The aging spine: The effect of cyclic loading, simulated degeneration and prolonged sitting on joint stiffness across age

by

Kristina May Gruevski

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# Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

<table>
<thead>
<tr>
<th>Role</th>
<th>Name</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>External Examiner</td>
<td>Dr. Greg N. Kawchuk, BSc, DC, MSc, PhD</td>
<td>Professor, Physical Therapy</td>
</tr>
<tr>
<td>Supervisor</td>
<td>Dr. Jack P. Callaghan, BPHE, MSc, PhD</td>
<td>Professor, Kinesiology</td>
</tr>
<tr>
<td>Internal Member</td>
<td>Dr. Steven L. Fischer, BSc, MSc, PhD</td>
<td>Assistant Professor, Kinesiology</td>
</tr>
<tr>
<td>Internal Member</td>
<td>Dr. Richard P. Wells, BSc, MEng, PhD</td>
<td>Professor Emeritus, Kinesiology</td>
</tr>
<tr>
<td>Internal-external Member</td>
<td>Dr. Philip L. Bigelow, BSc, MHSc, PhD</td>
<td>Associate Professor, Public Health and Health Systems</td>
</tr>
</tbody>
</table>
Author’s Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.
Statement of Contributions

Study I: Study design, data collection, analysis and written summary were completed by Kristina Gruevski. Dr. Jack Callaghan contributed to the study design, analysis approach and written summary. Mamiko Noguchi contributed to approaches used in the study design and data collection. Dr. Chad Gooyers contributed to the data analysis approach.

Study II, III, IV: Study design, data collection, analysis and written summary were completed by Kristina Gruevski. Dr. Jack Callaghan contributed to the study design, analysis approach and written summary.
Abstract

**Background:** Low back pain is estimated to have a lifetime prevalence as high as 84%, and both the severity and frequency of low back pain reporting have a dependency on age. The nucleus pulposus and annulus fibrosis of the intervertebral disc undergo significant structural and compositional changes with increases in age. As the Canadian working population ages, an understanding of mechanical properties of spine tissue across age is needed to understand pain generating pathways and functional changes. The aim of this thesis was to determine if spine stiffness changes with age and to determine how the mechanical properties of the osteo-ligamentous spine and the annulus contribute to these changes in different loading scenarios. The thesis implemented both *in-vitro* (Studies I and II) and *in-vivo* (Studies III and IV) approaches to meet the objectives of the global thesis question.

**Study I:** The effect of age and a cyclic loading protocol on the stiffness in porcine functional spine units (FSUs) was explored in study I. A total of 40 FSU specimens, with 21 young (aged 6-8 months) and 19 mature (aged 1.5-8 years) were cyclically loaded at 1 Hz to a range of motion of 8.5 degrees in flexion and extension around the midpoint of each specimen’s neutral zone for 3000 cycles with 1400 N of compression. Neutral zone stiffness was reduced in all specimens following the cyclic loading protocol, indicating no significant differences in temporal responses to repetitive loading across age. However, mature specimens were found to have greater neutral zone stiffness at both the C34 and C56 levels compared to younger specimens. This baseline differences between older and younger spines may alter load distributions in the disc and predispose mature discs to different types of injuries compared to younger specimens.

**Study II:** The aim of study II was to isolate stiffness changes in isolated samples of the annulus in response to simulated aging. Low pH in the disc caused by lactic acid has been linked with cell death in the nucleus, discogenic pain and is a hypothesized initiator of disc degeneration. A total of 79 multilayer samples of porcine annulus fibrosis tissue obtained from young (aged 6-8 months) spines were immersed in one of four pH and concentration controlled solutions of lactic acid in phosphate buffered saline (PBS) for a duration of 6 hours. The solutions included: (i) pH 7.2 PBS, (ii) pH 3.5 Lactic acid in PBS (15 mmol/L), (iii) pH 6 Lactic acid in PBS (15 mmol/L) or (iv) pH 7 Lactic acid in PBS (15 mmol/L). Following immersion, Specimens were biaxially loaded in tension in both the circumferential and axial directions to 20% strain at a rate of 2%/cycle for 100 cycles. The results of the study showed that circumferential peak stress was significantly higher in C56 specimens immersed in pH 3.5 solution compared to other solution groups. Circumferential stiffness was higher in the C56 specimens in a low pH 3.5 environment compared to the other solution groups. Exposure to a low pH environment altered the mechanical properties of the annulus fibrosis, including higher peak stress and increased stiffness. These changes demonstrate that the annulus is a contributor to increased spine stiffness.
changes with age. Furthermore, discs with accumulated lactic acid also have an altered mechanical environment that could put older discs at greater risk of annulus damage, such as delamination or fissures in the tissue.

Study III: The purpose of study III was to determine the effect of age on lumped passive trunk stiffness, postures and discomfort responses during prolonged seated exposures. Participants in Studies III and IV were collected in the same session and shared a common cohort of 34 participants across younger and older age groups, with average (standard deviation) ages of 23.8 (5.0) years and 63.7 (3.9) years, respectively. Passive torso stiffness was measured in flexion before and after sitting continuously (90 minutes) while completing a controlled task on a desktop computer. Discomfort was reported to be higher among older adults in the neck, right shoulder and middle back regions during the prolonged sitting protocol compared to younger adults. There were no significant differences in passive torso stiffness between older and younger adults in flexion postures representing 10%, 20% and 30% of maximum. However, during the sitting protocol, younger adults adopted 19 degrees more flexion compared to older adults. Differences in seated postures across age may be explained by changes to passive tissues in older adults that affect the end range of functional motion, which may have implications for acute pain development during sitting.

Study IV: The aim of study IV was to determine the effect of participant age, prolonged sitting and lift type on peak thoracic, lumbar, hip and knee postures and ratings of perceived effort. A secondary purpose was to quantify the effect of age on baseline lumbar range of motion about the mediolateral axis. All lifting tasks were floor to knuckle lifts and included, (i) 7 kg symmetrical, (ii) 4.5 kg symmetrical and (iii) 4.5 kg asymmetrical (box located 45 degrees to participant right). Lifting tasks were completed before and after the prolonged sitting protocol. The results of the study demonstrated lower peak lumbar flexion angles following 90 minutes of continuous sitting compared to prior to sitting. While there was no age-related difference noted in response to the prolonged sitting protocol, reduced peak flexion during the lifting tasks following sitting could represent swelling of the intervertebral disc in response to static sitting. Older adults adopted 12 degrees less lumbar flexion during the performance of all lifting tasks compared to younger adults. Older adults had reduced maximum range of motion about the mediolateral axis in the flexion direction compared to younger adults. However, when peak lumbar angles during lifting were expressed as a percentage of maximum flexion, angles were similar between groups with an average 71% and 65% among young and mature participants respectively. This could indicate that functional range of motion in the spine is reduced in older adults, with high flexion tasks entering a zone of higher stiffness.

General Conclusions: Together, the findings from this thesis indicate that osteo-ligamentous functional spine units and the annulus increase in stiffness with age providing a mechanistic understanding of age-related mechanical changes to disc tissue. These changes may partially contribute to the reduction in maximum range of low back motion observed in older adults. Lumped passive stiffness was not significantly different at low flexion postures, but, maximum
range of spine motion and peak flexion angles during high flexion tasks were reduced with increasing age. Higher stress in the posterior annulus of aged specimens could predispose older adults to greater risk of annulus disruption and could be a potential source of discogenic low back pain.
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Dedication

This thesis is dedicated to Elizabeth Gruevski. Your passion for education and lifelong learning continues to inspire me!
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1. Introduction

1.1 Overview

Both the number of working years and the average chronological age (in years) of Canadian workers are increasing. The proportion of Canadians aged over 55 years has increased from 22% to 32% between 1976 and 2010 (Carrière & Galarneau, 2011). This demographic change in Canada and other industrialized nations may partially explain the aging workforce. The employment of Canadian adults aged 55 years and older has increased between 1997 and 2010; among men from 30.5% to 39.4% and among women from 15.8% to 28.6% (Carrière & Galarneau, 2011). Similar trends have been reported among American civilian (non-military) workers where the labour force participation of those over 55 years was 13.1% in 2000, increasing to 19.5% in 2010 and is projected to represent 25.2% of the labour force by 2020 (Toossi, 2012). In December of 2006, the Ontario Human Rights code was revised to end mandatory retirement in the province of Ontario (OHRC, 2015). At the other end of the working age spectrum, the minimum age to perform part-time work in Ontario is 14 years (MOL, 2015). For some members of the population, work life will begin at an early age and it is unclear for how long it will continue given the demographic shifts of the working population. Furthermore, it is unclear how increased cumulative exposures in combination with biological changes due to aging will alter work capacity and risks of work related musculoskeletal injury.

Low back injuries represent a substantial proportion of lost time claims and low back pain is experienced by a majority of the population. In 2014, 17% of all injury claims in Ontario involved the low back, representing the greatest proportion of total claims for any body location.
(WSIB, 2014). The leading demographic group most affected by low back injuries were 45 to 49 year old males (WSIB, 2014). Low back pain is estimated to have a lifetime prevalence between 59-84% (Papageorgiou et al., 1995; Cassidy et al., 1998). There is also evidence of an age-dependency in low back pain reporting. A systematic review of non-specific neck and back pain among adults reported that with increases in chronological age, the prevalence of mild pain decreased but the prevalence of severe back pain increased (Dionne et al., 2006). The point prevalence of low back pain (previous 1 month) was shown to have a peak in reporting among male and female respondents between the ages of 45 to 59 years (Papageorgiou et al., 1995).

1.2 Neutral zone and age-related changes

In 1992, Panjabi described a region of joint displacement characterized by high flexibility and joint laxity around a neutral position called the neutral zone (Panjabi, 1992a; Panjabi, 1992b). The author hypothesized that this quantity of displacement was a superior metric to characterize joint stability compared to range of motion in a joint (Panjabi, 1992b). In the case of an unstable joint, the central nervous system would compensate by implementing muscular co-contraction to stabilize the joint (Panjabi, 1992a). Clinical joint instability in the spine has been defined as failure to maintain relationships between the vertebrae under physiological load resulting in damage to nerve roots, incapacitating deformity to a structure or pain (White and Panjabi, 1978). In a recent review, the strength of this theory has been described as its ability to link passive components of the spine structure to the neuromuscular system (Oxland, 2016).

Structural changes occur in the spine throughout maturation and aging that may alter the joint stiffness in the disc. There is evidence of microscopic changes that occur in the annulus that may affect its material properties. The bridging network in the interlamellar matrix of the
annulus becomes more developed with increasing age (Melrose et al., 2008; Schollum et al., 2008), the nucleus and annulus undergo an increase in cross-linking of collagen fibres (Pokharna and Phillips, 1998), and the type of collagen distribution changes and water content decreases (Antoniou et al., 1996). These results suggest that the passive stiffness of the disc may change with age and therefore may alter injury or pain pathways depending on age.

1.3 Global objectives and specific questions

The global question of this thesis explored how spine stiffness changes with aging and whether the osteo-ligamentous spine and the annulus contribute to these changes in different loading scenarios. This question was investigated using four different studies. Studies I and II used a porcine model of the cervical spine to represent the aging lumbar spine in humans while studies III and IV were collected from older (45-69 years) and younger (18-35 years) participants. Three of the studies included a measure of passive stiffness as a dependent measure including; stress-stretch modulus (MPa) of the annulus (Study II), stiffness (Nm/degree) of the osteo-ligamentous spine (study I) and lumped passive torso stiffness (Nm/%flexion) (study III). Study IV explored alterations in movement patterns in response to a protocol designed to increase lumped torso stiffness (Beach et al, 2005).

Age-related joint changes are difficult to isolate as they are a function of both cumulative exposures and structural and compositional changes of joints. Furthermore, disc degeneration is closely associated with increases in age. In order to parse out these factors, either a cadaveric approach or an animal model is needed. Given that the average age of human donors is often beyond working age, procurement of a young population would not be viable using a cadaveric approach. Furthermore, in a review of 600 lumbar disc specimens, previous work has reported that by the age of 50 years, 97% of specimens had macroscopic signs of disc degeneration
(Miller et al., 1988). Study I explored the effect of a cyclic loading protocol on osteoligamentous stiffness across older (1.5-8 years) and younger (6-8 months) porcine specimens. Study II implemented environmental changes to simulate an aspect of aging disc degeneration in the annulus in young porcine specimens. The stiffness of an osteo-ligamentous and an intact joint is partially explained by the mechanical properties of its constituent components. Study III investigated passive spine stiffness as affected by age or sex while also investigating if changes occur differently over a prolonged bout of sitting. Study IV explored if movement patterns are altered in response to passive spine stiffness. A flow chart of the inter-connection between the four investigations is depicted in Figure 1.

![Flow chart of the inter-connection between the four investigations](image)

**Figure 1** Overview of the global objectives and specific linking questions between the four studies in this thesis
1.4 Hypotheses

The global purpose of this thesis was to determine if spine stiffness changes with aging and to determine how the mechanical properties of the osteo-ligamentous spine and the annulus contribute to these changes in different loading scenarios. In-line with the global purpose, the following null hypotheses were tested in each of the four studies. Specific alternative hypotheses and an accompanying rationale are detailed in Chapters 3-6.

1. There will be no effect of age or loading on disc height, neutral zone range or neutral zone stiffness in osteo-ligamentous porcine spines (study I)
2. Simulated aging will have no effect on the mechanical properties of isolated porcine annulus layers across radial location or cycle (study II)
3. Prolonged sitting, sex and age will have no influence on discomfort responses, postures or lumped passive torso stiffness (study III)
4. There will be no effect of age, sex, prolonged sitting or lift type postures or perceived effort during a lifting task (study IV)
2. Literature Review

2.1 The study of aging

Age is a difficult variable to deconstruct for injury related potential as it includes co-
variates such as tissue tolerance, cumulative exposures, experience, physical task demands and
the presence of health-related issues. Cross-sectional investigations of *age* and longitudinal
investigations of *aging* often categorize workers into age ranges that vary widely. Age ranges
are either banded across 3-6 categories (Breslin et al., 2003; Smith et al., 2014; Broerson et al.,
1996; Lipscomb et al., 2008; Morassaei et al., 2013; Smith & Berecki-Gisolf, 2014; Smith et al.,
2013; Chau et al., 2009; Peek-Asa et al., 2004) or compared dichotomously in two categories of
relative younger and older workers (King et al., 2009; Siow et al., 2011; Zwerling et al., 1996).
The definition of “older worker,” in the literature ranges from over 25 years (Siow et al., 2011),
over 45 years (Chau et al., 2009; Morassaei et al., 2013; Kenny et al., 2008) over 50 years
(Broerson et al., 1996), over 55 years (Smith et al., 2014; Smith & Berecki-Gisolf, 2014; Smith
et al., 2013; Peek-Asa et al., 2004; King et al., 2009) and over 60 years (Lipscomb et al., 2008).
The World Health Organization has defined “older worker” as someone who is over 45 years
(WHO, 1993). The World Health Organization held a study group on the topic of Aging and
Working Capacity in December 1991 in Helsinki, Finland (WHO, 1993). As part of the
recommendations to member states, the study group determined; “In order to identify and
measure age-related effects associated with modifiable lifestyle and environmental factors,
research should as far as possible incorporate designs that allow the separation of these effects
from the effects of biological aging” (WHO, 1993, p 35). The following sections will explore
the influence of age and its co-variates and will be considered in light of several limitations with
a summary depicted in Table 1.
2.2 Epidemiological literature and the influence of age on workplace injuries

2.2.1 The severity of injuries to older workers

Several investigations have reported that the severity of musculoskeletal injuries increase with age (Breslin et al., 2003; Smith et al., 2014; King et al., 2009; Peek-Asa et al., 2004) while others report that severity peaks at middle age (Smith & Berecki-Gisolf, 2014; Smith et al., 2014). The length of time away from work following an injury is often used to estimate the severity of a claim. A cross-sectional investigation of self-reported work-related musculoskeletal injury claims among physiotherapists and occupational therapists in the United States, reported no differences as a function of age across the total number of claims (King et al., 2009). However, when only injuries that require a minimum of a half day off work were considered (a measure of increased severity), an age effect appeared where therapists over 55 years were more likely to sustain a more severe injury compared to therapists under 55 years (King et al., 2009). A similar trend was reported in a population of materials handlers, where the incidence of work-related low back injuries was similar across age groups (Peek-Asa et al., 2004). However, workers over 45 years had a greater number of days off work following an injury to the low back compared to workers less than 45 years (Peek-Asa et al., 2004). Sex differences in the severity of injury across age groups have also been demonstrated. Short-term disability claims due to work-related musculoskeletal injuries were analyzed to determine the effect of age on the duration of absence following an injury in British Columbia, Canada (Smith et al., 2014). Work absence following an injury increased with increasing age among men, with the highest age category over 55 years (Smith et al., 2014). There was a different pattern for female workers, where there was an inverted “U” shaped relationship and female workers between 35-54 years
had the longest absence following an injury (Smith et al., 2014). Other criteria such as healthcare costs and the degree of physical impairment have been used to estimate injury severity. Serious musculoskeletal injuries have previously been defined as claims resulting in a minimum of 10 days absence from work and/or a minimum healthcare expenditure of $610 Australian dollars (Smith & Berecki-Gisolf, 2014). The risk of serious injury was highest among workers aged 25-44 years, and the risk was lower among workers 15-24 years and over 45 years (Smith & Berecki-Gisolf, 2014). In a recent study examining injury claims in Ontario, Canada, permanent impairment was defined as a condition that would not change over a period of 12 months with or without medical treatment, such as chronic pain (Breslin et al., 2003). The study reported that adult workers over 25 years had a higher rate of permanent impairment following an injury claim caused by a musculoskeletal injury compared to young adults (20-24 years) or adolescent (15-19 years) workers (Breslin et al., 2003). Based on these results, there is evidence of age-related differences of musculoskeletal injury severity across different industries.

2.2.2 The frequency of injuries to older workers

The frequency of injury as a function of age generally demonstrates that there is not a clear increase in risk with increases in age (King et al., 2009; Smith et al., 2013; Peek-Asa et al., 2004; Lipscomb et al., 2008; Smith & Berecki-Gisolf, 2014). Several cross-sectional investigations have attempted to quantify the effect of age on the frequency of work-related musculoskeletal injuries across a variety of industries. Among unionized carpenters in the state of Wisconsin, USA, workers between the ages of 30-39 years were at highest risk of reporting a low back injury at work (Lipscomb et al., 2008). Among a cohort of materials handlers, there was no difference in low back injury reporting as a function of age (Peek-Asa et al., 2004). In a cross-sectional investigation of physiotherapists and occupational therapists, there were no
differences in self-reported work-related musculoskeletal injuries between workers under 55 years and those over 55 years (King et al., 2009). In a study investigating severe musculoskeletal time loss claims, the risk of injury was shown to be highest among workers aged 25-44 years (Smith & Berecki-Gisolf, 2014). While cross-sectional studies provide a valuable snapshot of claim risk across age, the true injury risk is likely underestimated given the healthy worker effect (Punnett, 1996).

2.2.3 The healthy worker selection effect

The healthy worker effect and health selection into less demanding jobs may influence injury statistics. The healthy worker selection effect is a bias that may occur in cross-sectional studies when only current workers are investigated. By only considering the current working population, less healthy workers may be removed from the cohort of interest (due to injury, self-selection to a different job, or health problems), making the remaining cohort a survivor population rather than representative of the general population (Punnett, 1996; Östlin, 1988). This means that existing cross-sectional estimates of injuries to older workers likely underestimate the actual risk of injury. Longitudinal studies that follow individual workers over time (Östlin, 1988) and asking workers to recall past injuries (Punnett, 1996) are suggested methods of mitigating this bias. There is some evidence of health selection into less demanding jobs. A study attempted to capture this self-selection response, and while the investigation did not stratify by age, a range between 25-74 years was included (Östlin, 1988). Workers were categorized as “stable” if they had always worked in light occupations and workers were categorized as “movers” if they had switched from heavy to light occupations. The results of the study indicated that movers were more likely to have musculoskeletal disorders and impaired working capacity compared to stable workers (Östlin, 1988). Further evidence has been
provided in a cohort of occupational therapists and physiotherapists, where older workers (over 55 years) were 2.5 times more likely to report that they changed jobs due to pain symptoms compared to workers under 55 years (King et al., 2009).

2.3 Other age related co-variates that influence injury risk

2.3.1 Experience

Experience increases with age and has been shown to alter the type of work and task demands. There is some evidence that young workers are employed in a more casual capacity compared to older workers even when they are experienced. Among health care workers in British Columbia Canada, experience was defined as a minimum of 1.5 years of work in the same position with younger workers defined as under 25 years and older workers defined as over 25 years (Siow et al., 2011). The results of the study reported that 9% of young experienced workers had full time jobs whereas 45% of older experienced workers had full time jobs (Siow et al., 2011). Some research has explored if experience has a protective effect to decrease injury risk. The literature suggests that experience changes how workers complete tasks. In an investigation of experienced (over 40 years) and less experienced (under 40 years) steel workers observed during night shifts, experienced workers exhibited greater planning of coil inspections and greater communication with technical staff compared to more inexperienced workers (Pueyo et al., 2011). The authors suggest that the experience of workers both enables and causes greater planning at work (Pueyo et al., 2011). There is evidence to suggest that the effect of age on injury risk has a dependency on the type of injury being investigated. Smith and colleagues (2013) conducted a longitudinal investigation exploring if age determines the risk of certain types of injuries differentially over time. The results of the investigation found differences in the types of injuries each age category was at particular risk of sustaining; where younger worker
were at higher risk of open wound injuries and middles ages (35-44 years) were at highest risk of musculoskeletal injuries (Smith et al., 2013). Similar findings have been reported where healthcare workers under 25 years were found to be at an increased risk of cut and puncture injuries and a lower risk of musculoskeletal injuries (Siow et al., 2011). Cooks and food service workers in the health care sector were also found to be at lower risk of injuries caused by contusions, burns, allergies and percutaneous injuries with increases in worker age (Alamgir et al., 2007). These results suggest that experience and cumulative exposures to some job demands may influence musculoskeletal injury risk.

2.3.2 Physical demands and capacity

Occupational demands may represent a greater proportion of capacity as workers age and furthermore, the occupational demands may influence worker capacity. An analysis of a health and retirement survey conducted among male employees aged 51-61 years were compared in two categories of demanding occupations; (i) construction workers and (ii) blue collar workers (Peterson and Zewerling, 1998). Blue collar workers included armed forces, machine operators, precision production, mechanics/repair, farming/fishing/forestry and service employees (Peterson and Zewerling, 1998). The results of the study concluded that construction workers were more likely to sustain a musculoskeletal injury compared to blue collar workers (Peterson and Zewerling, 1998). Among a cohort of workers between 51-61 years, heavy lifting was most associated with injuries sustained at work requiring medical treatment or interference with work activities (Zwerling et al., 1996). A longitudinal investigation was conducted examining worker capacity as a function of age and work task demands (Savinainen et al., 2004). Workers were followed between 1981 and 1997. The authors were interested to see if highly physically demanding work would have either a training effect to increase physical capacity among
workers, or if it would cause physical capacity to decline with worker age. The physical demands of work were categorized as either high workload or low workload and physical capacity was defined according to measurements of heart rate during a full shift in addition to laboratory measures which included; a crude estimate of spine flexibility which was defined as the increase in length of the spine during maximal forward flexion measured in mm, maximum handgrip strength, trunk extension and flexion strength were also measured. With the exception of handgrip strength, improvement in physical capacity was more likely in 16 years among the low workload group compared to the high workload group. Flexibility of the spine was found to increase over time in the high workload group. Isometric trunk extension strength was more likely to decrease among the high workload group whereas it was equally likely to increase or decrease among the low workload group. In a study comparing health status of white collar and construction workers (plumbers, carpenters, painters, plasterers and bricklayers) in Germany, back pain was measured qualitatively by asking workers if they experienced pain in the back extensor muscles (Arndt et al., 1996). There was a greater prevalence of low back complaints among the construction workers compared to the white collar workers. The results of these investigations indicate that occupations with high physical demands lead to a decline in physical capacity of workers and increase in pain reporting rather than a training effect.

2.3.3 Health status and lifestyle

The overall health of a worker has previously been shown to change with age and can have an impact on injury risk and the severity of an injury. Generally, the number of health complaints has been shown to increase with increasing age (Broerson et al., 1996; Smith et al., 2014). In a descriptive survey from the Netherlands, older workers had increased general health complaints, but have similar work-related health complaints compared to younger workers.
With the exception of depression, the presence of chronic conditions increased with increasing age of workers, with the oldest category over 55 years (Smith et al., 2014). The biggest differences in prevalence as a function of age were found in coronary heart disease, osteoarthritis and diabetes (Smith et al., 2014). Furthermore, the authors estimated that chronic conditions (diabetes, osteoarthritis, and heart disease) explain 11-28% of the effect of age on the severity of injury (Smith et al., 2014). The health status and severity of injury has also been shown to influence the chance of a second workplace injury. Workers diagnosed with disc degeneration, needed to be hospitalized, or took off at least a month with a first workplace injury to the back were at greater risk of reporting a second musculoskeletal injury to the back (Lipscomb et al., 2008).

Table 1 Summary of musculoskeletal injuries across age and co-variates

<table>
<thead>
<tr>
<th>Factor</th>
<th>Direction</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Severity of musculoskeletal injury claims</td>
<td>Increase with increases in age</td>
<td>Breslin et al., 2003</td>
</tr>
<tr>
<td></td>
<td>• Over 25 years</td>
<td>Smith et al., 2014</td>
</tr>
<tr>
<td></td>
<td>• Males – over 55 years</td>
<td>King et al., 2009</td>
</tr>
<tr>
<td></td>
<td>• Over 55 years</td>
<td>Peek-Asa et al., 2004</td>
</tr>
<tr>
<td></td>
<td>• Over 45 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peaks in middle age</td>
<td>Smith &amp; Berecki-Gisolf, 2014</td>
</tr>
<tr>
<td></td>
<td>• Females – 35-54 years</td>
<td>Smith et al., 2014</td>
</tr>
<tr>
<td></td>
<td>• Between 25-44 years</td>
<td></td>
</tr>
<tr>
<td>Frequency of musculoskeletal injury claims</td>
<td>No difference across age under 45 years compared to over 45</td>
<td>King et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Higher frequency between 25 years and 44 years</td>
<td>Lipscomb et al., 2008</td>
</tr>
<tr>
<td>Highly physical occupations and older workers</td>
<td>Increased injury claims among construction workers</td>
<td>Peterson and Zewerling, 1998</td>
</tr>
<tr>
<td></td>
<td>• Reduced capacity over time among those in physical jobs</td>
<td>Savinainen et al., 2004</td>
</tr>
<tr>
<td>General health complaints/chronic diseases</td>
<td>Arndt et al., 1996</td>
<td>Broerson et al., 1996</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>• Increased low back pain reporting among construction workers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Increase with increases in age (with the exception of depression)</td>
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</tbody>
</table>

2.4 Anatomy of the lumbar spine and age-related changes

2.4.1 The vertebrae and facet joints

The vertebrae of the spine are composed of a thin shell of cortical bone containing a matrix of cancellous bone (Bogduk, 2012). Each vertebra is comprised of three components; (i) the vertebral body, (ii) the pedicle and (iii) the posterior elements (Figure 2A). The cancellous trabeculae of the vertebral bodies are oriented vertically and horizontally to withstand compressive loading (Figure 2B). The pedicles are hollow cylindrical structures that transmit load between the vertebral body and the posterior elements. The posterior elements facilitate muscle attachment sites for spine extensors and include the superior and inferior surfaces of the facet joints.
2.4.1.1 Age related changes

There are documented age-related changes to the bone density and trabecular architecture of the vertebral body from both cadaveric (Wang et al., 2013; Mosekilde et al., 1987; Mosekilde, 1988) and *in vivo* investigations (Hanson and Roos, 1986; Riggs et al., 1981). Bone mineral content of the lumbar vertebrae undergoes a linear decline with increases in age among both men (Riggs et al., 1981) and women (Hanson and Roos, 1986; Riggs et al., 1981). After the age of 35 years, it is estimated there is a 1% decrease per year among women and this bone loss begins prior to menopause (Hanson and Roos, 1986). Other work has demonstrated no difference in the rate of bone loss in the lumbar vertebrae (L1 to L4) in post-menopausal women compared to pre-menopausal women (Riggs et al., 1981). Using cylindrical (7 mm x 5 mm) samples of trabecular bone excised from the L1 level from human donors, the age-related change in ash density (surrogate measure for bone density) was measured alongside age-related differences in mechanical properties of the samples (Mosekilde et al., 1987). The results of the study showed that the peak stress at failure decreased between 75-80% and 90-96% in the vertical direction and horizontal directions respectively between the ages of 20 and 80 years across sex (Mosekilde et
The age-related decline in ash density across the same age categories was lower than the decreases in strength (48-50%) (Mosekilde et al., 1987). The authors suggest that age-related changes in architecture of the trabeculae may explain the difference in trabecular strength (Mosekilde et al., 1987). In a follow up study, slices of trabecular bone (400 µm thick) were obtained from the L3 vertebral body from 23 donors, and both the thickness and distance between each trabecular lattice was measured across age (Mosekilde, 1988). The results of the study demonstrated that there was no significant change in vertical trabecular thickness as a function of age but showed age-related declines in horizontal trabecular thickness (Mosekilde, 1988). Furthermore, there were significant increases in the distance between the trabeculae in both the horizontal and vertical directions, but the mean values were greater in the horizontal direction, with increases in distance from 500 µm to 1500 µm between the ages of 20 and 80 (Mosekilde, 1988). More recent work has shown regional differences in peripheral and central regions of the trabecular architecture with increasing age and disc degeneration (Wang et al., 2012). Together, these results suggest that both bone density and trabecular architecture change as a function of age among healthy adults, with implications for the mechanical properties of the trabeculae.

