The characteristics of tendon pathology and its pathogenesis as detected by ultrasound tissue characterisation

Submitted as part of a Doctor of Philosophy degree (PhD)

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Notice 1

General declaration for thesis based on conjointly published and unpublished work

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes three original papers published in peer reviewed journals and two unpublished publications. The core theme of the thesis is the utilisation of ultrasound tissue characterisation in the understanding of tendon pathology. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Physiotherapy, Faculty of Medicine, Nursing and Health Sciences under the supervision of Professor Jill Cook

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research

In the case of Chapters three to seven, my contribution to the work involved the following:

Thesis Chapter	Publication title	Publication status	Nature and extent of candidate's contribution
3	Tendons structure changes after maximal exercise in the thoroughbred horse: Use of ultrasound tissue characterisation to detect in vivo tendon response	Published	Developed study design, performed data collection and statistical analysis, preparation of manuscript and as guarantor of the manuscript
4	Australian football players' Achilles tendons responds to game loads within two days: an ultrasound tissue characterisation (UTC) study	Published	Developed study design, performed data collection and statistical analysis, preparation of manuscript and as guarantor of the manuscript
5	Achilles tendon structure improves on UTC imaging over a five month pre-season in elite Australian football players	Returned for revision	Developed study design, performed data collection and statistical analysis, preparation of manuscript and as guarantor of the manuscript
6	Pathological tendons maintain sufficient aligned fibrillar structure on ultrasound tissue characterisation (UTC)	Returned for revision	Developed study design, performed data collection and statistical analysis, preparation of manuscript and as guarantor of the manuscript
7	Structural integrity is decreased in both Achilles tendon in people with unilateral Achilles tendinopathy	In press	Developed study design, performed data collection and statistical analysis, preparation of manuscript and as guarantor of the manuscript

Signed:

Date:

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Publications

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Rosengarten SD, Cook JL, Bryant AL, Cordy JT, Daffy J & **Docking SI** (2015). Achilles tendon response in football players using ultrasound tissue characterisation. *British Journal of Sports Medicine.* 49(3) pg. 183-7 (Chapter four)

Docking SI, Rosengarten SD, Daffy J & Cook JL (2014). Structural integrity is decreased in both Achilles tendons in people with unilateral Achilles tendinopathy. *Journal of Science and Medicine in Sport. In press* (Chapter seven)

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Noteworthy references

van Weeren R (2013). Tendon injury: The switch from curative to preventative medicine. *Vet J.* 193(3) pg. 274-5.

- Referred to findings of paper in same issue as a 'landmark paper' and an 'important breakthrough'

Conference proceedings

Docking SI & Cook JL (2014). Treat the doughnut, not the hole: The pathological Achilles and patellar tendon has sufficient amounts of normal tendon structure. *Accepted to Be Active 2014 Sports Medicine Australia Conference. Canberra*
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**Shortlisted for the British Journal of Sports Medicine prize*

van Ark M, **Docking SI**, van den Akker-Scheek I, Rudavsky A, Rio E, Zwerver H & Cook J (2014). Does the patellar tendon respond to 5 days of loading during a volleyball tournament. *3rd International Scientific Tendinopathy Symposium. Oxford*

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Wong A, **Docking SI,** Cook J & Gaida (2014). Does type I diabetes affect Achilles tendon response to a 10km run? *3rd International Scientific Tendinopathy Symposium. Oxford*

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Docking SI, Rosengarten S, Daffy J, van Schie HTM & Cook JL (2013). The role of Ultrasound Tissue Characterisation in the management and prevention of tendinopathy in athletes. *Asics Conference of Sport and Medicine in Sport. Phuket*

Docking SI, van Schie JTM, Daffy J, Rosengarten S & Cook JL (2012). Bilateral changes in unilateral Achilles tendinopathy quantified using ultrasound tissue characterisation. *2nd International Scientific Tendinopathy Symposium. Vancouver*
**Awarded best paper award for the conference*

Van Schie HTM, **Docking SI**, Daffy J, Praet SE, Rosengarten S & Cook JL (2012). Ultrasound tissue characterisation, an innovative technique for injury-prevention and monitoring of tendinopathy. *2nd International Scientific Tendinopathy Symposium. Vancouver*

Rosengarten S, **Docking SI,** van Schie JTM, Daffy J & Cook JL (2012). Tendon response in Achilles tendon of Australian football players using ultrasound tissue characterisation. *2nd International Scientific Tendinopathy Symposium. Vancouver*

Invited presentations

Docking SI, Rosengarten S & Cook J (2013). Ultrasound tissue characterisation monitoring of tendinopathy. *Australasian Musculoskeletal Imaging Group conference. Sydney.*

Abstract

Tendinopathy is the clinical presentation of pain and dysfunction resulting in prolonged periods of reduced activity. While not directly related, tendon pain is associated with structural pathology. However, the primary histopathological event and the pathogenesis of tendon pathology is not fully understood, with numerous theoretical models proposed. Consistent within all these models is that loading the tendon beyond its capacity (overload) is critical in the development of pathology and possibly pain. Our understanding of how load affects the structural integrity of the tendon and contributes to the pathogenesis is poor due to limitations associated with conventional imaging modalities (ultrasound and magnetic resonance imaging).

Ultrasound tissue characterisation (UTC) is a novel imaging modality that captures a three-dimensional ultrasound image, allowing for the quantification of tendon structure based on the stability of pixels brightness over contiguous transverse images. Four echo-types are discriminated that have been validated against histopathological specimens in horses. Quantification of these echo-types have been used to test the efficacy of various treatments in horses and humans, and test the diagnostic accuracy in detecting Achilles tendinopathy in humans. Little research has been performed using UTC to understand how tendons respond to load and the pathogenesis of tendon pathology.

The aim of this thesis was to investigate the pathogenesis and features of tendon pathology as characterised by UTC. As load is critical to the development of tendon pathology, the nature and temporal sequence of changes in the normal tendon in response to loading were investigated in both the short- (Chapters three and four) and medium-term

(Chapter five). The appearance of normal and pathological tendons determined by UTC were investigated to ascertain whether the pathological tendon lacked sufficient amounts of aligned fibrillar structure and whether tendon dimensions were related to the amount of disorganisation (Chapter six). Finally, tendon structure, as quantified by UTC, was examined in the symptomatic and contralateral asymptomatic tendon in patients suffering from unilateral Achilles tendinopathy compared to normal tendons (Chapter seven).

This thesis found evidence that the normal tendon is responsive to load, both in the short- (approximately 48hrs) and medium-term (five months). While definitive statements cannot be made as to whether these observed changes affect the health of the tendon (ie presence of pain and dysfunction), it suggests that the tendon is sensitive to load and that it may affect the structural integrity of the tendon. Furthermore, the pathological tendon exhibited a greater mean cross-sectional area of aligned fibrillar structure than a structurally normal tendon. With a significant relationship observed between tendon dimensions and the amount of disorganisation, tendon thickening may be an adaption to maintain sufficient levels of aligned fibrillar structure in the pathological tendon.

The findings of this thesis contributes to our understanding of the responsiveness of the normal tendon to load and provides insight into how the normal tendon transitions towards pathology. Despite the presence of disorganisation, the pathological tendon appears to maintain sufficient structure to tolerate load by increasing tendon dimensions. An improved understanding of the pathogenesis and the tendons response to load will lead to improvements in treatments and outcomes for those with tendinopathy.

Acknowledgements

To my supervisor, mentor and dear friend, Jill Cook. It has been an amazing journey and to think it all started with a random email and a Sunday coffee. Thank you for your endless support, your advice in teaching me how to be a researcher and being a role model by simply being yourself. While I end this journey with a degree, I have ended up with much more than that thanks to your guidance and friendship. I am eternally grateful to you and look forward to the next part of this journey.

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To all those people who have in some small way helped me get to this point. My grandparents, uncles, aunties, cousins and friends. Even the nay-sayers and the critics. Thank you.

Dedication

To Mamma and Nanna Doc. Thank you for all your loving support.

You are both greatly missed. I'll be sure to see you in the front row at the graduation ceremony.

I am finally getting that floppy hat.

Chapter one
Context and outline of Chapters

Tendinopathy is an overuse injury that results in pain and loss of function in a tendon (ie inability to perform energy storage tasks) (Rio et al, 2014). It affects a broad spectrum of individuals, from the young elite athlete to the older sedentary individual, resulting in a reduction in performance and physical activity participation (de Jonge et al, 2011b; Hagglund et al, 2011; Lysholm & Wiklander, 1987; Zwerver et al, 2011). In comparison to traumatic injuries, such as anterior cruciate ligament rupture, the prevalence and associated morbidity might be underreported due to the heterogeneity of the condition (Magnan et al, 2014) and difficulties in quantifying the magnitude of the problem (Clarsen et al, 2013). While tendinopathy is associated with tendon pathology, there is not a direct relationship between the presence/severity of pain and the presence/extent of tendon pathology.

Numerous studies have observed tendon pathology in approximately 10-20% of asymptomatic individuals (Giombini et al, 2013; Malliaras & Cook, 2006), suggesting that the terminology used when discussing pain and structure are critical. Tendinopathy is the clinical condition that describes “the combination of pain, swelling and impaired performance” (Maffulli et al, 1998). Since this term was

first described, it has been suggested that tendinopathy should be used independent of structural abnormalities (Malliaras & Cook, 2006). It is frequently described as localised pain that increases with greater load (ie single leg hopping is more painful for the Achilles tendon than calf raises)(Cook et al, 2002; Rio et al, 2014). Tendon pathology, or tendinosis, relates to structural abnormalities observed histologically or using clinical imaging modalities independent of clinical symptoms. These terms will be used throughout the thesis.

The histopathological features of the pathological, degenerative tendon have been extensively described in the literature. Yet, the primary histological change that leads to tendon pathology has yet to be ascertained, leading to a number of theoretical models for the pathogenesis of tendon pathology and tendinopathy (Abate et al, 2009; Cook & Purdam, 2009; Fu et al, 2010; Leadbetter, 1992; Rees et al, 2014). These models differ in relation to the primary histological event leading to pathology, the key features of tendon pathology and how pathology relates to pain. However, every model recognises that overload is critical in the development of pathology and pain with considerable evidence supporting this concept (Ristolainen et al, 2014; van der Worp et al, 2012; Visnes & Bahr, 2013).

This thesis aims to examine the characteristics of tendon pathology and its pathogenesis as detected by a new imaging modality, ultrasound tissue characterisation. As load is critical in the development of tendon pathology, the response of the normal tendon to load will be investigated to improve our understanding of the early changes observed within the tendon. The features of tendon pathology will also be explored, examining the mean cross-sectional area of aligned fibrillar structure in the pathological and normal tendon. While tendinopathy and tendon pathology occurs within the upper limb and other sites of the lower limb, this thesis will focus primarily on the Achilles and patellar tendon.

1.1- Outline of Chapters

This thesis contains eight Chapters, the contents are:

- Chapter two details existing knowledge on the features of the pathological tendon, proposed models for the pathogenesis of tendon pathology, clinical imaging of tendons and the response of the tendon to mechanical stimuli before providing justification for the studies included in this study. The aims of this study will also be outlined.
- Chapter three is a prospective study examining short-term changes (days) in response to maximal exercise in the normal superficial digital flexor tendon of thoroughbred horses.
- Chapter four is a prospective study examining short-term changes (days) in response to maximal exercise in the normal Achilles tendon in elite male Australian football players.
- Chapter five is a prospective study examining the medium-term changes (months) in response to pre-season training in the normal Achilles tendon of elite male Australian football players.
- Chapter six is a cross-sectional study investigating the features of the pathological Achilles and patellar tendon, specifically in relation to the mean cross-sectional area of aligned fibrillar structure and disorganised structure.
- Chapter seven is a case-control study that examines tendon structure in the symptomatic and contralateral asymptomatic tendon in patients suffering from unilateral Achilles tendinopathy compared to a normal Achilles tendon.

- Chapter eight summarises the findings of the Chapters in relation to the proposed models of tendon pathology, exploring the clinical implications of the findings and provides direction for future research in this area.

This thesis is submitted by publication with three Chapters published in peer-reviewed journals and two in submission (invited for revision). As such, there is slight repetition, especially relating to the methods used, between Chapters.

Chapter two
General introduction

Tendons have two primary functions; they can be positional (transmit force for muscle to bone to position limbs), or they can store and release energy to assist in locomotion or replace ligaments (Benjamin & Ralphs, 1996). While some tendons can be both positional and energy storage, each tendon has a distinct and suitable matrix for its required function (Benjamin, 2002). The matrix of energy storage tendons in horses have distinct differences in the composition and structure compared to positional tendons (Birch, 2007; Birch et al, 2008). As the tendons composition and structure is dependent on its functional requirements, changes in habitual loads will alter the tendon structure (Benjamin, 2002). Recent research on the structure and composition of energy storage tendons, such as the Achilles and patellar tendons in humans and the superficial digital flexor tendon (SDFT) in horses, and how mechanical stimuli affects these tendons will be reviewed. Before this, the structure of normal tendons and tendon pathology are appraised.

2.1- Normal tendon structure and composition

Tendon is a highly organised dense connective tissue that is comprised of the specialised fibroblasts, termed tenocytes, and the extracellular matrix (ECM). The ECM comprises of a fibrillar (collagen) and non-fibrillar components (water, proteoglycans, glycoproteins etc) surrounding the cell. The amorphous gel-like substance, comprised predominately of water and proteoglycans, within the ECM is termed the ground substance. This section reviews the structure and composition of normal tendons and tendon pathology in the context of these components.

2.1.1- Tenocytes

Tendon cells (tenocytes) are long, slender cells with spindle-shaped nuclei and very little cytoplasm that are arranged longitudinally between collagen fibrils (fig 2.1)(Benjamin & Ralphs, 2000; Benjamin & Ralphs, 1996). These specialised fibroblasts are the cellular machinery of the tendon and are responsible for the production and maintenance of the ECM (Birch et al, 2008; Hayem, 2001; Riley, 2008). Alterations in gene expression and ECM turnover are regulated by chemical and mechanical stimuli, as well as direct communication between adjacent cells. Tenocytes communicate with other tenocytes within their longitudinal row and adjacent rows via gap junctions (Benjamin & Ralphs, 2000; Hayem, 2001; Young et al, 2009). The potential mechanisms by which the tenocyte detects mechanical stimuli are discussed in later sections (section 2.4 and 2.5).

2.1.2- Collagen

The ECM of tendon is predominately comprised of water (~50-70%) and unidirectional type I collagen fibrils (50-80% of dry weight) (Dowling et al, 2000; O'Brien, 1997; Riley et al, 1994; Samiric et al, 2009). Types II, III and V collagen are also found in smaller quantities and play a role in the formation and/or cross-linking of collagen fibrils. The hierarchical structure of the tendon is similar to muscle and is separated into collagen fibrils, collagen fibres and subfascicles, which when grouped together form fascicles surrounded by endotenon (fig 2.2)(Kastelic et al, 1978). Endotenon is loose connective tissue that contains blood and lymphatic vessels, nerves, and cells that appear to have tenocyte precursor-like properties (Tempfer et al, 2009). The arrangement of collagen fibrils into fascicles allows the tendon to withstand tensile strains and provide elasticity to the tendon (Dowling et al, 2000).

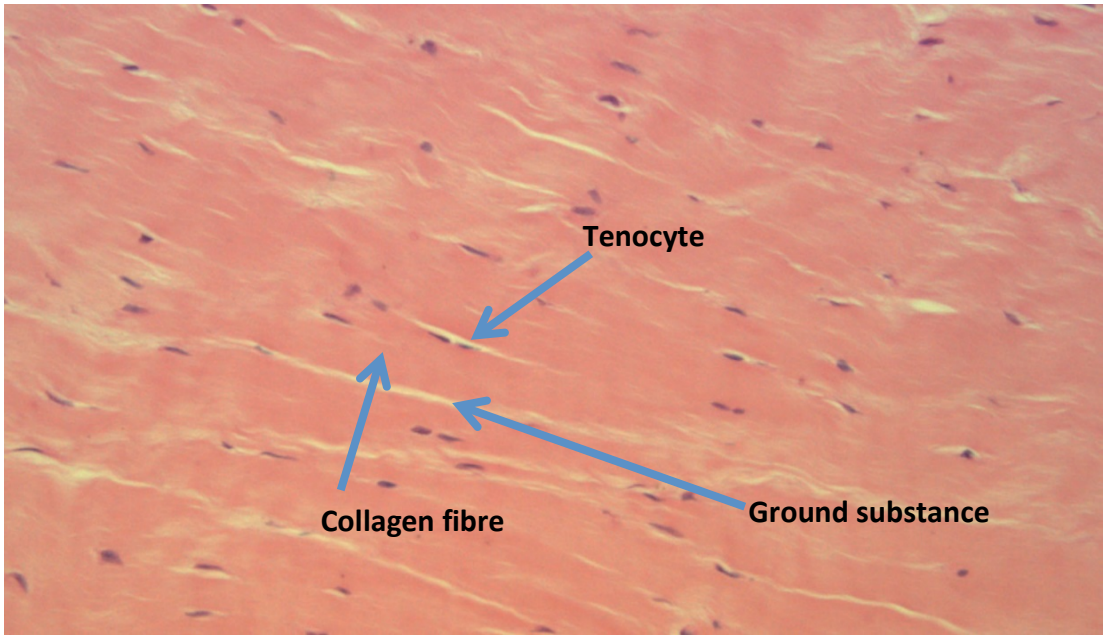

Figure 2.1- Histological specimen of a normal human quadriceps tendon (light microscopy, stained with haematoxylin and eosin). Long, slender cells are observed in between collagen fibres with inconspicuous ground substance (reproduced from Docking (personal communication) with permission).

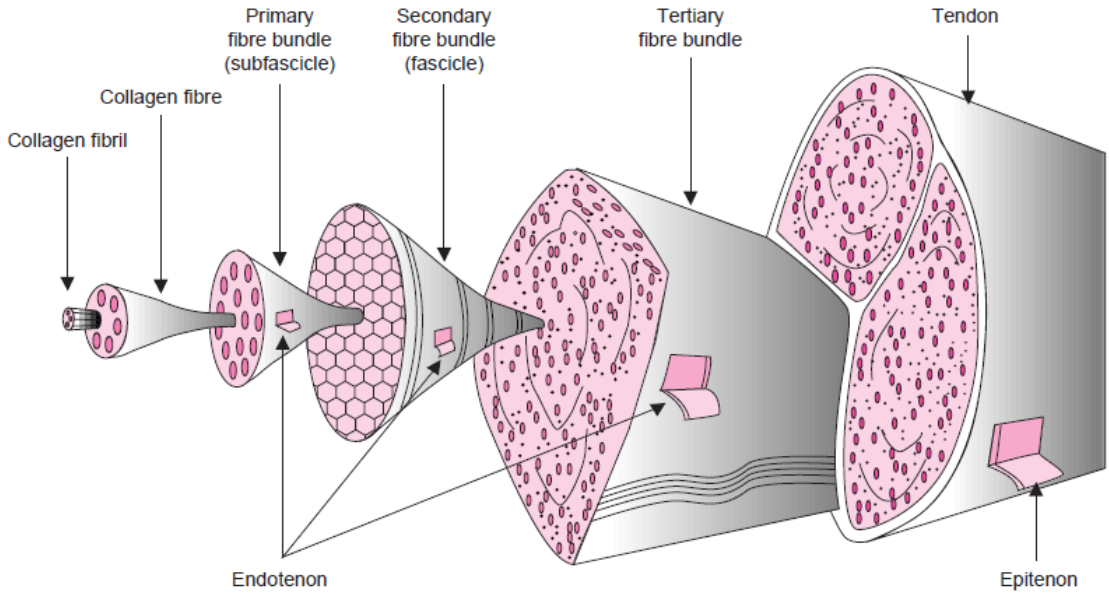

Figure 2.2- The hierarchical organisation of tendon structure from collagen fibrils to the entire tendon (reproduced from Kannus (2000) with permission).

2.1.3- Proteoglycans

The remaining 20-50% of dry matter of the ECM is composed of non-collagenous proteins such as proteoglycans (PGs). The amount and type of PGs is dependent on the mechanical forces that are applied to the tendon. In tensile regions, such as the midsubstance of the Achilles tendon, there are large concentrations of the small leucine-rich PG (SLRP), decorin, as well as smaller amounts of other SLRP's (biglycan and fibromodulin)(Rees et al, 2009; Samiric et al, 2004a; Waggett et al, 1998). These SLRP's are horseshoe shaped and located around type I collagen fibrils and are important in the regulation of fibrillogenesis (fig 2.3)(Henry et al, 2001). The presence of decorin has been suggested to be one mechanism by which lateral fusion of collagen fibrils is inhibited leading to a higher population of small diameter collagen fibrils (Rees et al, 2009; Watanabe et al, 2005). SLRP's have one or two glycosaminoglycan (GAGs) side chains (Parkinson et al, 2011).

