some cases the tears continued to the mid-sagittal section and migrated further to be identified in the opposite para-sagittal section. Other corresponding and associated degenerative changes also accompanied the disc lesions in the annulus fibrosis. Indeed, when analyzing the Annulus Fibrosis alone, significant increases in disc degeneration score were observed in the disc lesion compared with the control groups. Because the grading system we used incorporated three sections and considered the annular tears as one component of four in grading DD, these findings were minimized in the overall disc degeneration scoring. Still, this is evidence of distinct differences of this ovine cervical spine DD model as compared to the ovine lumbar spine where progression of DD throughout the IVD is commonly seen.

While the control group did not have such annular tearing, appreciable degenerative changes were also observed. It may be possible that the surgical exposure itself could have been responsible for initiating a cervical spine DD process resulting in the changes visualized. Alternatively, the implantation of the tantalum beads to mark and later identify the spinal level discs visualized during the sham neck surgeries could have been involved in the subsequent degenerative process. In studying the pathogenesis of intervertebral DD, rapid biochemical changes derived from inflammation and injury to the functional spinal unit have been previously found to initiate and continue towards DD (Saal, 1995; Adams, Lama, Zehra, & Dolan, 2015; Ziran et al., 1994). Indeed, previous study in the lumbar spine DD model did not use sham surgery of exposure of the anatomy in the control group, but rather just used age and gender matched controls who didn't have any surgery (Colloca et al., 2007). Future work should consider histologically grading normal sheep cervical spine intervertebral discs (not exposed to a sham neck surgery) to determine if mild degeneration is commonplace, rare, or non-existent in normal animals not exposed to any sham neck surgery.

While the overall grading data utilized herein (Gries et al., 2000) do not show a significant difference in the overall disc degeneration grades between the disc lesion and control groups it is important to point out that significant differences were observed between groups in the annulus fibrosis only at the site of the target lesion. This observation was expected as this was the site of the induced annular injury in the disc lesion group and localized mild degenerative changes in the annulus fibrosis would be expected. As stated, unlike previously observed in the lumbar spine, we did not observe rapid progression of intervertebral disc degeneration throughout and into adjacent areas. Because according to the Gries grade criteria some of the control cases had a mild grade 1.5-2, it appears that the annulotomy produces a mild degeneration that is much

less degenerate (cellularly as well as maintenance of a good disc height on histology view of mid sagittal sections) compared to the lumbar annulotomy sections previously reported. The control group cases also showed a mild degeneration but without large tears in the annulus and more lamellar splitting and widening. Future research may consider extending the amount of annular damage imparted to the discs at the time the lesions are created to determine if greater injury results in a more pronounced or extensive degenerative change as compared to controls.

Although we allowed a minimum of five months to allow the degenerative changes to mature in the current study consistent with conservative durations for lumbar DD maturation, it may be likely that longer durations are needed for ovine cervical spine disc lesions to mature and progress. Future research should examine this research question as well. Perhaps greater mobility in the cervical spine as compared to the ovine lumbar region, changes in loading mechanics, and/or biochemical differences of these different spinal regions could play a role individually or in combination in preventing the progression of DD in the ovine cervical spine as has been previously found in the ovine lumbar spine. These variables remain to be investigated.

The disc height and axial loading properties of the healthy spine can be attributed to the water molecules, which bind tightly to the proteoglycans, present within the nucleus pulposus of the disc and inner annulus fibrosus. The observed mild degenerative changes observed may be attributed to changes in the proteoglycan or collagen or water composition of the disc following injury or exposure. However, since a biochemical analysis was not conducted, and histology scoring were not held specific to the lesion site, further analysis will focus on site-specific matrix/cellular changes resulting from cervical annulotomy in future work. Other possible mechanisms of DD could be

attributed to inflammatory properties and/or the release of trophic factors and inflammatory cytokines (Podichetty, 2007; Risbud & Shapiro, 2014) although there is no direct evidence of this in the current study.

In vivo animal models like the cervical DD model proposed herein provide the means to model living phenomena such as the development and consequences of DD and other pathologies (Panjabi, 1998) yet are limited in their generalization to the human spine. Previous investigations have demonstrated anatomical, biomechanical and biochemical similarities between sheep and human intervertebral discs (Reid et al., 2002; Wilke, Kettler, & Claes, 1997; Wilke, Kettler, Wenger, & Claes, 1997). Thus, the ovine degenerative disc model is a promising model to investigate biomechanical responses to dorsoventral mechanical excitation for dynamic spinal stiffness assessment and responses to spinal manipulation. Indeed, this is the first study to develop, evaluate and utilize an ovine degenerative disc model for *in vivo* biomechanical comparisons of dynamic spinal stiffness and spinal manipulation outcomes in the cervical spine.

CHAPTER 4

PART 2 – BIOMECHANICAL TESTING

Introduction

From in situ cadaveric models today we better understand the decreased load sharing capabilities of degenerated discs. Indeed, DD has been found to result in decreased hydrostatic pressure, strength, and elastic modulus (flexibility) that causes increased stress concentrations in the outer annulus fibrosus of the disc (Hutton & Adams, 1987; Adams, 1995; Skrzypiec et al., 2007). Accordingly, numerous investigators have attempted to better understand the relationships between DD, spinal function, and pain (Kawchuk et al., 2001). Unfortunately, most clinical assessments to this extent are invasive or have the potential for harm, such as ionizing radiation, thus precluding their use in large populations. Furthermore, while diagnostic imaging techniques including X-ray and MRI are valuable in providing visual images of degenerative lesions, they do not provide information about the functional status of the spine's degenerative state. The ability to quantify in-vivo spine segment motion (displacement) and stiffness (force/deformation) in response to forces is thus considered to be of clinical significance in terms of both diagnosis and treatment of spinal disorders including neck pain. The extent to which disc degeneration produces abnormal spinal motion patterns and forces in the cervical spine *in vivo* however, are yet to be determined.

Our ability to noninvasively quantify dynamic spinal stiffness has been previously demonstrated in the lumbar spine (Colloca et al., 2009) where we have identified significant increases in stiffness and subsequent decreases in the vertebral motion response in those with DD (Colloca et al., 2007). This background work provided preliminary evidence of the feasibility of this work, at least in the lumbar spine, and if

they are to be replicated in the cervical spine will represent a novel in vivo animal model, one that currently does not exist.

Efforts to quantify the biomechanical function of cervical DD is also important because it will for the first time provide objective evidence of the force deformation response and intersegmental mobility of the cervical spine in vivo. Knowledge of spinal biomechanics resulting from cervical DD will thus provide useful information to the literature base. Indeed, preventing neck pain while important, is of little use if the predisposing biomechanical factors leading to disc degeneration are not appreciated. The ability to quantify in vivo spine segment motion and stiffness (force/deformation) among degenerated cervical spines as proposed herein will allow us to finally answer the question of the biomechanical fate of cervical DD.

The objective of Part 2 of this study was to quantify and compare animals in the disc lesion and control groups using a validated dynamic spinal stiffness assessment technique. Just as our previous work has demonstrated significant increases in dynamic spinal stiffness in animals with lumbar intervertebral disc degeneration, we hypothesized that disc lesions would be found to cause increased dynamic spinal stiffness as compared with the control group.

Methods

Biomechanical Examination. The sheep were returned to the surgical facility a minimum of five months following initial cervical spine surgery described in Part 1 of this study by which time it was expected that the intervertebral discs would show approximately 30-40% degeneration of the disc,(9) as assessed radiologically by cervical spine x-ray examination. Biomechanical testing (described below) then commenced. A custom, computer-controlled mechanical testing apparatus (Figure 10)

was used to generate mechanical excitation force-time profiles with varying force amplitude, duration and frequency. The apparatus is comprised of a linear voice coil actuator (model LA25-42, BEI Technologies Inc., Ashford, Kent, UK) and a programmable, pulse width modulated servo amplifier, voice coil drive controller (model VCA100, BEI Kimko Magnetics, San Marcos, CA). The voice coil has a continuous stall force of 84 N and total stroke of 25.4 mm. A 750 N load cell (Transducer Techniques, Temecula, CA) and a $\pm$ 25 mm linear variable displacement transducer (LVDT, model S1D, Instruments & Control, Inc., Branford, CT) was used to measure the actuator force and displacement signals, respectively. Force and displacement signals were amplified using a dual channel, digital programmable gain amplifier (model PGA204, gain = 1000, Burr-Brown, Tucson, AZ). General anesthesia was maintained after endotracheal