There are three articulating surfaces between two vertebrae, the intervertebral disc and two plane synovial joints, or the facet joints (Bogduk, 2012). The main functions of the facet joints are to resist forward shear and rotational movements (Adams et al., 2013). There is evidence of age-related changes to the facet joints including the development of osteoarthritis with increases in age (Videman et al., 1990; Butler et al., 1990). In a study investigating 68 patients between the ages of 15 to 75 years suffering from low back pain, magnetic resonance images were captured to detect changes to the intervertebral disc and computed tomography
scans were used to detect the presence of facet joint osteoarthritis (Butler et al., 1990). The authors categorized discs dichotomously as either healthy or degenerated (Butler et al., 1990). The results of the study indicated that disc degeneration was detected in the absence of facet osteoarthritis, but facet osteoarthritis occurred only when degenerative changes in the disc were present among patients without chronic diseases affecting the vertebral bodies (Butler et al., 1990). The results of this investigation suggest that facet joint osteoarthritis is a secondary outcome of disc degeneration. There is some evidence that facet joint loading changes in the presence of degenerated discs. Using cadaveric segments from 29 donors between the ages of 19 to 92 years, neural arch compressive load bearing was estimated following 2 hours of compressive creep loading in 2 degrees of extension (Pollintine et al., 2004). The results suggested that among non-degenerated spines, neural arch load bearing was estimated to be below 20% but increased to 49% among specimens from donors over 70 years (Pollintine et al., 2004). It is hypothesized that when disc height is reduced (either through degenerative changes or creep loading), the loading on the facet joints change and this may have implications for the initiation of osteoarthritis (Butler et al., 1990).

### 2.4.2 The intervertebral disc

The intervertebral discs are comprised of three main components; (i) the endplate, (ii) the annulus fibrosis and (iii) the nucleus pulposus (Figure 3A). In adults, the intervertebral disc is completely avascular and receives nutrients via diffusion to a limited extent through the annulus but primarily through the endplate (Rajasekaran et al., 2004; Gu et al., 1999). The nucleus is composed of water, and two structural components including collagens and proteoglycans (Urban and Maroudas, 1981). Proteoglycans are negatively charged molecules that imbibe fluid, resist its expulsion contributing to the high swelling pressure of the disc (Urban et al., 1979) and
enable resistance to compressive loading. Type II collagen is predominant in the nucleus, representing 95% of the total collagen content in the nucleus after age 5 (Antoniou et al., 1996). The endplate is comprised primarily of hyaline cartilage; and functions to anchor the annulus to the vertebrae and contain the nucleus of the disc superiorly and inferiorly (Bogduk, 2012). The annulus is comprised of sheets of type I and type II collagen organized into concentric rings around the nucleus, which provide the tensile strength of the disc (Figure 3B). As a percentage of total collagen, type II collagen represents 20% and 35% in anterior and posterior regions of the annulus respectively (Antoniou et al., 1996). There are 15 to 25 distinct layers of collagen fibres in the annulus of a given disc (Marchand and Ahmed, 1990) with some incomplete layers that do not span the full circumference (Schollum et al., 2008; Marchand and Ahmed, 1990). The mechanism of the incomplete layers follow one of two patterns; either two layers merge into one around an incomplete layer or an adjacent layer splits and surrounds an incomplete layer (Marchand and Ahmed, 1990). The collagen fibres in the annulus are oriented in bundles 30 degrees to the horizontal (Marchand and Ahmed, 1990) and are arranged in an alternating oblique and counter-oblique pattern (Yu et al., 2015) (Figure 4).
Figure 3 Anterior view of a porcine functional spine unit including two adjacent vertebral bodies and the intervening disc between them. Two endplates can be seen between the vertebrae and the disc (A). A cross section of a porcine disc depicting the nucleus pulposus (injected with radio opaque barium sulfate and blue dye) and the annulus fibrosus (B) and a schematic representation of the nucleus and annulus depicting the orientation of the annular layers. Image obtained from (Bogduk, 2012) (C).

Figure 4 Schematic depicting the orientation of collagen fibre orientation between lamellae in a cross-section of the annulus). Image obtained from Bogduk, 2012.
2.4.2.1 Age related changes

There are structural changes to the nucleus of the intervertebral disc as a function of age and maturation that have implications for its ability to bear load. A recent study used intervertebral discs from 12 lumbar spines obtained from donors aged 12 weeks to 79 years to investigate the effect of age on changes to the structural components of the disc (Antoniou et al., 1996). Water content in the nucleus decreased with increases in age and the difference in water content became more pronounced with increases in age (Antoniou et al., 1996). Levels of aggrecan (a proteoglycan) decreased with increases in age and the decline began between the ages of 5-15 years (Antoniou et al., 1996). Human fetal discs contain notochordal cells, which disappear prior to reaching adulthood (Boos et al., 2002; Urban et al., 2000). Notochordal cells produce proteins, collagens and proteoglycans that differ from mature disc cells (Urban et al., 2000). Some authors have hypothesized that the disappearance of these cells from the nucleus is associated with the transition from a gelatinous nucleus to a more fibrous solid (Zhao et al., 2007). Between the ages of 3 and 10 years, there is an increase in cell death in the nucleus and between the ages of 11 and 16 years, clefts and radial tears become visible (Boos et al., 2002).

There are several structural changes that occur between annular layers and within a single layer during maturation. The number of incomplete layers in humans is estimated to be 10% higher in older specimens (donors aged between 53-76 years) compared to younger specimens (donors aged between 18-29 years) and is highest in the posterolateral region of the annulus (Marchand and Ahmed, 1990). The thickness of each individual layer increases with increasing age in humans (Marchand and Ahmed, 1990) and ovine specimens (Schollum et al., 2008). In addition to the gross structural changes, recent work has documented microscopic changes to the annulus as a function of age that may have implications for its ability to bear load. Translamellar...
cross bridges represent a fibre network comprised of type VI collagen and contain proteoglycans that connect adjacent layers together in the annulus and are thought to prevent delamination during compressive or torsional loading (Melrose et al., 2008; Schollum et al., 2008). The presence of this network of fibres is present in both juvenile and mature annular samples, but has shown to be more developed in mature specimens (Melrose et al., 2008; Schollum et al., 2008). The number of cross-bridges between lamellae and the number of lamellae with cross-bridges increase with age comparing newborn intervertebral discs to samples obtained from a 4 or 6 year old sheep (Melrose et al., 2008). The increase of these cross-bridges is hypothesized to represent an adaptation to increased loading that occurs with increases in maturity (Melrose et al., 2008).

There are also age-related structural changes that occur within the collagen fibres of a single lamellar layer. Cross-linking of collagen fibres has been shown to increase in both the annulus and the nucleus with increases in age (Pokharna and Phillips, 1998). Recent work has examined microscopic changes attributable to age within a single annular layer loaded in tension (perpendicular to fibre orientation) (Schollum et al., 2010). It was shown that there were larger separations between the fibres of mature ovine specimens compared to a more diffuse and even separation of fibres in the juvenile samples when stretched in tension (Schollum et al., 2010). The authors attribute the increased space between fibres to increased adhesion between the collagen fibres, which under higher loads may lead to the formation of clefts (Schollum et al., 2010). A summary of age related spine tissue changes can be found in Table 2.
<table>
<thead>
<tr>
<th>Region</th>
<th>Age-related changes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebrae</td>
<td>Bone mineral content</td>
<td>Riggs et al., 1981, Hanson and Roos, 1986, Mosekilde et al., 1987</td>
</tr>
<tr>
<td></td>
<td>• Linear decrease with increases in age</td>
<td></td>
</tr>
<tr>
<td>Trabecular architecture</td>
<td>• Age related declines in horizontal trabecular thickness</td>
<td>Mosekilde, 1988</td>
</tr>
<tr>
<td></td>
<td>• Increased space between trabeculae in both horizontal and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vertical directions</td>
<td></td>
</tr>
<tr>
<td>Facet joints</td>
<td>• Osteoarthritis increases with increases in age</td>
<td>Videman et al., 1990, Butler et al., 1990</td>
</tr>
<tr>
<td></td>
<td>• Osteoarthritis hypothesized as a secondary outcome of</td>
<td>Pollintine et al., 2004</td>
</tr>
<tr>
<td></td>
<td>disc degeneration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(altered loading due to reduction in disc height)</td>
<td></td>
</tr>
<tr>
<td>Intervertebral disc</td>
<td>Nucleus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Decreased water with increases in age</td>
<td>Antoniou et al., 1996</td>
</tr>
<tr>
<td></td>
<td>• Decreases in aggrecan (a proteoglycan) with increases in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>age after 15 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Disappearance of notochordal cells with maturation</td>
<td>Boos et al., 2002, Urban et al., 2000</td>
</tr>
<tr>
<td></td>
<td>• Increased cross-linking of collagen fibres with increases</td>
<td>Pokharna and Phillips, 1998</td>
</tr>
<tr>
<td></td>
<td>in age</td>
<td></td>
</tr>
<tr>
<td>Annulus fibrosis</td>
<td>• An estimated 10% more incomplete annular layers with</td>
<td>Marchand and Ahmed, 1990</td>
</tr>
<tr>
<td></td>
<td>increases in age</td>
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</tbody>
</table>
2.4.2.2 Disc degeneration

There are significant structural and cellular changes in the intervertebral disc as a function of age, with many changes difficult to separate from disc degeneration. It is unclear if disc degeneration is part of natural aging (An et al., 2004; Brinjikji et al., 2014) or a separate process (Adams and Roughley, 2006; Rajasekaran et al., 2004; Adams et al., 2000) in part because the presence of disc degeneration increases with increasing age (Butler et al., 1990; Videman et al., 1995; Miller et al., 1988). In a review of images compiled from previous studies, 600 lumbar disc specimens obtained from human donors were analyzed for degenerative findings (Miller et al., 1988). It was reported that by the age of 50 years, 97% of all specimens exhibited degenerative changes (Miller et al., 1988). The earliest degenerative changes are reported to begin in the second or third decade of life (Boos et al., 2002; Miller et al., 1988).

Degenerative changes in excised \textit{(in vitro)} disc material are typically graded macroscopically and identify changes in the nucleus, annulus, (Thompson et al., 1990; Galante, 1967) endplate and vertebra (Thompson et al., 1990). In the Thompson grading system, each part of the disc is graded according to the level of degenerative change ranging from I, no degenerative changes and up to grade V, representing the most severe degeneration (Thompson et al., 1990). In particular, the intervertebral disc (nucleus, annulus and endplate) is rated as

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<tr>
<td>- More developed translamellar bridging network with increases in age</td>
<td>Melrose et al., 2008 Schollum et al., 2008</td>
</tr>
<tr>
<td>- Increased cross-linking of collagen fibres with increases in age</td>
<td>Pokharna and Phillips, 1998</td>
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</table>
grade I if the nucleus has the appearance of a transparent gel, the annulus has clearly visible lamellae and the endplate has a uniform thickness (Thompson et al., 1990). Grades V discs are characterized by visible clefts in the nucleus or annulus and abnormal hardening of the endplate (Thompson et al., 1990). More recently, grading systems have been developed to identify age related and/or degenerative changes using histologic samples (Boos et al., 2002). The guide provides a classification system to assess the quality of intervertebral disc cells, clefts/tears, granular changes, mucoid degeneration, cell death, scar formation and tissue defects (Boos et al., 2002). The classification system also provides a grading scheme for changes to or the presence of endplate deficits including; cellular organization, cartilage disorganization, cartilage cracks, microfracture, new bone formation, bony sclerosis, scar formation and tissue defects (Boos et al., 2002).

Magnetic resonance imaging (MRI) is considered the gold standard method of identifying in vivo degenerative changes (Emch and Modic, 2011). This is because a loss in nuclear signal can be detected using MRI and this change typically precedes a decrease in disc height which can be measured through radiographs (Frobin et al., 2001). A grading scheme developed by Pfirrmann et al. (2001) used MRI images to classify changes in gross morphology of disc structures in vivo. The system ranges from grade I representing a homogeneous bright white disc image, a clear distinction between the annulus and nucleus, normal disc height and hyperintense signal intensity to grade V representing a black disc image, no distinction between the annulus and nucleus, collapsed disc space and hypointense signal intensity (Pfirrmann et al., 2001).

Other documented degenerative changes in the literature include decreased disc height (Battie et al., 2004; Frobin et al., 2001; Videman et al., 1995), annular bulging (Battie et al., 2004; Videman et al., 1995), lowered signal intensity in the disc (Battie et al., 2004; Frobin et al., 2001;
Videman et al., 1995) and changes to endplate diffusion (Rajasekaran et al., 2004; Frobin et al., 2001). A more recent grading scheme categorizes qualities of endplate diffusion to the disc rather than structural changes (Rajasekaran et al., 2004). Following the injection of a contrast agent, this method successfully identified a difference between degenerative change and natural aging where endplate permeability decreases with aging, but has been shown to increase with degenerative changes (Rajasekaran et al., 2004). The initiation of disc degeneration is not well understood but has been related to the nutrition of the disc, genetics and mechanical loading.

The nutrition of the intervertebral disc changes throughout the life course and may be linked to the initiation of degeneration. Fetal intervertebral discs are fully vascularized (Boos et al., 2002; Hassler, 1969). Blood vessels penetrating the intervertebral discs have been reported among infant and child donors, but between the ages of 10 (Boos et al., 2002) or 13 years (Hassler, 1969) have completely receded. Between ages 3 and 10 years, there is a dramatic decrease in the number of blood vessels in the endplate of human donors (Boos et al., 2002). As adult discs are completely avascular (Urban et al., 2000; Boos et al., 2002; Hassler, 1969), endplate diffusion is believed to be the main pathway for nutrients (glucose) and metabolic waste (lactic acid) to reach or be eliminated from the nucleus in the process of glycolysis (Rajasekaran et al., 2004). It is hypothesized that when the blood vessels of the disc recede into the endplate, the diffusion distance increases from the centre of the disc and this increased distance may have implications for the viability of cellular processes related to disc metabolism (Horner and Urban, 2001). Previous work has demonstrated that among cultured nuclear cells harvested from bovine discs, the greater the distance from the nutrient source in an isolated chamber, the more cell death was reported (Horner and Urban, 2001). Decreased pH in the intervertebral disc is linked to low back pain symptoms. Previous work has demonstrated that patients with
discogenic pain have lowered pH in both the central region of the nucleus (Kitano et al., 1993) and the annulus (Keshari et al., 2008). The pH of the central region of the discs of symptomatic patients undergoing either lumbar fusion or discectomy was an average of 6.65 (0.07) compared to 7.14 (0.04) among non-asymptomatic patients (Kitano et al., 1993). In a more recent investigation, spectral analysis was utilized to isolate peaks representing lactate levels in annular samples surgically removed from either discogenic pain patients or non-symptomatic scoliosis patients undergoing a fusion procedure (Keshari et al., 2008). The results of the investigation revealed greater lactate peaks in the pain group (Keshari et al., 2008). There is evidence that cells in the nucleus are unable to survive in acidic environment. After 12 days of incubation all cells from bovine nucleus cultured at a pH of 6.0 were dead regardless of the oxygen or glucose concentration (Horner and Urban, 2001). This cell death would compromise the integrity of the matrix of the nucleus and perhaps the interlamellar matrix.

There is some evidence of a genetic predisposition for disc degeneration. Spine MRI images were compared between 115 male identical twins that had differences between materials handling experience, sedentary occupations, exercise, smoking and vibration exposure (Battie et al., 1995a). The authors estimated that genetics and early development explained 75% of degenerative changes in the upper lumbar region (levels T12 to L4) and 50% in the lower lumbar region (levels L4 to S1) (Battie et al., 1995a). In a similar investigation by the same group, 20 identical twins were investigated for signs of degeneration as identified by MRI with the results estimating that 26-72% of variability in degenerative changes are explained by being identical twins (Battie et al., 1995b).
Two hypothesized mechanical pathways that accelerate disc degeneration are (i) the overload hypothesis and (ii) the hypomobility hypothesis (Stokes and Iatridis, 2004). The overload hypothesis refers to damage accumulation in the tissues of the disc caused by an environment of either high loads or excessive motions (Stokes and Iatridis, 2004). The hypomobility hypothesis postulates that a lack of disc movements leads to tissue level weakening and degeneration (Stokes and Iatridis, 2004). Support for both hypotheses can be found in work by Videman and colleagues (1990) which investigated the relationship between degenerative changes and occupational history in cadaveric spines obtained from male donors. The results of the investigation suggested that sedentary work and heavy occupations were more closely related to grade III disc degeneration compared to mixed work or occupational driving (Videman et al., 1990). Other authors have shown that among twin pairs discordant for occupational exposure, heavy occupational lifting was associated with greater degeneration in the upper lumbar levels (T12-L4) and sedentary work was associated with lesser degeneration in the upper lumbar region (Battie et al., 1995a). However, in general, lower levels of the lumbar spine show more severe degenerative changes than higher levels (Butler et al., 1990; Videman et al., 1995; Miller et al., 1988). There is evidence that cellular processes in the nucleus respond to loading which supports the hypomobility hypothesis. To simulate the effect of weightlessness on the intervertebral disc, tail suspension for a duration of 2 weeks and 4 weeks in rats was compared to a control group (Hutton et al., 2002). There was a 35% reduction in the disc proteoglycan content of 4 week suspended rats compared to the control group (Hutton et al., 2002). This suggests that loading can influence the synthesis of proteoglycans and the subsequent swelling pressure of the disc (Hutton et al., 2002). Evidence to support the overload hypothesis has been simulated in vitro using cadaveric material across a variety of ages (Adams et al., 2000) and
using young and healthy porcine specimens (Callaghan and McGill, 2001). Endplate injuries have been induced during physiologic loading conditions in cadaveric specimens from donors aged between 19 and 87 years (Adams et al., 2000). The results of the study showed that endplate damage led to a reduction in pressure in the nucleus, which resulted in a reduction in disc height and inward buckling of the annulus (Adams et al., 2000). Compression in combination with repeated flexion and extension has been shown to induce herniations among young porcine specimens (Callaghan and McGill, 2001). These results together provide support for the overload hypothesis of disc degeneration. A summary of the nutritional, genetic and mechanical pathways that are hypothesized to contribute to disc degeneration is depicted in Figure 5.
Figure 5 Summary of the hypothesized initiators of disc degeneration. Possible connections between pathways have been omitted for clarity.
2.4.2.3 Age, degeneration and links to pain

Despite the links between aging and structural changes in the disc, the relationship between structural changes in the disc and perceived pain is not strong. Gross structural changes such as disc height narrowing and signal intensity loss have limited association with pain symptoms (Videman et al., 1990; Brinjikji et al., 2014). A recent review compared the radiographs of asymptomatic people to patients with disc degeneration across age (Brinjikji et al., 2014). The review reported that over 50% of asymptomatic people between the ages of 30-39 have disc degeneration (characterized by height loss or disc bulging) (Brinjikji et al., 2014). Up to 90% of individuals aged over 60 years are reported to have disc degeneration without pain symptoms (Brinjikji et al., 2014). However, recent evidence has demonstrated biologic markers other than structural failure that may be more closely associated with pain. Elevated levels of lactic acid in the intervertebral disc have been noted among patients with discogenic pain compared to asymptomatic patients (Kitano et al., 1993; Keshari et al., 2008). Other investigations have shown evidence of nerve ingrowths in the inner annular layers among patients with discogenic pain (Freemont et al., 1997) and in a mouse model (Miyagi et al., 2014). A recent investigation collected biopsy samples of lumbar discs from cadaveric donors without a history of low back pain and compared the nerve ingrowth to disc samples from people with chronic pain undergoing spinal fusion surgery (Freemont et al., 1997). It was found that 68% of patients with low back pain had nerve ingrowths into the inner third of the annulus fibrosus and the nucleus pulposus, with some nerves accompanied by blood vessels (Freemont et al., 1997). Discs from patients without a history of low back pain were found to have nerve ingrowths that only extended to the outer third of the annulus (Freemont et al., 1997). Further, using a mouse model of aging, mice that were missing osteonectin, a matrix protein important for tissue...
remodelling and has previously shown to be reduced in aging and degeneration, were compared to age matched wild type mice (Miyagi et al., 2014). The study used three age groups including young (6-7 weeks), middle aged (5-7 months) and mature (22-24 months) mice and compared nerve ingrowths in the disc across age and genotype (Miyagi et al., 2014). The results of the study showed that the mature mice missing osteonectin has significantly longer nerve fibre lengths per slice in the inner disc compared to other ages (Miyagi et al., 2014). Furthermore, there were more herniations present in these mice suggesting that nerve ingrowths could be a cause of discogenic pain in herniated or degenerated spines (Miyagi et al., 2014).

2.4.3 Animal models to represent human aging

There is no universally accepted animal model to replicate aging or degenerative processes in humans (Alini et al., 2008; An et al., 2004). In order to make results relevant to aging, a recent review has recommended that the age of the animal is important, as young developing animals may not be relevant to older humans or even a skeletally mature adult (Alini et al., 2008). When selecting an animal model, it has been suggested that there should be a baseline similarity between the selected species and humans in terms of anatomy, cellularity, maturity, nutrition and loading (An et al., 2004). Previous in vitro work has used a porcine model to study the aging spine (Park et al., 2005; Lundin et al., 2000). There are geometric and mechanical similarities between young cervical porcine specimens and young human lumbar spines (Yingling et al., 1999). The structure of the layers of the annulus has been shown to change with increases in age. An investigation was completed to determine changes in annular structure in cadaveric lumbar discs, comparing annular samples from younger donors aged between 18-29 years to older donors aged between 53-76 years (Marchand and Ahmed, 1990). The thickness of layers was found to increase significantly among older specimens that were
found to be more than double the size of the younger specimens (Marchand and Ahmed, 1990). Similar thickness changes have been reported using an ovine model of aging where samples obtained from the outer third of the anterior annulus measured an average (SD) 184 (45) μm and 320 (58) μm among juvenile and mature specimens respectively. Figure 6 depicts annular samples obtained from a juvenile (aged approximately 6 months) and a mature (aged 3 years) porcine spine. The lamellae are thicker in the mature specimen, similar to age-related change documented among human donors. Animals that retain notochordal cells in the nucleus (such as in pigs) are recommended to provide insights to the annulus rather than the nucleus (Alini et al., 2008). The notochordal cells eventually disappear during development (approximately age 10) in human discs (Roughley, 2004).
2.5 Spine functional age-related changes

2.5.1 Muscular strength and architecture

There is evidence of a decline in isometric strength, maintenance of endurance and changes in architecture of the lumbar extensor muscles with increases in age. Isometric back extensor strength declines when tested in both prone (Sinaki et al., 2001; Champagne et al., 2009) and standing (Yasserli et al., 2007; Singh et al., 2011; Singh et al., 2013) positions with increases in age. A recent investigation tested standing extensor maximum voluntary
contraction before and after a fatigue protocol as a function of age comparing younger (18-25 years) and older participants (55-65 years) (Yasserli et al., 2007). Electromyography was recorded at the level of L1 and L4-L5 to target the longissimus dorsi and multifidus muscles respectively (Yasserli et al., 2007). Participants completed an MVC followed by maintaining (within 5%) 30, 50 and 70% of maximum torque recorded followed by a post-fatigue maximum trial. Age was shown to significantly reduce torso extension strength by approximately 27% across sex. There were no significant differences in the rate of decline of median power frequency as a function of age indicating that there was similar endurance as a function of age despite decreases in absolute extensor strength between groups. Endurance time (seconds spent within target zone) was not significantly different between age groups. A similar study tested 20 males with an average (SD) age of 22.8 (3.1) years and 16 males with an average age of 72.8 (4.7) years (Champagne et al., 2009). Endurance time was measured using a modified version of the Biering-Sørenson test where participants were in a semi prone position (45 degrees) with the head, arms and trunk (superior to the iliac crests) unsupported. Isometric extensor strength was tested before and after the fatigue protocol. The results showed there was not a significant difference in endurance time between age categories. The isometric strength was significantly lower among the older participants; however, there was no interaction effect as a function of the fatigue protocol. There is some evidence of a sex difference in extensor strength as function of age. Using an isometric dynamometer to measure back extensor strength and comparing participants aged 30 to 39 years to those aged 80 to 89 years, strength decreased by 64% for males and 50% among females (Sinaki et al., 2001). There is also evidence of changes in muscle architecture and composition as a function of age (Singh et al., 2011; Mannion et al., 2000; Hiepe et al.,
A recent investigation collected ultrasound images of the extensor muscles at the level of L3 in standing and in 50% flexion among participants aged between 20 to 35 years and over 65 years (Singh et al., 2011). The angle of the extensor muscles at the level of L3 were measured with respect to the aponeurosis of the muscle (Singh et al., 2011). The results of the study showed that there was a smaller fibre angle among older participants in both standing and half flexion position (Singh et al., 2011). Previous work has noted an increased presence microscopic architectural changes associated with pathological fibres (i.e. signs of denervation) with age and these changes began between 31-40 years (Mannion et al., 2000). Younger (22.5 (1.4) years) and mature (55.3 (3.6) years) participants performed a modified Biering-Sørenson test (sustained back extensor exercise) inside an MR scanner with images captured at the L3-L4 level (Hiepe et al., 2015). The results of the study showed reduced type II fibres and an increase in fat infiltration in mature lumbar extensors compared to younger participants (Hiepe et al., 2015).

2.5.2 Lumbar lordosis and range of motion

The standing lordosis of the spine decreases with increases in age. Standing radiographs were captured at baseline and after 10-13 years had elapsed among a total of 53 participants with the age range at baseline between 50 to 77 years (62 to 88 years at follow-up) (Takeda et al., 2009). Lumbar lordosis was measured using the Cobb’s method (angle between perpendicular lines drawn from the upper endplate of L1 and the lower endplate of L5). A total of 13 segmental disc angles (from T5 to S1) were measured. The results of the study showed that average lumbar lordosis significantly decreased between baseline and follow-up from 33.1 (14.0) degrees to 25.4 (16.5) degrees. In a different study examining lordosis from a group of young male participants (average age of 27), the standing lordosis angles (measured using Cobb’s
method between L1 and superior edge of S1) were found to be an average of 63 (15) degrees (De Carvalho et al., 2010). The loss of lordosis with increasing age is hypothesized to be in part a result of anterior wedging as the segmental angles between L1-L2, L2-L3, L3-L4 and L4-L5 were found to have increased anterior wedging (not significant in the other 9 levels) (Takeda et al., 2009).