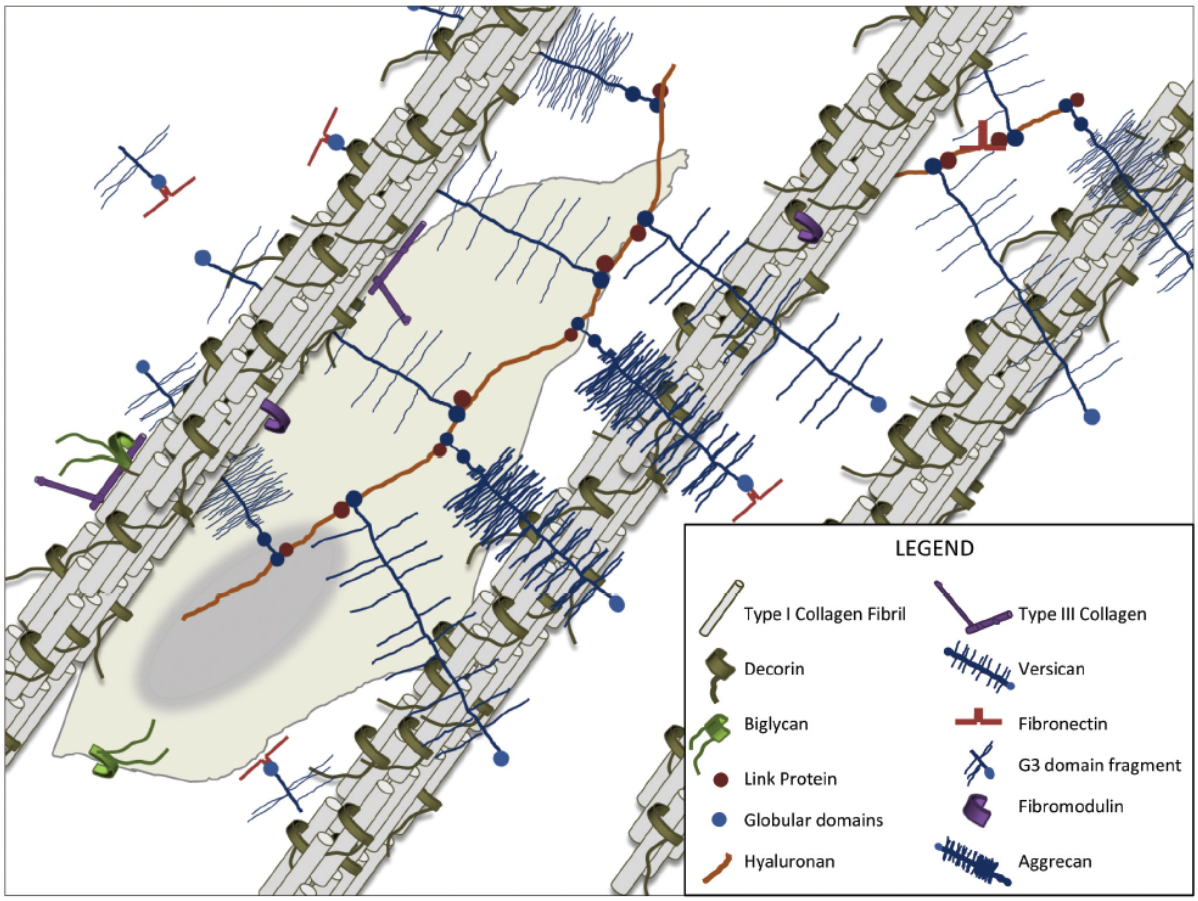

Figure 2.3- Interaction of proteoglycans and other matrix macromolecules within the extracellular matrix of the tensile regions of normal tendon (reproduced from Parkinson et al (2011) with permission).

Large aggregating PGs, such as aggrecan and versican, are present in small concentrations throughout the tendon. However, in areas where the tendon is compressed (ie insertion into bone or where the tendon wraps-around bone), these PGs are observed in higher concentrations (Benjamin & Ralphs, 1998; Okuda et al, 1987; Vogel et al, 1993). Due to the large number of negatively charged GAG side chains comparative to the SLRPs, these PGs bind water and assist in stress dissipation of compressive and shear forces (fig 2.3)(Rees et al, 2000; Samiric et al, 2004a; Wren et al, 2000a). The presence of these PGs results in a subsequent decrease in permeability that slows the dissipation of fluid and allows the tissue to maintain its fluid pressure for a longer time, resulting in a stiffened ECM that protects it from being disrupted or damaged (Wren et al, 2000a). Versican has also been implicated in modulating cell proliferation and influencing cell shape and motility (Rees et al, 2009).

2.1.4- Blood vessels and nerves

The tendon has frequently been described as avascular and aneural with few blood vessels and nerves present within the central portion of the tendon. When present, blood vessels are arranged longitudinally within the endotenon accompanied by nerves and lymphatic vessels (Elliott, 1965; Hess et al, 1989; Jozsa et al, 1991). Intra-fascicular tendon cells receive their nutrition from diffusion, and are dependent of the PGs and water present.

The four components of the tendon (cell, collagen, PGs and blood vessels) all have a role in allowing the tendon to withstand high levels of applied mechanical stresses. However, pathology alters these components and the tendon is susceptible to tendinopathy and/or rupture.

2.2- Histopathological features of tendon pathology

While there is still considerable debate on the aetiology and pathogenesis of tendon pathology, the histopathological features of end-stage or degenerative pathology have been extensively described within the literature.

2.2.1- Tenocyte

The tenocyte within the pathological tendon exhibit an altered phenotype, which has been described as chondrocytic (Khan et al, 1996). These cells appear rounded in shape with rounded nuclei and a conspicuous increase in size of cytoplasm (fig 2.4)(Astrom & Rausing, 1995; Cook et al, 2004a; Jarvinen et al, 1997; Khan et al, 1999; Shalabi et al, 2002). This appearance suggests an increase in the metabolic activity of the cell and an upregulation in matrix protein production (Dowling et al, 2000; Smith & Webbon, 1996). There is an

increase in the number and density of cells, and while areas of apoptosis and cell death have been reported in tendon pathology (fig 2.4)(Cook et al, 2004a; Jarvinen et al, 1997; Khan et al, 1999; Scott et al, 2005; Yuan et al, 2002) few studies (if any) have reported a decrease in cell numbers overall. The source of the increased cells remains elusive. Likely sources could be peritendinous structures, including the interfascicular matrix, an unidentified pool of stem cells or division of resident cells.

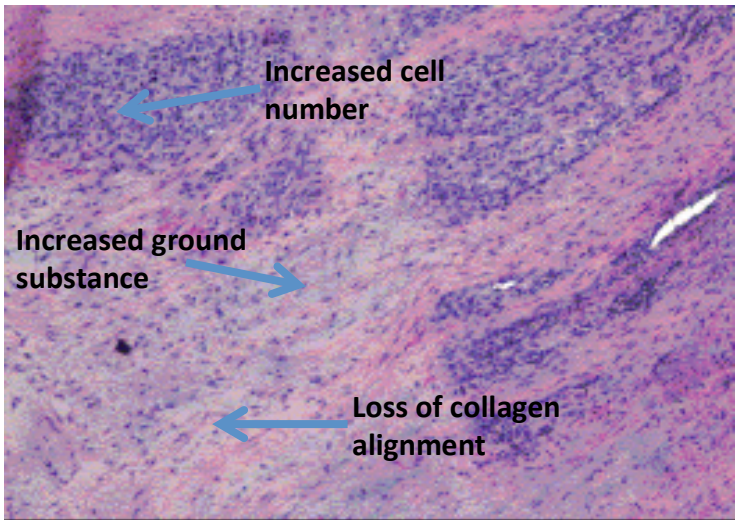

Figure 2.4- Histopathological appearance of a human degenerative tendon (light microscopy, stained with haematoxylin and eosin). Note the markedly increased cell numbers, increased ground substance, the loss of collagen evidenced by the loss of longitudinal rows of cells (adapted from Cook & Purdam, (2009) with permission).

2.2.2- Proteoglycans

The pathological tendon has been described as mucoid degeneration (Józsa et al, 1990; Khan et al, 1999), partly due to the gelatinous appearance of the tissue due to the increase in, and altered profile of, PGs within the tendon (fig 2.4). Studies have reported increased PG content, directly (Parkinson et al, 2010; Samiric et al, 2009) or indirectly via increased concentrations of associated GAG side chains (Benazzo et al, 1996; Birch et al, 1998; Movin et al, 1997). These changes, driven by the tenocyte, relate to significant increases in gene expression and content of the larger PGs aggrecan and versican (Parkinson et al, 2010; Samiric et al, 2009). The synthesis and degradation rates of these PG are both upregulated in pathology, leading to rapid turnover of the ground substance (Parkinson et al, 2010; Samiric et al, 2004a; Samiric et al, 2004b). This alteration in the PG profile results in an increase in water content within the tendon due to the hydrophilic nature of the GAGs associated with aggrecan and versican (Magnusson et al, 2003).

A number of review articles have attempted to provide rationale for this alteration in ground substance in tendon pathology. Increases in GAG concentration and resulting water content, have been shown to increase the number of large diameter tendon fibrils in a tissue culture with no changes in collagen content. This increase in GAG content resulting in an increase in fibril and tendon size may be a quick response of the tendon to overload to reduce stress and protect the tendon from further injury (Cook & Purdam, 2009; Magnusson et al, 2003).

2.2.3- Collagen

The pathological tendon exhibits a loss of the hierarchical tendon structure, with tendon bundles separated by clefts of increased ground substance (fig 2.4)(Jarvinen et al, 1997; Khan et al, 1999; Paavola et al, 2002). Coinciding with alterations in fibre alignment is an increase in type III collagen that form thinner fibres that are poorly organised (de Mos et al, 2007; Eriksen et al, 2002; Jarvinen et al, 1997; Maffulli et al, 2000b; Paavola et al, 2002; Riley, 2005; Riley et al, 1994). With the extracellular collagen arrangement in disarray, the area of tendon has diminished capability to transmit tensile force (Magnusson & Kjaer, 2003).

2.2.4- Blood vessels and nerves

The infiltration of blood vessels within the pathological tendon has been well documented. These vessels have been reported to be randomly oriented throughout the tendon (Jarvinen et al, 1997; Kannus & Józsa, 1991; Khan et al, 1999; Kraushaar & Nirschl, 1999; Williams, 1986), while others report the increased vessels are aligned parallel to tendon fibres (Maffulli et al, 2000a). There is considerable evidence that suggests that blood vessels are associated with areas of matrix disorganisation, suggesting that the infiltration of blood vessels may be opportunistic (Ingber, 1991; Pufe et al, 2005; Yang et al, 2010). Weinberg et al (1998) found that Doppler flow on ultrasound (indicative of blood flow/neovascularisation) was only observed in tendons that contained an area of disorganisation and were not observed in tendons that were thickened with aligned fibrillar structure.

2.3- The pathogenesis of tendon pathology and tendinopathy

Histological examination of human pathological tendon have been confined to end-stage degenerative pathology taken from surgical specimens; few studies have investigated the early stages of pathology due to difficulties in accessing tissue in humans. Evidence for the primary change within the tendon that drives tendon pathology is therefore lacking, although animal studies provide some insight. As a result, several hypotheses on the pathogenesis of tendon pathology, and more importantly the primary histological change within the tendon, have been proposed. This next section will describe existing models of tendon pathology and discuss strengths and weaknesses of each model. These models can be broadly categorised as collagen tearing, inflammation or cell activation, based on the initial step in the development of tendon pathology. It should be noted that these models are not necessarily mutually exclusive.

2.3.1- Collagen tearing based hypotheses

A number of models have suggested changes in the fibrillar matrix, specifically microtrauma to the collagen fibrils, is the primary histopathological event in the development of tendon pathology. Abate et al (2009) described the 'iceberg theory' based on epidemiological evidence and basic science studies in humans and animals. While long-term exercise within a physiological range can be beneficial to the tendon, relative overload may induce discontinuation of the fibrillar matrix. This repetitive strain and overload may break cross-links between collagen fibrils, termed microruptures, and tissue denaturation. Similarly, other models of tendon pathology have also suggested that microtrauma to the collagen fibres are the primary event in the development of pathology (Arnoczky et al, 2007; Fu et al, 2010). In the 'iceberg theory', the alteration in the fibrillar matrix in combination

with other conditions (eg further overloading, inadequate cell metabolism and blood supply) lead to the failure of the healing mechanisms within the tendon and lead to degradation of the ECM, neovascularisation and nerve proliferation. Despite substantial changes to tendon structure it can remain below the 'tip of the iceberg' and be asymptomatic.

A limitation of the 'iceberg theory' is while the presence of disorganisation within the tendon on imaging, such as ultrasound (US) and magnetic resonance (MR) imaging, may be associated with tendon pain, the painful tendon does not always exhibit changes in structure and vice versa. Malliaras & Cook (2006) reported that 16% of patellar tendons scanned in a volleyball cohort had a painful tendon (provoked by a single leg decline squat) with normal imaging on US as determined by an experienced musculoskeletal sonographer. Similarly, painful patellar tendons were observed to have a wide range of presentations on US from completely normal to diffuse thickening with diffuse changes in echogenicity to thickened tendons with focal areas of hypoechogenecity (table 2.1)(Malliaras et al, 2010). High proportions of asymptomatic tendon pathology have been observed in a wide range of tendons (table 2.1)(Achilles, patellar, quadriceps and rotator cuff tendons)(Cook et al, 2001; Fredberg et al, 2008; Giombini et al, 2013; Malliaras et al, 2010; Yamamoto et al, 2010), especially in populations that specifically load these tendons (ie patellar tendon pathology in basketball players)(Cook et al, 1998). While pain was most frequently observed in the tendons with focal areas of hypoechogenecity, the concept that there needs to be considerable structural changes within the tendon to cause pain is not supported by the literature.

Table 2.1- Relationship between grey scale ultrasound and patellar tendon pain (reproduced from Malliaras et al (2010) with permission)

	Pain (% of all painful tendons)	No pain (% of all non-painful tendons)
Normal grey scale	91 (30%)	295 (59%)
Diffuse thickening	84 (27%)	114 (23%)
Hypoechoic	134 (43%)	92 (18%)

The key aspect of collagen tearing based theories is that the primary event within the tendon that leads to pathology is microtrauma or tearing of the collagen fibrils. While microtrauma to collagen may occur in later stages of pathology, the concept that normal collagen can tear *in vivo* without significant changes in the non-collagenous matrix does not appear to be supported (Screen et al, 2005b). Changes in collagen structure appear to be proceeded by changes in cellularity and the ground substance (Cook et al, 2004a). Similarly, the longevity of collagen within tendon appears to suggest that collagen microtrauma is not a significant feature as it does not result in collagen turnover (Heinemeier et al, 2013; Thorpe et al, 2010).

2.3.2- Inflammation

There was a long held belief prior to the 1980's that pain and pathology within the tendon was driven by a traditional inflammatory cascade. However, Puddu et al (1976) first questioned the role and presence of inflammatory cells in tendon pathology with a number of other studies showing absent or minimal inflammation within degenerative lesions (Alfredson et al, 2001; Alfredson et al, 2000). While these studies are limited by investigating only one aspect of inflammation (ie upregulation of Prostaglandin E2), other investigations have shown little infiltration of inflammatory cells when using immunohistochemistry (Khan et al, 1996; Pecina et al, 2010). With little evidence showing an inflammatory process in the pathological tendon and traditional treatments, such as rest, ice and anti-inflammatory medication being unsuccessful (Almekinders & Temple, 1998), the concept of tendinitis was abandoned (Khan et al, 2002). The view of tendon pathology shifted from being inflammatory to that of a degenerative process. Yet, the notion that inflammation has a primary role in tendon pathology is still considered possible by a number of clinicians and researchers. This revisiting of inflammation in tendon pathology has been due to increasing evidence for the presence of inflammatory cytokines and cells and the infiltration of blood vessels. These features will be discussed in terms of the relevance to tendon pathology.

Alterations in biochemical mediators, specifically those that are observed in systemic shock, has led investigators to suggest that inflammation is a critical component in the development of tendon pathology (Fredberg & Stengaard-Pedersen, 2008; Rees et al, 2014). The upregulation and presence of inflammatory cytokines (TNF-α, IL-1β, COX-2, IL-6, TGF-β) have been reported in the pathological tendon in numerous studies (Fenwick et al, 2001;

Gaida et al, 2012; Gotoh et al, 1997; Legerlotz et al, 2012). However, these cytokines have been shown to be produced by tenocytes in normal tendon in response to mechanical stimuli, both over- and under-stimulation (table 2.2). Similarly, microdialysis studies of the peritendinous space have reported increases in inflammatory cytokines, such as PGE2, IL-6 and thromboxane B2, in response to exercise suggesting that these cytokines are driven by the tenocyte in response to mechanical stimuli (Langberg et al, 1999a). The upregulation of inflammatory cytokines in response to mechanical stimuli may drive the infiltration of inflammatory cells observed in the overloaded or pathological tendon.

Table 2.2- Summary of the literature investigating alterations in inflammatory cytokines in response to mechanical stimuli in normal cell/tissue cultures. PGE2, prostaglandin E2; IL-6, Interleukin 6; TGF- β, transforming growth factor beta; TNF-α, tumour necrosis factor alpha; PDGF, platelet-derived growth factor; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; TGF-α, transforming growth factor alpha; VEGF-A, vascular endothelial growth factor A; VEGF-C, vascular endothelial growth factor C

↑ denotes an increase in this cytokine

↔ denotes no change in this cytokine

Study	Mechanical stimulus	Inflammatory cytokine
Almekinders et al (1995)	25% maximal strain, 1 Hz frequency, motion 12hrs on and 12hrs off for 72 hrs	↑ PGE2
Almekinders et al (1993)	25% strain, 0.17 or 1 Hz for 3hrs	↑ PGE2
Skutek et al (2001a)	5% strain, 1 Hz for 1hr	↑ IL-6 ↔ TGF-β ↔ TNF-α ↔ PDGF
Skutek et al (2001b)	5% strain, 1 Hz for 1hr	↑ TGF-β ↑ PDGF
Li et al (2004)	8% or 12% strain, 0.5 Hz for 4hrs	↑ PGE2 ↑ Leukotriene B4
Wang et al (2003)	8% strain, 0.1, 0.5 or 1 Hz for 4hrs	↑ PGE2 ↑ COX-1 ↑ COX-2
Mousavizadeh et al (2014)	10% strain, 1 Hz for 24hrs	↑ COX-2 ↑ TGF-α ↑ VEGF-A ↑ VEGF-C
Bayer et al (2014)	De-tension for 1,2,4 and 6 days	↑ TGF-β ↑ COX-1 ↑ COX-2

Conflicting results have been published on the presence of inflammatory cells, such as neutrophils, macrophages and mast cells. Artificially induced tendon pathology, such as collagenase injection or surgical lacerations, can result in the infiltration of inflammatory cells likely due to the macroscopic disruption of the tendon and its vascularity (Cetti et al, 2003; Sugg et al, 2014). However, inflammatory cells are not isolated to the ruptured or torn tendon (Appendix D). Studies that have compared ruptured tendon to pathological non-ruptured tendon have reported increased inflammatory and endothelial cells (cells lining the inferior surface of blood vessels) in the non-ruptured tendon (Kragsnaes et al, 2014; Schubert et al, 2005). Similarly, an inverse relationship has been reported in two studies between inflammatory/endothelial cells and the size of the tear (Matthews et al, 2006; Millar et al, 2010). It appears that the presence of blood vessels and inflammatory cells are related.

The infiltration of blood vessels observed in the pathological tendon has also been proposed as evidence of an inflammatory process (Rees et al, 2014). Interestingly, investigators who have reported inflammatory cells in tendon have reported that these cells are located around blood vessels. While blood vessels, as detected by Doppler US, are a common feature of tendon pathology they are frequently observed in the late stages of tendon pathology (Weinberg et al, 1998). Similarly, only moderate associations have been observed between Doppler signal and the presence and location of pain, and the asymptomatic tendon exhibits Doppler signal (Cook et al, 2004b; de Jonge et al, 2014; Divani et al, 2010; Gisslen & Alfredson, 2005). These findings suggest that the infiltration of blood vessels and accompanying nerves are not the primary source of pain nor is inflammation critical in the development of pain and pathology.

While it may appear that aspects of inflammation are critical in the development of tendon pathology, the tenocyte and its responsiveness to mechanical stimuli may be central to this process. The upregulation and increase in inflammatory cytokines appear to be driven by the tenocyte in response to mechanical stimuli. These cytokines may function as an autocrine/paracrine cell signalling pathway rather than an inflammatory process. Critically, these findings suggest that the tendon cell is important in responding to mechanical stimuli and may drive tendon pathology.

2.3.3- Cell-driven pathology

Leadbetter (1992) was the first to propose that tendon pathology is driven by an initial cell-matrix response to mechanical overload. He proposed that an appropriate mechanical stimuli results in a response from the cell altering the ECM. When the mechanical stimuli are excessive, a heightened cell-matrix response can lead to tendon degeneration and subsequently tissue failure (fig 2.5). While the features of tendinosis were described (changes in cell number and phenotype, in-growth of small blood vessels, collagen fibre disorganisation and microtears), the initial changes in the matrix as a result of cell response were not described.

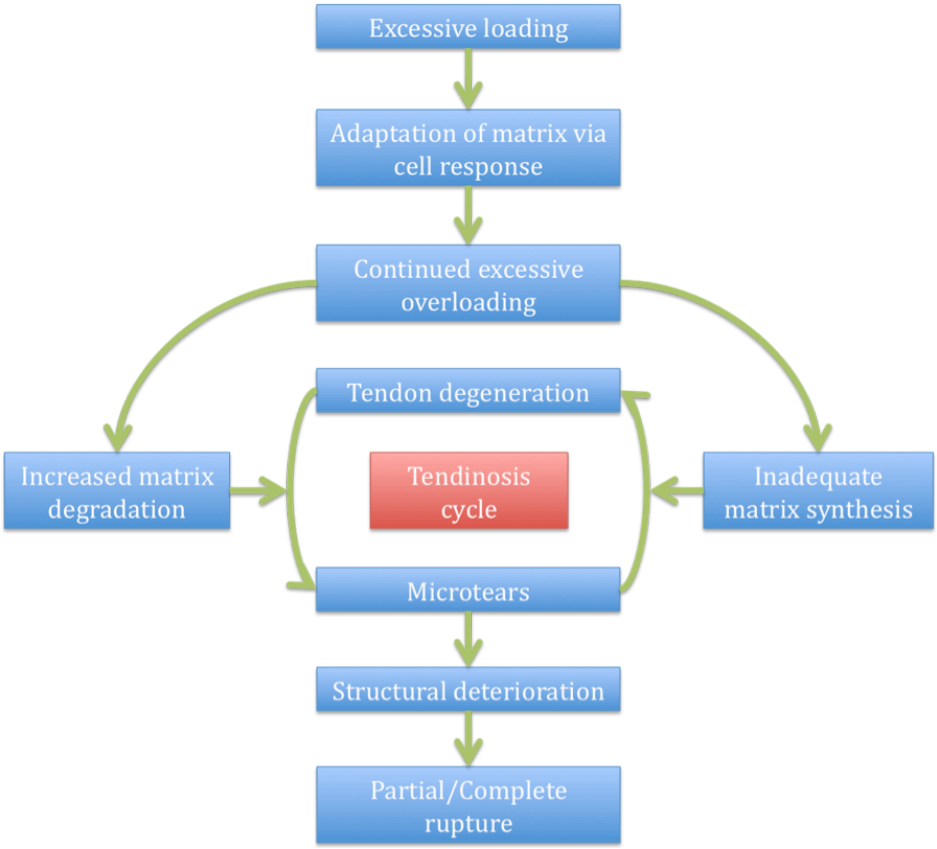

Figure 2.5- Flowchart of the tendinosis cycle as proposed by Leadbetter (1992). Overloading of the tendon leads to alterations in the matrix that the predisposes the tendon to collagen tearing and partial/complete rupture of the tendon (adapted from Leadbetter (1992) with permission).