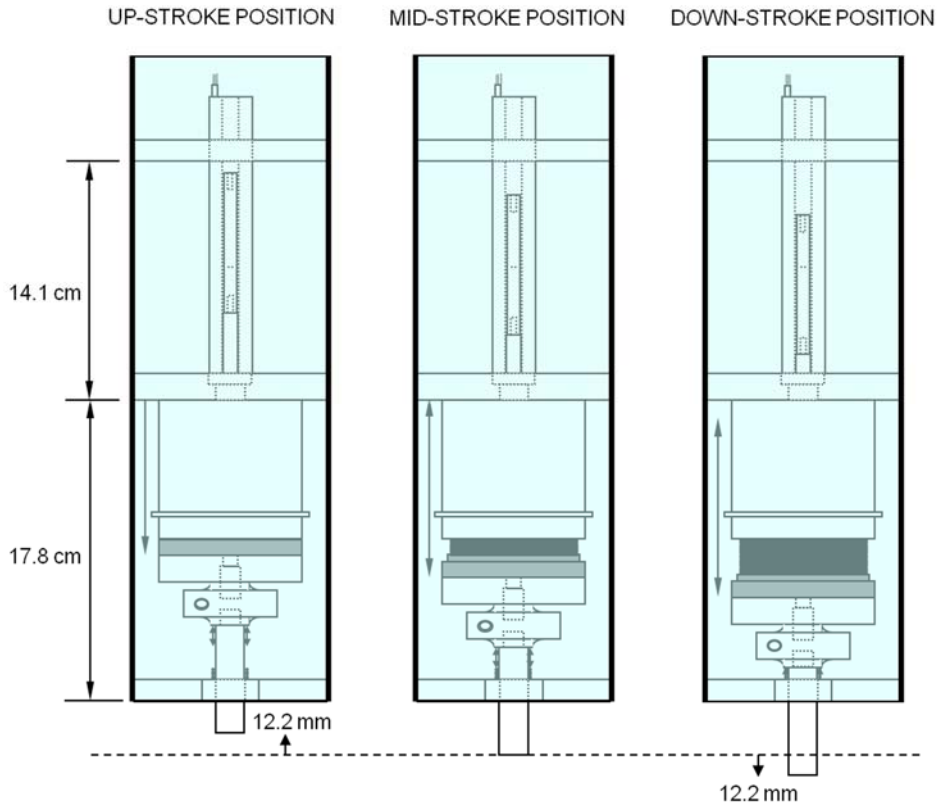

Figure 10. Schematic illustration of the computer controlled voice coil actuator used for biomechanical testing demonstrating dimensions and stroke distance.

intubation by 2.5% halothane and monitored by pulse oximetry and end tidal CO_2 measurement during biomechanical testing. Animals were ventilated and the respiration rate was linked to the tidal volume keeping the monitored $C0_2$ between 40-60 mmHg by a registered veterinary nurse at the research facility.

The anesthetized sheep were stabilized prone on an operating table, which included a rigid (wood) support beneath the abdomen (just caudal to the ribcage) (Figure 11). The support is designed to orient the long axis of the sheep spine parallel to the operating table and perpendicular to the load actuator and secondarily to stabilize the trunk (Figure 11). The compliance of the load frame+actuator+table+wooden support was 0.0069 mm/N. Foam blocks were placed on either side of the sheep abdomen to further stabilize the trunk along medial-lateral axis. An adhesive earthing pad was applied to the groin skin for electrocautery. With the animals in this standardized prone-lying position, the target spinous process was radiologically identified and a 1.5 cm region of the bony prominence of the target spinous process was exposed using electrocautery and the ligamentum nuchae removed for access to the cervical spine. Using the dynamic mechanical testing apparatus, dorsoventral (DV) forces were then applied directly to the exposed target spinous process via a 12.7 mm-diameter stainless-steel indenter rod equipped with a slotted tip that cradles the exposed spinous process. This minimizes problems associated with the actuator sliding off the sheep spinous processes, which are more slender than their human counterpart. DV forces (~13 N preload to ~48 N peak) were applied at swept-sine excitation frequencies ranging from 0.5 to 20 Hz of 2.0 Hz. Peak forces are approximately 10% of the mean animal body weight, consistent with the magnitude of posteroanterior forces used by clinicians in the treatment and assessment of cervical spine disorders (Latimer, Lee, & Adams, 1998). The applied force and DV displacement response was recorded at 2500

samples/second using a 16 channel, 16-bit data acquisition system (MP150, Biopac Systems, Inc., Goleta, CA). Force and displacement signals were recorded during the oscillations for a total of 22 seconds to examine dynamic spinal stiffness among the groups.

Data Analysis. A custom MatLab program was used to process the load, displacement, and acceleration signals. A peak detector was used to identify peak-peak responses for each of the mechanical excitation frequencies examined. DV Dynamic stiffness (peak-peak force/peak-peak displacement, N/mm) was determined from the force-time profiles of the periodic excitation protocols at 32 discrete frequencies for the 22 second swept-sine mechanical excitation protocol (Figure 12).

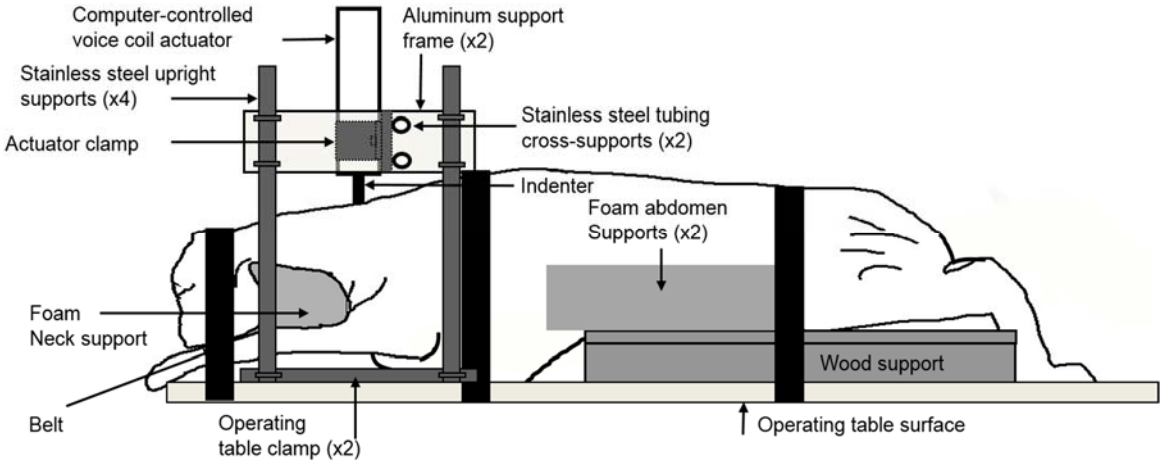

Figure 11. Experimental setup for mechanical testing shows the prone position of the sheep on the operating table and positioning of the computer controlled biomechanical testing unit positioned on the cervical spine at the level of C4. In addition, two tri-axial, dynamic accelerometers are located at C3 and C4 to record spinal motions.

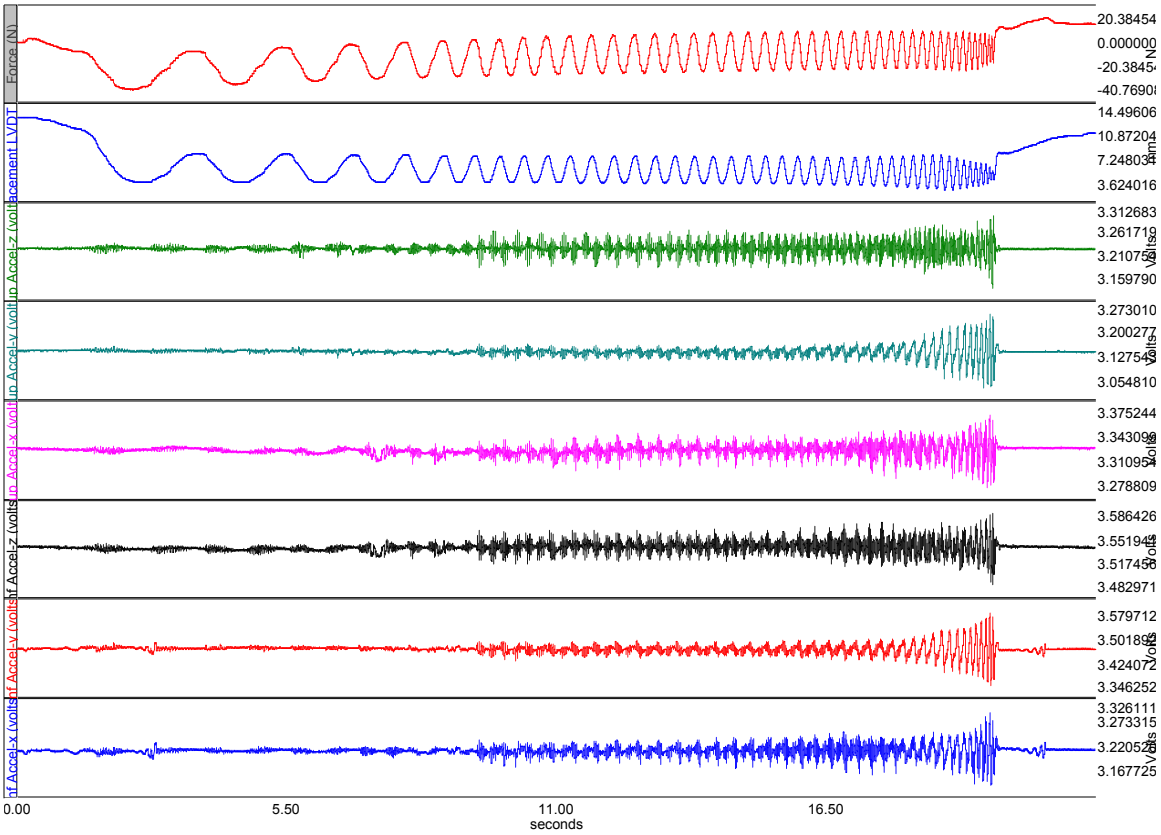

Figure 12. Data acquisition illustrating a single 23 second trial of chirp oscillation. Independent variables (top to bottom) of force, displacement, and six channels of acceleration data from each of two tri-axial accelerometers are shown. From these data the biomechanical variables and those calculated from the raw data are organized and compared at 32 distinct mechanical excitation frequencies after averaging the 6 trials administered in each of the two groups.