There is evidence from cadaveric samples (Taylor and Twomey, 1980) and human investigations (Dvorak et al., 1995; McGill et al 1999; Taylor and Twomey, 1980) that the range of motion in flexion and extension of the lumbar spine decreases with increases in age. An investigation tested 104 volunteers between the ages of 20 and 70 years using a device linkage of 6 potentiometers at the skin overlying the level of T12-L1 and the sacrum (Dvorak et al., 1995). Relative to quiet standing, the results of the study showed that maximum flexion among the 20-29 year old participants was an average of 75 (13.1) degrees and this was reduced to 59.5 (6.2) degrees among those aged over 50 years. Range of motion was also significantly reduced in right and left lateral bend and right and left axial twist as a function of age. There were no sex differences in range of motion. Similar results were shown in a study that compared spine kinematics of older adults (average of 69 years) to a database of younger participants (McGill et al., 1999). Kinematics were collected using a 3 space Isotrak system with the source located on the sacrum and the sensor located at the 12th thoracic vertebra. All lumbar angles were normalized to quiet standing while participants completed maximum flexion, lateral bend and axial twist trials (bilaterally). Trials were time normalized from 0-100% of motion because motions were self-selected in pace. Peak velocity (degrees/second) was found to be significantly lower among the older participants for all movements except left lateral bend, and bilateral axial twist. Peak displacement was significantly lower for the older participants compared to young
participants in lateral bend and flexion whereas axial twist values were not different across age. Peak flexion was found to be an average of 23 degrees less among older participants compared to younger participants. In a different investigation, total range of motion in degrees was measured between standing flexion and extension and left and right axial twist across three age categories; 18-35, 36-59 and over 60 years using a lumbar spondylometer and lumbar rotameter (Taylor and Twomey, 1980). The devices use a protractor affixed to the sacrum with the end of a moveable lever arm affixed to the skin above the first lumbar vertebrae to measure flexion and extension and a separate device using the same principle was developed for use to track rotational motions in axial twist (Twomey and Taylor, 1979). The greatest range of motion in flexion and extension was found among female and males between 18 and 35 years with an average (SD) of 42 (6.3) degrees and 42 (6.7) degrees for men and women respectively (Taylor and Twomey, 1980). The smallest range of motion was found in the over 60 years group where total lumbar range of motion (flexion and extension) was 30 (6.9) degrees and 28 (6.2) degrees for males and females respectively. Similar results of decreasing range of motion with increasing age were found in cadaveric spines (Taylor and Twomey, 1980). A summary of age-related functional changes can be found in Table 3.
Table 3 Summary of functional changes to the spine associated with increases in age

<table>
<thead>
<tr>
<th>Functional Factor</th>
<th>Age-related change</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Pain</td>
<td></td>
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<tr>
<td>Disc degeneration</td>
<td>• Gross structural changes do not have a strong relationship to pain</td>
<td>Brinjikji et al., 2014 Videman et al., 1990</td>
</tr>
<tr>
<td></td>
<td>• Acidic discs are linked to pain symptoms</td>
<td>Kitano et al., 1993 Keshari et al., 2008</td>
</tr>
<tr>
<td></td>
<td>• Nerve in-growths into the inner third of the annulus associated with discogenic pain</td>
<td>Freemont et al., 1997 Miyagi et al., 2014</td>
</tr>
<tr>
<td>Population reporting/non-specific</td>
<td>• Severity of low back pain reporting increases with increasing age</td>
<td>Dionne et al., 2006</td>
</tr>
<tr>
<td></td>
<td>• Peak prevalence (previous 1 month) of low back pain highest in those between 45 to 59 years</td>
<td>Papageorgiou et al., 1995</td>
</tr>
<tr>
<td>Extensor muscles</td>
<td>• Strength decreases with increases in age</td>
<td>Sinaki et al., 2001 Champagne et al., 2009</td>
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<tr>
<td></td>
<td></td>
<td>Yasserli et al., 2007 Singh et al., 2011</td>
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<td>Singh et al., 2013 Mannion et al., 2000</td>
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<td>Hiepe et al., 2015</td>
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<tr>
<td></td>
<td>• No differences in muscular endurance across age</td>
<td>Yasserli et al., 2007 Champagne et al., 2009</td>
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<td></td>
<td>• Changes in muscle architecture with increases in age</td>
<td>Singh et al., 2011 Mannion et al., 2000</td>
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<tr>
<td></td>
<td></td>
<td>Hiepe et al., 2015</td>
</tr>
<tr>
<td>Lumbar lordosis</td>
<td>• Decreases during standing postures</td>
<td>Takeda et al., 2009</td>
</tr>
</tbody>
</table>
Lumbar range of motion

- Decreases in both flexion and extension
  
  Taylor and Twomey, 1980
  Dvorak et al., 1995
  McGill et al 1999

2.6 Literature review general summary

Age is a challenging variable to deconstruct as its relationship to injury and capacity varies with several other factors. The frequency of musculoskeletal injuries is difficult to assess across age cross-sectionally. Based on the epidemiological evidence, workers under the age of 25 years sustain less severe musculoskeletal injuries than workers over 25 years (King et al., 2009; Peek-Asa et al., 2004; Smith et al., 2014; Smith & Berecki-Gisolf, 2014; Breslin et al., 2003). A sound understanding of age-related anatomical, functional and tissue-level changes is important when considering injury across a variety of ages. The intervertebral disc in particular undergoes significant structural and compositional changes with age and maturation that are difficult to separate from disc degeneration. In the nucleus, water content and proteoglycan concentration decrease with increasing age (Antoniou et al., 1996) and notochordal cells disappear with maturation (Boos et al., 2002; Urban et al., 2000). In the annulus, the number of incomplete collagen layers increases (Marchand and Ahmed, 1990) and the thickness of individual layers increase with increasing age (Marchand and Ahmed, 1990; Schollum et al., 2008). Despite structural changes in the disc with increases in age, there are limited links between low back pain and structural changes in the spine (Videman et al., 1990; Brinjikji et al., 2014). Increased lactic acid in the disc (Kitano et al., 1993; Keshari et al., 2008) and nerve fibres penetrating the inner third of the annulus (Freemont et al., 1997; Miyagi et al., 2014) are associated with increased discogenic pain. There are also functional changes with increasing age
such as reduced muscular strength (Sinaki et al., 2001; Champagne et al., 2009; Yasserli et al., 2007; Singh et al., 2011; Singh et al., 2013) and lumbar range of motion (Dvorak et al., 1995; McGill et al, 1999; Taylor and Twomey, 1980). In considering this body of literature, the prevalence and severity of work injury change across age and are accompanied by cellular and functional changes in the spine.
3. Study I: The effect of age on the mechanical properties of porcine intervertebral discs following a cyclic loading protocol

3.1 Introduction

The annulus fibrosis serves an important load bearing role in the intervertebral disc and undergoes structural and chemical changes with age and maturation. The structure circumferentially surrounds the nucleus pulposus of the disc and is composed of layers of collagen fibres embedded in an interlamellar matrix. Previous work has shown increased thickness of annular layers and a greater prevalence of incomplete layers with increases in age of specimens from human donors (Marchand and Ahmed, 1990). Furthermore, clefts between collagen fibres within a single annular layer have been noted with increasing age in porcine (Park et al., 2005) and ovine (Schollum et al., 2010) specimens. Water content and proteoglycan concentrations have also been shown to decrease with increases in age (Antoniou et al., 1996). These changes have implications for the load bearing capacity of the whole disc structure.

Despite documented structural and chemical changes to the annulus with age, previous work has reported a weak relationship between age and tensile modulus of the annulus. While the annulus undergoes complex three-dimensional loading during work related tasks, resistance to tension is an important function of the structure. Early work completed by Galante (1967) tested multilayer samples of annulus material collected from human donors of a variety of ages, but only from tissue samples having a degenerative grade of 1 to isolate age from macroscopic degenerative changes. The results of the study suggested that the tensile elongation (in mm) of specimens increased with maturation (until 26 years) after which it remained constant across age (Galante, 1967). More recently, the material properties in non-degenerate multi-layered
specimens from human donors (26 to 53 years) were shown to have a weak linear correlation with increases in age where there was a stiffer tensile modulus, lower failure stress, and lower failure strain in the specimens from older donors compared to younger donors (Ebara et al., 1996). In single layer non-degenerate specimens from human donors, no relationship was demonstrated between donor age and tensile properties of the specimens (Holzapfel et al., 2005). While this work has investigated the innate mechanical properties as a function of age in human spines, a mechanistic understanding of how a loading protocol influences the tensile properties of specimens of different ages has not previously been explored.

Exposure to cyclic loading or vibration has previously been shown to alter the material properties of young porcine annulus samples (Gregory and Callaghan, 2012) and introduce microstructural clefts in the tissue (Gooyers et al., 2015). In a study conducted by Gregory and Callaghan (2012), annulus specimens excised from functional spine units exposed to vibration were found to have a larger toe region and the vibration exposure was hypothesized to disrupt elastin fibres or proteoglycans embedded in the single lamellar layers tested (Gregory and Callaghan, 2012).

The formation of clefts and fissures in the annulus are implicated in both pain generating pathways and propagation of structural damage to the disc. An in-growth of nerve fibres and blood vessels in the inner third of the annulus fibrosis has been documented among discs removed from patients suffering from chronic low back pain (Freemont et al., 1997; Peng et al., 2005). The presence of substance P, a neurotransmitter associated with nociceptive fibres has been detected in painful discs (Peng et al., 2005; Freemont et al., 1997) suggesting the disc and annulus can be a source of low back pain. The margin of annular fissures has been qualitatively identified as a site of ingrowth for nerves and blood vessels in painful discs (Peng et al., 2005).
Tears in the annulus result in up to a 46% reduction of local pressure, relative to the pressure of the nucleus (Stefanakis et al., 2012). Furthermore, proteoglycan density was reduced within a 2 mm region of the fissure and the area of proteoglycan depletion surrounding a fissure increased with increasing donor age (Stefanakis et al., 2012). The loss in proteoglycans reduces the swelling pressure of the disc. The reduction in pressure at the site of annular tears has been hypothesized to create a low pressure micro-environment conducive to the ingrowth of small blood vessels (otherwise occluded in higher pressure environments) and nociceptive fibres (Stefanakis et al., 2012).

A mechanistic understanding of the influence of age on the loading response of spinal tissues is essential to the prevention of low back injury throughout the life course. It is unclear how the known age-related structural changes influence the tissue-level response of functional spine units to cyclic loading. The aim of this investigation was to quantify the effect of age on stiffness, neutral zone range, geometric properties and disc height in functional spine units. A secondary objective was to qualitatively compare injuries between young and mature specimens.

3.2 Hypotheses

1. The number of disc herniations will be higher in adolescent compared to mature functional spine units.

With increases in age, the water content in the nucleus of human discs decreases (Antoniou et al., 1996). In line with the assertions of previous authors (Tampier et al., 2007), the reduction in water content may decrease the hydraulic pressure gradient (Stefanakis et al., 2014) implicated in delamination of the annulus and a contributing factor in herniation injuries.

2. a. Disc height will decrease following cyclic loading.
2. b. Disc height will be lower in adolescent specimens.

Previous work has demonstrated a reduction in disc height following cyclic compressive loading with no influence of level (Gooyers and Callaghan, 2015). Lumbar porcine specimens from adolescent (~6 months) and mature (~3 years) specimens demonstrated greater height loss following axial compression in adolescent compared to the mature and degenerated samples (Park et al., 2005).

3. a. There will be a greater neutral zone range and a reduced neutral zone stiffness following the loading protocol.
3. b. There will be a higher neutral zone stiffness and a smaller neutral zone range in mature specimens.
3. c. There will be no effect of level on neutral zone range or stiffness.

Previous work has demonstrated decreased stiffness in lumbar osteo-ligamentous spines obtained from human donors following repetitive bending (Adams and Dolan, 1996). An in-vivo investigation of continuous lifting has previously demonstrated a biphasic trend where lumped torso stiffness decreased in the first 30 minutes of a continuous lifting task, followed by a trend of increased stiffness (Parkinson et al., 2004). In vivo measurements of lumped passive stiffness during upright sitting has shown that participants aged 22-48 years had significantly lower low back stiffness compared to those aged between 52-68 years (Shojaei et al., 2016).

### 3.3 Methods

#### 3.3.1 Specimens and preparation

A total of 23 porcine cervical spines (11 mature, 12 young) were obtained post-mortem and stored at -20 degrees Celsius. A total of 40 vertebrae-disc-vertebrae functional spine units were dissected with the distribution of specimens depicted in Figure 7. The young spines were obtained from pigs aged between 6 – 8 months at the time of sacrifice while the mature spines
were obtained from sows aged between 1.5 – 8 years. An animal model of the human lumbar spine was selected in order to control for diet, lifestyle and to promote homogeneity in tissue samples. Previous work has documented similarities in the geometric and structural characteristics between young porcine cervical spines compared to young and healthy human lumbar spines (Yingling et al., 1999). The similarities between mature porcine cervical and human lumbar spines in the literature are outlined in section 2.4.3. A total of 40 functional spine units were tested and analyzed for neutral zone range, neutral zone stiffness and qualitative count of injury. A subset of 20 FSUs (10 young, 10 mature) were compared for geometric properties. A total of 12 FSUs obtained from the C56 level (6 young, 6 mature) were analyzed for disc height measurements.

Figure 7 Distribution of functional spine units across age and cervical level
Spines were thawed at room temperature overnight; all muscle tissue was removed and the spines were dissected into osteo-ligamentous functional spine units (FSUs). Horizontal cuts through the levels C23, C45, C67 and through the superior and inferior facet capsules permitted a maximum of 2 FSUs per spine at the level of C34 and C56. The exposed superior and inferior discs of functional spine units from each age group were visually inspected and met a macroscopic grade I (non-degenerate) criteria (Galante, 1967). A 0.5 mL volume of contrast medium consisting of a solution of radio-opaque barium sulfate, Coomassie Brilliant Blue Dye (Thermo Fisher Scientific, Waltham, MA, USA) and distilled water (2:1:2 ratio) was injected through the right anterolateral aspect of each specimen (Callaghan and McGill, 2001). Sagittal plane radiographs at baseline confirmed injection of the solution into the nucleus. Six nickel plated steel pins (size 20 silk pin, 0.8 mm diameter) were inserted into the superior and inferior endplates of each FSU (3 superior, 3 inferior). The pins were located in the midline of the disc and two lateral positions (Figure 8) in order to aid in the localization of the endplate during surface laser scans of the anterior annulus surface. Specimens were secured to aluminum cups using woodscrews penetrating the superior and inferior endplates. Specimens were further secured to the aluminum cups using non-exothermic dental plaster (Denstone, Miles, Southbend, IN, USA) (Figure 8).
3.3.2 Protocol

A custom servohydraulic testing system (model 8872, Intron Canada, Burlington, ON, CAN) was used to apply the cyclic loading protocol. The system has been customized to permit the application of compressive loads by the servo-hydraulic system, while simultaneously an independent brushless servomotor (model AKM23D, Kollmorgen/Danaer Motion, Rad USA) attached in series to a torque cell (model T120-106-1K, SensorData Technologies Inc., Sterling heights, MI, USA) applies sagittal plane rotations (Callaghan and McGill, 2001). The testing
The apparatus was designed to align the centre of rotation during testing to the centre (horizontal and vertical position) of the intervertebral disc prior to testing (Callaghan and McGill, 2001).

An outline of the loading protocol is depicted in Figure 9. The superior aluminum cup was bolted to the actuator of the testing system while the inferior cup translated freely on a bearing tray. A preload was applied to all specimens consisting of a compressive load of 300 N for a duration of 15 minutes. During the preload, the servomotor was programmed to identify an angular position within each specimen’s neutral zone by minimizing the flexion/extension moment magnitude while also mitigating post-mortem disc swelling. Following the preload, the neutral zone, a region of laxity in a joint (Panjabi, 1992a, 1992b), was defined about the flexion and extension plane. In a single passive test, specimens underwent a minimum of three cycles of flexion and extension in displacement control at a rate of 0.5 degrees per second under 300 N of compression. The applied moment (Nm) and angular displacement (degrees) were sampled at 25 Hz.
A 2-dimensional blue semi-conductive laser displacement sensor (model LJV7080, Keyence, Osaka, Japan) was used in conjunction with a linear encoder (LS 328-C, Heidenhain,
Schaumburg, IL, USA) to record 3-dimensional scans of the anterior surface of intervertebral disc specimens. A two-dimensional cross-section of the surface was collected in slices every 40 μm, as the laser was pushed at a rate of 3 mm/s along a linear guide (NSK Ltd., Tokyo, Japan) using a linear servomotor (Tolomatic, Hamel, MN, USA, model: AKM22E-BNPNC-00) located at a right angle from the specimen. The specimens were positioned at the extension limit posture and held in this position for scanning. Scans were collected before and after the cyclic loading protocol.

Specimens were exposed to 1400 N of compression and cyclically loaded at 1 Hz to a range of motion 8.5 degrees in flexion and extension around the midpoint of each specimen’s neutral zone for 3000 cycles (50 minutes). Previous work has shown that 1400 N of compression replicates the completion of light material handling in vivo (Nachemson et al., 1981). This protocol has previously been shown to induce disc herniations following 4400 cycles (Tampier et al., 2007).

3.3.3 Dependent measures

3.3.3.1 Geometric properties

Select geometric properties were measured manually using digital calipers and compared between age and level of specimen. The endplate width, endplate depth, spinal canal width, spinal canal depth and vertebral height were measured on the superior and inferior surfaces of each functional spine unit (Figure 10). The average of the superior and inferior measurements was reported and compared across age and level (C34, C56).
Figure 10 Top panel: Vertebral height was measured between adjacent endplates and averaged between 2 levels of each FSU. Bottom panel: Dimensions of the disc (black lines) and the spinal canal (white lines), including width (dashed line) and height (solid line) were measured on the superior and inferior surfaces of exposed ends of each FSU and averaged.

3.3.3.2 Disc height

Three-dimensional surface scans of the anterior aspect of the disc were processed using a custom Matlab program to measure disc height. A median spatial filter was applied to surface scans, with enough area surrounding the disc pins captured to avoid image distortion using the same procedure previously published (Gooyers and Callaghan, 2015). The difference in height
between the two central pins on the endplate was used to calculate disc height in mm (Figure 11). Disc height before and after the loading protocol are presented from specimens obtained from the C56 level.

![Image of FSUs with metallic pins](image1.png)

**Figure 11** The anterior surface of FSUs were scanned before and after the loading protocol with metallic pins (white arrow) inserted in the upper and lower endplates (A). A 3-dimensional surface rendering of a specimen in an extended posture, with upper and lower endplate pins indicated by the two white arrows (B)

### 3.3.3.3 Neutral zone range and stiffness

The endpoints of the neutral zone were determined through a method outlined by Thompson and colleagues (2003) and previously applied to FSU data (Noguchi et al., 2015).
fourth-order polynomial was fit to the moment-angle curves of the last two cycles, and the fit moment data was differentiated with respect to angle. The angles (in flexion and extension) corresponding to an instantaneous stiffness of ±0.05 Nm/deg represented the flexion and extension limits of the neutral zone. The neutral zone range (degrees), calculated as the absolute sum of the length of the flexion and extension limits was reported. The slope of the line fit to the moment-angle data represented neutral zone stiffness (Nm/degree).

3.3.3.4 Injuries

Injuries to the disc, endplate and annulus were determined following the loading protocol using sagittal plane radiographs (taken before and after cyclic loading) and macroscopic dissection of the disc documented with photographs. Injuries will be documented according to the three types of annular tears; circumferential, radial or rim lesion (Vernon-Roberts et al., 2007; Adams and Roughley, 2006) and percent differences in injuries will be compared (qualitatively) between age groups.

3.3.4 Statistical analysis

A three way (age x level x loading) mixed general linear model with repeated measures on loading (pre/post) will be applied to neutral zone range (degrees) and neutral zone stiffness (Nm/degree). A two way (age x level) general linear model was applied to all geometric properties. A two-way (age x loading) mixed general linear model with repeated measures on loading (pre/post) was applied to disc height. The number of injuries were compared qualitatively across and level. All statistical analyses were computed using SAS studio (version 9.4, SAS Institute Inc., Cary, NC), with a significance level (α) of 0.05. Tukey’s post hoc tests were used to detect significant effects.
3.4 Results

Two types of herniations were observed in the study, ring lesions and radial fissures (Table 4, Figure 12). A total of 7 herniations were observed, with a higher prevalence in younger compared to older specimens. Specifically, 24% of younger FSUs herniated while 11% of mature samples herniated.

Table 4 Summary of observed disc injuries across age following cyclic loading

<table>
<thead>
<tr>
<th>Herniation type</th>
<th>Specimen Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
</tr>
<tr>
<td>Ring</td>
<td>2</td>
</tr>
<tr>
<td>Fissure</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
</tr>
<tr>
<td>Percent of all specimens tested</td>
<td>23.8%</td>
</tr>
</tbody>
</table>

Figure 12 Representative partial herniation injuries observed in the study; including ring (A) and fissure (B)
There was a main effect of age on endplate dimensions and vertebral height, while there were no significant differences across age or level in spine canal width or depth (Table 5). The greatest difference in dimension was in vertebra height, where the vertebral height in mature specimens was an average 10 mm greater compared to younger specimens. There was no effect of level across any geometric measure.

Table 5 Summary of vertebral geometric properties across age. Significant differences across age are denoted by A,B.

<table>
<thead>
<tr>
<th>Geometric Properties (mm)</th>
<th>Specimen Age</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Mature</td>
</tr>
<tr>
<td>Endplate Width</td>
<td>35.7 (2.2)^A</td>
<td>39.7 (2.3)^B</td>
</tr>
<tr>
<td>Endplate Depth</td>
<td>25.2 (1.6)^A</td>
<td>28.6 (1.6)^B</td>
</tr>
<tr>
<td>Spinal Canal Width</td>
<td>17.4 (1.0)^A</td>
<td>19.1 (2.6)^A</td>
</tr>
<tr>
<td>Spinal Canal Depth</td>
<td>12.6 (2.0)^A</td>
<td>13.2 (1.5)^A</td>
</tr>
<tr>
<td>Vertebral Height</td>
<td>16.2 (2.1)^A</td>
<td>26.9 (2.5)^B</td>
</tr>
</tbody>
</table>

There was a main effect of loading on disc height in C56 specimens. Specifically, disc height was reduced following the loading protocol from an average 13.2 (2.7) mm to 12.2 (3.0) mm following cyclic loading (p=0.0024). There were no significant differences in disc height detected across age (p=0.1695).

Neutral zone range was significantly affected by age, level and time. Specifically, there was a main effect of level on neutral zone range, where specimens excised from the C34 level had a greater neutral zone range compared to specimens from the C56 level, with mean (standard deviation) values of 5.2 (1.5) degrees compared to 4.2 (1.2) degrees respectively (p=0.0061) (Figure 13). Neutral zone range was significantly affected over time across age (p<.0001). Post-hoc analysis revealed young specimens significantly decreased the neutral zone range following cyclic loading (p<.0001). In contrast, neutral zone range did not significantly change in mature specimens following the cyclic loading protocol (p=0.7584). Prior to the cyclic loading protocol,
younger specimens had a significantly higher neutral zone range compared to mature specimens (p=0.0087) while after the cyclic loading protocol, there was no significant difference between age categories (p=0.0322).

Neutral zone stiffness was significantly affected by age, level and loading. Neutral zone stiffness was calculated as the slope of the line fit to the moment angle data between the flexion and extension limits. It was found that all R² values were between 0.95 and 1.00. There was a main effect of loading, where stiffness was significantly higher prior to the cyclic loading protocol compared to following the loading protocol, with average (standard deviation) values, 0.20 (0.11) Nm/degree compared to 0.17 (0.11) Nm/degree respectively (p=0.0004) (Figure 14). There was a significant interaction between age and level to significantly affect neutral zone

Figure 13 Neutral zone range across specimen age prior to and following cyclic loading
stiffness ($p=0.0099$). Post-hoc analysis revealed that mature specimens had higher neutral zone stiffness at both the C34 ($p=0.0110$) and C56 ($p<.0001$) levels. In younger specimens, there was no significant difference in stiffness between the C34 and C56 level ($p=0.9215$). In contrast, a significant difference between spinal level was detected in the mature specimens; where specimens obtained from the C56 level had significantly higher stiffness compared to specimens obtained from the C34 level ($p=0.0162$).

![Neutral zone stiffness across specimen age and cervical level](image)

**Figure 14 Neutral zone stiffness across specimen age and cervical level**

### 3.5 Discussion

The results of this study indicate that FSU stiffness, range of motion and herniation injury prevalence were all significantly affected by age. Higher neutral zone stiffness and lower neutral zone range of motion were observed at baseline, indicating that age alters the mechanical environment of the intervertebral disc and may alter potential pathways for injury, pain development and may reduce functional range of motion in older adults.
It was hypothesized that neutral zone range would decrease and stiffness would increase with specimen age (hypothesis 3b), neutral zone stiffness would decrease and range would increase following cyclic loading (hypothesis 3a), while there would be no effect of cervical level (hypothesis 3c). The results of the study supported the hypothesized increased stiffness and reduced neutral zone range with increasing age, while the cyclic loading protocol was shown to reduce the neutral zone range of young specimens and neutral zone stiffness of all specimens. In contrast to our hypothesis, there was a significant effect of cervical level on neutral zone range and stiffness. The neutral zone range was found to be greater in specimens from the C34 level compared to the C56 level. Level and age significantly interacted to affect neutral zone stiffness, where, mature C56 specimens had higher stiffness compared to mature C34 specimens (with no level effect noted in younger specimens). Lower levels of the human lumbar spine show more severe degenerative changes than higher levels (Butler et al., 1990; Videman et al., 1995; Miller et al., 1988). The results of this study support lower vertebral levels may have different mechanical properties compared to higher levels with increased age. Neutral zone stiffness was higher in mature compared to young specimens and baseline neutral zone range was lower in mature compared to young specimens. This is consistent with measurements of lumped passive stiffness in humans, where passive lumbar spine stiffness increases with increasing age at 70% flexion postures (Shojaei et al., 2016). Functional range of motion in older adults has been shown to decrease with increasing age (Troke et al., 2001; Dvorak et al., 1995; McGill et al., 1999), indicating the osteoligamenous spine may be a contributor to alterations in functional range of motion and spine stiffness. Stiffness was found to decrease following the cyclic loading
protocol regardless of specimen age. This is consistent with previous work exposing human spines to repetitive bending (Adams and Dolan, 1996).

A greater proportion of disc herniations were observed in young specimens compared to mature specimens following the cyclic loading protocol, supporting hypothesis 1. It has been hypothesized by previous authors (Tampier et al., 2007; Yingling et al., 1999) that the reduction in human disc water content with increased age (Antoniou et al., 1996) decreases the pressure gradient between the annulus and nucleus that contributes to delamination (Stefanakis et al., 2014), making herniation injuries less likely as age increases. This hypothesis aligns with the results of the current investigation. However, previous published work has indicated that the prevalence of sub-catastrophic failure, such as disc disruption, clefts and tears in the annulus increase with increasing age (Butler et al., 1990; Videman et al., 1995; Miller et al., 1988) and can be a pain generating pathway (Peng et al., 2005; Peng et al., 2006; Freemont et al., 1997; DePalma et al., 2007). While not measured in the current investigation, it is possible that greater sub-catastrophic failure occurred in older specimens despite a lower frequency of herniations.