Similar to the tendinosis cycle, the continuum of tendon pathology described by Cook & Purdam (2009) proposed that the key driver of pathology in the initial stages is alterations in the tenocyte. The tenocyte is responsible for maintaining the ECM in response to mechanical stimuli. However, unlike the tendinosis cycle that is a unidirectional pathogenesis, the cell-driven pathogenesis proposes that the tendon can transition forward and back between normal and pathological states, especially within the early stages (Cook & Purdam, 2009). These stages, termed *reactive, dysrepair* and *degeneration*, are not discrete *in vivo* but are discussed here in isolation (fig 2.6).

2.3.3.1- Reactive tendon pathology: an acute adaptation to overload

The first stage of pathology, reactive tendon pathology, is a pathological response to an acute overload (mechanical stimulation that exceeds the capacity of the tendon). On imaging, an increase in cross-sectional area is observed while collagen structure remains intact at this stage. The primary change within the tendon responsible for this increase in tendon dimensions is suggested to be tenocyte proliferation and activity resulting in increased protein production, specifically aggrecan and versican. These large hydrophilic PGs have been shown to be elevated in tendon pathology and can increase the tendons cross-sectional area by increasing the amount of bound water (Corps et al, 2006; Samiric et al, 2009). It was proposed that the increase in tendon dimensions driven by the cell and changes in the ground substance, is a quick but ultimately dysfunctional response of the tendon to overload in an attempt to reduce stress (force/unit area).

Unlike the collagen tearing theory, which suggests that degenerative changes are driven via inflammatory mediators, the pathological changes associated with reactive tendon pathology are driven by tenocytes in response to mechanical stimuli. How the tenocyte detects mechanical stimuli and converts it into a biochemical signal is discussed in section 2.4 and 2.5. Studies using animal models have supported the concept that changes associated with early tendon pathology, such as cell rounding and increased PG expression, are mediated via local stimulation of tenocytes rather than inflammatory cells or collagen tearing (Scott et al, 2007). As the reactive stage is characterised by a lack of fibrillar disorganisation, transition back to normal tendon structure is achievable with appropriate load modification (Cook & Purdam, 2009).

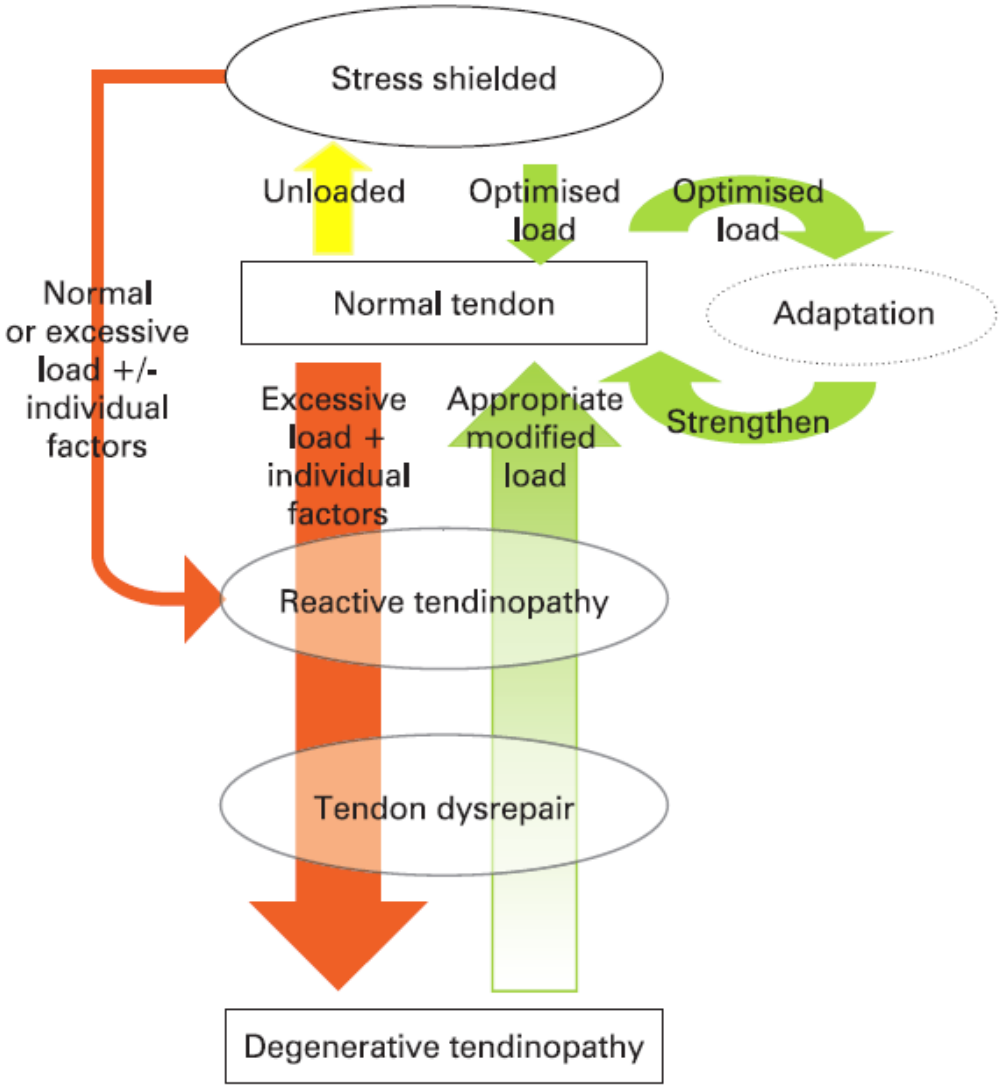

Figure 2.6- Continuum of tendon pathology as proposed by Cook & Purdam (2009). This model outlines the transition from normal through to degenerative tendon pathology and highlights the potential reversibility early in the continuum. Reversibility of pathology in the degenerative stage (adapted from Cook & Purdam (2009) with permission).

2.3.3.2- Tendon dysrepair: failed healing

If excessive load is continued, the tenocytes will continue to respond and further excess protein production will initiate breakdown of the aligned collagen structure. This stage is termed tendon dysrepair and was previously referred to as failed healing response of the tendon (Clancy, 1989). Histologically, more focal areas of matrix degradation are seen rather than diffuse changes seen in reactive tendon pathology. Similar to reactive tendon pathology, tenocytes appear chondrocytic (rounded and plump) and large PG expression is increased. However, the marked increase in ground substance causes separation of collagen fibrils and leads to the disorganisation of the collagenous matrix. Increases in type III collagen are also observed at this stage and result in a tendon less resilient to tensile strain (Maffulli et al, 2000b). As the collagen architecture has been altered and there is increased deposition of type III collagen, it has been hypothesised that although the pathological changes can be reversed, it is more difficult to return the ECM completely to normal due to the loss of collagen organisation.

2.3.3.3- Degenerative tendon pathology: end-stage pathology

Degenerative tendon pathology is end-stage pathology with marked changes in the ECM. This stage is characterised by extensive disorganisation of collagen and neovascularisation. These features have previously been described in the equine (Kobayashi et al, 1999; Strömberg, 1971; Strömberg & Tufvesson, 1969) and human literature (Khan et al, 1999; Maffulli et al, 2004). It has been proposed that the infiltration of blood vessels within the tendon is due to the large amount of ground substance and separation of collagen fibrils (Benjamin et al, 2007; Ingber, 2002). It is yet to be ascertained whether the presence of these vessels are beneficial or detrimental to tendon repair (Alfredson, 2005; Benjamin et al, 2007). However, Kraushaar & Nirschl (1999) found that vascularity was

associated with areas of significant collagen disorganisation suggesting that neovascularisation is not associated with improved healing.

Due to the extensive changes within the ECM, the tendons ability to return to normal structure is not possible. A loss of repair capacity may also be due to a loss of tenocytes due to cellular exhaustion, trauma or apoptosis. Focal areas of cell death have been identified in equine tendon pathology and have been implicated in the poor healing of these tendons (Hosaka et al, 2005a; Hosaka et al, 2005b).

2.3.4- Evidence for cell-driven tendon pathology in the horse

As it is not possible to obtain human tissue in early tendon pathology, evidence from animal studies are required. The horse provides a good model as it mimics human tendon pathology more closely than small animal models. A number of studies on equine tendon injury provide support for the cell-driven model of pathogenesis. Birch et al (1998) investigated subclinical superficial digital flexor tendon (SDFT) injuries that were diagnosed based on post mortem findings (central core discolouration) and analysed the extracellular components and compared them to normal tendons. These horses had no previous history of clinical tendon injury and showed no clinical signs prior to euthanasia. No significant differences in collagen content, percentage of type III collagen, collagen crosslinking and water content were observed however, GAG content within the central portion of the pathological tendon was significantly higher compared to normal tendon (Birch et al, 1998). The authors suggested that as collagen content did not differ, the assumption that increased collagen breakdown is the primary histopathological event in tendon injuries was no longer true.

Further support for the cell-driven model of pathogenesis is seen in horses that suffer from the hereditary disorder, degenerative suspensory ligament desmitis (DSLD)(Halper et al, 2006). The disease affects thoroughbreds, often leading to bilateral or quadrapedal lameness, and is characterised histologically by the accumulation of PGs within a number of connective tissues (Halper et al, 2006). Recent studies investigating tendon pathology in DSLD-affected horses reported accumulation of PGs within the matrix and disruption of collagen fibrils and the infiltration of blood vessels (Halper et al, 2006). These pathological features are similar to degenerative tendon pathology. As these lesions are often in present in horses without a history of excessive training or trauma, these pathological changes are due to the accumulation of PGs rather than microtrauma.

Changes in the unaffected limb in experimentally-induced animal models of tendon pathology may also provide support for a cell-driven pathogenesis. Williams et al (1984) observed that after collagenase injection, the control contralateral limb exhibited increased number of tenocytes that appeared less elongated and more rounded, similar observations were reported in mechanically induced tendon injury in the rabbit (Andersson et al, 2011). Changes in the cell phenotype may represent cell activation, leading to changes in the composition of the ECM through increased protein production (Smith & Webbon, 1996). The mechanisms of these changes in the contralateral limb is unknown (alteration in weight distribution between limbs and/or systemic drivers and/or central nervous system mediated changes), but it suggests that cellular changes are the primary histopathological event in the development of tendon pathology, not collagen disruption.

2.3.3.5- Evidence for cell-driven tendon pathology in humans

Due to the ethical issues in collecting tendon biopsies from humans in early stage tendon pathology, little basic scientific research has been conducted on the sequence of pathological changes. In the absence of tissues biopsies, imaging has been utilised to enhance knowledge of the pathogenesis of tendon pathology. Few studies have prospectively imaged the natural progression/history of tendons. Malliaras et al (2006) performed monthly US imaging of the patellar tendon in 58 volleyball players during the course of a competitive season (5 months). All scans were categorised into three groups based on the features observed on US; normal tendon (normal echogenicity and no thickening), diffuse thickening (diffuse changes in echogenicity accompanied by tendon thickening), and hypoechoic tendon (focal hypoechoic lesion). While these tendons were not classified based on the proposed continuum by Cook & Purdam (2009), inferences can be made retrospectively on the described classifications (normal, reactive and degenerative tendons, for the described classifications respectively).

From the transition matrix, patellar tendons that were normal or degenerative were likely to remain unchanged (probability = 0.78 and 0.81, respectively). Interestingly, those classified as reactive had a lower probability of remaining reactive (0.54) with a similar probability of tendons transitioning to normal or degenerative (0.26 and 0.2 respectively). While it provides little information on the histopathological features of tendon pathology, it supports the notion that tendons can transition along a continuum of pathological changes. Critically, reactive changes appear to be able to revert to normal or progress along the continuum, where degenerative tendons are unlikely to return to normal. This has implications in how we monitor tendon pathology with imaging and what is achievable when rehabilitating tendons.

One of the few studies evaluating early pathological changes histologically in humans was conducted in patellar tendon grafts of patients undergoing anterior cruciate ligament reconstruction (Cook et al, 2004a). Changes relating to the tenocyte (increased number and rounding) were the only pathological feature observed in isolation. An increase in ground substance and disruption of the collagen matrix were observed, but only in conjunction with pathological changes to the tenocyte. These findings are limited as it is a cross-sectional study, however it suggests that the cellular changes may be the primary pathological event of tendon pathology that lead to increases in ground substance, collagen disruption and neovascularisation (Cook et al, 2004a). With load being a critical factor in the development of tendon pathology, understanding how the cell detects and responds to load may advance our knowledge of the pathogenesis of tendon pathology.

2.4- Do tendons respond to load?

Tendons and their specialised cells (tenocytes) were long thought to be metabolically inert once the tendon was fully developed (Neuberger et al, 1951). This concept has taken hold due to considerable evidence suggesting that turnover of the ECM is minimal. Heinemeier et al (2013) investigated ^{14}C levels within normal tendons collected post-mortem. As a result of above ground nuclear testing in the 1950's, measurement of the ^{14}C isotope in biological tissue can provide information on the replacement rate or turnover of a specific tissue. The findings of this study showed that the core of the Achilles tendon contained similar levels of ^{14}C to atmospheric levels at the first 17 years of life, suggesting that the core of the tendon undergoes minimal turnover after skeletal maturity. Similar findings have been observed in the superficial digital flexor tendon of the horse based on the rate of aspartic acid racemisation (Thorpe et al, 2010).

When proteins are synthesised, the L-form of amino acids are used. Over time, these amino acids undergo spontaneous racemisation and convert to the D-form enantiomer (mirror image of the same molecule). As this is a time dependent step, measuring the ratio of the two enantiomers of aspartic acid allows information on the turnover of tissue to be assessed. Thorpe et al (2010) found that the half-life of SDFT was ~7.9 years, similar to the half-life of the positional common digital extensor tendon (~8.0 years). However, when the collagenous component of the SDFT was investigated in isolation a half-life of almost 200 years was observed, which equates to 0.25% of the collagenous matrix being turned over each year (Thorpe et al, 2010). It was suggested that the low rates of collagen turnover in the SDFT may be due to either a reduction in synthetic ability of the tenocyte because of a lack of nutrition or that the SDFT is protected from significant remodelling that may weaken the tendon. From the literature, turnover of collagen that is integrated into the matrix appears to be minimal in normal tendon.

Despite integrated collagen turnover appearing to be minimal, a number of studies have shown changes in collagen mRNA and protein expression in response to load (Langberg et al, 1999b; Miller et al, 2005; Olesen et al, 2007). Studies using micro-dialysis in the peritendinous tissue of the Achilles tendon in humans have reported elevations in a marker of collagen synthesis, collagen propeptide (PICP), 72 hours (hrs) post an endurance running exercise, with similar studies showing that PICP remained elevated over an 11 week training period (Langberg et al, 2007; Langberg et al, 1999b). Elevation of collagen protein synthesis rates were also demonstrated from Achilles biopsy samples 24-72hrs post-exercise. These findings seem to suggest that increased turnover of type I collagen occurs in response to

exercise, however, whether the newly synthesised collagen is integrated into the matrix is questionable.

The synthesis of collagen and integration into the tendon matrix is a complex pathway with numerous regulatory processes that are present through the process from mRNA translation to integration of the collagen fibres to the ECM (fig 2.7). A number of studies in tissues, including tendon, have demonstrated intracellular degradation of 10-40% of newly synthesised collagen (Humphries et al, 2008; Rennard et al, 1982; Steinberg, 1973). Similarly, Humphries et al (2008) described intracellular cleavage of type I procollagen in the

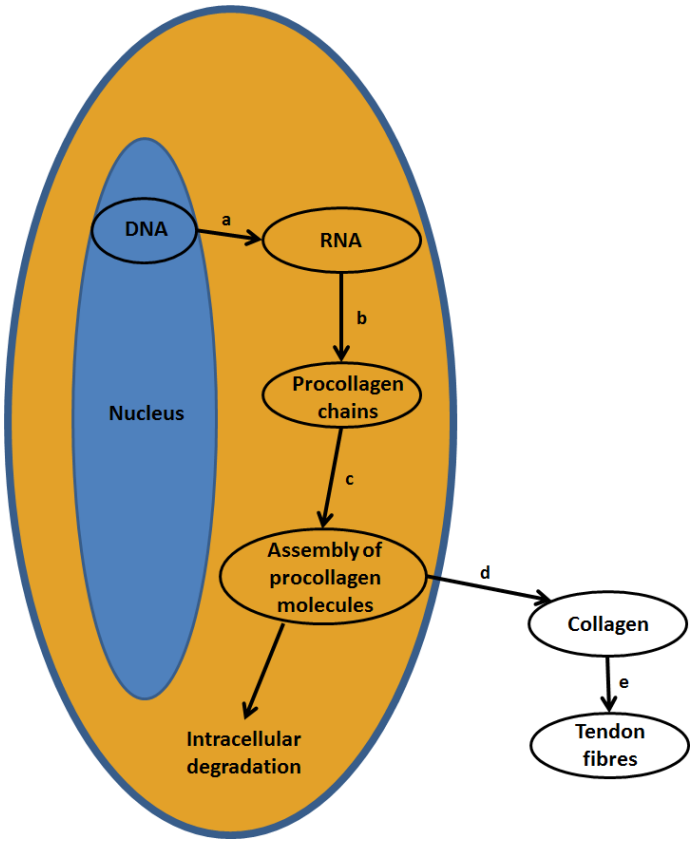

Figure 2.7- Schematic representation of the pathways of collagen production; major intracellular and extracellular steps are indicated. Each step requires a number of enzymes and regulatory mechanisms to progress from DNA to integrate tendon fibres. a) gene transcription, b) mRNA translation and protein synthesis, c) modification of amino acids and addition of carbohydrate, d) extracellular secretion of procollagen molecules and cleavage of 'procollagen' extensions, e) assembly and cross-linking of collagen (adapted from Rennard et al (1982) with permission).

absence of fibropositors (actin-stabilised plasma membrane processes required to organise the newly assembled collagen fibrils). These findings suggested that the cell can control and inhibit collagen fibril integration into the matrix. What was interpreted as an increase in collagen turnover from the studies after exercise may not be the case. Increases in PICP may be indicative of increased synthesis of procollagen, yet may not result in integration of newly synthesised collagen or turnover of the collagenous matrix.

Despite the lack of turnover in the collagenous matrix of tendon, non-collagenous matrix (eg PGs and GAGs) are degraded and synthesised at a faster rate. Thorpe et al (2010) observed a significantly shorter half-life for the non-collagenous component of the SDFT in horses (half-life of 2.18 years). There are two major classes of PGs within the ECM of the tendon, which have been shown to have different pathways for their synthesis and breakdown (Samiric et al, 2004b). Small leucine rich PGs (SLRPs) are the predominant PG within normal tendon (section 2.1.3). Because of its strong affinity to collagen molecules, SLRP's have a significantly longer half-life (~20 days) than the larger PGs (Samiric et al, 2004b). The larger PGs, such as aggrecan and versican, are synthesised within days of loading (Koob et al, 1992; Robbins et al, 1997) and catabolised rapidly (half-life ~2 days)(Samiric et al, 2004b). Despite ECM turnover appearing to be minimal, the tenocyte does respond to changes in mechanical stimuli with a number of systems that control whether these proteins are integrated into the matrix.

2.5- How cells sense load

The ability of the cell to detect and respond to the mechanical environment is critical to tissue homeostasis. Bone, a collagenous tissue like tendon, remodels when mechanically challenged, resulting in an increase or decrease in cortical bone mass. Frost (1987) proposed the 'mechanostat' mechanism; bone strains above a certain level (or 'set point') produced a response that increased the amount of cortical bone while bone strains below a certain level elicited a response that decreased the amount of cortical bone. Recent studies have suggested that a similar mechanism may occur for soft tissue structures such as tendon. Lavagnino & Arnoczky (2005) used *in vitro* collagen gel matrices with tendon cells and demonstrated that expression of catabolic or anabolic genes was dependent on mechanical strain. When the collagen matrix was released from tension, the matrix contracted in size and there was a down-regulation in type 1α1 collagen mRNA with an upregulated expression of collagenases, suggesting a catabolic state within the matrix (Lavagnino & Arnoczky, 2005). However, when tension was re-applied to the matrix the level of expression of mRNA was reversed, shifting to an anabolic state. This suggested that tenocytes have the ability to detect and respond to mechanical stimuli or the lack of it.

While there is extensive evidence that tenocytes respond to load, the exact mechanism by which tenocytes detect mechanical stimuli is poorly understood (Donnelly et al, 2010). The tenocyte has a number of structures and mechanisms that allows the cell to detect mechanical stimuli, such as their internal cytoskeleton, cellular projections (cilia), cell-cell communication and local/circulating chemical messengers. As the cytoskeleton physically links the internal compartment of the cell to the ECM, it is likely to have a critical role in detecting mechanical stimuli and converting it into a biochemical response.

