CERVICAL SPINE MENISCOIDS AND THEIR POTENTIAL ROLE IN NECK PAIN AND ITS MANAGEMENT

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B Physiotherapy (Hons I)

Thesis submitted for the degree of

Doctor of Philosophy (Physiotherapy)

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This is to certify that the thesis entitled *Cervical Spine Meniscoids and their Potential Role in Neck Pain and its Management* submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (Physiotherapy) is in a form ready for examination.

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Declaration

I, Scott F. Farrell, hereby declare that the work contained within this thesis is my own and has not been submitted to any other university or institution as a part or a whole requirement for any higher degree, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I certify that the work embodied in this thesis contains published papers of which I am the lead author. I have included a written statement, endorsed by my supervisor, attesting to my contribution to these joint publications. I have included a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to these joint publications (Appendix A).

In addition, ethical approval from The University of Newcastle Human Research Ethics Committee and/or Hunter New England Research Ethics Committee was granted for the five studies presented in this thesis. Written informed consent was gained prior to data collection and human tissue was bequeathed in accordance with appropriate legislation (see Appendices B and C). Ethical approvals for all studies are included in Appendices D-G.

I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Scott F. Farrell

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Date _____

Supervisor Statement

I, Professor Darren A. Rivett, attest that Research Higher Degree candidate Scott F. Farrell was the lead author of the following publications:

Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2015). Formic acid immersion does not affect the morphometry of cervical zygapophyseal joint meniscoids – a methodological study for anatomical dissection. *Anatomical Science International 90* pp57-63. DOI: 10.1007/s12565-014-0248-8.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2015). The anatomy and morphometry of cervical zygapophyseal joint meniscoids. *Surgical and Radiologic Anatomy 37* pp799-807. DOI: 10.1007/s00276-014-1406-3.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Immunohistochemical investigation of nerve fibre presence and morphology in elderly cervical spine meniscoids. *The Spine Journal*. DOI: 10.1016/j.spinee.2016.06.004.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (*in press*). Morphology of cervical spine meniscoids in individuals with chronic whiplash

associated disorder: a case-control study. *Journal of Orthopaedic and Sports Physical Therapy*.

Acknowledgement of Work Undertaken at the University of Otago

Studies 1-4 required access to human cadavers, a dissecting room and histology processing facilities. At the time these studies were undertaken, the anatomy facilities of The University of Newcastle were not available as the anatomy department was undergoing building renovations. My Co-supervisor Dr. Jon Cornwall was at the time affiliated with the Department of Anatomy at the University of Otago. Dr. Cornwall negotiated access to University of Otago cadavers, dissection facilities and technical support on my behalf. Dr. Cornwall directly supervised my performance of the dissection and histology components of these studies, in conjunction with my Principal Supervisor Professor Darren A. Rivett and Co-supervisor Dr. Peter G. Osmotherly.

Scott F. Farrell

Signed _____

Date 3/2/16

_____

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Publications and Presentations Arising from the Work in this Thesis

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Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2015). The anatomy and morphometry of cervical zygapophyseal joint meniscoids. *Surgical and Radiologic Anatomy 37* pp799-807. DOI: 10.1007/s00276-014-1406-3.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Immunohistochemical investigation of nerve fibre presence and morphology in elderly cervical spine meniscoids. *The Spine Journal*. DOI: 10.1016/j.spinee.2016.06.004.

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Published Abstracts

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Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2014). The anatomy and morphometry of the meniscoids of the lateral atlantoaxial joints. *Annals of Anatomy 196* (S1) p86. DOI: 10.1016/j.aanat.2014.05.035.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (2015). Cervical meniscoid morphology in whiplash associated disorder: a preliminary comparative analysis. *Australian Physiotherapy Association Conference Abstract E-book* p42. URL: http://www.physiotherapy.asn.au/DocumentsFolder/CONFERENCE2015/APA%202015%20Abstracts%20Final.pdf.

Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2016). Lateral atlantoaxial joint capsules but not meniscoids contain neurofilament heavy reactive axons. For publication in *Clinical Anatomy*.

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Published Papers

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Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2015). Can E-12 sheet plastination be used to visualise intra-articular spinal meniscoids? *Clinical Anatomy 28* p943. DOI: 10.1002/ca.22520.

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List of Abbreviations

ANOVA	Analysis of variance
BMI	Body mass index
CGRP	Calcitonin gene-related peptide
DAB	Diaminobenzidine
DESS	Double echo steady state
ICC	Intraclass correlation co-efficient
IQR	Interquartile range
LRT	Likelihood ratio test
MRI	Magnetic resonance imaging
MVA/MVC	Motor vehicle accident/motor vehicle collision
NF-H	Neurofilament heavy
NSNP	Non-specific neck pain
OR	Odds ratio
Pan-NF	Pan-neurofilament
PGP 9.5	Protein gene product 9.5
R^2	Co-efficient of determination
SD	Standard deviation
SP	Substance P
T1 VIBE	T1-weighted volumetric inter-polated breath-hold examination
T2 SPACE	T2-weighted sampling perfection with amplification-optimised contrast using different angle evolutions
WAD	Whiplash associated disorder

β	Regression co-efficient
η^2	Eta-squared

Abstract

The overall aim of the work presented in this thesis was to explore the clinical significance of cervical spine meniscoids in neck pain. Cervical spine meniscoids are folds of synovial membrane that extend between the articular surfaces of joints throughout the cervical spine. These structures are thought to function to improve joint congruence and to ensure the lubrication of articular surfaces with synovial fluid. However, little is known about the role of cervical spine meniscoids in neck pain, as understanding of their morphology is not comprehensive. This body of work comprising five studies sought to investigate the pathoanatomical capacity of cervical spine meniscoids by exploring their morphology and innervation, as well as by investigating meniscoid morphology *in vivo* in a symptomatic population.

Study 1 tested a novel method of facilitating gross dissection of cervical spine meniscoids from 12 lateral atlantoaxial and cervical zygapophyseal joints excised from four cadavers. This investigation was necessary as the bony congruence and extensive ligamentous attachments of the articular pillar make disarticulation of cervical zygapophyseal joints difficult, requiring considerable force to separate joint surfaces, and potentially damaging the delicate cervical spine meniscoids enclosed within. Such damage may jeopardise the accuracy of morphological assessment of cervical spine meniscoids undertaken using dissection. The study found that formic acid demineralisation of cadaveric cervical spines did not alter the morphometry of cervical spine meniscoids. This validated the use of this technique as a viable means of facilitating disarticulation of the lateral atlantoaxial and cervical zygapophyseal joints, by allowing the softened bone to be cut away with a scalpel, such that the joint surfaces

could be separated with minimal force. This technique was then utilised in Studies 2 and 3.

Study 2 explored the morphology and histology of lateral atlantoaxial joint meniscoids in 12 cadavers using gross dissection and light microscopy. The study resolved points of contention in previous research, including cervical spine meniscoid prevalence and patterns of composition and morphometry. An association was found between articular cartilage degeneration and fibrous meniscoid composition, suggesting a possible link between meniscoid morphology and articular pathology of the lateral atlantoaxial joint.

The morphology and histology of cervical zygapophyseal joint meniscoids were investigated in Study 3 in 12 cadavers using gross dissection and light microscopy. Consistent with Study 2, Study 3 also noted an association of fibrous meniscoid composition with articular cartilage degeneration, providing further evidence of a potential relationship between cervical spine meniscoid morphology and articular pathology. Meniscoid size was not found to vary with spinal level, position in joint, articular degeneration or sex in an elderly population.

The innervation of cervical spine meniscoids was explored in Study 4 to determine the capacity for meniscoids to generate nociceptive input. This was undertaken using immunohistochemistry with antibodies to neurofilament heavy and pan-neurofilament to identify both myelinated and unmyelinated nerve fibres in 77 cervical spine meniscoids excised from 12 cadavers. Unmyelinated nerve fibres were identified within the bodies of two lateral atlantoaxial joint meniscoids composed of adipose tissue. Myelinated and unmyelinated fibres were observed within joint capsules adjacent to 14

cervical spine meniscoids. These latter findings provide evidence of potential sensory innervation of lateral atlantoaxial and cervical zygapophyseal joint capsules. The identification of nerve fibres exclusively within bodies of two adipose meniscoids perhaps suggests that meniscoid composition may influence the innervation status of cervical spine meniscoids.

The fifth and final study investigated cervical spine meniscoid morphology in a living population with known cervical spine pathology. This was undertaken using magnetic resonance imaging to visualise cervical spine meniscoids in 20 people with chronic whiplash associated disorder (WAD) and 20 age and sex-matched pain-free controls. Cervical spine meniscoids were found to be smaller in the lateral atlantoaxial joints and were more frequently fibrous in composition at the dorsal aspect of cervical zygapophyseal joints of the WAD group. It is postulated that such differences may be the result of altered cervical spine kinematics secondary to pain and hypomobility associated with WAD, and could plausibly serve to perpetuate patient symptoms.

The body of work comprising this thesis extends current understanding of the clinical significance of cervical spine meniscoids. The question of the prevalence of these structures has been addressed through convergent findings of dissection and imaging studies, thus refuting previous reports that cervical spine meniscoids are rare in adults. Patterns of cervical spine meniscoid morphological variation have been explored in elderly cadavers, noting an association between composition and evidence of articular pathology. Nerve tissue has been identified within cervical spine meniscoids (albeit uncommon) and adjacent joint capsules that is potentially nociceptive in function. Cervical spine meniscoids have been studied in a living population with cervical spine

pathology, with results illustrating morphological differences between the meniscoids of people with WAD and a pain-free population. Cumulatively, these findings provide preliminary evidence that cervical spine meniscoids may feasibly be of clinical significance in neck pain, possibly by altering segmental biomechanics or through generating nociceptive input.

Chapter 1: INTRODUCTION

Background to Neck Pain

Neck pain is a common and burdensome condition that poses a significant challenge to healthcare systems around the world (Fejer, Kyvik, & Hartvigsen, 2006). Despite decades of clinical research, a clear and comprehensive understanding of musculoskeletal neck pain and its management remains elusive. Appreciation of the role that anatomical structures may play in this condition is expanding (Bogduk, 2011a), however neck pain still creates difficulties for physiotherapists, medical doctors and surgeons managing patients suffering the condition (Ho & Howard, 2015).

Neck pain is a broad term encapsulating a wide variety of clinical presentations. The International Association for the Study of Pain defines neck pain as:

"*...pain perceived arising from anywhere within the region bounded superiorly by the superior nuchal line, inferiorly by an imaginary transverse line through the tip of the first thoracic spinous process, and laterally by sagittal planes tangential to the lateral borders of the neck"* (p11) (Merskey & Bogduk, 1994).

Neck pain may be acute or chronic in nature (Bogduk & McGuirk, 2006). It may be related to occupational, postural or overuse factors, a particular traumatic event, such as whiplash associated disorder (WAD), or can be insidious in onset (Ho & Howard,

2015). Pain may be located solely in the neck itself, or associated with pain in the head, shoulder or upper limb (Ho & Howard, 2015; Hoy *et al.*, 2014a). Occasionally, a specific pathoanatomical cause of the patient's symptoms may be identified, such as radiculopathy or myelopathy, however in many presentations, a specific pathoanatomical diagnosis cannot be reached (Borenstein, 2013; Frohna & Della-Giustina, 2011). This is termed *non-specific, idiopathic* or *mechanical* neck pain (NSNP).

Burden to Society

Neck pain is a prevalent health condition (Cote *et al.*, 2008; Fernandez-de-las-Penas *et al.*, 2011; Hogg-Johnson *et al.*, 2008; Hoy, Protani, De, & Buchbinder, 2010). In a comprehensive systematic review, The Bone and Joint Decade 2000–2010 Task Force on Neck Pain and Its Associated Disorders reported the twelve-month prevalence of neck pain in the general adult population to be 30-50%, and 21-42% in children and adolescents (Hogg-Johnson *et al.*, 2008). The review found that the ability of individuals to undertake functional activities was limited by pain in 2-11% of people experiencing neck pain (Hogg-Johnson *et al.*, 2008). In the working population, approximately 5% of workers each year are predicted to develop persistent or recurrent neck pain and, depending on occupational factors, up to 10% of workers will experience at least one incident of neck pain that limits function (Cote *et al.*, 2008). More recently, Fernandez-de-las-Penas *et al.* (2011) reported the twelve-month prevalence of neck pain to be 19.5% (95% CI 18.9 to 20.1) in a survey of 29,478 Spanish adults. The point prevalence of neck pain globally has been estimated at 4.9% (95% CI 4.6 to 5.3) by Hoy

et al. (2014a) in a comprehensive review as part of the Global Burden of Disease 2010 collaborative studies (Hoy *et al.*, 2014b; Murray & Lopez, 2013).

The economic burden of neck pain is significant (Cote *et al.*, 2008; Hogg-Johnson *et al.*, 2008; Hoy *et al.*, 2010; Kleinman *et al.*, 2014). In Australia, an estimated 640,000 people suffer chronic neck pain annually, at an estimated cost of AUD$1.14 billion to the community in direct (medical care) and indirect (loss of productivity) expenses (Access Economics Pty Ltd, 2007; Blyth *et al.*, 2001; Blyth, March, & Cousins, 2003). The economic burden of WAD is of particular concern, constituting approximately 45% of motor vehicle accident (MVA) personal injury insurance claims, and approximately 27% of total MVA personal injury insurance claim costs (Motor Accidents Authority, 2009). In the Netherlands in 1996, neck pain was found to be responsible for USD$686.2 million in direct and indirect expenses, which formed 1% of total healthcare costs for that year, or 0.1% of the national gross domestic product (Borghouts, Koes, Vondeling, & Bouter, 1999). More recently in the United States of America, the combined annual direct and indirect costs of back and neck pain were found to be have increased nationally by approximately 65% (adjusted for inflation) during the period 1997 to 2005 (Martin *et al.*, 2008), supporting suggestions that the prevalence and economic burden of neck pain have grown in recent decades (Hogg-Johnson *et al.*, 2008; Holm *et al.*, 2008).

Prognosis

An episode of acute musculoskeletal neck pain often resolves in a period of several weeks, however a substantial portion of patients will experience recurrence of their pain

at a later date, or their symptoms may not resolve completely (Carroll *et al.*, 2009; Ho & Howard, 2015; Hoy *et al.*, 2010; Leaver *et al.*, 2013). A systematic review by Hoy and colleagues (2010) estimated that 33-65% of people suffering neck pain will experience recurrence within one year, and Carroll *et al.* (2009) estimated that 50-85% of people with neck pain will not report complete resolution 1-5 years later. Approximately one quarter of all people that sustain a whiplash injury in an MVA will never completely recover (Sterling et al., 2012; Sterling, Jull, & Kenardy, 2006). Risk factors for developing chronic neck pain include prior neck pain or neck injury, female sex, low back pain, poor self-reported health, psychosocial problems, anxious personality traits, increased body mass index, smoking and limited physical exercise (Carroll *et al.*, 2008a; Carroll *et al.*, 2009; Fejer *et al.*, 2006; Ho & Howard, 2015; Kaaria, Laaksonen, Rahkonen, Lahelma, & Leino-Arjas, 2012; Leaver *et al.*, 2013; Nilsen, Holtermann, & Mork, 2011).

Aetiology

Any structure of the cervical spine that is innervated may theoretically be a source of neck pain (Bogduk, 2011a; Evans, 2014). Anatomical structures that have been shown to be innervated include the intervertebral discs, the vertebral bodies, the zygapophyseal joints, the longitudinal ligaments and the neck muscles (Bogduk & McGuirk, 2006; Curatolo *et al.*, 2011).

Neck pain may result from trauma to the cervical spine or may be idiopathic (Frohna & Della-Giustina, 2011). Signs and symptoms can be a result of local or systemic pathological processes. Benign mechanical disorders of the musculoskeletal system

have been estimated as the source of approximately 90% of neck pain episodes (Borenstein, 2013), with the remaining 10% being caused by a variety of medical conditions including tumours, infections, fractures and inflammatory diseases (Bogduk, 2011a; Bogduk & McGuirk, 2006).

A major challenge in the management of NSNP and WAD is that, similar to many low back pain presentations, a specific pathoanatomical source of the patient's pain often cannot be determined and a definitive diagnosis frequently cannot be reached (Evans, 2014; Jull, Sterling, Falla, Treleaven, & O'Leary, 2008). By definition, the aetiology of NSNP is not well understood. In acute short-term neck pain without radiculopathy or myelopathy, it has been suggested that muscle, ligament or joint strains are potential sources of patient symptoms (Bogduk & McGuirk, 2006; Evans, 2014). Altered neuromuscular control, decreased cervical muscle strength and endurance, articular degeneration, altered biomechanics, overuse and postural factors have all been suggested as potential contributing factors in patients who suffer long-term mechanical neck pain. However it is unclear if these features occur secondary to the pain, rather than constitute actual primary causes of the pain (Bogduk & McGuirk, 2006; Jull *et al.*, 2008).

Cervical Spine Meniscoids

One potential source of neck pain has been theorised to be associated with the existence of meniscoid structures within the cervical zygapophyseal, lateral atlantoaxial and atlanto-occipital joints (Inami *et al.*, 2000; Inami, Shiga, Tsujino, Okado, & Ochiai, 2001; Kos, Hert, & Sevcik, 2002; Kos & Wolf, 1972; Mercer & Bogduk, 1993; Webb *et*

al., 2011a). Meniscoids are folds of synovial membrane that project into the joint cavity of synovial articulations (**Figure 1**) throughout the spinal column (Webb *et al.*, 2011a). These structures are thought to compensate for the incongruence of the articular surfaces of the enclosing joints, and to protect and lubricate the articular cartilages of these joints during normal gliding movements (Mercer & Bogduk, 1993).

Meniscoids have been theorised to contribute to neck pain through several different mechanisms, including: mechanical entrapment of the structures between the articular surfaces (Kos *et al.*, 2002; Kos & Wolf, 1972; Zukschwerdt, Emminger, Biedermann, & Zettel, 1955); extrapment of the meniscoid outside of its normal resting position between the joint surfaces (Bogduk & Jull, 1985); meniscoids acting as a catalyst for fibrous tissue proliferation and joint hypomobility (Jones *et al.*, 1989); or through being traumatically crushed between the joints surfaces in a 'whiplash' type mechanism of injury (Bogduk, 1999; Kaneoka, Ono, Inami, & Hayashi, 1999; Schonstrom, Twomey, & Taylor, 1993). These theories and their supporting evidence will be discussed in detail in the following chapter.

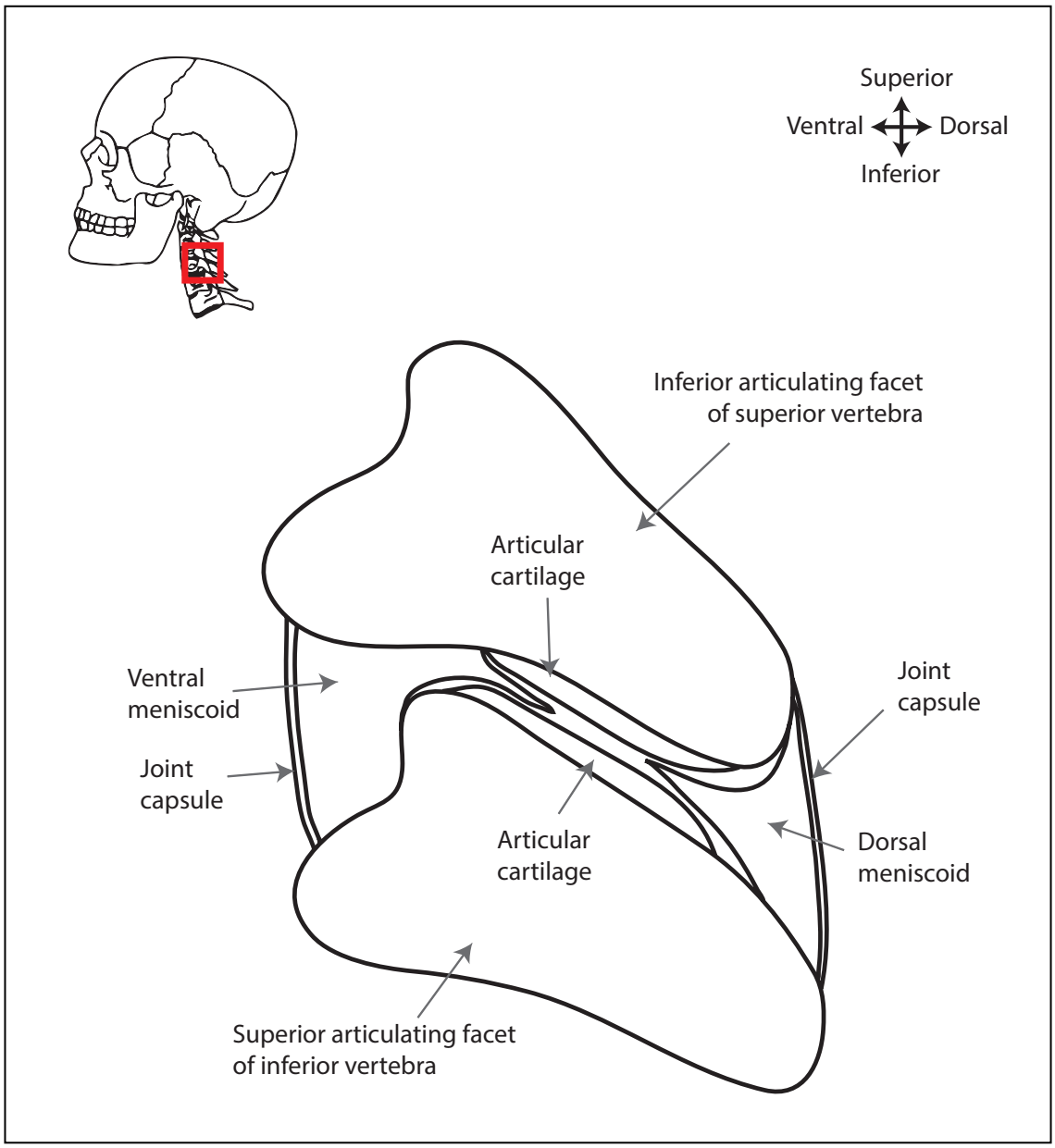


Figure 1 Schematic representation of sagittal section through mid-cervical spine zygapophyseal joint, demonstrating dorsal and ventral meniscoids. Red rectangle indicates location of cross section. Modified from Friedrich *et al.* (2008) and Webb *et al.* (2011a).

It is plausible that cervical spine meniscoids are pathoanatomical contributors to chronic neck pain, in both NSNP and WAD. Pathological changes affecting meniscoid morphology could form potential mechanisms for the production and maintenance of pain. However, as the following chapter will illustrate, a comprehensive understanding does not exist regarding cervical meniscoid anatomy and morphology, as previous studies of cervical spine meniscoid anatomy undertaken using cadavers have at times yielded inconsistent results. To date, the morphology of these structures has not been examined in a living population with cervical spine pathology. This is largely due to the historical inability to investigate these structures in living subjects, as the size and location of cervical spine meniscoids meant that the only means of visualising meniscoids was through cadaveric dissection. However with recent advances in magnetic resonance imaging (MRI) technology, cervical spine meniscoids can now be imaged *in vivo* and as such the morphological characteristics of these structures in a population with cervical spine pathology can be explored.

Further investigation of cervical spine meniscoids as potential pathoanatomical contributors to neck pain is therefore necessary, given the substantial burden of neck pain to society, incomplete appreciation of cervical spine meniscoid anatomy, and recently developed ability to examine these structures in living people. As the next section of this chapter will outline, this thesis comprises a series of studies that seek to expand current understanding of the clinical significance of the cervical spine meniscoids.

Rationale of Thesis

Aims

The overall aim of this thesis is to explore the potential clinical significance of the cervical spine meniscoids, as it relates to neck pain. This will be undertaken by:

- Exploring the anatomy of cervical meniscoids in cadavers to clarify their morphology, including their innervation;
- Investigating meniscoid pathoanatomy *in vivo* in a symptomatic population.

Significance

Both NSNP and WAD are responsible for substantial community burden worldwide, detracting from quality of life and productivity (Cote *et al.*, 2008). Current knowledge of the morphology of the cervical spine meniscoids and their potential pathoanatomical role in neck pain is not comprehensive. Facilitating an appreciation of the anatomy of cervical spine meniscoids, including possible morphological changes in neck pain and also their innervation, may enhance our understanding of the pathophysiology of neck pain. In turn, this may be valuable in the management of this burdensome condition, by providing a basic science foundation to underpin diagnostic practices and approaches to treatment.

Outline

This thesis will consider the clinical significance of the cervical meniscoids. It comprises a literature review followed by a series of papers (both published articles and manuscripts under review) which describe the series of five studies undertaken, the findings of which are consolidated in a concluding discussion chapter. The chapter outlines are as follows:

- Chapter 1 provides introductory information regarding neck pain and details the aims, structure, scope and significance of the thesis;
- Chapter 2 will review the current understanding of the morphology of the cervical meniscoids and their potential role in pathology, in the form of a critical appraisal of previously published basic science and clinical research;
- Chapter 3 will detail Study 1, an experimental study examining the effect of formic acid immersion upon meniscoid morphometrics, in order to validate the use of this technique as a means of facilitating meniscoid dissection (Farrell, Osmotherly, Rivett, & Cornwall, 2015c);
- Chapter 4 will report Study 2, an anatomical study using gross dissection of cadavers to investigate aspects of lateral atlantoaxial joint meniscoid morphology identified as requiring clarification in Chapter 2 (Farrell, Osmotherly, Cornwall, & Rivett, 2016b);
- Chapter 5 will report Study 3, also an anatomical study using gross dissection of cadavers to examine points of contention in previous studies of cervical zygapophyseal joint meniscoid clinical anatomy (Farrell, Osmotherly, Cornwall, & Rivett, 2015a);

- Chapter 6 will detail Study 4, an investigation of the presence of nerve tissue within the meniscoids of the lateral atlantoaxial and cervical zygapophyseal joint meniscoids in cadavers (Farrell, Osmotherly, Cornwall, & Rivett, 2016a). A structure that is innervated may theoretically be a source of pain, and as such the presence of nerve fibres within the cervical meniscoids may be clinically significant, however this aspect of cervical meniscoid morphology is relatively unexplored;
- Chapter 7 will report Study 5, a clinical study employing MRI to compare the radiological morphology of the cervical meniscoids and potentially associated degenerative changes *in vivo* in a chronic neck pain population to that of a pain-free control group;
- Chapter 8, the final chapter, provides a summary and concluding discussion of the key findings of the five studies, as relevant to the potential role of meniscoids in cervical spine pain and pathophysiology, with recommendations for future research and clinical practice.

Scope

Many structures in the cervical spine are capable of pain generation, however this thesis focuses upon the cervical spine meniscoids. Whilst lumbar and thoracic spine meniscoids have also been suggested to play a potential role in musculoskeletal back pain, the scope of this thesis is limited to the meniscoids of the cervical spine and their potential role in neck pain. Further, this thesis examines the clinical significance of the cervical spine meniscoids as anatomical entities, and does not assess these structures from a biomechanical perspective.

LITERATURE REVIEW

This section will examine the research to date regarding cervical spine meniscoids and their potential clinical relevance. First, evidence regarding meniscoid prevalence and morphology will be explored, including their size, shape and composition, as well as the influence of location, age, sex and articular degeneration on meniscoid structure. Second, previous research examining cervical meniscoid innervation will be discussed. Finally, evidence regarding theories of the clinical significance of cervical meniscoids will be reviewed.

Overview of Cervical Spine Meniscoids

Meniscoids are folds of synovial membrane that project into the joint cavity of spinal synovial articulations, and have been described in the cervical, thoracic and lumbar spines (Engel & Bogduk, 1982; Mercer & Bogduk, 1993; Schulte, FIiller, Struwe, Liem, & Bullman, 2010). The term 'meniscoid' arises from the structures' similar appearance to the crescent-shaped meniscus of the knee, however they may also be referred to as synovial folds (Webb *et al.*, 2011a) or intra-articular inclusions (Mercer & Bogduk, 1993).

Cervical spine meniscoids were first described by Henle in 1855 (Dörr, as cited in Webb, Collins *et al.* 2011), and were the subject of several cadaveric morphological studies through the 20th century, largely in European literature (De Marchi, 1963; Dörr,

1958; Penning & Toendury, 1963; Schmincke & Santo, 1932; Tondury, 1940; Zaccheo & Reale, 1956). The anatomy of cervical spine meniscoids and their role in pathology has not been extensively studied. Previous research undertaken includes dissection, cadaveric sectioning, imaging and histological studies, which in many respects have yielded contrary results.

Prevalence of Cervical Spine Meniscoids

A number of studies have described the cervical spine meniscoids, with conflicting findings reported regarding their prevalence (Bland & Boushey, 1990; Fletcher, Haughton, Ho, & Yu, 1990; Friedrich *et al.*, 2008; Inami *et al.*, 2000; Mercer & Bogduk, 1993; Yu, Sether, & Haughton, 1987). Meniscoids have been reported by some authors as being commonly found through the lateral atlantoaxial and cervical zygapophyseal joints (Bland & Boushey, 1990; Friedrich *et al.*, 2008; Hu, Ma, & Wang, 2006; Inami *et al.*, 2000; Mercer & Bogduk, 1993), yet other researchers have suggested that these structures are uncommon (Yu *et al.*, 1987) or even non-existent (Fletcher *et al.*, 1990; Kawabe, Hirotani, & Tanaka, 1989) in adult populations.

The variation in prevalence reported by the authors of previous work may be a product of methodological differences between the studies. It is possible that studies that have used gross dissection may have overlooked very small meniscoids either as a simple oversight or through inadvertently damaging them whilst disarticulating the joint. Similarly, studies that have used sectioning (Fletcher *et al.*, 1990; Yu *et al.*, 1987) may overlook meniscoids if the section does not transect the meniscoid. For example, Schmincke and Santo (1932) used coronal sections to study cervical spine meniscoids,

which, given that the structures appear to be located at the ventral and dorsal poles of a joint, could quite plausibly result in meniscoids not being detected (Mercer & Bogduk, 1993).

Morphology of Cervical Spine Meniscoids

Composition

Cervical spine meniscoids are composed of an outer layer of synovial membrane and a sub-synovial core (Friedrich *et al.*, 2007; Inami *et al.*, 2000; Webb *et al.*, 2011a). The sub-synovial core has consistently been shown to comprise either adipose tissue, fibrous tissue, or mixed fibroadipose tissue (Friedrich *et al.*, 2008; Inami *et al.*, 2000; Mercer & Bogduk, 1993; Tang, Liu, Yang, Peng, & Liao, 2007), as well as a vascular network (Mercer & Bogduk, 1993; Tang *et al.*, 2007).

Adipose meniscoids are composed of adipocytes, loose connective tissue and blood vessels, enclosed by collagen anchoring the structure to the joint capsule (Ibatullin, Zaitseva, Chudnovskii, & Chudnovskaia, 1987; Mercer & Bogduk, 1993; Tang *et al.*, 2007). Mercer and Bogduk (1993) found this type of meniscoid to be covered entirely by a cell-rich adipose type synovial membrane, which is in agreement with the findings of Inami and colleagues (2000) and Engel & Bogduk (1982). Fibrous meniscoids are composed of fibrous tissue (continuous with the joint capsule) and blood vessels, enclosed by fibrous synovium (Mercer & Bogduk, 1993; Tang *et al.*, 2007). Fibroadipose meniscoids consist of a combination of adipose and fibrous dense

connective tissue, as well as blood vessels, encapsulated in synovium (Inami *et al.*, 2000; Mercer & Bogduk, 1993). These structures feature a core of adipose tissue supported by collagen and covered in fibrous tissue that extends from the fibrous joint capsule to the apex of the structure, which is almost entirely collagenous in composition (Inami *et al.*, 2000; Mercer & Bogduk, 1993). They are enclosed by synovium, which has been shown to be cell-rich adipose type at the basal region, and cell-poor fibrous type at the apex (Inami *et al.*, 2000; Mercer & Bogduk, 1993).

Specific detail regarding the depth and arrangement of overlying synoviocytes has not been extensively investigated in previous research. Variation in the architecture or arrangement of the synovial membrane lining different types of meniscoid may offer insight into the biomechanical characteristics of these structures, and could have clinical implications for musculoskeletal conditions. In a cadaveric study examining cervical meniscoids, Chang and colleagues (1992) described the overlying synovial membrane as comprising two to three layers of synoviocytes, but did not comment on consistency or variation of this arrangement. Fibrous capsular rim meniscoids were reported by Mercer and Bogduk (1993) to be covered in fibrous type synovium. However, Inami and colleagues (2000) reported the same fibrous meniscoids to be covered in cell rich (i.e. adipose) type synovium at the bases, and by cell-poor (fibrous) synovium at the apices (Engel & Bogduk, 1982). Thus, there appears to be contention in previous research regarding the specific arrangement of synoviocytes in this form of meniscoid, which may have clinical significance.

Variation between the vascular networks of dorsal and ventral meniscoids has been reported in a study by Schonstrom, Twomey and Taylor (1993), who found that dorsal meniscoids had "*more plentiful*" (p887) vascular networks than ventral meniscoids in an

autopsy study of the upper cervical spines of trauma victims. Kawabe *et al.* (1989) also reported variation in vascular network density in a dissection study of child and adult cadavers. They reported only two types of meniscoid – "*…one rich in blood vessels …and another composed mainly of fibrous tissue"* (p571) – implying that fibrous meniscoids have lower vascular density than the other type of meniscoid described. Interestingly, fibrous meniscoids have been reported to be more frequent at the dorsal aspect of joints (see '*Location in Joint*' below), which suggests that Kawabe and colleagues' findings are contrary to those reported by Schonstrom, Twomey and Taylor (1993). Methodological explanation of this discrepancy is difficult to determine, as the dissection protocol used by Kawabe et al. (1989) is not comprehensively described in the paper.

A small number of authors have reported occasional findings of cartilage (Yu *et al.*, 1987) and of chondrocytes using safranine-O fast green and alcian blue staining (Chang *et al.*, 1992), however these reports are inconsistent and require further investigation to validate these findings (Webb *et al.*, 2011a).

Shape and Size

Cervical spine meniscoids are reported as being crescent-shaped, elliptical, semicircular, rectangular, or leaf-like in shape (Inami *et al.*, 2000; Kos *et al.*, 2002; Mercer & Bogduk, 1993; Zaccheo & Reale, 1956). It has been suggested that meniscoid shape appears to vary to conform to the surrounding joint surfaces (Mercer & Bogduk, 1993).

Where cervical spine meniscoids have been described, they have been variously described as consisting of either three (Inami *et al.*, 2000; Kos *et al.*, 2002; Mercer & Bogduk, 1993; Tang *et al.*, 2007; Zaccheo & Reale, 1956) or four (Yu *et al.*, 1987) types of morphological classification, based upon size, shape and composition. The conflicting findings of the previous descriptive anatomy studies are possibly a result of many of the earlier investigations being undertaken by sectioning of cadaveric tissue in only the sagittal plane, which may have lead to limited appreciation of the three dimensional structure of the meniscoids when compared to the multidimensional dissections of Mercer and Bogduk (1993) and Inami and colleagues (2000).

Location in Joint

The ventral meniscoids of the cervical zygapophyseal and lateral atlantoaxial joints have been shown to be larger than the dorsal meniscoids in a number of studies (Inami *et al.*, 2000; Webb, Darekar, & Rassoulian, 2011b; Webb, Darekar, Sampson, & Rassoulian, 2009). Several authors have reported fibrous meniscoids to be most frequently found at the dorsal aspect of a joint, and adipose meniscoids to more commonly found at the ventral aspect of a joint (Friedrich *et al.*, 2008; Giles, 1986; Inami *et al.*, 2000; Webb *et al.*, 2009; Webb, Rassoulian, & Mitchell, 2012). This has been speculated to be related to postural or biomechanical factors, however no studies have been undertaken or biomechanical models proposed exploring distribution of forces through the ventral and dorsal aspects of the lateral atlantoaxial or cervical zygapophyseal joints.

Location in Spine

Cervical spine meniscoid characteristics appear to vary between different cervical vertebral levels, however this has not been extensively investigated in the literature, and there is some contention between previously published studies.

Zygapophyseal Joints

Conflicting findings exist regarding the morphology of cervical spine meniscoids located in the zygapophyseal joints. Mercer and Bogduk (1993) reported the meniscoids of the cervical zygapophyseal joints to consist primarily of two classifications: fibroadipose meniscoids, a mixture of fibrous and adipose tissues, and capsular rims, fibrous thickenings of the joint capsule. They also noted a small proportion of zygapophyseal joints contain fat pads, however this was in just seven of 176 meniscoids examined. This is consistent with Inami *et al.* (2000) who also found fibroadipose and fibrous meniscoids to be most prevalent in the cervical zygapophyseal joints, however their study found a greater proportion of primarily adipose structures than Mercer and Bogduk (1993) (27 of 134 meniscoids examined). In contrast, Yu *et al.* (1987) reported zygapophyseal joints to most commonly lack a protruding meniscoid, with less than one quarter of joints featuring a meniscoid of fibrous and adipose tissues.

Lateral Atlantoaxial Joints

The lateral atlantoaxial joints differ from the zygapophyseal joints in form and in function, as the biconvex articular surfaces facilitate rotation, allowing the lateral atlantoaxial joints to provide approximately 40% of total cervical rotation range of motion (Bogduk & Mercer, 2000; Nordin & Frankel, 2012). Consequently, the meniscoids at this level appear to vary in their morphology when compared to the meniscoids of the lower spinal levels. Previous work has noted the meniscoids of the lateral atlantoaxial joints to be composed of adipose tissue (Friedrich *et al.*, 2008; Ibatullin *et al.*, 1987), or of a blend of fibrous and adipose tissues (Mercer & Bogduk, 1993; Yu *et al.*, 1987). This may have clinical implications in acute torticollis (see '*Entrapment Theory*' later in this section), a condition characterised by a sudden and painful restriction of rotation motion (Kawabe *et al.*, 1989).

Influence of Sex Upon Cervical Spine Meniscoid Morphology

The influence of sex upon the anatomy of the cervical zygapophyseal joint meniscoids has only been explicitly investigated in one study. Friedrich and colleagues (2008) used MRI to generate reference data for *in vivo* cervical meniscoid properties in 56 healthy volunteers (23 males, 33 females), and found that sex did not influence meniscoid size, tissue composition or prevalence.

However, in a study of the meniscoids of the lateral atlantoaxial joints through sectioning of cadaveric tissue, Webb and colleagues (2012) found meniscoid

dimensions to generally be larger in male specimens compared to females. This expands upon work previously undertaken by the authors (Webb *et al.*, 2011b) that found correlation of some anthropometric measures with variation in lateral atlantoaxial joint meniscoid morphometry. Sex has been shown in a previous study to influence cervical spine anthropometrics (Vasadava, Danaraj, & Gunter, 2008), so it could be suggested that anthropometric variation between sexes may lead to associated differences in cervical spine meniscoid characteristics. However, the influence of sex upon these structures needs to be further investigated.

Influence of Age Upon Cervical Spine Meniscoid Morphology

Cervical spine meniscoids may change in prevalence and morphology with increasing age, however there is little quality evidence to support this notion, as dissection studies examining meniscoid structure across age groups have suffered from low sample sizes and poor representation of younger age groups in the samples (Fletcher *et al.*, 1990; Yu *et al.*, 1987). This limitation is inherent to cadaveric research, as donors are typically of older age at death (Cornwall, Perry, Louw, & Stringer, 2012; Stiles & Cornwall, 2011). An *in vivo* study using MRI (Friedrich *et al.*, 2008) has suggested that the prevalence of cervical spine meniscoids decreases with increasing age, which is consistent with the findings of a previous dissection study (Fletcher *et al.*, 1990). This could feasibly be the result of articular degeneration leading to regressive changes in meniscoid size, such as secondary to decreased joint space.

Kos et al. (2002) suggested that meniscoid size increases as a person ages, however studies by a number of authors (Fletcher *et al.*, 1990; Inami *et al.*, 2000; Tang *et al.*,

2007; Yu *et al.*, 1987) indicate the opposite to be true, and studies by Friedrich et al. (2008) and Webb et al. (2009) reported age to have no influence on meniscoid dimensions. Further investigation is required using larger sample sizes with better distribution of specimens across the age span to clarify the influence of age upon the presence and size of cervical meniscoids. Such investigations likely need to be undertaken using living people to effectively capture sufficient data from younger age groups.

Influence of Articular Degeneration Upon Cervical Spine Meniscoid Morphology

Findings regarding the influence of joint degeneration upon cervical spine meniscoid morphology have been conflicting. Inami *et al.* (2000) reported zygapophyseal joint degeneration to be associated with an increased frequency of thin, fibrous meniscoids. On the other hand, another dissection study (Yu *et al.*, 1987) and an *in vivo* MRI study (Friedrich *et al.*, 2008) found joint degeneration to be associated with thick fibroadipose and thin fibroadipose meniscoids respectively.

Kos et al. (2002) reported that cervical spine meniscoid size increases with increasing articular degeneration, however more recent studies by Webb *et al.* at the lateral atlantoaxial joints (2009; 2012) and Friedrich *et al.* through the entire cervical spine (2008) found meniscoid size to have no relationship to articular degeneration. The reason for the discrepancy between Kos *et al.* (2002) and the other two studies may be related to methodology or objective measurement techniques used, however Kos and colleagues' paper was published in Czech, and the English summary provides limited

detail regarding the assessment of cartilage degeneration and measurement landmarks used in the study.

It should also be noted that the studies by Yu *et al*. (1987) and Friedrich *et al*. (2008) specifically excluded participants with neck pathology or pain, whereas Inami *et al*. (2000) and Kos *et al*. (2002) did not describe if cadaver medical records were screened for cervical pathology. Therefore, as the subjects of the above studies were either free of neck pain or had an undescribed neck pain history, the clinical applicability of these findings to a degenerative joint disease population is questionable.

Imaging and Radiological Identification of Cervical Spine Meniscoids

MRI has been shown to be a reliable method of imaging cervical spine meniscoids in a study comparing microimaging with corresponding MR images (Friedrich *et al.*, 2007). The same researchers have also investigated characteristics of cervical spine meniscoids in a healthy population (mean age 42 +/- 17 years) using MRI (Friedrich *et al.*, 2008). Webb and colleagues went on to use MRI to study lateral atlantoaxial joint meniscoid morphometrics in a healthy population (2009; 2011), however no study to date has explored meniscoid morphology in a population with cervical pathology.

The validation of MRI as a means of imaging cervical spine meniscoids is critical to improving our understanding of their morphology in both healthy and pathological populations, as it allows for examination of meniscoid morphology in living subjects. Dissection studies are often inherently limited by the typically advanced age of cadavers and absence of comprehensive knowledge regarding their medical histories. Therefore, imaging meniscoids in living subjects allows meniscoid morphology to be examined

across different age groups, and in pathological and healthy populations. This could address gaps in current understanding of cervical spine meniscoid morphology and pathoanatomy, and facilitate potential radiological clinical applications (Taylor & Taylor, 1996).

Innervation of Cervical Spine Meniscoids

The innervation of the cervical spine meniscoids has not been well investigated in comparison to the lumbar spine meniscoids (Inami *et al.*, 2001). Inami and colleagues (2001) demonstrated substance P (SP), calcitonin gene-related peptide (CGRP), protein gene product (PGP) 9.5 and β-III tubulin immunoreactive nerve fibres, suggestive of C-fibre nociceptive function (Barrett, Barman, Boitano, & Brooks, 2012a; Benarroch, 2011; Henry, 1982), in 10 cervical spine meniscoids extracted from five patients undergoing laminectomy.

This is consistent with findings published by Giles and Harvey (1987) and Giles and Taylor (1987) investigating meniscoid innervation in the lumbar spine. Giles and Harvey (1987) demonstrated the presence of SP immunoreactive nerves in three of four lumbar zygapophyseal joint meniscoids tested, and Giles and Taylor (1987) reported small, myelinated neurons coursing through the meniscoid tissue not associated with blood vessels; both findings are suggestive of nociceptive nerve fibre presence (Nathan, 1977). However, a study by Gronblad and colleagues (1991) used antibodies to SP, CGRP, PGP 9.5 and galanin to investigate lumbar zygapophyseal joint meniscoid innervation. The authors of this study suggested that nerve fibres in the meniscoids were

more likely to be responsible for local vasoregulation, rather than nociception, due to the absence of sensory neuropeptide immunoreactivity on examination.

In summary, limited information is available regarding the innervation of cervical spine meniscoids. Further investigation of the presence of nerves in the cervical spine meniscoids is required to determine whether these structures are a potential source of pain. Only one study has been undertaken on this topic, which had a small sample size (n = 5) and was conducted using tissue excised from a pathological sample (Inami *et al.*, 2001). Further work should include non-clinical populations, larger sample sizes and meniscoids from a comprehensive range of locations in the spine to facilitate an understanding of these structures.

Function of Cervical Spine Meniscoids

The cervical spine meniscoids have been suggested to be passive occupiers of space, serving to improve the congruity of the joint surfaces, assist in distribution of force through joints, and ensure the lubrication of articular cartilage as the joint surfaces separate during normal gliding movements (Chang *et al.*, 1992; Mercer & Bogduk, 1993; Webb *et al.*, 2011a; Yu *et al.*, 1987). Furthermore, a proprioceptive role for the cervical spine meniscoids may be plausible, given their location between weight bearing surfaces and the likelihood that they are innervated (Inami *et al.*, 2001; Webb *et al.*, 2011a).

Critical Evaluation of Methods Used in Previous Studies of Cervical Spine Meniscoids

Many of the previous studies exploring cervical spine meniscoid anatomy have been undertaken using cadaveric material (Chang *et al.*, 1992; De Marchi, 1963; Dörr, 1958; Giles, 1986; Hu *et al.*, 2006; Inami *et al.*, 2000; Kawabe *et al.*, 1989; Kos *et al.*, 2002; Mercer & Bogduk, 1993; Penning & Toendury, 1963; Schmincke & Santo, 1932; Tang *et al.*, 2007; Tondury, 1940; Webb *et al.*, 2012; Yu *et al.*, 1987; Zaccheo & Reale, 1956), as prior to the development and validation of sophisticated imaging modalities (e.g. MRI), dissection was the only means by which meniscoid anatomy could be studied. The two methodologies employed to explore cervical spine meniscoid anatomy in cadaveric material are gross dissection (Chang *et al.*, 1992; Inami *et al.*, 2000; Kawabe *et al.*, 1989; Kos *et al.*, 2002; Mercer & Bogduk, 1993; Tang *et al.*, 2007; Yu *et al.*, 1987) and tissue sectioning (De Marchi, 1963; Dörr, 1958; Giles, 1986; Penning & Toendury, 1963; Schmincke & Santo, 1932; Webb *et al.*, 2012; Yu *et al.*, 1987), and both of these methodologies have particular advantages and limitations.