The endplate dimensions and vertebral height were greater in mature specimens compared to younger specimens. This is consistent with previous work where the endplate area of porcine lumbar discs was significantly higher in mature (2-3 years) compared to younger (4 months) samples (Lundin et al., 2000). Pigs aged between 6 – 8 months are analogous to adolescent humans in skeletal development (Yingling et al., 1999). Given the estimated life expectancy of pigs can be up to 15-20 years (Shankland, 2011), it is likely that the sow specimens used in the current study represent a combination of maturation and aging compared to the market hog specimens. However, degenerative changes in human discs (radial tears) begin prior to the completion of growth, at age 11-16 years (Boos et al., 2002), demonstrating that the
process of age/degeneration and maturation are difficult to fully separate in the absence of simulated aging scenarios.

It was hypothesized that disc height would be lower in adolescent specimens (hypothesis 2b) and disc height would decrease following the cyclic loading protocol (hypothesis 2a). Disc height at the C56 level was not affected by age, but was affected by the loading protocol, supporting hypothesis 2a, and giving cause to reject hypothesis 2b. There was no significant age related change in disc height. This contrasts with previous work that has demonstrated a significantly greater reduction in disc height in young specimens following compressive axial loading on mature (3.4 years) and young (6 months) porcine lumbar segments (Park et al., 2005). However, the difference may be explained by disc degeneration, specifically disc height loss. Clinically, one aspect of disc degeneration includes disc height loss (Brinjikji et al., 2014). Spines in the mature group from Park and colleagues (2005) were excised from sows that experienced greater than 7 deliveries in order to ensure degenerative changes in the disc and vertebrae. In contrast, specimens in the current study were required to meet a grade I criteria of degeneration. Following the cyclic loading protocol, disc height was significantly reduced in the current study, which is consistent with previous work (Gooyers and Callaghan, 2015).

The results of the study can be considered in light of a few limitations. The age of the sow specimens was estimated from the abattoir in the age range between 1.5 to 8 years. Given that pigs can live up to 15-20 years (Shankland, 2011), the spines obtained likely represent a combination of maturation and aging. However, the time course of porcine aging has not been systematically investigated. All of the spines from the mature group were obtained from sow spines whereas the market hogs were a mix of male and female pigs. The study utilized a porcine model of the cervical spine to as a surrogate for the human lumbar spine. While there
are geometric similarities between human and porcine FSUs, (Yingling et al., 1999) the applicability of the results to the study to human spines is limited by the model implemented.

3.6 Conclusion

   Mechanical properties of functional spine units were investigated across the age of specimens in response to cyclic loading. The results of the study indicate greater stiffness and reduced range of motion in aged porcine cervical spines compared to adolescent spines, altering the mechanical environment of the disc, which may predispose older specimens to sub-catastrophic failure pathways and constrain functional range of motion in older adults. Fewer herniation injuries were observed in mature specimens compared to younger specimens, however, older specimens may be more predisposed to clefts and fissures in the annulus, which are a potential pain generating pathway. An understanding of how mechanical properties of the spine change in response to age and loading are important to understanding spine injuries throughout the life course.
4. Study II: The effect of simulated aging and degeneration on the material properties of the porcine annulus fibrosis

4.1 Introduction

While the initiation of disc degeneration is not fully understood, its prevalence increases with increasing age (Butler et al., 1990; Videman et al., 1995; Miller et al., 1988). In a study that macroscopically graded degenerative changes from over 600 discs, 97% of specimens from donors over 50 years exhibited degenerative changes (Miller et al., 1988). Early signs of degeneration have been reported as early as the second decade of life (Boos et al., 2002; Miller et al., 1988). The initiation of disc degeneration is thought to be related to a genetic predisposition (Battie et al., 1995a; Battie et al., 1995b), nutritional factors (Urban et al., 2000) and mechanical loading (Adams et al., 2000). While the link between degeneration and aging is not completely understood, recent evidence has identified differences in endplate diffusion to identify two separate processes of aging and degeneration (Rajasekaran et al., 2004).

Age and degeneration have been shown to alter the patterns of loading on the intervertebral disc and the annulus fibrosis. Recent evidence has shown a greater pressure concentration gradient between the nucleus and the annulus among degenerated specimens and this difference has been hypothesized to increase the propensity for delamination of the annulus (Stefanakis et al., 2014). Inward buckling of the annulus has been shown to increase most notably in extension (Heuer et al., 2008) when the nucleus is removed (Heuer et al., 2008; Goel and Kim, 1989). When the annulus buckles under compression, the interlamellar matrix may be prone to propagation of injury through delamination. Recent evidence using a needle puncture model of degeneration in rabbits has shown that the interlamellar matrix strength (between
adjacent layers) is reduced in degenerated discs compared to healthy control discs (Gregory et al., 2014). Using an ovine model of the spine and 3 different age groups, the intralamellar matrix strength (between collagen bundles in a single layer) had greater elastic stiffness in young compared to older specimens (Stewart et al., 2017). Furthermore, single layer annulus samples obtained from older ovine discs have reduced peak stress at failure compared to younger samples (Stewart et al., 2017).

Increased lactic acid in the disc has been associated with cell death in the nucleus, discogenic pain, is a hypothesized initiator of disc degeneration, but there has been no link established in the literature between lactic acid and mechanical changes in the annulus. Decreased pH has been found in the nucleus and the annulus of patients suffering from discogenic pain (Kitano et al., 1993; Keshari et al., 2008). Specifically, a pH of 6.65 (0.07) in painful discs was recently compared to a pH of 7.14 (0.04) among asymptomatic patients (Kitano et al., 1993). A spectral analysis identified a higher lactic peak among symptomatic patients compared to asymptomatic controls (Keshari et al., 2008). Impaired metabolite transfer, leading to an accumulation of lactic acid in the disc, has been hypothesized as an initiator of disc degeneration (Horner and Urban, 2001). Previous work has documented increased cell death in cultured bovine nucleus cells in a medium with a pH of 6.0 compared to a pH of 7.4 (Horner and Urban, 2001). Interlamellar matrix strength has been shown to be 30% lower in degenerated discs compared to control discs in a lapine puncture model (Gregory et al., 2014). However, the specific aspect of degeneration responsible for the reduction of interlamellar strength was not determined and attributed to the degenerative cascade in general. The purpose of this study was to determine if a simulated age-related degenerative change (an acidic environment) influences the material properties of young isolated annular specimens.
4.2 Hypotheses

1. a. Peak stress will decrease over time (stress-relaxation response).
   b. Peak stress will decrease in the low pH compared to a neutral environment.
   c. Samples excised from the posterolateral region of the annulus will have a greater reduction in peak stress compared to those from the anterolateral region.
   d. There will not be an effect of spinal level on peak stress.

Previous work has demonstrated a significant stress-relaxation response in isolated annulus tissue over 100 cycles of tensile loading (Gooyers and Callaghan, 2016). It is hypothesized that the low pH environment will change the material properties and mimic the response of cyclic fatigue damage that has been noted previously (Gregory and Callaghan, 2012; Iatridis et al., 2004). Further, it is expected that the effect of a low pH environment will be greater in specimens from the posterolateral annulus given that this is often the site of injury (Tampier et al., 2007).

2. a. The stretch–stress modulus will be lower in the acidic compared to a neutral environment.
   b. Specimens excised from the posterior region of the disc will not have a different stretch–stress modulus compared to those from the anterolateral region.
   c. The stress-stretch modulus will not differ across spinal level.

It is expected that the effect of a low pH environment will decrease S-S modulus compared to control conditions due to disruption of elastin fibres in the annulus (Gregory and Callaghan, 2012). The results of previous work have been mixed with respect to S-S modulus of annulus tissue across anterior and posterior regions. Some work has shown no difference in stiffness across regions (Karakolis, 2014; Gregory and Callaghan, 2010; Fujita et al., 1997) while other work has shown higher stiffness in single layer samples excised from the anterior side of the disc (Skaggs et al., 1994).
3. a. Specimens exposed to the acidic environment will have a higher stretch and lower stress at the end of the toe region compared to specimens in the neutral solution.
b. Specimens excised from the posterolateral region will have a higher stretch and lower stress at the end of the toe region compared to anterolateral specimens.
c. The stretch and lower stress at the end of the toe region will not be affected by spinal level.

Previous work has demonstrated a larger toe region in single layer annulus samples exposed to vibration, attributed to elastin disruption (Gregory and Callaghan, 2012). Given that the posterior annulus is the primary site of disc injury (Tampier et al., 2007), it is hypothesized that mechanical disruption will be more likely to occur in the posterior region.

4. a. Specimens will have greater increases in thickness and mass in the posterior compared to anterior region of the disc.
b. Specimens immersed in acidic solutions will increase in thickness and mass less than control solutions.
c. The percent change in mass and thickness will not be affected by spinal level.

Proteoglycan concentration is primarily responsible for the swelling pressure of the disc (Urban et al., 1979). Isolated samples of annulus fibrosis have been shown to swell in aqueous solutions (Han et al., 2012; Gruevski et al., 2016) with a regional (superficial, deep) dependency (Han et al., 2012). Previous work has demonstrated a higher concentration of proteoglycans in younger compared to older human discs (Antoniou et al., 1996).

4.3 Methods

4.3.1 Specimens and dissection

A total of 26 cervical spines were obtained fresh-frozen from immature (aged ~6 months) porcine specimens and stored at -20 degrees Celsius. An a priori sample size calculation (where $\alpha=0.05$, $1-\beta=0.8$, $d=0.707$) determined a minimum of 64 annulus specimens were required to be immersed in one of four lactic acid or control solutions. A total of 79 annulus specimens were dissected from two vertebral levels (C34, C56) with a maximum of four annulus samples
dissected from each disc level (2 anterolateral, 2 posterolateral) (Figure 15). Specimens were thawed at room temperature for a minimum of 12 hours prior to dissection. A summary of the distribution of annulus samples across solution and location is depicted in Table 6.

![Figure 15 Location of the annulus dissection from (A) the anterior side of the disc and (B) posterior side of the disc](image)

**Table 6** Summary of the distribution of tested annulus samples (total = 79) across radial disc location (anterior or posterior), cervical level (C34, C56) and solution

<table>
<thead>
<tr>
<th>Solution group</th>
<th>Anterior</th>
<th></th>
<th>Posterior</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C34</td>
<td>C56</td>
<td>C34</td>
<td>C56</td>
</tr>
<tr>
<td>Acid (pH = 3.5)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Acid (pH = 6.0)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Neutralized acid (pH = 7.0)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>PBS (pH = 7.2)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Cervical spines were dissected into 2 functional spine units (FSUs) to preserve the discs at the levels of C34 and C56. Specimens were further dissected using a handsaw to cut through the right and left pedicles to expose the posterior annulus (Figure 15B). The full annulus was removed on both the anterior and posterior sides via cuts parallel to the endplate on either side of the intervertebral disc using a scalpel. Between 3 and 5 annular layers, with an average (standard
deviation) of 3.2 (0.4) layers were dissected from an intermediate region of the annulus (between layers 3-8) while viewed through a stereoscopic optical zoom microscope (Nikon SMZ 1000, Nikon Instruments Inc., Melville, NY, USA). Baseline specimen dimensions were approximately 10 mm (circumferential) x 5 mm (axial) with an approximate baseline thickness of 1 mm. Specimens were dissected to be longer in the circumferential dimension (Figure 16A) in order to identify the orientation of specimens following immersion (section 4.3.2). The thickness of specimens was measured at baseline and following immersion using a laser displacement sensor (Omron ZX-LD40L Smart Sensor, Omron Canada Inc., Toronto, ON, CAN) using a 40 mm (±10 mm) sensing distance and a resolution of 2 µm. Following immersion, specimens were cut to testing dimensions (5 mm x 5 mm) (Figure 16B). Specimen mass was measured at baseline and following immersion (prior to cutting to testing dimensions) using a digital scale to a resolution of 0.01 mg (XS205 Analytical Balance, Mettler Toledo, Mississauga, ON, Canada).
Figure 16 Annulus specimen (A) cut to immersion dimensions and (B) testing dimensions.

4.3.2 Exposure solutions

4.3.2.1 Overview

Specimens were randomized into one of four solutions including; (i) pH 7.2 Phosphate buffered saline (PBS), (ii) pH 3.5 Lactic acid in PBS (15 mmol/L), (iii) pH 6 Lactic acid in PBS (15 mmol/L) or (iv) pH 7 Lactic acid in PBS (15 mmol/L) for a duration of 6 hours. Specimens were immersed in cryotube vials containing 2 mL of test solution (Figure 17A). Sealed vials were stored at 37 deg Celsius in a water bath (Microprocessor Controlled 280 Series, Thermo Scientific, Millcreek, OH, USA) (accuracy of ± 0.1 deg) containing distilled water (Figure 17B). The rationale and preparation of the solutions are discussed in sections 4.3.2.2 and 4.3.2.3, respectively. Following immersion in the exposure solution, specimens were rinsed with PBS,
dried with a Kimwipe, thickness and mass were measured, cut to testing dimensions and underwent mechanical testing (section 4.3.3). A summary of the four solutions can be found in Table 7.

![Figure 17 Specimens immersed in cryotube vial containing 2 mL of testing solution (A) and sealed vials immersed in heated water bath (B)](image)

Table 7 Summary of pH and lactic acid concentrations of the 4 testing solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>pH</th>
<th>Lactic acid concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffered saline (PBS)</td>
<td>7.2</td>
<td>0</td>
</tr>
<tr>
<td>Lactic acid in PBS (low pH)</td>
<td>3.5</td>
<td>15</td>
</tr>
<tr>
<td>Lactic acid in PBS (low pH, biological range)</td>
<td>6.0</td>
<td>15</td>
</tr>
<tr>
<td>Lactic acid in PBS (neutralized)</td>
<td>7.0</td>
<td>15</td>
</tr>
</tbody>
</table>

4.3.2.2 Rationale

The target concentration and pH of each solution was determined through reviewing existing literature on human intervertebral discs and pilot work to extract lactate from samples of porcine disc tissue. There was no literature that reports lactate concentrations from porcine discs. Lactate dehydrogenase (the conjugate base of lactic acid) was extracted from post-mortem porcine annulus tissue to determine; (i) the baseline lactate concentration of post-mortem porcine
annulus across age, (ii) if the lactate concentration of an annulus can be shifted through immersion in a lactic acid solution and (iii) the duration of exposure to a lactic acid solution required to initiate a shift in lactate. The following sections detail the rationale for selecting the target pH and concentration of solutions and the methods implemented for the metabolite extraction.

4.3.2.2.1 Solution design

Phosphate buffered saline (PBS) represented a control solution for the 3 lactic acid solutions. Phosphate buffered saline was selected as a control solution as it has a salt concentration and pH that mimics the human body. The pH 7 lactic acid in PBS solution was a control for pH compared to the pH 3.5 and pH 6 lactic acid solutions. Specifically, the neutralized (pH 7) lactic acid solution was included to determine whether any observed effects in the study can be attributed to low pH, or a property of lactate.

The target concentration of lactic acid used in the current study was designed to replicate values found in human disc specimens and previous work simulating disc degeneration using a porcine model. It was assumed that lactic acid and lactate (conjugate base) existed in equilibrium in all solutions (and the concentrations were equal). Previous work measuring lactate concentrations in the human annulus (Bartels et al., 1998) and nucleus (Diamant et al., 1968) have reported concentrations ranging from 2-16 mmol/L (tissue water) to 5-15mmol/kg (wet weight) respectively. There is no literature reporting the lactate concentration in porcine discs. However, previous work using a porcine model simulated disc degeneration by mixing a sodium lactate solution (concentration 15 mmol/L) with hydrochloric acid in nuclear material harvested from living pigs (Iwabuchi et al., 2001). In the present study, the concentration of lactic acid was controlled to be 15mmol/L in all lactic acid solutions.
The pH 6 lactic acid in PBS solution in the current study was intended to represent a biological acid condition while the pH 3.5 lactic acid in PBS solution was designed to provide a mechanistic understanding of the effect of low pH. The target pH of each of the acidic solutions was based on values reported in the human nucleus and previous work simulating disc degeneration using porcine and bovine models. Several authors have measured the pH of the nucleus across different pain and damage (herniation, nerve root damage) conditions in human discs. The range of pH values in the human nucleus across all conditions (damage, pain and control) was reported to be; 5.7-7.5 (Diamant et al., 1968; Kitano et al., 1993; Nachemson, 1969). In the annulus, previous work has attributed higher lactic acid peaks among patients experiencing discogenic pain (Keshari et al., 2008). This work provides evidence of increased lactic acid in the annulus (not isolated in the nucleus). While not measured directly, the results of the Keshari et al. (2008) study also implies a lower pH in the annulus given the linear relationship between lactate concentration and pH. Work by Diamant and colleagues (1969) demonstrated a linear relationship between lactate and pH in the nucleus, where, as the lactate concentration increases, the pH decreases. Previous work has simulated disc degeneration in porcine discs using pH 6 and pH 3.5 sodium lactate solutions to determine the effect on nerve conduction velocity (Iwabuchi et al., 2001) and other work has cultured isolated bovine nucleus cells in a pH 6 medium (Horner and Urban, 2001).

4.3.2.2.2 Lactate dehydrogenase extraction

The lactate dehydrogenase extraction and subsequent fluorometric measurements were completed across 3 conditions; on (i) immature samples, (ii) mature samples and (iii) immature samples following immersion in a pH 6 15mmol/L lactic acid solution for a duration of 3 hours and 6 hours. The first two tests were completed to determine the baseline lactate concentration
of immature and mature porcine samples. The third condition was completed to confirm if a 3-hour or 6-hour exposure to a pH 6 15 mmol/L lactic acid solution in PBS could initiate a shift in lactate. It has previously been shown that a majority of the uptake of fluid in full disc (Pflaster et al., 1997; Costi et al., 2002) and isolated annulus (Gruevski et al., 2016) samples occurs within the first 60 minutes of immersion. A total of 3 cervical spines were obtained from 2 immature (aged ~6 months) and 1 mature (aged 1.5-8 years) porcine specimens and stored at -20 deg Celsius.

The protocol used to extract lactate from samples of annulus has been completed previously on muscle tissue (Tupling et al., 2001) and blood (Bedbrook, 2010). Samples of annulus were dissected from the anterior and posterior region of the spine by excising the full structure using cuts parallel to the end plate of the disc. Dissection took place following 12 hours of specimen thaw at room temperature. Cross sections of the full disc (15-25 layers) measuring approximately 5 cm x 5 cm were weighed and stored at room temperature under a vacuum for 48 hours. It was determined that the weight of the specimens did not change after 48 hours of vacuum storage, representing the dry weight of all samples. Dried annulus tissue was crushed using a mortar and pestle followed by teasing out tissue using a straight blade to achieve a flake or powder-like consistency. Samples had an average (standard deviation) mass 22.4 (4.22) mg and were placed in pre-weighed microcentrifuge tubes. The extraction was completed over a duration of 120 minutes by adding 0.5 M perchloric acid to each tube. Specimens were centrifuged for 10 minutes at 15000 G at 0 deg Celsius. The supernatant was removed, frozen at -90 deg Celsius and neutralized with 2.3 M potassium bicarbonate. Tubes were centrifuged for 10 minutes at 15000G at 0 deg Celsius and the supernatant was removed.
Following the extraction, samples were prepared for calculating lactate concentration using a fluorometric procedure (Tupling et al., 2001; Bedbrook, 2010). The supernatant from each lactate extraction of the annulus was mixed with a reagent containing Hydrazine, Glycine and NAD'. In addition, the reagent was added to test tubes containing distilled water and a lactate standard. A baseline measurement from the spectrofluorophotometer (RF-1501; Shimadzu, Columbia, MD) was taken from all the samples. Dilute lactate dehydrogenase (L2625-50KU, Sigma-Alderich, Oakville, ON, Canada) was added to all test tubes and placed in the dark for a duration of 60 minutes. A second measurement was taken from the spectrofluorophotometer following the reaction. The change in fluorometric readings represented the fluorescence of NADH, which is proportional to the annulus lactate concentration (Bedbrook, 2010). Known concentrations and fluorometric readings from (i) the distilled water sample (where lactate concentration = 0) and (ii) the lactate standard were used to calculate the slope of the linear relationship (between lactate concentration and fluorometric readings). Using this linear relationship and the measured spectorfluorophotometer readings of the unknown solution (obtained from annulus samples), the lactate concentration was calculated in mmol/L wet weight.

To quantify differences in the baseline concentration of lactate across age, an unpaired two tailed t-test was with significance level (α) of 0.05 was completed on mature samples (n=15) and juvenile samples (n=4). To determine if lactate concentration was shifted in juvenile samples through immersion in a lactic acid solution for either 3 or 6 hours, a 1 way general linear model was applied to compare concentrations in samples at (i) baseline, (ii) 3 hours of immersion and (iii) 6 hours of immersion. General linear model analysis was computed using...
SAS studio (version 9.4, SAS Institute Inc., Cary, NC), with a significance level (α) of 0.05. A Tukey-Kramer post-hoc test was used to detect significant effects across immersion duration.

The concentration of lactate was not affected by age but increased in market hog annulus following immersion in lactic acid. A summary of the lactate extraction results can be found in Table 8. The results of an unpaired two tailed t-test showed no significant difference in baseline lactate concentrations in the annulus between sow and market hog samples (p=0.799). Significant increases in lactate concentrations were found comparing immersion conditions (p<.0001). Post-hoc analysis revealed significant increases in lactate concentration in the 3 hour and 6 hour immersion conditions compared to baseline values (p<.0001). However, there were no significant differences in lactate concentration comparing immersion in a pH 6 15 mmol/L lactic acid solution for either 3 hour or 6 hours (p=0.0572).
Table 8 Summary of lactate extractions from post-mortem porcine annulus samples. T-test results indicate no significant difference between lactate concentrations of sow specimens compared to market hog samples at baseline (p=0.799). Results from the general linear model demonstrate significant differences between solution condition (p<.0001). Post-hoc differences (a,b) indicated the immersion conditions significantly increased lactate compared to baseline values (p<.0001) but the concentration did not differ between immersion durations (p=0.0572)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Level</th>
<th>Location (A=Anterior, P=Posterior)</th>
<th>Lactate concentration (mmol/L)</th>
<th>Average (standard deviation) lactate concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market hog (n=4)</td>
<td>C45</td>
<td>A</td>
<td>0.74</td>
<td>0.63 (0.25)</td>
</tr>
<tr>
<td></td>
<td>C56</td>
<td>A</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C56</td>
<td>P</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C67</td>
<td>A</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Sow (n=15)</td>
<td>C23</td>
<td>A</td>
<td>1.21</td>
<td>0.79 (0.28)</td>
</tr>
<tr>
<td></td>
<td>C23</td>
<td>A</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C23</td>
<td>A</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>A</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>A</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>P</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>P</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C45</td>
<td>A</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C45</td>
<td>A</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C56</td>
<td>A</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C56</td>
<td>A</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C56</td>
<td>P</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C56</td>
<td>P</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C67</td>
<td>A</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C67</td>
<td>A</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Market hog (n=7)</td>
<td>C45</td>
<td>A</td>
<td>1.60</td>
<td>1.68 (0.19)</td>
</tr>
<tr>
<td>3 hours of</td>
<td>C56</td>
<td>A</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>immersion (n=4)</td>
<td>C56</td>
<td>P</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C67</td>
<td>A</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>6 hours of</td>
<td>C23</td>
<td>A</td>
<td>1.84</td>
<td>1.84 (0.16)</td>
</tr>
<tr>
<td>immersion (n=3)</td>
<td>C34</td>
<td>A</td>
<td>1.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>P</td>
<td>2.11</td>
<td></td>
</tr>
</tbody>
</table>

The results of the pilot work indicate that porcine discs do not change lactate concentration across age and immersion in a lactic acid solution can significantly increase the concentration of lactate in annulus tissue. Generally, the concentration of lactate in post-mortem
porcine annulus tissue were lower than those reported from human discs in the literature (Bartels et al., 1998; Diamant et al., 1968). Specifically, baseline concentration of lactic acid ranged on average between 0.63 to 0.79 mmol/L in market hog and sow spines respectively compared to 2-16 mmol/L in human annulus (Bartels et al., 1998). Immersion in a pH 6 15 mmol/L lactic acid in PBS solution for a duration of either 3 or 6 hours significantly increased lactate concentration in samples compared to baseline. Previous work has shown that a majority of the uptake of fluid occurs within first 60 minutes of immersion (Gruevski et al., 2016; Costi et al., 2002), but it could take up to 4 hours before functional spine units (i.e. full joint) plateau in terms of fluid (saline) absorption (Costi et al., 2002). Given these results, samples in the study II of this thesis were immersed in the 4 respective solutions (Table 7) for a duration of 6 hours prior to mechanical testing.

4.3.2.3 Preparation

Lactic acid in PBS solutions were mixed by adding a commercially produced lactic acid to PBS and neutralizing with sodium hydroxide to achieve the target pH. The PBS solution was made according to the proportions presented in Table 9. The pH of PBS was adjusted by adding between 1-2 drops of 0.5 M HCl to reduce the pH to 7.2, monitored using a digital pH meter (Mettler Toledo SevenEasy S20 pH Meter, Mississauga, Ontario, Canada). Purchased lactic acid (Sigma-Aldrich, Oakville, ON, Canada) had an 85% concentration at room temperature and was added to the PBS. A 1 M sodium hydroxide (NaOH) solution was used to neutralize all lactic acid solutions while being monitored by a digital pH meter. To ensure the final concentration of test solutions was 15 mmol/L, the required volume of lactic acid was added to less than the final volume of PBS, the solution was neutralized and brought to final volume with the remaining
PBS. For example, 2.4 mL of lactic was added to 120 mL of PBS, was neutralized to pH = 6 using 1 M NaOH and brought to volume with PBS so the final volume was 200 mL, making a 15 mmol/L pH 6 solution. All test solutions were mixed at room temperature. It was confirmed that there would be no effect of temperature on the pH of each solution by heating all solutions in a beaker while measuring the pH continuously. The pH was recorded at 37 deg Celsius (immersion temperature), 30 deg Celsius (mechanical testing temperature) as well as room temperature and was shown to be stable in all instances (Table 10).

Table 9 Overview of the proportions included in phosphate buffered saline solutions

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 mL (1L)</td>
<td>Distilled water</td>
</tr>
<tr>
<td>2.33 g</td>
<td>Sodium phosphate dibasic</td>
</tr>
<tr>
<td>0.18 g</td>
<td>Sodium phosphate monobasic</td>
</tr>
<tr>
<td>9 g</td>
<td>Sodium chloride</td>
</tr>
</tbody>
</table>

Table 10 Measured pH of test solutions across temperature

<table>
<thead>
<tr>
<th>Temperature (degrees Celsius)</th>
<th>Phosphate buffered saline (PBS, pH = 7.2)</th>
<th>15 mmol/L Lactic acid in PBS (pH = 3.5)</th>
<th>15 mmol/L Lactic acid in PBS (pH = 6)</th>
<th>15 mmol/L Lactic acid in PBS (pH = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient (25.5-27)</td>
<td>7.17</td>
<td>3.50</td>
<td>6.02</td>
<td>7.00</td>
</tr>
<tr>
<td>30</td>
<td>7.18</td>
<td>3.50</td>
<td>6.01</td>
<td>6.99</td>
</tr>
<tr>
<td>37</td>
<td>7.18</td>
<td>3.51</td>
<td>6.02</td>
<td>6.99</td>
</tr>
</tbody>
</table>

4.3.3 Mechanical testing

Following 6 hours of immersion in an exposure solution, specimens were rinsed with PBS, dried with a Kimwipe, thickness and mass were measured, cut to testing dimensions and underwent mechanical testing. Specimens were biaxially loaded in tension in both the circumferential and axial directions using a BioTester apparatus (BioTester 5000, CellScale, Waterloo, ON, CAN). Isolated specimens were oriented such that the circumferential and axial
directions of loading represented hoop and longitudinal stress on an intact disc, respectively. Specimens were mounted in the BioTester apparatus by puncturing the tissue using five sharpened tungsten rakes along each side. The loading protocol consisted of a pre-load, pre-conditioning and loading cycles. The preload implemented an initial load of between 10-15 mN of force in both circumferential and longitudinal directions to ensure uniform biaxial tension at the initiation of testing. The pre-conditioning phase loaded specimens to 10% strain at a rate of 1%/s for a total of 5 cycles. A total of 100 cycles of biaxial tensile loading were completed in displacement control to 20% strain at a rate of 2%/s. The full duration of the protocol was 35 minutes. Actuator displacement and force was sampled at 30 Hz for the full duration of the loading protocol. Specimens were hydrated with distilled water through an ultrasonic humidifier using a protocol developed previously in our lab (Gruevski et al., 2016).