The cytoskeleton plays a critical role in providing mechanical strength to the cell, determining the shape and consistency of the cell, cell motility and adherence of the cell to the ECM (Ingber, 1997). In the 1990's, Donald Ingber first described the 'tensegrity' model in an attempt to understand mechanotransduction and the role of cell deformation and the cytoskeleton (Ingber, 1991; Ingber, 1997,2008). In the tensegrity model, the internal

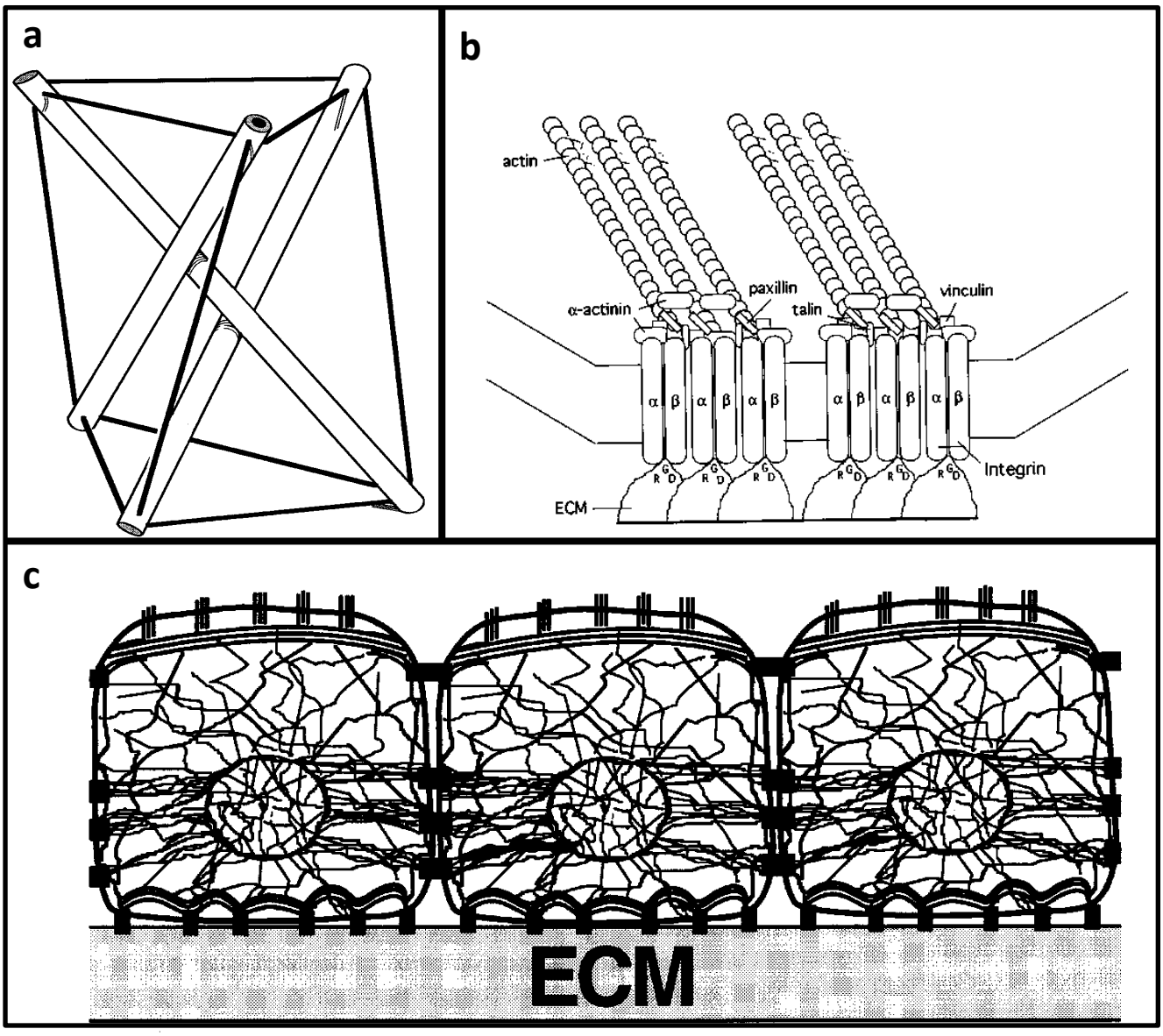

Figure 2.8- a) Schematic representation of the tensegrity model of the cell. These structures contain a series of isolated compression-resistant struts (white struts) that resist the pull of surrounding tissue elements (black cables). b) Cytoskeletal framework of the focal adhesion complexes connecting the cytoskeleton to the extracellular matrix. c) The intracellular cytoskeleton interconnects with the underlying extracellular matrix (adapted from Ingber et al (1997) with permission).

cytoskeletal structure forms a network of struts and cables (fig 2.8a), where forces applied to the cell by the surrounding ECM deform the cytoskeleton via attachments to the ECM (fig 2.8c). The cell is able to detect mechanical stimuli as it has numerous attachments to the ECM via integrins that bind the cell to specific ECM proteins (Chiquet, 1999; Hynes, 1992). Integrins primarily form indirect connections to actin microfilaments of the cytoskeleton via mediator proteins, such as paxillin, vinculin and talin (fig 2.8b)(Ingber, 1997). Because of the cytoskeleton's close association with the ECM, it suggests that it may have a vital role in the detection of mechanical stimuli.

A number of studies have shown the importance of the cytoskeleton in controlling gene expression of key matrix proteins in tendon using cell cultures. Lavagnino et al (2005) observed that under constant tensile strain mRNA expression of type I collagen was observed, however when the cytoskeleton is disrupted with cytochalasin D the expression of type I collagen was undetectable, while upregulation of collagenase mRNA expression was observed. Stress deprivation has been observed to increase expression of catabolic enzymes as there is a disproportionate balance between stress within the cell and externally (Arnoczky et al, 2008; Lavagnino & Arnoczky, 2005). The balance between tension within and external to the cell appears to be important in the cell being able to detect load and control the expression of catabolic and anabolic proteins.

Recently, the presence of primary cilia has been observed on tenocytes. Primary cilia are immotile microtubule based sensory organelles that may have a critical role in detecting mechanical stimuli and converting it into a biochemical response (Donnelly et al, 2010; Gardner et al, 2011). These cytoplasmic extensions are orientated parallel to the collagen fibres and appear to act as cantilevered beams that have a rigid attachment to the ECM,

which bend and deflect in response to mechanical stimuli. The deflection of cilia is weakly correlated to the amount of tensile strain, with the cilia deflecting 2.7° for every 1% strain (Lavagnino et al, 2011). Donnelly et al (2010) reported that 64% of tenocytes in normal tendon exhibited a primary cilia, with this percentage possibly under-estimated due to the cell cycle (cilia are reabsorbed prior to mitosis) and limitations due to histological section.

Interestingly, a nine-fold variation in cilia length was observed in normal tendon, possibly due to cells being in differing phases of the cell cycle, however the authors suggested that differing mechanical forces throughout the tendon may also be responsible (Donnelly et al, 2010). Gardner et al (2011) exhibited a significant increase in cilia length (159%) after 24 hrs of stress deprivation suggesting that the tenocytes use cilia to increase their mechanosensitivity and seek mechanical stimuli in a stress deprived state. This increase in cilia length was reversed after 24 hrs of 3% cyclic strain. As cilia have direct connections to the ECM and transmit mechanical stimuli to the internal cytoskeleton of the cell, they are a strong candidate as one of the mechanisms in which tenocytes detect and respond to mechanical stimuli.

2.6- Imaging and its role in tendinopathy

This section will discuss the features of normal and pathological tendons on conventional imaging (US and MR imaging), the accuracy and sensitivity of imaging in the detection of clinical tendinopathy, limitations relating to conventional imaging and introduce ultrasound tissue characterisation as a new modality in the imaging of tendons. Previous research investigating the response of the tendon to load using clinical imaging modalities will also be discussed.

2.6.1- Features of normal and pathological tendon on imaging

Imaging allows for the visual representation and assessment of tendon structure. Normal tendon contains uniform alignment of collagen fibres, little water and ground substance (section 2.1). Using US, the parallel arrangement of normal collagen fibres generates a single US reflection and allows visualisation of the tendon fibres (fig 2.9)(Rasmussen, 2000). Similarly, the relationship between water and collagen results in a strong dipole interaction and little signal being observed on MR imaging in the normal

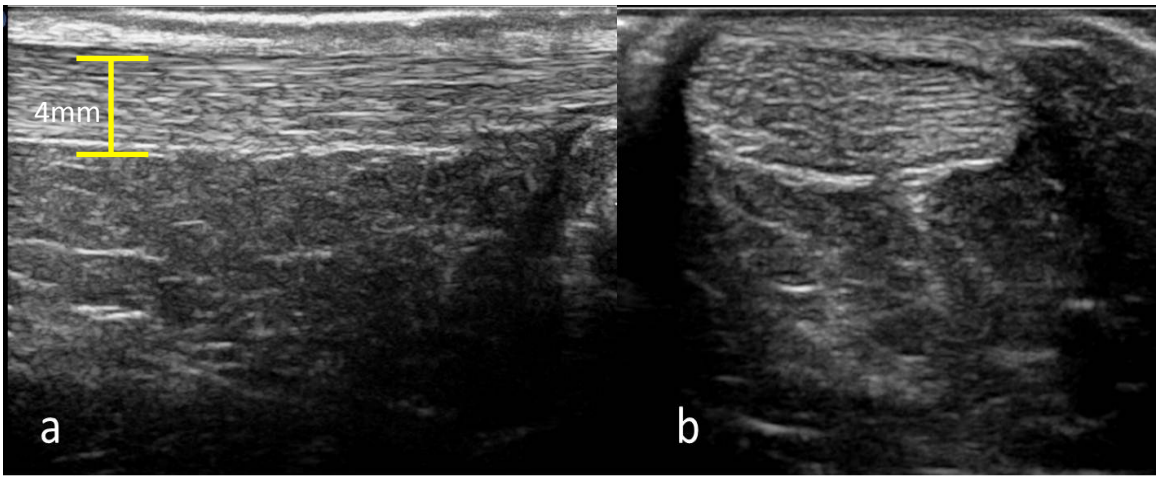

Figure 2.9- Ultrasound of a healthy Achilles tendon. (a) Longitudinal and (b) transverse ultrasound images of the mid Achilles tendon. The normal tendon texture appears homogeneous with parallel echogenic lines reflecting the internal fibrillar structure. The yellow lines represent the superficial and deep border of the tendon.

tendon (fig 2.10)(Calleja & Connell, 2010; Weinreb et al, 2014). Finally, normal tendons are relatively avascular especially when observed with colour Doppler US (Ohberg et al, 2001).

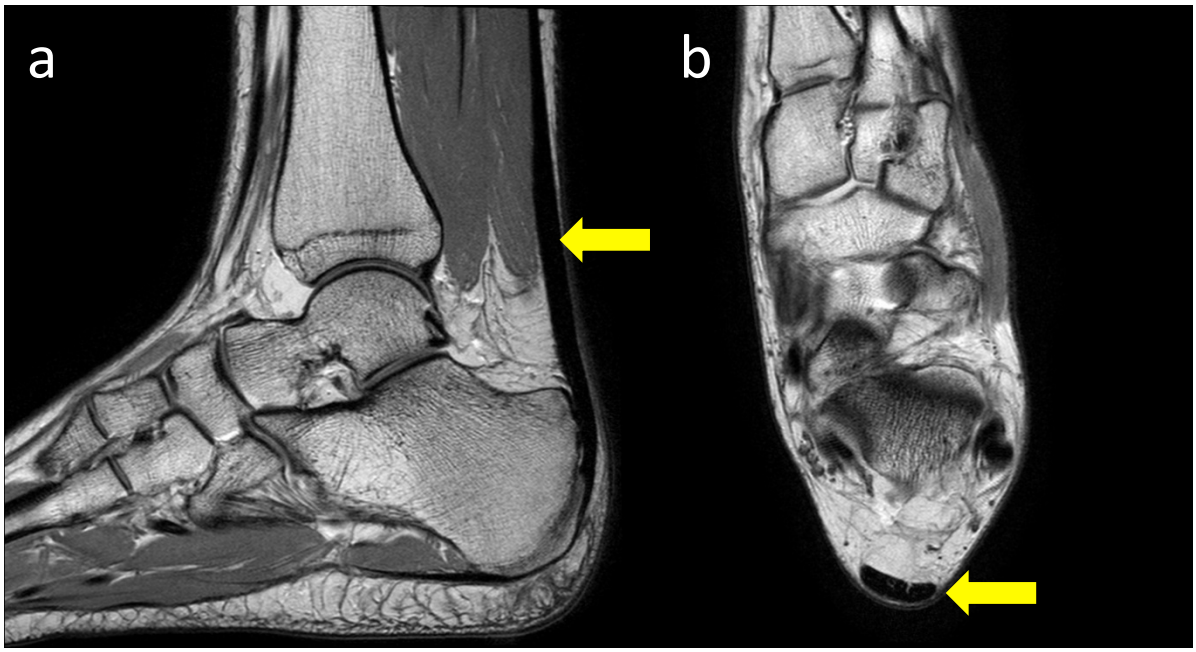

Figure 2.10- Magnetic resonance imaging of the normal Achilles tendon. (a) Sagittal and (b) Axial Proton-density sequence MR image showing a normal Achilles tendon (arrow) with uniform thickness and predominantly low signal intensity

Four main histopathological changes are observed in tendon pathology (section 2.2). While changes in cell number and phenotype are beyond the resolution of clinical imaging modalities, the consequence of these active metabolic cells can be detected. The increase in large PGs and bound water can be observed on imaging, which have been described as increases in tendon dimensions and heterogeneous or diffuse changes in echogenicity (Cook & Purdam, 2009; Malliaras et al, 2010). Fibrillar disorganisation and lack of parallel aligned fibres generates multiple reflections and shadowing, which is represented by an area of hypoechogenecity on US (fig 2.11)(Rasmussen, 2000). On MR imaging, this alteration in fibrillar alignment and increase in water content results in an increase in intratendinous

signal (fig 2.12)(Calleja & Connell, 2010; Weinreb et al, 2014). Neovascularisation within the pathological tendon can be imaged with colour and power Doppler US imaging (Boesen et al, 2006; du Toit et al, 2008).

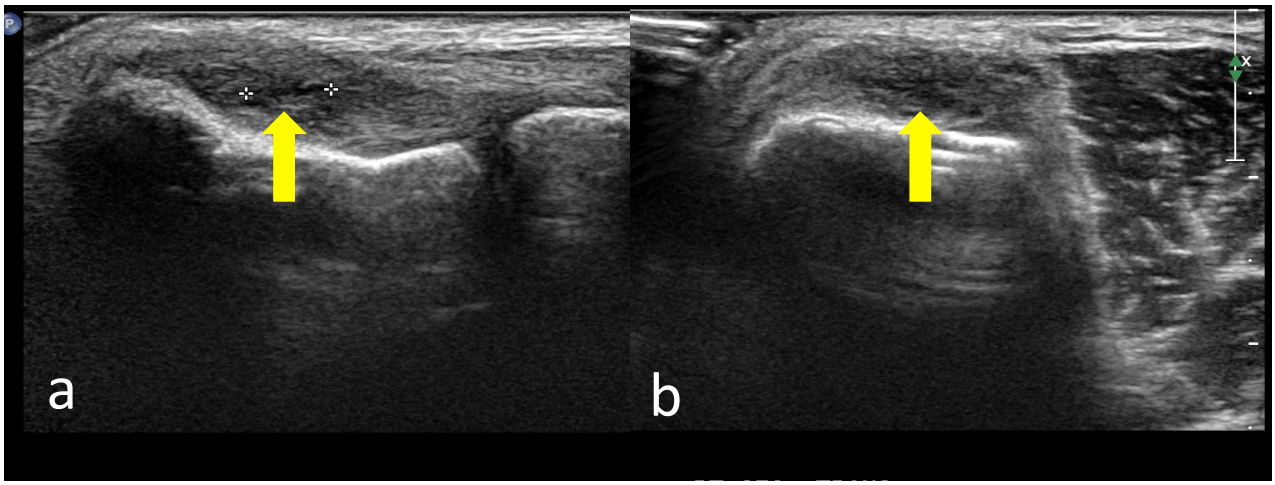

Figure 2.12- Ultrasound image of a pathological common extensor tendon of the elbow. (a) Longitudinal and (b) transverse image reveal a focal intratendinous hypoechoic lesion (yellow arrow)

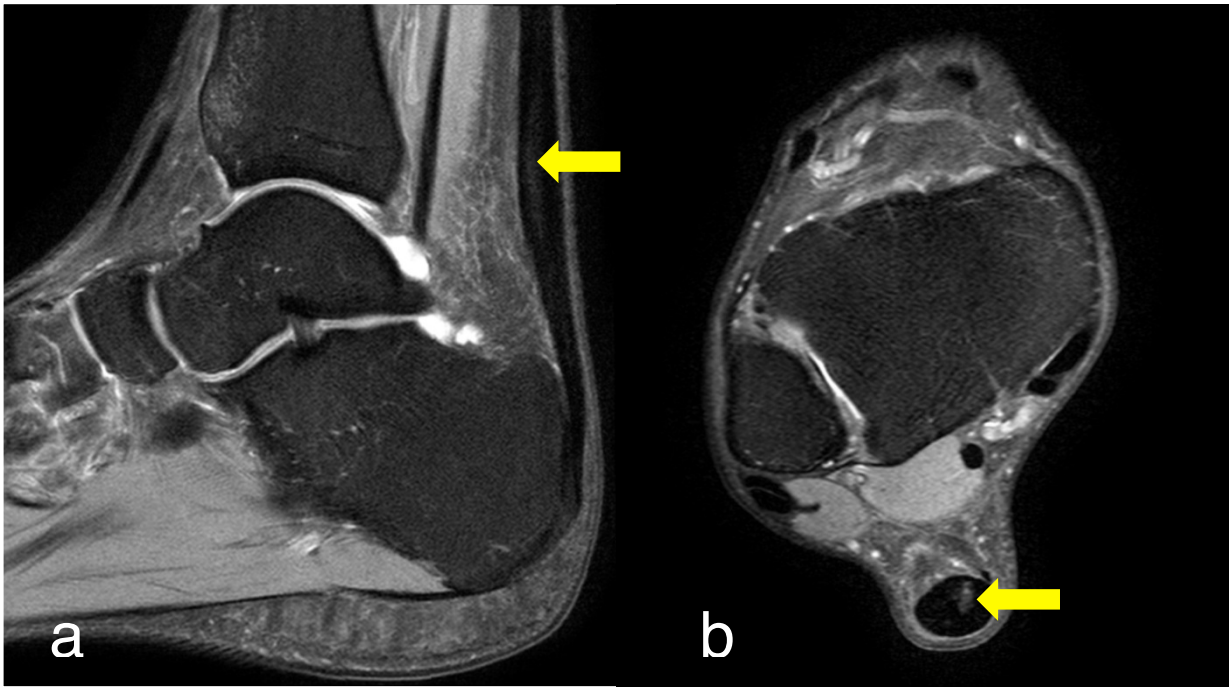

Figure 2.11- Magnetic resonance imaging of an abnormal Achilles tendon. (a) Longitudinal and (b) transverse image revealing increased signal intensity (yellow arrow) within the midsubstance of the tendon

2.6.2- Accuracy and sensitivity of conventional imaging modalities

The diagnosis of Achilles and patellar tendinopathy can be complex due the dissociation between pain and pathology, and there are few clinical diagnostic tests for tendinopathy. Numerous studies have investigated the accuracy (number of correct imaging diagnoses, both abnormal and normal imaging, divided by the total number of cases), sensitivity (number of correct abnormal imaging diagnoses divided by the total number of symptomatic cases) and specificity (number of correct normal imaging diagnoses divided by the total number of asymptomatic cases) of a number of imaging modalities in detecting clinical tendinopathy. These findings have consistently shown that both US and MR imaging have good-to-excellent accuracy (0.63-0.83 and 0.68-0.70, for US and MR imaging respectively), with varying sensitivity (0.68-0.87 and 0.50-0.57, for US and MR imaging respectively) and specificity (0.49-1.00 and 0.50-0.82, for US and MR imaging respectively) in detecting clinical Achilles and patellar tendinopathy (Kainberger et al, 1990; Khan et al, 2003; Nehrer et al, 1997; Warden et al, 2007). Most studies have dichotomised normal and abnormal tendons based on different criteria, and findings on imaging modalities with the general consensus being that a battery of criteria results in superior diagnostic accuracy (table 2.3). Critically, caution is required when interpreting the results of these studies as asymptomatic participants are frequently included and may over-estimate the accuracy and sensitivity of imaging in detecting clinical tendinopathy. Participant selection in these studies is critical and future studies need to reflect the use of imaging in the clinical setting (ie use of imaging to differentiate tendinopathy from other pain conditions in the region).