Load and displacement data were tabulated for each of the six trials at each frequency for each animal. From the load and displacement data, stiffness was calculated at each mechanical excitation frequency as DV Dynamic Stiffness (peak-peak force/peak-peak displacement, N/mm). Data analysis of the dependent variables for in vivo mechanical testing are shown in Table 6. In this manner, the biomechanical effect of the cervical disc lesion on dynamic lumbar spine stiffness was evaluated by comparing the disc lesion and control groups using a one-factor repeated measures

analysis of variance (ANOVA) for each of the 32 mechanical excitation frequencies examined.

Table 6. Raw data tabulating the sample size, frequencies examined, and number of trials to calculate the number of data points examined during data processing and analysis examine the effect of the independent variable, DD, upon dependent variables of dynamic spinal stiffness of the ovine cervical spine.

Independent Variable	**n**	**Frequencies Examined**	**Trials**	**Data Points Examined**
Disc Lesion Group	15	32	6	2880
Control Group	15	32	6	2880
Total				**5760**

Determination of Sample Size. Using dynamic spinal stiffness assessment data for the lumbar spine, a power analysis determined that 15 animals with cervical disc lesions (and 15 controls) would provide valid statistical data. Analysis of several key biomechanical parameters (vertebral displacement, mechanical stiffness, and vertebral acceleration response) measured from 10 normal disc sheep in a previous study (Keller & Colloca, 2007) showed that ≥15 animals were required to detect a 20% change in these parameters. The justification for the 20% change was based on several factors, including the ability to detect changes associated with key research questions being addressed, namely:

1. biomechanical response to different mechanical loading profiles;
2. biomechanical response to varying mechanical oscillation stimulation frequency;
3. effects of disc degeneration on these biomechanical responses.

The following statistical *Power Analysis* was used to determine the appropriate sample size (*n*) for within group and across group comparisons:

$$n = (Z^*\text{sd}/E)^2$$

where Z = critical value = 1.96 (α = 0.05); sd = standard deviation of the parameter of interest and E = error or difference to be assessed (Table 7).

Table 7. Sample results obtained for mechanical stiffness, (N/mm) at 2 Hz.

Mean	sd	E	n	Mean Difference
15.27903	5.65245	1.527903	53	(10% group mean difference)
15.27903	5.65245	2.291854	23	(15% group mean difference)
15.27903	5.65245	3.055806	13	(20% group mean difference)

Stiffness combines two separate measurements (force and deformation) and therefore has the greatest potential error) of the biomechanical variables. As shown, 13 animals are required to establish statistical significance for differences of 20% or greater. In the normal disc animals, we found up to a 19%, 3-fold change, and 2-fold change or difference related to questions 1-3, respectively. Other calculations yielded slightly higher/lower *n* values depending on the parameter examined, so 15 animals were deemed to be most appropriate. Based on the normal disc results, an experimental disc degeneration protocol was previously undertaken in the lumbar spine 15 animals. Analysis of the data indicated that there is about a 15-20% difference in the biomechanical response of the degenerated disc vs. normal disc animals. This finding supported the group sizes of 15 in the study design.

Results

Dynamic DV spinal stiffness ranged from 4.33 N/mm (1.8 Hz) to 7.69 N/mm (10 Hz) for animals with disc lesions and 3.62 N/mm (1.8 Hz) to 6.00 N/mm (13.1 Hz) for control animals, respectively (Figure 13). Dorsoventral stiffness was significantly increased at 31 of 32 mechanical excitation frequencies ($p<.05$) among animals with cervical disc lesions (all frequencies mean = 7.32 N/mm) compared with control animals (all frequencies mean = 5.47 N/mm). Table 8 provides dynamic stiffness ANOVA results with p-values for significant trials. Considering all 32 mechanical excitation frequencies examined, this represents a mean increase in cervical spine stiffness of 34% in the disc lesion group. The large effect sizes determined from these data lend credibility to the high practical significance of these results.

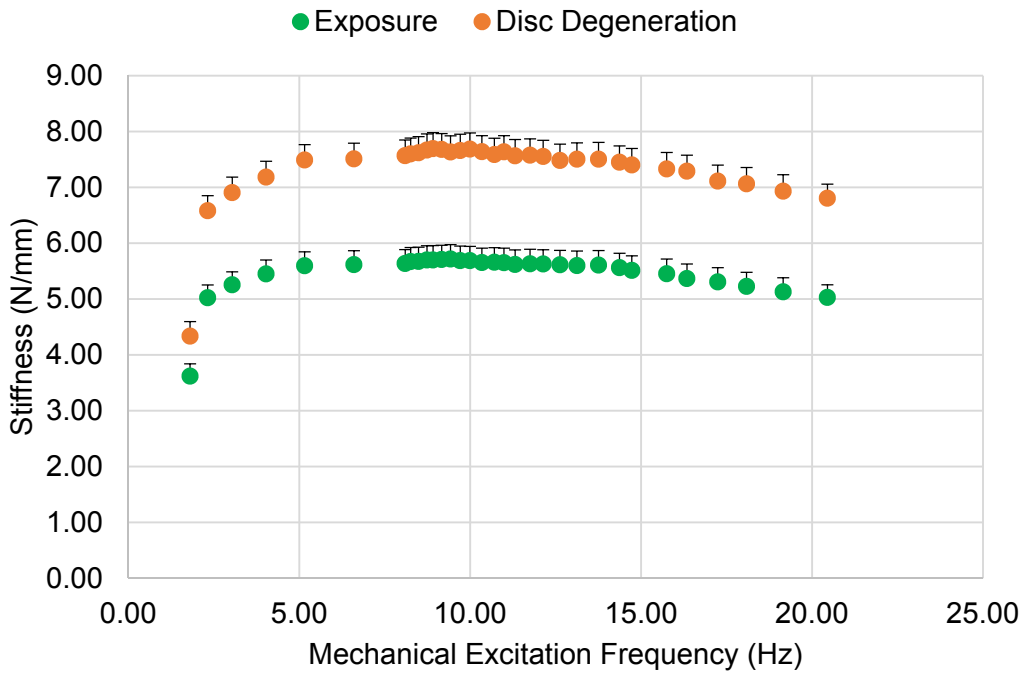

Figure 13. Mean dynamic spinal stiffness values are shown for the disc lesion and control groups at each of the 32 mechanical excitation frequencies examined. Error bars represent standard deviations of the mean.

Table 8. Dynamic stiffness results of the one-factor ANOVA and effect sizes comparing the disc lesion and control groups for each of the 32 mechanical excitation frequencies analyzed. Mean and standard error values are shown for each of the 32 mechanical excitation frequencies (Freq) examined. Significant trials are notated with an asterisk (*) and Cohen's d and effect size values are shown.

	Control		Disc Lesion						
Freq	Mean	Std Error	Mean	Std Error	F [1,28]	p value	Sig Trials	Cohen's d	Effect Size r
1.80	3.62	0.22	4.33	0.26	1.33	0.26		1.63	0.83
2.32	5.02	0.23	6.58	0.27	4.39	0.05	*	2.96	0.95
3.04	5.25	0.24	6.90	0.28	4.67	0.04	*	3.06	0.95
4.03	5.45	0.25	7.18	0.29	4.82	0.04	*	3.11	0.95
5.15	5.60	0.25	7.49	0.28	5.26	0.03	*	3.24	0.96
6.60	5.61	0.25	7.51	0.28	5.30	0.03	*	3.25	0.96
8.10	5.64	0.25	7.57	0.28	5.49	0.03	*	3.31	0.96
8.26	5.67	0.25	7.60	0.29	5.65	0.03	*	3.36	0.96
8.49	5.67	0.26	7.62	0.29	5.46	0.03	*	3.31	0.96
8.73	5.70	0.26	7.67	0.29	5.46	0.03	*	3.30	0.96
8.91	5.70	0.26	7.69	0.29	5.51	0.03	*	3.32	0.96
9.17	5.71	0.26	7.68	0.28	5.43	0.03	*	3.30	0.96
9.43	5.71	0.26	7.63	0.29	5.20	0.03	*	3.23	0.96
9.71	5.69	0.26	7.66	0.29	5.45	0.03	*	3.30	0.96
9.99	5.69	0.26	7.69	0.29	5.49	0.03	*	3.32	0.96
10.34	5.65	0.26	7.64	0.29	5.50	0.03	*	3.32	0.96
10.71	5.66	0.26	7.59	0.30	5.25	0.03	*	3.24	0.96
10.99	5.65	0.26	7.64	0.29	5.36	0.03	*	6.26	0.96
11.31	5.62	0.26	7.56	0.29	5.15	0.03	*	3.21	0.96
11.75	5.63	0.26	7.58	0.29	5.10	0.03	*	3.19	0.96
12.14	5.63	0.26	7.55	0.29	4.95	0.03	*	3.15	0.96
12.63	5.61	0.26	7.48	0.29	4.82	0.04	*	3.10	0.96
13.12	5.60	0.26	7.50	0.29	4.95	0.03	*	3.15	0.96
13.76	5.61	0.26	7.50	0.30	4.90	0.04	*	3.13	0.96
14.37	5.56	0.26	7.45	0.29	4.63	0.04	*	3.04	0.96
14.73	5.51	0.26	7.40	0.30	4.74	0.04	*	3.08	0.96
15.75	5.45	0.26	7.33	0.30	4.71	0.04	*	3.07	0.96
16.34	5.37	0.26	7.29	0.29	4.92	0.04	*	3.14	0.96
17.24	5.30	0.26	7.11	0.29	4.45	0.04	*	2.98	0.96
18.08	5.23	0.25	7.06	0.29	4.70	0.04	*	3.06	0.96
19.16	5.13	0.25	6.93	0.30	4.54	0.04	*	3.01	0.96
20.45	5.03	0.23	6.80	0.25	4.29	0.05	*	2.93	0.97