4.3.4 Dependent Measures

Dependent measures of interest included peak stress (MPa) at 20% stretch, the stretch (%) and stress (MPa) at the end of the toe region and the stress-stretch modulus. Engineering stress was calculated as the measured tensile force divided by the cross-sectional area of the sample (following 6 hours of immersion) in the plane normal to the applied force. The cross-sectional area calculation included 35% of each sample’s apron width to improve specimen stress estimates (Eliaghi et al., 2009). Actuator displacement and force were filtered with a dual pass second order Butterworth filter with a low-pass frequency cut-off of 10 Hz (Gooyers and Callaghan, 2016). Individual loading cycles were identified using actuator displacements.

The stress and stretch at the end of the toe region were identified using a linear fit and inverse slope technique used previously (Nelson-Wong et al., in press). A line was generated connecting the first and last point of a stretch cycle. Subsequently, the inverse negative slope of
the line used to connect the two sides of the loading cycle was used to solve for the equations of the lines from the line connecting the first and last point back to the loading curve. The longest distance between these two curves was considered the end of the transition zone (end of the toe region). In order to confirm this process was successful in identifying the linear region of the curve, regression lines were fit to the right side of the curve (beyond the transition zone). The $R^2$ values were found to be between 1 and 0.97. The stress-stretch modulus was determined by calculating the slope of the regression line equation fit to the linear portion of the stress-stretch curve in both the circumferential and axial directions. Peak stress was calculated at cycles 1, 50 and 100. The S-S modulus, end of toe region stress and end of toe region stretch were reported in cycle 1. The percent change in mass and thickness of specimens before and after the immersion were compared across all solution groups.

4.3.5 Statistical analysis

A 3-way (solution x level x location) general linear model was completed on the change in mass (%), change in thickness (%), end of toe region stress (MPa) in cycle 1 (circumferential, axial) and the end of toe region stretch in cycle 1 (%) (circumferential, axial) and stress-stretch modulus (MPa) in cycle 1 (circumferential, axial). Two 4-way (solution x level x location x cycle) mixed general linear models with repeated measures on cycle (cycles 1, 50 and 100) were completed on peak stress (MPa) at 20% in both loading directions. All statistical analyses were computed using SAS studio (version 9.4, SAS Institute Inc., Cary, NC), with a significance level ($\alpha$) of 0.05. Data containing repeated measures (cycle) were fit using a model with a covariance structure accounting for sphericity based on the recommendations of a statistics and actuarial science consultant at the University of Waterloo. Omega squared calculations (Keppel, 1982) were completed for all significant interactions. Further analyses were completed on interactions
accounting for greater than 1% of the total variance (Andrews et al., 2012). Tukey-Kramer post-hoc tests were used to detect significant effects.

4.4 Results

4.4.1 Change in Mass and Thickness

There was a main effect of solution (p<.0001) and disc location (p=0.0010) on the percent change in mass of specimens, while there was no significant effect of disc level (p=0.0561) on percent change in mass. Post-hoc analysis revealed specimens immersed in the Acid 3.5 solution had a significantly lower percent change in mass compared to all other solution conditions (p<.0001). Specifically, specimens immersed in the Acid 3.5 condition increased mass an average of 28.6% after 6 hours of immersion compared to a greater than 91% increase in all other solutions. The percent change in mass observed in the Acid 6 condition was significantly (15%) lower compared to the increase observed in the specimens immersed in the PBS condition (p=0.0298). A summary of the percent change in mass across solution is depicted in Figure 18. There was a main effect of location on the percent change in mass, where samples obtained from the posterior location of the disc increased in mass an average 13% more compared to specimens obtained from the anterior location of the disc (Table 11).
The percent change of thickness in specimens was significantly affected by solution (p<.0001) and location (p=0.0009) whereas there was no significant effect of disc level (p=0.2509). The effect of solution was driven by a significantly lower increase in thickness among specimens immersed in the Acid 3.5 condition with a 29% increase compared to greater than 77% in the other solution conditions (p<.0001) (Figure 19). The change in thickness was not significantly different between specimens immersed in the Acid 6, Neutralized or PBS solutions (p>0.6774). The change in thickness was dependent on the radial location of the spine, where specimens obtained from the posterior region of the disc had a 17% greater increase compared to those excised from the anterior region (Table 11). Images of specimen thickness
before and after immersion captured through a stereoscopic optical zoom microscope are depicted in Figure 20.

![Graph showing percent change in thickness across solution condition](image)

**Figure 19** Percent change in thickness across solution condition. Post-hoc results are depicted in lowercase letters.

<table>
<thead>
<tr>
<th>Location</th>
<th>Change in Mass (%)</th>
<th>Change in Thickness (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior</td>
<td>Posterior</td>
<td></td>
</tr>
<tr>
<td>Acid 3.5</td>
<td>74.3 (29.4)</td>
<td>60.4 (26.9)</td>
<td></td>
</tr>
<tr>
<td>Acid 6</td>
<td>87.0 (41.0)</td>
<td>77.3 (37.3)</td>
<td>p=0.0010</td>
</tr>
<tr>
<td>Acid Neutralized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td></td>
<td></td>
<td>p=0.0009</td>
</tr>
</tbody>
</table>

**Table 11** The change in mass and thickness across radial disc location. Significant differences across radial disc location are denoted by A,B.
Figure 20 Annulus specimens before immersion and following 6 hours of immersion in PBS (bottom left) and Acid 3.5 (bottom right) solutions

4.4.2 Peak Stress

There was a significant level by solution interaction to affect peak circumferential stress (p=0.0067) (Figure 21). Post-hoc analysis revealed specimens obtained from the C56 location immersed in the Acid 3.5 solution had higher peak stress compared to specimens immersed in Acid 6, Neutralized and PBS solutions across both levels (p<0.0026). Specimens obtained from the C34 level and immersed in the pH 3.5 solution had higher peak stress in the circumferential direction compared to specimens obtained from C56 level immersed in the pH 6 solution (p=0.0200) but was not different from specimens at either level or solution group (including C56 specimens in Acid 3.5 solution (p=0.1177)). There were no significant differences in the
circumferential peak stress in the Acid 6, Neutralized or PBS solutions across both cervical levels (p>0.4137).

![Graph showing peak circumferential stress across level and solution group](image)

**Figure 21 Peak circumferential stress across level and solution group**

A significant interaction between location and cycle affected peak circumferential stress (p=0.0292) (Figure 22). Peak stress in specimens obtained from the posterior location was significantly higher compared to specimens from the anterior location in cycle 1 (p=0.0048), cycle 50 (p=0.0011) and cycle 100 (p=0.0017). There was a significant stress-relaxation response where stress was significantly reduced in the anterior location across each cycle (p<0.0001) and in the posterior location across each cycle (p<0.0001). There was no significant difference between the peak stress of anterior specimens at cycle 50 compared to peak stress of posterior specimens at cycle 100 (p=0.5137).
There was a significant 3 way interaction of radial disc location, cervical level and cycle on the axial peak stress ($p=0.0018$). At the C34 level, there was no significant difference in peak stress between samples excised from the anterior and posterior locations at cycle 1 ($p=1.000$), cycle 50 ($p=0.9585$) or cycle 100 ($p=0.9319$) (Figure 23A). In contrast, at the C56 level, specimens excised from the posterior region of the disc had significantly higher peak stress compared to samples from the anterior region of the disc at cycle 1 ($p=0.0110$), cycle 50 ($p=0.0286$) and cycle 100 ($p=0.0293$) (Figure 23B). Peak stress at each time point was significantly different from each other at the C34 level in anterior ($p<.0001$) and posterior ($p<.0001$) specimens. At the C56 level, peak stress at each time point was significantly different from each other in anterior ($p<.0001$) and posterior ($p<.0001$) specimens. While not depicted graphically in Figure 23, post hoc analysis comparing levels C34 and C56 obtained from the

Figure 22 Peak circumferential stress across radial disc location and cycle

There was a significant 3 way interaction of radial disc location, cervical level and cycle on the axial peak stress ($p=0.0018$). At the C34 level, there was no significant difference in peak stress between samples excised from the anterior and posterior locations at cycle 1 ($p=1.000$), cycle 50 ($p=0.9585$) or cycle 100 ($p=0.9319$) (Figure 23A). In contrast, at the C56 level, specimens excised from the posterior region of the disc had significantly higher peak stress compared to samples from the anterior region of the disc at cycle 1 ($p=0.0110$), cycle 50 ($p=0.0286$) and cycle 100 ($p=0.0293$) (Figure 23B). Peak stress at each time point was significantly different from each other at the C34 level in anterior ($p<.0001$) and posterior ($p<.0001$) specimens. At the C56 level, peak stress at each time point was significantly different from each other in anterior ($p<.0001$) and posterior ($p<.0001$) specimens. While not depicted graphically in Figure 23, post hoc analysis comparing levels C34 and C56 obtained from the
anterior region demonstrated no significant difference in peak stress at cycle 1 (p=0.7886), cycle 50 (p=0.9845) or cycle 100 (p=0.9858). In samples obtained from the posterior region of the disc, there was also no significant differences observed between levels at the same time point; at cycle 1 (p=0.8247), cycle 50 (p=0.9971) or cycle 100 (p=0.9989).
Figure 23 Peak axial stress in specimens dissected from (A) C34 and (B) C56 levels across radial location and cycle.
4.4.3 Toe Region Stress and Stretch

There was a significant interaction between solution and radial disc location to affect circumferential stress at the end of the toe region (p=0.00280) (Figure 24). Post-hoc analysis revealed that end of toe region stress was significantly higher in posterior specimens immersed in the Acid 3.5 solution compared to all other conditions and locations (p<0.0036), including specimens obtained from the anterior location immersed in Acid 3.5 solution. There were no significant differences across solution condition within a location, or across solutions between location (p>0.0852).

Figure 24 Circumferential stress at the end of the toe region across radial disc location and solution in cycle 1

Solution and cervical level interacted to significantly affect the circumferential stress at the end of the toe region (p=0.0008) (Figure 25). The post-hoc analysis showed that specimens
excised from the C56 level and immersed in the Acid 3.5 solution had significantly higher circumferential stress at the end of the toe region compared to all other solution groups across both levels (p<0.0005) and specimens obtained from the C34 level immersed in Acid 3.5 solution (p=0.0134). Specimens obtained from the C34 level and immersed in the Acid 3.5 solution had significantly higher stress at the end of the toe region compared to C56 specimens immersed in the Acid 6 solution (p=0.0287).

![Graph showing circumferential stress at the end of the region across level and solution in cycle 1](image)

**Figure 25** Circumferential stress at the end of the region across level and solution in cycle 1

In the axial direction, there was a main effect of location to affect the stress at the end of the toe region (p=0.0016). Specifically, specimens obtained from the posterior location had an average 0.01 MPa higher stress at the end of the toe region compared to specimens obtained from the anterior side of the disc (Figure 26). There was no main effect or interaction involving solution group or level on end of toe region stress in the axial direction (p>0.0548).
There was a main effect of radial location on the end of toe region stretch ratio in both the circumferential ($p=0.0178$) and axial ($p=0.0006$) directions. Specifically, the stretch ratio at the end of the toe region was significantly higher in specimens obtained from the posterior region compared to the anterior region in both the circumferential and axial directions (Table 12).

**Table 12** Stretch ratio during cycle 1 at the end of the toe region in both the circumferential and axial directions across radial disc location. Significant differences across radial location are denoted by A,B.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Location</th>
<th>Anterior</th>
<th>Posterior</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumferential</td>
<td>1.099 (0.008)$^A$</td>
<td>1.103 (0.007)$^B$</td>
<td>0.0178</td>
<td></td>
</tr>
<tr>
<td>Axial</td>
<td>1.098 (0.008)$^A$</td>
<td>1.104 (0.007)$^B$</td>
<td>0.0006</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 26** Axial stress at the end of the toe region in cycle 1 stretch across radial disc location
4.4.4 Linear Region S-S Modulus

The range of $R^2$ values for the linear region of all specimens in cycle 1 was between 0.97 and 1.0 in both the circumferential and axial directions, confirming the identification of the linear elastic region. The slope of the regression line was used to report the S-S modulus in the linear region (i.e. stiffness).

There was a significant solution by level interaction to affect circumferential stiffness ($p=0.0059$) (Figure 27). Post-hoc analysis revealed that specimens obtained from the C56 level and immersed in the Acid 3.5 solution had significantly higher stiffness compared to specimens across both levels in the other solutions ($p<0.0235$). There was no significant difference between the stiffness of specimens obtained from the C34 or C56 levels immersed in the Acid 3.5 condition ($p=0.0914$). There was no difference in the stiffness of C34 specimens immersed in the Acid 3.5 solution compared to the other solutions and levels ($p>0.1520$). There was a main effect of radial location to affect the circumferential stiffness in the linear region ($p=0.0001$). Specimens obtained from the posterior region had an average stiffness (standard deviation) of 3.00 (1.00) MPa compared to 2.30 (0.79) MPa in anterior samples.
There was a significant interaction between radial disc location and cervical level to affect axial stiffness ($p=0.0051$) (Figure 28). Post-hoc analysis revealed that specimens excised from the posterior region at C56 had significantly higher axial stiffness compared to specimens excised from the anterior location from both cervical levels ($p<0.0396$). There was no difference in axial stiffness in specimens obtained from the anterior location across level ($p=0.2552$) and there was no differences in stiffness between levels in specimens obtained from the posterior region ($p=0.1237$). There was no effect of solution on the axial stiffness of specimens ($p=0.6024$).
4.5 Discussion

The results of the study demonstrate that a low pH environment affects the mechanical properties of isolated annulus fibrosis tissues. Contrary to our hypotheses linked to mechanical properties, the low pH environment resulted in greater circumferential stiffness, higher peak stress and higher toe region stress in C56 specimens. These results suggest that lactic acid in the disc stimulates a mechanism that increases stiffness in the annulus and may partially explain the increased stiffness in functional spine units in older specimens observed in study I. Given that lactic acid accumulation in the disc is a hypothesized initiator of the degenerative/aging cascade, the higher stresses noted in response to lactic acid exposure suggest that lactic acid may have the potential to increase risk of injury through clefts in the annulus. Recent work has demonstrated
that sub-acute levels of cyclic loading create sub-catastrophic disc injuries (Gooyers et al., 2015; Schollum et al., 2018) and this injury risk may be higher among older specimens.

It was hypothesized that peak stress would decrease over time (hypothesis 1a), decrease in a low pH solution (hypothesis 1b), posterolateral samples will have a greater stress-relaxation response compared to anterolateral samples (hypothesis 1c), while there will be no effect of cervical level (hypothesis 1d). The results of the study supported the hypothesized stress-relaxation response (hypothesis 1a) of specimens. In contrast to our hypotheses, peak stress in samples interacted with level, solution, radial disc location and responded differently in the circumferential and axial directions, not providing support for hypotheses 1bcd. Solution condition significantly interacted with cervical level to affect peak circumferential stress. In particular, it was shown that specimens dissected from the C56 level and immersed in the Acid pH 3.5 condition had significantly higher peak stress compared to specimens in all other solution groups. Specimens dissected from the C34 level immersed in the Acid 3.5 condition had peak stress values that were not statistically different from the C56 group but had lower peak stress on average. The porcine cervical spine has a lordotic curve; where the relative sagittal angle between the C5 and C6 vertebrae is greater than the angle between the C3 and C4 vertebrae. Given the difference in angle of collagen attachment to the endplate between cervical levels, it is possible that the attachment details of collagen across level are differentially affected by exposure to a lactic acid solution. Previous work has investigated the effect of targeted degradation of post-mortem annulus tissue in the radial direction (Perie et al., 2006; Smith et al., 2008). Enzymatically digested proteoglycans in post-mortem bovine annulus tissue demonstrated a reduction in confined compressive modulus compared to a non-exposed group of specimens (Perie et al., 2006). When loaded in tension, radially cut specimens treated with an
enzymatic degradation of elastin demonstrated a significant reduction in (radial) linear modulus compared to untreated samples from human donors (Smith et al., 2008). While this work tested specimens in the radial direction (between multiple layers), it provides evidence of a relationship between the chemical composition and material properties of the annulus. The current study provides evidence of a link between lactic acid and material properties of the annulus when loaded in tension replicating the physiologic orientation of the disc.

In the axial direction, peak stress was affected by cycle, cervical level and radial disc location with all specimens demonstrating a significant stress-relaxation response. Specifically, at the C34 level, there was no difference between radial locations at any time point, whereas, in the C56 level, the posterior samples had higher peak stress at all time points. Peak stress in the circumferential direction had a dependency on radial disc location and cycle. Peak stress was higher at cycles 1 and 100 in specimens obtained from the posterolateral compared to the anterior disc location, consistent with previous work investigating multilayer annulus samples (Gooyers and Callaghan, 2016). Specimens demonstrated a significant stress-relaxation response across level. When the magnitude of peak stress at cycle 100 was normalized to cycle 1, the peak stress in cycle 100 was reduced in anterior and posterior samples by 68% and 63% respectively compared to 45% and 49% in Gooyers and Callaghan (2016). The greater relaxation response in the current study may be explained by differences in the loading protocols between studies. The current study utilized a 20% stretch (circumferential-axial) directions whereas Gooyers and Callaghan (2016) implemented a 12-20% stretch (circumferential-axial).

It was hypothesized that specimen stiffness would be lower in the low pH environment (hypothesis 2a) while stiffness would not be affected by radial disc location (hypothesis 2b) or cervical level (hypothesis 2c). In contrast to these hypotheses, circumferential stiffness increased
in specimens immersed in the Acid 3.5 solution and dissected from the C56 level and circumferential stiffness was higher in the posterior region compared to the anterior region of the disc. A regional dependency on axial stiffness was also demonstrated where there was higher stiffness in specimens obtained from the posterior region at the C56 level. Previous work investigating the effect of chemically-induced collagen crosslinking in bovine annulus fibrosis tissue (Chuang et al., 2007) demonstrated similar results to those reported in the current study. Specifically, Chuang and colleagues (2007) immersed annulus samples in either PBS or Genipin (chemical used to increase collagen cross-linking) for a duration of 2 days and tested for both markers of increased collagen cross-linking as well as stiffness of specimens in tension. The results of the study indicated the procedure increased collagen cross-linking and circumferential stiffness, with no effect on linear stiffness in the axial direction (Chuang et al., 2007). It is possible that exposure to a lactic acid solution increases stiffness in the circumferential direction through a collagen cross-linking mechanism. Previous work has shown that collagen cross-linking in the annulus and nucleus is related to increases in age (Pokharna and Phillips, 1998) and clinical findings have reported increases in lumped passive flexion stiffness with increasing age (Shojaei et al., 2016). While more work is needed to confirm that lactic acid leads to increases in collagen cross-linking, it is possible that the treatment of annulus tissue with lactic acid may be an effective model to simulate age-related mechanical changes.

It was hypothesized that there would be lower relative increases in thickness and mass in low pH environments (hypothesis 4b) and in anterior samples (hypothesis 4a), while there would be no effect of cervical level (hypothesis 4c). The results of the study supported these hypotheses as specimens absorbed less fluid in a low pH environment compared to neutral pH environments, and this uptake of fluid had a regional dependency on radial disc location, but not
cervical level. Specifically, specimens immersed in the PBS, Neutralized and Acid 6 solutions increased in mass and thickness on average more than 92% and 77% respectively. The Acid 3.5 solution however, average increases were only 29% in both mass and thickness. The Acid 6 solution had a significantly lower change in mass compared to the increase observed in the specimens immersed in the PBS condition. However, there were no significant differences in the Neutralized condition compared to the PBS condition, indicating that differences in percent change and thickness are driven by pH and not a property of lactate. Previous work has shown that the intact human (Pflaster et al., 1997) and ovine (Costi et al., 2002) intervertebral disc tissues have a propensity to swell in aqueous solutions in the absence of load. Work from our lab has demonstrated that unloaded porcine annular samples isolated from the disc structure nearly double in mass and thickness when submerged in normal saline (Gruevski et al., 2016), similar to the results from this study using PBS. In the current study, exposure to a low pH solution prevented the typical swelling behaviour of the annulus when immersed in aqueous solutions. Previous work testing a lactic acid solution with a lactate concentration above 15 mmol/L and pH below 6.8 has shown to reduce the production rates of proteoglycans in the nucleus of bovine discs (Ohshima and Urban, 1992). The effect of low pH may have implications for the swelling pressure of the disc, disc height and buckling of the annulus. The posterior region of the disc was shown to imbibe more fluid compared to the anterior region of the disc increasing in mass and thickness by more than 12% and 16% respectively. Previous work examining regional differences in proteoglycan content in human discs has demonstrated higher content in the posterior annulus compared to the anterior region (Iatridis et al., 2007). Given that proteoglycans regulate the ability for disc material to imbibe fluid (Urban et al., 1979), the greater increase in mass and thickness in the posterior compared to anterior region
observed following immersion in the present study may be explained by proteoglycan concentration.

Toe region stress and stretch were hypothesized to be lower and higher, respectively, in specimens immersed in a low pH solution (hypothesis 3a) and excised from the posterolateral region (hypothesis 3a) while there would be no effect of cervical level (hypothesis 3c). In contrast to our hypotheses, the stretch and stress at the end of the toe region were significantly affected by cervical level, radial disc location and solution. In the circumferential direction, solution condition interacted separately with cervical level and radial location to affect stress at the end of the toe region. Specifically, stress at the end of the toe region was found to be higher in specimens immersed in the Acid 3.5 condition obtained from both the C56 level and the posterior region of the disc (separately interacting with solution condition). In the axial direction, there was no effect of solution condition, but specimens obtained from the posterior region has higher stress at the end of the toe region compared to the anterior region. The regional difference in toe region stretch, while statistically significant, was small in magnitude, where the posterior samples had a higher stretch at the end of the toe region compared to anterior samples. The end of the toe region in tensile tests of the annulus is thought to be related to the uncrimping of collagen fibres in a relaxed state. There is some evidence to suggest a regional difference in stretch at the end of the toe region in the intralamellar matrix between the anterior posterior locations (Gregory and Callaghan, 2012).

The results of the study can be considered in the context of a few limitations. This study utilized a lactate concentration in each test solution based on the concentration range found in human discs and applied them to a porcine model of the spine. Based on our pilot work, the baseline lactate concentration in porcine discs is lower compared to those reported from human
discs. Furthermore, the low pH Acid solution used a pH of 3.5, which is a value that is much lower than the pH that would occur in a living disc. However, these test conditions were intended to contribute to a mechanistic understanding of the link between lactic acid and the material properties of the annulus fibrosis. During immersion, specimens were stored at the internal body temperature of pigs (37 degrees Celsius) but tested mechanically at 30 degrees Celsius. This was due to the physical distance between the heat lamp and the CCD camera. Based on pilot work, the pH of test solutions was stable in the temperature ranges tested in this study.

4.6 Conclusions

While previous work has demonstrated a link between lactic acid, cell death in the nucleus and discogenic pain, this is the first study to link lactic acid and mechanical changes in the annulus. Low pH was shown to reduce the propensity of annulus tissues to imbibe fluid compared to immersion in neutral pH solutions. These changes demonstrate that the annulus is a contributor to increased spine stiffness changes with age. Furthermore, discs with accumulated lactic acid may adapt through a cross-link mechanism that results in higher peak stress and stiffness in the circumferential direction that could put older discs at greater risk of annulus damage, such as delamination or fissures in the tissue.
5. Study III: Postural, discomfort and passive tissue responses as a function of age and sex in response to seated exposures

5.1 Introduction

Prolonged sitting has been linked with a potential for increased risk of acute low back pain (Alperovitch-Najenson et al., 2010; Anderson et al., 1974; Callaghan et al., 2010) and is common in many occupations across age and industry. These results are supported by transient low back pain development among otherwise young and healthy participants exposed to a prolonged (2 hour) bout of lab simulated sitting in either an office (Callaghan et al., 2010; Schinkel-Ivy et al., 2013) or automotive (Gruevski et al., 2013) seat. According to the National Household survey, between 38-40% of Canadian workers employed as bus drivers, subway operators, general transit operators, taxi drivers, chauffeurs, survey interviewers and statistical clerks were aged over 55 years in 2011 (Galarneau, 2011); representing a large proportion of the workforce in jobs requiring prolonged seated exposures.

The loss of lordosis that occurs in sitting (De Carvalho et al., 2010; Keegan et al., 1953; Makhsous et al., 2003) may increase strain on the posterior elements of the spine. During sitting, young males have been shown to adopt postures that approach maximum flexion range of motion (De Carvalho et al., 2010; Dunk and Callaghan, 2005). The neutral zone hypothesis represents a range of laxity in an osteoligamentous joint (Panjabi, 1992a, 1992b) and more recent work has extended the concept to represent the lumped neutral zone of the low back in vivo (Scanell and McGill, 2003; Gallagher, 2014). If seated postures occur outside the flexion limit of the neutral zone, this would impose greater strain on the posterior elements of the spine.
There are documented changes to the lumbar lordosis and range of motion of the spine that may influence how older adults tolerate prolonged seated exposures. Evidence from the literature has demonstrated that the range of motion in flexion and extension of the lumbar spine decreases with increases in age among isolated specimens from human donors (Taylor and Twomey, 1980) and in vivo (Dvorak et al., 1995; McGill et al. 1999; Taylor and Twomey, 1980). There is also evidence that older adults stand in less extended postures (Takeda et al., 2009) compared to younger adults (De Carvalho et al., 2010) in part due to anterior wedging of the disc (Takeda et al., 2009). It is unclear how these changes will influence seated postures, discomfort responses or stiffness across age. Previous work has demonstrated that participants with hypolordosis in standing (reduced extension), sit in a more flexed posture and further from the flexion limit of the neutral zone compared to control participants (Scannell and McGill, 2003).

The purpose of the study was to determine the effect of age on lumped passive trunk stiffness, lumbar postures and discomfort responses during prolonged seated exposures. A secondary purpose was to determine sex specific responses.

5.2 Hypotheses

1. a. It is expected that lumped passive stiffness will increase after the prolonged sitting exposure.
1. b. Lumped passive torso stiffness will be higher in male participants compared to female participants.
1. c. Participants over 45 years will have greater lumped passive torso stiffness compared to those between 18 and 35 years.

Previous work has demonstrated increases in transition zone lumped passive flexion stiffness in both male and female young, healthy participants following one hour of sitting without a backrest (Beach et al., 2005) and in an automotive seat (De Carvalho and Callaghan, 2011). The breakpoints at the end of each transition zone were found to be shifted toward a reduced range of
motion in males (an indicator of increased stiffness) after 2 hours of sitting while there was no difference among female participants (Beach et al., 2005). A recent investigation that measured baseline passive lumped torso stiffness in flexion from an upright torso position demonstrated a main effect of age where participants over 52 years had significantly greater lumped torso stiffness compared to participants under the age of 48 years (Shojaei et al., 2016).

2. a. It is expected that discomfort will increase over time in response to prolonged sitting.
2. b. Discomfort reporting will be higher in participants over 45 years.
2. c. There will be no difference in discomfort reporting between sexes.

Previous work has reported increases in acute discomfort reporting following continuous sitting exposures without a backrest over time (Skinkel-Ivy et al., 2013). On a population scale, it has been reported that the prevalence of non-specific low back pain reporting increases with increasing age (Dionne et al., 2006).