Table 2.3- Summary of the literature investigating the accuracy, sensitivity and specificity of ultrasound and magnetic resonance imaging in detecting clinical Achilles and patellar tendinopathy and tendon pathology

Study	Tendon	Participants	Reference/gold-standard criteria	Imaging	Imaging criteria used	Accuracy	Sensitivity	Specif
Imaging modalities compared to presence of pain								
Kainberger et al (1990)	Achilles	73 symptomatic (54 males, mean age 38 years) 24 healthy volunteers (17 males, mean age unknown)	Presence of Achilles tendon pain: Defined as pain at the posterior part of the heel on flexion movement of the foot	Grey scale ultrasound	Dichotomous classification (normal/abnormal) based on: Tendon thickness Hypoechoic signals Hyperechoic signals Echogenic spots with acoustic shadowing indicating calcification Peritendinous lesions	0.75	0.72	0.8
Khan et al (1997)	Patellar	15 patients with abnormal imaging (0 males, mean age 23.7). 23 tendons assessed 15 patients with normal imaging (0 males, mean age 24.4). 23 tendons assessed	Presence of patellar tendon pain: Past history of anterior knee pain related to activity that caused her to miss games or modify training.	Grey scale ultrasound	Dichotomous classification (normal/abnormal) based on: Hypoechoic region observed in both longitudinal and transverse scans Fusiform swelling	Data not provided to calculate accuracy	0.26	Data provi to calcu specif

Study	Tendon	Participants	Reference/gold-standard criteria	Imaging	Imaging criteria used	Accuracy	Sensitivity	Specif
Khan et al (2003)	Achilles	45 patients (27 males, mean age 42).	Presence of Achilles tendon pain: Pain on activity and morning stiffness	Grey scale ultrasound + colour and power Doppler Note: 85 tendon assessed 57 symptomatic 28 asymptomatic	Dichotomous classification (normal/abnormal) based on: Tendon thickening above 6mm Alterations in echogenicity Presence Doppler signal	0.66 Neither colour nor power Doppler enhanced accuracy	0.80 Neither colour nor power Doppler enhanced sensitivity	0.4

Study	Tendon	Participants	Reference/gold-standard criteria	Imaging	Imaging criteria used	Accuracy	Sensitivity	Specif
Warden et al (2007)	Patellar	30 symptomatic patients (20 males, mean age 27 years) 33 asymptomatic	Presence of patellar tendon pain: Pain on at least 1 of jumping/landing, running or changing direction Patellar tendon pain on palpation Symptoms sufficient to affect exercise activity for >6 months VISA score of <80	Magnetic resonance	Dichotomous classification (normal/abnormal) based on: Altered signal intensity within patellar tendon on both proton-density weighted and STIR* images	0.70	0.57	0.8
				Grey scale ultrasound	Dichotomous classification (normal/abnormal) based on: Presence of hypoechoic region Fusiform swelling	0.83	0.87	0.8
				Grey scale ultrasound with colour Doppler	Dichotomous classification (normal/abnormal) based on: Presence of vascularity	0.83	0.70	0.9

* Sagittal short inversion time inversion-recovery sequence

Study	Tendon	Participants	Reference/gold-standard criteria	Imaging	Imaging criteria used	Accuracy	Sensitivity	Specif
Nehrer et al (1997)	Achilles	36 symptomatic patients (26 males, mean age of 43) No control group	Presence of Achilles tendon pain: History of pain and localisation of pain on palpation	Grey scale ultrasound	Dichotomous classification (normal/abnormal) based on: Tendon thickening above 6mm Alterations in echogenicity	N/A No control group	0.58	No co gro
				Magnetic resonance Note: 50 tendon assessed 34 symptomatic 16 asymptomatic	Dichotomous classification (normal/abnormal) based on: Increased signal on FSTIR**	0.68	0.95	0.5

** Sagittal fast short tau inversion recovery

Study	Tendon	Participants	Reference/gold-standard criteria	Imaging	Imaging criteria used	Accuracy	Sensitivity	Specif
Imaging modalities compared to surgical findings								
Kalebo et al (1992)	Achilles	37 Achilles tendons from 30 patients chosen for surgical treatment (19 males, mean age 35)	Presence of partial tear on surgery	Grey scale ultrasound	Dichotomous classification (normal/abnormal) based on: Tendon discontinuity Focal sonolucencies Local thickening Local oedema	0.95	0.94	1.0
Paavola et al (1998)	Achilles	80 Achilles tendons from 79 patients undergoing for surgical treatment (60 males, mean age 42)	Surgically verified findings: Complete rupture Partial rupture Tendinosis Peritendinitis/tendinitis Insertitis Retrocalcaneal bursitis	Grey scale ultrasound	Normal/Abnormal Complete rupture Partial rupture Tendinosis Peritendinitis/tendinitis Insertitis Retrocalcaneal bursitis	No control group	0.96 0.96 0.73 0.43 0.83 0.60 0.75	No co gro

Most studies have compared imaging to a clinical diagnosis (Khan et al, 2003) with few studies comparing findings on imaging to surgical or histological observations. A number of studies have suggested variable sensitivity (0.33-1.00) and good-to-excellent accuracy (0.91-0.95) of imaging compared to surgical findings (degenerative tendon pathology, partial or full tears) (Kalebo et al, 1992; Movin et al, 1998; Smith et al, 2011; Vlychou et al, 2009; Westacott et al, 2011). Conversely, Adams et al (2010) reported subscapularis tears on MR imaging that were confirmed by arthroscopy in 16 patients, yet 28 patients with subscapularis tears identified arthroscopically had normal imaging on MR. Similar findings have been demonstrated in the Achilles tendon where US diagnosis correlated with surgical findings in ~80% of cases (Astrom et al, 1996; Paavola et al, 1998)(table 2.3), with US imaging limited in its ability to differentiate between partial Achilles rupture and local degenerative lesions (Paavola et al, 1998). Shalabi et al (2002) found that intratendinous signal observed on dynamic contrast-enhanced MR imaging correlated with the severity of histopathological changes. Histopathological comparison to US has shown that areas of hypoechogenecity contained significant tendon pathology, with areas adjacent subjectively described as 'normoechoic' also exhibiting pathological changes to a lesser extent (Movin et al, 1998). Caution is advised extrapolating the accuracy and sensitivity findings for tendon pathology as the majority of studies investigated partial tears that required surgery.

There is little consensus on which modality is best suited for imaging tendons. Westacott et al (2011) performed a systematic review and reported that the sensitivity of MR imaging for detecting gluteal tendon tears ranged from 0.33-1.00, whereas specificity remained high (0.92-1.00). US was found to be consistently more sensitive (0.79-1.00) than

MR imaging, suggesting that US may be used as first-line imaging modality for evaluating gluteal tendon tears. Similarly, previous investigations have revealed that US demonstrates higher sensitivity but lower specificity compared to MR imaging in detecting clinically symptomatic rotator cuff tendinopathy (Weinreb et al, 2014). However, MR imaging has been reported to be superior in the evaluation of various degenerative changes in the Achilles tendon yet still relies on subjective interpretation (Neuhold et al, 1992; Warden et al, 2007).

Conventional imaging modalities are criticised for their reliance on subjective interpretation of images (Klauser et al, 2010; van Schie et al, 2010). Research has been limited to classifying the tendon as abnormal or normal or a subjective grading score based on a myriad of pathological features and their severity. Quantification of tendon structure has been limited to measurements relating to tendon dimensions (antero-posterior diameter or cross-sectional area) and the percentage cross-sectional area of the hypoechoic lesion. Tendon research may be improved with new imaging techniques that address these limitations.

2.6.3- Ultrasound tissue characterisation

Ultrasound tissue characterisation (UTC) is a new imaging modality originally developed in the equine athlete to address some of the limitations previously outlined (van Schie et al, 2003). It utilises conventional US, which is mounted within a customised apparatus (fig 2.13). The tracking unit comprises an ultrasound mount, an acoustic stand-off pad and an in-built motor which automatically moves the transducer along the tracking unit. This configuration ensures that transducer tilt angle is standardised as it is a frequent source of variability and factor in the reduced repeatability of US. Along with improved

repeatability and standardisation of scanning parameters (ie transducer gain, depth and focal point), the tracking unit allows the creation of a 3-dimensional US image of the tendon. Transverse US images are captured every 0.2mm over a 12cm region (600 transverse images), with a 3-D US data block created by compounding these contiguous images. Based on this data block, the dynamism of the echopattern is quantified using dedicated algorithms that have been matched to histopathological specimens.

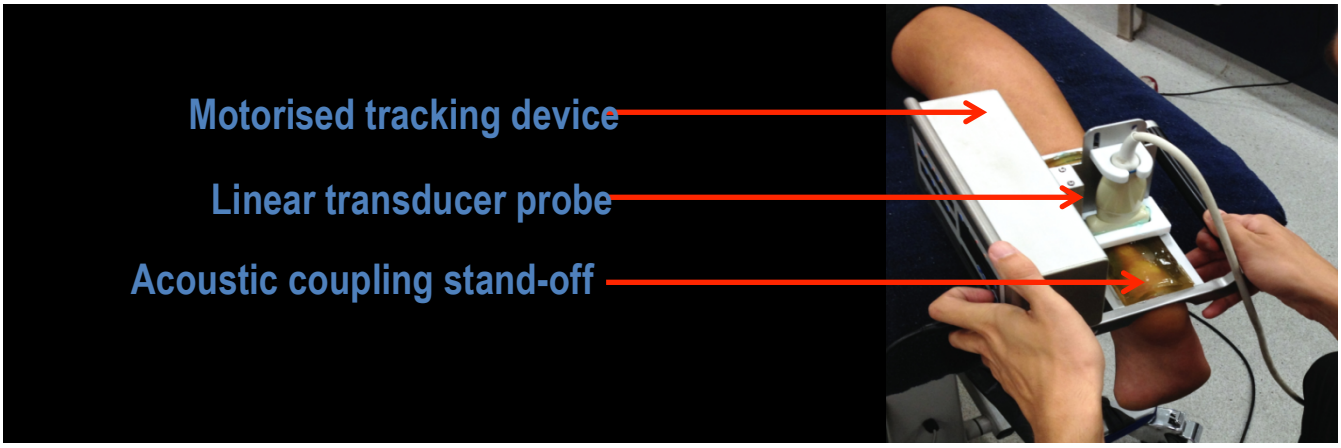

Figure 2.13- Ultrasound tissue characterisation tracking unit

These algorithms quantify the dynamics of grey levels of corresponding pixels in contiguous images. Based on their stability/degree of persistence, four different pixels types and resulting echo-types are discriminated and related to stages of ultrastructural integrity (fig 2.14 and 2.15). These echoes can be separated into two groups; echo-types I and II correspond with a single US reflection from one large structure where the resulting echo is stable over contiguous transverse images. Echo-types III and IV relate to echoes that are generated by multiple interfaces by more than one smaller structure and are characterised by a lack of stability over contiguous images (van Schie et al, 2010). Echo-type I corresponds with intact, continuous and aligned fibres and fasciculi, echo-type II represents less

continuous and/or more wavy fibres and fasciculi, echo-type III represent a mainly fibrillar matrix and echo-type IV represents complete disintegration, with tendon tissue replaced by an amorphous matrix and fluid (Cadby et al, 2013; van Schie et al, 2003). Previously, these echo-types have been matched to histopathological specimens in horses. Changes in the echopattern have been able to discriminate different tissue types (ie normal, necrotic, granulation and fibrotic tissue), yet basic grey level statistics could not (van Schie et al, 2000; van Schie et al, 2003).

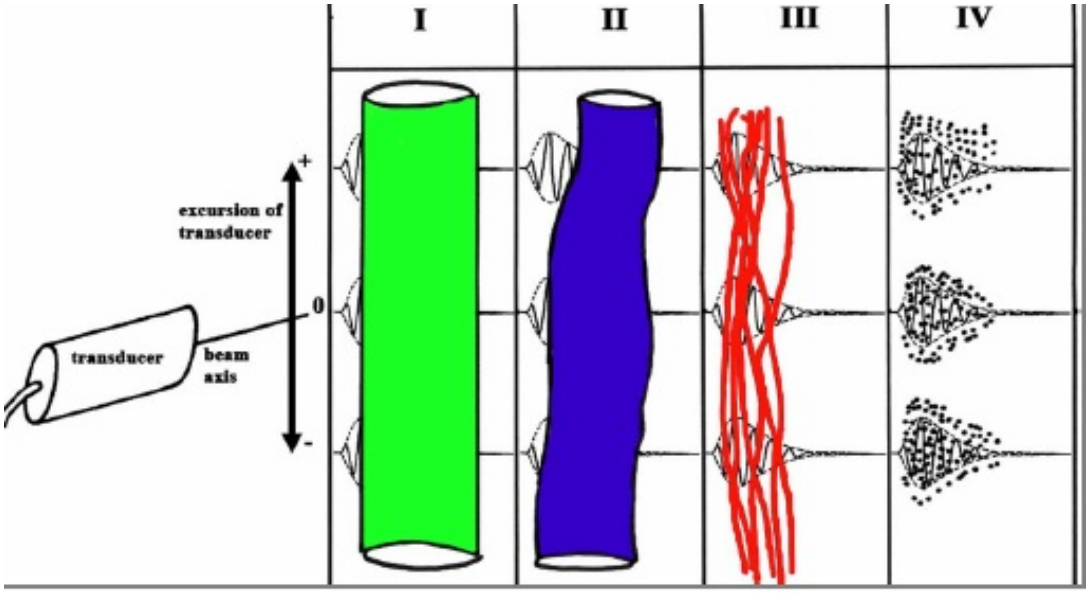

Figure 2.14- Schematic representation of the four echo-types discriminated by ultrasound tissue characterisation (adapted from van Schie et al (2010) with permission)

Van Schie et al (2000) collected superficial digital flexor tendons from horses with eight different stages of pathological tissue (Appendix E) with corresponding contiguous transverse US images collected using a similar customised scanning apparatus described above. When first-order grey level statistics were compared between the differing tissue types, late granulation tissue, early fibrotic tissue, late fibrotic tissue and scar tissue were not appreciably different from normal tissue. From this, semi-quantification of the stability

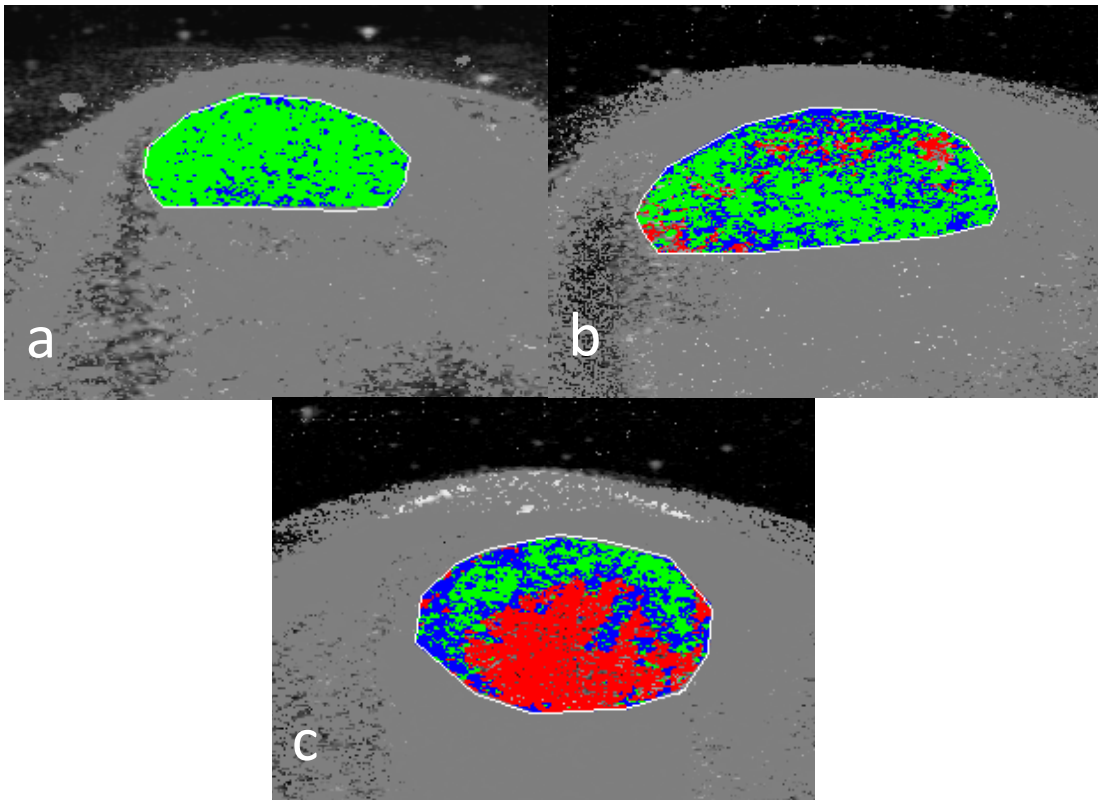

Figure 2.15- Ultrasound tissue characterisation of the Achilles tendon midsubstance. (a) Normal Achilles tendon characterised by a high proportion of echo-type I (green pixels) representing aligned tendon bundles. (b) Reactive tendon pathology due to tendon thickening and the presence of diffuse speckling of echo-type II (blue pixels) and echo-type III (red pixels). (c) Degenerative tendon pathology indicated by significant focal area of echo-type III (red pixels).

echopattern over contiguous images was performed for each tissue type and expressed as correlation (echo-type I), waviness (echo-type II) and entropy (echo-type III). Cluster analysis revealed that the differing tissues exhibited unique and distinct echopatterns suggesting quantification of the tendon based on the stability of pixel brightness more accurately represents the internal architecture of the tendon (van Schie et al, 2003). It is important to the note that these studies have some limitations due to the small sample size and that the aspects of the imaging technique differ to the current technique (refer to section 4.6 for further explanation). However, the overall components and concepts of UTC, including the quantification of the echo-types in these studies is similar to that used in this thesis.

To date, UTC has primarily been used to characterise an experimental model of tendon pathology in horses and monitor tendon structure in response to various treatments. Cadby et al (2013) created a surgical lesion within the SDFT in horses, where UTC detected a decrease in echo-type I and II coinciding with an increase in echo-type III and IV. Further investigations based on this surgical model reported a reduction in lesion length and size propagation with cast immobilisation compared to controls (David et al, 2012) and improved structure on UTC after platelet-rich plasma compared to saline injection (Bosch et al, 2011b).

Early research in humans utilising UTC investigated whether the UTC echopattern could differentiate the painful human Achilles tendon from the asymptomatic tendon. van Schie et al (2010) compared the UTC echopattern of 26 midsubstance Achilles tendinopathy patients to matched controls. The structure related echoes (echo-type I and II) were significantly decreased in the symptomatic tendon, coinciding with an increase in echo-type III and IV representing increase structural disorganisation. Based on an arbitrary threshold (75% echo-type I and II), an accuracy of 0.83 was reported, partly due to six asymptomatic tendons containing higher percentages of echo-type III and IV than the threshold (≥25%) (van Schie et al, 2010).

Similar to research performed in the horse, UTC has been used to test the efficacy of various treatments for Achilles tendinopathy in humans. de Vos et al (2012) demonstrated no change in tendon structure on UTC after a 16-week eccentric exercise protocol for midsubstance Achilles tendinopathy, despite improvements in pain and function. Contrary to research in the surgical model in horses, participants with midsubstance Achilles tendinopathy demonstrated similar improvements in tendon structure in response to

platelet-rich plasma as a placebo saline injection (de Jonge et al, 2011a). The reason for these conflicting findings may be due to differences in species, that the surgical experimental model is not a true representation of the naturally-occurring condition or differences in the exercise protocol that was performed concurrently (eccentric exercise in humans vs progressive increase in time walking in horses). While the ability to quantify the tendon echopattern makes UTC an ideal tool to test the efficacy of various treatments, its high repeatability may allow for the detection of subtle changes in structure in response to maximal load.

2.6.4- Detection of tendon response using imaging

Few studies have attempted to detect short- or medium-term changes in tendons in response to load using MR and US imaging. These modalities are reliant on measuring tendon dimensions (antero-posterior (AP) diameter or cross-sectional area (CSA)), visualisation of blood flow (Doppler signal on US) or subjective interpretation of fibre alignment (section 2.6.4). These outcome measures may not be sensitive enough to detect a short-term likely small response from the tendon to load.

2.6.4.1- Changes in tendon dimensions

Few studies have investigated changes in tendon dimensions in response to load or exercise in the medium-term (ie months). Conflicting results have been published in the literature where the dimensions of the normal tendon increase (Kongsgaard et al, 2007; Seynnes et al, 2009) or remain the same in response to load (Carroll et al, 2011; Reeves et al, 2003a; Standley et al, 2013)(table 2.4). These studies frequently use controlled heavy strength resistance training with no studies investigating changes in tendon dimensions in response to sport-specific training. These conflicting results may be due to the participants

recruited for these studies, with increases in tendon dimensions reported in the studies that investigated young (~20 years old) men, suggesting that the responsiveness of the tendon to mechanical load may be dependent on age and sex.

Similarly, studies investigating the response of the tendon to load in the short-term have reported conflicting results. Freund et al (2011) reported no changes in Achilles tendon thickness on MRI within 72 hours after a half or full marathon, with similar results reported in female badminton players immediately after a game (Fredberg et al, 2007). In contrast, Grigg et al (2009) reported an immediate decrease in AP diameter of the Achilles midsubstance tendon in in response to eccentric exercise, yet remained unchanged after concentric exercise. This short-term change in tendon thickness following eccentric exercise recovered to baseline levels ~2.5 hrs post-exercise. Similar findings have been reported in the patellar tendon in response to weighted squat exercise (Wearing et al, 2013b). The decrease in tendon thickness was proposed to be a loss of water produced by loss of the normal crimp pattern of tendon fibrils (Hannafin & Arnoczky, 1994; Lanir et al, 1988). With the straightening of tendon fibres a lateral compressive force is generated which forces fluid out of the interfibrillar space (Cheng & Screen, 2007; Lanir et al, 1988). Interestingly, the reduction in Achilles tendon thickness was not as large in individuals who were overweight or had a symptomatic Achilles tendon (Grigg et al, 2012; Wearing et al, 2013a). Similarly, this response was also demonstrated in the abnormal patellar tendon (Wearing et al, 2015). It was proposed that changes in the ECM (ie increases in large aggregating PGs such as aggrecan) may have altered matrix interactions with water and impeded fluid flow.

Table 2.4- Summary of literature investigating changes in tendon dimensions in response to various exercise/loading

Study	Imaging modality	Tendon	Participants	Exercise
Increase in tendon dimensions				
Kongsgaard et al (2007)	MRI	Patellar tendon	12 participants (All males) Mean age = 24.6	12-weeks, 3 sessions per week 8 x 10 at 70% 1RM with 3 mins rest *Contralateral side* 12-weeks, 3 sessions per week 36 x 10 at load equalling the amount of work to the work performed by the contralateral leg with 30 secs rest
Seynnes et al (2009)	Ultrasound	Patellar tendon	15 participants (All males) Mean age = 20	9-weeks, 3 sessions per week 10 x 4 at 80% of 1RM with 2 mins rest
No change in tendon dimensions				
Carroll et al (2011)	MRI	Patellar tendon	12 participants (8 males) Mean age = 67	12-weeks, 3 sessions per week 5 x 2 at mean of 74% 1RM with 2 mins rest
Reeves et al (2003a)	Ultrasound	Patellar tendon	9 participants (4 males) Mean age = 74	14-weeks, 3 sessions per week 10 x 2 at 80% 5RM with 3 mins rest
Standley et al (2013)	MRI	Patellar tendon	9 participants (No males) Mean age = 70	12-weeks, 3-4 sessions per week 20-45 mins on cycling ergometer at 60-80% of heart rate reserve.

Potential reasons for these conflicting results may be due to the differing imaging techniques used (ie MR versus US imaging), differing participants used and differences in when tendon dimensions were measured post-exercise.

2.6.4.2- Changes in tendon structure

Evaluating internal tendon structure, except for tendon dimensions, using conventional imaging has previously been limited to subjectively grading criteria based on changes in echogenicity. Prospective studies have evaluated the absence/presence of tendon abnormalities on imaging over the course of months and years. Giombini et al (2013) imaged the Achilles, patellar and quadriceps tendon in asymptomatic elite fencers over the course of three years. All tendons that were abnormal on US and power Doppler remained abnormal. A small percentage (1.45%) of normal tendons appeared abnormal on follow-up. Similarly, Malliaras et al (2010) reported that the normal and degenerative patellar tendon were likely to remain unchanged over the course of a volleyball season. These grading systems are based on gross changes in tendon structure and are likely not be sensitive enough to detect the potential subtle changes in response to load.