Many of the early studies of cervical spine meniscoid morphology were undertaken using tissue sectioning (De Marchi, 1963; Dörr, 1958; Giles, 1986; Penning & Toendury, 1963; Schmincke & Santo, 1932; Tondury, 1940). This involves fixing and/or freezing the tissue, and then slicing the tissue into sections (usually several millimetres in thickness) using a microtome or a bandsaw. Sectioning has been undertaken in sagittal (De Marchi, 1963; Dörr, 1958; Giles, 1986; Penning & Toendury, 1963), coronal (Penning & Toendury, 1963; Schmincke & Santo, 1932) and transverse (Giles, 1986) planes. Webb *et al.* (2012) argue that an advantage of sectioning over

dissection as a means of investigating meniscoid morphology is that sectioning allows the detection of small meniscoids that may be overlooked or disregarded in gross dissection.

However, there are two significant limitations to sectioning as a method of examining cervical spine meniscoid morphology. First, meniscoids can only be detected and examined if they fall within the section taken. Therefore, studies that have used coronal or transverse sections may have missed or provided a sub-optimal depiction of meniscoids that are generally located at the ventral and dorsal poles of a joint. Second, cervical meniscoid structure is only observable in the plane of the section. This means that only a limited, single-dimensional view of a meniscoid is provided by sectioning, and except in the application of the sophisticated three-dimensional image reconstruction software used by Webb *et al.* (2012), a quantitative understanding of the multi-planar morphological structure of the meniscoid as a whole is difficult to attain through sectioning (Mercer & Bogduk, 1993). Third, due to the thickness of the blade cutting the sections, a small amount of tissue may be destroyed during the cutting process for each section (Cook & Al-Ali, 1997), potentially detracting from the accuracy of morphological assessment.

In contrast, the advantage of gross dissection as a means of studying cervical spine meniscoid morphology is that it provides a three-dimensional appreciation of their morphology, which is key to developing our understanding of their normal structure and function within the context of a cervical synovial joint (Inami *et al.*, 2000; Mercer & Bogduk, 1993). Gross dissection of the zygapophyseal joint meniscoids of cadaveric specimens is often undertaken using a combination of sharp and blunt dissecting

techniques. Skin, muscles and overlying soft tissue are removed from the spine, and the lateral atlantoaxial or zygapophyseal joints are disarticulated to allow access to the meniscoids located within. For lateral atlantoaxial joints, this may be done through simply excising the joint capsule (Chang *et al.*, 1992; Tang *et al.*, 2007), such that the atlas can be separated from the axis. For the zygapophyseal joints, after excising the joint capsule, disarticulation of the adjacent vertebrae is often restricted by bony congruency and extensive connective tissue attachments between the bones. Overcoming this restriction by forceful separation of the joint surfaces may inadvertently damage or displace the delicate intra-articular meniscoids, which may in turn jeopardise the accuracy of study findings. This is a potential limitation of gross dissection as a methodology for the study of cervical spine meniscoid morphology. Authors have addressed this issue in previous studies by using a dental burr to drill caudally through the inferior articular process of the superior vertebra down to the level of the articular cartilage, such that it can be excised from the joint capsule and gently removed, preserving the meniscoids and inferior articular surface and allowing their study (Inami *et al.*, 2000; Mercer & Bogduk, 1993). Other authors have not explicitly addressed the issue of meniscoid preservation, which may constitute a source of methodological error in their study findings (Kos *et al.*, 2002; Yu *et al.*, 1987).

A novel way to address the difficulty disarticulating a zygapophyseal joint posed by bony congruence may be the use of formic acid demineralisation to soften bony tissue. Once softened, bone could be cut away with a scalpel or drilled away with a dental burr, allowing the joint capsule to be excised and joint surfaces separated with minimal force. There is a paucity of information on the application of formic acid demineralisation as a means of facilitating gross dissection for morphological study of anatomical structures.

Demineralisation is commonly used in the preparation of tissue for histological sectioning. This process has been shown to potentially lead to compromise of tissue ultrastructure and interfere with immunohistochemical investigation, as well as inhibit gene expression profiling. This is reported to occur when too high a concentration of demineralising solution is used, if the demineralising chemical reaction occurs at too high a temperature, or if demineralisation is undertaken for too long a time period (Belanger, Copp, & Morton, 1965; Callis & Sterchi, 1998; Charman & Reid, 1972; Hunter & Nikiforuk, 1954; Kiviranta, Tammi, Lappalainen, Kuusela, & Helminen, 1980; Leong, 2009; Shibata, Fujita, Takahashi, Yamaguchi, & Koji, 2000; Waissbluth, Chan, Chen, McIntosh, & Daniel, 2013; Wilson, 1994).

Considering these well documented microscopic adverse effects, it is plausible also that formic acid demineralisation may alter meniscoid structure on a macroscopic level, possibly shrinking or destroying the tissue, which would form a significant source of error in a morphological study. The effect of formic acid demineralisation upon the morphometry and appearance of the cervical meniscoids needs to be determined before formic acid demineralisation could be used as a tool to facilitate zygapophyseal joint meniscoid dissection.

Theories of Cervical Spine Meniscoid Involvement in Neck Pain

A number of theories exist regarding potential roles of the cervical spine meniscoids in cervical pathology. The ‘Entrapment’ and ‘Extrapment’ theories suggest means by which cervical meniscoids may be involved in the aetiology of acute locked neck (torticollis). Proliferation of fibrous tissue arising from cervical spine meniscoids has

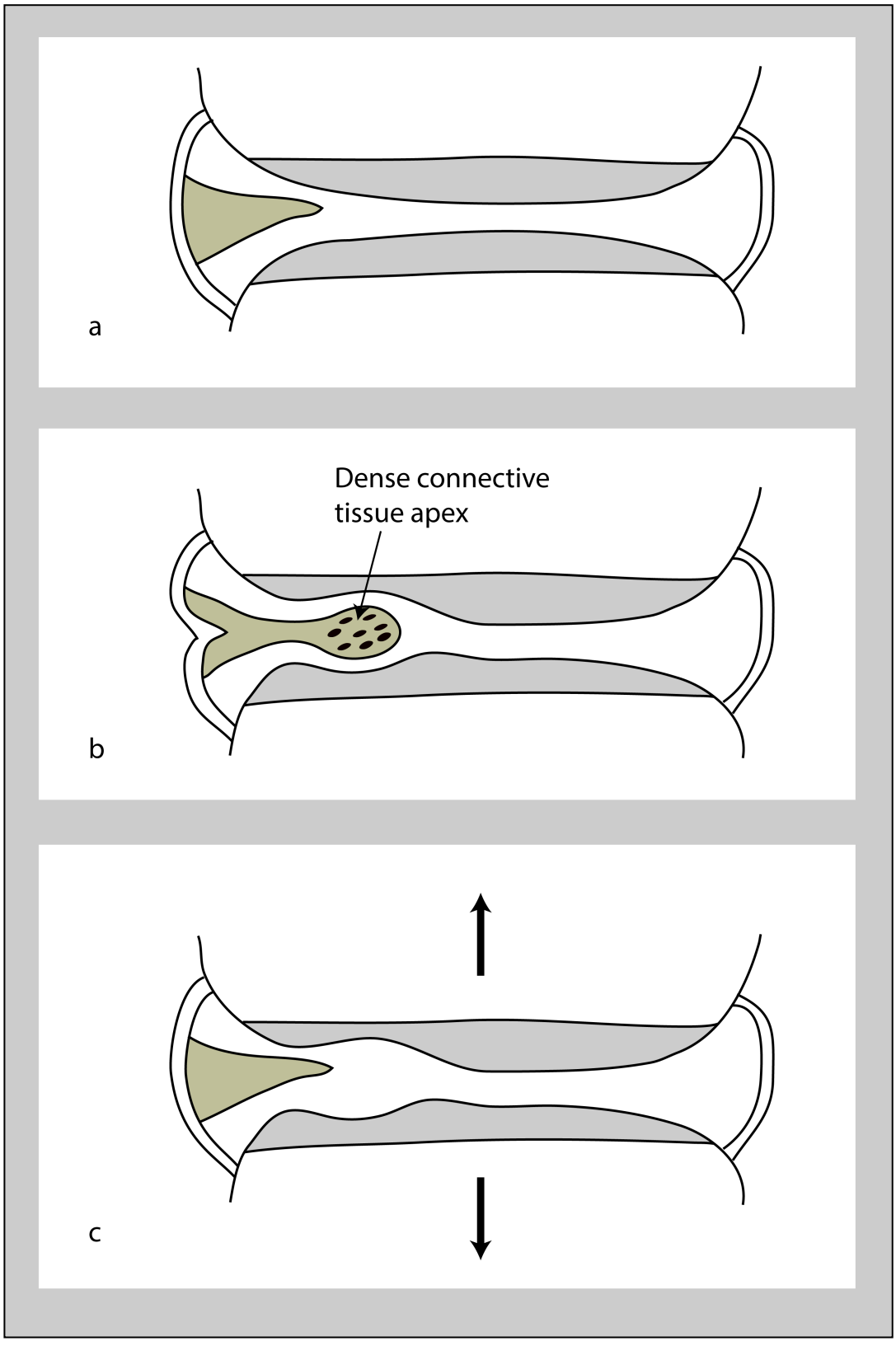
also been suggested as a pathological process leading to joint hypomobility (Jones *et al.*, 1989), and there is evidence implicating cervical spine meniscoids as structures potentially involved in zygapophyseal joint pain in WAD.

Entrapment Theory

Zukschwerdt *et al.* (1955) and Kos and Wolf (1972) proposed that cervical spine meniscoids may play an integral role in acute locked neck (torticollis). They hypothesised that during an abnormal movement, a meniscoid may move out of its normal position and further into the joint cavity, becoming pinched between the joint surfaces (**Figure 2**), thus leading to articular cartilage indentation about the dense connective tissue apex and lodging the meniscoid in an entrapped position (Bogduk & Jull, 1985; Evans, 2002; Webb *et al.*, 2011a). This results in pain, joint hypomobility, and secondary muscle spasm, possibly though mechanical compression of the meniscoid and distension of the joint capsule.

This hypothesis has been suggested to account for the immediate pain relief following manipulative therapy that is reported anecdotally. Bogduk and Jull (1985) and Kos *et al.* (2002) suggest that rotational or distraction based manual therapy such as mobilisation or manipulation gaps the joint surfaces, releases the meniscoid and therefore restores normal joint motion. The weakness of this hypothesis is that it is purely speculative and based on inferences of descriptive studies of meniscoid morphology. The effect of manual therapy upon meniscoid position in a spinal joint has not been examined, and the theory has not been tested in a clinical population (Webb *et al.*, 2011a).

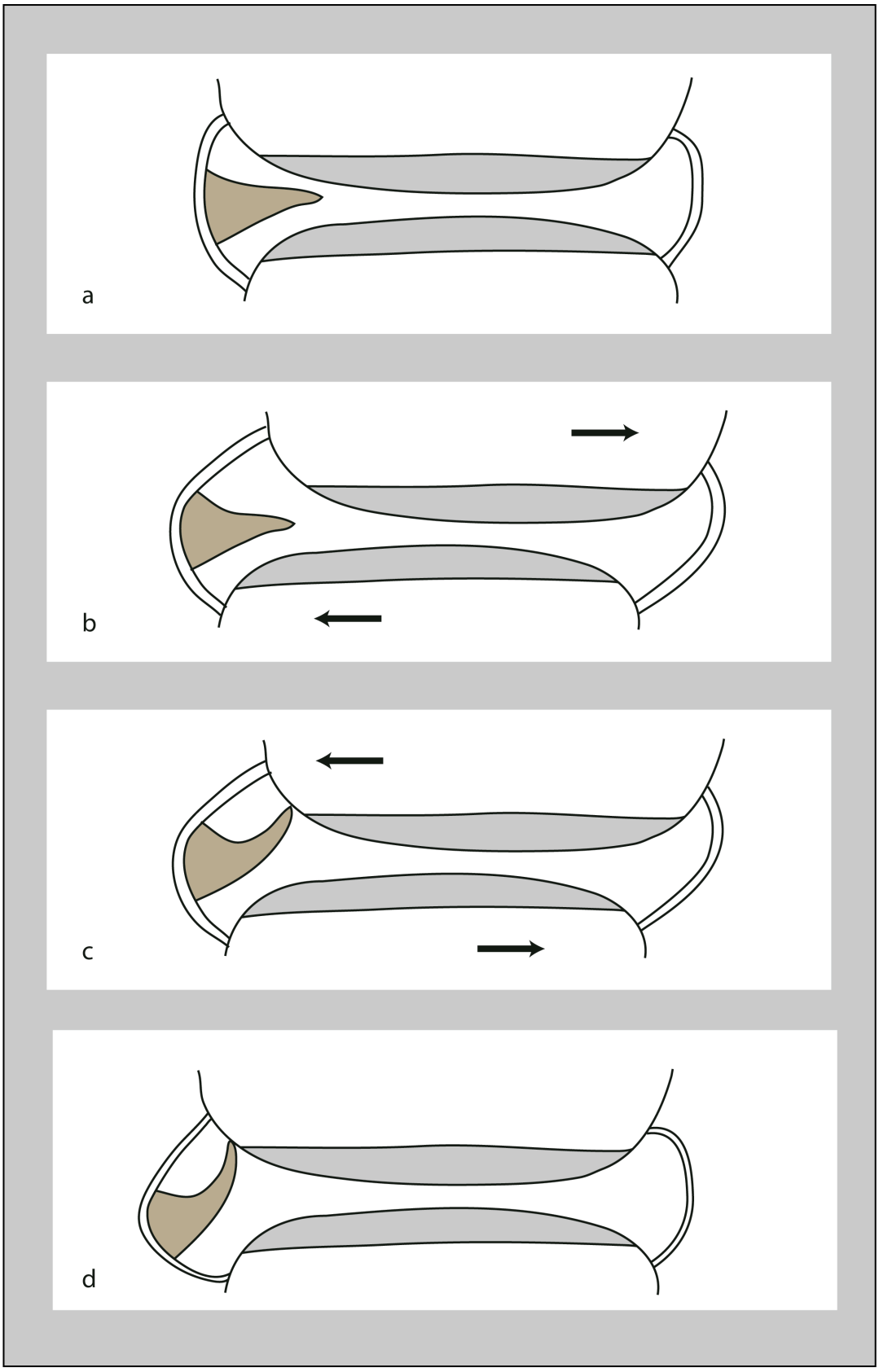
Figure 2: Following page – Schematic representation of cervical spine meniscoid Entrapment Theory. (a) Meniscoid *in situ* between articular surfaces (b) during an abnormal movement, the meniscoid moves further into the joint cavity, and becomes pinched between the joint surfaces. Articular cartilage indentation occurs about the dense connective tissue apex, lodging the meniscoid into an entrapped position (c) manual therapy treatment such as manipulation or mobilisation may gap the joint surfaces, releasing the trapped meniscoid. Modified from Webb, Collins, Rassoulain and Mitchell (2011).


a
Dense connective
tissue apex
b
c

Extrapment Theory

Bogduk and Jull (1985) proposed an alternate theory to the Entrapment Theory regarding the role of meniscoids in acute torticollis – the Extrapment Theory. They hypothesised that during excessive or poorly coordinated movement, a meniscoid may slide out of its position between the joint surfaces, causing meniscoid deformation, capsular distension and secondary muscle spasm (**Figure 3**) (Bogduk & Jull, 1985; Webb *et al.*, 2011a). This theory however is also based on inferences from cadaveric studies, and has been criticised as it does not account for the joint hypomobility associated with acute locked neck or back (Jull, 1986; Webb *et al.*, 2011a), although it is arguable that pain and muscle spasm could lead to decreased cervical spine range of motion, without an explicit intra-articular mechanical block to movement.

Figure 3: Following page – Schematic representation of cervical spine meniscoid Extrapment Theory. (a) Meniscoid *in situ* between articular surfaces (b) during movement such as cervical rotation, the articular surfaces move apart and reduce the area of contact (c) on return to the neutral position, the meniscoid fails to re-enter the joint cavity (d) the meniscoid is deformed and the joint capsule distends, leading to pain. Modified from Webb, Collins, Rassoulain and Mitchell (2011).


a
b
c
d

Fibrous Tissue Proliferation

Jones *et al.* (1989) propose that proliferation of the fibrous tissue comprising a meniscoid may lead to intra-articular fibrous adhesions, and in turn, long-term joint hypomobility. Mercer and Bogduk (1993) expand on this theory by suggesting that fibrous tissue proliferation may arise from fibroadipose meniscoids in immobile or under-used zygapophyseal joints in a similar fashion to previously observed fibrous tissue proliferation and adhesions in immobilised knee joints (Akeson, Amiel, & Woo, 1980; Enneking & Horowitz, 1972). In a review paper, Webb *et al.* (2011a) describe the increasing merit of this theory with respect to cervical zygapophyseal joint hypomobility, by noting evidence of a fibrous tissue proliferation process occurring in the zygapophyseal joints of the lumbar spine following surgical fixation (Baker Wde, Thomas, & Kirkaldy-Willis, 1969; Cramer, Fournier, Henderson, & Wolcott, 2004; Ginsburg, Daley, Cramer, & Henderson, 2000).

Whiplash Associated Disorder

Whiplash associated disorder is defined by the Quebec Task Force on Whiplash Associated Disorders as "*an acceleration-deceleration mechanism of energy transferred to the neck that results in soft tissue injury that may lead to a variety of clinical manifestations including neck pain and its associated symptoms*" (Spitzer *et al.*, 1995). WAD occurs as a result of trauma, typically during an MVA rear-end collision. During such an event, there is no direct impact to the head or neck; the injury to the cervical spine occurs due to the inertial response of the head on the neck, when the energy from

the collision is transferred from the trunk to the cervical spine (Bogduk & McGuirk, 2006).

Of all of the theories of cervical spine meniscoid involvement in neck pain, it is their potential role in WAD that has the strongest evidence base. The cervical zygapophyseal joints have been implicated as organic sources of pain in WAD by a comprehensive evidence base, comprising autopsy, biomechanical and clinical studies (Bogduk, 2011b). As intra-articular structures, the meniscoids are plausible contributors to zygapophyseal joint pain after MVA, as the following sections will outline.

Biomechanics of WAD

In 1999, a cineradiography study of healthy volunteers using a rear-end collision simulation sled device demonstrated potential mechanisms of zygapophyseal joint injury in WAD (Kaneoka *et al.*, 1999). The study showed that during a rear impact, the trunk rises upwards towards the head, compressing the cervical spine into an S-shape (**Figure 4**) in the sagittal plane. During this S-shaped compression, the lower cervical vertebrae move into extension, about an instantaneous axis of rotation that is altered such that the inferior articular facet of the superior vertebra drives downward into the superior articular facet of the inferior vertebra, potentially resulting in damage to the zygapophyseal joints (**Figure 5**) (Bogduk, 2011b; Kaneoka *et al.*, 1999; Siegmund, Winkelstein, Ivancic, Svensson, & Vasadava, 2009). As cervical spine meniscoids are positioned between the zygapophyseal joint surfaces, it is plausible that they may be contused or torn as the articular facets are driven into each other.

Figure 4: Following page – Schematic depiction of S-Shaped deformation of cervical spine following rear impact collision, illustrating altered segmental motion of lower cervical vertebrae, leading to impaction of zygapophyseal joints and distraction of anterior structures. C2 to C7 – second to seventh cervical vertebrae; ZJ – zygapophyseal joint. Modified from Kanoeka *et al.* (1999) and Bogduk (2011).

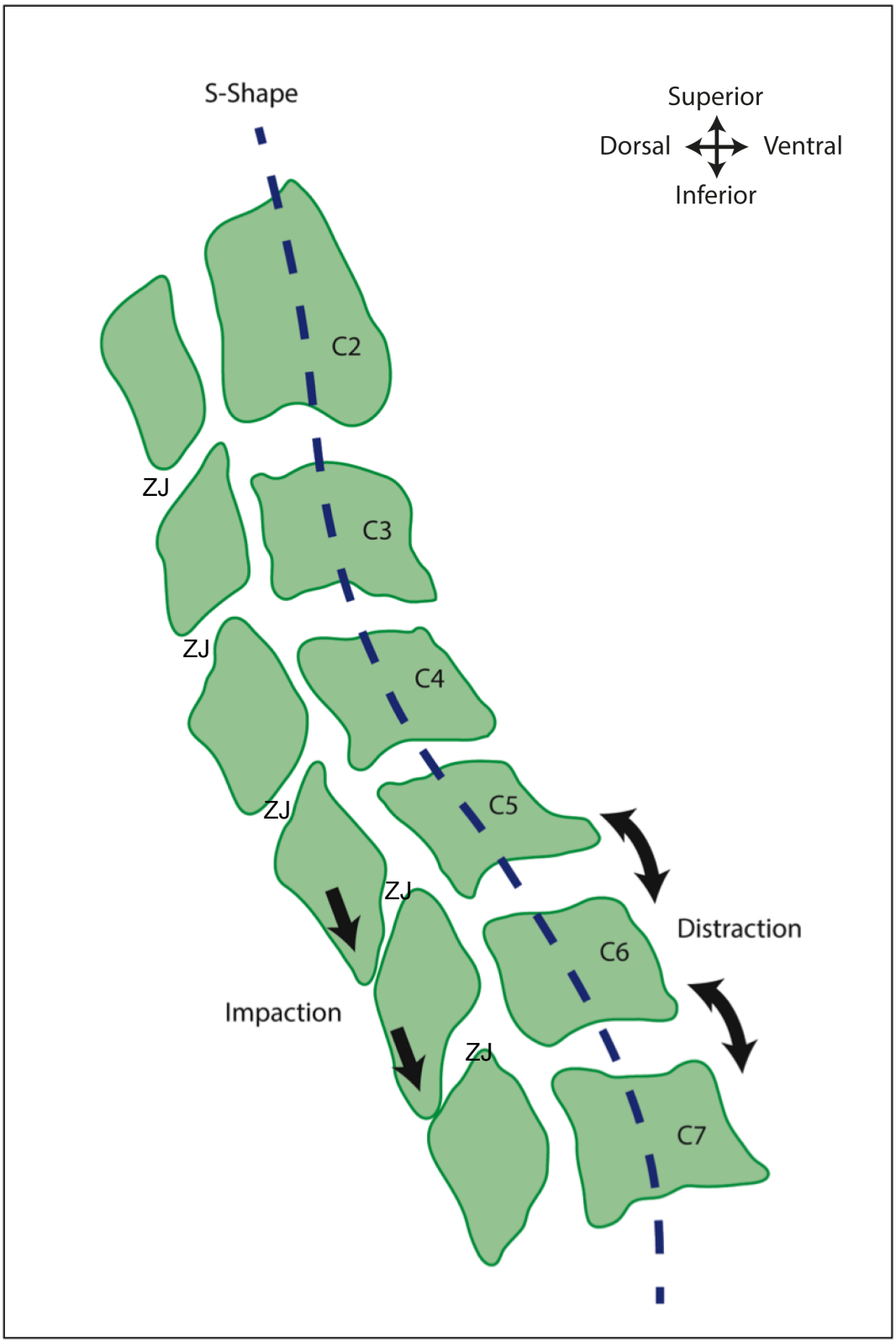
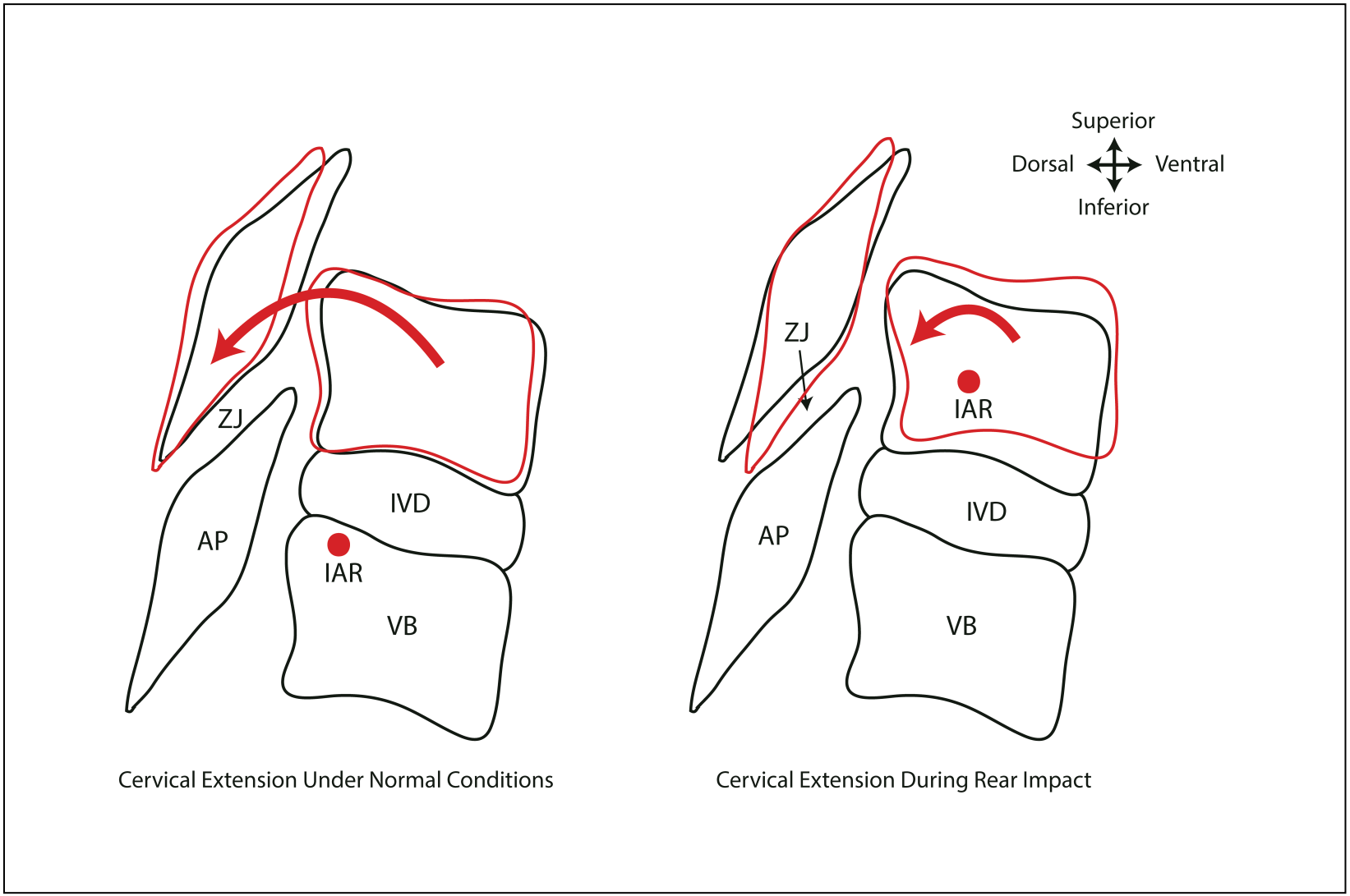

S-Shape
Superior
Dorsal
Ventral
Inferior
C2
ZJ
C3
ZJ
C4
ZJ
C5
ZJ
Distraction
C6
Impaction
ZJ
C7

Figure 5: Following page – Motion of lower cervical spine vertebrae about their instantaneous axis of rotation (IAR) under normal conditions and during a whiplash motion in a rear impact collision. Note that under normal conditions, as the superior vertebra undergoes sagittal rotation in a posterior direction, the zygapophyseal articular surfaces glide upon each other in a smooth motion. During the whiplash scenario, the IAR moves superiorly such that the inferior articular facet of the superior vertebra drives downwards into the superior articular facet of the inferior vertebra. AP – articular pillar; IVD – intervertebral disc; VB – vertebral body; ZJ – zygapophyseal joint. Modified from Kanoeka *et al*. (1999) and Bogduk (2011).



Cervical Extension Under Normal Conditions

Cervical Extension During Rear Impact

Autopsy Research

Damage to cervical spine meniscoids through trauma has been reported in autopsy studies of MVA and blunt head trauma victims (Jonsson, Bring, & Rauschning, 1991; Schonstrom *et al.*, 1993; Taylor & Taylor, 1996). Each of the studies reported bruising or haematoma of cervical spine meniscoids in patients following death due to trauma, which suggests these structures may well be a potential source of neck pain in a whiplash or post-trauma population. However, these studies can only be generalised to a more extreme trauma context, as the injury was of sufficient magnitude to result in death. Other signs of tissue damage within a zygapophyseal joint in a MVA post-mortem population include tears of the zygapophyseal joint capsule, intra-articular haemorrhage, tears of the annulus of the intervertebral discs, articular sub-chondral fractures, and articular pillar fractures (Bogduk, 2011b; Jonsson *et al.*, 1991; Taylor & Taylor, 1996; Taylor & Twomey, 1993).

Chronic Zygapophyseal Joint Pain in Clinical Research

Clinical research has demonstrated the zygapophyseal joints to be a source of ongoing pain in a WAD persistent pain population (Bogduk, 2011b), through the use of selective medial branch anaesthetic blocks. This diagnostic procedure involves anaesthetising the nerves innervating a suspected painful zygapophyseal joint with a small amount of local anaesthetic. A positive result is the complete relief of a patient's neck pain. False-positive responses can be controlled for by the use of comparative anaesthetic blocks or placebo-controlled anaesthetic blocks.

A review by Bogduk in 2011 examined the use of medial branch anaesthetic blocks as a means of exploring the prevalence of zygapophyseal pain in a persistent pain population. Bogduk commented that studies of patients with persistent neck pain due to WAD, and studies of persistent neck pain patients that may not have a history of WAD, suggests the prevalence of pain arising from a zygapophyseal joint is approximately 50% of persistent neck pain sufferers (Barnsley, Lord, Wallis, & Bogduk, 1995; Lord, Barnsley, Wallis, & Bogduk, 1996a; Yin & Bogduk, 2008). Given that medial branch anaesthetic blocks have been validated as an effective means of diagnosing zygapophyseal joint pain (Barnsley, Lord, & Bogduk, 1993; Bogduk, 2011b; Lord, Barnsley, & Bogduk, 1995), there is strong clinical evidence implicating the zygapophyseal joints as sources of persistent neck pain in WAD. It is plausible that the damage to the cervical spine meniscoids demonstrated by the previously discussed biomechanical and autopsy studies may contribute to the ongoing zygapophyseal pain in WAD established by the above clinical research.

Literature Review Summary

Evidence to date suggests that cervical spine meniscoids are composed largely of fibrous tissue, adipose tissue or a combination of the two, lined with an outer layer of synovium, and contain a network of blood vessels. They appear to be commonly found throughout the cervical spine, and are crescent-shaped, elliptical or semicircular. Ventral meniscoids appear to be more frequently composed of adipose tissue, and dorsal meniscoids more frequently composed of fibrous tissue, however the reason for this pattern is uncertain. MRI is a valid method of imaging meniscoids. During a whiplash

injury in a MVA, cervical spine meniscoids are likely to be damaged, and potentially contribute to zygapophyseal joint pain as a demonstrated source of neck pain in WAD.

There is not clear evidence regarding the influence of spinal level, age, sex or articular degeneration upon the composition and form of the cervical meniscoids. The presence of nerve tissue within cervical meniscoids has not been extensively investigated, and structural characteristics of the meniscoids of a healthy sample have not yet been compared to those of a pathological sample.

Research Aims:

a. To determine if demineralisation can be used to facilitate study of meniscoid morphology by gross dissection.
b. To explore and clarify the morphology of the meniscoids of the lateral atlantoaxial joints, including influencing factors such as spinal level, age, sex and articular cartilage degeneration.
c. To explore and clarify the morphology of zygapophyseal joint meniscoids, including influencing factors such as spinal level, age, sex and articular cartilage degeneration.
d. To determine *in vivo*, using MRI, morphological differences between cervical meniscoids of people suffering WAD and those who are healthy.
e. To explore the presence of nerve tissue in cervical meniscoids in a non-clinical population.

Chapter 3: STUDY 1 – FORMIC ACID DEMINERALISATION IN MENISCOID DISSECTION

Gross dissection of cadaveric tissue is a useful means of undertaking morphological examination of anatomical structures as it grants investigators an appreciation of a structure of interest in three dimensions (Mercer & Bogduk, 1993). Examination of cervical spine meniscoids using dissection requires disarticulation of the enclosing zygapophyseal joints. This process is potentially problematic due to the congruence of the complex bony architecture of the articular pillars; this can necessitate considerable force being used to disarticulate the zygapophyseal joints and provide access to the meniscoids. It is conceivable that such force could inadvertently damage the delicate meniscoids, and in turn jeopardise the accuracy of morphological measures undertaken. As noted in Chapter 2 (Literature Review), this issue is a potential source of methodological error applicable to previous studies of cervical spine meniscoid morphology.

A novel way to address this challenge is through the use of formic acid demineralisation, a technique commonly utilised in histopathology to allow sectioning of bony tissue for mounting on a microscope slide, to soften the bone of the vertebral column. This allows the softened bone to be cut away with a scalpel, allowing the joint surfaces to be separated with minimal force. However, the impact of this process upon the morphometry and morphology of the cervical spine meniscoids in cadaveric tissue has not been empirically investigated.

The first study presented in this thesis (Study 1) sought to address this question, and has been published as original research:

Farrell, S. F., Osmotherly, P. G., Rivett, D. A., & Cornwall, J. (2015). Formic acid demineralization does not affect the morphometry of cervical zygapophyseal joint meniscoids. *Anatomical Science International*, *90*, 57-63, DOI 10.1007/s12565-014-0248-8. (See Appendix H)

Human research ethics approval and research governance documentation can be found in Appendices C, D, I and J. Characteristics of cadavers included in the study can be seen in Appendices K and L, and bequeathal documentation can be seen in Appendix B.

Introduction

Background

Anatomical investigation of soft tissue structures that have intimate physical relationships with bony elements is potentially difficult, as access to these often delicate structures may be limited by congruent joint surfaces or adjacent anatomical structures. Such soft tissue structures are also at risk of damage if they are removed by force. Examples of such soft tissue structures include the intra-articular meniscoids of the zygapophyseal joints, or the intra-articular meniscus of the temporomandibular joint. Demineralisation can facilitate dissection of soft tissue structures in inaccessible locations by softening surrounding bone so that it can be easily removed without risking

damage to the soft tissue structure of interest. This is valuable when undertaking anatomical research of these structures using gross dissection, as inadvertent tissue damage can jeopardise the validity and reliability of the experimental findings.

Demineralisation

Demineralisation is commonly employed in histopathology and is utilised across a wide scope of scientific enquiry, from dentistry to astrobiology to paleontology (Ehrlich, Koutsoukos, Demadis, & Pokrovsky, 2008; Leong, 2009; Wilson, 1994). In histopathology, demineralisation refers to the removal of biominerals from tissue, such as hydroxyapatite crystals from bone, and is necessary to prepare calcified tissue for histoprocessing so that sections may be easily cut from a tissue block containing bone (Ehrlich *et al.*, 2008; Leong, 2009). Demineralisation may be achieved through the use of mineral acid decalcifying agents, such as formic acid, or the use of chelating agents, such as ethyl-diamine-tetra-acetic acid (Belanger *et al.*, 1965). Mineral acids act more quickly than chelating agents, however chelating agents offer greater preservation of histological structure and may successfully preserve elements within the tissue for investigations such as immunohistochemistry (Fejerskov, 1971; Leong, 2009; Shibata *et al.*, 2000). A number of authors have reported potential compromise to tissue ultrastructure, immunohistochemical investigation and gene expression profiling when using mineral acid demineralising agents (Belanger *et al.*, 1965; Charman & Reid, 1972; Hunter & Nikiforuk, 1954; Kiviranta *et al.*, 1980; Leong, 2009; Shibata *et al.*, 2000; Waissbluth *et al.*, 2013). Despite this, demineralisation using formic acid remains a viable option for researchers who do not want to undertake procedures such as immunohistochemistry as part of their investigation.

Formic Acid Demineralisation in Morphological Investigation

Formic acid demineralisation is commonly used to prepare tissue for histological sectioning to study soft tissue structures such as ligaments or menisci (Gao, Oqvist, & Messner, 1994; Leong, 2009). Resnick (1976) used formic acid demineralisation to allow sectioning of the carpal bones of six cadavers with rheumatoid arthritis to study pathological changes, while Spouge (1984) used formic acid demineralisation of pig mandibles to study epithelial residues of the periodontal ligament, allowing individual blocks of tooth, mandible and periodontal tissue to be cut before sectioning for morphological study.

The use of acids for demineralisation can potentially damage the ultrastructure of soft tissue (Leong, 2009). This may occur if too high a concentration of demineralising solution is used, if the demineralising chemical reaction occurs at too high a temperature, or demineralisation is undertaken for too long a time period (Callis & Sterchi, 1998; Leong, 2009; Wilson, 1994). These factors must all be considered when planning research utilising acid demineralisation for anatomical or histological investigation, as tissue degradation has the potential to detract from the accuracy of study findings.

While there is considerable research detailing the use of demineralisation in histopathology, there are no publications describing or evaluating how soft tissues are affected by extended-immersion demineralisation such as that used to assist studies of gross morphology. In particular, there are no guidelines to indicate how long demineralisation may proceed before the morphometry of soft tissue is compromised. In

light of the potential usefulness of formic acid demineralisation to facilitate dissection of structures in bony regions, and taking into account the risk of damage to tissue ultrastructure by acid immersion, the purpose of this study was to examine the influence of one common method of demineralisation on the morphology of an intra-articular soft tissue of interest. Demineralisation to soften surrounding bone to assist the removal of these structures may be of use to researchers interested in studying cervical or similar meniscoid morphology by dissection. The meniscoids of the zygapophyseal and lateral atlantoaxial joints were selected as a tissue model for this experiment as their dissection can be difficult because of the congruence of surrounding bones. We aimed to examine whether cervical meniscoids were degraded or their dimensions altered by immersion in formic acid over time.

Materials and Methods

Embalmed cadaveric material was sourced from the Department of Anatomy at the University of Otago (Dunedin, New Zealand). The cadavers were embalmed in accordance with departmental protocol using ethanol and water based preparations. Human tissue was bequeathed under the New Zealand Human Tissues Act (2008). Institutional ethical approval was granted for the study, and the study was performed in accordance with the ethical standards set by the Declaration of Helsinki.

Tissue Preparation

Cervical spine blocks were harvested from four cadavers (mean 74.25 years [standard deviation 9.00], two male), extending from the occiput to the C7 vertebrae. The skin, muscles and soft tissue overlying the vertebral column were removed using sharp and blunt dissection. A bone saw was used to cut along the longitudinal axis of each spine through the laminae and pedicles, such that four columns of zygapophyseal joints were produced (randomly allocated by random number generator, three left sided, one right sided). Three randomly selected individual zygapophyseal joints from each spine were removed by sawing through the articular pillar in the plane of the zygapophyseal joint lines, producing 12 isolated joints to comprise the sample. The cervical levels included were C1-2 (one left, one right), C2-3 (two left), C3-4 (two left, one right), C4-5 (three left, one right) and C5-6 (one left), and were determined by random sampling.

Each zygapophyseal joint was then disarticulated using a scalpel, forceps, and surgical microscope (Op-Mi 6, Carl Zeiss, Jena, West Germany). Using a technique described by Mercer and Bogduk (1993), a dental burr (Beaver Ace Dental Micro Engine, Osada Electric Co. Ltd., Tokyo, Japan) was used to remove bone from the inferior articular facet of the superior vertebra, down to the level of articular cartilage. Each joint capsule was excised near its attachment onto the inferior articular facet of the superior vertebra, such that it could be gently disarticulated, leaving the joint capsule and meniscoids intact in situ on the articular surface of the superior articular facet of the inferior vertebra. The final product of the dissection of each zygapophyseal joint comprised the inferior joint surface, its underlying bone, any included meniscoids and the joint capsule (excised along its superior margin). Due to variation in joint surface area according to

cervical level, the volume of tissue to be studied ranged from approximately 700 mm^3 to 2300 mm^3.

Eleven of the 12 joints dissected contained ventral and dorsal meniscoids, and one joint contained no meniscoids. Each of the 22 identified meniscoids were inspected using a surgical microscope. Sketches and notes were made regarding meniscoid appearance and location upon each joint surface, and each joint was photographed using a digital camera (Canon Powershot G10 14.7 megapixel camera, Canon Inc, Tokyo, Japan) mounted on a dissecting microscope (Olympus SZX7, Olympus Inc, Tokyo, Japan). A scale bar was included in each photograph, and small notches were made in the underlying bony tissue to indicate ventral-dorsal and medial-lateral orientation, to facilitate recalibration of repeat photographs later in the study.

Demineralisation Protocol

Joints were immersed in formic acid (5%) solution as described by Skinner, Hickmon, Lumpkin, Aronson, and Nicholas (1997) in individual containers for 32 days on a bench top at room temperature. The formic acid solution (5%) was created by diluting concentrated formic acid with distilled water. Formic acid solution was replaced every day for each specimen jar. At days 10, 15, 20, 21, 23, 25, 28, 30 and 32 chemical testing for decalcification was performed using 1 mL 5% ammonium hydroxide, 1 mL 5% ammonium oxalate, and 1 mL 5% formic acid demineralising solution.

Measurement of Meniscoid Characteristics and Size

On alternate days each joint was photographed using the camera mounted on the dissecting microscope and the assessments of meniscoid appearance and location in joint repeated. The single assessor was blinded to donor, joint level and previous results.

The digital photographs of the joint surfaces and overlying meniscoids taken on days 0, 4, 18 and 32 were measured using Adobe Photoshop (Adobe Systems Inc., San Jose, CA). The days on which measures were performed were based upon a study run in parallel with this trial, whereby tissue blocks were paraffin mounted and able to be sectioned after four days. Days 18 and 32 are two and four weeks after this time, respectively, to enable investigation as to whether formic acid immersion may have affected the morphometry of the meniscoids in the parallel trial. Measures included meniscoid surface area in mm^2, meniscoid surface area as a proportion of zygapophyseal joint surface area, and length of meniscoid protrusion into joint using the method described by Inami *et al.* (2000) (**Figure 6**). This was achieved using Adobe Photoshop, which calculated the distances and areas in pixels which were then converted into mm or mm^2 using a scale bar included in each photo. Using this technique, measurement of length could be achieved precise to ±0.01 mm, and measurement of surface area precise to ±0.04 mm^2. Measurement of digital photographs in this manner has been validated by Akhavan, Merguerian, Grady, DiSandro, and Shnorhavorian (2014), who reported strong correlations between intra-operative caliper measures and measurement of digital photographs in a surgical context. Each of the three measures (meniscoid surface area in mm^2, meniscoid surface area as a proportion of articular cartilage surface area, and meniscoid length in mm) for days 0, 4, 18 and 32

for all specimens were repeated three times over a three-week period, to test intra-rater reliability using intraclass correlation co-efficients (ICCs), with one week separating repeated assessments.

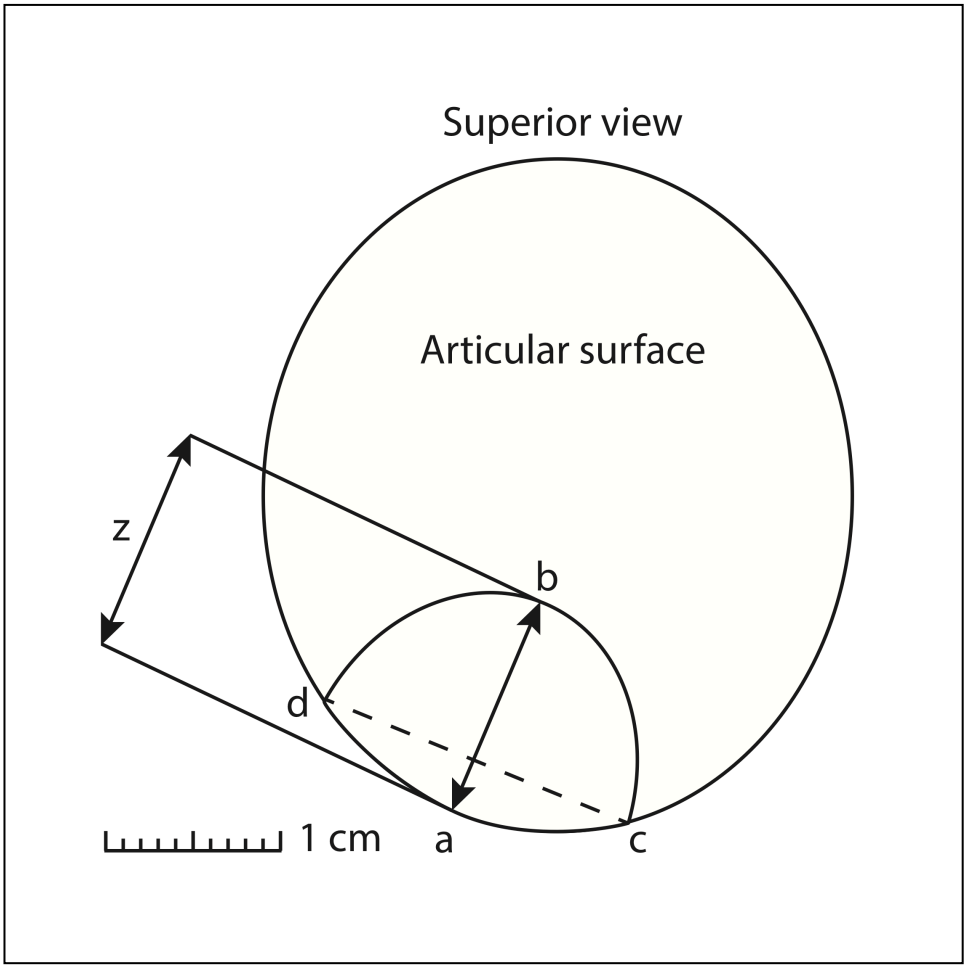


Figure 6: Measurement of meniscoids. Distance (z) was the measurement of the widest point perpendicular to the baseline (cd) that connected the bilateral ends of the meniscoid. (a) Base of meniscoid connecting to capsule, (b) free border of meniscoid. Adapted from Inami, Kaneoka, Hayashi, and Ochiai (2000).

Data Analysis

Measurements were recorded onto a Microsoft Excel (Microsoft, Redmond, WA) spreadsheet. Data were interpreted using descriptive statistics, then analysed with repeated measures ANOVA and ICCs using IBM SPSS (IBM, Armonk, NY) data analysis software, using $p < 0.05$ as the level of significance.

Results

Measures of Size

Repeated measures ANOVA indicated no significant differences between measures of ventral or dorsal meniscoid surface areas, either in mm^2 or as a proportion of the total zygapophyseal joint surface area, across the duration of the experiment (**Figure 6**). Similarly, no significant differences were found between ventral or dorsal meniscoid length using the measurement method described by Inami *et al.* (2000) across the duration of the experiment (**Table 1**).

Table 1: Means, standard deviations, and results of repeated measures ANOVA for each measure of morphometry throughout the experiment. N = 11 samples for each day that measures were taken.