3. a. It is expected that seated lumbar flexion will be greater in male participants compared to female participants.
3. b. Seated lumbar flexion will increase over time.
3. c. Participants under 45 years will sit in greater lumbar flexion compared to participants over 45 years.

Previous work has demonstrated that female participants adopt seated postures characterized by low lumbar flexion (upright sitting posture) and greater anterior rotation of the pelvis compared to male participants (Dunk and Callaghan, 2005) while seated without a backrest. There is evidence in the literature that with increases in age, standing lordosis decreases (Takeda et al., 2009) and spine range of motion decreases (Dvorak et al., 1995; McGill et al 1999; Taylor and Twomey, 1980). Among participants with hypolordotic (reduced lordosis) standing postures; these participants sat with greater lumbar flexion (further outside the neutral zone) compared to participants with a lumbar lordosis within a “normal” range (Scannell and McGill, 2003).
Previous work examining prolonged seated exposures have demonstrated an increase in posterior pelvic rotation and increased lumbar flexion over time (Callaghan et al., 2010).

5.3 Methods

5.3.1 Participants

Studies III and IV were collected in the same session and shared a common cohort of participants. An *a priori* sample size calculation (G*Power version 3.1.3, Kiel, Germany) using an effect size of 0.707, power (1-β) of 0.8 and α=.05 determined a minimum of 28 participants were required for adequate power. A total of 34 participants were collected and Figure 29 depicts the distribution of participants in the study. Informed written consent was obtained prior to testing and the study was approved by the University of Waterloo Office of Research Ethics. Older participants were defined as those aged over 45 years (WHO, 1993) and younger participants defined as individuals between 18 and 35 years. The upper limit of participant age included those aged 69 years, as 18-69 years was the validated age range for the physical activity screening tool used in the study. The average (standard deviation) of participants included in the study by age category was; 23.8 (5.0) years and 63.7 (3.9) years in young and mature groups respectively. Students and administrative staff from the university population were considered eligible for either age group. Participants were also recruited through a local recreation centre and clubs in the community. The inclusion criteria required that all participants had no pain or low back injury in the past year requiring a visit to a medical doctor or lost time at work. All participants had no professional lifting experience and were considered to be novice lifters. In order to control variability in regular levels of physical activity and anthropometry, a screening procedure (in addition to the inclusion criteria) was implemented prior to collections.
Participants were screened for both health status during physical activity and to confirm moderate levels of regular exercise. The Physical Activity Readiness Questionnaire (PAR-Q) was completed by all participants to ensure no contraindications (such as a heart condition or joint pain) to physical activity prior to participation. A copy of the PAR-Q survey can be found in Appendix A.1. Isometric training has been shown to increase passive trunk stiffness over a 6 week period, demonstrating a relationship between physical activity and lumped passive torso stiffness (Lee and McGill, 2015). As an inclusion criteria for this study, participants were required to be moderately active (at work, recreation, home) according to the International Physical Activity Questionnaire (I-PAQ) (I-PAQ, 2016; Ainsworth et al., 2000). The definition of moderate physical activity according to the I-PAQ was; achieving 600 to 3000 MET-minutes per week across all exercise intensities, completed for a minimum of 10 minutes per bout.

Participants were asked to evaluate the previous 7 days of activity, indicate the number of days per week spent exercising in each category of physical activity (vigorous, moderate and walking) and include the duration of activity each day. The definition of walking, moderate and vigorous
activities are equivalent to 3.3 METs, 4.0 METs and 8 METs respectively. The I-PAQ scoring protocol was used to categorize participant’s activity as low, moderate or high. Participants meeting the moderate criteria were able to participate in the study. The last question in the I-PAQ survey asked participants to estimate the number of hours per weekday spent sitting. Sitting behaviour was not used as part of the screening procedure, but this information was collected and summarized. Previous work has documented the test-retest reliability of the survey and its validity (Brown et al., 2004; Craig et al., 2003). A copy of the I-PAQ short self-assessment survey is included in Appendix A.2 and the scoring protocol can be found in Appendix A.3.

Several anthropometric measurements including waist circumference, Body Mass Index (BMI) and standing height have been linked to changes in seated spine posture and range of motion and were included as control factors in this study. A longitudinal investigation (16 year follow-up) has demonstrated that on average, BMI increases with increasing age (Savinainen et al., 2004). Obesity (defined according to BMI) has been shown to reduce seated and standing forward flexion range of motion in the thoracolumbar spine compared to controls within the normal weight range of BMI (Gilleard and Smith, 2007). Therefore, in the present study, participants with a body mass index (BMI) within a normal or overweight range (between 18.5 kg/m² and 29.9 kg/m²) (WHO, 1995) were recruited in order to avoid obesity as a co-variant with age. While there is no direct link between altered lumbar posture and waist circumference, previous work has attributed differences in thoracolumbar posture across BMI categories to the apposition of the torso against the thighs (Gilleard and Smith, 2007). Given that BMI does not account for weight distribution, waist circumference was an inclusion criteria in the study. A waist circumference equal to or below 102 cm for men and 88 cm for women represent a high
action value for overall health and has been shown to identify individuals with a BMI over 30 kg/m$^2$ with a 2% rate of misclassification (Lean et al., 1995). Participants in the current study were required to meet a minimum of one or both of the BMI and waist circumference criteria to participate in the study. Previous work has demonstrated that differences in seated postures in vehicle seats may be partly explained by standing height differences (Reed et al., 2000). In this study, participants within each sex (i.e. female participants between age groups) were paired with similar heights between age categories. A summary of the screening procedures for study III and IV collections can be found in Table 13.

**Table 13 Summary of participant screening procedure for the study III, IV collection**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>• Between 18-35 years or 45-69 years</td>
</tr>
<tr>
<td>Low back pain/injury status</td>
<td>• No back pain/injury in the past 12 months requiring either (i) a visit to a medical doctor or (ii) time away from work</td>
</tr>
<tr>
<td>Professional lifting experience</td>
<td>• No professional lifting experience (i.e. construction or material handling)</td>
</tr>
<tr>
<td>BMI/Waist circumference</td>
<td>• Participants were required to have a BMI within the normal or overweight range (between 18.5 kg/m$^2$ and 29.9 kg/m$^2$) and/or</td>
</tr>
<tr>
<td></td>
<td>• Participants with a BMI &gt;30 kg/m$^2$ were eligible for the study if the waist circumference measured ≤102 cm for males and ≤88 cm for females</td>
</tr>
<tr>
<td>Physical activity</td>
<td>• No major contraindications to exercise (PAR-Q)</td>
</tr>
<tr>
<td></td>
<td>• Moderately active as defined by the I-PAQ survey (600-3000 MET-min/week)</td>
</tr>
</tbody>
</table>
5.3.2 Protocol

5.3.2.1 Overview

Studies III and IV were collected in the same session and shared a protocol, outlined in Figure 30. In each session, participants were asked to complete 3 static calibration poses, a total of 12 lifts, sit continuously for 90 minutes and complete 2 side-lying passive stiffness tests. The details of the lifting tasks and maximal postures are discussed in greater detail in study IV. The following sections outline the protocol for the side-lying passive spine stiffness test and the prolonged sitting protocol relevant to study III.
Figure 30 Overview of shared protocol for studies III and IV. Participants were asked to complete a total of 12 lifts during the session, sit continuously for 90 minutes and complete a side lying lumped passive spine stiffness test in both flexion and extension.
5.3.2.2 Side Lying Passive Stiffness

A customized frictionless table was used to measure the passive moment angle relationship about the flexion-extension axis at baseline and following 90 minutes of continuous sitting. The apparatus has been previously published (McGill et al., 1994) and has been used to characterize the passive stiffness of the trunk (Scannell and McGill, 2003; Beach et al., 2005; Parkinson et al., 2004; De Carvalho and Callaghan 2011). The table consists of a lower body mount, an upper body mount and a Plexiglas tray that contains nylon ball bearings to permit free motion between the two surfaces (upper body mount and bearing tray) (Figure 31). Participants were asked to lie on the right side in the apparatus, stacking the hips on top of each other in order to prevent out of plane motion. The pelvis, lower extremities and upper body were secured using adjustable nylon straps attached to the upper and lower body mounts to isolate motion about the low back. To prevent axial rotation of the spine, participants were asked to wrap their arms around a vertical bar attached to the upper body mount. A pillow was supplied to each participant to ensure horizontal alignment of the spine. In order to isolate the passive contributions of the trunk, muscle activity was maintained below 5% maximum voluntary contraction (Gallagher, 2014) in all successful trials. If muscle activity exceeded 5%, the trial would be indexed to only include the passive portion of the trial. Participants were asked to relax all trunk and low back musculature and to avoid tensing up during the passive trials. A maximum of 3 trials in both the flexion and extension direction were collected.
The passive stiffness test involved the experimenter pulling the participant through a range of motion in series with a load cell in both the flexion and extension directions in separate trials. The upper body mount and lower body mounts were initially connected using wooden dowels while the participant was secured into position. After the participant was secured, the upper body mount and lower body mount were detached allowing for unconstrained motion of the upper body mount on the nylon ball bearing surface. A uniaxial load cell (MLP-500-CO, Transducer Techniques, Temecula, California, USA) was attached in series via a cable to the upper body mount and the handle used by the experimenter to pull participants in the flexion and extension directions. Two guiding bars were bolted in to the top surface of the upper body mount in order to aid in maintaining a perpendicular alignment of the handle during the pulling phase of the passive stiffness test. The pace of each pull was guided by a metronome at 60 beats per minute. The distance between a point digitized on the load cell (tracked by a cluster attached to the handle) represented the location of force application and a point digitized at the skin...
surface overlying the space between the 4th and 5th lumbar vertebrae (tracked with a cluster located at L1) represented the joint about which movement occurred and the moment arm of the applied force.

5.3.2.3 Prolonged Sitting

Participants were asked to complete a transcribing task on a desktop computer while seated in a backless chair for a duration of 90 minutes. Previous work has documented that among a population of office workers, the longest continuous sitting event was an average of 98 minutes in an 8 hour work day (Ryan et al., 2011). Desk height, seat pan height and reach envelopes were customized for each participant according to established guidelines for light work (Canadian Standards Association, 2012). Participants were asked to sit naturally during the prolonged sitting protocol but were not permitted to cross legs (Figure 32).
Figure 32 Participants were asked to complete a transcribing task on a desktop computer while sitting continuously for 90 minutes

5.3.3 Dependent Measures

5.3.3.1 Body Discomfort

Participants were asked to rate musculoskeletal discomfort using a 100 mm visual analog scale (VAS) at 15 minute intervals during the prolonged sitting protocol in addition to a baseline VAS, for a total of 7 surveys. The scale was anchored with 0 mm representing no discomfort and 100 mm representing extreme discomfort. Participants were asked to rate musculoskeletal discomfort in 12 different body locations including: the neck, left and right shoulders, upper back, middle back, low back, pelvis, sacrum, left and right buttocks and left and right thighs. The discomfort survey tablet interface schematically depicted each body location and each rating.
was completed by dragging an on-screen toggle switch manually between anchor points to indicate levels of discomfort. The baseline survey results were removed from all subsequent ratings in order to isolate any changes in discomfort prior to the collection from changes induced by the protocol over time. In addition, the baseline removed peak discomfort across all time points within a single body location was analyzed. A depiction of the discomfort survey interface can be found in Appendix B.1.

5.3.3.2 Kinematics
5.3.3.2.1 Motion Capture (Studies III and IV)

A four camera-bank optoelectronic motion capture system (Optotrak Certus System, Northern Digital Inc., Waterloo, ON, Canada) was used to track the relative motion of the thorax, lumbar spine and pelvis, left thigh, left shank and left foot of each participant for both studies III and IV. The capture volume, including the desk used for lifting and typing and the area surrounding the frictionless table, was calibrated dynamically using a 16-marker calibrated cube, with a root mean squared (RMS) marker position error (between camera banks) no greater than 0.43 mm. The global coordinate system of the collection space was defined by collecting a static trial of the cube (Optotrak Certus System, Northern Digital Inc., Waterloo, ON, Canada) in the capture volume, with an RMS error less than 0.26 mm in all collections. A total of 6 custom made rigid body clusters, with either 4 or 5 infrared light emitting Smart Markers (Optotrak Certus System, Northern Digital Inc., Waterloo, ON, Canada) affixed to each cluster, were secured to each body location using double sided tape. Each segment was defined by landmarks located distally and proximally to the cluster location (Table 14). A calibrated 4 Marker Digitizing Probe (Northern Digital Inc., Waterloo, ON, Canada) was used to establish the relationship between digitized anatomical landmarks and the local coordinate system of tracked
marker clusters. Anatomical landmarks were palpated and virtually digitized by holding a calibrated probe tip on the skin surface above each landmark to designate the end points of each segment. Marker trajectories were sampled at 50 Hz.

Table 14 Summary of tracking markers and digitized segment endpoints

<table>
<thead>
<tr>
<th>Cluster Location</th>
<th>Digitized Points</th>
<th>Segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>9th Thoracic vertebra</td>
<td>Right and left acromion, right and left 12th rib</td>
<td>Thoracic</td>
</tr>
<tr>
<td>1st Lumbar vertebra</td>
<td>Left and right 12th rib, and left and right iliac crests</td>
<td>Lumbar</td>
</tr>
<tr>
<td>Sacrum</td>
<td>Right and left anterior superior iliac spines, right and left posterior superior iliac spines</td>
<td>Pelvis</td>
</tr>
<tr>
<td>Lateral aspect of the left thigh</td>
<td>Left greater trochanter of the femur, left lateral and medial knee joint lines</td>
<td>Left thigh</td>
</tr>
<tr>
<td>Lateral aspect of the left lower leg</td>
<td>Left lateral and medial knee joint lines, left lateral and medial malleoli</td>
<td>Left shank</td>
</tr>
<tr>
<td>Lateral aspect of the left foot</td>
<td>Left lateral and medial malleoli, head of first and fifth metatarsal</td>
<td>Left foot</td>
</tr>
</tbody>
</table>

5.3.3.2.2 Data Analysis (Study III only)

An Euler angle decomposition sequence (flexion/extension, lateral bend and axial twist) was calculated in Visual3D (C-Motion Inc, Visual3D Standard v4.96.4, Germantown, MD, USA) for the lumbar spine relative to the pelvis and thoracic spine relative to the pelvis. Marker locations were used to construct a three-dimensional rigid link segment model in Visual 3D. A 5 second static calibration pose with the participant standing in anatomical position was collected to establish the local coordinate systems using digitized markers and the reference posture for all the monitored segments. The local coordinate systems of each segment were constructed according to ISB guidelines (Wu and Cavanaugh, 1995), with the positive X axis pointing anteriorly, Y pointing superiorly and Z pointing to the right. In the event of missing data points, an interpolation procedure was implemented using a 3rd order polynomial with a maximum gap.
of 0.2 seconds of data (10 frames). (Howarth and Callaghan, 2010). A 4th order Butterworth filter with a 3 Hz cut-off frequency (Howarth et al., 2009) was applied to raw marker trajectories using 50 padding points at the beginning and end of each trial using the reflection method (Howarth and Callaghan, 2009).

Angles were calculated during prolonged sitting and passive stiffness trials. An Euler angle decomposition sequence was calculated in Visual3D (C-Motion Inc, Visual3D Standard v4.96.4, Germantown, MD, USA) for the lumbar spine relative to the pelvis and thoracic spine relative to the pelvis using a flexion/extension, lateral bend and axial twist sequence. Average angles (lumbar and thoracic) about the mediolateral axis were calculated in 15 minute intervals over the duration of the 90 minute sitting protocol. In addition, the average lumbar angle about the mediolateral axis during quiet standing was subtracted as a bias from lumbar angles recorded during the prolonged sitting protocol.

5.3.3.3 Passive Stiffness
5.3.3.3.1 Surface Electromyography Collection Procedure

Surface electromyography (EMG) was sampled bilaterally from; the erector spinae at the level of 9th thoracic vertebra, erector spinae at the level of the 3rd lumbar vertebra, rectus abdominus and external oblique. The skin overlying each muscle site was shaved and cleansed with alcohol to promote adhesion of the electrode surface to the skin. Disposable Ag-AgCl surface electrodes (Blue Sensor, Medicotest Incorporated, Ølstykke, Denmark) were attached to the skin surface in line with the muscle fibre orientation, with an inter-electrode distance of 2 cm. Signals were differentially amplified and band pass filtered (10 - 1000 Hz; CMRR > 115 dB at 60 Hz; input impedance ~10GΩ; AMT-8, Bortec Biomedical Ltd., Calgary, AB, Canada). EMG signals were digitized with a 2000 Hz sampling frequency by a data acquisition board with 16
bits of resolution and a dynamic range of ±2V controlled by First Principles (Northern Digital Inc., Waterloo, ON, Canada).

Participants were asked to complete a series of exercises at a maximum voluntary intensity for normalization purposes. For the erector spinae muscles, participants were asked to lie prone on a table with the anterior superior iliac spine cantilevered over the edge of the table. The investigator provided manual resistance as the participant extended the torso (Dankaerts et al., 2004; McGill, 1992; Schinkel-Ivy et al., 2013). For the abdominal muscles, participants performed a series of manually resisted abdominal contractions including; trunk flexion, right lateral bend, left lateral bend, right axial twist and left axial twist (Dankaerts et al., 2004). A demonstration of each exercise was provided prior to maximum effort exercises. A supine rest trial was collected to represent quiet activity across all channels.

5.3.3.3.2 Data analysis

The data processing procedure for electromyography signals collected during passive stiffness trials involved noise removal, normalization procedure and down sampling. The noise removal procedure was implemented in all trials and included; (i) the removal of any bias in each signal, (ii) a high pass 4th order zero lag Butterworth filter with a 30 Hz cutoff frequency (Drake and Callaghan, 2006) (to remove heart rate contamination), (iii) a 60 Hz (between 59 and 61 Hz) 4th order Bandstop filter (Mello et al., 2007) to remove electromagnetic hum. Signals were then full wave rectified and a 4th order zero lag Butterworth filter with a 2.5 Hz cutoff frequency (Brereton and McGill, 1998) were applied to all EMG channels and trials. The normalization procedure utilized the noise removed MVE and supine rest trials to express the muscle activity during passive trials as a percentage of maximum effort. The normalization procedure involved;
(i) detecting the minimum signal from each muscle during the rest trial and subtracting this value from all other trials, (ii) detecting the peak signal achieved during the MVE trials and expressing activity during passive trials as a percentage of the maximum. The normalized muscle activity during all passive trials was then down sampled to 50 Hz in order to permit comparisons to kinematic data. If muscle activity exceeded 5% during the trial, the trial was cut off at that point as it was considered to no longer represent passive tissue properties.

The flexor and extensor reaction moments during the passive stiffness tests were calculated based on the calibrated outputs of the load cell multiplied by the perpendicular distance between the location of the load cell and the estimated location of the L4-L5 joint. Angles where normalized on a scale of 0 to 100%, where the first frame of the first pull (with the participant lying in the jig) representing 0%, while 100% was represented by the maximum flexion angle recorded while standing. An exponential model was fit to the moment and normalized angle data according to equation 1a, where y represents moment, x represents flexion angle and A and B were the coefficients of the model (Figure 33A). Moments were differentiated with respect to angles and evaluated for each frame according to the formula in equation 1b and consistent with the methods used by previous authors (McGill et al., 1994; Parkinson et al., 2004) to calculate instantaneous stiffness at each value of x (percent flexion) (Figure 33B). The average (standard deviation) of A and B from all exponential models in the study were 2.28 (1.87) and 0.04 (0.03) respectively. Instantaneous stiffness is reported at select values of flexion in the neutral zone including, 10%, 20% and 30% flexion.

\[ y = Ae^{Bx} \]  \hspace{1cm} \text{Equation 1a}

\[ \frac{dy}{dx} = AB e^{Bx} \]  \hspace{1cm} \text{Equation 1b}
Figure 33 Moment-angle curve with exponential fit during passive stiffness trial (A) Instantaneous stiffness plotted with flexion angle (B)
5.3.5 Statistical analysis

A two-way general linear model (sex x age) was applied to the general characteristics of the study participants including: standing height (m), BMI (kg/m²), waist circumference (cm), physical activity (MET minutes/week) and sitting exposures (hours). Two way general linear models (sex x age) were applied to baseline removed peak discomfort in each of the 12 body locations (%). Three way (age x sex x time) mixed general linear models with repeated measures on time condition (15, 30, 45, 60, 75, 90 minutes) was applied to baseline removed discomfort (%) in 12 body locations, averaged lumbar angles (degrees), averaged thoracic angles (degrees) and averaged lumbar angles relative to standing (degrees). A three way (age x sex x time) mixed general linear model with repeated measures on time (pre/post sitting) were applied to the instantaneous stiffness at 10%, 20% and 30% of maximum standing flexion. All statistical analyses were computed using SAS studio software (version 9.4, SAS Institute Inc., Cary, NC), with a significance level (α) of 0.05. Tukey-Kramer post hoc tests were used to detect significant effects.

5.4 Results

5.4.1 General Characteristics

There were no significant differences across age in height, BMI, waist circumference or physical activity (p>0.0916), indicating successful matching across age groups. There was a significant difference in sitting behaviour between age groups (p=0.0048), where younger participants sat for an average 2.4 hours longer per day (in a weekday) compared to the mature group. Significant sex differences were observed where males had greater height, BMI and waist circumference compared to female participants (p<0.0101). There were no sex specific
differences in physical activity. A summary of the general characteristics of participants are included in Table 15.

Table 15 Summary of the general characteristics of study 3 and 4 participants. Superscript letters indicate significant sex differences while superscript numbers indicate significant age differences.

<table>
<thead>
<tr>
<th>Screening Measure</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young (n=9)</td>
<td>Mature (n=9)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.62 (0.07)A</td>
<td>1.60 (0.06)A</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>22.8 (1.8)A</td>
<td>24.0 (2.7)A</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>72.0 (8.2)A</td>
<td>78.2 (6.1)A</td>
</tr>
<tr>
<td>Physical activity (MET-minutes/week)</td>
<td>2309 (566)</td>
<td>1969 (762)</td>
</tr>
<tr>
<td>Sitting duration per day (hours)</td>
<td>8.7 (3.1)$^1$</td>
<td>5.7 (1.6)$^2$</td>
</tr>
</tbody>
</table>

### 5.4.2 Peak Averaged Discomfort

Averaged peak discomfort across any given time point was found to be affected by the age of participants. Higher peak discomfort was reported among older adult participants in the neck (p=0.0010), right shoulder (p=0.0139) and mid-back (p=0.0119). The magnitude of the difference in these body locations exceeded the criteria for clinical significance of over 9 mm (Kelly, 1998). In fact, peak averaged discomfort reported by older adults exceeded the clinical criteria in the neck, left shoulder, right shoulder, upper back, mid back and low back compared to only the low back on average among younger participants. There was no main effect of sex on peak discomfort. In the right and left gluteal regions, there was a significant interaction effect between sex and age that trended toward significance (p<0.0421). Specifically, the results of the general linear model indicated a significant interaction effect for both the right and left gluteal regions, however, adjusted p-values obtained from the post-hoc analysis did not detect any differences between group means. Peak discomfort values are depicted in Figure 34.
Figure 34 Baseline removed peak averaged discomfort across participant age. Significant differences between age groups are indicated by (*). The red line on the graph indicates the minimum VAS score for clinically significant discomfort (Kelly, 1998).

### 5.4.3 Time-varying Discomfort

Time-varying discomfort was significantly affected by age, sex and time. In the neck, discomfort was found to be significantly affected by age, with values on average (standard deviation) 11.5 (3.4)% in older adults compared to 1.4 (4.9)% among younger adults (p=0.0060). Discomfort values significantly increased over time in the neck (p=0.0106), left shoulder (p=0.0153) and low back (p<.0001) (Table 16). Post-hoc analysis revealed that relative to values reported in the first 15 minutes, discomfort increased significantly in the neck and left shoulder after 75 and 60 minutes of the sitting protocol respectively compared to after 45 minutes in the low back. There was no effect of age, sex or time on time-varying discomfort in the upper back, sacrum, left buttock, right buttock, left thigh and right thigh.
Table 16 Time-varying discomfort across body location. Significant differences in discomfort across time compared to the first 15 minute block are denoted by A,B,C.

<table>
<thead>
<tr>
<th>Body Location</th>
<th>Discomfort</th>
<th>Time (minutes)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck (%)</td>
<td>3.2 (8.0)A</td>
<td>5.5 (10.2)AB</td>
<td>7.6 (13.0)AB</td>
<td>5.9 (10.7)AB</td>
<td>8.1 (12.0)B</td>
<td>8.5 (12.9)B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Shoulder</td>
<td>1.4 (3.7)A</td>
<td>3.6 (7.6)AB</td>
<td>4.7 (10.2)AB</td>
<td>5.6 (11.5)B</td>
<td>5.3 (10.7)AB</td>
<td>6.0 (11.9)B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Back (%)</td>
<td>2.3 (8.3)A</td>
<td>5.3 (9.5)AB</td>
<td>7.2 (11.2)B</td>
<td>8.6 (12.6)B</td>
<td>9.6 (13.7)B</td>
<td>11.3 (15.7)C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There were significant time by age interactions to affect discomfort in the right shoulder (p=0.0086) (Figure 35) and middle back (p=0.0022) (Figure 36) regions. In the right shoulder of young participants, there was no significant difference between averaged discomfort scores at any time point (p>0.9999). In contrast, right shoulder discomfort in older adults increased significantly after 60 (p=0.0085), 75 (p=0.0158) and 90 minutes (p=0.0008) compared to values reported in the first 15 minutes. The right shoulder discomfort values reported by older adults after 60 and 90 minutes were also significantly higher than shoulder discomfort reported after 30 minutes of sitting (p<0.0465). Comparing age related difference across time points, there were no significant age related differences in the first 75 minutes (p>0.2430) of the protocol, however, after 90 minutes, older adults reported significantly higher right shoulder discomfort compared to younger adults (p=0.0284). In the middle back, there were no significant difference between averaged discomfort scores among young participants at any time point (p>0.9741). Among older adults, discomfort reported after 90 minutes was significantly higher compared to discomfort reported at 15, 30, 45, and 60 minutes of prolonged sitting (p<0.0047). Middle back discomfort in older adults became significantly higher compared to the first 15 minutes of the
sitting protocol after 75 minutes of exposure (p=0.0060). There were no significant age related difference any time point (p>0.1763).

Figure 35 Time-varying discomfort in the right shoulder across age
There was a significant interaction between sex and age to affect discomfort in the pelvis (p=0.0259) (Figure 37). Post-hoc analysis revealed that pelvic discomfort in female participants demonstrated no significant difference between discomfort scores at any time point (p=1.0000). In contrast, among male participants, pelvis discomfort significantly increased after 45, 60, 75 and 90 minutes compared to the first 15 minutes of prolonged sitting (p<0.0268). There were no significant sex related differences any time point (p>0.0863).
5.4.4 Angles

There was a main effect of age on postural variables during the prolonged sitting protocol (thoracic and lumbar angles) (Table 17). Specifically, there was greater lumbar and thoracic flexion during sitting among younger adults compared to older adults in both lumbar (p<.0001) and thoracic angles (p=0.0060). The same effect was observed when lumbar angles were expressed relative to standing postures (p<.0001) where younger adults sat with greater than 19 degrees more flexion compared to older adults. There was no effect of time or sex on sitting postures.

Figure 37 Time-varying differences in pelvis discomfort across sex
Table 17 Average angles about the mediolateral axis across age. Significant differences across age are denoted by A,B. Thoracic and lumbar angles represent the average Euler angle about the mediolateral axis during the sitting protocol (where flexion is negative).