The development of new imaging techniques utilising existing imaging modalities have been developed to allow for semi-quantitative analysis of changes in internal architecture of the tendon. Off-resonant saturation MR imaging has been used in normal and tendinopathic Achilles to assess the amount of free and bound water within the tendon and found substantial increases in the off-resonance saturation ratio (OSR) in tendinopathic tendons (Grosse et al, 2013). Syha et al (2014) reported significant increases in OSR within the various segments of the Achilles tendon (ie calcaneal insertion, midsubstance and musculotendinous junction) after a 6.6 km running exercise. Interestingly, the same

alteration in free-bound water was not observed after a 15 minute rope skipping exercise with an increase in OSR only observed at the midsubstance less than that observed after the post-running exercise, suggesting that this response may be dependent to the amount of load. Unfortunately, specifics on exactly when these changes occurred post-exercise and the time-course of when they return to baseline levels were not described.

2.6.4.3- Changes in blood flow

Ultrasound with Doppler has been used clinically as a number of studies have shown increased Doppler activity, relating to the infiltration of blood vessels, in disorganised degenerative tendons (Cook et al, 2005; Divani et al, 2010; Ohberg et al, 2001). Boesen et al (2011) investigated changes in Doppler signal before and after two badminton games in the Achilles and patellar tendon. A significant increase in Doppler signal was observed at the patellar insertion of the dominant patellar tendon. All other sites scanned showed no significant differences in blood flow, yet a trend towards significance was reported. Interestingly, ~35% of all the tendon regions scanned contained abnormal blood flow with only 2 of the 14 players scanned having normal flow in all the tendons examined. It is difficult to ascertain from these results whether changes in blood flow occur in response to exercise only in the pathological tendons.

The use of real-time contrast-enhanced US (intravenous injection of contrast microbubbles where the first harmonic signals are detected with an US probe) has allowed detection and imaging of micro-vessels previously beyond the resolution of conventional colour Doppler. Pingel et al (2013a) described an increase in the microvascular volume immediately after a 1 hr run that returned to normal 24 hrs post-exercise. Individuals with Achilles tendinopathy had a higher microvascular volume at baseline, yet a similar temporal

sequence in response to exercise (Pingel et al, 2013a). It was proposed that these changes may play a role in the early stages of pathology, yet it is unclear how these changes in vascularity could lead to a loss of aligned tendon structure.

Changes in tendon mechanical properties have been investigated in response to load and are discussed in Appendix F.

2.7- Summary

There a numerous theories on the pathogenesis of tendon pathology due to our limited understanding of the critical factors in the development of tendon pathology and how tendon pathology progresses. With considerable evidence suggesting that the normal tendon responds to load, studies investigating changes in tendon structure in response to load may provide evidence on the pathoaetiology of tendon pathology. The studies of this PhD utilised a new imaging modality, ultrasound tissue characterisation (UTC), which can quantify the ultrasound echopattern and reliably quantify subtle changes in response to exercise. Similarly, understanding the features of the pathological tendon as quantified by UTC may provide evidence as to what are the critical factors in the pathogenesis of tendon pathology.

2.8- Aims and Hypothesis

The primary aim of this thesis is to investigate the features of the pathological tendon as detected by UTC to provide insight into the pathogenesis of tendon pathology. As load has been shown to be critical in the development of tendon pathology and tendinopathy (McCrory et al, 1999; Visnes & Bahr, 2013), understanding how the normal tendon responds to load may provide evidence as to how tendon transitions to becoming pathological. The specific aims of the thesis are:

1. To examine the nature and short-term changes (days) in the UTC echopattern in response to maximal exercise in the normal superficial digital flexor tendon of thoroughbred horses (Chapter 3).
2. To examine the nature and short-term changes (days) in the UTC echopattern in response to maximal exercise in the normal Achilles tendon of elite male Australian football players (Chapter 4).
3. To examine the nature and medium-term changes (months) in the UTC echopattern in response to pre-season training in the normal Achilles tendon of elite male Australian football players (Chapter 5).
4. To investigate the mean cross-sectional area of aligned fibrillar structure (echo-types I and II) and disorganised fibrillar structure (echo-types III and IV) as quantified by UTC in the pathological and normal Achilles and patellar tendon (Chapter 6).
5. To investigate the potential relationship between disorganised fibrillar structure echo-types III and IV) as quantified by UTC and tendon dimensions (AP diameter and total mean cross-sectional area (Chapter 6).

6. To examine tendon structure in the symptomatic and contralateral asymptomatic tendon in patients suffering from unilateral Achilles tendinopathy and a control group of normal tendons using UTC (Chapter 7).

The hypotheses of each study are outlined in each individual Chapter.

Chapter three
Tendon structure changes after maximal exercise in the thoroughbred horse: Use of ultrasound tissue characterisation to detect in vivo tendon response

Docking SI, Daffy J, van Schie HTM & Cook JL

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"The response of tendon cells to changing loading conditions has significant implications in unravelling the aetiopathogenesis of tendinopathy."

Arnoczky et al (2007)

3.1- Declaration for thesis Chapter three

In the case of Chapter three, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Tendon structure changes after maximal exercise in the thoroughbred horse: Use of ultrasound tissue characterisation to detect in vivo tendon response	70%

The following co-authors contributed to the work:

Name	**Nature of contribution**
Daffy, J	Study design and preparation of manuscript
Van Schie, H	Input into the methodology used and preparation of manuscript
Cook, J	Senior supervision of study design, data collection, statistical analysis and preparation of manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature		**Date**

Main Supervisor's Signature		**Date**

3.2- Abstract

Studies on the response of the superficial digital flexor tendon in the thoroughbred horse to mechanical stimuli have thus far been limited to *in vitro* studies in cell cultures and have focused primarily on gene expression of critical matrix proteins. It is difficult to translate these results to the equine athlete and the *in vivo* tendon. This study investigated changes in tendon structure in response to maximal exercise, using ultrasound tissue characterisation (UTC) to scan the superficial digital flexor tendon prior to, and after competitive racing. UTC uses contiguous transverse ultrasound images to assess the dynamics of the echopattern, which has a close relationship with changes in the 3-D ultra-structure of the tendon.

Using UTC, it was possible to detect subtle changes in the dynamics of the echopattern with a reduction in pixels that represent aligned and integer collagen tendon bundles on day 1 and 2 post-race when compared to pre-race ($p < 0.05$). The echopattern of these tendons returned to baseline on day 3. This change in echopattern was not seen in control horses. It can be concluded that the superficial digital flexor tendon responded to maximal exercise in the short-term that can be detected using UTC.

3.3- Introduction

The superficial digital flexor tendon (SDFT) of the horse accounts for the majority of tendon injuries – reducing performance, requiring prolonged recovery periods and predisposing to re-injury. Reported injury incidence ranges from 0.58 - 9.1 tendon injuries per 1000 race starts (Lam et al, 2007; Pinchbeck et al, 2004; Williams et al, 2001; Wilson & Robinson, 1996), however, these figures may be an under-estimation as they only account for injuries that occur during a race meeting. Kasashima et al (2004) reported that 11% of thoroughbreds suffered from a tendon injury in their career. This may also be an under-estimate as post mortem studies have reported pathological tendon lesions in 26.1% of the sample population (Webbon, 1977).

Horses with a tendon injury can require expensive interventions and up to 12 months to recover; even then, not all return to their previous level of racing (Fortier & Smith, 2008; Marr et al, 1993; Smith & Webbon, 2005). What predisposes some horses to SDFT injury is unknown and research into understanding how the tendon responds to mechanical stimuli is limited. Molecular biology techniques (eg cell culture, microdialysis) have demonstrated that the tendon responds to both loading and stress deprivation (Langberg et al, 2001; Lavagnino & Arnoczky, 2005; Skutek et al, 2001b). Research in both human and animal tendon indicates that changes in the expression of matrix proteins and enzymes, such as cytokines, matrix metalloproteinases, collagen and PGs, are critical to this process (Hosaka et al, 2005a; Langberg et al, 2001; Langberg et al, 1999b; Lavagnino & Arnoczky, 2005; Skutek et al, 2001b). However, it is difficult to translate these findings to how the *in situ* tendon reacts to mechanical stimuli.

Ultrasonography (US) is a valuable diagnostic tool for clinicians as it is non-invasive, portable and provides images of tendon structure. The size and structure of the tendon can be assessed qualitatively or (semi)quantitatively (Avella et al, 2009; Genovese et al, 1986; Smith et al, 1994) and echogenicity can be quantified with first-order grey level statistics. Ultrasonography has been used to assess the response of the tendon to long-term exercise. Avella et al (2009) scanned 263 event horses over two National Hunt seasons and reported no changes in cross sectional area (CSA) of the SDFT, with similar results observed in other studies (Birch et al, 1999). In contrast, Gillis et al (1993) performed analysis on first-order grey level statistics of ultrasound images and tracked echogenicity over a four-month training period; they noted a trend for decreased echogenicity possibly due to changes in the tendon structure and composition. This study used skeletally immature horses and its application to the adult equine athlete is questionable as mature SDFTs may have a decreased capacity to adapt to mechanical stimuli (Smith & Goodship, 2008; Stanley et al, 2007).

Comparison of echogenicity between serial US scans is difficult as minor changes in amplifier gain, transducer tilt and displacement affect the repeatability (van Schie et al, 2000; van Schie et al, 1999). Ultrasound also has limits of resolution, every US image is a mixture of structural reflections and interfering echoes: only relatively large structures, like secondary tendon bundles (fasciculi), generate reflections, while smaller entities, such as fibrils and cells, will result in interference, each with their specific dynamism in real-time US (van Schie & Bakker, 2000; van Schie et al, 2001). The dynamism of echopatterns over contiguous images is strongly related to changes in the 3-dimensional ultra-structural integrity of tendons, but this is not captured in still 2-dimensional US images.

Ultrasound Tissue Characterisation (UTC) was developed to address these limitations, with standardised instrumental settings and collection of transverse US images at even distances creates a 3-D ultrasound data-block. In this block, UTC-algorithms quantify the dynamics of echopatterns over contiguous images by intensity and distribution of relative grey levels of corresponding pixels. UTC is based on the close relationship between dynamics of echopatterns and 3-D ultra-structure of collagenous matrices with histomorphology of tendon tissue specimen as reference (van Schie et al, 2003). As a consequence of standardised data-collection and analysis routines, UTC has shown a high intra- and inter-observer reliability and subtle changes over serial scans can be detected with high reproducibility (van Schie et al, 2010). Previously, it has been used for monitoring the progression of tendon lesions and for objective evaluation of repair processes in response to various treatments (Bosch et al, 2011a; van Schie et al, 2009).

The aim of this study was to evaluate the short-term tendon reaction in response to maximal exercise (competitive flat racing) by quantifying tendon structure using UTC. We hypothesized that a tendon response to exercise will be detected by UTC when compared to a group of control horses.

3.4- Materials and methods

3.4.1- Horses

Thirteen thoroughbred horses (nine males, four females; mean ± SD age 3.8 ± 0.6 years, mean race starts 4.3± 3.1) currently in full race training were recruited from a single racing stable that were at the time of the study in full race training. These horses varied in previous race experience (mean ± SD race starts 4.3 ± 3.1) and had not participated in a race

in the week before the first scan (previous race start ranged from 1 to 37 weeks with three horses previously unraced). Horses had neither signs of lameness nor clinical/ultrasound signs of tendon pathology nor had any history of tendon injuries. Horses were involved in races at one of either two turf racecourses within Victoria under the control of Racing Victoria Limited. Horses in the race group competed over a race distance ranging from 1000-2085 m (mean ± SD distance 1482 m ± 326.5) in varying track conditions (7 horses on dry track condition, six horses on wet track conditions). Each horse was scanned using UTC no more than 8 hrs prior to racing and repeat scanned 1, 2 and 3 days post-race.

Five thoroughbred horses (3 male, 2 female; mean ± SD age 3.8 ± 1.1 years, mean ± SD race starts 4 ± 4.3), housed in the same racing stable but not racing over a week period and not partaking in training faster than a canter, were used as controls and UTC scanned daily over four days. Participation in a race prior to data collection ranged from 4 days to 72 weeks with one horse previously unraced. There was no significant difference between the race and control group in age or previous race starts using a Student's *t* test.

This study was conducted in compliance with NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and approved by the Monash University Animal Welfare Committee (Application number- SOBSB/2010/65)(Appendix G).

3.4.2- Ultrasound tissue characterisation

Structural integrity of the SDFT was quantified using UTC (van Schie et al, 2001; van Schie et al, 2003). The configuration consists of a linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000; Teratech) that is mounted in a tracking device with motor-drive and built-in acoustic-coupling stand-off (UTC Tracker, UTC Imaging). Prior to each scan, the palmar aspect of both forelimbs was shaved and coupling gel applied to ensure

maximum contact. The tracking device was then placed on the palmar aspect of the limb parallel to the long axis of the tendon, ensuring the mid-metacarpal portion of the tendon was located at the middle of the 12 cm scanning window of the tracking device (ie the transducer was positioned 6 cm proximal to the mid-metacarpal region). A clear US image of the SDFT was established and with standardized transducer tilt angle, gain, focus and depth, the ultrasound transducer moved automatically along the tendon's long axis, recording transverse images at even distances of 0.2 mm over a 12 cm scanning window. By compounding contiguous grey scale transverse images, a 3-D US data-block was created and dedicated UTC algorithms (UTC2010; UTC Imaging) quantified the dynamism of echopatterns by means of relative intensity and distribution of grey levels of corresponding pixels in contiguous images (over 25 images (4.8 mm)). Based on their stability/degree of persistence, four different pixel types and resulting echo-types were discriminated and related to stages of ultra-structural integrity.

This ultra-structural information is visualised tomographically in three planes of view and in 3-D and then quantified by means of the calculation of respective percentages of total pixels in the region of interest that is selected around the border of the SDFT (ROI). Each scan was checked to ensure no artefacts were present due to poor contact or movement and no signs of pathology on grey scale were evident (ie hypoechoic lesions). Three ROIs were selected around the contour of the SDFT at the mid-metacarpal region of the tendon over a distance of 1 cm. Contours were then interpolated between these defined ROIs allowing for the quantitative analysis of the echopattern over the 1 cm region. All scans and UTC analyses were performed by a single trained researcher (SID). The researcher was blinded to horse, limb and date of scan by saving scans as randomly

generated numbers prior to the analysis of UTC scans. Reliability of measures was determined by rescanning 10 limbs (inter-scan reliability) and re-measuring the echopattern within 10 scans (intra-scan reliability) on two occasions (table 3.1).

3.4.3- Statistical method

Data was analysed for normality; as the data was not normally distributed and because of the differences in group size, more conservative non-parametric tests were used. No Bonferroni adjustment was made as this is a pilot study and the alpha level was set at 0.05 (Perneger, 1998). Differences between limbs in race and control horses were analysed with related-samples Wilcoxon signed rank test. There were no differences between limbs in all horses, therefore further analysis was limited to the right SDFT to limit the possibility of Type I errors due to multiple statistical analyses (Appendix H). Differences in the proportion of all echo-types of the right SDFT between the race and control group on day 0 were examined with an independent-samples Mann-Whitney *U* test to determine differences between the two groups at baseline.

Differences in the median over the four day data collection were analysed using a related-samples Friedman's test for all four echo-types in the race and control groups. If a significant difference in the median was observed, post-hoc (Wilcoxon signed rank test) analysis was performed to identify differences between days 1, 2 and 3 and day 0.

3.5- Results

All tendons scanned prior to racing were normal on grey scale ultrasound and no changes in grey scale ultrasound were seen over the 3 days subsequent to the race. In particular, no focal hypoechoic lesions within the SDFT or clinical signs of tendon injury (ie

swelling, heat and pain on palpation) were observed throughout the data collection period in both the race and control group. Using UTC, no significant difference was observed between the race and control groups in the proportion of all echo-types on day 0 (p= 0.104, 0.587, 0.217, 0.374 for each echo-type respectively).

Changes were observed in the proportion of the echo-types I, II and III in the race group across the four days (p= 0.031, 0.001, 0.004 respectively). Echo-type IV did not vary significantly throughout the four day collection period (p= 0.903). In contrast, the control tendons showed no significant change over the four days for all four echo-types (p= 0.075, 0.468, 0.184, 0.552 respectively, table 3.1, fig 3.1).

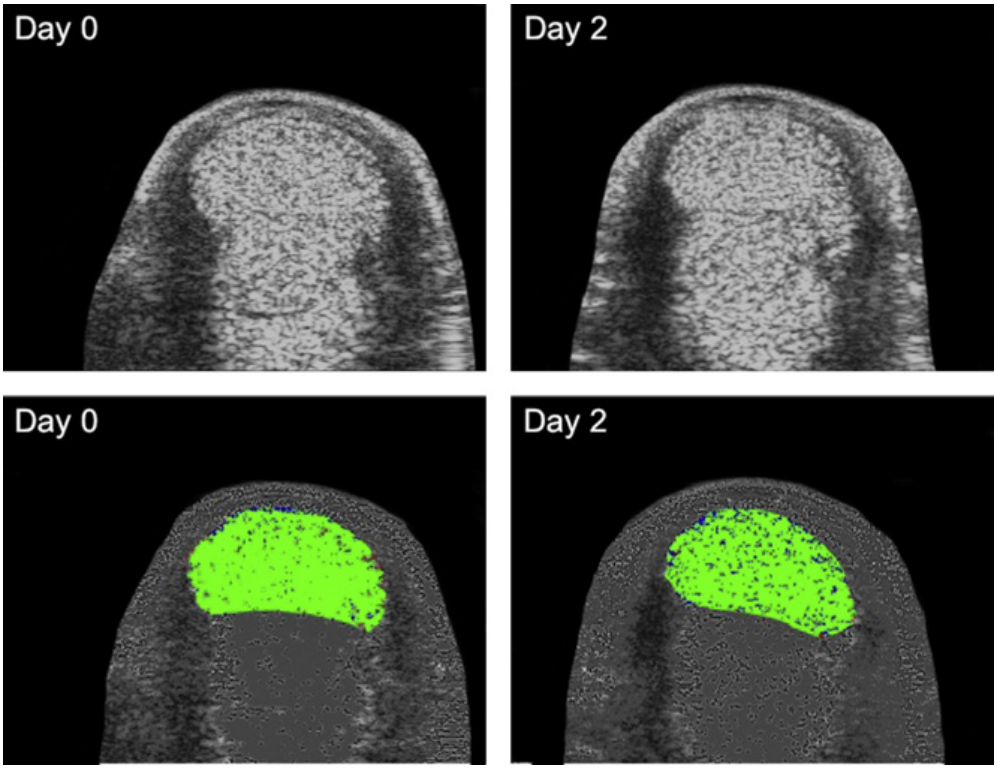

Figure 3.1- Grey scale transverse ultrasound images of the right SDFT, and UTC analysis of the same images, on days 0 and 2 of a horse in the race group. No hypoechoic lesions are seen on grey scale ultrasound on days 0 and 2. UTC analysis shows an increase of echo-type II (blue pixels) diffusely spread throughout the tendon on day 2 in comparison to day 0. Green = echo-type I, Blue = echo-type II, Red = echo-type III, Black = echo-type IV. (Note - Ultrasound images were cropped around the surface of the skin to remove background noise)

Table 3.1- Echopattern distribution of the right superficial digital flexor tendon.

		Race group (*n* =13)	Control tendons (*n* =5)		
Echo-type	**Time**	**Median (IQR)**	**Median (IQR)**	**Intra-scan ICC**	**Inter-scan ICC**
I	Day 0	93.9 (3.8)	91.3 (2.8)		
	Day 1	91.7 (5.3)*	91.3 (3.6)	0.80	0.72
	Day 2	90.2 (5.3)***	92.3 (2.2)		
	Day 3	92.2 (5.8)	93.3 (2.5)		
II	Day 0	2.2 (2.3)	2.9 (3.6)		
	Day 1	4.8 (2.8)**	3.7 (2.6)	0.96	0.84
	Day 2	4.7 (3.0)**	3.3 (1.9)		
	Day 3	2.4 (2.0)	2.0 (0.9)		
III	Day 0	0.4 (0.7)	0.8 (0.8)		
	Day 1	0.8 (0.9)	0.8 (0.4)	0.88	0.92
	Day 2	0.9 (0.8)***	0.7 (0.4)		
	Day 3	0.6 (0.6)	0.6 (0.3)		
IV	Day 0	2.8 (4.0)	5.1 (2.9)		
	Day 1	2.0 (4.0)	3.5 (2.2)	0.86	0.94
	Day 2	3.6 (3.1)	3.1 (2.9)		
	Day 3	3.3 (3.5)	4.1 (2.6)		

Relative proportion (interquartile range) of the right SDFT in race and control groups for all echo-types. Reliability interclass coefficients (ICC) within scan (intra-scan) and between scans (inter-scan) are within acceptable limits.

* p< 0.05, ** p< 0.01, *** p< 0.001 compared to day 0 within same group.

In the race group, a significant reduction in relative distribution of echo-type I (p= 0.033) was accompanied by a significant elevation in echo-type II on day 1 (p= 0.008), that remained at day 2 when compared to day 0 (p= 0.001 and 0.003 respectively). A non-significant trend was observed for echo-type III on day 1 (p= 0.064), but this echo-type was significantly elevated day 2 post-race (p= 0.001). The increased proportion of echo-type II and III were seen scattered throughout the tendon rather than located within a focal area (fig 1). All three echo-types that showed variation throughout the data collection period returned to baseline on day 3.

3.6- Discussion

As injury to the SDFT is an overuse injury, understanding how the tendon responds to exercise is important. Tendon injuries occur primarily during or after high intensity training or competitive racing (Singer et al, 2008) and the response of the *in situ* tendon to maximal exercise has not been investigated previously. In this study, significant differences in the echopattern of the SDFT were observed in the race group of horses. As mentioned, UTC quantifies the dynamics of echopatterns by means of relative intensity and distribution of grey levels of corresponding pixels in contiguous images. In other words, pixels with a high persistence of the relative brightness over 25 images, classified as echo-type I, can be related to collagen that is hierarchically organized into secondary tendon bundles (fasciculi) that run in parallel with the tendon long axis over distances of at least 4.8 mm (van Schie & Bakker, 2000). The significant reduction of echo-type I and corresponding elevation in echo-types II and III on days 1 and 2 post-race suggest a change in tendon ultra-structure as a result of participation in a competitive race. This change in echopattern was not seen in the control group and suggests that the change is in response to high-intensity mechanical

stimuli. As a practical implication when training, this study suggests that repeat maximal exercise should be avoided until the tendon has reached homeostasis, which appears to happen within 72 hrs. Exposing the tendon to maximal load within this 72 hrs period may predispose the tendon to injury as the tendon may not have recovered sufficiently from the previous loading bout.