Variable	Day 0 Mean (SD)	Day 4 Mean (SD)	Day 18 Mean (SD)	Day 32 Mean (SD)	Repeated Measures ANOVA	Partial η^2
VSA Proportional (%)	19.82 (8.18)	19.70 (8.75)	20.06 (8.57)	19.89 (8.42)	p = 0.76	0.03
VSA Real (mm^2)	31.58 (14.14)	32.21 (15.10)	32.46 (14.75)	31.76 (14.85)	p = 0.23	0.14
DSA Proportional (%)	21.17 (8.25)	20.89 (7.76)	21.36 (8.26)	21.42 (8.97)	p = 0.61	0.05
DSA Real (mm^2)	34.17 (16.02)	34.77 (16.93)	34.88 (16.64)	33.93 (16.17)	p = 0.24	0.13
Ventral Meniscoid Length (mm)	3.54 (1.45)	3.37 (1.08)	3.55 (1.20)	3.56 (1.27)	p = 0.43	0.08
Dorsal Meniscoid Length (mm)	3.18 (1.24)	3.21 (1.11)	3.37 (1.26)	3.31 (1.26)	p = 0.35	0.10

Legend: VSA Proportional – ventral meniscoid surface area (as % joint surface area); VSA Real – ventral meniscoid surface area in mm^2; DSA Proportional – dorsal meniscoid surface area (as % joint surface area); DSA Real – dorsal meniscoid surface area in mm^2.

Intra-rater Reliability

Intra-rater reliability testing of the above measures of meniscoid size demonstrated a high degree of reliability for all measures, with ICCs greater than 0.9 for all morphological measures undertaken (**Table 2**).

Table 2: Results of intra-rater reliability testing for each measure of meniscoid size, for experiment days 0, 4, 18 and 32. For all meniscoids, each measure was repeated three times, with one week separating each measurement session.

Measurement / Day	ICC	95% CI
VSA Proportional		
0	0.996	0.988-0.999
4	0.990	0.972-0.997
18	0.989	0.971-0.997
32	0.953	0.877-0.986
VSA Real		
0	0.996	0.986-0.999
4	0.994	0.984-0.998
18	0.995	0.985-0.998
32	0.991	0.977-0.997
DSA Proportional		
0	0.991	0.977-0.997
4	0.982	0.951-0.995
18	0.984	0.956-0.995
32	0.990	0.974-0.997
DSA Real		
0	0.996	0.988-0.999
4	0.990	0.973-0.997
18	0.992	0.977-0.997
32	0.995	0.987-0.999
Ventral Meniscoid Length		
0	0.979	0.944-0.994
4	0.961	0.898-0.988
18	0.952	0.867-0.986
32	0.931	0.736-0.982
Dorsal Meniscoid Length		
0	0.969	0.915-0.991
4	0.974	0.931-0.992
18	0.943	0.842-0.983
32	0.933	0.827-0.980

Legend: VSA Proportional – ventral meniscoid surface area (as % joint surface area); VSA Real – ventral meniscoid surface area in mm^2; DSA Proportional – dorsal meniscoid surface area (as % joint surface area); DSA Real – dorsal meniscoid surface area in mm^2; ICC – interclass correlation co-efficient; CI – confidence interval.

Descriptive Changes

Review of notes and photographs taken through the dissecting microscope indicated that the shape of all meniscoids identified remained consistent across the experimental period. The borders of five of the meniscoids became noticeably more ragged (at days 14, 16, 16, 18 and 30 respectively) across the experiment duration, however the other 17 meniscoids remained consistent in their appearance. The meniscoids that became more ragged were: the ventral and dorsal meniscoids from a left atlantoaxial joint in a 67 year old male; a dorsal meniscoid from a left C2-3 zygapophyseal joint in a 87 year old female; a ventral meniscoid from a right C3-4 zygapophyseal joint in a 74 year old male; and a dorsal meniscoid from a left C4-5 zygapophyseal joint in a 67 year old male. **Figure 7** illustrates the appearance of a single joint surface and associated meniscoids across the period of the experiment.

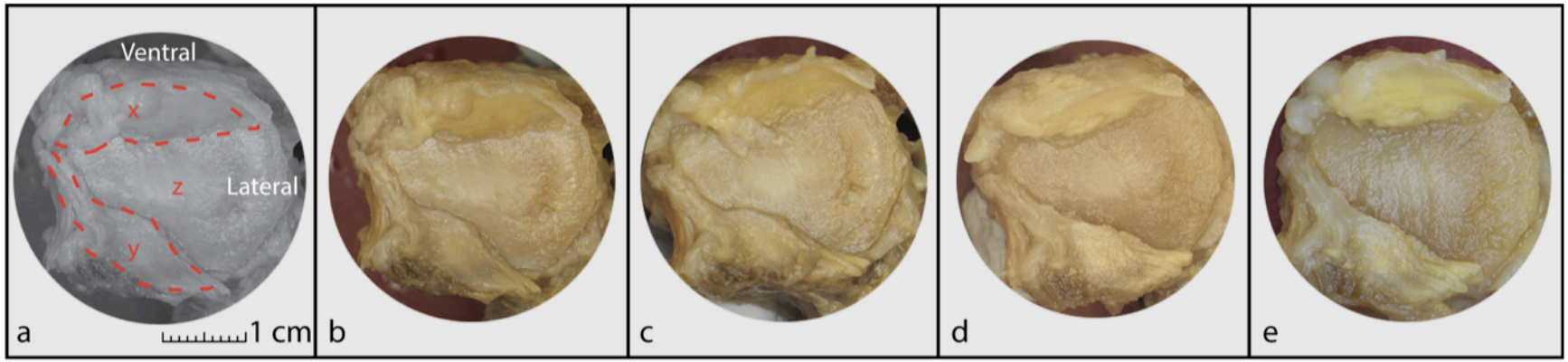


Figure 7: Timeline of meniscoids immersed in 5% formic acid demonstrating consistent morphometry over the period of immersion. (a) Meniscoid edges outlined with red dashed lines in a superior view of a right atlantoaxial joint showing position and orientation. (x) ventral meniscoid, (y) dorsal meniscoid, (z) articular surface. (b) to (e) Timeline of meniscoids (b) 0 days, (c) 4 days, (d)18 days, (e) 32 days.

Decalcification Testing

Ammonium hydroxide and ammonium oxalate testing of the formic acid demineralising solution in each specimen jar for decalcification yielded the following results: three specimens were completely demineralised by day 10, two by day 15, two by day 16, one by day 18, two by day 23, one by day 25, and one by day 32. In total, all 12 specimens were fully demineralised over the 32-day study period.

Discussion

This is the first study to assess soft tissue structures undergoing prolonged immersion in formic acid in order to determine whether tissue degradation occurs that affects structure morphometry. Results suggest that immersion of cervical meniscoids and associated zygapophyseal articular surfaces in 5% formic acid solution over a 32-day period did not significantly alter the surface area or dimensions of the meniscoids. These findings support the use of formic acid demineralisation of bony tissue to facilitate anatomical investigation of meniscoids or similar soft tissue structures by gross dissection, as even after 32 days in 5% formic acid the size and shape of these structures were not significantly altered.

The 32-day duration period for this experiment is significant, as it provided sufficient time for complete demineralisation of all the tissue blocks. This was confirmed by the chemical testing for decalcification undertaken as part of the study, which revealed eight of the 12 joints were fully demineralised by Day 18, and all were fully demineralised by Day 32. This length of time is comparable to previous work reporting

results of formic and nitric acid demineralisation of bony tissue (Belanger *et al.*, 1965; Verdenius & Alma, 1958), as well as concurrent studies undertaken in the same laboratory by the authors using formic acid demineralisation. It is worth noting that larger tissue blocks would likely take longer to fully demineralise, however blocks identical to those used in this study were dissected by the authors after four days in 5% formic acid, including removal of the softened bone to access zygapophyseal meniscoids. This suggests that complete decalcification is not necessary to enable safe and easy removal of the bone, and we have demonstrated that the morphometry of structures such as meniscoids do not alter after up to 32 days immersed in 5% formic acid. The complete demineralisation of the bony tissue is relevant to the application of this technique in aiding studies involving gross dissection and tissue morphometry as it confirms that the bone would be soft enough to be cut with a scalpel.

Only minor changes in the appearance of some of the meniscoids' morphology were noted over time. The edges of five meniscoids became visibly more ragged over the period of the experiment, however these changes did not alter the morphometry of the structures. Three of these observations occurred after the joint had fully decalcified according to chemical testing. The other two meniscoids, which were the ventral and dorsal meniscoids of the same atlantoaxial joint, became noticeably ragged on Days 14 and 18, despite the joint being fully decalcified on Day 32. In the context of an anatomical study using demineralisation to facilitate removal of bone, the meniscoids that became ragged after the bone had demineralised would in fact have been removed from formic acid on the day demineralisation was complete, and therefore would not have been immersed in formic acid long enough for the changes to occur.

The early ragged changes noted in the two atlantoaxial meniscoids may be related to the composition of the structures, as meniscoids at this spinal level tend to be primarily of composed adipose tissue, whereas meniscoids at lower cervical levels are more likely to be composed of fibrous tissue (Kos *et al.*, 2002; Mercer & Bogduk, 1993), however this explanation is speculative. An alternative explanation is based on the observations of Callis and Sterchi (1998), who undertook a systematic review and an experimental study comparing various demineralising agents. They found that larger specimens were at risk of suffering morphological damage when demineralised using acid agents. The joint in which the two meniscoids of interest were located was the last specimen to reach complete demineralisation and one of the larger specimens in the sample, which suggests that Callis and Sterchi's (1998) finding of larger specimens being at risk of damage may be a plausible explanation for the ragged changes observed. It should be reiterated however that whilst the appearance of the two specimens changed (i.e. the edges of the meniscoids became visibly ragged), the morphometry remained consistent.

Limitations

Tissue shrinkage secondary to dehydration in ethanol embalming has been reported in muscle and nervous tissues (Brown, Reed, & Henry, 2002; Cutts, 1988), and as such the possibility of similar tissue shrinkage occurring in the specimens used in this study cannot be excluded. Therefore, it is uncertain if the results of this study can be generalised to studies of cervical meniscoids using fresh or non-ethanol embalmed cadavers.

Only one assessor undertook the measurements of the meniscoids, so whilst the intra-rater reliability of measures of surface area and protrusion length was high (ICC > 0.9, see Table 2), the reliability of the measures between raters cannot be determined.

As the meniscoids were semi-detached structures sitting on the zygapophyseal or atlantoaxial joint surfaces, they may have moved during handling and their position may have altered between photographs. This may have affected the photographic measures, however, given that the intra-rater reliability across the three measurement readings was high, and that repeated measures ANOVA indicated no significant differences between any measurements, it is unlikely that variation in meniscoid position has significantly detracted from the accuracy of the photographic measures.

The cadavers included in the study were elderly, and age-related decreases in bone mineral density (Duque & Troen, 2009) may have influenced results of the study. It is unknown if the results would differ in younger specimens. Furthermore, as donor medical records were not available to the researchers, the influence of donor pathology upon the outcome measures is uncertain.

Conclusions

In conclusion, meniscoid dimensions were not significantly altered by extended-immersion in 5% formic acid demineralising solution. This suggests that extended-immersion demineralisation using formic acid can be safely used to facilitate study of cervical spine meniscoids, and possibly other similar structures, without altering their

morphometry. These findings may have implications for dissection studies of other soft tissue or meniscoid-type structures in different joints.

Summary: A possible source of methodological error applicable to previous gross dissection studies of cervical spine meniscoid morphology has been the considerable force that may be required to disarticulate the highly congruent zygapophyseal joints to access the intra-articular meniscoids. It is conceivable such force may inadvertently damage the delicate meniscoids, and in turn affect the accuracy of study findings. The results of Study 1 have established that formic acid immersion does not significantly alter the morphometry of the meniscoids. This technique can therefore be utilised to facilitate future research using gross dissection to investigate cervical meniscoid morphology by softening bone to allow easy disarticulation of spinal joints. Formic acid demineralisation was utilised in this manner during preparation of cadaveric spinal tissue in Studies 2 and 3 (Chapters 4 and 5).

Chapter 4: STUDY 2 – MORPHOLOGY AND MORPHOMETRY OF LATERAL ATLANTOAXIAL JOINT MENISCOIDS

The lateral atlantoaxial joints are recognised sources of musculoskeletal spinal pain (Aprill, Axinn, & Bogduk, 2002; Bogduk, 2001, 2011a; Dreyfuss, Michaelsen, & Fletcher, 1994; Star, Curd, & Thorne, 1992). These joints differ in morphology from the zygapophyseal joints with respect to the size, orientation and topography of their articulating surfaces (Bogduk & Mercer, 2000; Cattrysse *et al.*, 2008; Koebke & Brade, 1982; Mercer, 2004). These contrasting features allow for different biomechanical and functional capabilities at these joints, which make a substantial contribution to available cervical rotation range of movement (Mercer, 2004; White & Panjabi, 1990). The lateral atlantoaxial joints are anatomically and functionally different from the zygapophyseal joints, and as such, the meniscoids within these joints will be approached as entities distinct from the zygapophyseal joint meniscoids.

As established in Chapter 2 (Literature Review), the morphology of the lateral atlantoaxial joint meniscoids requires further investigation due to a number of factors. There have been conflicting findings regarding meniscoid morphological variation between the sexes (Friedrich *et al.*, 2008; Webb *et al.*, 2012), which may be of clinical significance as females have been suggested to have poorer prognoses in neck pain conditions (Carroll *et al.*, 2009; Carroll *et al.*, 2008b). There are also conflicting data on the prevalence of meniscoids at the lateral atlantoaxial joints, as previous studies have

reported prevalence rates ranging from 0% to 97% of joints containing meniscoids (Chang *et al.*, 1992; Kawabe *et al.*, 1989; Mercer & Bogduk, 1993; Tang *et al.*, 2007).

Furthermore, the relationship between articular cartilage degeneration and lateral atlantoaxial joint meniscoid composition has been investigated in only one study (Friedrich *et al.*, 2008) and requires further exploration given the potential association of articular cartilage degeneration with pathology (Felson, 2013; Kemp, Burns, & Brown, 2008). Friedrich *et al.* (2008) used MRI to examine articular cartilage degeneration and meniscoid composition, but this is an indirect method to assess these variables and is limited by the resolution of imaging technology. Gross dissection and light microscopy are techniques that permit articular cartilage degeneration and meniscoid histology to be directly visualised, and as such may allow for more thorough exploration of the morphological characteristics of structures within and around these joints.

Essential to understanding a structure's potential role in pathology is a thorough appreciation of its normal anatomy and function. With respect to the lateral atlantoaxial joint meniscoids, this understanding is not comprehensive and their morphology requires further clarification. Study 2 therefore sought to explore patterns of morphological variation in the lateral atlantoaxial joint meniscoids and associated degenerative changes in the articular cartilage.

This work has been published as original research:

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z. (See Appendix M)

Human research ethics approval and research governance documentation can be found in Appendices C, D, I, and J. Characteristics of cadavers included in the study can be seen in Appendices K and L, and bequeathal documentation can be seen in Appendix B.

Introduction

Cervical spine meniscoids, also referred to as synovial folds, are invaginations of the synovial membrane that project into the joint cavity of synovial joints throughout the vertebral column (Mercer & Bogduk, 1993; Webb *et al.*, 2011a). It has been suggested that they compensate for the incongruence of the articular surfaces of structures such as the lateral atlantoaxial or zygapophyseal joints, assist in distribution of forces passing through the joints (Lang, 1993), and protect and lubricate the articular cartilage of these joints during normal gliding movements (Kos *et al.*, 2002; Mercer & Bogduk, 1993; Webb *et al.*, 2011a). Cervical meniscoids are nominated as potential sources of pain through mechanical entrapment or extrapment (Bogduk, 2005; Kos *et al.*, 2002; Kos & Wolf, 1972; Lang, 1993; Mercer & Bogduk, 1993), or during degenerative or traumatic cervical pathology including whiplash associated disorder (Bogduk, 2011b; Friedrich *et al.*, 2008; Schonstrom *et al.*, 1993; Taylor & Taylor, 1996).

The morphology of the meniscoids of the lateral atlantoaxial joints has been the subject of previous studies (Chang *et al.*, 1992; Friedrich *et al.*, 2008; Kos *et al.*, 2002; Mercer

& Bogduk, 1993; Tang *et al.*, 2007; Webb *et al.*, 2011b; Webb *et al.*, 2012). This is due to the unique anatomy of these joints and their substantial functional significance, as they account for approximately 40 degrees of cervical axial rotation to each side (White & Panjabi, 1990). Lateral atlantoaxial joint meniscoids have been described as being located at the ventral and dorsal poles of the joint, being generally crescent-shaped, and composed of varying quantities of adipose and fibrous tissue (Kawabe *et al.*, 1989; Mercer & Bogduk, 1993; Tang *et al.*, 2007). However, conflicting findings have been reported regarding their prevalence and histological composition (Kawabe *et al.*, 1989; Mercer & Bogduk, 1993; Tang *et al.*, 2007; Yu *et al.*, 1987), and the influence of age and articular degeneration upon meniscoid morphology is unclear (Friedrich *et al.*, 2008; Kos *et al.*, 2002). In addition, it is unclear whether sex may affect lateral atlantoaxial joint meniscoid morphology. Friedrich *et al.* (2008) found no clear association between sex and morphology using MRI, whereas Webb *et al*. (2012) reported the lateral atlantoaxial joint meniscoids to generally be larger in male specimens than female specimens in a study using cervical spine sections.

Integral to determining a structure's potential role in pathology is a sound understanding of its normal anatomy and function. In the case of the lateral atlantoaxial joint meniscoids, previous research has yielded conflicting results regarding patterns of their normal morphological and morphometrical variation, such as the influences of sex and articular cartilage degeneration upon meniscoid size and composition. Because these structures are of potential clinical significance in neck pain (Friedrich *et al.*, 2008; Kawabe *et al.*, 1989; Mercer & Bogduk, 1993; Tang *et al.*, 2007; Webb *et al.*, 2011a), and since chronic mechanical neck pain is a costly and burdensome condition (Fejer *et al.*, 2006), it is essential that the normal morphology and morphometry of the lateral

atlantoaxial joint meniscoids is further clarified. The aim of this study was to explore the form and composition of lateral atlantoaxial joint meniscoids, investigating the influence of age, sex and articular degeneration upon the morphometry and composition of these structures.

Materials and Methods

Twelve adult cervical spines, from donors aged 69 to 93 years (mean 81.5 years, standard deviation 7.3, 6 female), were sourced from the body bequest program at the Department of Anatomy at the University of Otago. Tissue was embalmed in accordance with departmental protocols using ethanol and water based solutions. Specimens were included in the sample if the lateral atlantoaxial joints from the cadaver were intact.

Ethics Statement

Written informed consent was obtained from donors prior to death, and their bodies were donated in accordance with the Human Tissue Act (2008) of New Zealand. Institutional ethics approval was obtained from The University of Newcastle Human Research Ethics Committee and dissection was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

Dissection Procedure

Overlying muscles and soft tissue were removed from each spine by dissection to the level of bone and joint capsules. A scalpel was used to disarticulate the atlas from the occiput. A dental burr (Beaver Ace Dental Micro Engine, Osada Electric Co. Ltd., Tokyo, Japan) was then used to drill through the lateral aspects the posterior arch of the atlas, at either side of the posterior tubercle, to allow removal of the posterior portion of the bone. A bone saw (Kam-Lok Professional Model 20-A, Kasco/SharpTech Corp., St Louis, MO) was used to cut along the longitudinal axis of the spine through the laminae and the pedicles, to allow the lateral atlantoaxial and zygapophyseal joints to be removed as single columns. Either the left or right atlantoaxial joint of each donor was included in the study through a process of random allocation using a random number generator. Even numbers of left and right joints made up the sample.

Over four days, the tissue was then partially demineralised by immersion in formic acid (5%) solution on a shaker table at room temperature. This allowed the atlantoaxial joints to be cut away from the lower cervical vertebrae by slicing through the articular pillar in the plane of the C2-3 zygapophyseal joint line. Demineralisation in this way has been shown to not alter the morphometrics of cervical meniscoids (Farrell *et al.*, 2015c).

Dissection of each atlantoaxial joint was then undertaken using a surgical microscope (Op-Mi 6, Carl Zeiss, Jena, West Germany) by excising the joint capsule near its attachment to the atlas, so that the joint could be gently disarticulated by lifting off the atlas. This left any enclosed meniscoids intact *in situ* on the superior articular facet of the axis.

Examination of each identified meniscoid was undertaken using a surgical microscope. A four point grading system (Wang, Yu, & Haughton, 1989) was employed to assess articular cartilage degeneration: Grade I cartilage covers the entire joint surface with uniform thickness; Grade II cartilage covers the entire joint surface with erosions; Grade III cartilage incompletely covers the joint surface with underlying bone exposed; Grade IV denotes an absence of joint cartilage. Meniscoid appearance and location within the joint were noted, and each joint was photographed using a digital camera (Canon Powershot G10 14.7 megapixel, Canon Inc., Tokyo, Japan) mounted on a dissecting microscope (Olympus SZX7, Olympus Inc., Tokyo, Japan) using a Canon LA-DC58K conversion lens (Canon Inc., Tokyo, Japan) and an Olympus DF PL 0.75X microscope lens (Olympus Inc., Tokyo, Japan).

Adobe Photoshop (Adobe Systems Inc., San Jose, CA) was used to measure the digital photographs of the joint surfaces and overlying meniscoids. Measurements undertaken included meniscoid surface area expressed as a percentage of the surface area of the superior articular facet of the axis, and the length of meniscoid protrusion into the joint as per the method proposed by Inami *et al*. (2000) (**Figure 8**). The distances and areas were first measured in pixels and then converted into millimetres (mm) and mm^2 using a scale bar included in each photo, precise to ±0.01 mm for measurement of length, and to ±0.04 mm^2 for surface area. The measurement of meniscoid surface area and length of protrusion in this manner using Adobe Photoshop has been shown to have high intra-rater reliability (Farrell *et al.*, 2015c). Measurement of digital photographs using pixel counting with imaging software was validated by Akhavan *et al.* (2014), who found strong correlations between measurement of digital photographs and intra-operative caliper measures in a surgical study.

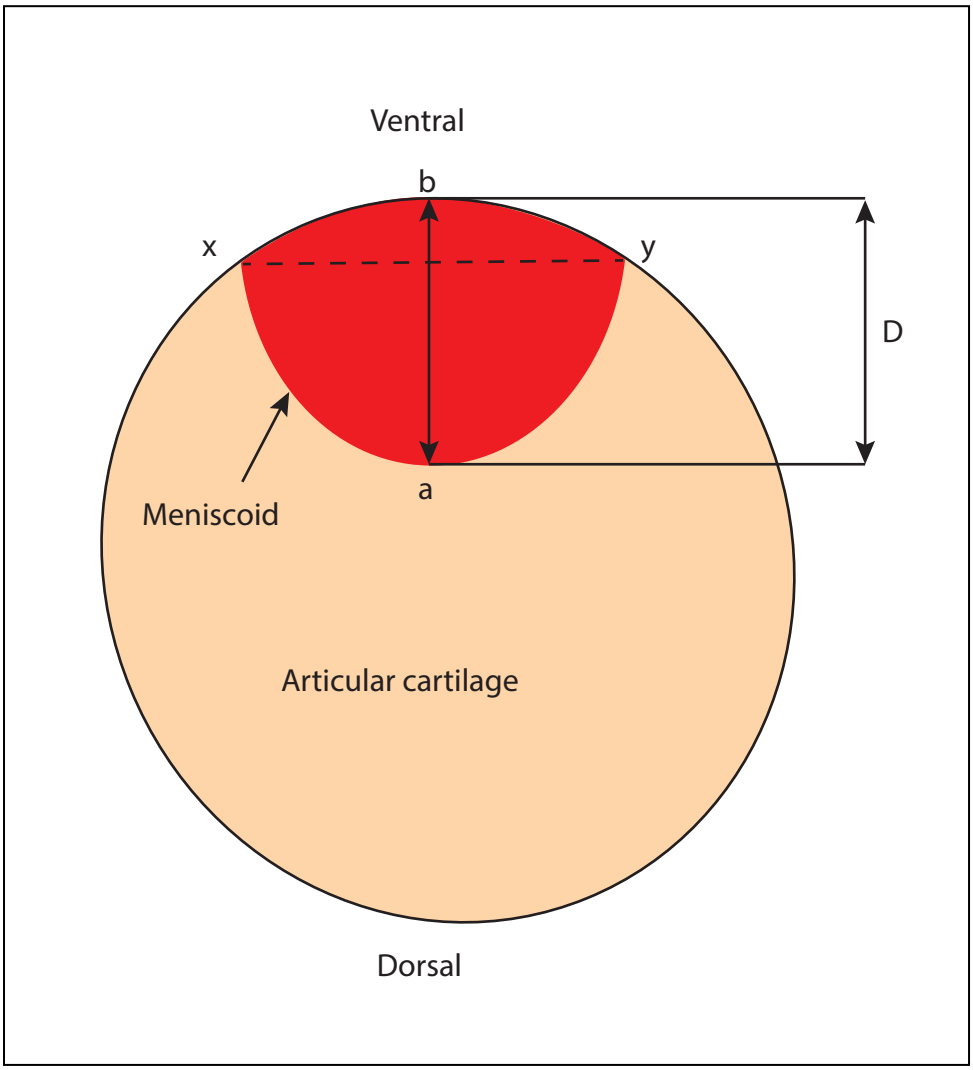


Figure 8: Measurement of meniscoid protrusion length. Superior view of atlantoaxial joint surface, meniscoid illustrated in red. Distance (D) is the measurement of the widest point perpendicular to the baseline (xy) that connects the bilateral ends of the meniscoid as they intersect the joint margins. (a) Free border of the meniscoid, (b) meniscoid base connecting to joint capsule. Modified from Inami *et al*. (2000).

Histological Processing

Specimens were washed in 70% ethanol and then rinsed for 1 hour. Each sample was embedded in paraffin and sectioned at 5 μm in the sagittal plane through the midline of identified meniscoids. Three joints (25% of sample) of sufficient size to permit multiple sections were chosen for five sections to be acquired in order to examine the histological composition of the meniscoids along the breadth of their structure. Sections were then mounted on microscope slides, stained with haematoxylin and eosin, and examined with a light microscope (Olympus CH30, Olympus Inc., Tokyo, Japan).

Data Analysis

Data were analysed descriptively and with non-parametric techniques. Kruskal-Wallis and Wilcoxon rank-sum tests were used to explore relationships between categorical and continuous variables (i.e. meniscoid composition and size, meniscoid location and size, donor sex and meniscoid size, articular cartilage degeneration and meniscoid size). Relationships between two continuous variables (i.e. meniscoid size and donor age) were explored using Spearman's correlation rho. The association between meniscoid composition and articular cartilage degeneration (absence or presence thereof) was examined using chi squared testing. The significance level was set at $p < 0.05$.

Results

Ventral and dorsal meniscoids were found in all twelve joints dissected. Two ventral meniscoids were damaged in processing, and were not included in the morphological or histological analysis.

Meniscoid Morphology and Histology

Each meniscoid featured a base, which arose from the joint capsule and extended between the joint surfaces to form an apex. In keeping with the classification systems applied by Inami *et al.* (2000) and Tang *et al.* (2007), three types of meniscoid were identified based upon their histological composition: adipose type, fibrous type, and fibroadipose type (**Figure 9**).

Seven meniscoids (32%) were classified as adipose type. These meniscoids were elliptical or crescent-shaped structures that were located primarily at the ventral aspect of the joints (six of the seven). The free borders of these meniscoids were clean and tapered. Histologically, these meniscoids were primarily made up of adipose tissue and lined with adipose type synovium demonstrating layers of synoviocytes of varying depths (**Figure 10a**). These were generally between one to two layers deep, though in one specimen they were up to five synoviocytes deep. Blood vessels were noted traversing each of these meniscoids.

Figure 9: Following page - Photographs of meniscoids upon articular surfaces of two disarticulated right atlantoaxial joints. Superior view showing surface of superior articular facet of axis in each specimen. (a) Adipose type meniscoids (black arrows) located at ventral and dorsal aspects of joint. (b) Fibrous type meniscoid (black arrowhead) and fibroadipose type meniscoid (black arrow) located at dorsal and ventral aspects of joint respectively. ac = articular cartilage.

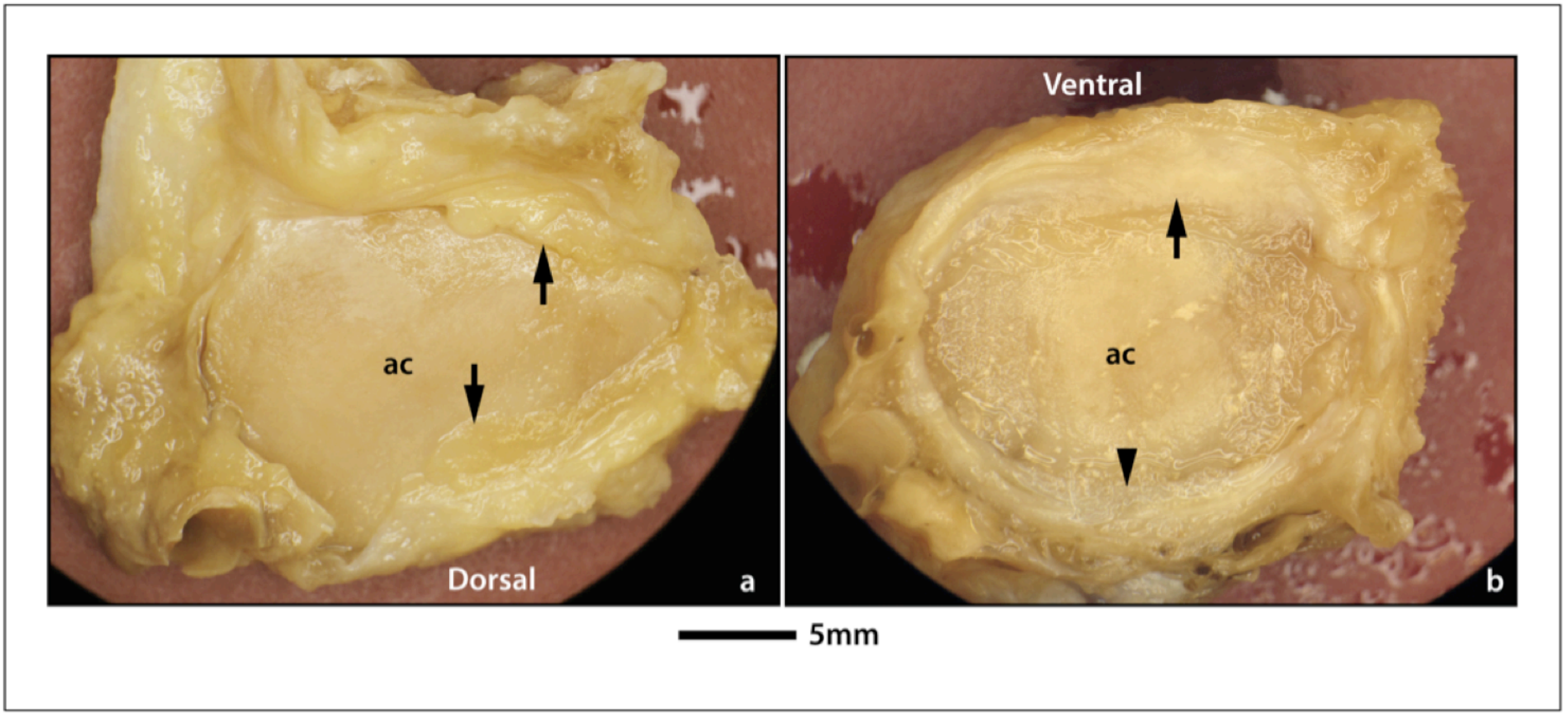
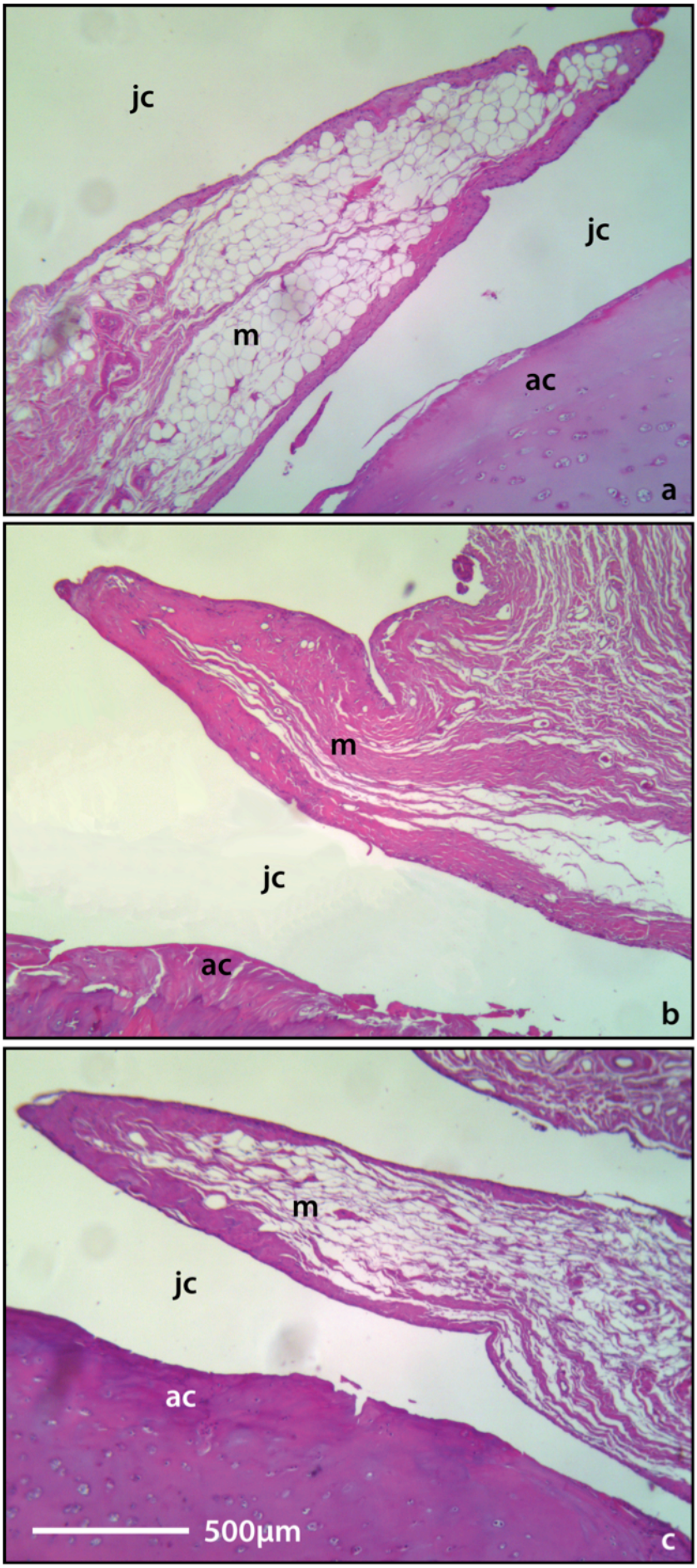

ac
Dorsal
a
Ventral
ac
b
5mm

Figure 10: Following page - Sagittal sections of atlantoaxial joint meniscoids photographed through a light microscope, illustrating different histological characteristics: (a) adipose type meniscoid composed primarily of adipocytes; (b) fibrous type meniscoid composed primarily of dense irregular connective tissue; (c) fibroadipose type meniscoid composed of fibrous and adipose tissue. ac = articular cartilage; jc = joint cavity; m = meniscoid. Haematoxylin and eosin, x4 magnification.


jc
jc
m
ac
a
m
jc
ac
b
m
jc
ac
500µm
c

Nine meniscoids (41%) were classified as fibrous type. These were crescent, semi-circular or irregular in shape, and were located primarily at the dorsal aspect of the joints (seven of the nine). The structures tapered to protruding free edges that in three specimens were observed to be ragged in appearance. Histologically, these meniscoids were primarily composed of irregular dense connective tissue covered with fibrous type synovium lined by varying numbers of synoviocytes (**Figure 10b**). This was recorded as between two and three layers in six specimens, up to four layers in two specimens, and zero to one layer in one specimen. Blood vessels were noted traversing all but one of these meniscoids.

Six meniscoids (27%) were classified as fibroadipose type. These meniscoids were crescent-shaped and found at both the ventral and dorsal aspects of the joint (two ventral, four dorsal). Five of the six meniscoids demonstrated clean edges and one was ragged in appearance. Histologically, these meniscoids were composed of an adipose tissue core, surrounded by outer layers of dense irregular connective tissue with small amounts of loose connective tissue and adipocytes dispersed throughout the structure, such that the proportions of adipose and fibrous tissues were approximately equal (**Figure 10c**). The overlying synovium was fibrous in type and generally contained two to three layers of synoviocytes. Five layers of synoviocytes were observed in one specimen. Blood vessels were noted traversing each of these meniscoids.

Of the three joints sectioned five times, each meniscoid demonstrated consistency in composition across the breadth of its structure. Three of these meniscoids were classified as adipose type, one was classified as fibrous type, and two were classified as fibroadipose type.

Size

Median meniscoid surface area and protrusion length, arranged according to various factors, can be seen in **Table 3** and **Table 4**. No significant difference was detected between the median meniscoid surface areas of adipose, fibrous and fibroadipose type meniscoids ($p = 0.31$), nor between the median protrusion lengths of the three meniscoid types ($p = 0.86$) (**Table 3**). No significant difference existed between meniscoids located at the ventral or dorsal aspects of their joints, with respect to surface area ($p = 0.25$) or protrusion length ($p = 0.13$) (**Table 4**).

Influence of Age

The distribution of different donor ages across the three meniscoid classifications was consistent (**Table 3**). No relationship was found between donor age and meniscoid surface area (Spearman's rho = 0.11, $p = 0.64$) or meniscoid protrusion length (Spearman's rho = 0.13, $p = 0.56$).

Table 3: Characteristics of meniscoid classifications.

Characteristic	Adipose Type	Fibrous Type	Fibroadipose Type
Quantity	7	9	6
Location (number ventral/dorsal)	6/1	2/7	2/4
Surface area – as proportion (%) of joint surface area (median (IQR))	15.05 (8.83-19.32)	18.29 (9.11-20.36)	21.03 (13.99-21.77)
Protrusion length (mm) (median (IQR))	2.69 (2.15-3.06)	2.94 (2.11-3.15)	2.55 (2.05-2.69)
Sex (number male/female)	4/3	4/5	3/3
Age (years) (median (IQR))	82 (73-83)	84 (74-89)	81 (77-85)
Cartilage degeneration Grade I/Grade II/Grade III	6/1/0	3/5/1	5/0/1

Legend: IQR - Inter-quartile range; Cartilage degeneration as per rating scale described by Wang et al. (1989): Grade I - cartilage covers entire joint surface with uniform thickness; Grade II - cartilage covers joint surface with erosions; Grade III - cartilage incompletely covers joint surface with underlying bone exposed.

Table 4: Meniscoid size (surface area and protrusion length) by location, gender and cartilage degeneration.

Characteristic		**Surface area (as % joint surface area) (median (IQR))**	**Protrusion length (mm) (median (IQR))**
Location			
	Ventral	13.03 (8.83-21.31)	2.32 (1.37-2.69)
	Dorsal	18.81 (14.59 – 21.26)	2.83 (2.19-3.28)
Sex			
	Male	20.36 (18.18-21.77)	2.94 (2.68-3.4)
	Female	13.99 (9.1-19.33)	2.23 (1.74-2.69)
Cartilage degeneration			
	No cartilage degeneration (Grade I)	16.62 (9.1-21.77)	2.46 (2.05-3.06)
	Cartilage degeneration (Grade II or III)	18.81 (13.1-20.84)	2.82 (2.34-3.06)

Legend: IQR - Inter-quartile range; Cartilage degeneration as per rating scale described by Wang et al. (1989): Grade I - cartilage covers entire joint surface with uniform thickness; Grade II - cartilage covers joint surface with erosions; Grade III - cartilage incompletely covers joint surface with underlying bone exposed.

Influence of Sex

Sex was evenly distributed between the three classifications (**Table 3**). Median meniscoid surface area and protrusion length for males and females is reported in **Table 4**. There was no significant difference between males and females for meniscoid surface area ($p = 0.07$), however a significant difference existed between the sexes in meniscoid protrusion length ($p = 0.04$).

Influence of Articular Cartilage Degeneration

The three classifications of meniscoid were associated with different patterns of cartilage degeneration (**Table 3**). Adipose type (six of seven) and fibroadipose type (five of six) meniscoids were more frequently associated with Grade I cartilage degeneration, whereas of the nine fibrous type meniscoids, three were associated with Grade I, five with Grade II, and one with Grade III cartilage degeneration ($p = 0.05$).

Median meniscoid surface area as a proportion of articular surface area and length in millimetres can be seen in **Table 4** for joints with and without cartilage degeneration. No significant difference in meniscoid surface area or meniscoid length was detected between joints free of cartilage degeneration and joints with cartilage degeneration ($p = 0.54$ and 0.31, respectively).

Discussion

This morphological study of the meniscoids of the lateral atlantoaxial joints indicates distinct patterns in the composition and nature of these structures. Meniscoids were composed of fibrous tissue, adipose tissue, or a combination of the two, were larger in males than females, and demonstrated variation in composition depending upon articular cartilage degeneration and position in joint.

Meniscoids were found in all the adult atlantoaxial joints studied. This is consistent with some previous investigations (Kos *et al.*, 2002; Webb *et al.*, 2011b; Webb *et al.*, 2009; Webb *et al.*, 2012; Yu *et al.*, 1987) but is dissimilar to others (Chang *et al.*, 1992; Kawabe *et al.*, 1989; Mercer & Bogduk, 1993; Tang *et al.*, 2007) that report frequencies of between 0 and 97%. Webb *et al*. (2012) considered these differences to be the result of the different methodologies employed, as small meniscoids may have been overlooked in studies using gross dissection. Interestingly, the authors of some dissection studies (Inami *et al.*, 2000; Mercer & Bogduk, 1993; Tang *et al.*, 2007) suggest the opposite to be true, that studying meniscoid morphology by sectioning of cadaveric tissue may lead to structures being overlooked due to variation in meniscoid location. This study found two meniscoids in each joint dissected (dorsal and ventral), which is consistent with prevalence rates reported by studies that used MRI (Webb *et al.*, 2011b; Webb *et al.*, 2009) and sectioning of cadaveric lateral atlantoaxial joints (Webb *et al.*, 2012), indicating that careful dissection is a valid method to examine these structures.

The present study observed three different patterns of meniscoid histological composition: adipose, fibrous and fibroadipose. This is similar to the findings of Tang *et al.* (2007) and Farrell *et al.* (2015a) who used light microscopy to investigate meniscoid histology in cadavers and Friedrich *et al.* (2008) who used MRI signal intensity to extrapolate meniscoid tissue types, but in disagreement with those of Mercer and Bogduk (1993) who reported meniscoids in the lateral atlantoaxial joints of cadaveric specimens to be almost exclusively of fibroadipose classification using light microscopy. This disagreement may be the result of the different strategies of classification of meniscoid composition employed by the studies, as Friedrich *et al*. (2008) describe adipose type meniscoids as being composed primarily of adipose tissue, fibrous type meniscoids as being primarily composed of fibrous tissue, and fibroadipose meniscoids as being a mixture of the two; Mercer and Bogduk (1993) have classified any meniscoid at this level containing fibrous and adipose tissue as fibroadipose, regardless of the proportions of each tissue type observed. Previous authors (Farrell *et al.*, 2015a; Inami *et al.*, 2000; Tang *et al.*, 2007) have described an equivalent classification strategy to that of Friedrich *et al*. (2008), and based upon the patterns of histological composition observed in the present study, the authors of this paper agree this strategy appears most appropriate. Furthermore, whilst agreement does exist between most authors regarding this classification strategy, it would be valuable for future research employing this strategy to undertake intra and inter-rater reliability analysis to formally assess its reproducibility.

Meniscoid length was generally between 2 and 4 mm (18 of 22), a finding consistent with previous studies of lateral atlantoaxial joint meniscoid morphology by Webb *et al*. (2012) and Mercer and Bogduk (1993). However, Friedrich *et al*. (2008) also measured

meniscoid length in an equivalent fashion in a morphological *in vivo* study using MRI, and found mean atlantoaxial joint meniscoid length to be approximately 5 mm. The larger measurements reported by Friedrich *et al*. may be indicative of changes in tissue size during the embalming process, or they may be a reflection of participant age from their younger sample population (16-92 years, mean 42 years) when compared to the older cadaveric specimens used by Webb *et al*. (2012) (75-102 years, mean 84 years), Mercer and Bogduk (1993) (>65 years) and this study (67-93 years, mean 81.5 years). There are few data on the influence of age upon meniscoid composition, however Webb *et al*. (2012), Friedrich *et al*. (2008), and the current study have found no relationship between meniscoid age and composition. Data on the influence of age on meniscoid morphometry and composition throughout adulthood therefore remain elusive, and further investigations comparing these variables across different age groups are required. Correlation of meniscoid anatomical features and tissue types has been reported for microanatomical sections compared with MRI (Friedrich *et al.*, 2007). However, to the authors' knowledge no study has been undertaken investigating meniscoid morphology in a large, age-stratified sample of children, adolescents and young adults using MRI to investigate patterns of meniscoid morphology across the lifespan.

Articular degeneration may influence the morphology of lateral atlantoaxial joint meniscoids, with the findings of this study indicating that articular cartilage degeneration of the lateral atlantoaxial joint is associated with fibrous meniscoid composition. This is in agreement with results reported by Inami *et al*. (2000) from dissection of C2-3 to C6-7 zygapophyseal joints meniscoids. It is not clear whether degenerative change elsewhere in the cervical spine, such as C2-3 intervertebral disc

degeneration, affects the morphology of the lateral atlantoaxial joint meniscoids. This may be of interest clinically and should be considered in future research.

The present study found there to be an even distribution of the three different types of meniscoid between the sexes, a result consistent with that reported by Friedrich *et al*. (2008). Results indicated meniscoid length to be significantly greater in males than females, a finding consistent with a study using cadaveric specimens by Webb *et al*. (2012). There was a trend for the meniscoids in males to be larger than those in females as a percentage of total articular surface area, however this trend was not significant (P = 0.07). Larger meniscoids in males may simply be a result of males often being larger than females, however anthropometric information regarding the cadavers such as height and weight was not available to enable further analysis on spinal morphometrics (Friedrich *et al.*, 2008; Webb *et al.*, 2011b).

Seven of the twelve dorsal meniscoids were fibrous in composition, compared to two of ten ventral meniscoids. This association of fibrous type meniscoids with dorsal joint position has been reported previously (Friedrich *et al.*, 2008; Inami *et al.*, 2000; Webb *et al.*, 2009; Webb *et al.*, 2012) and has been speculated to be a result of biomechanical factors, such as postural influences upon weight distribution through the joint or chronic mechanical impingement of meniscoids resulting in proliferation of fibrous tissue. Further research is necessary to explore the hypothesis that fibrous dorsal meniscoids are an adaptive result of greater distribution of weight through the dorsal aspect of the atlantoaxial joint.