Discussion

While there have been a number of studies of cervical disc degeneration using animal models *in situ* (Laing, Cox, Tetzlaff, & Oxland, 2011; Wang et al., 2006; Chiang et al., 2011) to our knowledge our study is the first animal model designed specifically to investigate both the *in vivo* mechanical and pathological consequences of surgically created cervical spine disc lesions. The fairly consistent increase in spinal stiffness measured across 31 of 32 frequencies examined demonstrated a fairly consistent mean 34% rise in the disc lesion group. Inasmuch, while widespread disc degeneration or more severe DD grading was not observed histologically, it is evident that disc lesions induced by annulotomy and the mild degenerative changes that ensued were found to be associated with increased dynamic spinal stiffness. Stabilization of the spinal motion segments observed in the cervical spine is consistent with our *in vivo* biomechanical findings of increased among those with DD in the lumbar spine.

The dynamic frequency-dependent stiffness behavior of the animal and human spine is modulated by intrinsic viscoelasticity of component tissues (ligaments, cartilage, bone, tendons, muscle) and load sharing provided by adjacent structures (e.g., head and thorax attachments). When such factors are combined with other features such as spinal curvature, the net effect is a complex structure-frequency-dependent mechanical behavior. Common methods of exciting a structure for the purpose of dynamic mechanical analysis use periodic, transient, or random or statistical methods (Keller & Colloca, 2007). Because the spine is a viscoelastic structure, its vibratory response is frequency dependent and thus our choice of using a swept-sine testing algorithm to mechanically excite the cervical spine at a number of different frequencies to observe its response. In this study, we found that the *in vivo* DV stiffness of the ovine spine was frequency dependent and varied more than 2-fold over the 1.8 Hz to 20.4 Hz mechanical

excitation frequency range examined. During DV cervical spine mechanical stimulation at the lowest frequency (1.8 Hz) was the only non-significant group comparison and this may be related to pre-conditioning deformation as it represents the first oscillatory loading cycle in the protocol. Further research at quasi-static loading profiles may confirm this notion. In addition, randomizing the mechanical excitation frequencies as opposed to administering only the chirp type profile as performed in the current study (delivering mechanical excitation frequencies from lowest to highest) would also be helpful in further exploring differences between groups at the lowest frequency.

The advent of advances in diagnostic imaging such as MRI have provided researchers and clinicians with impressive imagery of spinal anatomy that aid in diagnosis and treatment considerations. The extent to which the DD effects the patients symptomatic or state, or function, however cannot be ascertained by MRI technology. Inasmuch, quantification of the intersegmental function of the spinal joints are limited to either *in situ* cadaveric studies or invasive measures such as implantation of steel pins into the spine's spinous processes to mount motion sensors (Kaigle, Pope, Fleming, & Hansson, 1992; Kaigle, Holm, & Hansson, 1997). The invasive nature of this experimental setup enables it to only be performed in surgical candidates and precludes overwhelming majority of the neck pain population. The non-invasive biomechanical analysis of the cervical spine presented herein has been previously validated in the lumbar spine (Colloca et al., 2009), but no such model had yet been developed for the cervical spine.

Traditional concepts for treatment cervical DD have aimed at symptomatic relief by limiting motion in the cervical spine through instrumented spinal fusion and novel treatment strategies involving stem cells, growth factors, and gene therapy have the theoretical potential to prevent, slow, or even reverse disc degeneration. Knowledge of

spine segment motion patterns, forces and stiffness is also of fundamental interest to understanding the postural, time-dependent and dynamic response of the spine, the role of spinal implants in mechanical load sharing (Keller et al., 2002). At present, treatment options for degenerative disc disease remain suboptimal, and development of outcome measures are necessary to improve the current unpredictable nature of DD.

Understanding the biomechanical basis of cervical DD is essential for the development of treatment strategies that target the underlying mechanics of disc degeneration. Such strategies ideally aim to influence spinal function and thus quantifying the force displacement response and mobility of the cervical spine are innovative from both a diagnosis and evaluation of biomechanical outcomes perspective. This knowledge gained can be used in future research including spinal modeling for improved cervical spine diagnosis and biomechanical outcomes assessments in patients with neck pain. These novel findings would enable future research on the sustainability of interventions upon the function of the cervical intervertebral disc *in vivo*.

CHAPTER 5

PART 3 – BIOMECHANICAL RESPONSE TO TREATMENT

Introduction

As previously presented, neck pain and associated disorders including headache and arm pain and/or numbness are a common worldwide healthcare problem. A large proportion of the population who receive manual therapies have some degree of disc disease (Lisi et al., 2005). To influence the peripheral pain generator, patients with discogenic disease commonly undergo spinal manipulative therapy (SMT) with primary goals of reducing pain, normalizing loads and improving spinal mobility (Burton et al., 2000). A wide range of manual techniques have been developed providing clinicians with choices of force amplitude, speed, and vector among other variables of SMT delivery in patient care. Force-time characteristics, including the applied force magnitude, speed, and/or frequency, have therefore been attributed to the underlying mechanisms of SMT (Keller et al., 2002).

Both *in vitro* (Gal et al., 1997; Maigne & Guillon, 2000) and *in vivo* (Nathan & Keller, 1994; Keller et al., 2003) biomechanical studies have examined segmental and intersegmental displacements and vibration responses during SMT, but few (if any) studies have quantified SMT-induced spinal kinematics in the degenerated IVD. Previous study in the lumbar spine by our group using a similar ovine degeneration model determined that vertebral kinematics were dependent on mechanical excitation pulse duration, and were significantly reduced in animals with degenerated discs (Colloca et al., 2007). No study to our knowledge has quantified the motion response of the cervical spine during SMT comparing normal subjects to those with DD.

In Part 3 of this study, the objective was to examine motion responses of the cervical spine during SMTs delivered at two different force-time profiles and to assess

differences among those animals in the disc lesion and control groups. We hypothesized that there would be differences in intersegmental motion in the cervical disc lesion group compared to the control group consistent with the biomechanical changes of DD.

Methods

To simulate manual and mechanical SMT force-time profiles commonly applied to the cervical spine in clinical practice (Herzog, Conway, Kawchuk, Zhang, & Hasler, 1993; Colloca, Cunliffe, Pinnock, Kim, & Hinrichs, 2009; Symons, Wuest, Leonard, & Herzog, 2012), two mechanical pulse durations (t = 10, and 100 ms) at a constant DV force (~80 N) were administered to the target level in all animals with the mechanical testing apparatus presented in Part 2 of this study. Cervical spine stiffness and motion responses were monitored during the SMTs by means of the load cell and LVDT directly attached to the actuator of the biomechanical testing apparatus previously described in Part 2 of this study, and accelerometers (described below) attached across the targeted disc in each of the animals on adjacent spinous processes. The experimental setup is shown in Figures 14 and 15. To quantify intersegmental motions during SMTs, Ten-g piezoelectric tri-axial accelerometers (Crossbow Model CXL100HF3, Crossbow Technology, Inc., San Jose, CA) were attached to intraosseous pins rigidly fixed to the

spinous processes of the target level (C2, C3, or C4) under fluoroscopic guidance. The accelerometers are high frequency vibration measurement devices that feature low noise (300-μg rms), wide bandwidth (0.3 - 10,000 Hz) and low nonlinearity (<1% of full scale) and are precision calibrated by the manufacturer. The *x*-, *y*- and *z*-axes of the accelerometer were oriented with respect to the medial-lateral (ML), dorsoventral (DV)

Figure 14. Experimental setup showing positioning of the biomechanical testing apparatus, fixed frame, and tri-axial accelerometer placement.

and cranial-caudal or axial (AX) planes of the vertebrae, respectively, and were used to quantify adjacent segment vertebral accelerations to subsequently calculate intersegmental displacements. Equipment utilized, dependent variables processed, and calculations subsequently conducted to determine the dependent variable values are shown in Table 9.

Six SMT trials of each force-time profile (DV force amplitude and duration) were administered to the superior vertebra to the target (disc lesion or control) at the levels

ranging from C2-C4 to study the cervical spine's biomechanical response among animals with disc lesions and controls. In each case an approximate 10 N preload was applied, and the order in which the SMT thrusts were performed was randomly determined. During the SMT thrusts, the applied force and DV displacement response and acceleration channels were recorded at 2500 samples/second using the Biopac MP 150 data acquisition system previously cited.