The UTC algorithms were validated against pathological tendons that were exactly matched with the generated UTC-processed image. Echo-type I was generated by intact and aligned secondary tendon bundles (fasciculi), type II by less integer and/or more waving fasciculi, type III by mainly fibrillar matrix and type IV by mainly cellular matrix and/or free fluid. This study investigated tendons post-race and although histomorphological information of matching tendon specimens is lacking, it is reasonable that during the reaction to the race load an increase of echo-type II is indicative for (reversible) remodelling and/or swelling of secondary tendon bundles. Similarly, an increase of echo-type III is indicative of (reversible) loosening of the fibrillar matrix, most probably an increase in amount of ground substance inside the collagenous matrix without accumulation of free fluid (therefore no increase in echo-type IV is observed).

Further histological analysis is needed to determine the exact nature of the changes in ultra-structure and composition in the race group tendons. However, a recently proposed continuum of tendon pathology (Cook & Purdam, 2009) that suggests the tenocyte may drive the progression of tendinopathy, may provide a possible reason for the change in tendon ultra-structure in response to racing. This cell-driven pathogenesis of tendinopathy is in contrast to previous tendon pathology models that have suggested that micro-rupture within the collagen matrix is the primary histopathological event in the tendon matrix.

The cell-driven model proposed that a tenocyte driven reactive non-inflammatory response is the first response of the tendon to mechanical stimuli. The tenocytes respond by upregulating the production of large proteoglycans, such as aggrecan and versican, which bind water into the tendon matrix, thus increasing the total amount of ground substance. This diffuse thickening of the tendon is a short-term attempt to reduce tendon stress. This model was based on human clinical and basic science studies, but there is evidence for its application to equine tendon injuries.

Birch et al (1998) investigated the SDFT in horses with and without current or previous clinical signs of tendinopathy and showed no significant differences in collagen, percentage of type III collagen, collagen crosslinking and water content. However, glycosaminoglycan (GAG) content, the key water binding component of large proteoglycans, within the central portion of the pathological tendon was significantly higher compared to normal tendon (Birch et al, 1998). The authors suggested that as collagen content did not differ, increased collagen breakdown in the early stage of tendon injuries was unlikely. Support for this can be seen in horses suffering from the hereditary disorder called degenerative suspensory ligament desmitis that is characterised by the accumulation of proteoglycans (predominately aggrecan) in connective tissue (Halper et al, 2006; Plaas et al, 2011). It can be hypothesised from these findings that changes in GAG content within the ground substance can alter the structure of the tendon.

The echopattern of the race group tendons changed on days 1 and 2 post-race, followed by a return to baseline on day 3. Interestingly, aggrecan follows a similar pattern in its synthesis and catabolism. These large proteoglycans are upregulated rapidly with mRNA expression and protein synthesis increased at 24 hrs in response to mechanical load

(Robbins et al, 1997; Samiric et al, 2004b). Increases in ground substance can lead to separation of collagen fibrils while the structural integrity (ie parallel orientation of collagen fibres) of the tendon is not altered. Aggrecan also has a short half-life (1-2 days), providing a possible explanation to why the echopattern of the tendon returned to baseline on day 3 (Samiric et al, 2004b). As the echopattern was restored to baseline 3 days post-race, enzymatic breakdown and clearance of the ground substance is likely, rather than synthesis and integration of new collagen in response to microtears, which is a time-consuming process.

A number of previous studies have used US imaging to investigate changes in CSA and mean echogenicity of the SDFT over a long-term period (Avella et al, 2009; Birch et al, 1999; Gillis et al, 1995; Kasashima et al, 2002), these studies observed little change in CSA of the tendon. However, Gillis et al (1995) reported a non-significant increase in CSA and decrease in mean echogenicity of the SDFT over four months. It is difficult to make comparisons between this study and Gillis et al's findings as the previous study used skeletally immature thoroughbreds (5 two-year olds and 1 three-year old) and investigated long-term changes of the tendon (scanned twice weekly over four months) whereas the current study describes short-term response. It can also be suggested that the findings reported in Gillis et al's study does not represent the normal response of the tendon as two of the six horses used in the study developed a clinical tendon injury. To this date, all horses used in the current study have raced again (1 to 13 race starts) without any clinical signs of tendon injury suggesting the changes in ultrasound were a normal response, not early signs of tendon injury.

A non-significant trend was observed for change in echo-type I in the control horses. Despite these horses being restricted in their training intensity throughout the data collection period, they still were undertaking some exercise and subjecting the SDFT to tensile strain. This group was a pragmatic control group as it was not possible to control the training loads of these horses prior to the data collection period. These training loads prior to and during the data collection period may have been sufficient enough to elicit a response from some of these horses.

Recently, the concept of bilaterality of tendinopathy has been documented. Andersson et al (2011) showed increases in cellularity in the Achilles tendon of rabbits in both the exercised and contralateral unexercised limb. These bilateral changes were suggested to be central nervous system mediated as they were associated with increases in tenocyte expression of neurotransmitters such as substance P (Andersson et al, 2008). Similarly, the response in the race group of horses of this study was shown to be bilateral as no difference was seen between the two limbs over the data collection period. Despite there potentially being differences in the tensile strain imposed on left and right SDFT's depending on the gait cycle (races in this study were in an anti-clockwise direction, the right SDFT is often the non-lead tendon around the bends and may be subjected to larger tensile strains (McGuigan & Wilson, 2003)), tendon response may not be driven solely by mechanical stimuli, but by the tenocyte release of neurotransmitters and cytokines (TNF-α, IL-1α)(Hosaka et al, 2005a; Hosaka et al, 2002).

3.7- Conclusions

This paper showed a short-term response in the structural integrity of the *in situ* tendon in response to maximal exercise and demonstrated that the tendon responded maximally at 48 hrs post-race. Future studies should examine the exact nature of the changes in the extracellular changes responsible for the decrease in structural integrity post-race. Understanding these concepts may allow for the design of optimal exercise and training schedules and for screening methods to detect early or impending tendon injuries and hence reduce the impact of tendinopathy in the equine athlete.

3.8- Conflict of interest statement

Dr van Schie developed the UTC imaging used in this study and is a director of the imaging company. Dr van Schie provided support and guidance in the development of the methods and interpretation of the results. The data was collected and analysed without Dr van Schie's input and he did not influence the study findings in any way.

3.9- Acknowledgements

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Chapter four
Australian football players' Achilles tendon respond to game loads within two days: An ultrasound tissue characterisation (UTC) study.

Rosengarten SD, Cook JL, Bryant AL, Cordy JT, Daffy J & **Docking SI**

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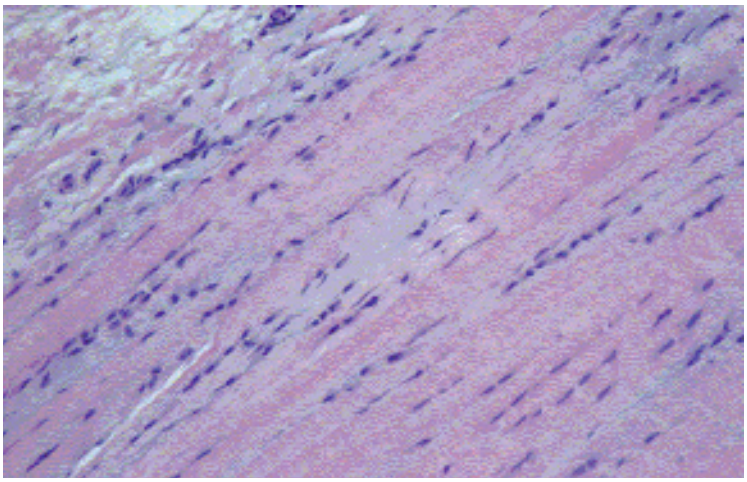

Adapted from Cook & Purdam (2009) with permission

"Tendons respond to load on a daily basis, well below what we can sense clinically, what the athlete experiences in terms of symptoms and what standard imaging devices can detect."

Cook & Purdam (2014)

4.1- Declaration for Thesis Chapter

In the case of Chapter four, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Australian football players' Achilles tendons respond to game loads within two days: An ultrasound tissue characterisation	65%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Rosengarten, S	Study design, data collection and preparation of manuscript
Cook, J	Study design, statistical analysis and preparation of manuscript
Bryant, A	Study design and preparation of manuscript
Cordy, J	Study design and preparation of manuscript
Daffy, J	Study design and preparation of manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature		**Date**

Main Supervisor's Signature		**Date**

4.2- Abstract

The Achilles tendon is a tissue that responds to mechanical loads at a molecular and cellular level. *In vitro* and *in vivo* studies have shown that expression of anabolic and/or catabolic proteins can change within hours of loading and return to baseline levels within 72 hours. These biochemical changes have not been correlated with changes in whole tendon structure on imaging. We examined the nature and temporal sequence of changes in Achilles tendon structure in response to competitive game loads in elite Australian football players.

Elite male Australian football players with no history of Achilles tendinopathy were recruited. Achilles tendon structure was quantified using Ultrasound Tissue Characterisation (UTC) imaging, a valid and reliable measure of intra-tendinous structure, the day prior to the match (Day 0), and then re-imaged on days 1, 2 and 4 post-game.

Of the 18 participants eligible for this study, 12 had no history of tendinopathy (NORM) and six had previous history of patellar or hamstring tendinopathy (TEN). Differences in baseline UTC echopattern were observed between the NORM and TEN groups, with the Achilles of the TEN group exhibiting an altered UTC echopattern, consistent with slightly disorganised tendon structure. In the NORM group, a significant reduction in echo-type I (normal tendon structure) was seen on day 2 ($p= 0.012$) that returned to baseline on day 4.

There was a transient change in UTC echopattern in the Achilles tendon as a result of an Australian football game in individuals without a history of lower limb tendinopathy.

4.3- Introduction

Tendinopathy – pain and dysfunction in the tendon - is a prevalent condition in athletes that is often associated with overload (Archambault et al, 1995; Scott et al, 2013). The pathoaetiology of tendon injury is currently unknown, with several theories differing in the proposed primary histopathological change (Abate et al, 2009; Cook & Purdam, 2009; Fu et al, 2010). Cross-sectional and prospective studies have demonstrated tendon pathology on imaging prior to the development of clinical symptoms (Cook et al, 1998; Giombini et al, 2013; Malliaras & Cook, 2006). Despite these observations, the response of the tendon to load and the early stages of tendinopathy are poorly understood.

Tendon is a mechanically responsive connective tissue that reacts to changes in load in both the short- (24-72hrs) and long-term (12wks-years)(Miller et al, 2005). Changes in tendon anabolic and catabolic cellular processes resulting in protein expression can be present the day after an acute bout of exercise (Maeda et al, 2010). These changes have been shown to return to normal levels as early as four days after exercise (Langberg et al, 2000), suggesting that transient changes exist in tendons as a response to loading. However, detecting these changes using conventional imaging have been limited due to a number of factors (eg spatial resolution, user-dependency, subjective measurements).

Few studies have reported short-term changes in imaging as a result of load. Previous studies showed conflicting results when using imaging as they have been confined to gross measurements of tendon dimension (cross-sectional area (CSA) or antero-posterior (AP) diameter). A number of studies have found no change or an increase in CSA in the short-term in response to increased loading (Arampatzis et al, 2010; Freund et al, 2012; Kubo et al, 2010). Short-term changes in AP diameter have been detected in the Achilles and

patellar after exercise; a decrease in AP diameter was reported immediately after exercise that returned to normal at 24 hrs (Grigg et al, 2012). These changes were proposed to be due to a loss of water within the tendon due to creep, however changes in intra-tendinous structure were not investigated.

Recently a novel imaging modality, Ultrasound Tissue Characterisation (UTC), utilising conventional B-mode ultrasound was introduced for the Achilles tendon (van Schie et al, 2010). UTC collects 600 contiguous transverse US images at 0.2mm intervals and renders a 3-dimensional image of the tendon allowing quantification of tendon structure by measuring the stability of pixel attributes (brightness) over the length of the scan. This scanning technique standardises operator-dependent parameters such as transducer tilt and angle, gain, and depth. van Schie et al (2010) reported high reproducibility with excellent intra- and inter- observer reliability (intraclass correlation coefficient > 0.92).

As UTC semi-quantifies tendon structure, it is an ideal research tool to objectively assess different rehabilitation modalities by monitoring tendon integrity. de Vos et al (2010) found that the midsubstance of the Achilles in tendinopathy patients did not improve on UTC after a 24 week eccentric loading program and injection therapy, despite improvements in clinical and functional outcomes. UTC has detected subtle structural changes in response to load in the superficial digital flexor tendon of the thoroughbred horse; an analogy for the Achilles tendon in humans (Docking et al, 2012).

This research aimed to investigate the presence and time course of short-term change in the Achilles tendon in response to load in elite Australian football players during an Australian Football League (AFL) game. Australian football is a fast paced sport involving repeated high intensity bursts of running (cumulative distances of up to 15 kilometres) as

well as jumping and cutting manoeuvres. Due to the physical demands of the sport, energy storage within the lower limb tendons are high. Based on previous studies (Docking et al, 2012; Langberg et al, 1999b), we hypothesised that a maximal bout of exercise (AFL game) will change the Achilles tendon echopattern on UTC imaging on days 1 and 2 with a return to baseline by day 4.

4.4- Methods

An entire elite male Australian football team (21 players, age 23.8 ± 3.01 years, mean ± SD) who were selected for an in-season competitive match were recruited for this study. Participants with a history of Achilles tendinopathy were excluded. Current or previous history of other lower limb tendinopathy (eg patellar tendinopathy), excluding the Achilles, was noted from club medical records. All participants were determined as fit and healthy by the club medical officer. The protocol was approved by the Monash University human ethics committee and all participants provided written informed consent prior to participating in the study (Appendix I).

4.4.1- UTC imaging

Achilles tendon structure was quantified using UTC imaging. UTC has been shown to be reliable in human tendons (van Schie et al, 2010), and in equine tendons has been validated against pathological specimens histologically (Bosch et al, 2011b; Docking et al, 2012; van Schie et al, 2009). A 7-10 MHz linear ultrasound transducer (SmartProbe 10L5, Terason 2000; Teratech) was mounted in a tracking device that moves the transducer automatically along the tendon's long axis recording transverse images at intervals of 0.2 mm over a 12 cm distance (600 axial images). The tracking device standardises transducer tilt, angle, gain, focus and depth.

Coupling gel was applied between skin, an integrated stand-off pad and the transducer to optimise contact prior to scanning the Achilles tendon. The participant was positioned standing on a raised level surface with the great toe and knee touching the wall in a standardised lunge position (fig 4.1). The tracking device was placed on the posterior surface of the Achilles region parallel to the long axis of the tendon. The transducer was aligned with the Achilles insertion at the posterior aspect of the calcaneus and the scan collected in a distal to proximal direction. All scans were taken by a single investigator (SID) who has four years' experience in UTC imaging.

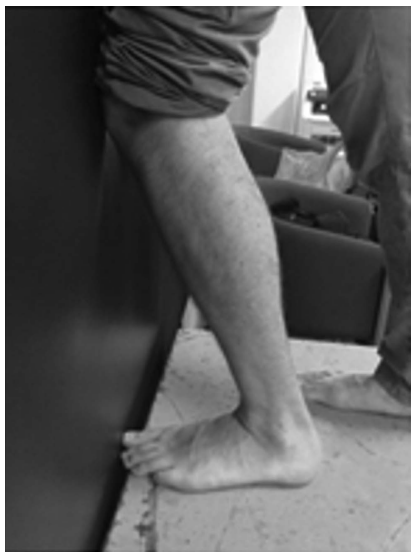

Figure 4.1- Participant in the lunge position with the ultrasound tissue characterisation tracking device placed over the Achilles tendon.

UTC analysis compounds consecutive transverse grey scale images creating a 3-D reconstruction (van Schie et al, 2000). Dedicated UTC algorithms (UTC2010; UTC imaging) quantify the dynamics of grey levels of corresponding pixels in contiguous images over a distance of 25 scans 4.8mm). Fundamental research revealed that the dynamics of grey levels were strongly related to the architecture and integrity of the histo-morphology of the tendon (van Schie et al, 2000). Four validated echo-types can be discriminated and related

to tendon integrity: echo-type I represents intact, continuous and aligned fibres and fasciculi, echo-type II represents less continuous and/or more wavy fibres and fasciculi, echo-type III represents a mainly fibrillar matrix and echo-type IV represents complete disintegration, with tendon tissue replaced by an amorphous matrix and fluid (refer to van Schie et al (2010) for further explanation of echo-types). These echo-types are quantified as relative percentages of the tendon in the region of interest (ROI).

Tendon structure was quantified by selecting a ROI, defined by the margin of the Achilles tendon in the transverse plane. A ROI was selected at the midsubstance of the Achilles (defined as 20mm proximal to the upper border of the calcaneus) and at regular intervals of 5mm over a distance of 20mm. The UTC software automatically interpolated contiguous ROI's between the defined ROI's selected by the investigator, creating a tendon volume in which the proportions of echo-types were quantified.

All participants were scanned with UTC imaging on the day prior to the match (Day 0), and then re-imaged on days 1, 2 and 4 post-game. The scan analysis was completed in a blinded fashion to participant, tendon pathology history, side and day of scan with scans assigned randomly generated numbers before being passed on for scan analysis. Results were decoded for statistical analysis by another investigator using the key.

4.4.2- Load monitoring

All players wore a GPS monitor (Catapult minimax, Catapult Sports) to monitor game loads (ie total distance covered for each player). Players did not undertake any other training in the four days of the study.

4.4.3- Intra-tester reliability

10 Achilles tendons in a sample population of similar demographics were repeat scanned to test repeated measure reliability. Tendon structure was quantified using the same methods as described above. Standard error of the measurement (SEM = standard deviation of population x √(1 – ICC)) was calculated. The ICC was calculated using a two-way mixed single measures (3,1) for absolute agreement between the repeated scans. The minimum detectable change (MDC = 1.96 x SEM x √2) was calculated with the results displayed in table 4.1.

4.4.4- Statistical analysis

Median and interquartile ranges were calculated for all four echo-types in both limbs on each of the four test days. Tests of normality using Kolmogorov–Smirnov test demonstrated that the data were not normally distributed. Hence, data were analysed using non-parametric statistics. No Bonferroni adjustment was made and was set at 0.05 as this is the first study looking at tendon response to load using this technology. It is reasonable to be comprehensive in the data analysis with the view of not missing potential important findings (Perneger, 1998).

At baseline, there was no difference between left and right Achilles for all participants in all four echo-types (Appendix J) hence, the left Achilles was randomly selected and subsequently used for all statistical tests to minimise the risk of Type 1 errors.

Difference in the overall echopattern was analysed in participants with and without a history of lower limb tendinopathy (excluding the Achilles) on day 0 using a Mann-Whitney U test. The median and IQR for all echo-types were plotted over the four days. In an attempt to reduce the number of statistical tests performed and minimise the risk of Type I errors, a

related samples Wilcoxon signed rank test was performed only if the changes in the echopattern on days 1, 2 and 4 were greater than the MDC in comparison to day 0. Linear regression modelling was used to identify potential interaction between distance covered in the game compared to changes in echopattern. Significance was set at $p< 0.05$, all analyses were conducted using statistical package, SPSS Version 20.0 (SPSS for Windows, SPSS Inc., Chicago, IL).

4.5- Results

Three participants were excluded based on a current/prior history of Achilles tendinopathy. Of the remaining 18 participants, six were noted to have had current or a previous history of lower limb tendinopathy excluding the Achilles (patellar or hamstring). All players were currently asymptomatic and were not taking any medication or interventions that may have had a systemic effect.

Baseline tendon structure was investigated in the TEN and NORM group as previous studies have suggested that tendinopathy at one site can alter lower limb biomechanics, which may overload other structures (Andersson et al, 2011; Edwards et al, 2012). Significant differences in baseline tendon structure on UTC imaging were observed between the TEN and NORM group. A significant increase in echo-type II was observed in the TEN group ($p= 0.032$), with no significant changes in echo-type I, III and IV ($p= 0.053$, 0.616, 0.053, respectively, table 4.1). As differences were observed between the two groups, changes in echopattern over the four days were analysed within group.

Table 4.1- Median and interquartile range (IQR) for all echo-types in the NORM and TEN group over the 4 days of the study

Echo-type	Day	NORM (n = 12) Median (IQR)	TEN (n = 6) Median (IQR)	Standard error of the measurement	Minimum detectable difference (%)
I	0	92.3 (2.77)	90.1 (2.39)		
	1	91.9 (4.34)	89.5 (3.83)	0.3	0.9
	2	90.8 (2.67)^*	89.6 (5.22)		
	4	91.6 (2.66)	92.1 (4.55)^		
II	0	6.9 (2.63)†	9.6 (2.20)†		
	1	7.1 (3.98)	7.8 (4.25)^	0.3	0.9
	2	8.6 (2.08)^*	10.0 (5.0)		
	4	7.3 (2.4)	7.5 (4.0)^		
III	0	0.22 (0.13)	0.14 (0.16)		
	1	0.24 (0.13)	0.41 (0.50)	0.09	0.3
	2	0.22 (0.15)	0.22 (0.17)		
	4	0.22 (0.20)	0.23 (0.32)		
IV	0	0.53 (0.40)	0.26 (0.27)		
	1	0.49 (0.49)	0.87 (3.19)^	0.21	0.6
	2	0.53 (0.30)	0.35 (0.28)		
	4	0.44 (0.61)	0.48 (0.59)		

†Significance difference between groups on day 0

^Greater than the MDC within group compared to day 0

*Significant difference within group compared to day 0

The NORM group demonstrated a change in echo-type I and II greater than the MDC on day 2 (table 4.1). Post-hoc analysis showed a significant reduction in echo-type I on day 2 in comparison to day 0 (p= 0.012), which returned to baseline on day 4 (p= 0.594, fig 4.2). This coincided with a significant increase in echo-type II (p= 0.013) on day 2 that returned to baseline on day 4 (p= 0.789, table 4.1). These changes in the UTC echopattern suggest that the Achilles midportion exhibited a loss of normal tendon structure two days post-maximal load that returned to baseline at day 4.