Clinical Implications

The patterns of variation of meniscoid morphology observed may be relevant to musculoskeletal neck pathology and potentially to diagnosis and intervention. It might be postulated that the association of fibrous meniscoid composition with articular cartilage degeneration may reflect a relationship between meniscoid morphology and biomechanical factors, such as excess joint loading leading to chronic inflammation and in turn fibrosis of meniscoids (Barr, Barbe, & Clark, 2004), or a potential relationship between meniscoid morphology and degenerative joint pathology, such as fibrosis of the meniscoids occurring in osteoarthritis of the lateral atlantoaxial joint.

The lateral atlantoaxial joints have been established as potential sources of head and neck pain (Aprill *et al.*, 2002; Bogduk, 2001, 2011a; Dreyfuss *et al.*, 1994; Star *et al.*, 1992), and osteoarthritis has been frequently described at the lateral atlantoaxial joints (Halla & Hardin, 1987; Harata, Tohno, & Kawagishi, 1981; Star *et al.*, 1992; Zapletal & de Valois, 1997). It is clear that articular cartilage degeneration is a significant feature of osteoarthritis (Felson, 2012, 2013; Kemp *et al.*, 2008), and it is known that biomechanical factors such as excess joint loading may contribute to the development of osteoarthritis (Felson, 2012). Sources of pain in osteoarthritis include innervated structures such as the joint capsule, ligaments, and sub-chondral bone (Felson, 2012), and taking into account that the cervical zygapophyseal joint meniscoids are suggested to be innervated (Inami *et al.*, 2001) and demonstrate morphological changes in presence of articular degeneration, it is plausible that meniscoids may be a source of pain in osteoarthritis of the lateral atlantoaxial joints.

Limitations

The cadavers included in the study were elderly, thereby limiting the ability to generalise findings across the lifespan and explore the relationships between age and morphology. Whilst information regarding donor cause of death was available, medical records did not detail whether donors suffered any form of neck pathology. In addition, resource constraints meant that only one side of each spine was included in the study. Whilst there was an even representation of left and right joints in the sample, we were not able to compare differences between left and right within the same cadaver. Mercer and Bogduk (1993) included a description of articular facet shape in their study of meniscoid anatomy, however these data were not collected in the present study.

Conclusions

Findings of this study further our knowledge of lateral atlantoaxial joint meniscoid anatomy, including meniscoid composition, size, and relationship to sex, position in joint and changes in the articular cartilage. Articular cartilage degeneration was found to be associated with fibrous type meniscoids, and adipose type meniscoids with intact cartilage. Dorsal meniscoids tended to be fibrous in composition, whereas ventral meniscoids were mostly adipose type. Meniscoids were significantly longer in males than females, but age, composition, joint position and cartilage degeneration had no significant relationship to meniscoid size. Further investigation is needed to determine the clinical significance of patterns of meniscoid morphological variation in the context of pathology and across the lifespan.

Summary

Study 2 has clarified the morphology of the lateral atlantoaxial joint meniscoids by addressing points of contention identified in the findings of previous studies. The prevalence of lateral atlantoaxial joint meniscoids noted in this study is in accordance with the high rates of prevalence reported by some authors (Mercer & Bogduk, 1993; Webb *et al.*, 2012), and refutes the assertion that meniscoids are less common (Chang *et al.*, 1992; Tang *et al.*, 2007) or non-existent at this level in adults (Kawabe *et al.*, 1989). The findings of this study demonstrated three histological classifications of meniscoid at the lateral atlantoaxial joint, an observation consistent with results reported by Chang *et al.* (1992) and Tang *et al.* (2007) but at odds with those of Mercer and Bogduk (1993) who reported meniscoids to be almost exclusively fibroadipose in composition at this level. Meniscoids were larger in males than females at the lateral atlantoaxial joint, a finding in agreement with Webb *et al.* (2012) but contrary to that of Friedrich *et al.* (2008) who found no significant difference in meniscoid protrusion length between sexes.

Study 2 also examined the association of meniscoid size and composition with articular cartilage degeneration, and is the only study to date to do so using dissection and histology at the lateral atlantoaxial joint. The association of articular cartilage degeneration with fibrous meniscoid composition may suggest a link between meniscoid structure and articular pathology, and as such warrants further investigation in a sample with known cervical spine pathology to further elucidate the role of these structures in musculoskeletal pain. This was undertaken in Study 5 and will be presented in Chapter 7. The next chapter will examine the morphology of the cervical

zygapophyseal joint meniscoids using dissection and histology, to investigate patterns of variation in meniscoid morphometry and composition in these joints.

Chapter 5: STUDY 3 - MORPHOLOGY AND MORPHOMETRY OF CERVICAL ZYGAPOPHYSEAL JOINT MENISCOIDS

Cervical zygapophyseal joints have been well-established as organic sources of neck pain in both clinical and experimental studies (Aprill & Bogduk, 1992; Aprill, Dwyer, & Bogduk, 1990; Bogduk, 2011a, 2011b; Bogduk & Marsland, 1988; Cooper, Bailey, & Bogduk, 2007; Manchikanti, Singh, Falco, Cash, & Fellows, 2008). Of the components comprising the zygapophyseal joints, the meniscoids have been proposed to be of possible significance in zygapophyseal joint-related musculoskeletal pain (Bogduk, 2011a, 2011b).

As discussed in Chapter 2 (Literature Review), the morphology of cervical zygapophyseal joint meniscoids is not well understood. Previous studies have reported conflicting findings regarding their prevalence and aspects of their morphology. For instance, Yu *et al.* (1987), Inami *et al.* (2000), Kos *et al.* (2002), and Mercer and Bogduk (1993) found meniscoids to exist in 25%, 77%, 100% and 100% of cervical zygapophyseal joints respectively, and Fletcher *et al.* (1990) reported that meniscoids do not exist in adult cervical zygapophyseal joints at all.

Consistent with lateral atlantoaxial joint meniscoids, cervical zygapophyseal joint meniscoids are known to be composed of adipose tissue, fibrous tissue, or a blend of fibrous and adipose tissue (Inami *et al.*, 2000; Mercer & Bogduk, 1993; Webb *et al.*,

2011a). Conflicting findings exist however regarding the relative proportions of these tissue compositions. Mercer and Bogduk (1993) found 5% of cervical zygapophyseal meniscoids to be composed of adipose tissue, whereas Inami *et al.* (2000) and Friedrich *et al.* (2008) respectively found 20% and 69% of cervical zygapophyseal joint meniscoids to be composed of adipose tissue.

The relationship between articular cartilage degeneration and cervical zygapophyseal joint meniscoid morphology requires clarification due to the potential contribution of articular cartilage degeneration to pathology (Felson, 2013; Kemp *et al.*, 2008), and as conflicting findings are reported in prior studies. Inami *et al.* (2000) found articular cartilage degeneration to be associated with increased frequency of thin, fibrous meniscoids, whereas Yu *et al.* (1987) and Friedrich *et al.* (2008) found joint degeneration to be associated with thick fibroadipose and thin fibroadipose meniscoids respectively. Kos *et al.* (2002) reported meniscoid size to increase with higher levels of articular cartilage degeneration, however Friedrich *et al.* (2008) found meniscoid size to have no association with articular degeneration. The reason for the variation in findings between previous studies is not clear, however it may be a result of the variety of methodologies utilised in these studies (MRI (Friedrich *et al.*, 2008); gross dissection (Inami *et al.*, 2000; Kos *et al.*, 2002); cryomicrotomy (Yu *et al.*, 1987)). Gross dissection and cryomicrotomy allow direct visualisation of meniscoids and articular cartilage, whereas imaging is dependent upon acquisition and interpretation of images in the assessment tissue morphology. Inami *et al.* (2000) and Kos *et al.* (2002) used light microscopy in the assessment of meniscoid composition, in contrast with the inference of composition undertaken by Yu *et al.* (1987) and Friedrich *et al.* (2008).

These differences in methodology could plausibly contribute to the conflicting findings of previous research.

No dissection study has examined the differences in cervical zygapophyseal joint meniscoid anatomy between the sexes. Friedrich *et al.* (2008) used MRI to examine cervical spine meniscoids in healthy volunteers, and found no difference in meniscoid prevalence, size or composition between the sexes. However Study 2 (Chapter 4) (Farrell *et al.*, 2016b), in concurrence with Webb *et al.* (2012), noted a difference in meniscoid size between men and women at the lateral atlantoaxial joint. As females are thought to have poorer prognoses in neck pain conditions than their male counterparts (Carroll *et al.*, 2009; Carroll *et al.*, 2008b), sex-related variation in cervical zygapophyseal joint meniscoid anatomy may be of clinical significance and warrants further investigation.

A thorough understanding of a structure's anatomy is necessary to permit recognition of pathoanatomical changes and allow appreciation of a potential role in pathology. For the cervical zygapophyseal joint meniscoids, this understanding is not comprehensive nor consistently described, and as such their anatomy requires further exploration. Study 3 therefore sought to investigate patterns of morphological variation in the cervical zygapophyseal joint meniscoids.

This work has been published as original research:

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Human research ethics approval and research governance documentation can be found in Appendices C, D, I and J. Characteristics of cadavers included in the study can be seen in Appendices K and L, and bequeathal documentation can be seen in Appendix B.

Introduction

Cervical meniscoids, or synovial folds, are invaginations of synovial membrane that protrude between the articular surfaces of synovial joints of the cervical spine, including the zygapophyseal joints (Mercer & Bogduk, 1993; Webb *et al.*, 2011a). It is hypothesised that these structures function to enhance the congruity of the joint surfaces and ensure the lubrication of the articular surfaces with synovial fluid (Mercer & Bogduk, 1993; Webb *et al.*, 2011a). They are also suggested to have a role in cervical spine pathology, including through their mechanical entrapment (Bogduk, 2005; Bogduk & Jull, 1985; Kos *et al.*, 2002; Kos & Wolf, 1972; Mercer & Bogduk, 1993), in articular degeneration (Bogduk & Engel, 1984; Friedrich *et al.*, 2008), or in cervical trauma such as whiplash associated disorder (WAD) (Bogduk, 2011b; Schonstrom *et al.*, 1993; Taylor & Taylor, 1996).

Zygapophyseal joint meniscoids are composed of fibrous tissue, adipose tissue, or a combination of the two (Inami *et al.*, 2000; Mercer & Bogduk, 1993). They are often crescent-shaped or semicircular, and located at the ventral and dorsal poles of a joint (Inami *et al.*, 2000; Webb *et al.*, 2011a). Previous authors have described the anatomy and morphology of the cervical zygapophyseal meniscoids, however these studies have, in many respects, yielded conflicting results (Fletcher *et al.*, 1990; Friedrich *et al.*, 2008; Hu *et al.*, 2006; Inami *et al.*, 2000; Kos *et al.*, 2002; Mercer & Bogduk, 1993; Yu *et al.*, 1987). In the cervical zygapophyseal joints of adults Yu *et al.* (1987) reported meniscoids to be rare and Fletcher *et al.* (1990) reported them to be non-existent, whereas Mercer and Bogduk (1993), Inami *et al.* (2000) and Friedrich *et al.* (2008) have all reported meniscoids to be common. Published patterns of morphological variation have also been conflicting, with the influence of articular degeneration (Friedrich *et al.*, 2008; Inami *et al.*, 2000; Kos *et al.*, 2002) and cervical spinal level (Friedrich *et al.*, 2008; Mercer & Bogduk, 1993) upon meniscoid morphological characteristics being described inconsistently in previous anatomical studies. Notably, the influence of gender upon cervical zygapophyseal joint meniscoid morphology has only been examined in one imaging study (Friedrich *et al.*, 2008) that reported gender to have no influence upon meniscoid size or composition, however a dissection study of the lateral atlantoaxial joint meniscoids (Webb *et al.*, 2012) reported cervical meniscoids to be larger in male than female specimens.

In order to properly understand the anatomy of the cervical zygapophyseal joint meniscoids, normal patterns of morphological variation must be clarified. From a clinical perspective, an understanding of normal cervical meniscoid anatomy is important as these structures have been reported to possess nociceptive fibres (Inami *et*

al., 2001) and may be involved in neck pathology (Bogduk, 2011b; Mercer & Bogduk, 1993; Webb *et al.*, 2011a). Knowledge of the pathoanatomical characteristics of these structures therefore cannot be fully appreciated without a clear understanding of their anatomy in a non-pathological population. The aim of this study was to explore the morphology and morphometry of the cervical zygapophyseal joint meniscoids, including investigation of patterns of variation in meniscoid structure associated with joint position, spinal level, articular degeneration and gender.

Materials and Methods

Twelve cervical spines from donors aged 69 to 93 years (mean 81.5 years, SD 7.34, 6 female) were sourced from the tissue banks of the Department of Anatomy, Otago School of Medical Sciences, University of Otago. Cadavers were embalmed in accordance with mortuary protocol using ethanol and water based solutions. Each donor signed an informed consent document prior to death as per the bequest protocols of the University and in accordance with the Human Tissue Act (2008) of New Zealand; ethics approval for the study was also obtained from The University of Newcastle Human Research Ethics Committee. The study was undertaken in accordance with the ethical standards set out in the Declaration of Helsinki. Specimens were included in the sample if their cervical zygapophyseal joints were intact.

Dissection Procedure

The muscles and overlying soft tissue were removed from each cervical spine, and each occiput was disarticulated from the atlas using a scalpel. A dental burr (Beaver Ace

Dental Micro Engine, Osada Electric Co. Ltd., Tokyo, Japan) was then used to remove the posterior arch of the atlas by removing the posterolateral aspects of the bone at either side of the spinous process. Using a bone saw (Kam-Lok Professional Model 20-A, St Louis, MO), cuts were made along the longitudinal axis of the cervical spine through the laminae and the pedicles, such that the zygapophyseal joints could be removed as a single column. The left or right zygapophyseal joints of each donor were randomly selected for inclusion, such that even numbers of left and right joints made up the sample.

The resulting columns of tissue were then partially demineralised by immersion in formic acid (5%) solution on a shaker table at room temperature over 4 days. Demineralisation was utilised as the complex bony architecture and congruence of adjacent vertebrae make disarticulation of the zygapophyseal joints particularly difficult, often requiring significant force to access the delicate meniscoids within the joint. Demineralisation softened bone, allowing bone surrounding the zygapophyseal joints to be easily removed, facilitating the gentle disarticulation of the joints with minimal force, reducing the risk of inadvertently damaging the meniscoids and in turn jeopardising the accuracy of study findings. Demineralisation has previously been shown to not influence the morphometrics of the cervical meniscoids (Farrell *et al.*, 2015c).

The columns were then removed from the formic acid and rinsed in distilled water. Using a razor blade, individual zygapophyseal joints were removed by slicing obliquely through the articular pillar in the same plane as the zygapophyseal joints, such that a block of tissue was produced comprising one joint: the inferior articular process of the

superior vertebra, and the superior articular process of the inferior vertebra. Each joint was stored in a single labelled specimen container.

Each demineralised zygapophyseal joint was dissected using a dental burr, surgical microscope (Op-Mi 6, Carl Zeiss, Jena, West Germany), forceps and scalpel. The dental burr was used to remove the demineralised bone of the inferior articular facet of the superior vertebra, down to the level of the articular cartilage and capsular attachments, in accordance with the method described by Inami *et al.* (2000) and Mercer and Bogduk (1993). Dissection of the joint was then undertaken using a surgical microscope, by carefully excising the zygapophyseal joint capsule near its attachment onto the inferior articular facet of the superior vertebra, such that the joint could be gently disarticulated by lifting away the articular surface, and the joint capsule and any meniscoids were left intact *in situ* on the articular surface of the superior articular facet of the inferior vertebra.

Each identified meniscoid was inspected using a surgical microscope. Articular cartilage was assessed using the grading system proposed by Wang *et al.* (1989): Grade I cartilage covers the whole joint surface with a uniform thickness; Grade II cartilage covers the whole joint surface with some erosions; Grade III cartilage incompletely covers the joint surface with underlying bone exposed; Grade IV is an absence of joint cartilage. Sketches and notes were made regarding meniscoid appearance and location within the joint, and each joint was photographed using a Canon Powershot G10 14.7 megapixel camera (Canon Inc., Tokyo, Japan) mounted on a dissecting microscope (Olympus SZX7, Olympus Inc., Tokyo, Japan) using a Canon LA-DC58K conversion lens (Canon Inc., Tokyo, Japan), an Olympus DF PL 0.75X microscope lens (Olympus

Inc., Tokyo Japan) and a Schott MC500 microscope light source (Schott Inc., New York City, NY).

The digital photographs of the joint surfaces and overlying meniscoids were measured using Adobe Photoshop (Adobe Systems Inc., San Jose, CA). Measures undertaken included meniscoid surface area as a proportion of the surface area of the superior articular facet of the inferior vertebra, and length of meniscoid protrusion into the joint using the method described by Inami and colleagues (2000) (**Figure 11**). This was achieved using Adobe Photoshop, which calculated the distances and areas in pixels, which were then converted into mm using a scale bar included in each photo.

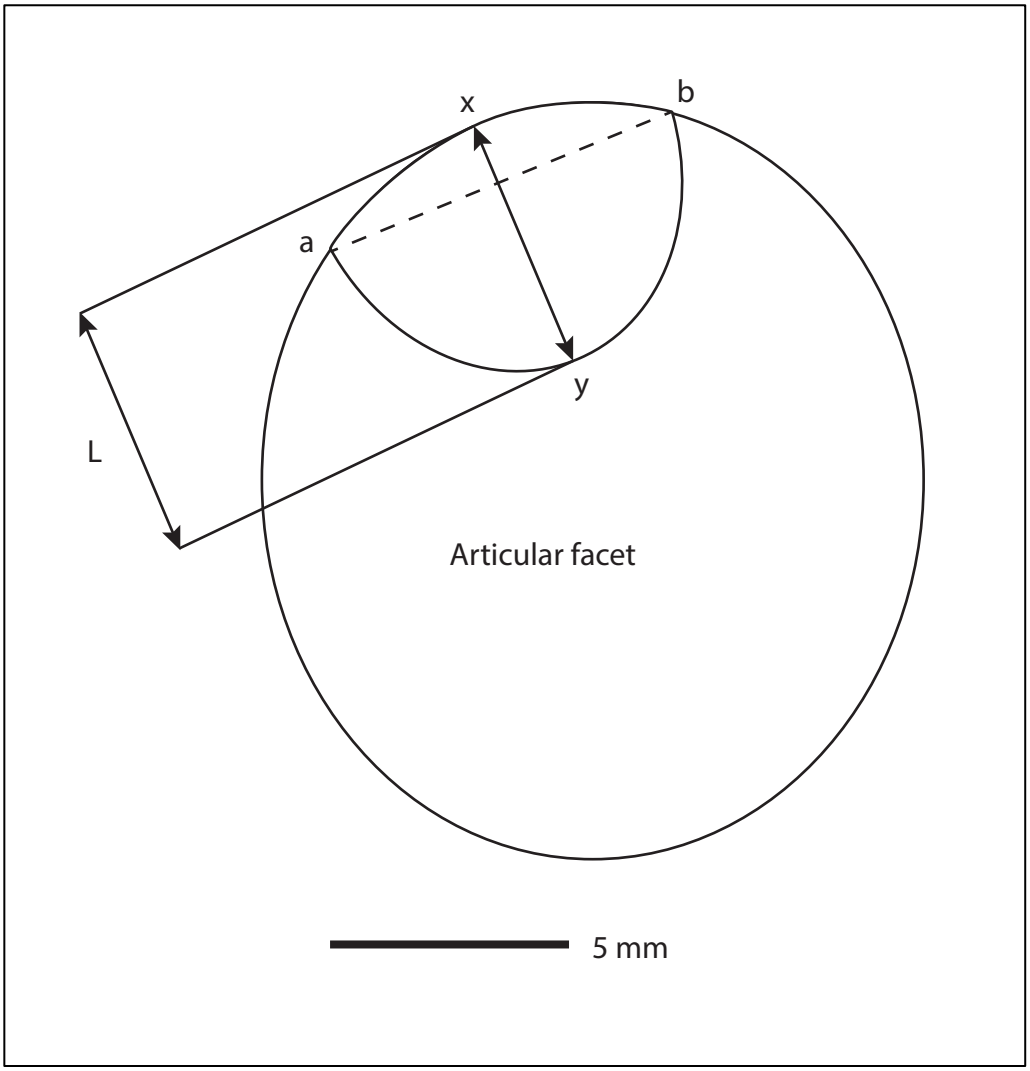


Figure 11: Measurement of meniscoids. Distance (L) was the measurement of the widest point perpendicular to the baseline (ab) that connected the bilateral ends of the meniscoid. (x) Base of meniscoid connecting to capsule. (y) Free border of the meniscoid. Adapted from Inami *et al.* (2000).

Histological Processing

Each specimen was immersed in 70% ethanol and rinsed for 1 hour. Tissue was paraffin mounted and a microtome was then used to take 5μm sections through the midline of identified meniscoids. Sections were then fixed on slides, stained with haematoxylin and eosin, and viewed under a light microscope (Olympus CH30, Olympus Inc., Tokyo, Japan).

Statistical Analysis

Data were analysed descriptively and with parametric or non-parametric techniques as appropriate, determined by Shapiro-Wilk testing. Relationships between categorical and continuous variables (meniscoid composition and size, donor gender and meniscoid size, spinal level and meniscoid size, cartilage degeneration and meniscoid size) were examined using Kruskal-Wallis tests for three groups, and Wilcoxon rank-sum tests for two groups. Spearman's correlation rho was used to examine relationships between two continuous variables (meniscoid size and donor age). One sample t-tests were used to investigate differences in size between ventral and dorsal meniscoids. Significance was set at $p < 0.05$.

Results

A total of 60 zygapophyseal joints were dissected. Four were excluded from morphological examination due to damage in processing (two C2-3, one C4-5, one C6-7). Of the remaining 56 joints, 32 contained ventral meniscoids (57%) and 44 contained dorsal meniscoids (79%). Eight (14%) joints did not contain a meniscoid, 28 (50%) contained both ventral and dorsal meniscoids, four joints (7%) contained a ventral meniscoid only and 16 joints (29%) contained a dorsal meniscoid only. A total of 76 meniscoids were identified and measured, however a technical issue during the processing of two joints (one containing a dorsal meniscoid and another containing ventral and dorsal meniscoids) meant that 73 meniscoids underwent histological examination.

Morphology and Histology

Each meniscoid featured a base and an apex. The base arose from the joint capsule and then extended into the joint cavity to form an apex. Three types of meniscoid were identified, based upon their histological characteristics – adipose type, fibrous type and fibroadipose type (**Table 5**; **Figure 12**).

Three (4%) meniscoids were classified as adipose type. These structures were composed primarily of adipocytes, and covered with adipose type synovium demonstrating one to three layers of synoviocytes (**Figure 13**). They were semicircular

or crescent-shaped, with a clean, well-defined free border extending into the joint cavity.

Table 5: Characteristics of meniscoid classifications on histological examination (n=73).

Characteristic	Adipose	Fibrous	Fibroadipose
Number	3	54	16
Location (number ventral/dorsal)	2/1	23/31	6/10
Spinal level			
C2-3	1	11	0
C3-4	0	14	4
C4-5	1	6	5
C5-6	0	14	6
C6-7	1	9	1
Gender (number male/female)	0/3	24/30	9/7
Surface area – as proportion (%) of joint surface area (median (IQR))	Ventral 20.17 (19.21-21.12) Dorsal 33.99	Ventral 16.24 (11.74-21.58) Dorsal 17.61 (12.9-30.22)	Ventral 29.35 (24.14-37.00) Dorsal 19.75 (10.31-33.58)
Protrusion length (mm) (median (IQR))	Ventral 3.55 (2.37-4.72) Dorsal 1.72	Ventral 3.55 (2.14-4.80) Dorsal 2.81 (2.13-4.94)	Ventral 3.54 (2.77-4.73) Dorsal 2.74 (2.33-3.63)
Cartilage degeneration Grade I/Grade II/Grade III/Grade IV	1/2/0/0	3/26/23/2	4/8/4/0

Legend: IQR **–** Inter-quartile range; Cartilage degeneration: Grade I - cartilage covers the whole joint surface with a uniform thickness; Grade II - cartilage covers the whole joint surface with some erosions; Grade III - cartilage incompletely covers the joint surface with underlying bone exposed; Grade IV - absence of joint cartilage. As per rating scale described by Wang *et al.* (1989).

NB: Microscope slide for two specimens not available, so histological classification of the three meniscoids that were observed and measured in these joints not presented in data

Figure 12: Following page - Photographs of meniscoids upon articular surfaces of three disarticulated zygapophyseal joints. Superior view showing surface of superior articular facet of inferior vertebra in each specimen. (a) Adipose type meniscoid (black arrow) located at ventral aspect of right C3-4 zygapophyseal joint. (b) Fibrous type meniscoid (arrowhead) located at dorsal aspect of zygapophyseal joint. (c) Fibroadipose type meniscoid (white arrow) located at dorsal aspect of left C4-5 zygapophyseal joint. ac = articular cartilage.

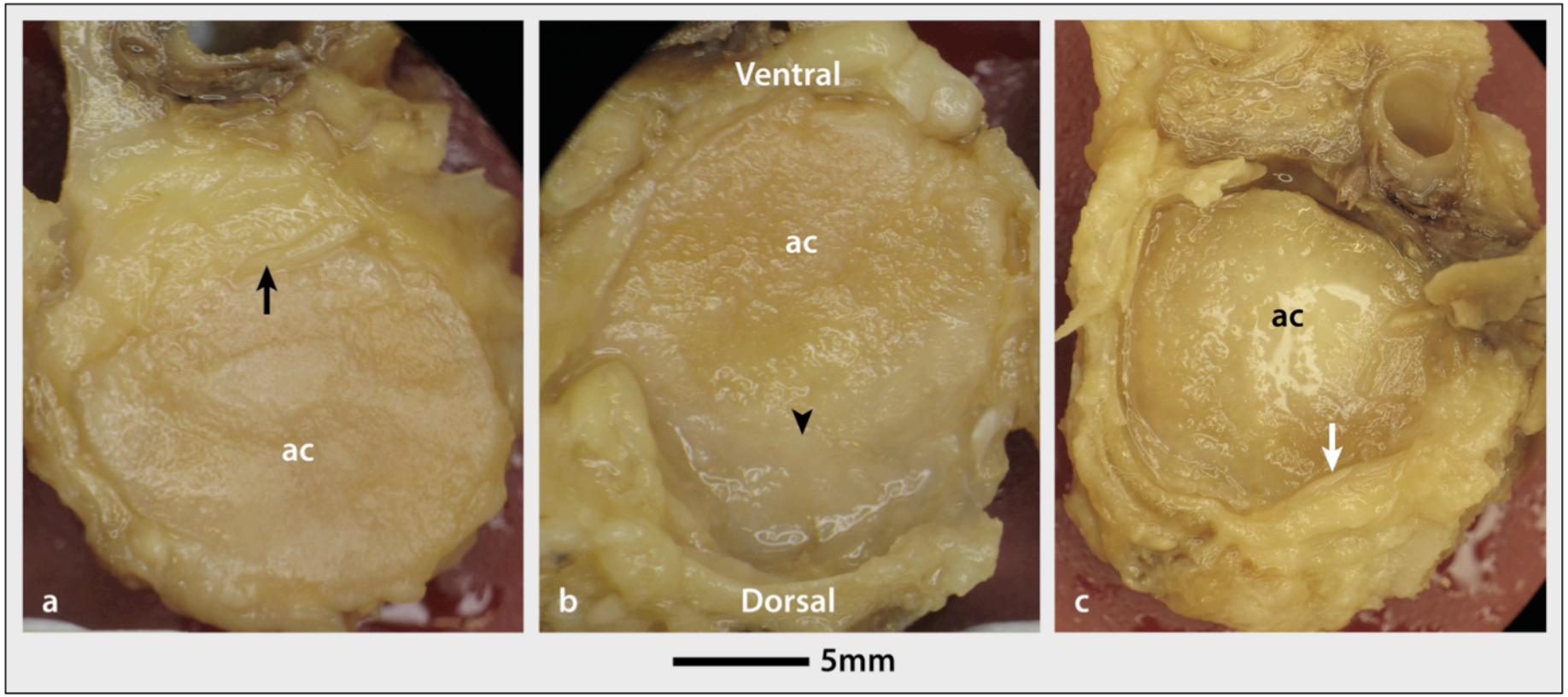
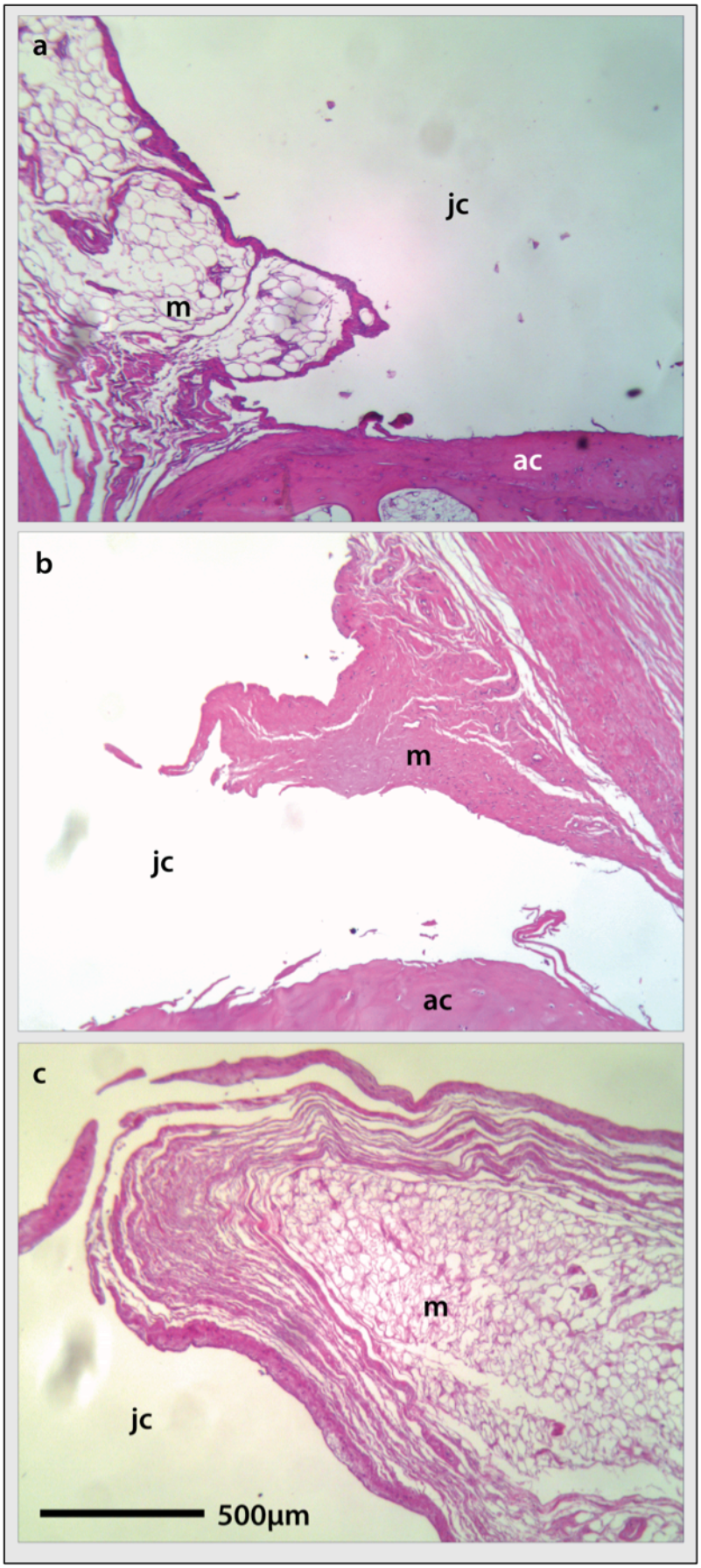

a
ac
Ventral
ac
b
Dorsal
ac
c
5mm

Figure 13: Following page - Sagittal sections of zygapophyseal joint meniscoids photographed through a light microscope, illustrating different histological characteristics. (a) Adipose type meniscoid composed primarily of adipocytes, located at ventral aspect of a right C2-3 zygapophyseal joint. (b) Fibrous type meniscoid composed primarily of dense irregular connective tissue, located at ventral aspect of a right C5-6 zygapophyseal joint. (c) Fibroadipose type meniscoid composed of fibrous and adipose tissue, located at ventral aspect of a right C4-5 zygapophyseal joint. ac = articular cartilage; jc = joint cavity; m = meniscoid. Haematoxylin and eosin, x4 magnification.


a
jc
m
ac
b
m
jc
ac
c
m
jc
500µm

Two of three adipose meniscoids were located at the ventral aspect of the joint. Blood vessels were noted within one of these meniscoids, identified as round or elongated structures of epithelial cells, often containing erythrocytes and surrounded by loose connective tissue.

Fifty-four (74%) meniscoids were classified as fibrous type. These meniscoids were composed of dense irregular connective tissue and lined with fibrous type synovium, featuring a variable number of layers of synoviocytes, ranging from one to five cells deep. These structures were elliptical, crescent-shaped, triangular or irregular in shape, and in 40 cases, demonstrated a frayed or ragged free border. Twenty-three of the fibrous meniscoids were located at the ventral aspect of the joint, and 31 were observed at the dorsal aspect. Blood vessels were noted traversing 25 of these meniscoids.

Sixteen (22%) meniscoids were composed of a mixture of fibrous tissue and adipocytes, and classified as fibroadipose meniscoids. Histologically, these structures featured a blend of adipose tissue, dense irregular connective tissue and loose connective tissue, lined with fibrous type synovium featuring variable depths of synoviocytes. These meniscoids were round, crescent-shaped or semi-circular, and possessed a clean, well-defined free border. Six of these meniscoids were located at the ventral aspect of their joint, and ten were located at the dorsal aspect. Blood vessels were noted traversing ten of these meniscoids.

Size

Median meniscoid surface area (calculated as a proportion of joint surface area) was 19.31% (IQR 12.87 – 27.1), and median meniscoid length was 2.88mm (IQR 2.2 – 4.32). Median meniscoid size can be seen arranged in relation to the various study factors in **Table 5** and **Table 6**. No significant difference existed between dorsal meniscoid surface area and ventral meniscoid surface area (mean difference 2.03%; -0.35 to 7.41 95% confidence interval [CI]). No significant difference existed between dorsal meniscoid protrusion length and ventral meniscoid protrusion length (mean difference 0.06mm; -0.70 to 0.81 95% CI).

Median ventral and dorsal meniscoid surface areas and protrusion lengths for adipose, fibrous and fibroadipose meniscoids can be seen in **Table 5**. A significant difference was detected between the three groups for ventral ($p = 0.02$) meniscoid surface area, however no significant differences existed between the groups for dorsal meniscoid surface area ($p = 0.54$), ventral meniscoid protrusion length ($p = 0.85$) or dorsal meniscoid protrusion length ($p = 0.43$).

Table 6: Meniscoid size (surface area and protrusion length) by location, spinal level, gender and cartilage degeneration (n = 76).

Characteristic		Surface area (as % joint surface area) (median [IQR])	Protrusion length (mm) (median [IQR])
Location	Ventral	18.52 (12.72-24.24)	3.52 (2.34-4.73)
	Dorsal	19.74 (12.91-30.46)	2.81 (2.16-4.00)
Spinal level			
C2-3	Ventral	11.74 (11.26-21.12)	2.37 (2.14-3.56)
	Dorsal	13.60 (12.90-30.08)	3.34 (2.07-4.94)
C3-4	Ventral	18.86 (13.55-27.72)	3.68 (2.18-4.51)
	Dorsal	13.2 (6.39-30.22)	2.72 (1.58-4.36)
C4-5	Ventral	17.82 (17.53-19.21)	4.72 (2.82-4.8)
	Dorsal	19.77 (10.16-33.86)	2.86 (2.53-5.04)
C5-6	Ventral	22.80 (13.18-27.91)	3.26 (2.22-4.61)
	Dorsal	20.38 (13.81-26.92)	2.71 (2.07-3.26)
C6-7	Ventral	16.01 (8.91-19.63)	3.22 (2.32-4.81)
	Dorsal	29.77 (16.15-36.37)	3.73 (1.97-4.77)
Gender			
Male	Ventral	15.08 (10.29-28.89)	2.80 (2.14-3.77)
	Dorsal	16.78 (12.90-26.10)	2.75 (2.21-3.73)
Female	Ventral	20.27 (13.31-24.14)	3.56 (2.37-4.8)
	Dorsal	25.39 (12.91-33.99)	2.86 (2.07-5.25)
Cartilage degeneration			
Grade I	Ventral	17.68 (14.49-21.68)	2.92 (2.18-4.10)
	Dorsal	11.86 (7.10-23.57)	2.78 (1.90-3.25)
Grade II	Ventral	19.33 (13.78-24.34)	2.92 (2.14-4.28)
	Dorsal	19.85 (12.90-30.22)	2.75 (2.07-4.08)
Grades III or IV	Ventral	18.62 (11.74-26.95)	3.63 (2.77-5.31)
	Dorsal	17.61 (13.6-34.01)	3.34 (2.21-4.97)

Legend: IQR **–** Inter-quartile range; Cartilage degeneration: Grade I - cartilage covers the whole joint surface with a uniform thickness; Grade II - cartilage covers the whole joint surface with some erosions; Grade III - cartilage incompletely covers the joint surface with underlying bone exposed; Grade IV - absence of joint cartilage. As per rating scale described by Wang *et al.* (1989)

Influence of Spinal Level

Median values of meniscoid surface area and protrusion length for both ventral and dorsal meniscoids at each spinal level are provided in **Table 6**. No significant difference existed between meniscoid surface areas across the different spinal levels for the ventral meniscoids ($p = 0.56$) or for the dorsal meniscoids ($p = 0.32$). There were also no significant differences detected between meniscoid protrusion lengths across the different spinal levels for the ventral meniscoids ($p = 0.70$) or for the dorsal meniscoids ($p = 0.73$). The distribution of the three meniscoid compositions across each of the spinal levels can be seen in **Table 5**.

Influence of Gender

Median ventral and dorsal meniscoid surface areas for males and females can be seen in **Table 6**. No significant differences existed between meniscoid surface areas of males and females for ventral ($p = 0.52$) or dorsal ($p = 0.37$) meniscoids. Median ventral and dorsal meniscoid protrusion lengths for males and females can be seen in **Table 6**. No significant difference existed between meniscoid protrusion lengths of males and females for ventral ($p = 0.44$) or dorsal ($p = 0.49$) meniscoids. The distribution of adipose, fibrous and fibroadipose meniscoids between the genders can be seen in **Table 5**.

Influence of Articular Cartilage Degeneration

The distribution of ratings of the severity of articular cartilage degeneration across the spinal levels can be seen in **Table 7**. The distribution of cartilage degeneration appears to be generally consistent across the spinal levels, with Grades II and III being the most common scores for each level, with the exception of C3-4 which featured Grades I and II as the most common scores at that level.

The distribution of the four cartilage degeneration ratings between the three meniscoid compositions can be seen in **Table 5**. All three adipose meniscoids were associated with Grades I or II cartilage, 54% of fibrous meniscoids were associated with Grades I or II cartilage, and 75% of fibroadipose meniscoids were associated with Grades I or II cartilage. Median surface area and protrusion length for both ventral and dorsal meniscoids can be seen arranged by cartilage degeneration score in **Table 6**. No significant difference existed between the various cartilage ratings with respect to ventral meniscoid surface area ($p = 0.99$), dorsal meniscoid surface area ($p = 0.23$), ventral protrusion length ($p = 0.62$) or dorsal protrusion length ($p = 0.45$).

Table 7: Distribution of zygapophyseal joint articular cartilage degeneration across the cervical spine levels C2-3 to C6-7 (n = 56).

Cartilage Degeneration	**C2-3**	**C3-4**	**C4-5**	**C5-6**	**C6-7**	**Total**
Grade I	1	2	1	1	0	5
Grade II	5	9	7	5	6	32
Grade III	4	1	3	6	4	18
Grade IV	0	0	0	0	1	1

Legend: Grade I - cartilage covers the whole joint surface with a uniform thickness; Grade II - cartilage covers the whole joint surface with some erosions; Grade III - cartilage incompletely covers the joint surface with underlying bone exposed; Grade IV - absence of joint cartilage. As per rating scale described by Wang *et al.* (1989)

Discussion

The findings of this study demonstrate distinct patterns of morphological variation within the meniscoids of the cervical zygapophyseal joints. Meniscoids were composed of adipose tissue, fibrous tissue, or a blend of adipose and fibrous tissues, and this composition appears associated with articular degeneration. Neither meniscoid size as a percentage of joint surface area nor protrusion length into the joint were significantly associated with position of the meniscoid within the joint, gender, spinal level, or articular degeneration.

Meniscoids were identified in 48 (86%) of the 56 joints included in the study. This figure is consistent with figures of cervical meniscoid prevalence reported in studies by Inami *et al.* (2000) (77%), Mercer and Bogduk (1993) (100%), and Kos *et al.* (2002) (100%). Friedrich *et al.* (2008) found meniscoids to be common throughout the zygapophyseal joints, however did not specify the proportion of joints containing meniscoids. These figures are in conflict with prevalences reported by Yu *et al.* (1987) (25%) and Fletcher *et al.* (1990) (0%). This discrepancy may be related to differences in the methodologies employed, as the latter two studies used cryomicrotomy with sagittal sectioning to examine the meniscoids, whereas the studies reporting higher figures of prevalence used gross dissection (Inami *et al.*, 2000; Kos *et al.*, 2002; Mercer & Bogduk, 1993) or MRI (Friedrich *et al.*, 2008). Considering the agreement in prevalence reported by the dissection and MRI studies, the possibility that the cryomicrotomy studies underestimated the prevalence of the meniscoids due to inherent differences in this methodology cannot be discounted.

The zygapophyseal joint meniscoids examined in the present study were primarily of fibrous (74%) or fibroadipose (22%) composition, with only 4% of identified meniscoids being of adipose classification. This is consistent with results reported by Mercer and Bogduk (1993) in their studies on cadavers. In contrast, Inami *et al.* (2000) reported that 20% of meniscoids identified in their dissection study were of adipose composition, and Friedrich *et al.* (2008) found 69% of zygapophyseal joint meniscoids investigated in their *in vivo* MRI study to be of adipose composition. The large proportion of adipose meniscoids reported in Friedrich and colleagues' study may be related to an average sample age that was much younger (mean age 42 years) than the elderly specimens that are typical of dissection studies. This possibly reflects age-related changes in meniscoid composition, as proposed by Bogduk and Engel (1984), suggesting adipose meniscoids may become fibrous in composition in response to long term mechanical stress or increasing joint degeneration. However, Friedrich *et al.* deduced meniscoid tissue composition based upon MRI signal intensities, whereas the dissection studies directly viewed tissue types using light microscopy, and as such, error associated with interpretation of tissue based upon MRI signal intensity is also a possible explanation for the discrepancy in findings.

Higher levels of articular cartilage degeneration appear to be associated with the presence of fibrous type meniscoids in this sample. Almost half of the fibrous meniscoids were associated with Grades III or IV cartilage degeneration, compared to a quarter of fibroadipose meniscoids and no adipose meniscoids. This pattern was also reported by Inami *et al.* (2000) and by Kos *et al.* (2002) and may plausibly infer a biomechanical or possibly pathological relationship between meniscoid composition and degenerative changes within the cervical zygapophyseal joints.

Meniscoids were generally between 2 mm and 4.5 mm long, with a surface area of approximately 12% to 30% of the zygapophyseal joint surface area. No significant differences were detected in either parameter of size on the basis of spinal level. Meniscoid surface area differed significantly between the classifications of meniscoid composition although the small number of adipose meniscoids compared to fibrous and fibroadipose meniscoids should be considered in the interpretation of this analysis. The position of a meniscoid at the ventral or dorsal pole of a joint did not influence any measured aspect of meniscoid dimensions. The overall distribution of meniscoid type did not appear to be related to their position within the joint.

Investigation of the association of gender with meniscoid size or composition has not been reported in previous dissection studies of the zygapophyseal joints. Meniscoid surface area was measured as a proportion of joint surface area to control for normal variation in spine size between larger and smaller specimens, as may be the case between males and females, and allow investigation of meniscoid surface area relative to the enclosing joint. No significant differences were detected between the meniscoids of male and female donors with respect to proportional meniscoid surface area or length. Distribution of the three meniscoid compositions between the genders was essentially similar, with the exception of one female specimen that possessed all three of the adipose type meniscoids (**Table 5**).

Clinical Implications

Cervical zygapophyseal joint meniscoids have been suggested as being involved in neck pain, however their precise role in musculoskeletal spinal pain is not well understood

(Bogduk, 2005; Bogduk & Jull, 1985; Kos *et al.*, 2002; Kos & Wolf, 1972; Mercer & Bogduk, 1993). The patterns of variation in meniscoid morphology observed may be relevant to musculoskeletal neck pathology as they could provide insight into biomechanical influences acting upon the zygapophyseal joints and their encapsulated meniscoids (Inami *et al.*, 2000), and in turn, the potential pathomechanical processes affecting these structures. For instance, the association of fibrous type meniscoids with increased cartilage degeneration may reflect an adaptive response to long-term biomechanical stress and/or mechanical degeneration of the articular structures, as in the case of osteoarthritis or degenerative cervical myelopathy.

Cervical meniscoids have been theorised to be involved in acute pain through their mechanical entrapment between the zygapophyseal joint surfaces leading to hypomobility and pain (Kos *et al.*, 2002; Webb *et al.*, 2011a), and through contusion in cervical trauma such as WAD (Bogduk, 2011b; Webb *et al.*, 2011a). The protrusion lengths noted in this study – IQR 2.2 to 4.32 mm, range 0.49 to 7.05 mm – suggest that larger cervical meniscoids may be of sufficient size to permit their impingement between the articular surfaces, and as such support the plausibility of these two theorised mechanisms of acute pain.

The present study and prior investigations of meniscoid anatomy have provided insight into morphological characteristics and patterns of the structures in cadaveric (Inami *et al.*, 2000; Kos *et al.*, 2002; Mercer & Bogduk, 1993) or non-pathological living (Friedrich *et al.*, 2008) populations. However to understand the role of the cervical meniscoids in a clinical context, further research is required to compare and contrast the morphological properties of meniscoids in pathological and healthy populations.

Limitations

The cadavers included in the study sample were elderly, thereby limiting the capacity to explore the relationship between age and meniscoid morphology or to generalise findings across the lifespan. Medical records accompanying the cadavers specified cause of death but did not detail whether donors suffered any form of neck pathology, therefore it is unclear whether findings were influenced by any pre-existing pathology. Furthermore, time and resource restrictions allowed only one side of each spine to be included in the study. Whilst the sample had an even representation of left and right joints, it was not possible to compare differences between left and right joints within the same spine.