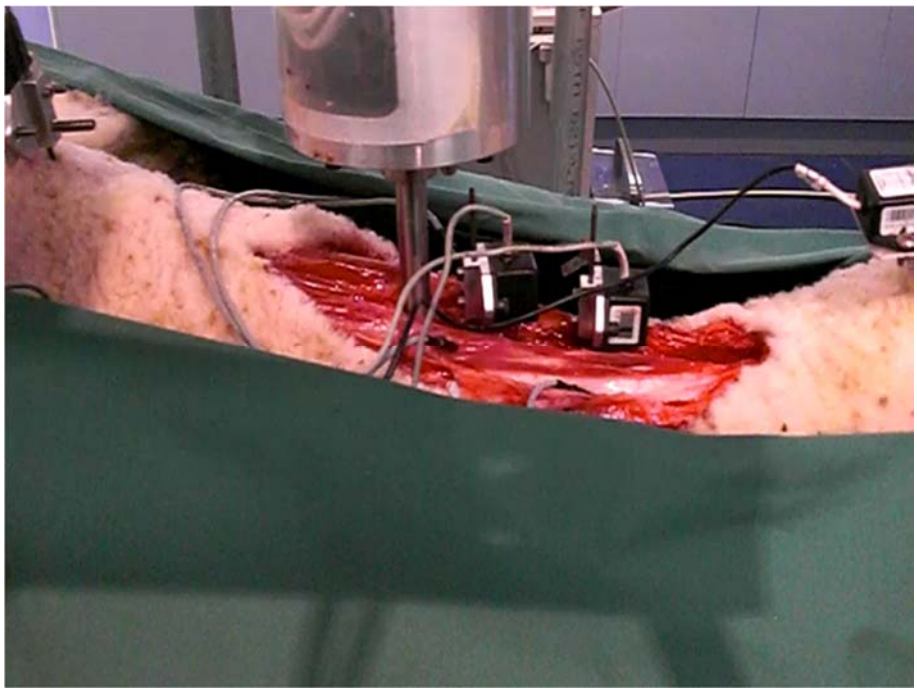

Figure 15. Experimental set-up showing segmental contact point for the actuator stylus in delivering the SMTs and adjacent segment monitoring using tri-axial accelerometers mounted to pins placed into the target spinous processes. (needle electromyographic electrodes shown are for data presented elsewhere).

Data Analysis. A custom MatLab program was used to process the load, displacement, and acceleration signals from the SMT trials. A peak detector was applied to identify peak-peak responses for each of the SMTs administered. Peak-to-peak acceleration signals were tabulated for each of the two sensors at adjacent

vertebrae. Resultant peak-to-peak intersegmental accelerations were then analyzed for the DV and axial (AX) planes for each of the trials. Intersegmental displacements were calculated from the acceleration time histories using trapezoidal numerical integration. Resultant peak-to-peak intersegmental displacements were derived for the DV and axial (AX) axes at each mechanical excitation frequency for each of the trials and compared among groups. Data analysis of the dependent variables for evaluating the biomechanical response to treatment herein Part 3 of this study are shown in Table 9. In this manner, the biomechanical effect of the cervical disc lesion on the cervical spine's motion response was evaluated by comparing the disc lesion and control groups using a 2 x 6 x 2 repeated measures analysis of variance (ANOVA). SMT type (10 ms vs. 100 ms) and trials served as the within-subjects factor whereas disc lesion versus control served as the between-subjects factor. Interaction effects were evaluated to determine the combined effects of factors on the dependent measure. Statistical significance was set at $p < 0.05$.

Table 9. Equipment utilized, dependent variables processed, and calculations subsequently conducted to determine the dependent variable values are shown. Force and displacement at the target level contacted by the instrumented stylus of the mechanical testing apparatus was obtained while simultaneous intersegmental acceleration data are collected and dependent values calculated.

Equipment	Dependent Variables Processed	Dependent Variables Calculated
Actuator Load Cell	Force (N)	Stiffness (N/mm)*
Actuator LVDT	Displacement (mm)	
Accelerometer	C3 DV Acceleration (m/s^2)	C3 DV Displacement (mm)
Accelerometer	C3 AX Acceleration (m/s^2)	C3 AX Displacement (mm)
Accelerometer	C4 DV Acceleration (m/s^2)	C4 DV Displacement (mm)
Accelerometer	C4 AX Acceleration (m/s^2)	C4 AX Displacement (mm)
		C3-C4 DV Acceleration Transfer (m/s^2)
		C3-C4 DV Displacement (mm)
		C3-C4 AX Acceleration Transfer (m/s^2)
		C3-C4 AX Displacement (mm)

Results

Actuator Displacements and Stiffness. For spinal displacements obtained at the actuator (at the segmental contact point of force application) there was a significant effect, $F(1,28) = 4.29$, $p = 0.048$, where greater mean displacements were observed for the control animals (6.93 mm, S.D. = 0.33 mm) compared to those with disc lesions (5.50 mm, S.D. = 0.25 mm) (Figure 16). No significant interactions were identified from this analysis. When considering the applied force into the equation of calculating stiffness ($k = F/d$), however, no significant difference in spinal stiffness was observed between the disc lesion and control groups.

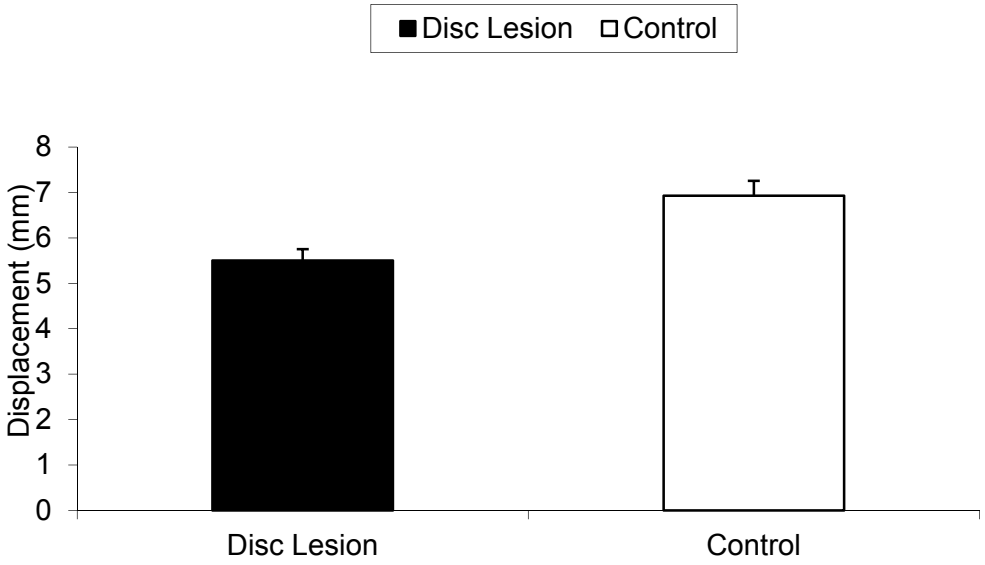

Figure 16. Comparison of mean cervical spinal displacements (mm) during all SMTs among disc lesion and control groups.

Studying the effect of SMT force-time profile on spinal displacements, there was a significant effect of SMT thrust type on displacement, $F(1,28) = 133.00$, $p = 0.001$, where 100 ms SMTs resulted in significantly greater mean spinal displacements than 10 ms thrusts, 9.29 (S.D. = 0.26) mm and 3.14 (S.D. = 0.07) mm, respectively (Figure 17). No

significant interactions were identified in this analysis indicating that force-time profiles cause different displacements in both control and disc lesion animals.

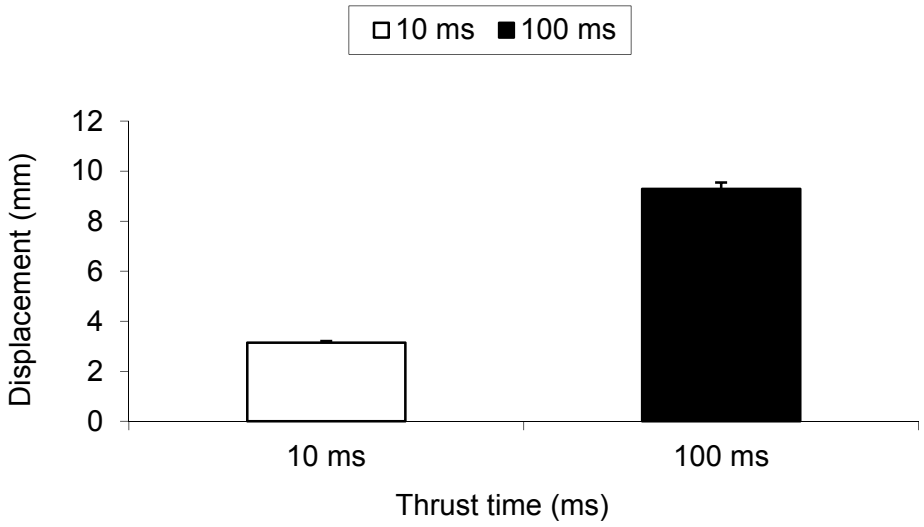

Figure 17. Comparison of SMT thrust type on mean cervical spine displacements.