<table>
<thead>
<tr>
<th>Dependent Measure</th>
<th>Participant Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young adults</td>
</tr>
<tr>
<td>Thoracic angle (degrees)</td>
<td>-3.1 (14.3)A</td>
</tr>
<tr>
<td>Lumbar angle (degrees)</td>
<td>5.5 (11.9)A</td>
</tr>
<tr>
<td>Lumbar angle relative to standing (degrees)</td>
<td>-28.6 (11.3)A</td>
</tr>
</tbody>
</table>

5.4.5 Passive Lumbar Stiffness

Following the analysis of EMG activity, select participants were unable to relax the musculature during passive stiffness trials. A subset of 24 of the 34 participants (11 mature, 13 young) were included in the passive stiffness analysis. Prolonged sitting and participant’s sex significantly affected passive flexion stiffness, while there were no significant effects of age detected. Stiffness was significantly higher following the prolonged sitting protocol at 20% flexion compared to prior to the prolonged sitting protocol (p=0.0474), whereas there were no significant time differences at 10% or 30% flexion (Table 18). Stiffness was significantly higher among male participants compared to female participants at 10%, 20% and 30% flexion (p<0.0165) (Table 19).

Table 18 Passive stiffness (Nm/% flexion) across relative flexion angle before and after the prolonged sitting protocol. Significant differences across time (pre/post) are denoted by A,B.

<table>
<thead>
<tr>
<th>Passive Stiffness</th>
<th>Time</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-sitting</td>
<td>Post-sitting</td>
</tr>
<tr>
<td>10% flexion (Nm/%)</td>
<td>0.07 (0.03)A</td>
<td>0.09 (0.06)A</td>
</tr>
<tr>
<td>20% flexion (Nm/%)</td>
<td>0.09 (0.05)A</td>
<td>0.12 (0.09)B</td>
</tr>
<tr>
<td>30% flexion (Nm/%)</td>
<td>0.12 (0.06)A</td>
<td>0.15 (0.10)A</td>
</tr>
</tbody>
</table>
Table 19 Passive flexion stiffness across sex and age. Significant differences across sex are denoted by A,B.

<table>
<thead>
<tr>
<th>Passive Stiffness</th>
<th>Female</th>
<th>Male</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% flexion (Nm/%)</td>
<td>0.04 (0.02)^A</td>
<td>0.09 (0.05)^B</td>
<td>0.0026</td>
</tr>
<tr>
<td>20% flexion (Nm/%)</td>
<td>0.05 (0.02)^A</td>
<td>0.12 (0.06)^B</td>
<td>0.0165</td>
</tr>
<tr>
<td>30% flexion (Nm/%)</td>
<td>0.07 (0.02)^A</td>
<td>0.18 (0.08)^B</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

5.5 Discussion

The results of this study indicate that older adults adopted less flexed postures during prolonged sitting exposures and developed higher levels of discomfort during sitting while there were no significant age related differences in passive spine stiffness at low flexion range of motion. Based on these findings, differences in discomfort development across age these differences in seated postures may be explained by increased passive tissues stiffness in older adults near the end range of functional motion, which may have implications for acute discomfort development during sitting.

It was hypothesized that older participants would sit in less flexion compared to younger participants (hypothesis 3c), flexion would be greater in males (hypothesis 3b) and sitting flexion would increase over time (hypothesis 3a). Sitting angles differed between younger and mature participants, providing support for hypothesis 3c. In contrast to our hypotheses, no differences were detected across time or sex. Younger adults adopted greater flexed postures compared to older adults during prolonged sitting exposures. When lumbar angles were expressed relative to maximum flexion (100%) and quiet standing (0%), younger adults adopted postures representing an average 60.9 (20.2)% of maximum flexion, while older adults adopted postures representing

127
33.4 (16.4)% of maximum flexion during sitting. There was no change in sitting postures over the course of the exposure and no lumbar and thoracic postural differences between male and female participants. Previous work has shown an increase in flexion posture during prolonged sitting in an automotive seat (Callaghan et al., 2010), which became significant after 30 minutes of exposure. Previous work has detected greater flexion postures in male participants compared to female participants during sitting on backless chairs, such as stability balls (Gregory et al., 2006) and stools (Dunk and Callaghan, 2005). It is possible that movement patterns during the sitting protocol may provide further insight into sex-specific responses.

Lumped passive stiffness was hypothesized to increase following prolonged sitting (hypothesis 1a), to be higher in male participants (hypothesis 1b) and higher in mature participants (hypothesis 1c). Lumbar passive stiffness in the low stiffness zone was significantly affected by the sitting protocol and participant sex, but not age. It was hypothesized that the current study would detect changes in stiffness at lower ranges of motion. While older adults did have higher passive stiffness on average compared to younger adults, the differences were not statistically different. Previous work has demonstrated differences in passive stiffness at 70% angular displacement in older adults compared to younger adults (Shojaei et al., 2016). It is possible that differences would have become significant in the current investigation at higher percentages of maximum flexion. Male participants were found to have higher passive stiffness compared to female participants at 10%, 20% and 30% flexion. The endpoint of the low stiffness zone has previously been shown to be right shifted (an indicator of increased stiffness) among males compared to female participants (Beach et al., 2005). While there were no differences detected in average postures between males and females during sitting in the current investigation, it is possible that females adopted a more dynamic sitting strategy compared to
males in the current investigation. Lumbar passive stiffness significantly increased at 20% flexion following the prolonged sitting protocol. Previous work has shown higher transition zone stiffness after sitting for 60 and 120 minutes compared to the baseline (Beach et al., 2005). The mechanism of increased passive stiffness during sitting has been hypothesized to be caused by disc swelling in response to static sitting strategies (Beach et al., 2005), similar to disc swelling that occurs during bedrest contributing to diurnal variation during waking hours (Eklund and Corlett, 1984). The results of the current investigation support this hypothesis and suggest that passive stiffness in the low flexion zone is affected. However, it is unclear which structures contribute to the composite passive stiffness curve through the range of angular displacement. Previous work has demonstrated sagittal migration of the intervertebral disc (identified by MRI imaging) during sitting exposures, which could also contribute to changes passive stiffness in response to sitting (Alexander et al., 2007). More recent work has demonstrated higher passive stiffness at the end range of spine flexion motion in response to a 6 week isomeric strength training program, suggesting the passive properties of muscle contribute to passive stiffness near the end range of motion (Lee and McGill, 2015).

It was hypothesized that discomfort would increase over the prolonged sitting protocol (hypothesis 2a), discomfort would be higher in mature participants (hypothesis 2b), while there would be no sex related differences in discomfort reporting (hypothesis 2c). Age was found to significantly affect discomfort in the neck, middle back and right shoulder regions. The middle back region had the highest levels of reported discomfort among older adults, reaching a peak of 24% compared to 7% among younger adults. In addition, there was evidence of temporal differences in discomfort reporting in the right shoulder and middle back between age groups, where older adults increased discomfort after 60 minutes and 90 minutes respectively compared
to younger adults that had no significant differences in discomfort. This result suggests that interventions aimed at preventing low back pain development during prolonged sitting, such as standing or walking breaks may be beneficial for older adults to occur earlier than 60 minutes. Discomfort in a total of 3 body locations significantly increased over time, including, the neck, left shoulder and low back. Among a population of office workers, previous work has reported the longest continuous sitting event was an average of 98 minutes in an 8 hour work day (Ryan et al., 2011). The results of the investigation support that acute pain develops during a prolonged sitting exposure that replicates a duration of sitting found in the workplace.

The results of the study can be considered in the light of some limitations. The baseline passive stiffness measurement takes place following a high flexion task (lifting) whereas the second passive stiffness test takes place immediately following the prolonged sitting protocol. To mitigate any creep effects of the lifting task on spine passive stiffness, participants were asked to stand during a 5 minute demonstration of how to safely enter and withdraw from the jig. Previous work has demonstrated that 50% of baseline flexion angle recovers after a duration of 2 minutes of seated recovery (using a backrest) following 20 minutes of maximal seated flexion (McGill and Brown, 1992). Therefore, it is unlikely that the order of the protocol affected the results of the study. The logistics of scheduling participants employed in a full-time capacity made it not possible to schedule collections at the same time each day. However, previous work (Eklund and Corlett, 1984) and a review (Adams et al., 1990) support that the rate of standing height loss decreases as the time after getting up increases. Given that participants were in the lab for over an hour prior to the passive stiffness test (consent, set-up, MVCs and demonstrations), it is likely that a majority of the diurnal changes in the spine had already occurred. A subset of participants were unable to relax core musculature during the passive
trials. Therefore, participant data included in the passive stiffness measures were not height matched. However, exercise level and waist circumference were controlled, and these variables have been shown to impact range of motion and passive stiffness. While participants were screened for moderate levels of physical activity, the intake I-PAQ survey did not discriminate between level of fitness, but rather intensity of physical activity. It is possible that training status influenced passive stiffness properties given the relationship between muscle strength and passive stiffness (Lee and McGill, 2015).

5.6 Conclusions

This is the first study to investigate differences in posture, stiffness and discomfort development across age in response to a prolonged sitting exposure. Older adults sat in less flexion compared to younger adults during prolonged sitting exposures. While passive stiffness was not found to be different in low stiffness regions, it may differ at higher levels of flexion. Older adults reported higher levels of discomfort during 90 minutes of sitting and reported discomfort earlier in the simulation compared to younger adults, indicating walking breaks or standing interventions may need to be implemented earlier in a bout of prolonged seated exposures.
6. Study IV: The effect of age, prolonged sitting and sex on posture and perceived effort during a lifting task

6.1 Introduction

The North American working population is aging (Carrière & Galarneau, 2011; Toossi, 2012) across all industries and trends of delayed retirement have been present in the Canadian working population since the 1990s (Carrière & Galarneau, 2011). Workers over 40 are represented in occupations with heavy physical demands including construction (Arndt et al., 1996; Petersen and Zwerling, 1998) and general labour and repair (Zwerling et al., 1996). Given that middle aged and older workers tend to sustain more severe musculoskeletal injuries at work compared to younger workers (Breslin et al., 2003; Smith et al., 2014; King et al., 2009; Peek-Asa et al., 2004; Smith & Berecki-Gisolf, 2014), an understanding of how strategies and joint changes across the life course affect the performance of occupationally relevant materials handling tasks is of importance to determine if age dependent injury prevention strategies are necessary.

Lifting is an occupationally relevant task that has been investigated widely for links to injury risk. In fact, among workers aged between 51 and 61 years, self-reported exposure to heavy lifting was the factor most strongly associated with occupational injury (Zwerling et al., 1996). In general, back extensor strength (Sinaki et al., 2001; Champagne et al., 2009; Yasserli et al., 2007; Singh et al., 2011; Singh et al., 2013) and low back range of motion (Dvorak et al., 1995; McGill et al 1999; Taylor and Twomey, 1980; Sullivan et al., 1994) decrease with increasing age. Comparisons between older and younger novice (Song and Qu, 2014a; Song and
Qu, 2014b; Boocock et al., 2015) and experienced (Chen et al., 2017) lifters have recently been completed. Novice older lifters (aged 43 – over 55 years) have lower peak trunk flexion during lifting compared to younger novice lifters (aged 20-31 years) in symmetric (Song and Qu, 2014a; Boocock et al., 2015) and asymmetric lifting (Song and Qu, 2014b). After 20 minutes of continuous lifting, younger participants (20-31 years) increased peak lumbar flexion by an average of 18% over the course of a 20 minute lifting protocol reaching almost maximal values of lumbar range of motion whereas older participants (43-54 years) increased peak lumbar flexion by an average of 4%, reaching 82% of maximum flexion range of motion (Boocock et al., 2015). Previous work has suggested that high flexion in lifting partially relies on passive stretch of muscle, ligaments or the intervertebral discs given the flexion-relaxation of the low back musculature (Dolan et al., 1994). Furthermore, older novice lifters (aged either 43-54 years or over 55 years) have been shown to have slower trunk angular velocity in extension compared to younger novice lifters (aged 20-31 years or 20-30 years) (Boocock et al., 2015; Song and Qu, 2014a, respectively).

The purpose of this study was to determine the effect of prolonged sitting, age and lift type on peak thoracic, lumbar, hip and knee postures and ratings of perceived effort during three different lifting tasks. A secondary purpose was to determine any sex specific responses.

6.2 Hypotheses

1. a. It is expected that ratings of perceived effort (RPE) during the lifting tasks will be greater following the prolonged sitting protocol.
1. b. RPE ratings will be greater in participants over 45 years.
1. c. It is expected that there will be no differences in RPE between sexes.
1. d. It is expected that there will be no difference in perceived effort between lifting tasks.
Previous work measuring overall RPE and RPE specific to the low back among experienced female materials handlers across relative younger (20-30 years) and older (over 50 years) age demonstrated no differences between age groups during a lifting task (Chen et al., 2017). In addition, the weights lifted between each age group were greater than those in the proposed study with an average 9.6 kg for the older group compared to 12.6 kg in the younger group (Chen et al., 2017). However, transient discomfort has previously been reported following continuous sitting exposures without a backrest over time (Skinkel-Ivy et al., 2013) and there could be an altered response of perceived effort for lifting following a bout of sitting.

2. a. It is expected that maximum lumbar and thoracic flexion will be lower and maximum hip and knee flexion will be higher after a prolonged bout of sitting.
2. b. It is expected that maximum lumbar and thoracic flexion will be lower and maximum hip and knee flexion will be higher among participants over 45 years compared to those under 35 years.
2. c. There will be no difference in maximum lumbar, thoracic, knee or hip flexion during the lifting task between sex.
2. d. There will be no difference in maximum lumbar, thoracic, knee or hip flexion across lift type.

Among experienced female manual materials handlers, participants over 50 years demonstrated increased left peak hip flexion (and a non-significant trend toward increased peak right hip flexion) across all lifting tasks compared to participants between 20-30 years (Chen et al., 2017). This trend has also been demonstrated among novice older lifters (aged 43 – over 55 years), having lower peak trunk flexion during lifting compared to younger novice lifters (aged 20-31 years) (Song and Qu, 2014a; Boocock et al., 2015).

3. a. It is expected that older adults will have a longer lift duration compared to younger adults.
3. b. It is expected that there will be no difference in lift duration across sex.
3. c. It is expected that the prolonged sitting exposure (time) will have no effect on lift duration.
3. d. It is expected that lift type will have no effect on duration of lift.

There is some evidence in the literature that trunk velocity during lifting tasks is lower in novice older lifters compared to younger lifters (Boocock et al., 2014; Song et al., 2014) and attributed to a safer lifting strategy among older adults compared to younger adults.

4. a. It is expected that older adults will have less range of motion in the mediolateral axis compared to younger adults.
4. b. It is expected that male and female participants will have no differences in range of lumbar motion.

Previous work has demonstrated that lumbar range of motion about the mediolateral axis decreases with increasing age (Dvorak et al., 1995; McGill et al. 1999; Taylor and Twomey, 1980) and no differences were reported between males and females (Dvorak et al., 1995).

**6.3 Methods**

**6.3.1 Participants**

A total of 34 moderately active participants were collected (17 young, 17 mature), with an average (standard deviation) age of participants; 23.8 (5.0) years and 63.7 (3.9) years respectively. All participants were novice lifters; defined as having no professional lifting experience with no low back pain or injury in the past 12 months with enough severity to result in time away from work or a visit to a doctor. Participants were paired by similar heights between age groups and met a BMI/waist circumference within a normal to overweight criteria in order to participate. This study shared a common participant cohort with study III. Full details of the participants and screening procedure can be found in section 5.3.1.
6.3.2 Protocol

6.3.2.1 Overview

Study IV shared a protocol with Study III, outlined in Figure 30. Participants were asked to perform 3 static calibration poses while standing, 12 box lifts, sit continuously for 90 minutes and perform 2 side-lying passive (flexion and extension) stiffness tests. The following sections outline the protocol for the lifting tasks and calibration poses relevant to study IV.

6.3.2.1 Lifting Tasks and Calibration Poses

Participants completed a series of static calibration poses at the beginning of the protocol. In addition to a 5 second quiet standing trial, participants were asked to slowly flex forward at the lumbar spine maximally and hold that position for a duration of 5 seconds. Participants were then asked to slowly extend the lumbar spine maximally and hold the position for a duration of 5 seconds. All calibration poses were completed while standing.

Participants were asked to complete a total of 12 box lifts; 6 prior to and 6 following 90 minutes of continuous sitting. Each lift consisted of the participant lifting the box from the floor to a table located 100 cm above the ground at a frequency of 2 lifts per minute. The box was returned to the starting position by the experimenter or research assistant. There were 3 randomized lifting tasks including; (i) 7 kg symmetrical, (ii) 4.5 kg symmetrical and (iii) 4.5 kg asymmetrical. Each lift task was completed 4 times; 2 repeats prior to the prolonged seated protocol and 2 repeats following prolonged sitting (total of 12 lifts). The starting foot position prior to each lift represented a maximum horizontal distance from the table that would ensure lifting task to be within the maximum acceptable lift conditions according to the NIOSH lifting guideline (Waters et al., 1993). For lifts (i) and (ii), the box was located directly in front of
participants in the starting position and for lift (iii), the box was located 45 degrees to the right of the starting position. Participants were asked to begin each lifting task facing forward with both feet inside the square taped off on the floor, and complete lift in a way they would approach the lift naturally (Figure 38). The frequency of lifts was constrained to 2 lifts per minute, while the duration of each lift (duty cycle) was self-selected by participants. Participants were asked to practice each lift type prior to the first block of lifting tasks (prior to sitting protocol).

![Figure 38 Box lift starting position for symmetrical (A) and asymmetrical tasks (B)](image)

**6.3.2.2 Lifting task rationale**

Each lifting task was designed to have a lift index (LI) value less than 1, representing a job/task not needing corrective action based on the NIOSH lifting equation (Waters et al., 1993). The NIOSH lifting equation is comprised of 6 multipliers and a load constant that estimate the recommended weight (RWL) of the object being lifted (Waters et al., 1993) according to Equation 2.
Equation 2

\[
RWL = 23kg \times \left( \frac{25}{H} \right) \times \left( 1 - (0.003 \times |V - 75|) \right) \times \left( 0.82 + \frac{4.5}{D} \right) \times \left( 1 - (0.0032 \times A) \right) \times FM \times CM
\]

The NIOSH equation is comprised of H, representing the horizontal distance from the midpoint of the feet to the hands, V is the distance from the hands to the ground, D is the distance travelled during the lift, A is the angle of asymmetry, FM and CM are the frequency and coupling multipliers respectively obtained from table values. The LI is the ratio between the weight of lift of interest compared to the Recommended Weight Limit (RWL) calculated from equation 2.

The estimated LI’s for lifts (i), (ii) and (iii) were 0.71, 0.46 and 0.53 respectively.

6.3.3 Dependent Measures

6.3.3.1 Ratings of Perceived Effort

The Borg CR-10 scale was administered following each lift type, for a total of 6 surveys per participant. While the scale was originally developed to evaluate effort during the performance of aerobic exercise, it has been utilized more generally to evaluate subjective responses to activities including short duration bouts of lifting or carrying (Borg, 1990, Chen et al., 2017). Participants were asked to rate the level of effort by circling the number that corresponds to the level of effort during each lift completed (i.e. after trial 2 of each lift type). The scale is anchored between 0, representing no effort to either 10 representing the heaviest object lifted and an option to select an effort level above what the person has previously experienced. A copy of the Borg survey can be found in appendix B.2.
6.3.3.2 Kinematics
6.3.3.2.1 Motion Capture

An optoelectronic motion capture system (Optotrak Certus System, Northern Digital Inc., Waterloo, ON, Canada) was used to track the relative motion of the thorax, lumbar spine, pelvis, left thigh, left shank, left foot and box trajectories during each lifting task. The capture volume was calibrated dynamically in the area surrounding the table including the starting position of each lifting task. A total of 6 rigid body marker clusters were affixed to each participant with 2 additional marker clusters affixed to each of the boxes used in the study (4.5 kg and 7 kg). A calibrated probe tip was used to digitize points on the skin surface overlying the anatomical landmarks representing the proximal and distal end points of each body segment tracked with each rigid body. A detailed table indicating the position of the rigid body and the anatomical endpoints of each segment can be found in Table 14. Marker positions were sampled at 50 Hz.

6.3.3.2.1 Data Analysis

Peak angles during the lifting tasks, average angles during the calibration poses and the duration of each lift were quantified from marker trajectories. An interpolation procedure was followed for any missing data points using a 3rd order polynomial with a maximum gap of 0.2 seconds of data (10 frames) (Howarth and Callaghan, 2010). A 4th order Butterworth filter with a 3 Hz cut-off frequency (Howarth et al., 2009) was applied to raw marker trajectories using 1 second of padding points at the beginning and end of each trial using the reflection method (Howarth and Callaghan, 2009). Marker positions were used to construct a three-dimensional rigid link segment model in Visual 3D (C-Motion Inc, Visual3D Standard v4.96.4, Germantown, MD, USA). An Euler angle decomposition sequence (flexion/extension, lateral bend, axial twist) was calculated in Visual3D for the thoracic spine relative to the pelvis, lumbar spine
relative to the pelvis, left thigh relative to pelvis and left lower leg relative to the left thigh. A 5 second static calibration pose with the participant standing in anatomical position was collected to establish the local coordinate systems using digitized markers and the reference posture for all the monitored segments. Local coordinate systems segments were constructed according to ISB guidelines (Wu and Cavanaugh, 1995), with the positive axes anterior (X), superior (Y) and to mediolateral to the right (Z).

Outcome variables of interest included: peak angles during the lifting tasks, lumbar angles during calibration poses, and the duration of each lift. Peak angles about the mediolateral axis were determined from the thorax relative to the pelvis, lumbar spine relative to the pelvis, left thigh relative to the pelvis and left lower leg relative to the thigh and reported during each lifting task. A total of 5 variables were reported from the calibration poses, including standing lumbar lordosis angles, maximum flexion lumbar angle, maximum extension lumbar angle, the absolute lumbar angle range of motion between standing and maximum flexion and the absolute lumbar angle range of motion between standing and maximum extension. Lift duration was defined according to the resultant displacement of box markers during each lift. An increase or decrease greater than 4 mm from the starting position on the floor represented the beginning of the lift, while a change greater or less than 4 mm from the table position represented the end of the lift.

6.3.4 Statistical analysis

A four way (age x sex x time x lift type) mixed general linear model with repeated measures on time (pre/post sitting) and lift type (7kg, 4.5kg symmetrical and 4.5kg asymmetrical) was applied to ratings of perceived effort, peak lumbar angles (degrees), peak
thorax angle (degrees), and the duration of lift (seconds). A four way (age x sex x time x lift type) mixed general linear model with repeated measures on time (pre/post sitting) and lift type (7kg and 4.5kg symmetrical) was applied to peak right hip and peak right knee angles (degrees). Given that marker data were only collected on the left side, peak angles from the left thigh and left knee were only calculated during the completion of symmetrical tasks. Two way general linear models (sex x age) were applied to lumbar spine maximum flexion (degrees), maximum extension (degrees), lumbar lordosis in standing (degrees), lumbar flexion range of motion (degrees) and lumbar extension range of motion (degrees). All statistical analyses were computed using SAS studio (version 9.4, SAS Institute Inc., Cary, NC), with a significance level (α) of 0.05. A Bonferroni corrected post-hoc test was used to detect significant effects.

6.4 Results

6.4.1 Ratings of Perceived Effort

There was a main effect of lift type on Borg RPE scores (p<.0001) (Table 20). Post-hoc analysis revealed that participants rated the asymmetrical and symmetrical 4.5kg lifting tasks with lower RPE scores compared to the 7kg lifting task (p<0.0005). Ratings of the asymmetrical and symmetrical 4.5kg lifting tasks were not significantly different (p=0.4027). There was no effect of sex, age or time on RPE values.

Table 20 Ratings of perceived effort across lift task. Significant differences across lift task are denoted by A,B.
6.4.2 Peak Angles

There was a main effect of age on peak lumbar angle during the lifting tasks (p=0.0029). Specifically, younger adults adopted greater than 12 degrees more peak flexion compared to older adults during the lifting tasks (Figure 39). There was a main effect of time on peak lumbar angles during the lifting task where peak lumbar flexion angles were significantly lower following 90 minutes of continuous sitting compared to angles achieved prior to sitting (p=0.0117). Specifically, averaged peak lumbar angles were an average (standard deviation) 3.8 (13.3) degrees prior to the sitting protocol compared to 6.3 (13.4) degrees following 90 minutes of sitting, where positive angles represent extension. There was a main effect of lifting task on peak lumbar angles (p<.0001). Post-hoc analysis revealed that the asymmetrical 4.5 kg lifting task elicited a greater peak lumbar flexion angle compared to both the 7 kg and 4.5 kg symmetrical lifting tasks. There were no significant differences between the 7 kg and 4.5 symmetrical tasks. There was no effect of sex on peak lumbar angles.
There was a main effect of lift type on the peak thoracic angle (p<.0001) (Table 21). Specifically, participants adopted significantly greater thoracic flexion angles while completing the 4.5 kg asymmetrical task compared to both the 7 kg and 4.5 symmetrical tasks. There were no significant differences between the 7 kg and 4.5 symmetrical tasks. There was no effect of time, age or sex on peak thoracic angles during the lifting tasks.

Table 21 Peak lumbar and thoracic angles across lifting tasks. Positive values represent spine extension and negative values represent flexion about the mediolateral axis. Significant differences across lift task are denoted by A,B.
There was no effect of sex, age, lift or time on peak left knee angles (p>0.1208). There was a significant sex by lift task interaction to affect peak hip angle detected by the general linear model (p=0.0313). However, pairwise comparisons of the interaction revealed no significant differences between groups (p>0.0612). There was no effect of age or time on peak hip angle.

6.4.3 Lift Duration

There was a main effect of lift type on the duration of lift (p<.0001). Post-hoc analysis revealed participants completed the asymmetrical 4.5 kg lift faster than either the 7 kg or 4.5 kg symmetrical lifts (Table 22). There was no significant difference in the average lift duration between the 7 kg or 4.5 kg symmetrical tasks. There was a significant sex by time interaction on the duration of lift (p=0.0321) (Figure 40). Pairwise comparisons revealed the interaction was driven by male participants; where lift durations were greater following a prolonged bout of sitting compared to durations prior to the prolonged sitting protocol. There were no significant differences in lift duration across age.

Table 22 Duration of lift across lift task. Significant differences across lift task are denoted by A,B.

<table>
<thead>
<tr>
<th>Lifting task</th>
<th>7 kg</th>
<th>4.5 kg symmetrical</th>
<th>4.5 kg asymmetrical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Lift (s)</td>
<td>1.7 (0.4)^A</td>
<td>1.7 (0.4)^A</td>
<td>1.4 (0.5)^B</td>
</tr>
</tbody>
</table>
6.4.4 Standing Lumbar Range of Motion

There was a main effect of age on both the maximum range of motion in flexion relative to standing ($p=0.0003$) and the angle achieved at maximum flexion ($p=0.0049$). Specifically, older adults achieved an average of 11 degrees less maximum flexion range of motion compared to younger adults. In contrast, there were no differences in maximum range of motion in the extension direction or in the lumbar lordosis angle during standing (Table 23). There was a main effect of sex on lumbar angles during maximum flexion ($p=0.0241$) where women achieved an
average (standard deviation) -13.2 (13.0) degrees of flexion compared to -2.9 (15.1) degrees among male participants.

Table 23 Maximum range of motion and standing postures across age. Significant differences across age are denoted by A,B.