Conclusions

The results of this morphological study of the cervical zygapophyseal joint meniscoids in cadavers suggest that meniscoid size is not influenced by gender, spinal level, position in joint, or articular degeneration. Meniscoid size may differ between histological classifications, however the low quantity of adipose meniscoids makes the strength of this finding questionable. There appears to be a relationship between meniscoid composition and articular cartilage degeneration, as fibrous type meniscoids were more frequently associated with increased degeneration. This pattern may be indicative of biomechanical influences upon meniscoid morphology, and as such, may have implications in the context of musculoskeletal neck pain. Further research is required to examine these morphological properties of the structures in a pathological population.

Summary

Study 3 has used cadaveric dissection and microscopy to re-visit the morphology of the cervical zygapophyseal joint meniscoids, with respect to details of contention in previous research. Patterns of variation in meniscoid size and composition have been examined with respect to sex, spinal level and articular cartilage degeneration, and the prevalence of meniscoids in the zygapophyseal joints has been clarified. This is due to the methodological advantages of dissection and microscopy, which permit direct visualisation of meniscoid morphometry, composition and articular cartilage degeneration. Small meniscoids that may have been overlooked in studies that used cryomicrotomy (Fletcher *et al.*, 1990; Yu *et al.*, 1987) were effectively detected by dissection under surgical microscope, evidenced by the larger meniscoid incidence reported in the present study.

The association of articular cartilage degeneration with fibrous meniscoid composition is of particular clinical interest, as this may infer a possible relationship between meniscoid morphology and a pathological process. This finding is consistent with the results of Study 2 (Chapter 4) at the lateral atlantoaxial joint, which supports the plausibility of cervical meniscoids being involved in musculoskeletal pain, given the established link between cartilage degeneration and articular pathology (Felson, 2012, 2013; Kemp *et al.*, 2008) and in turn cervical spondylosis (Engstrom & Deyo, 2015). To evaluate this suggestion, the morphology of the cervical zygapophyseal and lateral atlantoaxial joint meniscoids needs to be investigated in a population with known musculoskeletal cervical spine pathology in order to determine whether morphological

changes in meniscoids are associated with cervical spine pathology. This was undertaken in Study 5 and will be presented in Chapter 7.

Concurrent to morphological variation in the presence of articular degeneration, the capacity for cervical zygapophyseal and lateral atlantoaxial joint meniscoids to generate nociceptive afferent input is inherent to their proposed involvement in musculoskeletal neck pain. The next chapter details Study 4, which investigates the presence of nerve tissue within the meniscoids to examine the potential for these structures to be a source of pain.

Chapter 6: THE PRESENCE OF NERVE TISSUE IN CERVICAL SPINE MENISCOIDS

As noted by Bogduk (2011a) and Bogduk and McGuirk (2006), for a structure to be a source of nociceptive input and in turn a potential contributor to pain, it must be innervated. Nociceptive input arises from stimulation of nociceptors, and is transmitted through myelinated Aδ-fibres and unmyelinated C-fibres to the brain, where it may lead to the subjective experience of pain (Barrett, Boitano, Barman, & Brooks, 2012b; Barrett, Boitano, Barman, & Brooks, 2012c). The presence of nerve tissue within cervical spine meniscoids is therefore of interest when considering the potential clinical significance of these structures, however the nature of cervical spine meniscoid innervation is not well understood.

The existence of nerve tissue within cervical spine meniscoids has been previously examined in one study (Inami *et al.*, 2001), which found nerve fibres consistent with unmyelinated C-fibres within cervical spine meniscoids excised during laminoplasty. However no study has explicitly investigated the presence of myelinated fibres within cervical spine meniscoids, nor in a population without obvious cervical spine pathology.

Fast conducting myelinated Aδ-fibres are responsible for transmission of signals that may lead to well-localised, sharp pain in response to mechanical and thermal noxious stimuli (Barrett *et al.*, 2012c; Lawson, 2002). Aδ-fibres have also been implicated in chronic pain maladaptivity, as altered activation and processing of non-nociceptor A-

fibre input may result in allodynia (Dubin & Patapoutian, 2010; Woolf & Mannion, 1999), a clinical feature commonly described in chronic WAD (Greening, Dilley, & Lynn, 2005; Hubbard & Winkelstein, 2005; Lee, Davis, Mejilla, & Winkelstein, 2004; Moog, Quintner, Hall, & Zusman, 2002). Slow conducting unmyelinated C-fibres, in contrast, are responsible for the transmission of signals that may lead to poorly localised, burning pain. Innervation by C-fibres is of clinical interest as maladaptive functioning of C-fibre nociceptors in the form of decreased activation threshold leads to hyperalgesia (Dubin & Patapoutian, 2010; Woolf & Mannion, 1999), a common clinical feature of chronic neck pain presentations (Javanshir, Ortega-Santiago, Mohseni-Bandpei, Miangolarra-Page, & Fernandez-de-las-Penas, 2010; La Touche *et al.*, 2010; Lauche, Cramer, Langhorst, Dobos, & Gerdle, 2014; Wallin, Liedberg, Borsbo, & Gerdle, 2012).

Furthermore, examining the existence of myelinated and unmyelinated nerve fibres within cervical spine meniscoids may be of relevance for the medical management of meniscoid related neck pain, as pharmacologic intervention for pain targets mechanisms of nociception through Aδ- and C-fibre pathways (Woolf & Mannion, 1999). An appreciation of the characteristics of cervical spine meniscoid innervation is thus pertinent to understanding their clinical significance and potential role in pathology. The aim of Study 4 was to investigate the presence of both myelinated and unmyelinated nerve fibres within cervical spine meniscoids and adjacent joint capsules, in order to gain insight into the characteristics of the innervation of these structures.

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Human research ethics approval and research governance approvals can be seen in Appendices C, D, I and J. Characteristics of cadavers included in the study are detailed in Appendices K and L, and bequeathal documentation can be seen in Appendix B.

Introduction

Cervical spine meniscoids, also referred to as synovial folds, are invaginations of synovial membrane that lie between the articular surfaces of the lateral atlantoaxial and cervical zygapophyseal joints (Farrell *et al.*, 2015a, 2016b; Farrell, Osmotherly, Rivett, & Cornwall, 2015b; Farrell *et al.*, 2015c; Inami *et al.*, 2000; Mercer & Bogduk, 1993; Webb *et al.*, 2012). Meniscoid function has been hypothesised to improve the congruence of the articular surfaces, and ensure the lubrication of the articular surfaces with synovial fluid (Mercer & Bogduk, 1993; Webb *et al.*, 2011a). These structures have been nominated as potential contributors to pain arising from spinal joints (Bogduk, 2011b; Mercer & Bogduk, 1993; Webb *et al.*, 2011a).

Cervical spine meniscoids are frequently found to be present in lateral atlantoaxial joints (100% (Farrell *et al.*, 2016b; Webb *et al.*, 2011b; Webb *et al.*, 2012)) and cervical

zygapophyseal joints (77% – 100% (Farrell *et al.*, 2015a; Inami *et al.*, 2000; Kos *et al.*, 2002; Mercer & Bogduk, 1993)). These structures are composed of adipose tissue, fibrous tissue, or a mix of both adipose and fibrous tissues (Farrell *et al.*, 2015a, 2016b; Inami *et al.*, 2000; Webb *et al.*, 2011a). The mechanism of meniscoid involvement in pathology remains unclear due to uncertainty about their innervation and therefore potential to generate nociceptive input (Webb *et al.*, 2011a), and it is unclear whether meniscoid composition has any relationship to the presence of innervation.

Few studies have examined the innervation of meniscoids in any region of the spine to determine their potential to generate nociceptive input (Bogduk, 2011a; Bogduk & McGuirk, 2006; Webb *et al.*, 2011a). In the lumbar spine, Giles and Harvey (1987) demonstrated the presence of SP reactive nerve fibres in lumbar zygapophyseal joint meniscoids, and Giles and Taylor (1987) reported small, myelinated fibres coursing through lumbar spine meniscoid tissue; both findings suggestive of nociceptive potential (Nathan, 1977). Gronblad *et al.* (1991) however suggested that PGP 9.5 immunoreactive nerve fibres observed in lumbar spine meniscoids were more likely to be responsible for local vasoregulation, rather than nociception, due to their proximity to blood vessels. In the cervical spine, there has only been one study investigating innervation of meniscoids. Inami *et al.* (2001) described small nerve fibres immunoreactive to antibodies to SP, CGRP, β-III tubulin and PGP 9.5 in ten cervical spine meniscoids excised from five patients (mean age 53 years) during laminoplasty for cervical spine pathology, suggestive of innervation by C-fibres with likely nociceptive and vasoregulatory functions (Barrett *et al.*, 2012a; Benarroch, 2011; Henry, 1982).

A detailed understanding of the innervation of cervical spine meniscoids is important in order to effectively underpin evidence-based clinical management of cervical spine pain. The only previous investigation of cervical spine meniscoid innervation examined a small sample of meniscoids in surgical patients (Inami *et al.*, 2001), and it is therefore unclear whether meniscoid innervation is similar in a population that does not have obvious cervical pathology that requires surgery. Further, the existence of different nerve *types* in cervical spine meniscoid innervation is unknown. Both large (Aδ-) and small (C-) nerve fibres serve nociceptive functions (Barrett *et al.*, 2012c; Lawson, 2002), and maladaptive function of both these nerve fibre types has been implicated in chronic pain pathophysiology as contributors to hyperalgesia and allodynia (Dubin & Patapoutian, 2010; Voscopoulos & Lema, 2010; Woolf & Mannion, 1999). Understanding if and how meniscoids are innervated, and by what type of nerve, is important in regard to targeted therapeutic intervention for different mechanisms of nociception (Woolf & Mannion, 1999).

Given the potential clinical significance of cervical spine meniscoids (Mercer & Bogduk, 1993; Webb *et al.*, 2011a) and the substantial social burden of musculoskeletal neck pain (Cote *et al.*, 2008; Hogg-Johnson *et al.*, 2008; Holm *et al.*, 2008), the aim of this study was to explore the presence and morphology of innervation in cervical spine meniscoids to facilitate greater understanding of how these structures may contribute to spinal pain and pathology.

Materials and Methods

Cervical spine meniscoids from elderly cadavers were removed and processed using immunohistochemistry to identify nerve presence and morphology; meniscoid morphology was determined using histology (Farrell *et al.*, 2015a, 2016b). Antibody to neurofilament heavy (NF-H) was used to identify the presence of myelinated nerve fibres (Reinisch & Tschachler, 2005; Watanabe *et al.*, 2007), consistent with the presence of Aδ- or Aβ-fibres that conduct information on pain and kinesthesia, respectively (Barrett *et al.*, 2012b). Antibody to neurofilament (Pan-NF) was used as a pan-axonal marker to identify any nerves present. Pan-NF antibodies have previously been shown to identify both unmyelinated and myelinated nerve fibres (Dau & Wenthold, 1985; Hafidi & Romand, 1989; Lauria *et al.*, 2004; Lopez *et al.*, 2005).

Ethics Statement

Ethical approval to undertake this study was granted by The University of Newcastle Human Research Ethics Committee. Bodies were bequeathed in accordance with the Human Tissue Act (2008) of New Zealand, and written informed consent was obtained from donors prior to death. The study was undertaken in accordance with the ethical standards of the Declaration of Helsinki.

Dissection

The cervical spines of twelve cadavers were sourced from the University of Otago Department of Anatomy (mean [SD] donor age 82.9 [6.5] years, six female). Cadavers were embalmed using ethanol and water based solutions as per departmental protocol, and were included for assessment if their lateral atlantoaxial and cervical zygapophyseal joints were intact; exclusion criteria included previous surgery to the region that was macroscopically apparent.

Overlying skin and muscles were removed from each specimen to expose the skull and vertebral column and allow the occiput to be disarticulated from the atlas. A dental burr (Beaver Ace Dental Micro Engine, Osada Electric Co. Ltd., Tokyo, Japan) was used to drill through the posterior arch of the atlas at either side of the posterior tubercle, to allow removal of the posterior aspect of the bone. A bone saw was then used to cut along the longitudinal axis of the vertebral column through the laminae and pedicles, such that the cervical articular pillars could be separated from the vertebral bodies. Through a process of random allocation either the left or right articular pillar of each specimen was selected for inclusion in the study, such that an even number of left and right sides made up the sample.

Lateral atlantoaxial and cervical zygapophyseal (C2-3 to C6-7) joints were dissected using a surgical microscope (Op-Mi 6, Carl Zeiss, Jena, West Germany) by carefully cutting along the superior attachment of the joint capsule, such that each joint could be gently disarticulated by lifting the inferior articular facet of the superior vertebra away from the superior articular facet of the inferior vertebra. This left the joint capsule and

any enclosed meniscoids *in situ* upon the superior articular facet of the inferior vertebra.

Each identified cervical spine joint and meniscoid was examined using a surgical microscope and meniscoid presence and location within the joint was recorded. The superior aspect of the lateral edge of each meniscoid was marked with tissue ink and photographed using a Canon Powershot G10 14.7 megapixel camera (Canon Inc., Tokyo, Japan) to facilitate medial-lateral and superior-inferior orientation. Each meniscoid and adjacent joint capsule was then excised from its disarticulated joint using a scalpel, and stored in an individual specimen jar immersed in phosphate buffered saline with 0.05% sodium azide.

Immunohistochemistry and Histology

Cervical spine meniscoids were embedded in paraffin blocks, sectioned sagittally (5 μm) through their midpoint at right angles to their widest margins in the transverse plane (i.e. sagittally through their middle point), and mounted on microscope slides. Prior to immunohistochemistry, sections were dewaxed through a series of ethanol and xylene baths. Sections were then immersed in 3% methanolic hydrogen peroxide (10 minutes) before undergoing antigen retrieval using a laboratory microwave at 95°C in citrate buffer (pH 6.0) for 25 minutes, then left at room temperature to cool.

Sections underwent 30 minutes incubation with 10% bovine serum albumin (BSA) at 37°C, before being incubated with primary antibodies (1:3500 dilution in 2% BSA, Neurofilament H [NF-H] non-phosphorylated monoclonal antibody [catalogue no. SMI-32R], mouse raised, Covance Inc., Princeton, NJ; 1:3000 dilution in 2% BSA, Anti-

Neurofilament [Pan-NF] monoclonal antibody [catalogue no. SMI-312], mouse raised, Abcam, Cambridge, MA) for one hour at room temperature. Sequential sections were processed from each meniscoid: one section was incubated using antibody to NF-H and the next using antibody to Pan-NF. Positive (spinal nerve sections) and negative (adjacent meniscoid sections processed without the primary antibody) control sections were processed during each run; negative controls were processed for each individual meniscoid sample, while a single positive control was used for each individual run of sections.

Sections were then incubated with secondary antibody (EDL, undiluted, Dako Envision Link System, Glostrup, Denmark) for 30 minutes at room temperature, followed by exposure to diaminobenzidine for four minutes. Sections were then counterstained with Mayer's haematoxylin and dehydrated with a series of ethanol and xylene baths prior to cover slipping in DPX mounting medium (Thermo Scientific, Manor Park, United Kingdom) and examination by light microscopy (Olympus SZ-STS with SZ-40 lens, Olympus, Tokyo, Japan) using a digital microscope camera (Moticam 1000 1.3 MP USB 2.0, Motic Inc. Ltd., Causeway Bay, Hong Kong) and Motic Image Plus 2.0 software platform (Motic Inc. Ltd., Causeway Bay, Hong Kong). All sections were then assessed for staining to the primary antibodies and observed for histological composition based on the protocol of Farrell *et al.* (2015; 2016). Positively stained nerve fibres were assessed visually by counting the number of nerve fibre bundles present, number of fibres within bundles, location of nerve fibre bundles within the meniscoid or joint capsule, and proximity to blood vessels. Fibre diameter was measured using Adobe Photoshop by comparing linear measures of the study images

with a scale bar slide.

Results

Of the 72 lateral atlantoaxial and cervical zygapophyseal joints available, 67 were successfully disarticulated. Five joints could not be disarticulated due to extensive degenerative changes. Seventy-nine cervical spine meniscoids were identified in the remaining 67 joints, two of which were damaged in processing and could not be included in subsequent analyses, resulting in a final sample of 77 meniscoids. This meant 154 paired, sequential sections were processed successfully for either Pan-NF or NF-H and subsequently included in analysis (

Table 8).

Table 8: Positions of identified meniscoids within lateral atlantoaxial (LAA) and cervical zygapophyseal joints (n = 77).

Meniscoid Location	**LAA (n)**	**C2-3 (n)**	**C3-4 (n)**	**C4-5 (n)**	**C5-6 (n)**	**C6-7 (n)**	**Total**
Ventral pole	11	4	8	5	6	5	39
Dorsal pole	11	3	6	6	7	2	35
Other - Lateral edge - Spanning entire joint	 1			 1		 1	 2 1
Total	23	7	14	12	13	8	77

NF-H Immunohistochemistry

Positive staining to NF-H was found in 13 sections; almost exclusively axons identified by NF-H were less than 10 μm in diameter. Details of the joints in which these meniscoids were located, position of meniscoids within these joints and histological composition of meniscoids can be see in **Table 9**.

NF-H nerve fibres were observed in the fibrous tissue of joint capsules and fibrous and adipose tissue at the junction of joint capsules and bases of meniscoids (**Figure 14**; **Figure 15**). Such positively stained fibres were 2-10 μm in diameter, and were identified in bundles ranging from six to eight fibres (n = 22) to larger bundles of approximately 200 fibres (n = 4). In 10 specimens nerve fibre bundles were noted in close proximity to blood vessels. The numbers of NF-H immunoreactive fibre bundles observed in each specimen can be seen in **Table 9**. NF-H immunoreactive fibres were not observed within the meniscoid proper of any of the specimens examined.

Table 9: Characteristics of cervical spine meniscoids containing nerve fibres immunoreactive to antibodies to neurofilament heavy and pan-neurofilament, including location and numbers of positively stained nerve fibre bundles.

Donor	Joint	Location of Meniscoid	Histo.	Location of Nerves	NF-H +ve Nerve Fibre Bundles (n)	Pan-NF +ve Nerve Fibre Bundles (n)	Pan-NF +ve & NF-H -ve Nerve Fibre Bundles (n)
1	LAA	V	A	JC & MB	1	1	0
1	LAA	D	FA	JC	4	4	0
2	LAA	V	A	JC & MB	1	2	1
2	LAA	D	F	JC	1	2	1
3	LAA	D	F	JC	1	3	2
4	LAA	SJ	F	JC	1	1	0
5	LAA	D	F	JC	4	6	2
6	LAA	V	F	JC	1	2	1
7	LAA	D	FA	JC	5	5	0
8	LAA	D	F	JC	2	2	0
3	C2-3	V	F	JC	0	1	1
3	C3-4	V	FA	JC	1	5	4
8	C3-4	V	FA	JC	2	2	0
8	C5-6	D	FA	JC	2	2	0

Legend: Histo. – histological composition of meniscoid; NF-H – neurofilament heavy; Pan-NF – pan-neurofilament; LAA – lateral atlantoaxial joint; V – ventral; D – dorsal; SJ – spanning joint ventral to dorsal; A – adipose; FA – fibroadipose; F – fibrous; JC – joint capsule; MB – meniscoid body

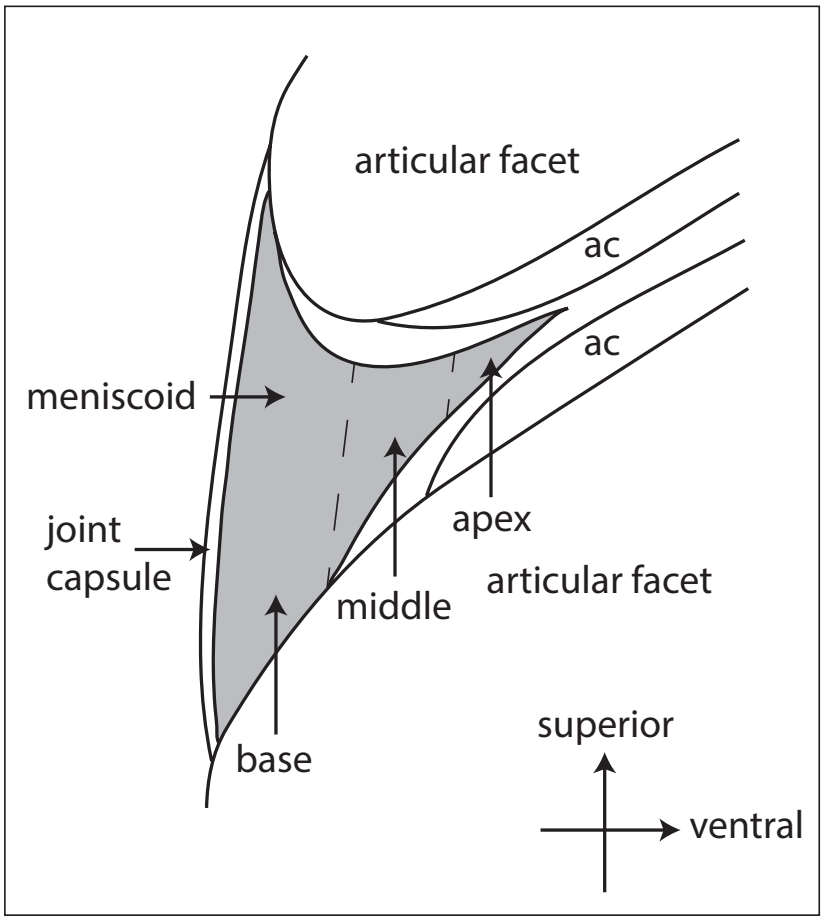


Figure 14: Schematic representation of sagittal section of cervical spine meniscoid at dorsal aspect of a zygapophyseal joint. Basal, middle and apical regions forming the body of a meniscoid are shown as per Webb *et al.* (2011a). ac = articular cartilage.

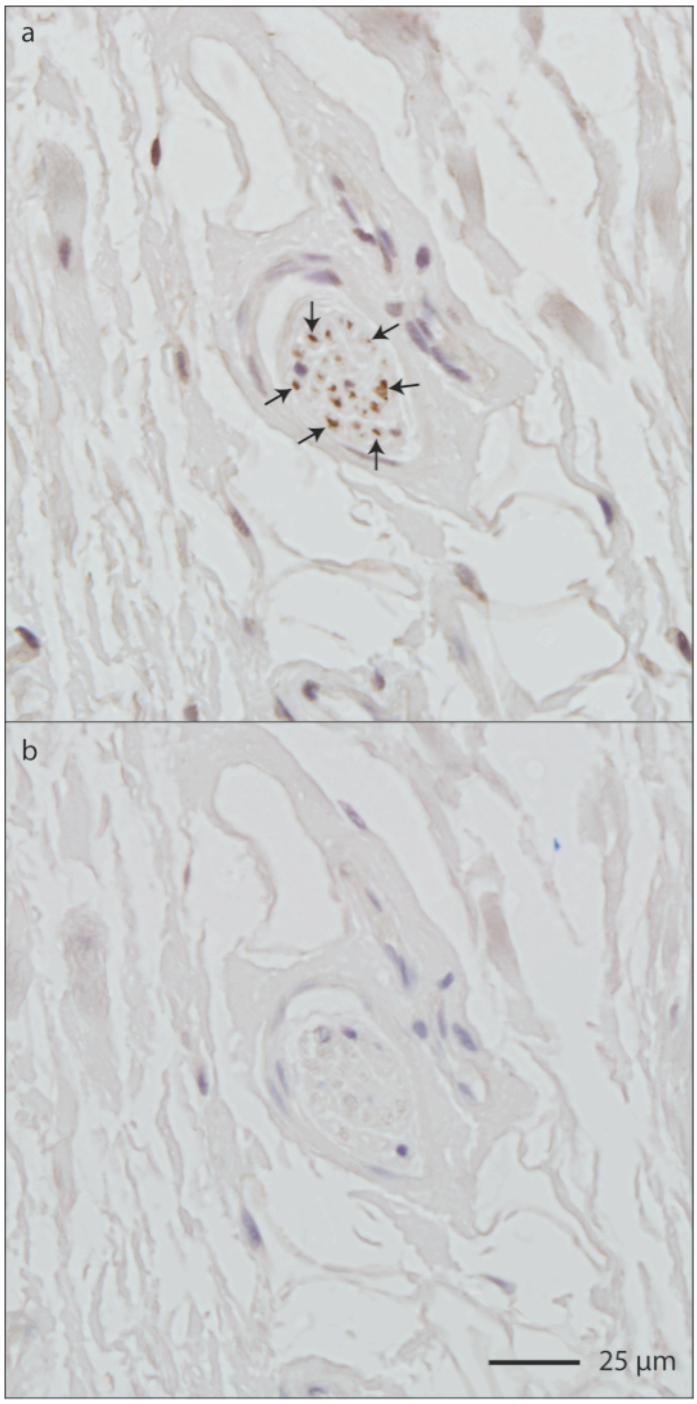


Figure 15: Nerve fibres immunoreactive to antibody to neurofilament heavy using diaminobenzidine immunohistochemistry located in the dorsal joint capsule of a lateral atlantoaxial joint from a 73-year-old female cadaver. Haematoxylin counterstaining. (a) Positively stained fibres (brown staining); (b) adjacent section processed as negative control.

Pan-NF Immunohistochemistry

Positive staining immunoreactive to Pan-NF was found in 14 sections. Details of the joints in which these meniscoids were located, position of meniscoids within these joints and histological composition of meniscoids can be see in **Table 9**.

Similar to NF-H, Pan-NF immunoreactive nerve fibres were primarily observed in the fibrous tissue of joint capsules (**Figure 16**). However, in two ventral meniscoids of lateral atlantoaxial joints, Pan-NF immunoreactive fibres were observed within the meniscoid (**Figure 16**). In one of these specimens, two small nerve fibre (approximately 1 μm diameter) bundles were noted in the base of the meniscoid in close proximity to blood vessels, and in the other a nerve fibre bundle was located in the middle region of the meniscoid body separate from blood vessels. These meniscoids were primarily adipose in composition.

Pan-NF positively stained fibres were observed in bundles ranging from six to eight fibres (n = 34) to larger bundles of approximately 200 fibres (n = 4) (**Figure 17**). In 11 specimens, nerve fibre bundles were noted in close proximity to blood vessels. The numbers of Pan-NF immunoreactive fibres observed in each specimen can be seen in **Table 9**.

Figure 16: Following page – Nerve fibres immunoreactive to antibody to pan-neurofilament (Pan-NF) using diaminobenzidine immunohistochemistry located in the ventral joint capsule and meniscoid of a lateral atlantoaxial joint from an 83-year-old male cadaver. Haematoxylin counterstaining. (a) Positively stained fibres (brown staining) within joint capsule; (b) section of joint capsule adjacent to a) processed as negative control demonstrating no staining; (c) sagittal section of meniscoid, red rectangle indicates location of Pan-NF immunoreactive fibres within body of meniscoid viewed at x4 magnification; (d) area of red rectangle from (c) viewed at x40 magnification demonstrating bundle of small nerve fibres.

a
25 µm
b
25 µm
c
500 µm
d
50 µm

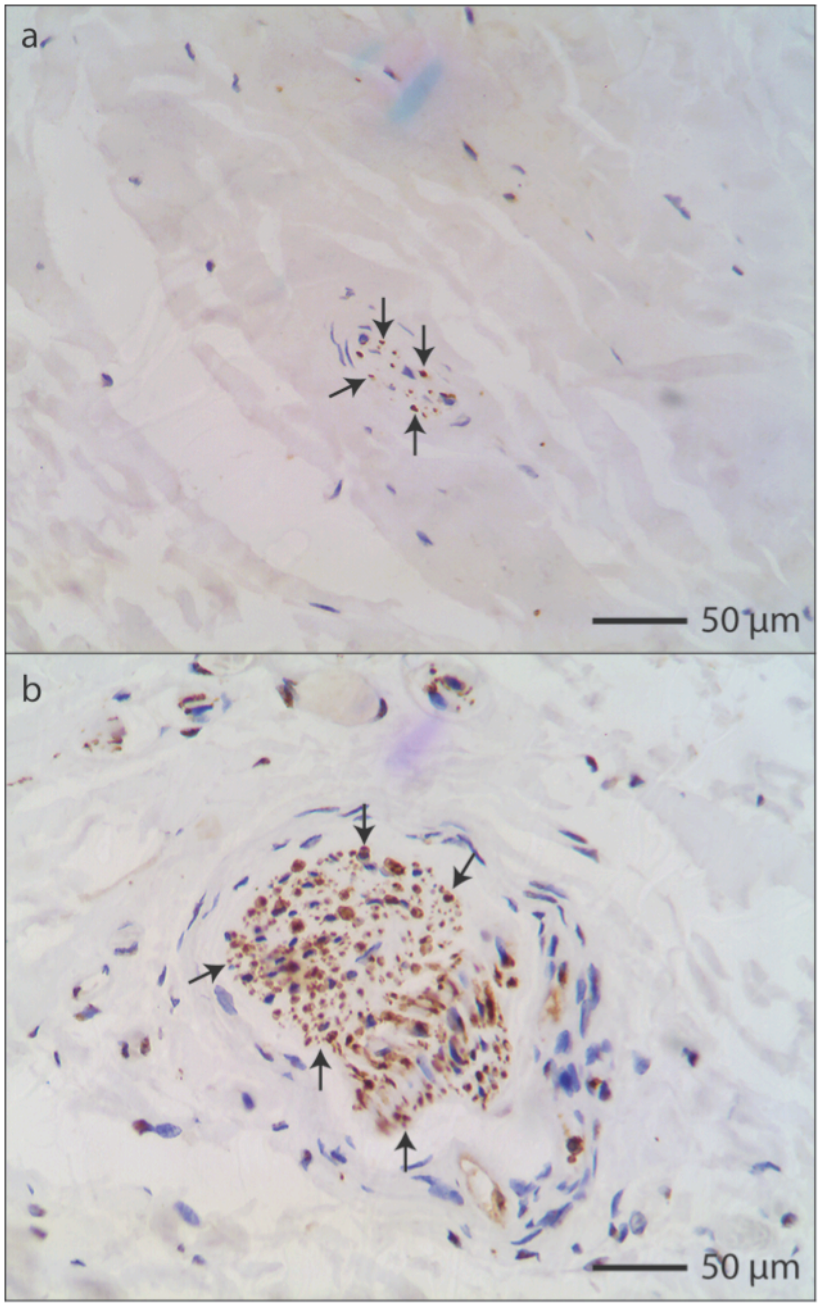


Figure 17: Nerve fibres immunoreactive to antibody to pan-neurofilament using diaminobenzidine immunohistochemistry with haematoxylin counterstaining (brown staining). (a) Small bundle of nerve fibres from dorsal aspect of a lateral atlantoaxial joint from an 83-year-old male cadaver; (b) large bundle of nerve fibres from dorsal aspect of a lateral atlantoaxial joint from a 77-year-old female cadaver.

Comparison of NF-H and Pan-NF Staining

In seven cervical spine meniscoids, all nerve fibre bundles were immunoreactive to both the NF-H and Pan-NF antibodies in adjacent sections (**Table 9**). In a further seven specimens, some nerve fibre bundles demonstrated positive immunoreactivity to Pan-NF antibody that were not immunoreactive to NF-H antibody. Six of these seven specimens contained multiple Pan-NF immunoreactive nerve fibre bundles within the sections, at least one of which stained positively for NF-H, and the remaining specimen contained a single Pan-NF immunoreactive nerve fibre bundle that did not stain positively to NF-H.

Histology

Each meniscoid featured basal, middle and apical regions (**Figure 14**). Cervical spine meniscoids displayed three patterns of histological composition consistent with previous descriptions (Farrell *et al.*, 2015a, 2016b; Inami *et al.*, 2000): primarily composed of adipose tissue, primarily composed of fibrous tissue, or of mixed composition (i.e. fibroadipose). Five (6.5%) meniscoids were primarily adipose tissue, 41 (53.2 %) meniscoids were fibrous, and 31 (40.3%) meniscoids were fibroadipose in composition. Small blood vessels were commonly noted traversing the basal regions of adipose and fibroadipose meniscoids, adjacent to the point of attachment to the fibrous joint capsule, however were less frequently observed in fibrous meniscoids.

Discussion

The present study has examined the existence of nerve tissue within cervical spine meniscoids and adjacent joint capsules in human cadavers using immunohistochemistry, finding some nerves in joint capsules of lateral atlantoaxial and cervical zygapophyseal joints, and very few nerves within the bodies of cervical spine meniscoids. NF-H immunohistochemistry identified bundles of fibres in some joint capsules, with no NF-H immunoreactive fibres in cervical spine meniscoids. Pan-NF immunohistochemistry showed nerve fibres in joint capsules and occasionally within the bodies of adipose meniscoids of lateral atlantoaxial joints. These findings have implications for our understanding of pain generation in the elderly cervical spine, with the low prevalence of nerves in cervical spine meniscoids suggesting their role in generation of nociceptive input in elderly neck pain may be limited.

Nerve fibres consistent with C-fibres have been previously reported within cervical spine meniscoids by Inami *et al.* (2001). No study however has explicitly investigated the presence of myelinated fibres within cervical spine meniscoids. In keeping with Inami *et al*. (2001), findings of the present study suggest that unmyelinated fibres – positive for Pan-NF but negative for NF-H – consistent with C-fibres exist within the bodies of some elderly cervical spine meniscoids. Furthermore, the present study also found that myelinated fibres – positive for both Pan-NF and NF-H – consistent in morphology almost exclusively with Aδ-fibres (Barrett *et al.*, 2012b) exist within adjacent joint capsules, but not within the bodies of cervical spine meniscoids.

Evidence of nerve fibres within the basal or middle regions, but not the apical region, of a cervical spine meniscoid was found in just two specimens. This incidence is considerably lower than that of Inami *et al*. (2001) who found multiple nerve fibres within the adipose tissue of every processed section from all ten cervical spine meniscoids excised from five people suffering cervical myelopathy undergoing laminoplasty. These observations may be related to the different samples of each study as the present study involved elderly cadavers (mean age 82.9 years) of unknown medical history, whereas Inami *et al.* (2001) studied a younger population (mean age 52.8 years) that all had pre-existing spinal pathology requiring surgery. Further, we examined the midpoint of each meniscoid with sequential 5 μm sections, whereas Inami *et al.* (2001) included multiple sequential sections of 50 μm in their analysis. Despite these methodological differences the low prevalence of nerves in meniscoids in this sample is significant because it suggests potential differences in meniscoid innervation between younger and elderly cervical meniscoids. This potentially has consequences for effective and targeted management of neck pain in an elderly demographic, with elderly meniscoids perhaps not being as densely innervated by nociceptors as meniscoids in young cervical spines or those spines which require surgery, such as those in the study by Inami *et al.* (2001).

Nerve fibre bundles were only observed within the bodies of adipose cervical spine meniscoids. Inami *et al*. (2001) found nerves to be present in the adipose and fibrous connective tissue basal regions of meniscoids examined in their study, but not in fibrous apical regions. Adipose meniscoids made up 6.5% of the specimens examined in the present study, with fibrous meniscoids representing more than half of the specimens and mixed fibroadipose composition forming the remainder. Inami *et al*. (2001) in contrast

reported 70.0% of meniscoids in their study to be composed primarily of adipose tissue, while the remainder were adipose with a fibrous apical region. It appears that nerve fibre bundles are located within the adipose tissue of cervical spine meniscoid bodies but not fibrous tissue, and the specimens studied by Inami *et al*. (2001) comprised a greater proportion of adipose tissue meniscoids than the present study, possibly due to age differences between the samples (Webb *et al.*, 2011a). This suggests that innervation of a cervical spine meniscoid may be associated with meniscoid composition. This finding is relevant because of the potential for imaging modalities such as MRI to accurately identify meniscoid composition (Friedrich *et al.*, 2008; Friedrich *et al.*, 2007), therefore aiding diagnosis of cervical pathology if innervation is shown to be related to meniscoid morphology. However, the low numbers of adipose meniscoids and nerves identified within meniscoids should be considered when interpreting these data, preventing any meaningful statistical analysis.

Another possible explanation for the disparity of frequencies of nerve fibres observed in the present study and Inami *et al*. (2001) may be related to reorganisation or 'sprouting' of sensory nerve fibres associated with tissue injury, inflammation and longstanding pain. It must be considered that Inami *et al.* investigated meniscoids excised from participants undergoing laminectomy for cervical myelopathy, removing the dorsal meniscoids at the affected spinal level. Sensory innervation may vary in the presence of pathology, as increased frequency of sensory nerve fibres has been observed in arthritic knee pain (Jimenez-Andrade & Mantyh, 2012), breast pain (Gopinath *et al.*, 2005), cancer-related pain (Jimenez-Andrade *et al.*, 2010) and non-healed fractures (Chartier *et al.*, 2014), and hypersensitivty of regenerating axonal sprouts is thought to contribute to the perpetuation of symptoms in chronic pain (Jimenez-Andrade & Mantyh, 2012;

Ropper, Samuels, & Klein, 2014). As all participants included in the study of Inami *et al*. had sufficient pain to warrant surgical intervention, and the neck pain statuses of the cadavers of the present study are unknown, it is plausible that the high nerve fibre frequency reported by Inami *et al*. represents sprouting of sensory nerve fibres in the context of longstanding pain and inflammation.

In the zygapophyseal joint capsules of the cervical and lumbar spines, both myelinated and unmyelinated nerve fibres have previously been described by studies employing immunohistochemistry (Giles & Harvey, 1987; Kallakuri, Li, Chen, & Cavanaugh, 2012; Kallakuri, Singh, Chen, & Cavanaugh, 2004), electron microscopy (Giles & Taylor, 1987; Giles, Taylor, & Cockson, 1986), silver or gold chloride impregnation (Giles & Taylor, 1987; McLain, 1994) and neurophysiological electrical stimulation (Chen, Lu, Kallakuri, Patwardhan, & Cavanaugh, 2006). The findings of the present study concur with those of Chen *et al*. (2006) who reported neurophysiological evidence of both Aδ- and C-fibres in the cervical zygapophyseal joint capsules of goats. The size of the myelinated fibres described in the joint capsules in the present study is consistent with Aδ- (most prevalent) and Aβ-fibres (which were very few in number), which transmit information regarding nociception and temperature, and touch and pressure respectively (Barrett *et al.*, 2012b; Barrett *et al.*, 2012c). Current results therefore corroborate the reported potential for joint capsules to be considered nociception-generating structures in the elderly cervical spine.

A large proportion of the small, Pan-NF positive nerve fibre bundles identified in the present study were located in close proximity to blood vessels, inferring a potential vasoregulatory function as suggested by Gronblad and colleagues (1991). In

concurrence with Inami *et al.* (2001) nerve fibres were also observed independent of blood vessels, and it is therefore likely that these structures represent nerves capable of nociception.

Interestingly, ten of the 14 cervical spine meniscoid specimens that demonstrated immunohistochemical evidence of nerve fibre presence were excised from lateral atlantoaxial joints. The reason for the disproportionate representation of this joint in the results is not clear. It is established that cervical zygapophyseal joint capsules are innervated (Kallakuri *et al.*, 2012; Kallakuri *et al.*, 2004), however in the present study the larger size of lateral atlantoaxial joint meniscoids, and in turn adjacent joint capsules, when compared to zygapophyseal joint meniscoids (Farrell *et al.*, 2015a, 2016b) may somewhat account for the high proportion of identified nerve fibres located in lateral atlantoaxial joints. This finding is consistent with suggestions of the lateral atlantoaxial joints as a possible source of headache (Aprill *et al.*, 2002; Bogduk, 2011a; Ehni & Benner, 1984).

Limitations and Future Research

The cadavers included in this study were elderly, which likely affects cervical spine meniscoid morphology (Farrell *et al.*, 2015a, 2016b), and limits the capacity to generalise these results to a younger population. Furthermore, whilst information regarding donor cause of death was available, accompanying medical records did not specify if donors suffered cervical spine pathology and only macroscopic inspection of specimens was used to exclude those samples which may have had obvious cervical spine pathology. Embalmed tissue can reduce the ability for antibody binding reactions

in immunohistochemistry (Haines & Chelack, 1991; Shi, Key, & Kalra, 1991), however our positive and negative control slides were consistent throughout the study and support the robustness of the methodology.

Of particular clinical interest is the capacity for cervical spine meniscoids to generate nociceptive input. Whilst antibodies to NF-H and Pan-NF provide insight into the presence of myelinated and unmyelinated nerve tissue (Dau & Wenthold, 1985; Hafidi & Romand, 1989; Lauria *et al.*, 2004; Lopez *et al.*, 2005; Reinisch & Tschachler, 2005; Watanabe *et al.*, 2007), staining with these antibodies is not specific to nerve fibres with nociceptive function, as is the case for antibodies to SP or CGRP (Barrett *et al.*, 2012a; Benarroch, 2011; Henry, 1982). This would have been desirable to distinguish more clearly between vasoregulatory and nociceptive nerve fibres to provide further insight into the possible contribution of these structures to nociceptive input, however pilot testing using these antibodies was unsuccessful, possibly due to the limitations of undertaking imunohistochemistry in cadaveric tissue noted above. The use of antibodies to SP and CGRP should be considered in future research on this topic.

Conclusions

This study has demonstrated the presence of NF-H and Pan-NF positive nerve tissue within the cervical spine meniscoids and joint capsules of elderly cadavers. The low nerve fibre prevalence in elderly cervical spine meniscoids suggests these structures may play a minimal role in cervical pain generation in this population, with the more frequently innervated joint capsules potentially more substantial contributors to nociceptive input. Findings indicate that both unmyelinated and myelinated nerve fibres

are present within lateral atlantoaxial and cervical zygapophyseal joint capsules, and that a small number of unmyelinated nerve fibres exist within meniscoids composed of adipose tissue. Nerves were noted both independent to and in close proximity to blood vessels, inferring potential sensory and vasoregulatory functions. Furthermore, the few nerves identified within meniscoids were only located in adipose tissue, similar to the single previous study on this topic, raising the possibility that innervation status may be related to meniscoid composition.

Summary

Study 4 has used immunohistochemistry to investigate the presence of myelinated and unmyelinated nerve fibres within the cervical spine meniscoids of cadavers. Whilst nerve fibres consistent with unmyelinated C-fibres have previously been described within cervical spine meniscoids (Inami *et al.*, 2001), no prior study has explicitly investigated the presence of myelinated fibres within cervical spine meniscoids.

Our findings indicate that cervical spine meniscoids in the elderly contain no myelinated nerve fibres, however myelinated fibres are present in fibrous and adipose tissue of the adjacent joint capsules. These fibres may represent Aδ-fibres and in turn serve a nociceptive function, and may feasibly give rise to neck pain in instances of capsular distension, as is hypothesised in the Entrapment and Extrapment theories. Unmyelinated nerve fibres were also noted in joint capsules, defined as nerve fibre bundles immunoreactive to Pan-NF but not to NF-H. A small number of nerve fibres likely to be unmyelinated nerves were found within the bodies of two adipose cervical spine meniscoids. This frequency of observation is considerably less than that reported

by Inami *et al.* (2001) and may be due to the methodological challenges posed by undertaking immunohistochemistry on embalmed tissue, inherent differences between the elderly sample of Study 4 and the group of young, cervical myelopathy patients of Inami *et al*. (2001), or perhaps an association between meniscoid composition and innervation status. Nevertheless, this finding confirms the plausibility of cervical meniscoids being innervated by C-fibres with potential nociceptive function.

Immunohistochemistry utilises the capacity of an antibody to bind to an antigen of interest in a tissue specimen (Haines & Chelack, 1991). When tissue is fixed during the embalming process, the ability for such reactions to occur may be compromised (Haines & Chelack, 1991; Shi *et al.*, 1991), which reduces the effectiveness of staining. This was a significant challenge for this study, as illustrated by the extensive pilot testing of different primary antibodies and immunohistochemical approaches outlined in Appendix O. The final immunohistochemical protocol can be seen in Appendices P and Q.

Studies 2, 3 and 4 have examined aspects of cervical spine meniscoid morphology in cadavers. Study 5 (Chapter 7) will investigate cervical spine meniscoid morphology in a living clinical population, in order to gain insight into potential pathoanatomical characteristics that may exist in cervical spine meniscoids as compared to a pain-free control population.

Chapter 7: MORPHOLOGY OF CERVICAL SPINE MENISCOIDS IN CHRONIC WHIPLASH ASSOCIATED DISORDER

Studies 2, 3 and 4 (Chapters 4, 5 and 6) have examined cervical meniscoid morphology in elderly cadavers. This involved the use of gross dissection, histology and immunohistochemistry to assess aspects of cervical spine meniscoid morphology, including morphometrics, composition, associated articular degeneration and presence of nerve tissue. However, these methods of enquiry are not compatible with a living sample and the presence of cervical spine pathology was not disclosed in available medical records for the donors.

To advance current understanding of the potential role of cervical spine meniscoids in pathology, the morphology of these structures must be examined in a population with known neck pain and compared with a pain-free population. Historically, this has only been achievable in people after death, due to the location of cervical spine meniscoids necessitating disarticulation or sectioning of a joint to allow meniscoids to be seen. However with recent advances in radiographic technology, cervical spine meniscoids have been visualised using MRI (Friedrich *et al.*, 2008; Friedrich *et al.*, 2007; Webb *et al.*, 2011b; Webb *et al.*, 2009), thus facilitating investigation of the morphology of these structures in a living clinical population.

As discussed in Chapter 2 (Literature Review), cervical spine meniscoids have been implicated as structures potentially damaged in a whiplash incident (Bogduk, 2011b; Kaneoka *et al.*, 1999; Webb *et al.*, 2011a). This is due to their location between articular surfaces making them vulnerable to damage during the abnormal compressive forces of a whiplash event (Bogduk, 2011b; Kaneoka *et al.*, 1999) and the substantial clinical evidence implicating cervical zygapophyseal joints as sources of nociceptive input in chronic WAD (Barnsley *et al.*, 1995; Bogduk, 2011b; Lord *et al.*, 1996a; Lord, Barnsley, Wallis, McDonald, & Bogduk, 1996b; Yin & Bogduk, 2008).

Study 5 sought to examine cervical spine meniscoid morphology in a living population with chronic WAD. Such data will advance understanding of the clinical significance of cervical spine meniscoids by comparing the size and composition of these structures to those in matched controls, in turn potentially providing insight into morphological differences that may have pathoanatomical implications.

This manuscript has been accepted for publication as original research:

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (*in press*). Morphology of cervical spine meniscoids in individuals with chronic whiplash associated disorder: a case-control study. *Journal of Orthopaedic and Sports Physical Therapy*.

Human research ethics approval and research governance approvals can be seen in Appendices E-G, R and S. The recruitment flyer, participant information sheet, consent form and safety screening form for this study can be found at Appendices T-W respectively.