Stiffness calculated from SMT thrusts also showed a significant effect of SMT force-time profile on spinal displacement, $F(1,28) = 103.97$, $p=0.001$. Ten ms thrusts resulted in greater stiffness (7.11 N/mm, S.D. = 0.22 N/mm) than 100 ms thrusts (4.29 N/mm, S.D. = 0.14 N/mm) (Figure 18). There was no significant interaction between the force-time profile and disc lesion status.

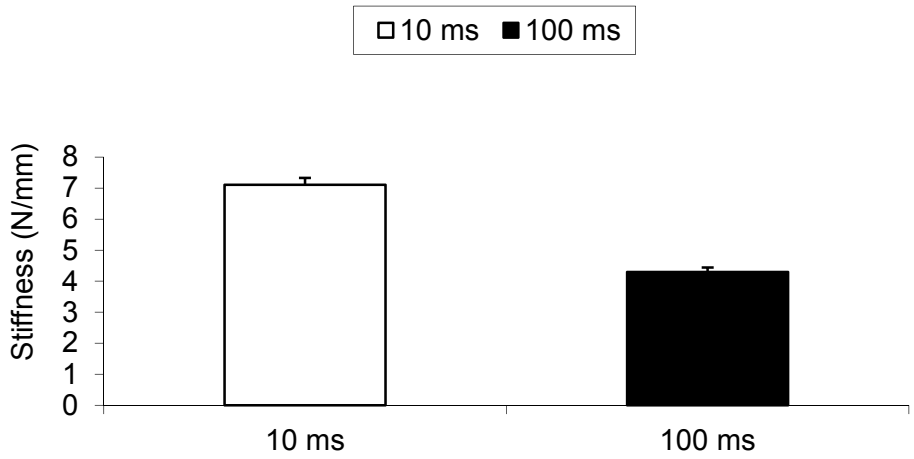

Figure 18. Comparison of SMT thrust type on mean cervical spine stiffness.

Dorsoventral Plane Accelerations and Displacements. Considering intersegmental motion response in the DV plane, data obtained from the superior mounted accelerometer revealed a significant effect of disc lesion upon acceleration response, $F(1,28) = 7.63$, $p=0.01$. (Figure 19). No significant interaction was noted indicating significantly increased accelerations observed among both SMT force-time profiles. No difference in accelerations were observed for the inferior mounted accelerometer among the disc lesion and control groups.

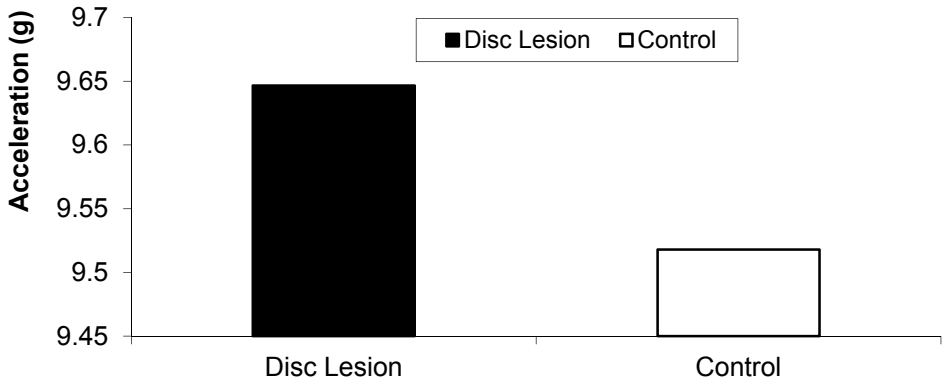

Figure 19. Group mean comparison of DV accelerations obtained from the superior accelerometer during SMTs.

In the DV plane, data recorded from both the superior and inferior mounted accelerometers revealed significant increases in spinal acceleration for 100 ms SMTs compared to 10 ms SMTs (p=0.001) (Table 10). Because no interaction was observed this difference was not affected by disc lesion grouping.

Table 10. Mean dorsoventral (DV) acceleration response for the superior (Sup) and inferior (Inf) mounted accelerometers (Accel) and repeated measures ANOVA results for within group comparisons of SMT force-time profile.

Accel	10 ms		100 ms		F	p
	Mean	S.D.	Mean	S.D.		
Sup DV	9.38	0.02	9.78	0.005	62.21	0.001
Inf DV	9.26	0.018	9.76	0.006	137.07	0.001

Displacements calculated from the superior and inferior mounted accelerometers revealed significantly increased motions for the 100 ms SMTs (M = 2.77 mm, SD = 0.10 mm), F(1,28) = 32.2, p=0.001, and (M = 3.88, SD = 0.13 mm) F(1,28) = 50.82, p0.001, respectively (Figure 20). No significant differences were observed between groups for displacements derived from the accelerometers evaluated individually or combined.

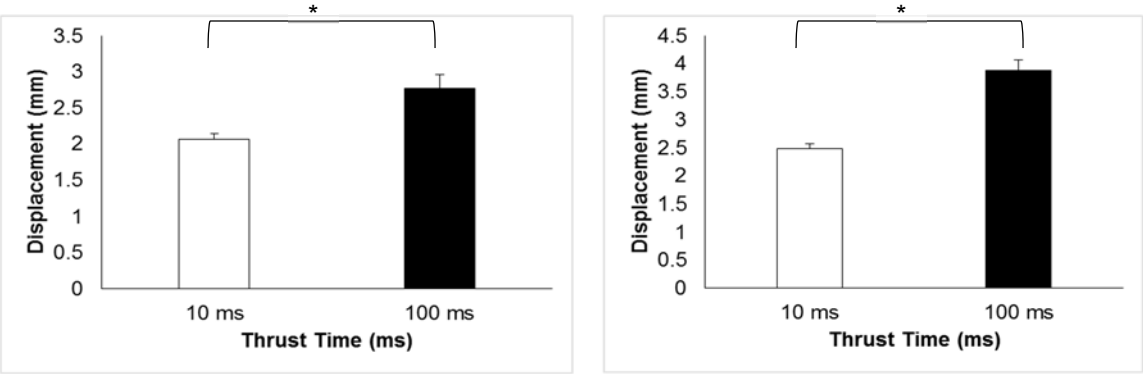

Figure 20. Mean displacements derived from the superior (left) and inferior (right) accelerometers comparing motion responses of 10 versus 10 ms SMT force-time profiles.

Axial Plane Accelerations and Displacements. No significant differences were observed when comparing the effect of disc lesion on SMTs in the Axial (AX) plane. Similar to the DV plane, data recorded from both the superior and inferior mounted accelerometers revealed significant increases in AX spinal accelerations for 100 ms SMTs compared to 10 ms SMTs (p=0.001) (Table 11). No significant interaction was noted. When acceleration transfer was calculated no significant differences were observed for any of the accelerometer planes between or within-groups.

Table 11. Mean axial (AX) acceleration response for the superior (Sup) and inferior (Inf) mounted accelerometers (Accel) and repeated measures ANOVA results for within group comparisons of SMT force-time profile.

Accel	**10 ms**		**100 ms**		**F**	**p**
	Mean	**S.D.**	**Mean**	**S.D.**		
Sup AX	8.38	0.053	9.7	0.007	113.23	0.001
Inf AX	8.42	0.058	9.8	0.006	101.36	0.001

Displacements calculated from the superior mounted accelerometer in the AX plane revealed significantly increased motions for the 100 ms SMTs (M = 3.63 mm, SD = 0.186 mm), F(1,28) = 12.53, p=0.001 (Figure 21). No significant differences were observed for displacements derived from the inferior mounted accelerometer, or between groups for displacements derived from the accelerometers evaluated individually or combined.

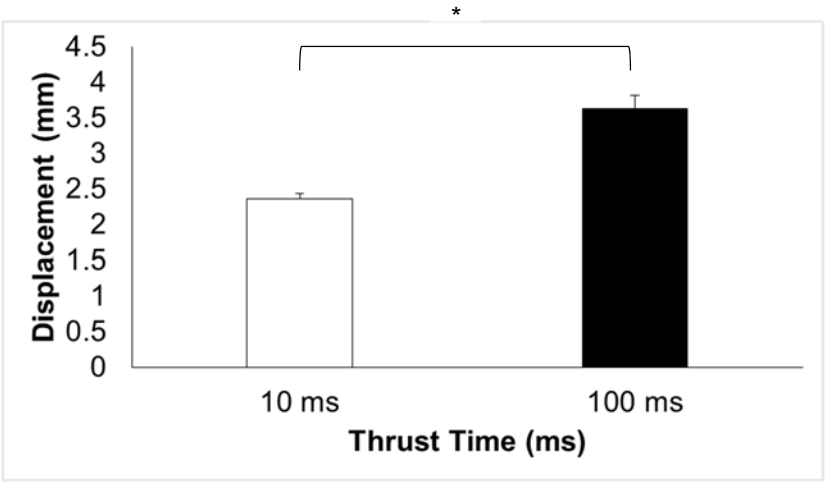

Figure 21. Mean displacements derived from the superior accelerometer comparing motion responses of 10 versus 10 ms SMT force-time profiles.