<table>
<thead>
<tr>
<th>Participant Age</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>Mature</td>
</tr>
<tr>
<td>Maximum flexion range of motion (degrees)</td>
<td>48.7 (10.4) (^A)</td>
</tr>
<tr>
<td>Maximum extension range of motion (degrees)</td>
<td>16.9 (7.0) (^A)</td>
</tr>
<tr>
<td>Standing lordosis (degrees)</td>
<td>33.8 (8.8) (^A)</td>
</tr>
<tr>
<td>Standing maximum extension (degrees)</td>
<td>51.2 (11.7) (^A)</td>
</tr>
<tr>
<td>Standing maximum flexion (degrees)</td>
<td>-14.9 (12.7) (^A)</td>
</tr>
</tbody>
</table>

6.5 Discussion

The results of the study demonstrate that age affected peak lumbar angles during lifting tasks while there were no differences in thoracic, knee or hip angles. Age did not affect ratings of perceived effort or duration of lifting tasks. Maximum flexion range of motion was reduced in older participants. In contrast to our hypothesis, there were no age specific differences in lifting responses following a prolonged bout of sitting. Despite differences in lumbar angles during lifting, when these values were expressed as a percentage of maximum flexion, younger and older adults adopted similar percentages of max flexion during lifting tasks, with average 71% and 65%, respectively. This could indicate that functional range of motion in the spine is reduced in older adults, with high flexion tasks entering a zone of higher stiffness.

It was hypothesized that older age (hypothesis 2b) and prolonged sitting (hypothesis 2a) would affect lift performance by reducing lumbar and thoracic flexion and increasing maximum hip and knee flexion while there would be no effect of sex (hypothesis 2c) or lift type
There was a main effect of peak lumbar and thoracic flexion angles across age during the lifting tasks with no age effect on left hip and knee angles. Expressed as a percentage of maximum flexion, peak lumbar angles during the lifting tasks reached an average 71% and 65% among young and mature participants respectively. While not measured in this study, it is possible that older adults used the upper extremity to reach the box to compensate for less spine flexion during the lifts given that participants were height matched. Reduced flexion in older adults during lifting tasks is consistent with previous work examining lumbar postures across age (Boocock et al., 2015; Song and Qu, 2014a; Song and Qu, 2014b). Based on the flexion-relaxation response of the low back musculature during high flexion tasks (Dolan et al., 1994), previous authors have suggested that the reduction in peak angles during lifting among older adults represents a protective lifting strategy, based on the reduction in flexion and reduction in reliance on passive stretch of muscle, ligaments or disc tissue during a lift. However, it is possible that increased passive stiffness in spine tissues are also a contributor to reductions in peak flexion angles during lifting tasks among older adults. Recent work has demonstrated increased passive and active spine stiffness in older adults compared to younger adults in flexion values of 70% range of flexion motion (Shojaei et al., 2016).

Peak lumbar flexion angles were reduced in lifting tasks that occurred after 90 minutes of continuous sitting compared to peak angles prior to prolonged sitting exposures. The average reduction was low, with 2.8 degrees less peak flexion during lifting following 90 minutes of continuous sitting across both mature and young participants. It was hypothesized that peak flexion would be reduced in older adults following the prolonged sitting protocol. This was not supported by the results of the study and may be related to the post-sitting passive stiffness test. Reductions in peak flexion during lifts following sitting in the current study are similar to the 1.8
degree reduction in peak lumbar flexion angle reported during lifting among young (mean age: 24.4) inexperienced lifters following 60 minutes of sitting (Howarth et al., 2009). The change in peak angle could be related to an increase in passive stiffness of the posterior elements of the spine in response to a prolonged bout of sitting as documented previously (Beach et al., 2005; study III). Peak flexion in the thoracic and lumbar spine were shown to be affected by lifting task with no difference in left knee or left thigh peak flexion angles. This effect was driven by greater flexion (>5 degrees) across participants while performing the 4.5 kg asymmetrical lifting task compared to the 7 kg and 4.5 kg symmetrical tasks. Previous work has reported similar trunk flexion angles during symmetrical (Song and Qu, 2014) and asymmetrical task transferring load away from the sagittal plane (Song and Qu, 2014b). There were no differences in peak angles between male and female participants.

It was hypothesized that prolonged sitting (hypothesis 1a) and increased age (hypothesis 1b) would increase ratings of perceived effort, while there would be no effect of sex (hypothesis 2c) or lifting task (hypothesis 2d). There were no differences in ratings of perceived effort (RPE) across time, age or sex of participants, while there was a main effect lift type on RPE. While there was a statistically significant increase in perceived effort in the 7 kg lift compared to the 4.5 kg lifts, all lifting tasks were perceived as the “very weak” and “weak” categories according to averaged RPE responses. This result suggests that the lifting tasks in the current study, designed with Lift Indices under 1, correspond to a low perceived effort among participants regardless of age. Previous work investigating age-related differences in RPE scores demonstrated no age related differences and “moderate” effort scores corresponding to an average (standard deviation) 10.18 (3.57) kg and 13.57 (4.03) floor to knuckle lift every 2 minutes among experienced female older and younger workers respectively (Chen et al., 2012).
However, there is some evidence of a sex dependency (Sinaki et al., 2001) in extensor strength reductions with increases in age (Sinaki et al., 2001; Champagne et al., 2009; Yasserli et al., 2007; Singh et al., 2011; Singh et al., 2013), and it is possible that age related sex differences in RPE would emerge with increases in box mass.

While the frequency of lifts in the study was constrained at 2 lifts per minute, the duty cycle, or, duration of lift was self-selected. The average duration across all lifts in the study was an average (standard deviation) 1.6 (0.4) seconds. There were no differences in lift duration across age, sex or time. Previous authors have attributed slower trunk velocity during lifts as an indicator of a safer and more controlled lifting strategy among older novice lifters compared to younger lifters (Boocock et al., 2015; Song and Qu, 2014). While the average lift duration for the asymmetrical task was statistically greater compared to the other lifting tasks, mean lift durations across lifting tasks were marginally different.

Lumbar range of motion was hypothesized to be lower in older adults (hypothesis 4a) with no significant sex-related difference (hypothesis 4b). Differences in lumbar range of motion were detected as a function of age but not sex, supporting hypotheses 4a and 4b. When flexion and extension ranges of motion were separated, the difference in range of motion was driven by a reduction in range of motion in the flexion direction, as there were no significant differences detected in extension range of motion across age groups. Previous work has examined maximum range of motion in both flexion and extension across age and demonstrated reductions in both the flexion and extension maximal range of motion between the ages of 16 and 90 years (Troke et al., 2001). Work that has grouped participants according to bands of age have indicated no differences in extension range detected between age bands including 20-29, 30-39, 40-49 or over 50 years, but age related flexion differences have been reported between the
ages of 20-29 and 30-39 years (Dvorak et al., 1995). Work by McGill and colleagues (1999) detected reductions in lumbar spine range of motion comparing participants with an average age of 69 years to those aged an average of 21 years.

The results of the study detected no significant differences in standing lordosis angles across age. Previous work investigating the sagittal radiographs over a 10 year follow-up period compared Cobb angles during standing (between upper endplate of L1 to the lower endplate of L5) reported that lordosis decreased over the 10 year observation period. The average baseline age in the work by Takeda and colleagues (2009) was 63 years compared to 63.7 (3.9) years in the current investigation. While there are notable technical differences in the measurement of lordosis angle between studies (comparing radiographs to motion capture angles), the reported lordosis angles were similar; with 33.1 (14.0) degrees and 34.4 (9.6) degrees in Takeda and colleagues (2009) and the current investigation, respectively. There were no age-related differences in lumbar lordosis angle between the young and mature groups in the current investigation. At the 10 year follow-up in the work by Takeda and colleagues (2009), the average age of participant increased to 75 years and the lordosis angle decreased to 25.4 (16.5) degrees, suggesting that age-related changes to standing lordosis may occur after the 6th decade of life. This is supported by the work of Lang-Tapis and colleagues (2011) that showed no differences in standing lordosis across an age range from 20 to 67 years when accounting for sex and obesity (Lang-Tapia et al., 2011).

The results of the study can be considered in light of some limitations. The time of the collection during the day was not controlled to account for diurnal variation in the disc and passive tissues of the spine, which may affect high flexion tasks, such as lifting. However, given a 60 minute set up and transportation to the lab, all collections took place more than 2 hours after
rising, a procedure that has been implemented in previous work investigating range of motion of spinal structures (Troke et al., 2001). Furthermore, a majority of the diurnal changes that occur in the spine take place in the morning (Eklund and Corlett, 1984; Adams et al., 1990), so it is unlikely diurnal changes affected the results reported. A total of 12 lifts were completed by each participant in the study. It is possible that the approach and performance of each lifting task would change with an increased number of tasks completed, however, it was an objective of the investigation to test the effect of the prolonged seated exposure (i.e. post time point), and therefore the number of lifts were limited. The second bout of lifting tasks (following prolonged sitting) were completed after the second passive stiffness trial. This order was selected in order to determine of passive tissue changes were observed across age following sitting. It is possible that the observed effects of the sitting/time variable in the current study are underestimated given the stretch in tissues during the passive tissue trials that may have occurred. All calibration postures were completed while standing. It is possible that by implementing a supine extensor max procedure and a seated flexion trial (Sullivan et al., 1994) some participants would have elicited greater maximum trials. However, the values reported in the current investigation are aligned with reports from previous work and the standing procedure has been implemented in other studies (Troke et al., 2001; Dvorak et al., 1995; McGill et al., 1999).

6.6 Conclusions

Peak angles during the lifting tasks in the current investigation show that older adults have reduced peak flexion compared to younger adults during lifting tasks. Some authors have interpreted the increased margin of safety between maximal flexion angles among older adults as a protective or safer lifting strategy; however, these results should be interpreted with caution given that the mechanical properties of passive tissues in older adults may be changing. This
could mean that despite a margin of safety relative to functional range of motion, older adults may be entering a range of motion representing a zone of high stiffness. While there was no significant age-specific response during lifting tasks following a prolonged sitting exposure, it is possible effects may be observed if the tasks occur immediately following sitting. The results of the study also suggest that range of motion decreases with increases in age and this decrease may be driven by changes in the flexion direction that precede any changes in the extension direction.
7. Synthesis of Contributions

7.1 Thesis overview and hypotheses revisited

The global purpose of this thesis was to explore how spine stiffness changes with age and whether the mechanical properties of the osteo-ligamentous spine and annulus contribute to these changes under different loading conditions. The effect of a cyclic loading protocol on stiffness in naturally aged porcine functional spine units was explored in study. This study contributes to a mechanistic understanding of how (i) maturation/age changes and (ii) cyclic loading influence joint stiffness across intervertebral discs. Based on a review of the literature, spine aging and disc degeneration are linked, but recent evidence has shown they may be separate processes. Given this coupling of age and degeneration, there is value in simulating single aspects of age in order to mechanistically understand the aging process. Study II of this thesis explored if a simulated aging change influenced the mechanical properties of the annulus across age (via solution). Acidic discs are a hypothesized initiator of aging/disc degeneration and have been associated with discogenic pain. This was the first study to explore if acidity affects annulus mechanics. Together, these studies attempted to determine how these changes contribute to spine (torso) stiffness changes across age (study III). Study III contributes to an understanding of changes in passive stiffness in low flexion postures across age and following a prolonged bout of sitting. Study IV explored if there is a relationship between a protocol designed to increase passive spine stiffness and postures assumed during a lifting task across participant age.

1. There will be no effect of age or loading on disc height, neutral zone range or neutral zone stiffness in osteo-ligamentous porcine spines (study I)
Baseline differences in neutral zone range and stiffness between older and younger spines alter the mechanical environment of the disc, contributing to changes in functional range of motion with age and may predispose mature discs to different types of injuries compared to younger specimens. The results of study I showed greater neutral zone stiffness, reduced neutral zone range and no change in disc height at baseline in mature specimens compared to younger specimens. Mature specimens had higher neutral zone stiffness at both the C34 and C56 levels compared to younger specimens. Comparing stiffness in mature specimens, C56 stiffness was higher compared to C34 while there was no significant difference in stiffness between the C34 and C56 levels in young specimens. Neutral zone stiffness was significantly higher prior to the cyclic loading protocol compared to following the loading protocol across both age categories, with average (standard deviation) values, 0.20 (0.11) Nm/degree compared to 0.17 (0.11) Nm/degree respectively. Specimens excised from the C34 level had a greater average neutral zone range 5.2 (1.5) degrees compared to specimens from the C56 level 4.2 (1.2) degrees. Young specimens significantly decreased the neutral zone range following cyclic loading, whereas, neutral zone range did not significantly change in mature specimens following the cyclic loading protocol. Disc height in C56 specimens was reduced following the loading protocol from an average 13.2(2.7) mm to 12.2 (3.0) mm following cyclic loading with no significant differences detected across age.

2. Simulated aging will have no effect on the mechanical properties of isolated porcine annulus layers across radial location or cycle (study II)

A low pH lactic acid solution increased annulus stiffness and increased peak stress on the posterior side of the annulus, demonstrating that the annulus is a contributor to increased spine stiffness with increasing age. The results of study II indicated higher S-S modulus (stiffness) and higher peak stress in the circumferential direction among samples immersed in the low pH (3.5)
lactic acid solution to simulate aging. Specimens dissected from the C56 location immersed in the Acid 3.5 solution had higher peak stress compared to specimens immersed in Acid 6, Neutralized and PBS solutions across both levels. Specimens obtained from the C34 level and immersed in the pH 3.5 solution had higher peak stress in the circumferential direction compared to specimens obtained from C56 level immersed in the pH 6 solution, with no significant differences in the circumferential peak stress in the Acid 6, Neutralized or PBS solutions across both cervical levels. Peak stress in specimens obtained from the posterior location was significantly higher compared to specimens from the anterior location in cycles 1, 50 and 100. Specimens dissected from the C56 level and immersed in the Acid 3.5 solution had significantly higher stiffness compared to specimens across both levels in the other solutions, while there was no significant difference between the stiffness of specimens obtained from the C34 or C56 levels immersed in the Acid 3.5 condition. There was no difference in the stiffness of C34 specimens immersed in the Acid 3.5 solution compared to the other solutions and levels. Specimens obtained from the posterior region had an average stiffness (standard deviation) of 3.00 (1.00) MPa compared to 2.30 (0.79) MPa in anterior samples.

3. Prolonged sitting, sex and age will have no influence on discomfort responses, postures or lumped passive torso stiffness (study III)

Compared to younger participants, mature participants were shown to have increased acute pain reporting in response to prolonged sitting in select body locations, low back and thoracic postures demonstrated less flexion during sitting and there were no differences in passive torso stiffness at low levels of flexion. In the right shoulder of young participants, there was no significant difference between averaged discomfort scores at any time point, while increasing significantly in older adults after 60 minutes sitting. In the middle back, there were no significant differences between averaged discomfort scores among young participants at any time point
while middle back discomfort in older adults became significantly after 75 minutes of sitting. There was greater lumbar and thoracic flexion during sitting among younger adults compared to older adults in both lumbar and thoracic angles. When lumbar angles were expressed relative to standing postures, younger adults sat in more than 19 degrees more flexion compared to older adults. There was no effect of time or sex on sitting postures. Stiffness was significantly higher following the prolonged sitting protocol at 20% flexion while no significant time differences were detected at 10% or 30% flexion. Stiffness was significantly higher among male participants compared to female participants at 10%, 20% and 30% flexion. While stiffness values on average were higher among older participants, there was no statistical difference across age.

4. There will be no effect of age, sex, prolonged sitting or lift type on postures or perceived effort during a lifting task (study IV)

Despite reduced functional range of motion in the lumbar spine among older adults, a high flexion task (floor to knuckle lifting) was performed at similar percentages of maximum flexion across age, suggesting that passive spine stiffness may be greater in older adults at higher ranges of motion. Mature participants were found to have significantly lower functional range of motion compared to younger participants. Older adults achieved an average 11 degrees less maximum flexion range of motion compared to younger adults. There were no differences in maximum range of motion in the extension direction or in the lumbar lordosis angle during standing. Women achieved greater maximum flexion with -13.2 (13.0) degrees compared to -2.9 (15.1) degrees among male participants. Participants rated the asymmetrical and symmetrical 4.5kg lifting tasks with lower RPE scores compared to the 7kg lifting task, but there was no effect of sex, age or time on RPE values. Younger adults adopted more than 12 degrees more
lumbar peak flexion compared to older adults during the lifting tasks. Peak lumbar flexion angles were significantly lower following 90 minutes of continuous sitting compared to angles adopted prior to sitting with average values of 3.8 (13.3) degrees prior to the sitting protocol compared to 6.3 (13.4) degrees following 90 minutes of sitting. The asymmetrical 4.5 kg lifting task elicited a greater peak lumbar flexion angle compared to both the 7 kg and 4.5 kg symmetrical lifting tasks. There were no significant differences between the 7 kg and 4.5 symmetrical tasks. There was no effect of sex on peak lumbar angles. Participants adopted significantly greater thoracic flexion angles while completing the 4.5 kg asymmetrical task compared to both the 7 kg and 4.5 symmetrical tasks. There were no significant differences between the 7 kg and 4.5 symmetrical tasks. There was no effect of time, age or sex on peak thoracic angles during the lifting tasks. There was no effect of sex, age, lift or time on peak left knee or hip angles.

7.2 Combined Implications

7.2.1 Overview

The results of this thesis are visually integrated into a framework of existing evidence from the literature in Figure 41. Evidence linking age to injury claims and low back reporting from the epidemiological data are highlighted in addition to aging-related factors affecting annulus, disc mechanics and functional range of motion. Contributions from this thesis appear with red text and arrows. The following sections will specifically address the combined implications of the thesis.
Figure 41 Summary of contributions of thesis to existing literature
7.2.2 Key findings

1. The annulus and disc are a source of increased passive stiffness in the spine with aging (study I and study II), but this effect may only have implications toward the end range of motion (study III).

The results of the thesis indicate that age has a significant effect on the mechanical properties of the disc which may partially explain reductions in spine range of motion with increasing age. The results of studies I and II support that the annulus and FSU increase stiffness with increasing age. There were trends of increased passive stiffness in low flexion ranges of motion in older compared to younger adults in study III, however, these differences were not significant. It is possible that differences were not detected because they occur at greater flexion ranges of motion, approaching functional end range. Previous work has demonstrated that lumped passive stiffness increases in older adults at 70% of maximum flexion posture (Shojaei et al., 2016). This is supported by the results of study I and study IV, where maximum range of motion about the mediolateral axis was reduced in older adults and specimens compared to younger adults and specimens. Previous work supports the reduction of total range of spine motion with increases in age (Troke et al., 2001; Dvorak et al., 1995; McGill et al., 1999).

A possible mechanism for the increased stiffness in the annulus may be related to lactic acid in the intervertebral disc. Based on the results, of study II, where circumferential S-S modulus increased in the low pH group, it is hypothesized that the increased stiffness observed in the annulus in response to a low pH lactic acidic solution may be due to an increased cross-linking mechanism. Increased cross-links in annulus tissue have been noted in post-mortem specimens treated with Genipin (Chuang et al., 2007), and further, have been shown to reduce neutral zone range of motion and neutral zone stiffness in treated functional spine units (Kirking et al., 2013). In fact, Genipin has been investigated and framed as a potential injectable treatment to
compensate for the degenerative cascade in degenerated discs (Hedman et al., 2006). The degenerative cascade includes, loss of disc height, loss of water content, increased disc bulging, lower joint stability. It is possible that lactic acid in the disc may stimulate a natural cross-linking mechanism to remodel the disc and stabilize the joint. However, this work did not measure collagen cross-links in the porcine specimens tested in either study I or study II, and therefore, more work is needed to test this hypothesis.

2. **Age alters the mechanical environment of the intervertebral disc and annulus with implications of different injury patterns across age.**

Qualitative injury data in study I demonstrated the frequency of disc herniations was lower in the older discs compared to the younger discs. Herniation injuries have a dependency on hydraulic pressure gradient of the disc (Stefanakis et al., 2014). Water content in human discs decreases with increasing age (Antoniou et al., 1996). Tears in the annulus cause up to 46% reductions in local pressure, caused by a loss of proteoglycans surrounding the damage, with more proteoglycans lost with increases in donor age (Stefanakis et al., 2012). This may provide insight into why the aged porcine annulus was less likely to exhibit a herniation in study I. However, study II demonstrated higher peak stresses in the posterior annulus in the aged specimens. This may predispose older specimens to sub-catastrophic damage to the disc, including annulus tears, clefts and delamination. This type of damage is of interest given that it is a source of discogenic low back pain (Peng et al., 2005; Peng et al., 2006; Freemont et al., 1997; DePalma et al., 2007).

3. **Age affects postures and acute pain development during high flexion, simulated occupational tasks (study III and IV).**

Older adults adopted less flexed postures in the lumbar spine during floor to knuckle lifts and prolonged sitting tasks compared to younger adults (study III and study IV). When normalized
to maximum flexion angles, the postures assumed by older adults during these high flexion tasks, were a lower percent of maximum flexion compared to younger adults. This suggests that reduced maximum functional range of low back motion in older adults does not fully explain changes in postures during high flexion occupational tasks across age. While no significant difference in passive torso stiffness were detected in low levels of flexion (study III), it is possible that age-specific differences in stiffness emerge in higher flexion and older adults self-select less flexed positions to minimize strain on the posterior elements of the spine. Previous work has demonstrated that in flexion postures (relative to standing) representing 70% of maximum flexion, older adults exhibit higher passive stiffness compared to younger adults (Shojaei et al., 2016). Recent work has suggested that the perception of stiffness, elicits a mechanism to avoid pain provocation and injury (Stanton et al., 2017). While the perception of stiffness was not measured in the current investigation, it is possible that a similar mechanism may have contributed to older adults constraining flexion postures during sitting and lifting in order to avoid pain provocation given that older adults were more predisposed to acute pain development in select body locations during the prolonged sitting exposure.

7.2.3 Thesis limitations

This thesis can be considered in light of some limitations. The maturation period of a pig during its life course (rather than a % of life expectancy) is not clear. Furthermore, the relationship between pig and human aging processes are not well understood. However, there is no widely accepted animal model for the aging human spine. The use of naturally aged tissue is important to understand mechanistically the process of aging. The link between in vitro and in vivo investigations is confounded by the activity level, the degenerative status and hydration of participant discs in studies III and IV. Ideally, sedentary participants would have been recruited
in order to reduce variability in training status of core musculature. Other spine tissues
including, the nucleus, ligaments and muscles undergo significant changes with increasing age
may also contribute to differences in spine stiffness and range of motion across age.
Specifically, imaging of the in-vivo spines of participants was not completed for studies III and
IV. Therefore, the degree of spine degeneration/aging in passive tissues among study
participants was not quantified. Changes in stiffness attributable to age in the in-vitro
investigations provide a mechanistic understanding of aging on disc tissue but do not explain
mechanical changes observed in the in-vivo collection. The “age” variable in studies III and IV
represents a combination of chronological age in addition to co-variates with age, such as,
cumulative exposures, lifestyle and health status when comparing young and mature groups.
However, effort was made in the screening procedure to minimize differences in exercise levels,
BMI/waist circumference, injury status and work history. The results of studies III and IV can
be understood as changes occurring in a moderately active, normal/overweight population with
no low back pain or history of professional lifting. The results observed may be affected by
different cohorts of older adults, such as sedentary adults or those employed in physically
demanding occupations.

7.3 Summary

On a population scale, there is evidence of older adults reporting greater severity and
frequency of low pain compared to younger adults. While there are known age related changes
to the structures surrounding the spine, the implications of these changes and the tissue-level
sources have not been systematically explored previously. This thesis provides evidence that
aging alters the mechanical properties of the annulus and intervertebral disc. These mechanical
changes may have implications for spine functional range of motion, postures during high
flexion tasks and acute pain development. In order to prevent musculoskeletal disorders throughout the life course, an understanding of how age affects spine mechanics is essential. This thesis provides evidence of differences in the performance of high flexion occupational tasks and pain development across age. Age-related differences in task performance, pain development and changes in mechanical disc properties indicate that age may be an important factor to consider in strategies to prevent workplace musculoskeletal injuries. As the Canadian working population ages, and an understanding of changes in spine mechanics across age is relevant to injury prevention strategies and to gain a more fundamental understanding of how passive components of the spine change across age and influence work related tasks.
References:


Kelly, A. M. (1998). Does the clinically significant difference in visual analog scale pain scores vary with gender, age, or cause of pain?. Academic Emergency Medicine, 5(11), 1086-1090.


Appendix A: Study 3 and 4 Physical Activity Surveys

A.1 Physical Activity Readiness Questionnaire (PAR-Q)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?</td>
<td></td>
</tr>
<tr>
<td>2. Do you feel pain in your chest when you do physical activity?</td>
<td></td>
</tr>
<tr>
<td>3. In the past month, have you had chest pain when you were not doing physical activity?</td>
<td></td>
</tr>
<tr>
<td>4. Do you lose your balance because of dizziness or do you ever lose consciousness?</td>
<td></td>
</tr>
<tr>
<td>5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?</td>
<td></td>
</tr>
<tr>
<td>6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?</td>
<td></td>
</tr>
<tr>
<td>7. Do you know of any other reason why you should not do physical activity?</td>
<td></td>
</tr>
</tbody>
</table>

If you answered YES to one or more questions:

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

Information Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity and it in cases after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME: ________________________________ DATE: ________________________________

SIGNATURE: ____________________________ WITNESS: ____________________________

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority): ____________________________

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

© Canadian Society for Exercise Physiology www.csep.ca/forms
A.2 International Physical Activity Questionnaire (I-PAQ)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ days per week

   □ No vigorous physical activities ➔ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   _____ hours per day
   _____ minutes per day

   □ Don’t know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.
3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

☐ No moderate physical activities ➔ Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don’t know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ days per week

☐ No walking ➔ Skip to question 7

6. How much time did you usually spend walking on one of those days?
The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

____ hours per day
____ minutes per day

☐ Don’t know/Not sure

This is the end of the questionnaire, thank you for participating.
A.3 I-PAQ Scoring Protocol

At A Glance
IPAQ Scoring Protocol (Short Forms)

Continuous Score
Expressed as MET-min per week: MET level x minutes of activity/day x days per week

Sample Calculation

<table>
<thead>
<tr>
<th>MET levels</th>
<th>MET-minutes/week for 30 min/day, 5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking = 3.3 METs</td>
<td>3.3<em>30</em>5 = 495 MET-minutes/week</td>
</tr>
<tr>
<td>Moderate Intensity = 4.0 METs</td>
<td>4.0<em>30</em>5 = 600 MET-minutes/week</td>
</tr>
<tr>
<td>Vigorous Intensity = 8.0 METs</td>
<td>8.0<em>30</em>5 = 1,200 MET-minutes/week</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2,295 MET-minutes/week</td>
</tr>
</tbody>
</table>

Total MET-minutes/week = Walk (METs*min*days) + Mod (METs*min*days) + Vig (METs*min*days)

Categorical Score- three levels of physical activity are proposed

1. **Low**
   - No activity is reported OR
   - Some activity is reported but not enough to meet Categories 2 or 3.

2. **Moderate**
   Either of the following 3 criteria
   - 3 or more days of vigorous activity of at least 20 minutes per day OR
   - 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day OR
   - 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 600 MET-minutes/week

3. **High**
   Any one of the following 2 criteria
   - Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week OR
   - 7 or more days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3000 MET-minutes/week

Please review the full document "Guidelines for the data processing and analysis of the International Physical Activity Questionnaire" for more detailed description of IPAQ analysis and recommendations for data cleaning and processing (www.ipaq.ki.se).
# Appendix B: Study 3 and 4 Discomfort and Ratings of Perceived Effort Surveys

## B.1 Discomfort Survey (100mm Visual Analog Scale)

<table>
<thead>
<tr>
<th>Body Part</th>
<th>No discomfort</th>
<th>Extreme discomfort</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Neck</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Left Shoulder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Right Shoulder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Upper Back</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Middle Back</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Lower Back</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Pelvis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Sacrum/Tail Bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Left Buttocks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Right Buttocks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Left Thigh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Right Thigh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>Description</td>
<td>Note</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>0</td>
<td>Nothing at all</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>Extremely weak</td>
<td>(just noticeable)</td>
</tr>
<tr>
<td>1</td>
<td>Very weak</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Weak</td>
<td>(light)</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Strong</td>
<td>(heavy)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Very strong</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Extremely strong</td>
<td>(almost max)</td>
</tr>
</tbody>
</table>

- Maximal