Introduction

Clinical, biomechanical and post-mortem studies have established the cervical zygapophyseal joints as a source of nociceptive input in chronic whiplash associated disorder (WAD) (Barnsley *et al.*, 1995; Bogduk, 2011b; Kaneoka *et al.*, 1999; Lord *et al.*, 1996a; Siegmund *et al.*, 2009; Uhrenholt, Grunnet-Nilsson, & Hartvigsen, 2002; Yin & Bogduk, 2008). These joints contain intra-articular folds of synovium, known as meniscoids (Farrell *et al.*, 2015b; Inami *et al.*, 2000; Mercer & Bogduk, 1993) which have been proposed as possible contributors to cervical spine pain including in people with chronic WAD (Bogduk, 2011b; Inami *et al.*, 2000; Inami *et al.*, 2001; Kaneoka *et al.*, 1999; Mercer & Bogduk, 1993; Siegmund *et al.*, 2009; Webb *et al.*, 2011a). Cervical spine meniscoids are known to be composed of fibrous tissue, adipose tissue or a mixture of fibrous and adipose tissue, and are thought to function to ensure the lubrication of articular surfaces during cervical spine movements (Farrell *et al.*, 2015a, 2016b; Inami *et al.*, 2000; Mercer & Bogduk, 1993).

Cervical spine meniscoids have been implicated as structures potentially damaged by the mechanical trauma of a whiplash event (Bogduk, 2011b). Kaneoka *et al.* (1999) and Grauer *et al*. (1997) found that shortly following a rear-impact motor vehicle collision (MVC) the lower cervical spine moves into extension and the upper cervical spine

moves into flexion, forming a characteristic S-shape. At the lower cervical vertebrae, the extension motion exceeds the physiologic limits of the joints and could plausibly induce sub-failure injuries to facet joint capsules, intervertebral discs, and spinal ligaments (Bogduk, 2011b; Grauer *et al.*, 1997). During this motion, the superior articular surfaces of the lower cervical zygapophyseal joints are driven inferiorly into the articular facets of the vertebrae below, potentially resulting in damage to the meniscoids that lie between the joint surfaces (Bogduk, 2011b; Kaneoka *et al.*, 1999; Siegmund *et al.*, 2009).

Consistent with this notion, autopsy studies have reported pathological findings, including contusions or tears, in cervical spine meniscoids in individuals post MVC and post blunt head trauma (Schonstrom *et al.*, 1993; Taylor & Taylor, 1996). Immunohistochemical examination of cervical spine meniscoids excised from patients post-surgery has demonstrated evidence of nerve fibre presence within meniscoids and, in turn, potential to generate nociceptive input (Inami *et al.*, 2001). Dissection studies of elderly cadavers have also noted an association between fibrous tissue meniscoid composition and articular cartilage degeneration (Farrell *et al.*, 2015a, 2016b; Inami *et al.*, 2000), inferring a possible relationship between meniscoid morphology and articular pathology. However, the morphological properties of cervical meniscoids have not yet been investigated in a living population with known cervical spine pathology such as WAD, and as such the clinical implications of the above findings are speculative.

Cervical spine meniscoids have been visualised and quantitatively assessed using magnetic resonance imaging (MRI) *in vivo* (Friedrich *et al.*, 2008). These authors examined cervical spine meniscoid morphometry and composition in a sample of pain-

free volunteers, and described three classifications of meniscoid composition (adipose; fibrous; mixed/fibroadipose), as well as meniscoid size at each cervical level. The present study seeks to extend upon this work and aims to explore the morphometry and composition of cervical spine meniscoids in a population experiencing chronic WAD, as compared to pain-free individuals, to extend understanding of the potential pathoanatomical role of cervical spine meniscoids in WAD.

Methods

Ethics Statement

Institutional ethical approval was obtained from Hunter New England Local Health District Human Research Ethics Committee (Ref. 13/09/18/4.09) and reciprocal approval granted by The University of Newcastle Human Research Ethics Committee (Ref. H-2014-0018). All participants gave informed written consent prior to participation, and the study was undertaken in accordance with the ethical standards of the Declaration of Helsinki to ensure that the rights of all participants were protected throughout the study process.

Study Population

Participants were sought using posters and flyers displayed at The University of Newcastle, Australia, and in medical and physiotherapy practices in the greater Newcastle region. Participants were also recruited through the Hunter Medical Research

Institute Research Register. Posters and flyers provided introductory information regarding the study, and people interested in participating contacted the research team to discuss eligibility and participation requirements. Eligibility for participation was determined through a telephone interview with a physiotherapist researcher (S.F.). Two groups of participants were recruited: the first experiencing WAD for a duration greater than 3 months (WAD group) and the second consisting of pain-free controls matched by age and sex to the chronic WAD participants (control group). Recruitment was undertaken over a 9-month period from November 2014 to August 2015.

Inclusion criteria for the chronic WAD group was Grade II WAD as per the classification of The Quebec Task Force on Whiplash-Associated Disorders (Spitzer *et al.*, 1995) (i.e., neck pain with decreased range of motion and point tenderness) of greater than 3 months' duration. Onset of neck pain following an MVC was necessary for inclusion in this group, and people who were experiencing headaches as a component of their WAD were also included. Inclusion criteria for the pain-free control group were matching the age (± 1 year) and sex characteristics of a member of the chronic WAD group, with no history of MVC, cervical trauma, or neck pain lasting greater than 2 weeks' duration, or recurrent headaches. Exclusion criteria for both groups consisted of history of cervical surgery, spinal fracture, specific cervical pathology (e.g., malignancy, radiculopathy), congenital cervical abnormalities, systemic inflammatory pathology or conditions affecting connective tissue, or inability to safely undergo MRI (e.g., cardiac pacemaker, aneurysm clip).

MRI Technique

MRI scanning of each of the participants was undertaken consistent with the method described by Friedrich *et al.* (2008) using a Siemens Magnetom Skyra 3.0 Tesla unit (Siemens AG, Munich, Germany) in the supine lying position using a 20-channel head and neck coil. Participants were positioned in the neutral cervical spine position, which was determined by careful visual inspection by an MRI radiographer, and supported in this position using foam wedges (**Figure 18**). Images were acquired in the sagittal plane using four sequences (T1-weighted volumetric inter-polated breath-hold examination [T1 VIBE] sequence; T2-weighted sampling perfection with amplification-optimised contrast using different angle evolutions [T2 SPACE] sequence; double echo steady state [DESS] sequence; T1 VIBE sequence with fat suppression). Images were taken in the sagittal plane as cervical spine meniscoids are located at the ventral and dorsal poles of their enclosing joints, and the sagittal plane is, in turn, the most useful orientation by which to view these structures in cross-section (Mercer & Bogduk, 1993).

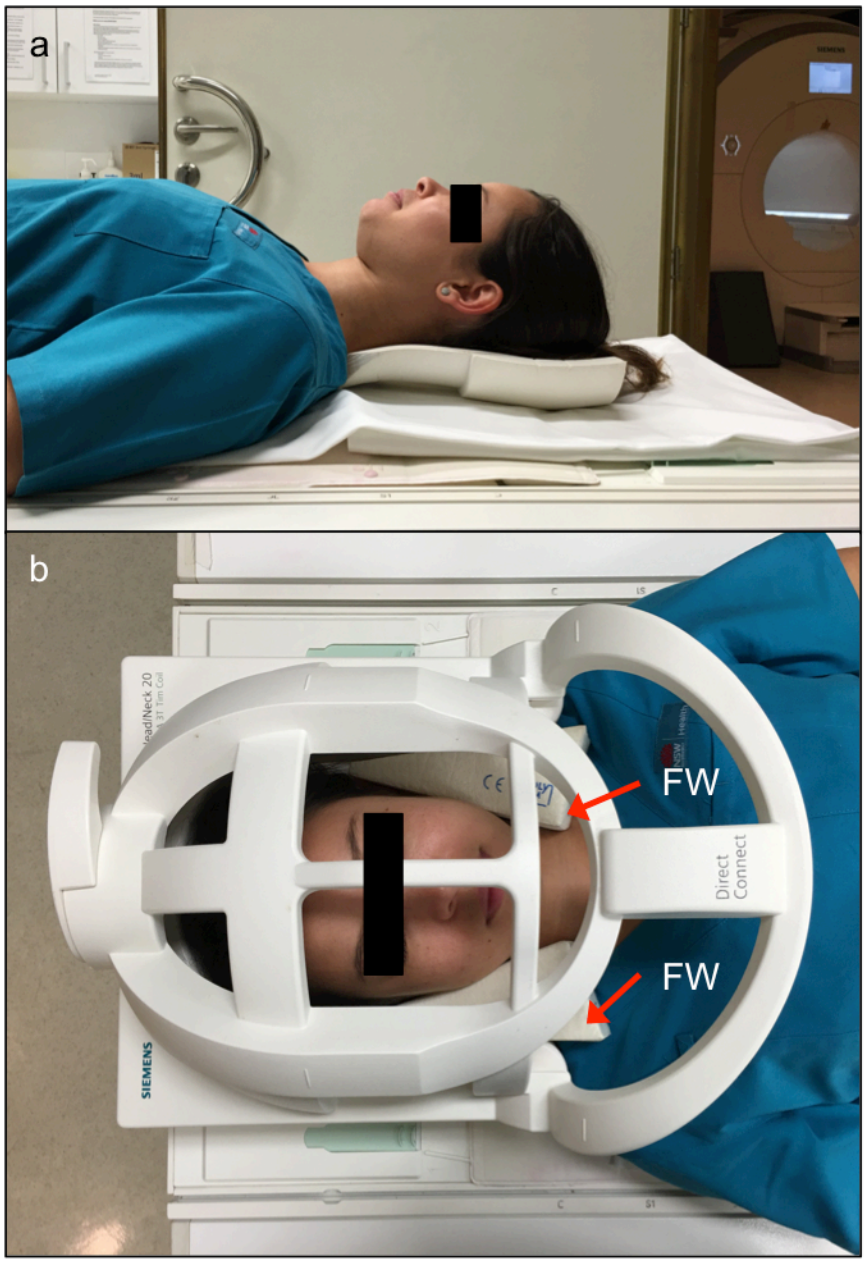


Figure 18: Participant MRI scanning position: a) lateral view of participant in supine lying in the neutral cervical spine position, with the head and neck coil removed to demonstrate the position; b) superior view with the head and neck coil in place, demonstrating foam wedges (FW) used to maintain participant position.

Protocol parameters for the T1 VIBE and DESS sequences were encoded in an anterior to posterior direction, with 200 x 200 mm^2 field-of-view, a 320 x 288 matrix, and a slice thickness of 0.6 mm (20% slice oversampling) with 120 slices per slab (90% slice resolution), to produce an isotropic resolution of 0.6 x 0.6 x 0.6 mm^3. Repetition time, echo time, flip angle, and bandwidth were 10.80 milliseconds, 3.41 milliseconds, 10°, and 240 Hz/pixel for the T1 VIBE sequence, 15.88 milliseconds, 5.40 milliseconds, 25°, and 200 Hz/pixel for the DESS sequence, and 10.80 milliseconds, 4.93 milliseconds, 10°, and 240 Hz/pixel for the T1 VIBE sequence with fat suppression. T2 SPACE images were acquired in 1 slab, with 1500 milliseconds repetition time, 136 milliseconds echo time, an echo train length of 88 with echo train duration of 322 milliseconds, 625 Hz/pixel bandwidth, 1.4 averages, phase encoding in the head-feet direction, a 250 x 250 mm^2 field-of-view (100% phase oversampling), a 320 x 288 matrix and a slice thickness of 0.6 mm (9.10% slice oversampling) with 88 partitions (98% slice resolution, 6/8 slice partial Fourier), which resulted in an isotropic resolution of 0.4 x 0.4 x 0.6 mm^3. Generalised auto-calibrating partially parallel acquisition with a parallel acquisition factor of 2 was used to produce image times of 8 minutes 30 seconds for T1 VIBE, 8 minutes 47 seconds for T2 SPACE, 5 minutes 37 seconds with DESS, 8 minutes 30 seconds for T1 VIBE with fat suppression, giving an approximate total scan time of 32 minutes.

Outcome Measures

Demographics including sex, age, height, and weight were collected for each participant. Height and weight were used to calculate body mass index (BMI), which was included as a potential independent variable of interest in meniscoid

morphometrical analyses to control for participant body size. For the WAD group, duration of WAD symptoms was recorded in years and months.

Images were reviewed by an anatomist and musculoskeletal physiotherapist researcher (S.F.) in consultation with an experienced specialist radiologist (P.L.). OsiriX v3.0.2 imaging software (Pixmeo, Geneva, Switzerland) was used to view and assess the study images. All outcome measures were assessed by a physiotherapist researcher (S.F.) who was blinded to participant identity and WAD/control allocation. Images were examined in the sagittal plane across all sequences concurrently, initially for the presence of meniscoids, after which the anterior-posterior size and histological composition were assessed, as per the methods outlined by Friedrich *et al.* (2008). Anterior-posterior size was measured on the T1 VIBE sequence from the base of the meniscoid at its attachment to the joint capsule, to the apex of the meniscoid protruding into the joint cavity (**Figure 19**). This was quantified in millimeters and, to account for normal variation in participant size, as a percentage of the anterior-posterior length of the articular surface of the inferior articular facet of the enclosing joint.

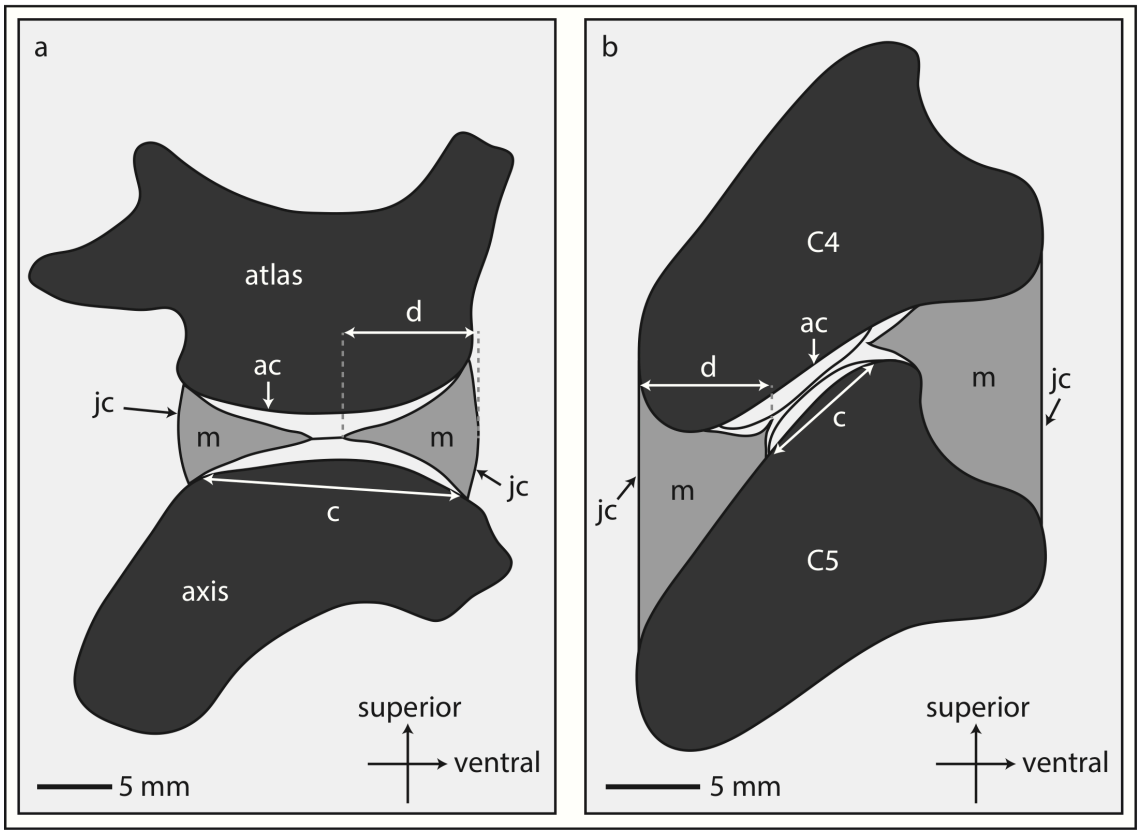


Figure 19: Measurement of cervical spine meniscoid protrusion length at (a) lateral atlantoaxial joint and (b) cervical zygapophyseal joint. m = meniscoid, ac = articular cartilage, jc = joint capsule, d = meniscoid protrusion length, c = articular cartilage length. Meniscoid length expressed in mm (distance d) and as a percentage of articular cartilage length (d ÷ c × 100). Modified from Friedrich *et al.* (2008).

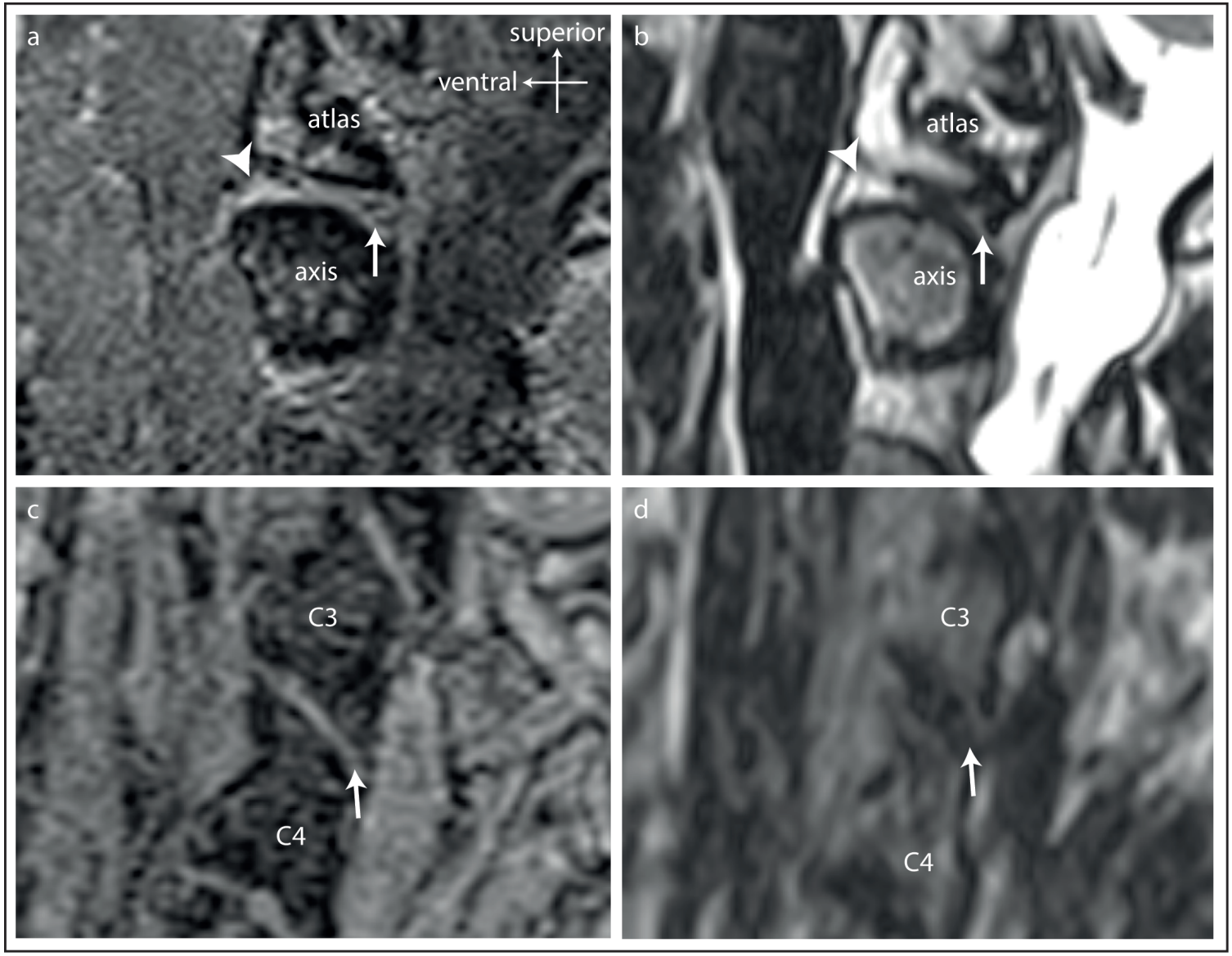
Meniscoid composition was assessed using the technique applied by Friedrich *et al*. (2008) by examining signal intensities on T1 VIBE and T2 SPACE sequences: meniscoids that were primarily hyperintense on T1 VIBE and hyperintense on T2 SPACE were classified as adipose in composition; meniscoids that were primarily hypointense on T1 VIBE and hypointense on T2 SPACE were classified as fibrous in composition; and meniscoids that were partly hyperintense and partly hypointense on T1 VIBE and partly hyperintense and partly hypointense on T2 SPACE were classified as fibroadipose (mixed) in composition (**Figure 20**). Interpretation of signal intensities as hyper- or hypointense was achieved by comparing the signal intensity of meniscoids to adjacent structures with clearly discernable compositions, such as subcutaneous adipose tissue for hyperintense signal, or paraspinal ligaments and muscles for hypointense signals.

Articular cartilage degeneration was assessed dichotomously as presence or absence of evidence of degeneration. Loss of joint space or presence of osteophytes was considered evidence of articular degeneration (Fujiwara *et al.*, 1999; Weishaupt, Zanetti, Boos, & Hodler, 1999).

Intra-rater reliability was tested on three randomly selected participants, with one week between first and second measurements. Reliability of the following was tested using the complete set of study images for these participants: assessment of meniscoid presence, measurement of meniscoid length, assessment of meniscoid composition, and identification of articular cartilage degeneration. Intrarater reliability testing assessed repeated measures of the physiotherapist researcher (S.F.), and interrater reliability testing compared measures of the physiotherapist researcher (S.F.) with those of a

specialist radiologist (P.L.).

Figure 20: Following page - Assessment of cervical spine meniscoid composition on sagittal images as per Friedrich *et al.* (2008) (a) T1-weighted volumetric inter-polated breath-hold examination (T1 VIBE) sequence lateral atlantoaxial joint (b) T2-weighted sampling perfection with amplification-optimised contrast using different angle evolutions (T2 SPACE) sequence lateral atlantoaxial joint (c) T1 VIBE sequence C3-4 zygapophyseal joint (d) T2 SPACE sequence C3-4 zygapophyseal joint. White arrowhead (a, b) denotes adipose meniscoid: primarily hyperintense on the T1 VIBE and T2 SPACE sequences; white arrow (a, b) denotes mixed fibroadipose meniscoid: partly hyperintense and partly hypointense on the T1 VIBE and T2 SPACE sequences; white arrow (c, d) denotes fibrous meniscoid: primarily hypointense on T1 VIBE and T2 SPACE sequences.


a
superior
ventral
atlas
axis
b
atlas
axis
c
C3
C4
d
C3
C4

Data Analysis

Stata 13.1 (StataCorp, College Station, TX) statistical analysis software package was used to analyse results. Shapiro-Wilk testing was used to examine distribution of continuous variables (meniscoid length, BMI). Data were analysed descriptively and explored using non-parametric techniques as appropriate to data distribution. Wilcoxon signed-rank testing was used to compare meniscoid length and BMI between WAD and control groups, and Spearman's rho used to examine correlation between BMI and meniscoid length. Conditional (fixed effects) logistic regression of age and sex-matched pairs was used to generate odds ratios (ORs) to explore the association between the dichotomous outcome variable of participant group (WAD or control) and predictor variables meniscoid composition (adipose, fibrous, or fibroadipose) and evidence of articular cartilage degeneration. Linear regression was used to examine association between meniscoid length and duration of WAD symptoms. Intra and interrater reliability was tested using two-way, absolute agreement intraclass correlation coefficients (ICCs). Significance was set at $p < 0.05$.

Results

Study Participants

Twenty people with Grade II WAD of duration greater than three months (mean [SD] age 39.3 [11.0] years, 10 female), and 20 corresponding age and sex-matched controls (mean [SD] age 39.1 [10.6] years, 10 female) volunteered to participate. A total of 80

lateral atlantoaxial and 400 cervical zygapophyseal joints (C2-3 to C6-7) were examined. Median (IQR) participant BMI for the WAD group was 24.12 kg/m^2 (21.33, 28.88) and for the control group 23.88 (21.19, 26.26). This difference was not statistically significant ($z = 0.56$, $p = 0.58$).

Presence of Meniscoids

Meniscoids were noted at the ventral and dorsal aspects of all lateral atlantoaxial joints in the WAD group. In one member of the control group (female, 57 years old) a ventral meniscoid could not be visualised in the left or right lateral atlantoaxial joints, however in all other participants in this group, ventral and dorsal meniscoids could be observed.

Meniscoids were identified in 159 (80%) and 154 (77%) zygapophyseal joints in the WAD and control groups respectively. In the WAD group, 237 meniscoids were identified and in the control group 218 meniscoids were identified. Some joints contained two meniscoids, some contained one meniscoid, and some did not contain a meniscoid (**Table 10**). Location of meniscoids within the enclosing joints for the 2 groups can be seen in **Table 10**.

Table 10: Location of cervical spine meniscoids within lateral atlantoaxial and zygapophyseal joints for chronic whiplash associated disorder (WAD) and control groups.

	Ventral and dorsal meniscoids		**Ventral meniscoid only**		**Dorsal meniscoid only**		**No meniscoid**	
	WAD	*Control*	*WAD*	*Control*	*WAD*	*Control*	*WAD*	*Control*
Lateral atlantoaxial joints (n)	40	38	-	-	-	2	-	-
Zygapophyseal joints C2-3 to C6-7 (n)	78	64	27	32	54	58	41	46

Meniscoid Length

The length of protrusion of meniscoids for the WAD and control groups, as well as results of accompanying Wilcoxon signed-rank tests can be seen in **Table 11**. At the lateral atlantoaxial joint, meniscoid protrusion was greater in the control group than the WAD group.

No significant correlation was found between meniscoid length in mm and BMI for lateral atlantoaxial joint meniscoids (WAD group: Spearman's rho = 0.10, p = 0.36; control group: Spearman's rho = -0.04, p = 0.70). Similarly, no significant correlation was observed for zygapophyseal joint meniscoids (WAD group: Spearman's rho = 0.07, p = 0.30; control group: Spearman's rho = 0.06, p = 0.34).

Table 11: Protrusion lengths of lateral atlantoaxial and cervical zygapophyseal joint meniscoids for chronic whiplash associated disorder (WAD) and control groups, expressed in mm in accordance with the method described by Friedrich *et al.* (2008), and as a percentage of inferior articular cartilage anterior-posterior length, with accompanying results of Wilcoxon signed-rank tests.

	WAD group median (IQR)	**Control group** median (IQR)	**p**
Lateral atlantoaxial joints			
Ventral (mm)	5.01 (4.27, 6.34)	6.07 (4.76, 6.80)	0.06
Dorsal (mm)	6.48 (5.80, 7.04)	7.24 (6.08, 8.49)	< 0.01
Ventral percentage (%)	32.83 (27.71, 38.39)	40.11 (29.97, 44.15)	0.01
Dorsal percentage (%)	40.79 (35.99, 45.28)	47.24 (40.91, 55.21)	< 0.01
Cervical zygapophyseal joints C2-3 to C6-7			
Ventral (mm)	4.12 (3.28, 5.03)	4.00 (3.02, 4.92)	0.67
Dorsal (mm)	2.56 (2.22, 3.13)	2.34 (1.91, 2.90)	0.16
Ventral percentage (%)	45.55 (32.55, 53.74)	42.93 (32.47, 52.38)	0.30
Dorsal percentage (%)	26.94 (22.60, 32.33)	25.03 (20.38, 32.59)	0.70

Meniscoid Composition

Distribution of meniscoid composition types between the two groups can be seen in **Table 12**. At the lateral atlantoaxial joint, logistic regression modeling indicated no significant association between participant group (WAD compared to control) and meniscoid composition (fibrous or adipose compared to fibroadipose) for ventral (fibrous: OR 1.61, p = 0.46; Likelihood Ratio Test [LRT] Chi-square [2] = 3.32, LRT p = 0.19) or dorsal meniscoids (adipose: OR 0.69, p = 0.65; fibrous: OR 0.63, p = 0.49; LRT Chi-square [2] = 0.64, LRT p = 0.73).

For zygapophyseal joint meniscoids, a significant association was noted between participant group and ventral meniscoid composition in that meniscoids fibrous in composition were more likely to belong to the control group (fibrous: OR 0.49, p = 0.02; LRT Chi-square [2] = 8.69, LRT p = 0.01). A significant association was also noted between participant group and dorsal meniscoid composition, in that meniscoids fibrous in composition were more likely to belong to the WAD group (fibrous: OR 2.38, p < 0.01; LRT Chi-square [2] = 9.02, LRT p = 0.01).

Table 12: Distribution (n [%]) of cervical spine meniscoid composition types between chronic whiplash associated disorder (WAD) and control groups.

Meniscoid composition	**WAD group**		**Control group**	
	Ventral	*Dorsal*	*Ventral*	*Dorsal*
Lateral atlantoaxial joints				
Adipose	2 (5.00)	3 (7.50)	0 (0.00)	4 (10.00)
Fibrous	8 (20.00)	9 (22.50)	5 (13.16)	11 (27.50)
Fibroadipose	30 (75.00)	28 (70.00)	33 (86.84)	25 (62.50)
Zygapophyseal joints C2-3 to C6-7				
Adipose	0 (0.00)	0 (0.00)	2 (2.08)	1 (0.82)
Fibrous	38 (35.85)	100 (76.34)	46 (47.92)	75 (61.48)
Fibroadipose	68 (64.15)	31 (23.66)	48 (50.00)	46 (37.70)

WAD Duration

Median (IQR) duration of WAD symptoms for the chronic WAD group was 10.0 (3.3, 19.5) years. No significant association was identified between WAD duration and lateral atlantoaxial joint meniscoid length as a percentage of inferior articular facet anterior-posterior length for ventral meniscoids ($\beta = 0.11$, $p = 0.29$, adjusted $R^2 < 0.01$). A negative association was noted between WAD duration and lateral atlantoaxial joint dorsal meniscoid length as a percentage of inferior articular facet anterior-posterior length ($\beta = -0.28$, $p = 0.04$, adjusted $R^2 = 0.08$).

For zygapophyseal joint meniscoids, no significant association was found between WAD duration and meniscoid length as a percentage of inferior articular surface length for dorsal meniscoids ($\beta = 0.07$, $p = 0.31$, adjusted $R^2 < 0.01$). For ventral meniscoids, a negative association existed that approached significance ($\beta = -0.25$, $p = 0.05$, adjusted $R^2 = 0.03$).

Articular Degeneration

All 80 of the lateral atlantoaxial joints examined were assessed as showing no evidence of articular degeneration. Of the 400 zygapophyseal examined, 81 of 200 joints in the control group demonstrated evidence of degeneration, compared to 66 in the WAD group. The association between articular cartilage degeneration and study group was not statistically significant (OR 0.67, $p = 0.09$, LRT Chi-square [1] = 2.95, LRT $p = 0.09$). A significant association was found between articular degeneration and meniscoid

composition, in that fibrous meniscoids were more likely to be identified in joints with evidence of degeneration compared to fibroadipose meniscoids (OR 2.97, $p < 0.01$, LRT Chi-square [2] = 30.97, LRT $p < 0.01$).

Intra and Inter-rater Reliability

The intra and inter-rater reliability of the various measurement methods employed can be seen in **Table 13**. ICCs for all variables assessed were consistent with 'good' or 'fair' reliability, as proposed by Fleiss (1986).

Table 13: Intraclass correlation co-efficients (ICCs) for intra and inter-rater reliability of outcome measures.

Variable measurement	ICC	95% Confidence interval
Intra-rater (n = 36 joints)		
Meniscoid presence	0.62	0.45, 0.75
Meniscoid length	0.81	0.68, 0.89
Meniscoid composition	0.88	0.80, 0.93
Presence of cartilage degeneration	0.75	0.57, 0.87
Inter-rater (n = 12 joints)		
Meniscoid presence	0.79	0.56, 0.90
Meniscoid length	0.89	0.73, 0.96
Meniscoid composition	0.41	0.06, 0.74
Presence of cartilage degeneration	0.69	0.26, 0.90

Discussion

The present study is the first to examine lateral atlantoaxial and cervical zygapophyseal joint meniscoid morphology in a living sample with known cervical spine symptoms. Chronic WAD was chosen as an appropriate cervical condition to study due to biomechanical (Kaneoka *et al.*, 1999) and autopsy (Schonstrom *et al.*, 1993; Taylor & Taylor, 1996) evidence inferring potential involvement of cervical spine meniscoids in the trauma of whiplash loading. While central nervous system and psychosocial factors are considered significant contributors to chronic WAD pathophysiology (Sterling, 2011), potential peripheral morphological changes have been noted in recent work (Eliott *et al.*, 2010; Elliott, 2011; Elliott *et al.*, 2011), suggesting further exploration of peripheral changes in WAD is warranted.

Cervical spine meniscoids were noted in all lateral atlantoaxial joints and 78.25% of cervical zygapophyseal joints examined. This figure is consistent with rates of prevalence noted in previous studies that utilised gross dissection (Farrell *et al.*, 2015a, 2016b; Farrell *et al.*, 2015c; Inami *et al.*, 2000), sectioning of cadaveric tissue (Webb *et al.*, 2012) and sheet plastination (Farrell *et al.*, 2015b). This consistency with other methods of examination suggests that MRI is a valid method of visualising cervical meniscoids *in vivo*.

Findings of the present study suggest that lateral atlantoaxial joint meniscoids are smaller in people with chronic WAD compared to people without cervical spine symptoms. This was demonstrated when meniscoid protrusion length was quantified both in millimeters and as a percentage of inferior articular surface anterior-posterior

length. This may suggest that in individuals with chronic WAD the meniscoids of the lateral atlantoaxial joints are subject to regressive changes. We hypothesise that such changes may be a result of postural or biomechanical contributors related to altered cervical spine kinematics in individuals with chronic WAD (Dall'Alba, Sterling, Treleaven, Edwards, & Jull, 2001; Woodhouse & Vasseljien, 2008). However, as linear regression modeling of meniscoid size as predicted by WAD duration explained little of the variance in the data, as indicated by low adjusted R^2 values for each model, results suggest that such regressive changes do not advance with time.

Composition of the meniscoids of the cervical zygapophyseal joints was found to vary between the chronic WAD and control groups. A fibrous dorsal meniscoid at these joints was 2.38 times more likely to belong to a participant from the chronic WAD group than the control group, as compared to a meniscoid of fibroadipose composition. Given existing evidence of decreased cervical range of motion in WAD (Dall'Alba *et al.*, 2001), this finding is consistent with the suggestion by Jones *et al.* (1989) that meniscoids may contribute to joint hypomobility through proliferation of fibrous tissue and development of intra-articular fibrous adhesions. Mercer and Bogduk (1993) and Webb *et al.* (2011a) suggest such adhesions may develop from meniscoids in immobile or under-used zygapophyseal joints in a similar fashion to fibrous tissue proliferation and adhesions in immobilised zygapophyseal and knee joints (Cramer, Henderson, Little, Daley, & Grieve, 2010; Enneking & Horowitz, 1972).

In agreement with prior studies undertaken by dissection (Farrell *et al.*, 2015a, 2016b; Inami *et al.*, 2000) and imaging (Friedrich *et al.*, 2008), the present study found evidence of articular cartilage degeneration to be associated with meniscoids composed

of fibrous tissue. This may suggest a potential biomechanical or even pathological relationship between cervical spine meniscoid morphology and articular degeneration of the zygapophyseal joints, such as fibrosis of cervical spine meniscoids in response to excess joint loading leading to chronic inflammation (Barr *et al.*, 2004; Farrell *et al.*, 2016b).

Clinical Implications

In a chronic WAD population, traditional clinical imaging and investigations may not reveal a clear structural source of symptoms (Curatolo *et al.*, 2011; Sterling *et al.*, 2011), though recent studies utilising MRI have reported peripheral changes in individuals with chronic WAD in the form of fatty infiltration of cervical muscles which are postulated as contributing to ongoing cervical spine dysfunction (Eliott *et al.*, 2010; Elliott, 2011; Elliott *et al.*, 2011). Such changes could potentially represent a pathoanatomical contribution to chronic WAD, and it is plausible that the morphological differences noted in the cervical spine meniscoids in the present study may similarly represent a contributor to chronic WAD pathoanatomy.

The key findings of the present study – that is, smaller meniscoids in lateral atlantoaxial joints and a higher frequency of fibrous meniscoids in the dorsal aspects of zygapophyseal joints in the chronic WAD group – highlight the possibility that morphological changes to the cervical spine meniscoids may occur as a consequence of the pain and hypomobility associated with WAD. As discussed above, decreased movement of a zygapophyseal joint over an extended period of time, such as in the case of WAD (Dall'Alba *et al.*, 2001; Woodhouse & Vasseljien, 2008), could plausibly lead

to development of fibrous adhesions associated with proliferation of fibrous tissue of an enclosed meniscoid (Jones *et al.*, 1989; Mercer & Bogduk, 1993). While speculative, this suggestion may lend support to current clinical guidelines for acute and chronic WAD which recommend range of motion exercises, advice to 'act as usual', and to avoid collar immobilisation (Motor Accidents Authority, 2014; TRACsa: Trauma and Injury Recovery, 2008).

The morphological differences noted between the chronic WAD and control populations could represent evidence of changes in lateral atlantoaxial and cervical zygapophyseal joints. It has been well established that the cervical zygapophyseal joints are a source of nociceptive input in individuals with chronic WAD (Barnsley *et al.*, 1993; Barnsley *et al.*, 1995; Bogduk, 2011b; Curatolo *et al.*, 2011; Lord *et al.*, 1995; Lord *et al.*, 1996a; Lord *et al.*, 1996b), and the results of the present study suggest that meniscoids may be involved in chronic WAD pathology. It must be acknowledged that these results should be considered as associations only, because the study design does not provide insight into causation as no data are available regarding the pre-injury morphology of participant cervical spine meniscoids. No evidence exists regarding the relationship between cervical spine meniscoid morphology and WAD prognosis (Walton *et al.*, 2013), and further research is therefore required to explore cervical spine meniscoid morphology over time in these populations.

Limitations

Measurement of morphometry of an anatomical structure in a single plane using MRI may be subject to error, due to the critical relationship between slice orientation and

participant position. This was minimised in the present study through the use of standardised participant positioning. Despite this, an element of random error may have remained inherent to the study due to variation in participant anthropometry. This error however, would not have been systematically different between the study groups, as indicated by the consistent IQRs for measurement of meniscoid protrusion length. Furthermore, the differences in median meniscoid protrusion length (1.1 mm, 0.7 mm) identified approach the lower limits of scan resolution (slice thickness 0.6 mm), which should be considered when interpreting results.

The accuracy of assessment of cervical spine meniscoid composition using MRI signal intensities, while based upon fundamental principles of MRI interpretation (Armstrong, Wastie, & Rockall, 2009; Gay & Woodcock, 2008; Yochum *et al.*, 2005) and the precedent of Friedrich *et al.* (2008) has not been examined empirically by directly comparing MRI results with physical examination, microscopy, or histology of meniscoid composition. However, the appearance of synovial plicae on MRI has been reported descriptively in the knee (Kosarek & Helms, 1999) and shoulder (Novak, Lee, & Saleem, 2009), and directly compared to microscopy in the elbow (Awaya *et al.*, 2001). Findings of all three of these studies were consistent with the classification strategy employed by Friedrich *et al*. (2008) and the present study, supporting the validity of this method. The interrater reliability ICC of 0.41 (95% confidence interval 0.06, 0.74) between the primary assessor (S.F.) and specialist radiologist (P.L.) falls close to the lower limit of fair-good reliability according to Fleiss (1986) and this should be considered when interpreting study findings. To empirically determine the accuracy of using MRI signal intensities to infer cervical meniscoid composition, future

research should directly compare meniscoid radiological appearance with microscopy in a sample of cadavers or people undergoing cervical spine surgery.

Exploration of relationships between cervical spine meniscoid morphology and chronic WAD clinical signs and symptoms was beyond the scope of this study. To enhance understanding of the potential role of cervical spine meniscoids in chronic WAD, further investigation is needed to determine whether the morphological differences noted in the present study are related to patient clinical signs and symptoms. This requires longitudinal studies involving patients with varying levels of pain-related disability following MVC.

Conclusions

Cervical spine meniscoids display morphological differences in a chronic WAD population compared to age and sex-matched controls. Meniscoids are smaller in the lateral atlantoaxial joints of people with chronic WAD, and at the dorsal aspect of cervical zygapophyseal joints, meniscoids appear to be more frequently fibrous in composition in people with chronic WAD. This information provides new insight into potential peripheral structural contributors to chronic WAD, and in turn advances our appreciation of the pathoanatomical processes underpinning this burdensome condition.

Summary

Study 5 has used MRI to examine the morphological characteristics of cervical spine meniscoids *in vivo* in a population with chronic WAD. Results suggest that cervical spine meniscoids are smaller in the lateral atlantoaxial joints of people with chronic WAD compared to age and sex-matched controls. Study 5 also found cervical spine meniscoids located at the dorsal aspect of zygapophyseal joints to be more frequently fibrous in composition in people with chronic WAD, compared to age and sex-matched controls.

Cervical spine meniscoid morphology has not previously been investigated *in vivo* in a population with cervical spine pathology. The findings of Study 5 provide for the first time an insight into morphological differences between cervical spine meniscoids of a population with known cervical spine pathology and those of matched, pain-free controls. The implications of these findings will be discussed in the context of the findings of Studies 1-4 in the next chapter, with respect to addressing the overarching aims of the thesis.

Chapter 8: SUMMARY AND CONCLUSIONS

Summary of Study Findings

The aim of this thesis was to explore the potential clinical significance of the meniscoids of the cervical spine, as it relates to neck pain. This has been undertaken by exploring the morphology and innervation of cervical spine meniscoids, and by investigating meniscoid pathoanatomy *in vivo* in a symptomatic population. This body of work comprised four studies examining the anatomy of these structures in cadavers, with respect to aspects of their morphology that had not been previously investigated, or that were the subject of disagreement in prior research. The fifth and final study examined cervical spine meniscoid morphology in a living population with WAD, by comparing morphological characteristics to those of an age and sex-matched pain-free population.

Study 1 (Chapter 3) validated a novel method of facilitating gross dissection of cervical spine meniscoids from the zygapophyseal and lateral atlantoaxial joints of cadavers. This study was necessary as disarticulation of cervical zygapophyseal joints is potentially problematic due to the congruence of the complex bony architecture of the articular pillars. This congruence can necessitate the utilisation of considerable force to disarticulate the zygapophyseal joints and provide access to the meniscoids, which could feasibly damage the delicate meniscoids in the process. Study 1 examined the effect of formic acid demineralisation upon the cervical spine meniscoids, and found

that this process did not alter the morphometry of these structures. This supported the use of this technique as a viable means of facilitating disarticulation of the cervical zygapophyseal joints, by allowing the softened bone to be cut away with a scalpel, such that the joint surfaces could be separated with minimal force. Formic acid demineralisation was therefore utilised in this manner during preparation of cadaveric spinal tissue in the subsequent dissection studies.

In Study 2 (Chapter 4), the morphology and morphometry of lateral atlantoaxial joint meniscoids was examined by investigating points of disagreement identified in the findings of previous studies. This was undertaken by gross dissection of cadavers and light microscopy with haematoxylin and eosin staining. Findings confirmed previous reports of lateral atlantoaxial joint meniscoid composition – adipose, fibrous and fibroadipose (Webb *et al.*, 2011a) – and support the previous high rates of meniscoid prevalence described by prior studies (Mercer & Bogduk, 1993; Webb *et al.*, 2012). Meniscoids were larger in males than females at this joint, and an association was found between articular cartilage degeneration and fibrous meniscoid composition, providing evidence of a possible link between meniscoid morphology and articular pathology of the lateral atlantoaxial joint.

The morphology and morphometry of cervical zygapophyseal joint meniscoids was similarly investigated in Study 3 (Chapter 5) by examining points of contention in the findings of prior investigations of these structures, as well as aspects that had not yet been formally investigated. This was undertaken by gross dissection of cadavers, application of the formic acid demineralisation dissection facilitation technique validated in Study 1, and light microscopy examination of zygapophyseal joint

meniscoid composition. The findings of this study indicate that cervical zygapophyseal joint meniscoid size was not influenced by sex, spinal level, location in joint, or articular degeneration. Consistent with the findings of Study 2, Study 3 noted an association of fibrous meniscoid composition with articular cartilage degeneration, providing further evidence of a potential relationship between cervical spine meniscoid morphology and articular pathology, and in turn a possible pathoanatomical contribution of cervical spine meniscoids to neck pain. Findings also confirmed prior reports of three distinct meniscoid composition classifications in the zygapophyseal joints (adipose, fibrous, fibroadipose).

Study 4 (Chapter 6) examined the nature of cervical spine meniscoid innervation in cadavers using immunohistochemistry. Antibodies to NF-H and Pan-NF were used to assess the presence of nerve fibres within cervical spine meniscoids and adjacent joint capsules. The study found NF-H immunoreactive nerve fibres within joint capsules adjacent to cervical spine meniscoids, but not within meniscoid bodies. Antibody to NF-H has been established as a marker for myelinated nerve fibres (Reinisch & Tschachler, 2005; Watanabe *et al.*, 2007). Consequently, the NF-H immunoreactivity observed in Study 4 could plausibly represent Aδ-fibres, indicative of nociceptive function, which in turn supports the Entrapment and Extrapment Theories of meniscoid involvement in acute torticollis through joint capsule distension. Pan-NF immunoreactive fibres were primarily found within joint capsules as well, however a small number of immunoreactive fibres were noted within the bodies of two adipose lateral atlantoaxial joint meniscoids. Antibody to Pan-NF has previously been shown to identify both myelinated and unmyelinated fibres (Dau & Wenthold, 1985; Hafidi & Romand, 1989; Lauria *et al.*, 2004; Lopez *et al.*, 2005), and as some Pan-NF immunoreactive fibres

were not reactive to NF-H in the same location in adjacent sections, it is plausible that such fibres are unmyelinated C-fibres which may serve a nociceptive function.

Study 5 investigated cervical spine meniscoid morphology in a living population with chronic WAD using MRI, by comparing morphological characteristics to those of an age and sex-matched pain-free population. In the chronic WAD group, cervical spine meniscoids were found to be smaller in the lateral atlantoaxial joints and were more frequently fibrous in composition at the dorsal aspect of cervical zygapophyseal joints. These morphological differences may be the result of altered cervical spine kinematics secondary to pain and joint hypomobility associated with WAD. This is the first study to examine cervical spine meniscoid morphology and morphometry in a living population with cervical spine pathology, and provides evidence for the meniscoids as structures of pathoanatomical interest in chronic WAD.