Discussion

At a minimum of five months follow-up, localized mild degenerative changes in the annulus fibrosis of the ovine cervical spine were identified in the disc lesion group that were significantly different than the control group. Although the model developed in Part 1 of the current study did not progress to widespread moderate or severe degenerative changes in the IVD, the annular degeneration observed in the disc lesion animals produced a model that allowed comparisons between disc lesion and control groups that were significantly different histologically enabling us to examine the dynamic stiffness of the cervical spine and compare differences among groups and further understand the cervical spine's motion response during treatment in the presence of DD. Indeed, the ovine cervical disc lesions were found to be associated with an increase in stiffening properties of the spine to DV forces that have also previously been reported in the lumbar spine (Colloca et al., 2007; Colloca et al., 2008). Decreased DV spinal displacements identified in the disc lesion group during the 10 ms and 100 ms SMTs are consistent with the increased stiffening reported in Part 2 of this study. The fact that the

control animals had significantly larger DV spinal displacements than those with disc lesions suggests differences in how the spine moves during manual interventions that are commonly administered to patients with or without DD, an important consideration for clinicians. Because patients with cervical DD comprise an appreciable number of patients presenting for manual therapy or chiropractic care, based on the knowledge gained herein, augmenting or decreasing applied forces or alternatively increasing the speed of applied SMTs are ways that clinicians can overcome the biomechanical consequences of cervical DD. Applying spinal manipulative forces that maximize the biomechanical motion response while minimizing the applied force to accomplish the intervention is prudent from a safety standpoint and efficient. Further research to explore these variables are necessary to better understand the clinical utility of augmenting forces to improve patient care which include both clinical outcomes and patient satisfaction.

Noteworthy were the differences in spinal acceleration response observed in the types of SMTs administered according to varying the speed of the impulse in the force-time profile. To this extent, we evaluated faster (10 ms) thrust, analogous to impulsive thrusts delivered by hand-held spinal manipulation devices (Keller et al., 2006), and longer duration (100 ms) thrusts that are commonly administered in manually delivered spinal manipulation to the cervical spine (Symons et al., 2012). Consistent with the greater energy delivered to the spine with the 100 ms thrusts, consistently, longer duration SMT force-time profiles were found to cause larger accelerations for both the DV and AX planes for both accelerometers used for recording in this study. The fact that larger accelerations and displacements were identified from the superior accelerometer in the cervical spine disc lesion group is consistent with previously reported findings of changes in spinal motion response resulting from SMT in the presence of DD in the

lumbar spine (Colloca et al., 2007; Colloca et al., 2012). Because the SMTs were delivered perpendicular to the spine it was expected that DV thrusts would differ between groups similar to the increases in dynamic spine stiffness observed in the disc lesion group observed in Part 2 of this study. The lack of significant differences between the disc lesion and control groups for axial plane accelerations may be due to their off axis coupling. The axial (caudal-cranial) plane in the current study represents a secondary or coupled plane of motion as opposed to the main DV plane only about 30 degrees offset axially of applied SMT force. Further research in applying SMT forces focusing more upon the axial plane may reveal differences between groups.

This is the first in vivo study demonstrating differences in vertebral kinematics among specimens with cervical spine disc lesions, an important finding for clinicians. Clinicians practicing SMT cognitively and kinesthetically gauge the amount of force they deem appropriate for a particular patient or condition based upon biomechanical (i.e., anatomical) and clinical (i.e., pain tolerance) variables alike. Knowledge that degenerated or ankylosed functional spinal units will undergo substantially less posteroanterior motion for a given spinal manipulative force as demonstrated in the current study provides clinicians with important biomechanical information that can be considered in clinical practice. Altering chiropractic technique application in the presence of identified cervical DD to utilize mechanical advantages such as increased speed of SMT may be helpful in optimizing the motion response of the cervical spine as a clinical outcome. Understanding that degenerative changes of the cervical spine are associated with increased spinal stiffness and reduced spinal motion responses should be taken into account in expectations for biomechanical outcomes for both patients and clinicians as well.

Measurement of vertebral movement using intra-osseous pins equipped with accelerometers (Keller et al., 2003; Colloca, Keller, & Gunzburg, 2004; Nathan & Keller, 1994) and other invasive motion measurement devices (Kaigle et al., 1997; Kaigle et al., 1992) has been previously shown to be a precise measure of spine segmental and intersegmental motion, but invasive procedures currently have limited clinical utility. Noteworthy, however, was our finding that increases in spinal accelerations in animals with disc lesions and longer duration force-time profiles were able to be observed biomechanically. The ability to non-invasively detect biomechanical changes in degenerated discs *in vivo* (without ionizing radiation) using an indenter over the spinous processes may have implications for the development of quantitative biomechanical spinal assessment strategies (Colloca et al., 2009).

It is important to point out that the animals examined in this study were anesthetized, which may have altered the mechanical responses (force-displacement) slightly. Most likely any effects of muscle tone on the force-displacement response of the spine were minimal, however, as the ligamentous spine is a highly damped structure (Keller et al., 2002). Other work (Keller, Colloca, Harrison, Moore, & Gunzburg, 2006) indicates that, while sustained supramaximal muscle stimulation increases the dorsoventral stiffness (force/displacement) of the ovine spine up to two-fold, the effects of low amplitude muscle stimulation were much less dramatic (less than 5% increase in DV stiffness). Therefore, the absence of muscle tone should have a minimal effect on the mechanical responses reported in this study.

The creation of spinal lesions in animal models consistent with clinically relevant spinal disorders are important first steps in understanding our ability to identify the functional status, and clinical correlates of the lesion and/or targets for spinal

manipulation or other types of treatments. Further work examining the dynamic mechanical response of the normal and degenerated cervical spine as well models of other spinal disorders will assist in the understanding both the etiology of cervical spinal disorders and putative effects of spinal manipulative therapy among different patient populations presenting for diagnosis and treatment of the neck. The ability to detect biomechanical differences in the presence of cervical spine disc lesions are an important first step in objectively discriminating spinal disorders. Further work aimed towards understanding the relationships between cervical DD, SMT, and disabling conditions such as neck pain and related disorders will serve to assist in better managing this patient population.

CHAPTER 6

GENERAL CONCLUSION

In this study, a novel ovine model of cervical intervertebral disc degeneration was developed and found to create significant localized mild degenerative changes in the residing annulus fibrosis tissue at the targeted spinal level at a minimum of 5 months following IVD scalpel injury. The disc lesions created were not found to progress to moderate-severe degenerative changes or to extend throughout other regions of the IVD as has been previously demonstrated in models of the ovine lumbar spine. Consequently, the overall disc degeneration grading system utilized herein (Gries et al., 2000) did not yield significant differences between the disc lesion and control groups in this ovine cervical spine degeneration model. Further complicating our group comparisons was the finding of mild degenerative changes in areas of the IVD in the control group who had received a sham neck surgery. These results clearly point to differences in utilizing the ovine cervical spine versus its lumbar spine as a model for DD. Notwithstanding, to our knowledge, this is the first ovine cervical spine DD model to be developed and evaluated biomechanically in vivo. The cervical spine DD model herein provided the ability to examine the in vivo dynamic stiffness of the cervical spine and spinal motion response of the cervical spine during spinal manipulative treatments in fulfillment of the study's aims.

Unique to this study design was the aim to specifically to investigate both the *in vivo* mechanical and pathological consequences of surgically created cervical spine disc lesions. The fairly consistent increase in spinal stiffness measured across 31 out of 32 of the frequencies examined demonstrated a fairly stable and approximate 34% mean increase in the disc lesion group. Inasmuch, while widespread disc degeneration or more severe DD grading was not observed histologically, it is evident that the localized

changes in the cervical spine annulus fibrosis in the animals with disc lesions were found to be associated with increased dynamic spinal stiffness across a wide range of mechanical excitation frequencies. The nearly 2-fold increase in *in vivo* DV stiffness of the ovine cervical spine is consistent with the spine's frequency dependence and necessity to challenge the spine at a range of mechanical excitation frequencies to comprehensively examine its biomechanical response. If confirmed in humans, these results likely provide a methodology to clinically evaluate the biomechanical function of the cervical spine in patients DD and associated neck pain and related spinal disorders, a leading cause of disability worldwide.

Likewise, the development of a new ovine model of cervical intervertebral disc degeneration enabled for the first time the ability to examine the motion response of the cervical spine during manual treatments commonly delivered by clinicians, namely spinal manipulative therapy. Decreased DV spinal displacements identified in the disc lesion group during the 10 ms and 100 ms SMTs are consistent with the increased stiffening reported in Part 2 of this study. Not only do disc lesions in this ovine cervical spine DD model increase the stiffness of the spine, they also influence the spine's kinematic response during treatment. The fact that the animals in the control group had significantly more spinal displacements than those with disc lesion group suggests differences in how the spine moves during manual interventions that are commonly administered to patients with or without DD, an important consideration for clinicians. Augmenting or decreasing applied forces or alternatively increasing the speed of applied SMTs are ways that clinicians can overcome the biomechanical consequences of cervical DD that may improve the safety, efficiency, and patient satisfaction in patient care in this population.