Limitations of Studies

As identified in Chapters 3, 4, 5 and 6, the cadavers included in the gross dissection and immunohistochemistry studies (Studies 1, 2, 3 and 4) were elderly, which could feasibly influence meniscoid anatomy and morphology, and limits the ability to generalise study findings to a younger population. Furthermore, whilst information regarding donor cause of death was available, departmental records did not detail whether donors suffered any form of neck pathology. Therefore, it is not clear if the morphological characteristics observed in these studies were influenced by cervical spine pathology. This is an inherent limitation to undertaking anatomical research using human cadavers, as bodies bequeathed to medical schools are typically elderly (Cornwall *et al.*, 2012).

Measurement of meniscoid morphometrics in studies 1, 2 and 3 may have been affected by tissue shrinking as a result of the embalming process. As identified in Chapter 3, tissue shrinkage secondary to dehydration in ethanol embalming has been reported in muscle and nerve tissues (Brown *et al.*, 2002; Cutts, 1988). As such, the possibility of similar tissue shrinkage occurring in the fibrous and adipose meniscoid specimens used in Studies 1, 2 and 3 cannot be excluded.

Study 4 used immunohistochemistry to examine the presence of nerve tissue in meniscoids excised from cadavers. Immunohistochemistry utilises the capacity of an antibody to bind to an antigen of interest in a tissue specimen (Haines & Chelack, 1991). When tissue is fixed during the embalming process, the ability for such binding reactions to occur diminishes (Haines & Chelack, 1991; Shi *et al.*, 1991), which reduces the effectiveness of immunohistochemical staining. This was a significant challenge in the development of the method used in Study 4, as illustrated by the extensive pilot testing of different primary antibodies and immunohistochemical approaches outlined in Appendix O. Primary antibodies specific to various aspects of neuron structure and function (SP, PGP 9.5, S100, β-III tubulin, nitric oxide synthase, tyrosine hydroxylase) were trialed unsuccessfully using immunofluorescence and DAB immunohistochemistry, before developing a successful technique using NF-H and Pan-NF primary antibodies. Whilst the method employed provided effective staining of our cadaveric specimens, the two primary antibodies used are not specific to nerve tissue with nociceptive function, as is the case for antibodies to SP or CGRP (Barrett *et al.*, 2012a; Benarroch, 2011; Henry, 1982). This information would have been desirable to

distinguish between vasoregulatory and nociceptive nerve fibres to provide further insight into the possible contribution of these structures to nociceptive input.

As noted in Chapter 7, the accuracy of assessment of cervical spine meniscoid composition using MRI signal intensities, as employed in Study 5, has not been examined empirically by directly comparing results with microscopy. Whilst this approach is based upon fundamental principles of MRI interpretation (Armstrong *et al.*, 2009; Gay & Woodcock, 2008; Yochum *et al.*, 2005) and the precedent set by Friedrich *et al.* (2008), it is not clear if this technique provides an accurate reflection of meniscoid composition *in vivo*, and this should be considered in the interpretation of study results.

Implications of Findings

The findings of this thesis have a number of implications. This includes implications for basic science enquiry as well as clinical practice.

Basic Science Implications

Study 1 (Chapter 3) has proposed and evaluated a novel method for facilitating dissection of the zygapophyseal joints using formic acid demineralisation to soften bone and allow the joints to be disarticulated with minimal force, without altering the morphometry of the enclosed meniscoids. This technique has implications for future research seeking to examine the morphology of zygapophyseal joint meniscoids or similar structures by gross dissection, as this technique can be employed to potentially

decrease the risk of inadvertently damaging meniscoids or other structures during disarticulation of a joint.

Clinical Implications

Fibrous Meniscoid Composition and Articular Cartilage Degeneration

Studies 2, 3 and 5 (Chapters 4, 5 and 7) found an association between cervical spine meniscoid morphology and evidence of articular pathology, in that fibrous meniscoids were primarily observed in joints demonstrating degenerative changes, and adipose meniscoids occurred in joints with intact cartilage. This finding provides evidence for potential involvement of cervical spine meniscoids in articular degenerative changes, as it is plausible that biomechanical factors such as excess joint loading, an established contributor to osteoarthritis (Felson, 2012), may lead to chronic inflammation and in turn fibrosis of enclosed meniscoids (Barr *et al.*, 2004). This theoretically implicates cervical spine meniscoids as structures affected by degenerative joint pathology such as osteoarthritis, given the significant role of articular cartilage degeneration in osteoarthritis pathophysiology, and the association of altered meniscoid morphology with articular degeneration (Felson, 2013; Kemp *et al.*, 2008).

The association of fibrous meniscoid composition with articular degeneration also supports the Fibrous Tissue Proliferation Theory of meniscoid involvement in spinal pain, as outlined in Chapter 2. Jones *et al.* (1989) proposed that proliferation of the fibrous tissue comprising a meniscoid may lead to intra-articular fibrous adhesions, and

in turn, long-term joint hypomobility. Mercer and Bogduk (1993) suggested that such fibrous tissue proliferation may arise from fibroadipose meniscoids in immobile or under-used zygapophyseal joints in a similar fashion to previously observed fibrous tissue proliferation and adhesions in immobilised knee joints (Akeson *et al.*, 1980; Enneking & Horowitz, 1972). In the context of cervical spine pain, under-use of a zygapophyseal joint could feasibly occur as a result of pain associated with degenerative pathology. The findings of Studies 2, 3 and 5 expand upon this theory, implying that the proposed fibrous tissue proliferation may also occur secondary to mechanical factors such as excess joint loading, a contributor to degenerative joint pathology (Felson, 2012). Fibrous tissue adhesions may plausibly lead to joint hypomobility, further perpetuating the fibrous tissue proliferation process, and ultimately altering cervical spine biomechanics by reducing segmental range of motion.

The Fibrous Tissue Proliferation Theory mechanism is of clinical significance in physiotherapy and musculoskeletal medicine practice. Joint hypomobility, altered cervical spine biomechanics and decreased range of motion are established deficits present in NSNP and WAD (Borenstein, 2013; Dall'Alba *et al.*, 2001; Lee, Nicholson, & Adams, 2003; Prushansky, Pevzner, Gordon, & Dvir, 2006; Sran, 2006), and understanding the pathoanatomical and physiological processes potentially underpinning these deficits is of value to clinicians. Furthermore, the Fibrous Tissue Proliferation Theory of meniscoid involvement in spinal pain provides a feasible mechanism by which mechanical treatments such as joint mobilisation or manipulation may deliver clinical benefit. It has been proposed that mobilisation or manipulation of a joint distracts articular surfaces, which could plausibly break any fibrous tissue adhesions restricting joint movement, and in turn restore segmental biomechanics and

spinal range of motion (Cramer *et al.*, 2004; Evans, 2002; McCarthy, Bialosky, & Rivett, 2015; Olsen, 2009).

Innervation of Cervical Spine Meniscoids

Cervical spine meniscoids have been previously shown to contain C-fibre free nerve endings, likely nociceptive in function (Inami *et al.*, 2001). This in turn implicates cervical spine meniscoids as potential sources of nociceptive sensory input. Study 4 (Chapter 6) noted evidence of unmyelinated fibres in a small number of cervical spine meniscoids, consistent in morphology with C-fibres. These fibres were noted both independent of blood vessels and in close proximity to vessels, suggestive of both sensory and vasoregulatory functions (Gronblad *et al.*, 1991; Inami *et al.*, 2001). Study 4 also found that cervical spine meniscoids do not however contain myelinated fibres and therefore no Aδ-fibre nociceptors.

Consequently, it appears that nociceptive input arising from cervical spine meniscoids is most likely to give rise to slow conducting, poorly localised, burning pain characteristic of C-fibre nociceptors, rather than the fast conducting, well-localised, sharp pain of Aδ-fibre nociceptors (Barrett *et al.*, 2012c; Lawson, 2002; Voscopoulos & Lema, 2010). Findings of Study 4 do however suggest that Aδ-fibre nociception could feasibly emanate from fibres within the joint capsule adjacent to a meniscoid, should an appropriate mechanical stimulus activate these nociceptors (Voscopoulos & Lema, 2010), due to the presence of myelinated fibres within the capsules of the cadavers studied.

In keeping with the Fibrous Tissue Proliferation Theory described in the previous section, chronic inflammation of the meniscoids may occur as a consequence of excess joint loading and in turn articular degeneration (Barr *et al.*, 2004; Felson, 2012). As C-fibre nociceptors can be triggered by noxious chemical stimuli, such as inflammatory markers inherent in the pathophysiology of articular degeneration (Benito, Veale, Fitzgerald, van den Berg, & Bresnihan, 2005; Bonnet & Walsh, 2005; Haywood *et al.*, 2003), it is plausible that cervical spine meniscoids may contribute to neck pain through nociceptive input. Furthermore, C-fibre and Aδ-fibres are both considered to be involved in nervous system maladaptations in chronic pain (Dubin & Patapoutian, 2010; Koltzenburg, Torebjork, & Wahren, 1994; Lawson, 2002; Voscopoulos & Lema, 2010; Woolf & Mannion, 1999). Decreased threshold for activation of C-fibre nociceptors can occur in chronic pain conditions (Dubin & Patapoutian, 2010; Koltzenburg *et al.*, 1994; Voscopoulos & Lema, 2010; Woolf & Mannion, 1999), and combined with altered activation and processing of non-nociceptor A-fibre input (Dubin & Patapoutian, 2010; Woolf & Mannion, 1999), is thought to result in hyperalgesia and allodynia. The findings of Study 4, as well as those of Inami *et al.* (2001), suggest that C-fibres are present within cervical spine meniscoids, and that A-fibres are likely present in adjacent joint capsules, and may plausibly be involved in nervous system maladaptations in chronic neck pain.

Entrapment and Extrapment Theories

Results of the studies forming this thesis are consistent with the Entrapment and Extrapment Theories of cervical spine meniscoid involvement in neck pain. Briefly, these theories hypothesise that during excessive joint motion, a cervical spine meniscoid

may be pinched between the articular surfaces of a joint (Entrapment Theory) or become dislodged from its position between the articular surfaces, and fail to re-enter the joint cavity causing capsular distension and in turn pain (Extrapment Theory) (Bogduk & Jull, 1985; Evans, 2002; Kos & Wolf, 1972; Webb *et al.*, 2011a; Zukschwerdt *et al.*, 1955). These theories have been proposed as pathoanatomical explanations for acute torticollis (Bogduk & Jull, 1985; Evans, 2002).

Length of meniscoid protrusion into the enclosing joint cavity was measured in Studies 2, 3 and 5 (Chapters 4, 5 and 7). These studies found median meniscoid lengths of approximately 3 mm at the lateral atlantoaxial and cervical zygapophyseal joints, with larger structures reaching 6 mm and 4 mm respectively. It is plausible that a structure of such a size may be compressed or caught between the articular surfaces of a spinal joint, and it is also plausible that a meniscoid of such a size, if dislodged from the joint cavity, may initially fail to re-enter the cavity spontaneously and would be large enough to cause considerable distension of the joint capsule. However this phenomenon is yet to be formally observed in a symptomatic population.

The association of fibrous meniscoid composition with articular cartilage degeneration, as discussed earlier in the chapter, may be the result of a fibrosis process affecting meniscoids subjected to excessive joint loading (Felson, 2012). Such potential mechanical compression or shearing forces are consistent with the mechanics of Entrapment Theory. Furthermore, the presence of both myelinated and unmyelinated fibres consistent with Aδ- and C-fibre nociceptors in the joint capsule adjacent to cervical spine meniscoids (Study 4) supports the plausibility of joint capsule distension leading to pain, and is therefore consistent with Entrapment and Extrapment Theories.

Findings of Study 5 (Chapter 7) suggest that morphological differences exist between the cervical spine meniscoids of people with chronic WAD and those of a pain-free population. Lateral atlantoaxial joint meniscoids were found to be smaller in people with chronic WAD compared to an age and sex-matched control group, and dorsal zygapophyseal joint meniscoids were more frequently fibrous in composition in those with WAD. These differences could potentially represent a pathoanatomical contribution to chronic WAD from the cervical spine meniscoids, as a consequence of the altered cervical spine kinematics secondary to the pain and hypomobility associated with the condition.

The direct implications of these differences for clinical diagnosis and intervention in WAD are not yet clear. Rather, the findings contribute to our understanding of the potential pathoanatomical underpinnings of chronic WAD, and add to the growing body of evidence of peripheral morphological changes associated with this challenging and burdensome condition (Eliott *et al.*, 2010; Elliott, 2011; Elliott *et al.*, 2011).

Future Research Questions

The findings of Study 5 demonstrate morphological differences between cervical spine meniscoids of people with chronic WAD with those of a pain-free population. It is not known if these differences exist in atraumatic chronic neck pain conditions such as NSNP, or in forms of neck trauma not related to a whiplash event, or if these

characteristics are specific to WAD. Further research is therefore needed examining meniscoid morphology in other chronic neck pain conditions, to further elucidate a possible pathoanatomical role of these structures.

It was also noted in Study 5 that in the chronic WAD group, dorsal zygapophyseal joint meniscoids were more likely to be fibrous in composition when compared to control participants. When considered in the context of the mechanics of a whiplash event (see Chapter 2), it is possible that meniscoids at the dorsal pole of a zygapophyseal joint may be particularly vulnerable to damage through compression between enclosing articular facets. Such damage could feasibly contribute to scar tissue formation and fibrosis, possibly accounting for the increased likelihood for dorsal meniscoids to be fibrous in composition in the chronic WAD group. Further investigation of this hypothesis may be valuable in enhancing our understanding of the pathomechanics of WAD.

Study 5 also found lateral atlantoaxial joint meniscoids to be smaller in the chronic WAD group than the control group. Due to the cross-sectional design of this study, inference cannot be made as to whether this difference is a result of the whiplash trauma and associated ongoing symptoms. Further investigation is therefore required regarding this morphological difference, particularly with respect to temporal development, association with patient presentation, and relationship to other theorised mechanisms of meniscoid involvement in neck pain.

As noted above in ‘Limitations’, the accuracy of assessment of cervical spine meniscoid composition using MRI signal intensities employed in Study 5 has not been examined directly by comparing results with microscopy. To empirically determine the accuracy

of using MRI signal intensities to infer cervical meniscoid composition, future research should directly compare meniscoid radiological appearance with microscopy in a sample of cadavers or people undergoing cervical spine surgery. This would validate this method of radiological assessment of meniscoid composition, and in turn guide future radiological examination of meniscoid morphology for research and potentially clinical purposes.

Finally, cervical spine meniscoid Entrapment or Extrapment Theories as possible mechanisms for acute torticollis require examination *in vivo*. The ability to visualise cervical spine meniscoids in living people through MRI has been established by Friedrich *et al.* (2007), Friedrich *et al.* (2008), Webb *et al.* (2011b), Webb *et al.* (2009) and now Study 5 (Chapter 7). MRI would therefore be an appropriate method by which future research could explore this theorised pathoanatomical mechanism in a clinical sample. Furthermore, rotation or distraction based manual therapy has been proposed as an intervention efficacious in addressing the entrapment of a meniscoid between articular surfaces, as suggested in the Entrapment Theory, by gapping the articular surfaces to allow a displaced meniscoid to return to its resting position (Bogduk & Jull, 1985; Evans, 2002; Kos *et al.*, 2002). If it is possible to identify 'entrapped' or 'extrapped' meniscoids with MRI, future research could investigate the effect of manual therapy upon meniscoid position.

Conclusions

This thesis consists of five studies that have investigated aspects of cervical spine meniscoid morphology, in order to advance current understanding of the potential

clinical significance of these structures. The morphology of cervical spine meniscoids has been examined in cadavers using gross dissection and light microscopy, with findings indicating an association between fibrous meniscoid composition and articular degeneration, suggestive of a possible relationship between meniscoid morphology and articular pathology. Immunohistochemical investigation of the innervation of cervical spine meniscoids has shown the presence of myelinated fibres consistent in morphology with nociceptive Aδ-fibres in adjacent joint capsules, but not within the meniscoids. Unmyelinated fibres were also noted in joint capsules and occasionally within meniscoid bodies, suggestive of potential nociceptive innervation by C-fibres. Finally, meniscoid anatomy has been examined *in vivo* in a pathological population, demonstrating distinct morphological differences when compared to a pain-free population, notably in the form of decreased meniscoid size in the lateral atlantoaxial joints of people with chronic WAD, and increased frequency of fibrous composition of the meniscoids of the dorsal aspects of cervical zygapophyseal joints in those with chronic WAD.

These findings provide evidence of morphological changes affecting cervical spine meniscoids in association with pathology, as well as a feasible capacity for these structures to give rise to nociceptive input through noxious stimuli. From a clinical perspective, it is therefore plausible that cervical spine meniscoids could contribute to neck pain, at least in some individuals. The insight gained from this work has significance for the practice of physiotherapy and musculoskeletal medicine, as it contributes to current knowledge and understanding of the pathoanatomical basis of pain originating from the cervical spine.

APPENDICES

Appendix A – Statements from co-authors relating to papers published

Statement from Darren A. Rivett relating to papers published with Scott Farrell

I, Darren A. Rivett, attest that Research Higher Degree candidate, Scott Farrell, contributed to the listed publications by contributing to the study conception and design, data acquisition, data analysis and interpretation, manuscript development and revision for publication.

Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2015). Formic acid immersion does not affect the morphometry of cervical zygapophyseal joint meniscoids – a methodological study for anatomical dissection. *Anatomical Science International 90* pp57-63. DOI: 10.1007/s12565-014-0248-8.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2015). The anatomy and morphometry of cervical zygapophyseal joint meniscoids. *Surgical and Radiologic Anatomy 37* pp799-807. DOI: 10.1007/s00276-014-1406-3.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Immunohistochemical investigation of nerve fibre presence and morphology in elderly cervical spine meniscoids. *The Spine Journal*. DOI: 10.1016/j.spinee.2016.06.004.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (*in press*). Morphology of cervical spine meniscoids in individuals with chronic whiplash associated disorder: a case-control study. *Journal of Orthopaedic and Sports Physical Therapy*.

Statement from Peter G. Osmotherly relating to papers published with Scott Farrell

I, Peter G. Osmotherly, attest that Research Higher Degree candidate, Scott Farrell, contributed to the listed publications by contributing to the study conception and design, data acquisition, data analysis and interpretation, manuscript development and revision for publication.

Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2015). Formic acid immersion does not affect the morphometry of cervical zygapophyseal joint meniscoids – a methodological study for anatomical dissection. *Anatomical Science International 90* pp57-63. DOI: 10.1007/s12565-014-0248-8.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2015). The anatomy and morphometry of cervical zygapophyseal joint meniscoids. *Surgical and Radiologic Anatomy 37* pp799-807. DOI: 10.1007/s00276-014-1406-3.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Immunohistochemical investigation of nerve fibre presence and morphology in elderly cervical spine meniscoids. *The Spine Journal*. DOI: 10.1016/j.spinee.2016.06.004.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (*in press*). Morphology of cervical spine meniscoids in individuals with chronic whiplash associated disorder: a case-control study. *Journal of Orthopaedic and Sports Physical Therapy*.

Statement from Jon Cornwall relating to papers published with Scott Farrell

I, Jon Cornwall, attest that Research Higher Degree candidate, Scott Farrell, contributed to the listed publications by contributing to the study conception and design, data acquisition, data analysis and interpretation, manuscript development and revision for publication.

Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2015). Formic acid immersion does not affect the morphometry of cervical zygapophyseal joint meniscoids – a methodological study for anatomical dissection. *Anatomical Science International 90* pp57-63. DOI: 10.1007/s12565-014-0248-8.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2015). The anatomy and morphometry of cervical zygapophyseal joint meniscoids. *Surgical and Radiologic Anatomy 37* pp799-807. DOI: 10.1007/s00276-014-1406-3.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Immunohistochemical investigation of nerve fibre presence and morphology in elderly cervical spine meniscoids. *The Spine Journal*. DOI: 10.1016/j.spinee.2016.06.004.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (*in press*). Morphology of cervical spine meniscoids in individuals with chronic whiplash associated disorder: a case-control study. *Journal of Orthopaedic and Sports Physical Therapy*.

Jon Cornwall

Signed _____

Date 21-01-2016

Scott Farrell

Signed _____

Date 1/2/16

Robert Callister

Faculty Assistant Dean (Research Training), Faculty of Health and Medicine

Signed _____

Date 1/2/16

Statement from Peter Lau relating to papers published with Scott Farrell

I, Peter Lau, attest that Research Higher Degree candidate, Scott Farrell, contributed to the listed publication by contributing to the study conception and design, data acquisition, data analysis and interpretation, manuscript development and revision for publication.

Farrell, S.F., Osmotherly, P.G., Cornwall, J., Lau, P., & Rivett, D.A. (*in press*). Morphology of cervical spine meniscoids in individuals with chronic whiplash associated disorder: a case-control study. *Journal of Orthopaedic and Sports Physical Therapy*.

Scott Farrell

Appendix B – University of Otago Department of Anatomy

Bequest Form Studies 1-4

Department of Anatomy **BEQUEST FORM B**
(Nov 2011)

REGISTRATION OF BEQUEST OF BODY FOR MEDICAL STUDY

PART A: to be completed by the donor

To:- The Licensed Anatomist
Department of Anatomy
Otago School of Medical Sciences

(A1) Having read the information sheet on Bequest of Bodies for Medical Study (Bequest Form A) **and ascertained that to the best of my knowledge none of my relatives is likely to object to the bequest**, I now confirm my wish to register the bequest of my body after death for Medical study under the conditions set out in that form and in accordance with the Human Tissue Act 2008. I accept that my remains will be cremated and the ashes scattered on **the rose garden at Andersons Bay Cemetery, Dunedin,** and I give permission for the School of Anatomy to retain, indefinitely, any material which may be of value for future teaching and/or research.

I am aware of the circumstances which may preclude my bequest being accepted upon my death, as outlined in Form A. I confirm that I have not lived in the United Kingdom, France or the Republic of Ireland between 1980 and 1996 for a cumulative 6 months or more. I understand that my estate should make alternative arrangements in case circumstances prevent my body from being accepted at the time of death.

I understand that a request for my ashes to be returned to my family will mean that the Department will not be able to utilise my body to its full potential. However, should my family request that my ashes be returned to them, they should do so in writing, no later than the time of my death. Requests received after this date will mean that the Department is unable to return all the ashes since some body parts will already be committed to teaching and research. However, the Department will endeavour to return the majority of my ashes to my family.

(A2) Please forward the "Instructions on Procedure to be followed at Death" (Bequest Form 'C'), and two copies of Bequest Form D directly **to me / my solicitor** (delete one of these).

Solicitor's name and address (if applicable):.....

.....

(A3) My full name is: (Please use block capitals)

Mr/Mrs/Miss/Ms .....

(A4) **Address** .....

.....

(A5) **Age** .....

(A6) **Date of Birth** ..... Please turn over

(A7) I am happy for the Department of Anatomy to obtain my medical records through my General Practitioner and/or Hospital Specialist. The Department may extract information from my records that is relevant to the anatomical examination of my body. I understand that my identity will be kept private at all times, and personal information will not be given to students.

YES / NO (delete one of these)

My General Practitioner is Dr .....

Address: .....

My Hospital Specialist is .....

Address .....

(A8) **Donor Signature** ..... **Date** .....

If the Donor is unable to sign, a person who has Power of Attorney may sign on their behalf:

Signature ..... **Date** .....

Name & Relation to Donor .....

PART B : to be completed by a member of the immediate family* or a close available relative*

(B9) Having read the information sheet on Bequest of Bodies for Medical Study (Bequest Form A), **and section A1 of this form**, I give my consent for the above named person to register their wish to bequeath their body to the Department of Anatomy at the Otago School of Medical Sciences.

Mr/Mrs/Miss/Ms .....

Address .....

Relationship to Donor .....

(B10) **Signature** ..... **Date** .....

* Immediate family or a close available relative relates to a person who has a close relationship or one of the following relationships to the Donor:
Spouse, civil union partner, de facto partner, child, parent, guardian, grandparent, brother, sister, stepchild, step-parent, stepbrother, stepsister

Appendix C – Correspondence, University of Otago Studies 1-4

5 February 2013

To whom it may concern,

Re: Scott Farrell, upcoming visit to the University of Otago during 2013

This letter is to inform Newcastle University of the approval given by the Department of Anatomy, University of Otago, for the visit of Scott to participate in research at this institution. Scott is to be supervised by myself while undertaking a dissection study here over an eight week period during 2013. Prof. Dave Grattan (head of department) and Prof. Mark Stringer (Professor of Anatomy) are aware of and have approved this visit.

This study will involve Scott performing dissection on donated cadaveric material within the department. The tissue will be utilized in accordance with the NZ Human Tissue Acts (1968, 2004). Such investigations do not require ethical approval at our institution as subjects are deemed to have provided informed consent prior to death.

Attached documents include a copy of the document that relates to informed consent for body donation, and code of conduct documents for students utilizing human material. We have no documentation for risk assessment or standard operating procedures for dissection room activities.

Kind regards,

Jon Cornwall

Dr. Jon Cornwall
Dip.Phty, BSc(Physiol.),
PGCertTertT, DMPhty(Manipulative Phty),
MSc(Anat.), PhD(Anat.).
Postdoctoral Fellow in Clinical Anatomy,
Department of Anatomy,
University of Otago,
Dunedin, New Zealand.
Ph: +64.3.470.4696

Appendix D – Notification of Ethics Committee Approval Studies 1-4

HUMAN RESEARCH ETHICS COMMITTEE

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Notification of Expedited Approval

To Chief Investigator or Project Supervisor:	**Professor Darren Rivett**
Cc Co-investigators / Research Students:	**Mr Peter Osmotherly** **Mr Scott Farrell**
Re Protocol:	**Descriptive anatomy of the cervical zygapophyseal meniscoids**
Date:	**06-Mar-2013**
Reference No:	**H-2013-0043**
Date of Initial Approval:	**06-Mar-2013**

Thank you for your **Response to Conditional Approval (minor amendments)** submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under **Expedited** review by the Ethics Administrator.

I am pleased to advise that the decision on your submission is **Approved** effective **06-Mar-2013**.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research, 2007, and the requirements within this University relating to human research.

Approval will remain valid subject to the submission, and satisfactory assessment, of annual progress reports. *If the approval of an External HREC has been "noted" the approval period is as determined by that HREC.*

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal *Certificate of Approval* will be available upon request. Your approval number is **H-2013-0043**.

If the research requires the use of an Information Statement, ensure this number is inserted at the relevant point in the Complaints paragraph prior to distribution to potential participants You may then proceed with the research.

Conditions of Approval

This approval has been granted subject to you complying with the requirements for *Monitoring of Progress*, *Reporting of Adverse Events*, and *Variations to the Approved Protocol* as detailed below.

PLEASE NOTE:
In the case where the HREC has "noted" the approval of an External HREC, progress reports and reports of adverse events are to be submitted to the External HREC only. In the case of Variations to the approved protocol, or a Renewal of approval, you will apply to the External HREC for approval in the first instance and then Register that approval with the University's HREC.

- ***Monitoring of Progress***

Other than above, the University is obliged to monitor the progress of research projects involving human participants to ensure that they are conducted according to the protocol as approved by the HREC. A

progress report is required on an annual basis. Continuation of your HREC approval for this project is conditional upon receipt, and satisfactory assessment, of annual progress reports. You will be advised when a report is due.

- ***Reporting of Adverse Events***

1. It is the responsibility of the person **first named on this Approval Advice** to report adverse events.
2. Adverse events, however minor, must be recorded by the investigator as observed by the investigator or as volunteered by a participant in the research. Full details are to be documented, whether or not the investigator, or his/her deputies, consider the event to be related to the research substance or procedure.
3. Serious or unforeseen adverse events that occur during the research or within six (6) months of completion of the research, must be reported by the person first named on the Approval Advice to the (HREC) by way of the Adverse Event Report form (via RIMS at https://rims.newcastle.edu.au/login.asp) within 72 hours of the occurrence of the event or the investigator receiving advice of the event.
4. Serious adverse events are defined as:
    - Causing death, life threatening or serious disability.
    - Causing or prolonging hospitalisation.
    - Overdoses, cancers, congenital abnormalities, tissue damage, whether or not they are judged to be caused by the investigational agent or procedure.
    - Causing psycho-social and/or financial harm. This covers everything from perceived invasion of privacy, breach of confidentiality, or the diminution of social reputation, to the creation of psychological fears and trauma.
    - Any other event which might affect the continued ethical acceptability of the project.

5. Reports of adverse events must include:
    - Participant's study identification number;
    - date of birth;
    - date of entry into the study;
    - treatment arm (if applicable);
    - date of event;
    - details of event;
    - the investigator's opinion as to whether the event is related to the research procedures; and
    - action taken in response to the event.

6. Adverse events which do not fall within the definition of serious or unexpected, including those reported from other sites involved in the research, are to be reported in detail at the time of the annual progress report to the HREC.

- ***Variations to approved protocol***

If you wish to change, or deviate from, the approved protocol, you will need to submit an *Application for Variation to Approved Human Research* (via RIMS at https://rims.newcastle.edu.au/login.asp). Variations may include, but are not limited to, changes or additions to investigators, study design, study population, number of participants, methods of recruitment, or participant information/consent documentation. **Variations must be approved by the (HREC) before they are implemented** except when Registering an approval of a variation from an external HREC which has been designated the lead HREC, in which case you may proceed as soon as you receive an acknowledgement of your Registration.

Linkage of ethics approval to a new Grant

HREC approvals cannot be assigned to a new grant or award (ie those that were not identified on the application for ethics approval) without confirmation of the approval from the Human Research Ethics Officer on behalf of the HREC.

Best wishes for a successful project.

Professor Allyson Holbrook

Chair, Human Research Ethics Committee

For communications and enquiries:
Human Research Ethics Administration

Research Services
Research Integrity Unit
The Chancellery
The University of Newcastle
Callaghan NSW 2308
T +61 2 492 18999
F +61 2 492 17164
Human-Ethics@newcastle.edu.au

RIMS website - https://RIMS.newcastle.edu.au/login.asp

Linked University of Newcastle administered funding:

Funding body	Funding project title	First named investigator	Grant Ref

Appendix E – Notification of Ethics Committee Approval Study 5

11 October 2013

Professor Darren Rivett
School of Health Sciences
HA15 Hunter Building
University of Newcastle

Dear Professor Rivett,

Re: Role of cervical spine meniscoids in whiplash associated disorder: Cervical meniscoids in WAD (13/09/18/4.09)

HNEHREC Reference No: 13/09/18/4.09
NSW HREC Reference No: HREC/13/HNE/371

Thank you for submitting the above protocol for single ethical review. This project was first considered by the Hunter New England Human Research Ethics Committee at its meeting held on **18 September 2013.** This Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research (2007)* (National Statement) and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. Further, this Committee has been accredited by the NSW Department of Health as a lead HREC under the model for single ethical and scientific review. The Committee's Terms of Reference are available from the Hunter New England Local Health District website: http://www.hnehealth.nsw.gov.au/Human_Research_Ethics.

I am pleased to advise that following acceptance under delegated authority of the requested clarifications and revised Information Statement and Recruitment Flyer by Dr Nicole Gerrand Manager, Research Ethics & Governance, the Hunter New England Human Research Ethics Committee has granted ethical approval of the above project.

The following documentation has been reviewed and approved by the Hunter New England Human Research Ethics Committee:

- For the Participant Information Statement (Version 2 dated 26 September 2013);
- For the Consent Form (Version 1 dated 28 August 2013); and
- For the Recruitment Flyer (Version updated 1 October 2013)

For the protocol: **Role of cervical spine meniscoids in whiplash associated disorder: Cervical meniscoids in WAD**

Approval has been granted for this study to take place at the following site:

- **Calvary Mater Newcastle**

Hunter New England Research Ethics & Governance Unit
(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 950 Facsimile (02) 49214 818
Email: hnehrec@hnehealth.nsw.gov.au
http://www.hnehealth.nsw.gov.au/research_ethics_and_governance_unit

Approval from the Hunter New England Human Research Ethics Committee for the above protocol is given for a maximum of **3** years from the date of this letter, after which a renewal application will be required if the protocol has not been completed.

The *National Statement on Ethical Conduct in Human Research (2007),* which the Committee is obliged to adhere to, include the requirement that the committee monitors the research protocols it has approved. In order for the Committee to fulfil this function, it requires:

- A report of the progress of the above protocol be submitted at 12 monthly intervals. Your review date is **October 2014.** A proforma for the annual report will be sent two weeks prior to the due date.

- A final report must be submitted at the completion of the above protocol, that is, after data analysis has been completed and a final report compiled. A proforma for the final report will be sent two weeks prior to the due date.

- All variations or amendments to this protocol, including amendments to the Information Sheet and Consent Form, must be forwarded to and approved by the Hunter New England Human Research Ethics Committee prior to their implementation.

- The Principal Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including:

    - any serious or unexpected adverse events

        - Adverse events, however minor, must be recorded as observed by the Investigator or as volunteered by a participant in this protocol. Full details will be documented, whether or not the Investigator or his deputies considers the event to be related to the trial substance or procedure. These do not need to be reported to the Hunter New England Human Research Ethics Committee

        - Serious adverse events that occur during the study or within six months of completion of the trial at your site should be reported to the Manager, Research Ethics & Governance, of the Hunter New England Human Research Ethics Committee as soon as possible and at the latest within 72 hours.

        - All other safety reporting should be in accordance with the NHMRC's Safety Monitoring Position Statement – May 2009 available at http://www.nhmrc.gov.au/health_ethics/hrecs/reference/_files/090609_nhmrc_position_statement.pdf

        - Serious adverse events are defined as:

            - Causing death, life threatening or serious disability.
            - Cause or prolong hospitalisation.
            - Overdoses, cancers, congenital abnormalities whether judged to be caused by the investigational agent or new procedure or not.

    - Unforeseen events that might affect continued ethical acceptability of the project.

- If for some reason the above protocol does not commence (for example it does not receive funding); is suspended or discontinued, please inform Dr Nicole Gerrand, as soon as possible.

Hunter New England Research Ethics & Governance Unit
(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 950 Facsimile (02) 49214 818
Email: hnehrec@hnehealth.nsw.gov.au
http://www.hnehealth.nsw.gov.au/research_ethics_and_governance_unit

You are reminded that this letter constitutes ethical approval only. You must not commence this research project at a site until separate authorisation from the Chief Executive or delegate of that site has been obtained.

A copy of this letter must be forwarded to all site investigators for submission to the relevant Research Governance Officer.

Should you have any concerns or questions about your research, please contact Dr Gerrand as per the details at the bottom of the page. The Hunter New England Human Research Ethics Committee wishes you every success in your research.

Please quote **13/09/18/4.09** in all correspondence

The Hunter New England Human Research Ethics Committee wishes you every success in your research.

Yours faithfully

For: Professor M Parsons
Chair
Hunter New England Human Research Ethics Committee

Hunter New England Research Ethics & Governance Unit
(Locked Bag No 1)
(New Lambton NSW 2305)
Telephone (02) 49214 950 Facsimile (02) 49214 818
Email: hnehrec@hnehealth.nsw.gov.au
http://www.hnehealth.nsw.gov.au/research_ethics_and_governance_unit

Appendix F – Notification of Ethics Committee Reciprocal Approval Study 5

HUMAN RESEARCH ETHICS COMMITTEE

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Notification of Expedited Approval

To Chief Investigator or Project Supervisor:	**Professor Darren Rivett**
Cc Co-investigators / Research Students:	**Conjoint Associate Professor Lindsay Rowe** **Mr Peter Osmotherly** **Mr Scott Farrell**
Re Protocol:	**Role of cervical spine meniscoids in whiplash associated disorder**
Date:	**03-Feb-2014**
HREC Reference No:	**H-2014-0018**
External HREC Reference No:	**13/09/18/4.09**
Date of Initial Approval:	**29-Jan-2014**

Thank you for your **Initial Application** submission to the Human Research Ethics Committee (HREC) seeking approval in relation to the above protocol.

Your submission was considered under **Expedited Review of External Approval** review by the Chair/Deputy Chair.

I am pleased to advise that the decision on your submission is **External HREC Approval Noted** effective **29-Jan-2014**.

In approving this protocol, the Human Research Ethics Committee (HREC) is of the opinion that the project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research, 2007*, and the requirements within this University relating to human research.

As the approval of an External HREC has been "noted" the approval period is as determined by that HREC.

The full Committee will be asked to note this decision at its next scheduled meeting. A formal *Certificate of Approval* will be available upon request. Your approval number is **H-2014-0018**.

PLEASE NOTE:
As the HREC has "noted" the approval of an External HREC, progress reports and reports of adverse events are to be submitted to the External HREC only. In the case of Variations to the approved protocol, or a Renewal of approval, you will apply to the External HREC for approval in the first instance and then Register that approval with the University's HREC.

Linkage of ethics approval to a new Grant

HREC approvals cannot be assigned to a new grant or award (ie those that were not identified on the application for ethics approval) without confirmation of the approval from the Human Research Ethics Officer on behalf of the HREC.

Best wishes for a successful project.

Professor Allyson Holbrook

Chair, Human Research Ethics Committee

For communications and enquiries:
Human Research Ethics Administration

Research Services
Research Integrity Unit
The Chancellery
The University of Newcastle
Callaghan NSW 2308
T +61 2 492 17894
F +61 2 492 17164
Human-Ethics@newcastle.edu.au

RIMS website - https://RIMS.newcastle.edu.au/login.asp

Linked University of Newcastle administered funding:

Funding body	Funding project title	First named investigator	Grant Ref
		,	

Appendix G – Notification of Ethics Committee Approval

Amendment Study 5

17 December 2014

Professor Darren Rivett
School of Health Sciences
HA15 Hunter Building
University of Newcastle

Dear Professor Rivett,

Re: Role of cervical spine meniscoids in whiplash associated disorder: Cervical meniscoids in WAD (13/09/18/4.09)

HNEHREC Reference No: 13/09/18/4.09
NSW HREC Reference No: HREC/13/HNE/371
NSW SSA Reference No: SSA/13/HNE/527

Thank you for submitting a request for an amendment to the above project. This amendment was reviewed by the Hunter New England Human Research Ethics Committee. This Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research (2007)* (National Statement) and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. Further, this Committee has been accredited by the NSW Department of Health as a lead HREC under the model for single ethical and scientific review.

I am pleased to advise that the Hunter New England Human Research Ethics Committee has granted ethical approval for the following amendment requests:

- For the addition of Dr Peter Lau as co-investigator; and
- For the Participant Information Statement (Version 3 dated 17 December 2014)

For the study: **Role of cervical spine meniscoids in whiplash associated disorder: Cervical meniscoids in WAD**

Approval has been granted for this study to take place at the following site:

- **Hunter New England Radiology, Calvary Mater Newcastle**

Approval from the Hunter New England Human Research Ethics Committee for the above study is given for a maximum of **3** years from the date of the approval letter of your initial application after

Hunter New England Human Research Ethics Committee
Locked Bag 1
New Lambton NSW 2305
Telephone: (02) 49214950 Facsimile: (02) 49214818
Email: HNELHD-HREC@hnehealth.nsw.gov.au
http://www.hnehealth.nsw.gov.au/research_ethics_and_governance_unit

which a renewal application will be required if the study has not been completed. The above study is approved until **October 2016.**

The *National Statement on Ethical Conduct in Human Research (2007)* which the Committee is obliged to adhere to, include the requirement that the committee monitors the research protocols it has approved. In order for the Committee to fulfil this function, it requires:

- A report of the progress of the above study to be submitted at 12 monthly intervals. Your review date is **October 2015.** A proforma for the annual report will be sent two weeks prior to the due date.

- A final report must be submitted at the completion of the above study, that is, after data analysis has been completed and a final report compiled. A proforma for the final report will be sent two weeks prior to the due date.

- All variations or amendments to this study, including amendments to the Information Sheet and Consent Form, must be forwarded to and approved by the Hunter New England Human Research Ethics Committee prior to their implementation.

- The Principal Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including:

    - any serious or unexpected adverse events

        - Adverse events, however minor, must be recorded as observed by the Investigator or as volunteered by a participant in this study. Full details will be documented, whether or not the Investigator or his deputies considers the event to be related to the trial substance or procedure.

        - Serious adverse events that occur during the study or within six months of completion of the trial at your site should be reported to the Ethics Officer of the Hunter New England Human Research Ethics Committee as soon as possible and at the latest within 72 hours.

        - Copies of serious adverse event reports from other sites should be sent to the Hunter New England Human Research Ethics Committee for review as soon as possible after being received.

        - Serious adverse events are defined as:

            - Causing death, life threatening or serious disability.
            - Cause or prolong hospitalisation.
            - Overdoses, cancers, congenital abnormalities whether judged to be caused by the investigational agent or new procedure or not.

    - Unforeseen events that might affect continued ethical acceptability of the project.

- If for some reason the above study does not commence (for example it does not receive funding); is suspended or discontinued, please inform Dr Nicole Gerrand, the Manager, Research Ethics and Governance Unit as soon as possible.

The Hunter New England Human Research Ethics Committee also has delegated authority to approve the commencement of this research on behalf of the Hunter New England Local Health District. This research may therefore commence.

Hunter New England Human Research Ethics Committee
Locked Bag 1
New Lambton NSW 2305
Telephone: (02) 49214950 Facsimile: (02) 49214818
Email: HNELHD-HREC@hnehealth.nsw.gov.au
http://www.hnehealth.nsw.gov.au/research_ethics_and_governance_unit

Should you have any queries about your project please contact Dr Nicole Gerrand as per the contact details at the bottom of the page. The Hunter New England Human Research Ethics Committee Terms of Reference, Standard Operating Procedures, membership and standard forms are available from the Hunter New England Local Health District website.

Please quote **13/09/18/4.09** in all correspondence.

The Hunter New England Human Research Ethics Committee wishes you every success in your research.

Yours faithfully

For: Professor M Parsons
Chair
Hunter New England Human Research Ethics Committee

Hunter New England Human Research Ethics Committee
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Appendix H – Journal Publication Study 1

Farrell, S.F., Osmotherly, P.G., Rivett, D.A., & Cornwall, J. (2015). Formic acid immersion does not affect the morphometry of cervical zygapophyseal joint meniscoids – a methodological study for anatomical dissection. *Anatomical Science International 90* pp57-63. DOI: 10.1007/s12565-014-0248-8.

Appendix I – Notification of Safety Committee Approval Studies 1-4

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

University Drive, Callaghan
NSW 2308 AUSTRALIA
HUMAN RESOURCE SERVICES
HEALTH AND SAFETY

Contact Person: Liz Pilgrim

Telephone 02-4921 6542
Fax 02-4921 6982
E-mail: liz.pilgrim@newcastle.edu.au

19 March 2013

TO: Professor D Rivett, School of Health Sciences
COPY TO: Professor Nick Talley, Pro Vice-Chancellor (Health)
Mr P Osmotherly, Mr S Farrell, School of Health Sciences
Ms R Gibbins, Human Research Ethics Officer
FROM: Ms Melissa Musicka, Senior Safety Officer (Laboratory/Research)
SUBJECT: SAFETY REVIEW NOTIFICATION

I wish to advise that the following Research Project based on the information provided, has been reviewed by the relevant Technical Committee/s and is subject to compliance with all the appended comments and there being no variation to the research processes that have been indicated in the application. If there is any variation to the protocol that affects the safety outcomes an additional application for safety review is necessary.

PROJECT TITLE	CHEMICAL	INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)	RADIATION	GRANTING BODY
THE ANATOMY AND HISTOLOGY OF THE CERVICAL ZYGAPOPHYSEAL MENISCOIDS **REF NO 10/2013**	NA	REVIEWED 15.03.2013	NA	NA

Where the research plan requires the use of a PC2 laboratory the safety approval is provided for work to be performed only in the PC2 laboratory/ies nominated on the Safety Review Application or where directed by the IBC. No other facility may be used for the procedures. If the research plan changes necessitating the use of equipment located within another laboratory the Safety Review Application - Variation must be completed and an additional review completed by the respective Committee/s prior to any work being performed in another laboratory.

In order to comply with the Work Health and Safety Act 2011 Chief Investigators must ensure that all, reasonably foreseeable, occupational health and safety risks arising out of their research activities are effectively controlled. A risk assessment must be completed to achieve this control. Effective controls follow on through the elimination (preferable), or minimisation, of these risks. Risk assessments are only validated once they have been signed and dated by the author and authorising supervisor. They must be reviewed annually and the review process needs to be documented (signed and dated).

Control measures for research activities must include (but are not limited to):

- A site (documented) orientation/induction to be given to all personnel when they are attending a facility/location for the first time. This induction will include; local rules (eating, drinking, storage of bags, standard of dress, hand washing and Personnel Protective Equipment [PPE] required etc), location of amenities, location of emergency procedures flipchart (which must contain correct contact information), rundown of evacuation procedure including the location of the assembly area.
- **Ongoing inductions as appropriate to ensure current legislative requirements are reinforced to all facility users.**
- Written standard operating procedures (SOP's) for equipment and processes
- Current SDS's must be readily available for all hazardous substances associated with the activity
- Training in SOP's for all personnel engaged in hazardous operations (with appropriate records)
- All documented control measures must be implemented.
- The minimum requirements for PPE in a laboratory are laboratory clothing (lab coat), protective eyewear (safety glasses), and closed shoes unless lesser requirements can be justified by a risk assessment AS/NZS 2243.1 Safety in laboratories Part 1: Planning and operational aspects.
- When ordering hazardous substances with a hazard rating of high or extreme, consideration should be given to using safer alternate reagents (where available) and only amounts required for the project should be purchased- to prevent unnecessary stockpiles of hazardous reagents.
- Any injury/incident occurring during the activity is reported via the University Online Incident Reporting System

Please ensure that all staff involved in working on this project is aware of the requirements of this review and a copy of this memorandum is to be kept in the Laboratory Safety Manual/s of the facility/s where the work is performed.

If you have any enquiries in relation to this safety review (implications) please do not hesitate to contact me.

MS MELISSA MUSICKA
SENIOR SAFETY OFFICER
LABORATORY/RESEARCH SAFETY

Appendix J – Permission Letter, University of Otago Department of Anatomy Studies 1-4

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND

10 April 2013

PERSONAL

VisStudent
Farrell
PH: Gen

Mr S Farrell
2/30 Andretta Avenue
Elemore Vale
NSW 2287
Australia

Dear Mr Farrell

The Department of Anatomy advised me that you have sought an opportunity to obtain work experience in the Department, as a Visiting Student, without remuneration.

I am pleased to offer you the Visiting Student work experience opportunity under the supervision of Dr Jonathon Cornwall in a research capacity from 27 March 2013 to 28 June 2013. The work experience will involve a project to identify the morphology of the cervical zygapophysial joint meniscoids. The purpose of this arrangement, which does not and should not be seen to establish an employment relationship between the University of Otago and yourself, is to allow you an opportunity to gain work experience and training in an area in which you have expressed an interest and are currently studying.

As previously stated this arrangement is without emolument. It is understood that you will continue to be self-supported. This arrangement will provide access to laboratory, library and computing facilities and there will be no requirement to pay bench fees.

You will be responsible to the Head of Department, Professor David Grattan, who will oversee your activities in the Department. You are required to observe all University Occupational Health and Safety regulations and policies, as well as the University's Ethical Behaviour Policy and Smoke-Free Policy, and to act in accordance with instruction from Dr Cornwall.

We are pleased to be able to assist you with this unique opportunity and reiterate again that in doing so we are not establishing an employment relationship with you. I trust that you will find the experience both challenging and rewarding and that you will acquire the experience and training which you seek. This arrangement may be terminated by the University without notice at any time.

The contents of this letter of offer and any attachments constitute the entire agreement between yourself and the University, and supersede all previous representations, negotiations, commitments and communications, either written or oral between the parties. Modification of these agreements will only be binding on the University where they have been formally offered by the Human Resources Division and accepted.

Human Resources Division / Te Wāhanga Matua Pūmanawa Tangata
PO Box 56, Dunedin / Ōtepoti 9054, New Zealand / Aotearoa
Tel / Waea +64 3 479 8269 • Fax / Waea whakaahua +64 3 479 8279 • Email / Īmēra human.resources@otago.ac.nz
www.otago.ac.nz

If you have any immediate questions regarding this arrangement you should contact Dr Cornwall on telephone +64 3 470 4696, or email *jon.cornwall@otago.ac.nz*. I would appreciate it if you could sign the attached copy of this letter and return it to me as soon as possible as confirmation of the arrangements described above.

Yours sincerely

Paul Hibbert
for Director of Human Resources Division

I confirm these arrangements subject to the above terms:

Signed: _____ Date: _____

cc Professor D.R. Grattan
Professor H.D. Nicholson

Appendix K – Characteristics of Cadavers Included in Studies 1-4

Number	Sex	Age at Death	Cause of Death	Embalming Fluid
O472	M	82	Renal failure	Crosado
O379	F	93	Gastrointestinal haemorrhage/chronic renal failure	Dodge Anatomical
O455	F	89	Pneumonia	Crosado
O450	F	69	Metastatic melanoma	Crosado
O539	M	67	Sudden cardiac death/coronary artery disease	Dodge Anatomical
O399	M	85	Pneumonia/renal failure	Dodge Anatomical
O383	M	67	Ischaemic bowel	Dodge Anatomical
O580	F	77	Metastatic small bowel cancer	Crosado
O511	F	84	Acute renal failure	Crosado
O572	M	79	Metastatic renal carcinoma	Crosado
O530	M	73	Sepsis/aspiration pneumonia	Crosado
O518	F	87	Pneumonia/congestive heart failure	Crosado
O555	M	83	Hepatocellular carcinoma	Crosado
O533	M	90	Pulmonary oedema/heart failure	Crosado
O521	F	73	Carcinomatosis	Crosado
O467	F	67	Metastatic adenocarcinoma lung	Crosado
O398	M	82	Metastatic melanoma	Crosado
O528	M	74	Large right intracerebral haemorrhage/hypertension	Crosado

Appendix L – University of Otago Department of Anatomy Embalming Fluid Recipe Studies 1-4

Crosado Mix Embalming Fluid Recipe, University of Otago

40 L 95% ethanol

10 L water

10 L glycerine

5 L 99% phenoxytol

1.25 L 37% formaldehyde in solution

Dodge Anatomical Mix Embalming Fluid Details (Dodge Anatomical, Dodge Co., Boston, MA)

A commercially available water-based embalming fluid. Also known as Michigan Mix.

Dodge metasyn arterial chemical (22%)

Dodge metaflow arterial conditioner (30.7%)

Dodge restorative humectant (7%)

Dodge mold X (3%)

Water

Due to commercial sensitivity, Dodge Co. does not provide further information regarding the individual ingredients.

Appendix M – Journal Publication Study 2

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Morphology and morphometry of lateral atlantoaxial joint meniscoids. *Anatomical Science International 91* pp89-96. DOI: 10.1007/s12565-015-0276-z.

Appendix N – Journal Publication Study 3

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2015). The anatomy and morphometry of cervical zygapophyseal joint meniscoids. *Surgical and Radiologic Anatomy 37* pp799-807. DOI: 10.1007/s00276-014-1406-3.

Full text version of final published article available at:

http://link.springer.com/article/10.1007/s00276-014-1406-3

Appendix O – Immunohistochemistry Pilot Testing Study 4

The following details a series experiments trialing different immunohistochemical techniques using a variety of antisera undertaken in the development of a method to be utilised in Study 4. After eight unsuccessful trials performed between September 2014 and February 2015, a successful method was developed that became the protocol employed in Study 4 (Appendix P).

Pilot 1

Day 1 30/09/2014

Using meniscoid (dorsal, specimen O530), block cut in sagittal plane from mid-section of meniscoid. Pilot testing undertaken as per protocol of Jobling Lab, School of Biomedical Sciences and Pharmacy, The University of Newcastle.

1. Wash fix out – 3 x 15 minutes 80% ETOH
2. Permeablise membranes – 3 x 15 minutes dimethyl sulfoxide (DMSO)
3. Dehydrate (to allow for polyethylene glycol (PEG) embedding)
4. Place in 1000 MW molten PEG in 46° oven until tissue sinks (2 hours)
5. Embed in 1450 MW molten PEG in a cryostat mould
6. Place in -20° freezer for 5 minutes
7. Remove from mould and mount on microtome chuck for sectioning (15 μm)
8. Place sections free floating in phosphate buffered saline (PBS)

9. Move sections into 10% normal donkey serum (NDS) – 30 minutes

10% NDS made by diluting NDS in antibody diluent (NaCl 0.3 M, Na_2HPO_4 7.5 M, NaH_2PO_4 2.5 M Na azide 0.05%)

10. Place in primary antisera (plus 10% NDS) diluted in antibody diluent at following concentrations overnight in humidity chamber
    a. Rat ∝ substance P (SP) 1:200
    b. Rabbit ∝ nitric oxide synthase (NOS) 1:1000

Day 2 01/10/2014

11. Wash off primary antisera – 3 x 15 minutes PBS
12. Place in secondary antisera for 2 hours in humidity chamber
    a. ∝ Rat fluorescein isothiocyanate (FITC) 1:50
    b. ∝ Rabbit Cyanine 3 (CY3) 1:50
13. Wash off secondary antisera – 3 x 15 minutes in PBS
14. Mount in buffered glycol on glass microscope slide with coverslip
15. View using fluorescence microscope

Outcome: nil effective staining identified, non-specific immunofluorescence noted.

Pilot 2

Day 1 08/10/14

Using meniscoid (dorsal, specimen O530), block cut in sagittal plane from mid-section of meniscoid. Pilot testing undertaken as per protocol of Jobling Lab, with addition of antigen retrieval.

1. Wash fix out – 3 x 15 minutes 80% ETOH
2. Permeablise membranes – 3 x 15 minutes DMSO
3. Dehydrate (to allow for PEG embedding)
4. Place in 1000 MW molten PEG in 46° oven until tissue sinks (2 hours)
5. Embed in 1450 MW molten PEG in a cryostat mould
6. Place in -20° freezer for 5 minutes
7. Remove from mould and mount on microtome chuck for sectioning (15 μm)
8. Place sections free floating in PBS
9. Antigen retrieval – microwave in citrate buffer (one and two minute subgroups)
10. Wash in PBS for 15 minutes
11. Move sections into 10% NDS – 30 minutes
12. Place in primary antisera (plus 10% NDS) diluted in antibody diluent at following concentrations overnight in humidity chamber
    a. Rat ∝ SP 1:200
    b. Rabbit ∝ NOS 1:1000

Day 2 09/10/2014

13. Wash off primary antisera – 3 x 15 minutes PBS
14. Place in secondary antisera for 2 hours in humidity chamber
    a. ∝ Rat FITC 1:50
    b. ∝ Rabbit CY3 1:50
15. Wash off secondary antisera – 3 x 15 minutes in PBS
16. Mount in buffered glycol on glass microscope slide with coverslip
17. View using fluorescence microscope

Outcome: Possible immunoreactive staining identified for both SP and NOS sections for 2 minute antigen retrieval subgroup, however moderate non-specific immunofluorescence present as well (Figure 1). Need to investigate with positive and negative control runs.

Pilot 3

Day 1 27/10/14

Using meniscoid (dorsal, specimen O530), block cut in sagittal plane from mid-section of meniscoid. Pilot testing undertaken as per protocol of Jobling Lab, with addition of antigen retrieval and negative controls (i.e. protocol without primary and/or secondary antisera as well as unlabelled sections).

1. Wash fix out – 3 x 15 minutes 80% ETOH

2. Permeablise membranes – 3 x 15 minutes DMSO
3. Dehydrate (to allow for PEG embedding)
4. Place in 1000 MW molten PEG in 46° oven until tissue sinks (2 hours)
5. Embed in 1450 MW molten PEG in a cryostat mould
6. Place in -20° freezer for 5 minutes
7. Remove from mould and mount on microtome chuck for sectioning (15 μm)
8. Place sections free floating in PBS
9. Antigen retrieval – microwave in citrate buffer two minutes
10. Wash in PBS for 15 minutes
11. Move sections into 10% NDS – 30 minutes
12. Place in primary antisera (plus 10% NDS) diluted in antibody diluent at following concentrations overnight in humidity chamber
    a. Rat ∝ SP 1:200
    b. Rabbit ∝ NOS 1:1000

<u>Day 2 28/10/14</u>

13. Wash off primary antisera – 3 x 15 minutes PBS
14. Place in secondary antisera for 2 hours in humidity chamber
    a. ∝ Rat FITC 1:50
    b. ∝ Rabbit CY3 1:50
15. Wash off secondary antisera – 3 x 15 minutes in PBS
16. Mount in buffered glycol on glass microscope slide with coverslip
17. View using fluorescence microscope

Negative controls:

- Rat ∝ SP primary antisera only
- Rabbit ∝ NOS primary antisera only
- ∝ Rat FITC secondary antisera only
- ∝ Rabbit CY3 secondary antisera only
- Unlabelled

Outcome: Possible immunoreactive staining identified for both SP and NOS sections, however moderate non-specific immunofluorescence present as well. Need to investigate with positive control runs.

Pilot 4

Day 1 31/10/14

Using section of spinal nerve excised from a study cadaver as a positive control for the presence of nerve tissue. Pilot testing undertaken as per protocol of Jobling Lab, with addition of antigen retrieval as per Pilot 2.

1. Wash fix out – 3 x 15 minutes 80% ETOH
2. Permeablise membranes – 3 x 15 minutes DMSO
3. Dehydrate (to allow for PEG embedding)
4. Place in 1000 MW molten PEG in 46° oven until tissue sinks (2 hours)
5. Embed in 1450 MW molten PEG in a cryostat mould
6. Place in -20° freezer for 5 minutes

7. Remove from mould and mount on microtome chuck for sectioning (15 μm)
8. Place sections free floating in PBS
9. Antigen retrieval – microwave in citrate buffer two minutes
10. Wash in PBS for 15 minutes
11. Move sections into 10% NDS – 30 minutes
12. Place in primary antisera (plus 10% NDS) diluted in antibody diluent at following concentrations overnight in humidity chamber
    a. Rat ∝ SP 1:200
    b. Rabbit ∝ NOS 1:1000
    c. Mouse ∝ NF-H 1:2500
    d. Rabbit ∝ Tyrosine hydroxylase (TH) 1:100

Day 2 03/11/14

13. Wash off primary antisera – 3 x 15 minutes PBS
14. Place in secondary antisera for 2 hours in humidity chamber
    a. ∝ Rat FITC 1:50
    b. ∝ Rabbit CY3 1:50
    c. ∝ Mouse aminomethylcoumarin (AMCA) 1:50
15. Wash off secondary antisera – 3 x 15 minutes in PBS
16. Mount in buffered glycol on glass microscope slide with coverslip
17. View using fluorescence microscope

Negative control:

- Unlabelled

Outcomes: Possible immunoreactive staining identified for all primary antibodies sections, however moderate non-specific immunofluorescence present as well including on unlabelled sections. Non-specific immunofluorescence significant limitation to fluorescence immunohistochemistry, so will pilot DAB immunohistochemistry.

Pilot 5

Day 1 6/11/14

Using meniscoid (dorsal, specimen O530), block cut in sagittal plane from mid-section of meniscoid, as well as fixed rat brain as positive control in kind from A/Prof. Paul Tooney. Pilot testing undertaken as per protocol of Tooney Lab, School of Biomedical Sciences and Pharmacy, The University of Newcastle.

1. Wash fix out – 3 x 15 minutes 80% ETOH
2. Permeablise membranes – 3 x 15 minutes DMSO
3. Dehydrate (to allow for PEG embedding)
4. Place in 1000 MW molten PEG in 46° oven until tissue sinks (2 hours)
5. Embed in 1450 MW molten PEG in a cryostat mould
6. Place in -20° freezer for 5 minutes
7. Remove from mould and mount on microtome chuck for sectioning (20 μm)
8. Place sections free floating in PBS
9. Wash in PBS for 15 minutes

Day 2 12/11/14

10. Sections free floating in PBS
11. Transfer to solution 49.55% methanol, 49.55% PBS, 0.9% hydrogen peroxide (H_2O_2) solution – 20 minutes
12. Remove PBS/H_2O_2 solution and add triton diluent (PBS + 0.1% triton X-100) – 15 minutes
13. Remove triton diluent and add 10% NDS – 30 minutes
14. Remove NDS and add primary antisera diluted in triton diluent containing 1% NDS
    a. Rabbit ∝ Protein Gene Product (PGP) 9.5 1:200
15. Place in humidity chamber and incubate on rocker in cold room for 48 hrs

Day 3 13/11/14

16. Remove primary antibody solution and wash in triton diluent – 3 x 10 minutes
17. Incubate sections for 1h at room temperature with secondary antibody diluted in triton diluent containing 1% NDS
    a. Donkey ∝ Rabbit 1:1000
18. Prepare the avidin-biotin complex (ABC) tertiary agent (Vectastain Elite Kit, Vector Laboratories, Burlingame CA): Mix 100 µl A + 100 µl B in 10 ml PBS. React for 30 minutes at room temperature)
19. Remove the secondary antibody and wash in PBS – 3 x 10 minutes
20. Remove PBS, add ABC – 1 hour
21. Wash in tris buffered saline (TBS) – 3 x 10 minutes

22. Make up the DAB solution:
    a. Open satchel containing DAB tablet (Sigmafast™ 3,3′-Diaminobenzidine, Sigma-Alrdich, St. Louis MO) in fume cupboard.
    b. Drop tablet into 500 µl of distilled water and crush submerged in water
    c. Make up to 20 ml with TBS and filter through Whatman #1 paper
    d. Add 20 mL of 30% H2O2 ([0.03%] to the DAB solution
23. Remove slides from TBS, drain away excess fluid, and place on ice
24. Place sections in DAB solution, allow exposure to up to three minutes watching for colour change. When section begins to turn brown, remove from DAB and place in distilled water
25. Mount on gelatin subbed glass microscope slide, allow to dry overnight

Day 4 14/11/14

26. Dehydrate:
    a. 70% ETOH – 5 minutes
    b. 95% ETOH – 5 minutes
    c. 100% ETOH – 2 x 5 minutes
    d. Xylene – 3 x 5 minutes
27. Coverslip – Ultramount (Dako Australia Pty. Ltd., North Sydney, Australia) mounting medium
28. View using light microscope

Outcomes: Some successful immunoreactive staining in the rat brain, but not in the meniscoid. Next step to re-attempt with antigen retrieval and addition of a second primary antisera.

Pilot 6

Using meniscoid sections cut on Day 1 of Pilot 5 that had been stored in PBS with 0.05% sodium azide, as well as fixed rat brain in kind from A/Prof. Paul Tooney. Pilot testing undertaken as per protocol of Tooney Lab with addition of antigen retrieval.

<u>Day 1 08/12/14</u>

1. Sections free floating in PBS
2. Antigen retrieval – microwave in citrate buffer two minutes
3. Transfer to solution 49.55% methanol, 49.55% PBS, 0.9% hydrogen peroxide (H_2O_2) solution – 20 minutes
4. Remove PBS/H_2O_2 solution and add triton diluent – 15 minutes
5. Remove triton diluent and add 10% NDS – 30 minutes
6. Remove NDS and add primary antisera diluted in triton diluent containing 1% NDS
    a. Rabbit ∝ PGP 9.5 1:200
    b. Rat ∝ Somatostatin 1:500
7. Place in humidity chamber and incubate on rocker in cold room for 48 hrs

Day 2 10/12/14

8. Remove primary antibody solution and wash in triton diluent – 3 x 10 minutes
9. Incubate sections for 1h at room temperature with secondary antibody diluted in triton diluent containing 1% NDS
    a. Donkey ∝ Rabbit 1:1000
    b. Donkey ∝ Rat 1:1000
10. Prepare ABC tertiary agent as per Pilot 5
11. Remove the secondary antibody and wash in PBS – 3 x 10 minutes
12. Remove PBS, add ABC – 1 hour
13. Wash in TBS – 3 x 10 minutes
14. Make up the DAB solution as per Pilot 5
15. Remove slides from TBS, drain away excess fluid, and place on ice
16. Place sections in DAB solution, allow exposure to up to three minutes watching for colour change. When section begins to turn brown, remove from DAB and place in distilled water
17. Mount on gelatin subbed glass microscope slide, allow to dry overnight

Day 3 11/12/14

18. Dehydrate:
    a. 70% ETOH – 5 minutes
    b. 95% ETOH – 5 minutes
    c. 100% ETOH – 2 x 5 minutes
    d. Xylene – 3 x 5 minutes

19. Coverslip – Ultramount (Dako Australia Pty. Ltd., North Sydney, Australia) mounting medium
20. View using light microscope

Outcomes: Successful staining noted in rat brain (brown staining of cells consistent in appearance with neuron cell bodies), however significant damage occurred to meniscoid sections substantially detracting from quality of slides on examination, difficult to determine if immunoreactive staining has occurred. This may be due to the damage occurring to sections during antigen retrieval process in microwave. Next pilot will trial antigen retrieval prior to sectioning.

Pilot 7

Using piece of cervical spine meniscoid (dorsal, specimen O530), block cut in sagittal plane from mid-section of meniscoid. Undertaken using sectioning protocol of Jobling Lab and DAB immunohistochemistry protocol of Tooney Lab. Antigen retrieval performed to blocks of tissue and greater sectioning thickness employed to attempt to reduce risk of damage to sections.

Day 1 15/12/14

1. Antigen retrieval – microwave in citrate buffer 2 minutes
2. Wash fix out – 3 x 15 minutes 80% ETOH
3. Permeablise membranes – 3 x 15 minutes DMSO
4. Dehydrate (to allow for PEG embedding)

5. Place in 1000 MW molten PEG in 46° oven until tissue sinks (2 hours)
6. Embed in 1450 MW molten PEG in a cryostat mould
7. Place in -20° freezer for 5 minutes
8. Remove from mould and mount on microtome chuck for sectioning (50 μm)
9. Place sections free floating in PBS
10. Transfer to solution 49.55% methanol, 49.55% PBS, 0.9% hydrogen peroxide (H_2O_2) solution – 20 minutes
11. Remove PBS/H_2O_2 solution and add triton diluent – 15 minutes
12. Remove triton diluent and add 10% NDS – 30 minutes
13. Remove NDS and add primary antisera diluted in triton diluent containing 1% NDS
    a. Rabbit ∝ NOS 1:1000
14. Place in humidity chamber and incubate on rocker in cold room for 48 hrs

Day 2 17/12/14

15. Remove primary antibody solution and wash in triton diluent – 3 x 10 minutes
16. Incubate sections for 1h at room temperature with secondary antibody diluted in triton diluent containing 1% NDS
    a. Donkey ∝ Rabbit 1:1000
17. Prepare ABC tertiary agent as per Pilot 5
18. Remove the secondary antibody and wash in PBS – 3 x 10 minutes
19. Remove PBS, add ABC – 1 hour
20. Wash in TBS – 3 x 10 minutes
21. Make up the DAB solution as per Pilot 5

22. Remove slides from TBS, drain away excess fluid, and place on ice
23. Place sections in DAB solution, allow exposure to up to three minutes watching for colour change. When section begins to turn brown, remove from DAB and place in distilled water
24. Mount on gelatin subbed glass microscope slide, allow to dry overnight

Day 3 18/12/14

25. Dehydrate:
    a. 70% ETOH – 5 minutes
    b. 95% ETOH – 5 minutes
    c. 100% ETOH – 2 x 5 minutes
    d. Xylene – 3 x 5 minutes
26. Coverslip – Ultramount (Dako Australia Pty. Ltd., North Sydney, Australia) mounting medium
27. View using light microscope

Outcomes: Tissue was more durable and withstood processing, however clear positive staining not evident on examination with light microscope. Some brown staining in form consistent with nerve tissue in spinal nerve, but not convincing.

Pilot 8

Using cervical spine meniscoid sections and spinal nerve sections cut from Pilot 7 that were stored in PBS with 0.05% sodium azide. Trialed 2 different primary antisera. Pilot

testing undertaken as per protocol of Tooney Lab with addition of antigen retrieval prior to sectioning as per Pilot 7.

Day 1 03/02/15

1. Wash sections – 3 x 15 minutes in PBS
2. Transfer to solution 49.55% methanol, 49.55% PBS, 0.9% hydrogen peroxide (H_2O_2) solution – 20 minutes
3. Remove PBS/H_2O_2 solution and add triton diluent – 15 minutes
4. Remove triton diluent and add 10% NDS – 30 minutes
5. Remove NDS and add primary antisera diluted in triton diluent containing 1% NDS
    a. Rabbit ∝ S100 1:1000
    b. Rabbit ∝ βIII tubulin 1:400
6. Place in humidity chamber and incubate on rocker in cold room for 48 hrs

Day 2 05/02/15

7. Remove primary antibody solution and wash in triton diluent – 3 x 10 minutes
8. Incubate sections for 1h at room temperature with secondary antibody diluted in triton diluent containing 1% NDS
    b. Donkey ∝ Rabbit 1:1000
9. Prepare ABC tertiary agent as per Pilot 5
10. Remove the secondary antibody and wash in PBS – 3 x 10 minutes
11. Remove PBS, add ABC – 1 hour

12. Wash in TBS – 3 x 10 minutes
13. Make up the DAB solution as per Pilot 5
14. Remove slides from TBS, drain away excess fluid, and place on ice
15. Place sections in DAB solution, allow exposure to up to three minutes watching for colour change. When section begins to turn brown, remove from DAB and place in distilled water
16. Mount on gelatin subbed glass microscope slide, allow to dry overnight

Day 3 06/02/15

17. Dehydrate:
    e. 70% ETOH – 5 minutes
    f. 95% ETOH – 5 minutes
    g. 100% ETOH – 2 x 5 minutes
    h. Xylene – 3 x 5 minutes
18. Coverslip – Ultramount (Dako Australia Pty. Ltd., North Sydney, Australia) mounting medium
19. View using light microscope

Outcomes: Clear positive staining not evident on examination with light microscope. Some brown staining in form consistent with nerve tissue (long axon-like structures with varicosities), but not clear enough to be convincing.

Pilot 9

Due to lack of success with pilot testing to date, supervisor Dr. Jon Cornwall reported he has previously successfully undertaken DAB immunohistochemistry using embalmed cadaveric tissue in his laboratory at University of Otago Departments of Anatomy and Physiology. Pilot 9 was undertaken in Histology Unit, University of Otago, New Zealand. Tissue used was spinal nerve root and deep cervical muscle excised from a study cadaver.

Protocol:

1. Tissue processed to wax overnight using Excelsior ES 2 unit (Thermo Scientific, Cheshire, UK).
2. Tissue embedded in paraffin, orientated such that cut surfaces lay along bottom of embedding dish.
3. Embedded tissue sectioned at 5 μm and mounted upon gelatin-coated slides (two on each slide), incubated at 37°C overnight
4. Dehydration and washing:
    a. Xylene – 3 x 2 minutes
    b. 100% ETOH – 2 x 2 minutes
    c. 95% ETOH – 2 minutes
    d. 70% ETOH – 2 minutes
    e. Tap water – 2 minutes
    f. Distilled water – 2 minutes

5. Immersion in 3% methanolic hydrogen peroxide to block endogenous perioxidase – 10 minutes
6. Further washing:
    a. Tap water – 2 minutes
    b. Distilled water – 2 minutes
    c. PBS – 2 minutes
7. Antigen retrieval: microwave for 25 minutes in 10% citrate buffer pH 6.0 to 95°C
8. Allow to cool to 37°C
9. Wash in PBS – 2 x 2 minutes
10. Delineate sections using peroxidase-antiperoxidase hydrophobic pen
11. Incubate with 10% bovine serum antibody (BSA) for 30 minutes to prevent nonspecific binding of the antibodies
12. Drain BSA from slides add primary antisera diluted in 2% BSA
    a. Mouse ∝ NF-H 1:3500
13. Incubate for 60 minutes at room temperature
14. Drain primary antisera and 2% BSA from slides.
15. Wash slides as follows:
    a. Rinse with PBS
    b. PBS-Tween (500 µL Tween per 1000 mL PBS) – 2 x 5 minutes on shaker table
    c. PBS – 2 minutes
16. Incubate for 30 minutes at room temperature with secondary antisera
    a. EnVision + Dual Link System (Dako North America Inc., Carpinteria, CA) undiluted

17. Drain secondary antibody from slides and wash slides as follows:
    a. Rinse with PBS
    b. PBS-Tween (500 μL Tween per 1000 mL PBS) – 2 x 5 minutes on shaker table
    c. PBS – 2 minutes
18. Mix DAB solution using Dako DAB system: 1 mL DAB Buffer to 1 drop of DAB Chromagen (Dako North America Inc., Carpinteria, CA)
19. Remove slides from PBS, drain away excess fluid, and place on white paper
20. Cover sections with DAB solution, allow exposure to three minutes.
21. Drain away DAB solution, rinse slides with distilled water to stop reaction
22. Wash slides as follows:
    a. Distilled water – 2 minutes
    b. Tap water – 2 minutes
23. Mayer's haematoxylin – 2 minutes
24. Wash in tap water – 2 minutes
25. Immerse 15 seconds in Scott's Tap Water
26. Wash in tap water – 2 minutes
27. Dehydrate in 100% ETOH – 3 x 1 minute
28. Clear slides in xylene – 3 x 1 minute
29. Mount each slide in DPX mounting medium (Thermo Scientific, Manor Park, United Kingdom) with glass coverslip
30. View using light microscope

Outcomes: successful staining of nerve fibres noted within muscle and spinal nerve. Minimal tissue damage or artifact noted. Negative controls do not have the same

staining. Pilot testing has therefore confirmed that this technique can be used for Study 4.

Appendix P – Detailed Immunohistochemistry Procedure Study 4

Tissue Preparation, Embedding, Sectioning and Mounting

1. Dissected meniscoids and control specimens (spinal nerve, cervical paraspinal muscle) stored individual specimen jars immersed in phosphate buffered saline (PBS) with 0.05% sodium azide
2. Each meniscoid cut in sagittal plane through its midline
3. Tissue (half meniscoids and control specimens) placed into individual labeled cassettes
4. Tissue undergoes processing to wax overnight using Excelsior ES 2 unit (Thermo Scientific, Cheshire, UK)
5. Tissue embedded in paraffin, orientated such that cut surfaces lay along bottom of embedding dish
6. Embedded tissue sectioned at 5 μm and mounted upon gelatin-coated slides (two on each slide), incubated at 37°C overnight

Preparation for Staining

7. Dehydration and washing – two minutes in each of the following:
    a. Xylene x 3
    b. 100% ethanol (ETOH) x 2
    c. 95% ETOH
    d. 70% ETOH
    e. Tap water

f. Distilled water

8. Ten minutes immersion in 3% methanolic hydrogen peroxide to block endogenous perioxidase
9. Further washing – two minutes in the following:
    a. Tap water
    b. Distilled water
    c. PBS

Antigen Retrieval

10. Microwave for 25 minutes in 10% citrate buffer pH 6.0 to 95°C
11. Allow to cool to 37°C

Protein Block

12. Wash in PBS two minutes x 2
13. Delineate sections using peroxidase-antiperoxidase hydrophobic pen
14. Incubate with 10% bovine serum antibody (BSA) for 30 minutes to prevent nonspecific binding of the antibodies
15. Drain BSA from slides

Primary Antibodies

Neurofilament H (NF-H) Non-Phosphorylated (SMI-32) Monoclonal Antibody (catalogue no. SMI-32R), mouse raised, Covance Inc., Princeton, NJ

Anti-Neurofilament (Pan-NF) Monoclonal Antibody (SMI-312), mouse raised, Abcam, Cambridge, MA

16. Incubate for 60 minutes at room temperature with primary antibody (NF-H 1:3500 dilution in 2% BSA; Pan-NF 1:3000 dilution in 2% BSA). NB: two sections mounted on each slide, one incubated with primary antibody, the other incubated with 2% BSA (no primary antibody) to serve as a negative control
17. Drain primary antibody and 2% BSA from slides
18. Wash slides as follows:
    a. Rinse with PBS
    b. Five minutes PBS-Tween (500 μL Tween per 1000 mL PBS) x 2 on shaker table
    c. Two minutes PBS

Secondary Antibody

EnVision + Dual Link System (Dako North America Inc., Carpinteria, CA) secondary antibody

19. Incubate for 30 minutes at room temperature with secondary antibody (undiluted).
20. Drain secondary antibody from slides.
21. Wash slides as follows:
    a. Rinse with PBS

b. Five minutes PBS-Tween (500 μL Tween per 1000 mL PBS) x 2 on shaker table
c. Two minutes PBS

Diaminobenzidine (DAB) Exposure

Dako DAB system: 1 mL DAB Buffer to 1 drop of DAB Chromagen (Dako North America Inc., Carpinteria, CA)

22. Remove slides from PBS, drain away excess fluid, and place on white paper.
23. Cover sections with DAB solution, allow exposure to three minutes.
24. Drain away DAB solution, rinse slides with distilled water to stop reaction.
25. Wash slides as follows:
    a. Two minutes distilled water
    b. Two minutes tap water

Counterstaining, Dehydrating and Clearing

26. Two minutes Mayer's haematoxylin
27. Wash two minutes in tap water
28. Immerse 15 seconds in Scott's Tap Water
29. Wash two minutes in tap water
30. Dehydrate in 100% ETOH 3 x one minute
31. Clear slides in xylene 3 x one minute

Coverslip

32. Mount each slide in DPX mounting medium (Thermo Scientific, Manor Park, United Kingdom) with glass coverslip

Appendix Q – Laboratory Recipes Study 4

Phosphate Buffered Saline (PBS) Recipe

1000 mL distilled water

10 PBS tablets

Dissolve PBS tablets in distilled water by stirring in beaker.

Mayer's Haematoxylin Recipe

50 g aluminum potassium sulfate

1000 mL distilled water

1 g haematoxylin

0.2 g sodium iodate

20 mL glacial acetic acid

Dissolve aluminum potassium sulfate in distilled water. Add and dissolve haematoxylin.

Add sodium iodate and acetic acid, bring mixture to boil, allow to cool, then filter.

Scott's Tap Water Recipe

5000 mL distilled water

10 g sodium bicarbonate

100 g magnesium sulphate

Citrate Buffer Recipe (10x concentrate)

29.4 g tri-sodium citrate

1000 mL distilled water

pH to 6.0

Formic Acid Recipe (5% Solution)

950 mL distilled water

50 mL concentrated formic acid

Appendix R – Hunter Medical Research Institute Research Register Approval Study 5

Hunter Medical Research Institute

Lot 1 Kookaburra Circuit,
New Lambton Heights

Locked Bag 1000, New Lambton,
NSW, Australia 2305

ABN 27 081 436 919

P +61 2 4042 0000
F +61 2 4042 0001

E info@hmri.com.au
W www.hmri.com.au
S www.hmri.com.au/social

18th March 2015

Professor Darren Rivett
Discipline of Physiotherapy
School of Health Sciences
Faculty of Health and Medicine
The University of Newcastle
CALLAGHAN NSW 2308

Dear Darren,

I would like to inform you of the HMRI Research Register Management Committee's decision to approve your request for volunteers from the Research Register to participate in the study **"Role of cervical spine meniscoids in whiplash associated disorder"**

The Register will approach up to a maximum of 200 selected members by mail on your behalf, inviting them to participate in the study. This letter will include an invitation to participate in the study, a copy of the participant information sheet, and a Study Response Form. It should be noted that the study consent form will not be included. It is the responsibility of the researcher to gain consent from study participants.

Register members will be asked to respond to the invitation by ticking a box on the Study Response Form and returning this to the Register in a pre-paid envelope. Members who wish to participate in the study will be told to expect a phone call from the research group within the following two weeks.

The names and contact details of those wishing to participate in the study will be forwarded to the researcher.

Please note: The researcher is expected to inform the Register of the names of those who accept the conditions of the study and participate, decline, are ineligible, complete the study or withdraw.

Researchers accessing participants via the HMRI Research Register are required to provide the Register with an annual progress report and a final report. The information from these reports will be used in the HMRI Annual Report and to update information about current HMRI affiliated research activities in the HMRI Research Register newsletter and website, as a means of maintaining members' interest in research activities. Individual researchers will have the opportunity to preview this material before publication.

If you have any further questions, please contact the HMRI Research Register Coordinator on 40420587.

Yours sincerely,

Trisha DAccione
Coordinator,
HMRI Research Register
P: 4042 0587
E: trisha.daccione@hmri.com.au

In partnership with our community

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

NSW GOVERNMENT Health
Hunter New England
Local Health District

Appendix S – Hunter New England Imaging Service Agreement

Study 5

Research/Clinical Trial Service Agreement

NSW GOVERNMENT | Health
Hunter New England Imaging

This is a Clinical Trial Service Agreement between Hunter New England Imaging and the Discipline of Physiotherapy, School of Health Sciences, University of Newcastle
Amended: 26 November 2013

Project Title:	ROLE OF CERVICAL SPIN MENISCOIDS IN WHIPLASH ASSOCIATED DISORDED December 2013 to June 2014 Ethics approval has been conditionally attained from HNE HREC
The purpose of this project is to investigate meniscoids in people with WAD using MI and to compare findings to healthy subjects. This would be the first study to image meniscoids in people suffering WAD and would provide valuable insight into the potential role of these structures in this costly and burdensome condition. Calvary Mater Newcastle Hospital	
Duration of Research/Trial	
Protocol Number:	HNE HREC Ref No: 13/09//18/4.09 NSW HREC Ref No: HREC/13/HNE
Examination/Procedure Requirements	Structural MRI
HNE Facility(s) where Examination/Procedure will be performed	MRI Unit, Radiology Department,
Scheduling	▪ Structural MRI's will be performed on the MRI Scanner at the Calvary Mater Newcastle Hospital ▪ Scheduling of these examinations will be negotiated between the Senior Radiographer, MRI, Calvary Mater Newcastle Hospital and the Discipline of Physiotherapy, School of Health Sciences, University of Newcastle. The Senior Radiographer, MRI, Calvary Mater Newcastle Hospital can be contacted on telephone 02 40144600 ▪ Twelve (12) Structural MRI Sessions will be conducted throughout the course of this research study with a maximum of 3 patients being scanned per 2 hour session on the MRI unit at Calvary Mater Newcastle Hospital ▪ The number of Structural MRI sessions per month will be upon negotiation wit the Senior Radiographer, MRI, Calvary Mater Newcastle Hospital and the Discipline of Physiotherapy, School of Health Sciences, University of Newcastle ▪ The availability of in-hours and out-of-hours scanning will be reviewed every three (3) months, with Hunter New England Imaging reserving the right to re-schedule research sessions out of hours. Structural MRI sessions may be suspended or postponed if a clinically urgent patient requires an MRI examination
Specific Requirements	▪ There is no requirement for the Division of Radiology, examination report for participants of this study. ▪ There is no requirement for images to be stored on the HNE LHD PACS ▪ There is no requirement for images to be sent to other devices ▪ There is no requirement for images to be anonymised ▪ The Discipline of Physiotherapy, School of Health Sciences, University of Newcastle will ensure that the Division of Radiology is aware that the patient is a participant of this Study. This may be in the form of a "Clinical Trial Patient Sticker" on the patients medical imaging
Image Management	▪ The researcher will provide the Division of Radiology with blank CD's and upon the completion of each session, the participa images will be burned
Financial Arrangements & Study Payments	an invoice for this study and payment details are noted on the invoice ▪ The Discipline of Physiotherapy, School of Health Sciences, University of Newcastle must ▪
Additional Notes	▪ **The researcher must advise Hunter New England Imaging <u>when the trial is 50% completed</u>.** ▪ **The researcher must ensure all patients in the trial are identified at the HNEI site**

Signature

Date

26/11/2013

Darrin Gray
A/Director, Hunter New England Imaging
A/Director, BreastScreen NSW Hunter New England

Prof Darren Rivett
Principal Investigator
Head of School

Date:

Appendix T – Participant Recruitment Flyer Study 5

DO MENISCOIDS IN THE NECK PLAY A ROLE IN NECK PAIN AFTER A WHIPLASH INJURY?

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

RESEARCH STUDY

www.newcastle.edu.au

Details

We are conducting a study using magnetic resonance imaging (MRI) to investigate the size, shape and make up of meniscoids (small soft tissue structures in the neck) in people suffering Whiplash Associated Disorder (WAD). We hope to compare findings between people with WAD and people without neck pain.

Who can volunteer?

We need 2 groups of participants aged 18-60 years:

- People that have suffered ongoing neck pain following a car accident

OR

- People that do not have chronic neck pain and have never been in a car accident

You will be reimbursed $20 to compensate for your time and travel costs.

This project has been approved by Hunter New England LHD Human Research Ethics Committee, Approval Number [13/09/18/4.09]. Chief investigator Professor Darren Rivett, Head of School of Health Sciences.

CONTACT

If you are interested or for further information and to find out if you are eligible please contact:
Scott Farrell (School of Health Sciences)
T: 4921 7374
Email: scott.farrell@newcastle.edu.au

Appendix U – Participant Information Sheet Study 5

Professor Darren A Rivett
BAppSc(Phty), GradDipManipTher,
MAppSc(ManipPhty), PhD

Head of School
School of Health Sciences
Faculty of Health and Medicine
University Drive, Callaghan
NSW 2308 Australia
Phone: +61 2 49217220
Fax: +61 2 49217053
Email: Darren.Rivett@newcastle.edu.au

Information Statement for the Research Project:
The role of the cervical meniscoids in Whiplash Associated Disorder.

Document Version 3; Dated 17/12/2014

You are invited to participate in the research project identified above which is being conducted by Prof Darren Rivett, Dr Peter Osmotherly, A/Prof Lindsay Rowe, Dr Peter Lau and Mr Scott Farrell from the Faculty of Health and Medicine at the University of Newcastle.

The research is part of Mr Scott Farrell's PhD program at the University of Newcastle, supervised by Prof Darren Rivett and Dr Peter Osmotherly from the School of Health Sciences.

Why is the research being done?

The purpose of the project is to investigate cervical meniscoids (small soft tissue structures in the neck) in people with whiplash associated disorder neck pain using magnetic resonance imaging (MRI), and to compare findings to people without neck pain.

Previous research has shown that these structures could be a potential source of the ongoing neck pain experienced by some people after motor vehicle accidents, so improving our understanding of these structures and how they may change in whiplash associated disorder neck pain may guide clinicians in managing this burdensome condition.

Who can participate in the research?

We are seeking two groups of people aged 18-60 years to participate in this research. The first group is people who are suffering neck pain due to whiplash following a motor vehicle accident. The second group is people who do not have ongoing neck pain and have not been in a motor vehicle accident.

If you are have had previous neck surgery or a fracture (broken bone) in your neck, then this study is not suitable for you. People who are claustrophobic or who have metal implants (such as a pacemaker) should not participate, as such people should not undergo MRI.

What choice do you have?

Participation in this research is entirely your choice. Only those people who give their informed consent will be included in the project. Whether or not you decide to participate, your decision will not disadvantage you.

If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data that identifies you.

What would you be asked to do?

If you agree to participate, you will be asked to:

- Attend the Radiology Department of the Newcastle Mater Hospital (Waratah) on one occasion to undergo a magnetic resonance imaging (MRI) scan of your neck.
- You will also be asked some basic demographic questions regarding age, gender, and history of whiplash associated disorder neck pain (if you are part of the whiplash group).
- The MRI scanner will be operated by a qualified radiographer, and the image will be interpreted by a specialist radiologist. The data gathered from the scans will include recordings of the size, shape, location and composition of the meniscoid structures within the joints of the neck.
- Participants will be given $20.00 reimbursement for travel and parking expenses.

How much time will it take?

The whole process should take less than one hour.

***What are the risks and benefits of participating*?**

There will be no direct benefit to you in participating in this research.

Once appropriately screened for contraindicated implants (e.g. pacemakers), there are no known side effects of MRI. Some people may experience a feeling of claustrophobia in the confined space of the MRI scanner, however if this occurs you can ask for the test to be stopped and be removed from the machine.

How will the information collected be used?

The results of the research will be reported and distributed via national and international conferences and peer reviewed publications. The data collected will also contribute towards Mr Scott Farrell's PhD thesis.

You will not be personally identified in any reports arising from the study.

A written summary of results of the study will be available to any interested participants on completion of analysis, write up and publication by contacting the research team.

***What do you need to do to participate*?**

Please read this Information Statement and be sure you understand its contents before you consent to participate. If there is anything you do not understand, or you have questions, please contact the research team.

If you would like to participate, please contact the researchers on (02) 4921 7374 or (02) 4921 7220 or by emailing scott.farrell@newcastle.edu.au to register your interest and undergo screening.

Further information

If you would like further information please contact Mr Scott Farrell on (02) 4921 7374 or Professor Darren Rivett on (02) 4921 7220.

Thank you for considering this invitation.

Professor Darren A. Rivett
Head of School
School of Health Sciences
Faculty of Health and Medicine

Mr Scott Farrell
PhD Candidate
School of Health Sciences
Faculty of Health and Medicine

Complaints about this research

This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Local Health District, Reference no. 13/09/18/4.09.

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Manager Research Ethics and Governance, Hunter New England Local Health District, Locked Bag 1, New Lambton NSW 2305, telephone (02) 49214950, email Hnehrec@hnehealth.nsw.gov.au

Appendix V – Participant Consent Form Study 5

Professor Darren A Rivett
BAppSc(Phty), GradDipManipTher,
MAppSc(ManipPhty), PhD

Head of School
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Email: Darren.Rivett@newcastle.edu.au

Consent Form for the Research Project:
The role of the cervical meniscoids in Whiplash Associated Disorder.

Document Version 1; Dated 28/08/2013

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to:

- Attend the Radiology Department of the Newcastle Mater Hospital (Waratah) on one occasion to undergo a magnetic resonance imaging (MRI) scan of my neck.
- Be asked basic demographic questions regarding age, gender, and history of whiplash associated disorder neck pain (if part of the whiplash group).

I understand that my personal information will remain confidential to the researchers.

I have had the opportunity to have questions answered to my satisfaction.

Print Name: _____

Signature: _____ **Date:** _____

Appendix W – MRI Safety Questionnaire Study 5

Office Use Only
RIS Sticker

HUNTER NEW ENGLAND
NSW HEALTH IMAGING

MRI SAFETY SCREENING QUESTIONNAIRE

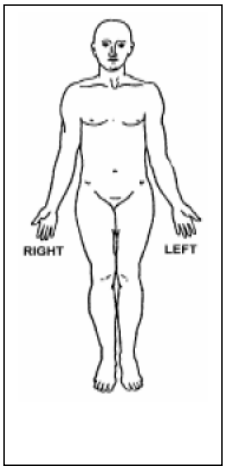
Patient Name: _____
Date of Birth: _____ **Weight**_____**kg/stone** **Height**_____**cm/feet inches**

WARNING: Due to the MRI system having a very strong magnetic field that is always on, it may be inappropriate/ hazardous for some individuals to have a scan. Therefore all questions must be answered accurately to determine your eligibility. Incorrect information could result in serious injury. If you do not fully understand any of the questions please ask for help.

Have you ever had an operation of any kind, at any time in your life? ❒Yes ❒No
List **all** operations, approximate dates, and mark the operation areas on the figure opposite.

_____
_____
_____
_____

Have you had an MRI examination before? ❒Yes ❒ No If yes, when and where? _____
_____



Do you have any of the following? (Please circle Yes or No for all questions)

Yes No Cardiac pacemaker/ Defibrillator/ Pacing wires
Yes No Artificial heart valve
Yes No Aneurysm clip
Yes No Ear implant (e.g. Stapes, Cochlear)/ Bionic ear
Yes No Vascular implant/ Stent/ Coil/ Filter/ Aortic graft
Yes No Neurostimulator or Drug infusion pump
Yes No Electronic or Magnetically activated implant or device
Yes No Brain shunt
Yes No Any other type of prosthesis/ implant (eye, penile etc.)

If you answered yes to any of these questions, please call JHH 49213396 Calvary Mater 40144603, to allow implants to be checked to assess your suitability for MRI

Yes No Joint replacement or artificial limb
Yes No Metal rods, plates or screws in/on bones
Yes No Metal/ Shrapnel/ Bullet injuries
Yes No Hearing aid

Yes No False teeth/ Removable bridgework
Yes No Body piercing
Yes No Tattoos
Yes No Nicotine or medication patches

Yes No Have you ever been a metal worker (e.g. welding, grinding, lathe)?
Yes No Have you ever had metal in your eye following an injury or operation? If yes, has it been removed? ❒Yes ❒No
Yes No Is there any possibility of metal in your body through injury or surgery, other than that stated on this sheet?

Yes No Do you have a history of kidney (renal) disease?
Yes No Have you ever had an allergic reaction to contrast media?

Yes No Do you have liver failure?
Yes No Are you diabetic?

Yes No Do you suffer from claustrophobia?
Yes No Is there any possibility you could be pregnant?

Yes No Are you breastfeeding?

Yes No Have you filled out and understood all questions on this form?

I hereby confirm that I have read, understood and correctly answered the above questions and agree to have an MRI and procedures necessary to complete the examination

Signature of person completing form: _____Date ___/ ___ /_____

Form completed by: ❒Patient ❒Relative ❒Doctor ❒Other (please specify) _____
Print name and contact number/ page number

WARNING: Before entering the scan room **all** metallic objects must be removed including watches, jewellery, hearing aids, wallet, credit cards, coins, keys, pens/pencils, scissors, mobile phones, hair pins, clips, piercings, clothing with metal e.g. bra, jeans, zips, studs. Lockers available.

MRI OFFICE USE ONLY
Form information reviewed by, and first time out identification: ....
Signature Designation
Second Time Out identification by:..... .....
Signature Designation

Appendix X – Journal Publication Study 4

Farrell, S.F., Osmotherly, P.G., Cornwall, J., & Rivett, D.A. (2016). Immunohistochemical investigation of nerve fibre presence and morphology in elderly cervical spine meniscoids. *The Spine Journal*. DOI: 10.1016/j.spinee.2016.06.004.

Full text version of published article available at:

http://www.thespinejournalonline.com/article/S1529-9430(16)30222-4/fulltext

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