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APPENDIX A

ANIMAL USER PERMITS AND ANIMAL ETHICS APPROVAL

ANIMAL USERS PERMIT

THE INSTITUTE OF MEDICAL & VETERINARY SCIENCE

CENTRAL NORTHERN ADELAIDE HEALTH SERVICE

ANIMAL ETHICS COMMITTEE

IMVS

Associated Organisations: RAH
TQEH
University of South Australia
Other companies/organisations

Organisation	IMVS	Issued By Division	TISSUE PATHOLOGY/ ACSR	Phone No.	23645	Permit No.
Surname	COLLOCA	Given Name	CHRISTOPHER	Title	DR	Email

Animal Intervention for which you request a permit.
Delete where not applicable:

(a) Peripheral venepuncture of small laboratory animals, for collection of blood required for assay, routine laboratory tests, or research programs approved by the AEC.

(b) Injection (S/C, I/Cr, I/V, or I/P) under appropriate anaesthesia or restraint, of drugs/substances as approved by the AEC.

(c) Other specific procedures: eg. surgery, tumour transplants, techniques associated with wildlife studies etc.
1)Spinal surgery, spinal manipulation and monitoring
2)
3)

Experience with these procedures: .....

PRINCIPLES

The underlying principle of the following guidelines is that the lives and welfare of animals, especially vertebrate animals, should be treated with respect and care. These guidelines cover all research in which animals have to be either killed or experimented upon. Although it is not possible to specify in advance precisely when the use of animals is justifiable, the impact of a proposed experiment on the lives and welfare of animals should be taken into account in deciding whether the proposal is of sufficient importance to be carried out. Accordingly, experiments on animals should not be performed except to seek knowledge that is new and significant, or to achieve essential objectives that cannot be gained in any other way. Experiments using animals should be conducted only when the aims of the research cannot be achieved by methods or techniques other than animal experimentation, and if animals are to be used for training/teaching purposes the educational objectives must be clearly stated and the lack of suitable alternatives to animal use must be justified.

1. At all times, animals used in experiments must be housed in appropriate conditions, and fed, watered, cleaned, handled and transported in a fashion least likely to cause stress or discomfort, and in accordance with accepted international standards.
2. All experiments on animals, surgical and non-surgical, must be carried out in a humane manner. Procedures likely to cause pain of more than trivial extent must not be carried out without the administration of an anaesthetic, the use of adequate and appropriate analgesia a suitable for that species, until the procedure is terminated.
3. In experiments not involving surgical interference or painful procedures requiring anaesthesia, every care should be taken to minimise the degree of discomfort or stress to which animals are subjected.
4. Experiments, conducted under anaesthesia, which involve serious disturbance of, or interference with, structure or function must be terminated while the animal remains anaesthetised. Any animal which is the subject of a recovery experiment must be killed painlessly if undue pain and suffering cannot be relieved quickly and adequately.
5. Experiments using animals should be designed to keep the numbers of animals to a minimum.
6. All animals used for experiments must be lawfully acquired, and their retention and use must be in strict compliance with the Code and State laws and regulations.
7. Wildlife studies, either observational or mildly interventionist (eg. capture and release) must be carried out in a way which minimises the impact on the animals, and the AEC needs to be given the opportunity to understand the methodology employed by such studies.

Copies of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004) are held by Heads of Divisions. Additional copies are available from the Head, Veterinary Services Division.

Permit holders must familiarise themselves with this Code.

I certify that the above applicant has received training* in the above techniques and in my opinion is competent to perform the procedures.	**Signed by Applicant** ..... **Date:** .....
Signed: ..... **Head of Division/Department of :**	**Animal Ethics Committee Executive Officer:** **Signed:** ..... **Date:** .....

* Training in particular techniques should be arranged through the Supervisor, Animal Care Facility Ph. 8222 3524

IMVS 113/F

IMVS

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Email: carol.hewitt@imvs.sa.gov.au

22nd July, 2008

24 JUL 2008

A/Prof Robert Moore
The Adelaide Centre for Spinal Research

APPLICATION FOR ANIMAL ETHICS APPROVAL

I am pleased to advise that your Project entitled 'Biomechanical Evaluation of Conservative and Surgical Spinal Interventions' has been given approval by the I.M.V.S./Central Northern Adelaide Health Service, Animal Ethics Committee for the period 10/07/08 to 31/01/09 with the proviso that the answer to Q3 be amended to include transport of the sheep to and from Gilles Plains and Frome Road. Housing at the Gilles Plains facility should be mentioned in the answer to Q20.4.

Total number of animals approved : Sheep Merino-cross 30

Project number 87/08 has been assigned to this application and should be used on all correspondence, animal orders and cage identification associated with this Project. Your animal user's permit number should also be quoted when ordering animals for this study.

It will be necessary for you to complete annually a brief progress report form for the purpose of review by the Committee and collation of statistical data for the responsible Minister.

I am obliged to point out that if it becomes apparent that the Project will continue for a longer period than is covered by this approval it will be necessary for you to seek a time extension from the AEC. Furthermore, if experience gained by yourself or others during your project demonstrates that the pain category or any other aspect of animal welfare is in fact different from that anticipated in your application form, the AEC must be informed at the earliest possible time.

I have enclosed a copy of the application, signed by the Chairman of the Animal Ethics Committee for your records.

Yours sincerely,

Advised all participants 25/7.

BHewitt

Carol Hewitt
Secretary
IMVS/Central Northern Adelaide Health Service
Animal Ethics Committee

APPENDIX B

ANIMAL MONITORING

Post-Operative Pain Scoring (General Observations)

Observation	**Score**	**Criteria**
Comfort “over the fence observation”		
	0	Awake, interested in surroundings, patient recumbent or standing ruminating (chewing cud). Eating
	1	Awake, standing or recumbent, not interested in surroundings, not chewing cud. Reduced appetite.
	2	Lethargic, depressed appearance, ears dropped, not chewing cud. Anorexia.
	3	Head down, very lethargic (ears stay drooped, not chewing cud. Teeth grinding (bruxism)
	4	Recumbent (no response when approached), fixed look and staring or eyes half closed, little response when gently prodded. (Note: such a case should be euthanized). Teeth grinding (bruxism).
Movement (following orthopedic procedures)		
	0	Normal ambulation, full weight bearing, no lameness.
	1	Slight lameness on operated limb, touching toe on all steps.
	2	Lameness on operated limb, touching toe on some (but not all) steps.
	3	Lameness on operated limb, not touching toe on all steps when walking voluntarily but will touch toe if herded. If an animal falls into this category, additional care may be necessary.

	4	Lameness on operated limb, not touching toe on all steps when walking voluntarily and even when herded. (If this degree of lameness continues despite treatment, such a case should be euthanized.
Flock Behavior		
	0	Normal (Moves with rest of flock)
	1	Mild changes: (lethargic or lags behind rest of flock if flock is moved but eventually voluntarily joins them.)
	2	Moderate changes: (lags behind rest of flock if flock is moved but eventually joins them if encouraged to do so)
	3	Severe changes: (No interest in rest of flock, always separated from them) (Note: such a case should be euthanized if there is no response to treatment.
Feeding Behavior / Appetite		
	0	Normal (Up at the feed bunk/trough with rest of the flock)
	1	Mild changes: (lags behind rest of flock if flock is moved but eventually voluntarily joins them)
	2	Moderate changes: (lags behind rest of flock if flock is moved but eventually joins them if encourage to do so, tolerates jostling)
	3	Severe changes: No interest in rest of flock, always separated from them. (Note: such a case should be euthanized if there is no response to treatment)

Observation	Score	Criteria
Respiration Rate (in shade)		
	0	Normal respirations compared to normal sheep in the pen.
	1	Noticeable increase in rate compared to normal sheep in pen, Such cases should be checked for infectious disease and treated if necessary.
	2	Hyperventilation compared to normal sheep in pen. Such cases should be checked for infectious disease and treated if necessary.
	3	Hyperventilation (with mouth breathing). Such cases should be checked for infectious disease and treated if necessary.
Total Maximum Score	17	

Criteria for euthanasia following general observations	
Level for continued treatment	> 6
Euthanasia Endpoints	a) ≥ 12 for more than 24 hours
	b) ≥ 9 for 48 hours

Postoperative Pain Scoring (Specific for spine surgery)

All animals undergoing spinal surgery will have a neurological examination by a veterinarian immediately postoperatively and then as required. Thereafter, animals were observed daily. Animals with a score of 3 or greater, were housed indoors and receive

physical therapy three times daily. Food and water were placed in close proximity at all times.

Neurological assessment following spinal surgery.

Score	**Criteria**
0	Walking w/o any detectable ataxia. Able to rise unassisted.
1	Walking, slightly ataxic on one hind limb. Able to rise unassisted.
2	Walking, slightly ataxic on both hind limbs. Able to rise unassisted.
3	Can rise with assistance but able to walk
4	Can rise with assistance and can stand but unable to walk.
5	Recumbent and unable to stand.
6	Recumbent and unable to stand. No voluntary movement of the hind limbs and unresponsive to deep pain.

Criteria for euthanasia following neurological assessment	
Level for continued treatment	1 or 2
Euthanasia Endpoints	a) 6 > 4 hours
	b) 5 > 2 days
	c) 3 or 4 > 3 days