Changes in the echopattern on UTC were observed over the four days in the TEN group that were greater than the MDC (table 4.1). *Post-hoc* analysis was performed for all changes greater than the MDC with no significant differences observed when compared to baseline (table 4.1).

The NORM group (13.4 ± 1.1 km, median ± IQR) and TEN group (12.9 ± 2.0 km, median ± IQR) covered similar distances during the game, with no correlation observed between distance covered during the game and change of echopattern on day 2 in the NORM group (R = 0.297, 0.237, 0.293 & 0.921, for all echo-types respectively).

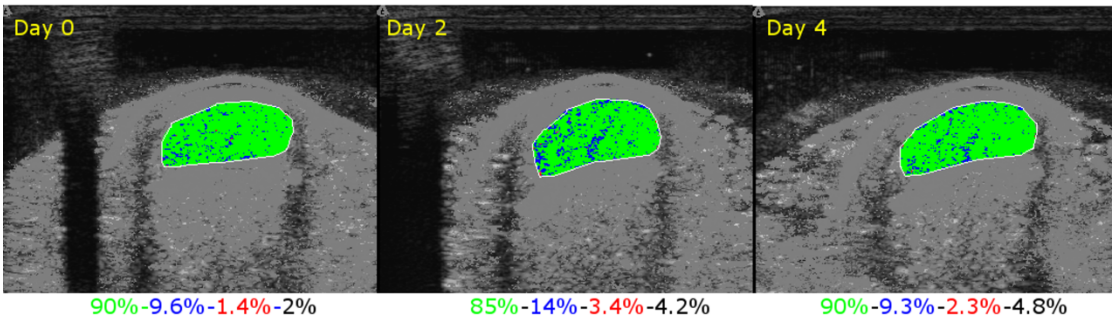

Figure 4.2- Example of a transverse ultrasound tissue characterisation (UTC) image of the Achilles tendon on days 0, 2 and 4. UTC analysis showing an increase of echo-type II diffusely spread throughout the tendon on day 2 in comparison to day 0. Green=echo-type I, blue=echo-type II, red=echo-type III, black=echo-type IV. (Note: ultrasound images were modified to remove all colour external to the Achilles tendon).

4.6- Discussion

This study demonstrated a loss of normal tendon structure on UTC imaging two days after maximal exercise in players with normal tendons and no history of tendinopathy. UTC imaging echo-types have been validated histologically against equine tendons, which have similar structural and compositional properties. The extrapolation of the UTC echo-types to structural features in human tendon is unknown (van Schie et al, 2000). As echo-type I indicates high stability in the grey scale pixels, it reflects homogeneity that corresponds with aligned tendon fibrils within the matrix. Echo-types II, III and IV represent increasing degrees of variability in grey scale pixel brightness. Slight separation and increased waviness of tendon fibrils are represented by echo-type II, disorganised fibrillar matrix correlated with echo-type III, with echo-type IV indicative of a more amorphous collagen structure.

A decrease in echo-type I coinciding with an increase in echo-type II observed on day 2 in comparison to baseline in the NORM group suggests that the normal tendon integrity has been negatively affected. The observation that echo-type III and IV were consistent across the four days indicates that there was no increase of disorganised fibrillar or amorphous matrix structure. As echo-type I and II returned to baseline by day 4, this observation supports the concept of a short-duration and fully reversible tendon response without loss of integrity of the collagen matrix. In response to high loads, this transient loss of normal tendon structure on UTC imaging may be considered normal. As it is not ethically possible to collect matching tendon biopsies from elite athletes for histology, the specific structural and extracellular matrix changes that lead to the alteration in echopattern observed in this study is, as yet, unknown.

The changes observed in the normal Achilles tendon over the course of the four days may be a result of a cell driven mechanism (Cook & Purdam, 2009), as the tenocyte is primarily responsible for remodelling of the extracellular matrix in response to mechanical stimuli (Banes et al, 1995). The continuum of pathogenesis, proposed by Cook & Purdam (2009), suggests that the tendon responds to overload by increasing the expression of large proteoglycans (aggrecan / versican). This results in an increase in bound water within the ground substance (Parkinson et al, 2010), which may lead to matrix disorganisation. Aggrecan has been shown to be upregulated rapidly (<24hrs), with 60% of this and other larger proteoglycans being degraded within three days (Parkinson et al, 2010; Robbins et al, 1997; Samiric et al). As the echopattern returns to baseline by day 4, deposition, enzymatic breakdown and clearance of proteoglycans may be responsible for the findings of this study.

Recent findings in a tendon explant model showed increases in bound water content and an increase in large-diameter tendon fibrils when the explant was incubated in phosphate-buffered saline, despite the dry weight of collagen remaining similar to the control (Screen et al, 2005b). This swelling of tendon fibres as well as the separation of tendon fibres was proposed to be mediated by proteoglycan interactions with bound water. Echo-type II is indicative for (reversible) tendon matrix remodelling and/or swelling of tendon fibres, suggesting that the findings of the current study may be explained by alterations in fibre diameter and separation due to increases in bound water and proteoglycan content.

The concept that the tendon is responsive to mechanical stimuli in a matter of days is not new and has been described by a number of authors. A number of studies have shown increases in markers of collagen synthesis and degradation collected from the tendon and

peritendinous space; these were elevated 24hrs post-exercise and remained elevated after 72hrs (Kjaer, 2004; Langberg et al, 1999b; Miller et al, 2005). However, alterations in the collagenous matrix are unlikely to explain the findings in this study as their restoration would take up to 11 weeks. As echo-types III and IV did not change over the four days post-exercise, it further supports that collagen fibre remodelling and integrity was not affected.

The reduction in normal Achilles tendon structure on UTC imaging at baseline between participants with (TEN) and without (NORM) a history of lower limb tendinopathy (other than Achilles tendons) may have substantial implications, yet needs further investigation. No participants in the TEN group had a history of Achilles symptoms or abnormality on imaging, yet exhibited a compromised Achilles tendon structure at baseline. Andersson et al(2011) described changes within the unloaded tendon (increase in cell number and neovascularisation) in a unilateral Achilles tendon overload model in rabbits. Unfortunately, the findings in other tendons were not reported. These changes coincided with increased expression of the neuropeptide, substance P, suggesting a systemic or central nervous system role in the development of pathology despite the absence of load. Systemic upregulation of substance P may lead to disorganisation of tendon structure and account for changes in the echopattern between the two groups. These changes may also be due to a genetic susceptibility (Schwellnus, 2013; September et al, 2007), changes in kinetic chain biomechanics adversely loading the Achilles (Edwards et al, 2012) or be related to systemic conditions (Gaida et al, 2009). Regardless of the mechanism, these findings warrant further investigation as confirmation and explanation of the underlying mechanism, that structural changes in one tendon affect other tendons of the body, is of considerable clinical importance if confirmed in further research.

This study demonstrated a transient change in Achilles tendon response on UTC imaging to a single game, however the effect of repeated tendon load over a season was not assessed. Without sufficient recovery time, repeat tendon loading may result in cumulative tendon matrix adaptation or degradation. Malliaras et al(2006) demonstrated that tendons can transition forward and back along the tendon pathology continuum (Cook & Purdam, 2009) over the course of a volleyball season. Monitoring tendons over a greater period of time with UTC may provide insight into variations in tendon morphology across the season. Furthermore it may lead to a better understanding of the 'point of no-return' where homeostatic capacities of the tendon are exceeded (Comin et al, 2013). Identifying critical changes in the tendon pathology continuum that lead to non-reversible degeneration may help in developing a prognostic criteria for decision making in the prevention and treatment of tendinopathy.

This pilot study had a number of limitations. A major limitation of this study was the small sample size in both groups. Also, this pragmatic study within the elite athletic environment did not allow standardised load across participants. In future, a standardised tendon specific loading protocol to induce a tendon response should be attempted. The clinical relevance of this tendon response needs to be determined, as the current study is unable to provide any insight whether this response is a pathological or adaptive response.

The results of this study need to be considered in the context of previous studies published using UTC (de Vos et al, 2012; de Vos et al, 2011; van Schie et al, 2010). The technique described in this study differs in five critical points; 1) the transducer is moved automatically by a mechanically-driven arm compared to manually; 2) the transducer captures images over 12cm compared to 9.6cm in previous studies; 3) the stability of

brightness over contiguous transverse images is quantified across 4.8mm versus 4.2mm; 4) UTC scans were performed in a standing lunge position in this study; 5) and the region of tendon selected for quantification. Caution is advised in using the UTC data from this study, or others, as reference data unless the scanning parameters are identical.

The sensitivity and reproducibility of UTC to detect and monitor these changes offers new insight into the pathophysiology of tendon responses and opens a pathway to investigate tendons and their response to loading. As understanding of the response of normal tendons to load improves, so will the opportunity to explore various pathological states, the processes involved in their pathogenesis and to design appropriate exercise protocols for prevention and rehabilitation.

4.7- Conclusion

This study showed the normal Achilles tendon may respond in the short-term (reduction in UTC echopattern corresponding with a loss of normal tendon structure) to exercise loads in individuals with no history of lower limb tendinopathy. These findings are preliminary in a small cohort, yet were similar to the results published in Docking et al (2012) who reported a similar tendon response on UTC in racehorses. A normal acute and transient response in tendons as a result of load application has been suggested from basic science research. This study suggests that UTC may be able to detect these changes in tendon structure in response to load, with the potential to elucidate the clinical relevance of this response. Future studies with greater numbers are indicated to further investigate the factors as well as studies to investigate long term effects of repeat application of bouts of exercise loads.

4.8- Acknowledgements

The author would like to thank the players and staff at the Carlton Football Club, Melbourne Australia for participation and assistance during this study.

4.9- Funding

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Chapter five
Achilles tendon structure improves on UTC imaging over a five month pre-season in elite Australian football players

Docking SI, Rosengarten S & Cook JL.

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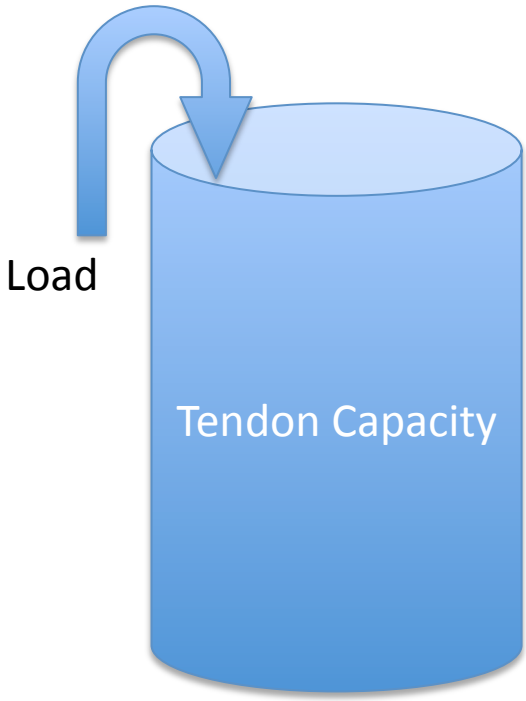


"Understanding how tendon tissue adapts to mechanical loading, and how and when this process is attenuated during tendinopathy, will contribute to our understanding of the pathogenesis of this condition."

Magnusson et al (2010)

5.1- Declaration for Thesis Chapter

In the case of Chapter four, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Achilles tendon structure improves on UTC imaging over a five month pre-season in elite Australian football players	70%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Rosengarten, S	Data collection and preparation of manuscript
Cook, J	Senior supervision of study design, statistical analysis and preparation of manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature		**Date**

Main Supervisor's Signature		**Date**

5.2- Abstract

Pre-season injuries are common and may be due to a re-introduction of training loads. Tendons are sensitive to changes in load, making them vulnerable to injury in the pre-season. This study investigated changes in Achilles tendon structure on ultrasound tissue characterisation (UTC) in response to cumulative training over the course of a five month pre-season in elite male Australian football players.

18 elite male Australian football players with no history of Achilles tendinopathy and normal Achilles tendons were recruited. The left Achilles tendon was scanned with UTC to quantify the stability of the echopattern. Participants were scanned at the start and completion of a five month pre-season.

15 players remained asymptomatic over the course of the pre-season. All four echo-types were significantly different at the end of the pre-season, with the overall echopattern suggesting an improvement in Achilles tendon structure. Three of the 18 participants developed Achilles tendon pain that coincided with a change in the UTC echopattern.

This study demonstrates that the UTC echopattern of the Achilles tendon improves over a five month pre-season training, representing increased fibrillar alignment. However, further investigation is needed to elucidate the intrinsic and extrinsic factors that are responsible for the development of pain and compromised UTC echopattern observed, potentially being targets for future injury prevention.

5.3- Introduction

Achilles tendinopathy in the elite athlete can affect the athlete's ability to participate in training and perform optimally, with severe cases leading to missed games (Gajhede-Knudsen et al, 2013). A critical factor in the development of Achilles tendinopathy is a high volume of Achilles tendon load, with middle- to long-distance runners 31 times more likely to develop Achilles tendinopathy before the age of 45 compared to a control group (healthy population)(Kujala et al, 2005). Magnusson et al (2010) proposed that the association between load and tendinopathy may be due to the tendon responding negatively to load and altering the extracellular matrix and structure of the tendon. However, tendons can respond positively to load depending on the type and frequency of load (Kubo et al, 2006).

Connective tissues can respond positively or negatively to mechanical stimuli. Frost (1987) proposed that bone strains above a certain point increased cortical bone while bone strains below a certain level decreased cortical bone. Tendon cells responded positively to static tension *in vitro* but a release from tension results in a catabolic state within the matrix (Lavagnino & Arnoczky, 2005). An anabolic state was evident when tension was re-applied to the matrix. Tenocytes have the ability to detect the presence or absence of mechanical stimuli and respond accordingly, however this has primarily been investigated in tissue or cell cultures.

Various imaging modalities have been used to investigate tendon response to load. Ultrasound or MR imaging have demonstrated changes in tendon size (AP diameter or tendon volume)(Grigg et al, 2009,2012; Syha et al, 2014; Wearing et al, 2013b), micro-vascular volume (Pingel et al, 2013b), and hydration level(Syha et al, 2014) in response to maximal exercise. Changes in tendon structure have previously been reported in-season

(Gisslen & Alfredson, 2005; Malliaras et al, 2006; Malliaras et al, 2010), however these findings have been limited to subjective interpretation of pathological changes on imaging with little ability to detect subtle changes that may represent adaptation or early pathological changes.

Ultrasound tissue characterisation (UTC) uses conventional ultrasound to construct a 3-dimensional image of the tendon after capturing 600 transverse images over a 12cm region (van Schie et al, 2010). Dedicated algorithms quantify the stability of pixel brightness over multiple transverse images into four echo-types, which reflect the structural integrity of the tendon. Echo-type I corresponds with high stability in grey scale pixels over contiguous images, where this homogeneity corresponds with aligned tendon fibrils within the matrix. Where there is increasing degrees of variability in grey scale pixels brightness, the tendon is classified as echo-type II, III or IV. Echo-type II represents slight separation and increased waviness of tendon fibrils, disorganised fibrillar matrix correlates with echo-type III, with echo-type IV indicative of a more amorphous structure (van Schie et al, 2010). These echo-types have previously been compared to histopathological specimens in horses (van Schie et al, 2003). Previously, transient changes in the UTC echopattern have been demonstrated in the short-term in response to one bout of maximal exercise. An altered UTC echopattern consistent with slightly disorganised structure within the Achilles tendon was observed two days after a competitive Australian football match that returned to baseline on day four (Rosengarten et al, 2015). The ability of UTC to quantify tendon structure, its high repeatability (based on minimum detectable differences and intra-class correlations)(Docking et al, 2014; van Schie et al, 2010) and ability to detect responses to load makes it an ideal tool for this study.

A high proportion of Achilles problems occur in the pre-season, possibly the result of the repeated high tendon loads during this period (Woods et al, 2002). This study aimed to investigate whether structure of the normal pain-free Achilles tendon, as quantified by UTC, changed over the course of an elite Australian football pre-season. Previous studies in this population have shown that the pre-season involves high training loads, where players on average cover over 10km per session and average training loads where greater compared to in-season (ie distance, distance covered above players aerobic threshold, sprint distance and an arbitrary measure of accelerations, decelerations and change of direction)(Colby et al, 2014). As Achilles tendinopathy has a higher prevalence in the pre-season and tendinopathy is associated with structural disorganisation, we hypothesised that the UTC echopattern would change negatively over the course of the pre-season.

5.4- Material and methods

Senior players (n = 38) from a single elite male Australian football team were invited to participate in this study. Players with current, or history of, pain in either Achilles or any other lower limb tendinopathy were excluded. Players were also excluded if they had any other injury that precluded them from participating fully in pre-season training at any point. Participants were assessed over the course of the five month pre-season (November to March) for the development of Achilles tendon pain by a trained sports physiotherapist (SR). Achilles tendon pain was defined as localised pain over the Achilles that was aggravated with loading of the Achilles tendon (ie sprinting, acceleration and decelerations) that lasted more than a week. The pre-season was selected as previous research in elite Australian football players has shown that average training loads are greater in this period compared

to in-season. The study protocol was approved by the Monash University human ethics committee and all participants provided written informed consent (Appendix I).

5.4.1- UTC imaging

A 5-10 MHz linear ultrasound transducer (SmartProbe 10L5, Terason 2000; Teratech) was mounted within a customised tracking device (UTC tracker; UTC imaging) allowing for transducer tilt, gain, focus and depth to be standardised (focus = 1.3cm, depth = 3cm). As part of the tracking device, a built-in motor drive automatically moved the transducer over the length of the tendon. Acoustic coupling gel was applied to the acoustic stand-off pad of the tracker to ensure maximum contact between the skin and transducer. Participants were scanned using UTC in a standardised position where the participant was positioned standing on a raised platform with the great toe and knee touching the wall in a standardised lunge position (Docking et al, 2014; Rosengarten et al, 2015). The tracking device was placed over the posterior aspect of the calcaneus perpendicular to the long-axis of the tendon. Once in a suitable position with no air bubbles between the skin and stand-off pad, the transducer travelled proximally capturing a transverse US image every 0.2mm over a scanning window of 12cm.

These contiguous transverse US images were compounded and a 3-D data block was generated. Tendon structure was quantified by dedicated UTC algorithms that assess the echopattern by means of relative intensity and stability of brightness of corresponding pixels over 25 transverse images (4.8mm). Four echo-types were generated based on the stability of the echopattern, which have been extensively described (van Schie et al, 2010).

Tendon structure was quantified by manually selecting a region of interest (ROI) around the margin of the Achilles tendon in the transverse plane. Regions of interest were

contoured at regular intervals no greater than 5mm over the entire length of the tendon (defined as the disappearance of the calcaneus to the musculotendinous junction). Contours were then automatically interpolated between the defined ROI's generating a tendon volume from which the proportion of echo-types were quantified. The four echo-types were quantified as a relative percentage over the length of the tendon.

The left Achilles tendon was arbitrarily selected prior to all testing and analysis. Participants were scanned with UTC in the first week of pre-season and in the last week of pre-season (defined as the week before the first match of the Australian Football League season). Scan analysis was performed in a blinded fashion to participant, date of scan and the development of pain by assigning each scan randomly generated numbers by another investigator. All scans and data analysis were performed by a single investigator with three years' experience (SID).

All data were analysed using statistical software (SPSS for Windows V.20.0, SPSS Inc, Chicago, Illinois, USA). As data were not normally distributed, a related-sample Wilcoxon signed rank test was performed investigating differences between the start and end of pre-season for each echo-type. The minimum detectable difference using the same technique for the same experienced investigator have previously been calculated and was used for this study (2.5%, 2.7%, 0.3% and 0.8% for the four echo-type, respectively)(Docking et al, 2014).

5.5- Results

Eleven participants were excluded due to a current or previous history of Achilles or any other lower limb tendinopathy, with a further nine excluded due to being unable to fully participate in pre-season training at some point. Eighteen participants (age (mean ± SD) 23.8 ± 3.6 years) were eligible for participation in this study. All participants had no Achilles

tendon pathology, defined as tendon thickening and the presence of a hypoechoic region, at baseline in either tendon.

Fifteen players remained asymptomatic over the course of the pre-season with significant differences in all echo-types observed at the end of the pre-season compared to the start (p= 0.001, 0.001, 0.001, 0.003, for each echo-type respectively, table 5.1). An increase in echo-type I coincided with a significant decrease in the remaining echo-types suggesting improvement in Achilles tendon structure. Differences in the echo-types were greater than the reported minimum detectable difference, except for echo-type IV.

Three players (age (mean ± SD) 25.3±2.3 years) developed symptoms in their left Achilles tendon during the pre-season. One of the players developed symptoms approximately two months into the pre-season, with continued pain and dysfunction for the rest of the season. The other two players developed symptoms three months into the pre-season, which resolved two weeks later with appropriate load management. Due to the small sample size, inferential statistics were not able to be performed on this group. Subjectively, age, seasons played at AFL level or baseline UTC echopattern did not appear to be different between participants who developed symptoms and those that remained asymptomatic. Interestingly, the UTC echopattern between the start and end of the pre-season did not appear to be substantially different and within the report minimum detectable difference (table 5.1). However, at the time of symptoms all three participants exhibited a UTC echopattern that was compromised and greater than the minimum detectable difference compared to their baseline scans (fig 5.1). The UTC echopattern of the participant with a focal area of disorganisation remained compromised, where in the participants with diffuse speckling their UTC echopattern resolved (fig 5.1).

None of the 18 included participants developed right Achilles tendon pain during the pre-season.

Table 5.1- Relative proportion for all four UTC echo-types of the Achilles tendon at the start and end of the pre-season.

	Non-painful group (n=15)		Painful group (n=3)	
	Start of pre-season	**End of pre-season**	**Start of pre-season**	**End of pre-season**
Echo-type I (%)	83.2 (78.8 - 91.0)	89.3 (82.9 - 93.4)*#	82.5 (81.0 - 91.0)	83.3 (77.6 - 92.0)
Echo-type II (%)	13.9 (7.9 - 18.5)	9.4 (5.5 - 15.2)*#	13.4 (8.1 - 16.9)	12.0 (6.6 - 15.3)
Echo-type III (%)	0.7 (0.2 - 1.3)	0.3 (0.1 - 0.8)*#	0.7 (0.3 - 1.4)	0.6 (0.4 - 3.2)
Echo-type IV (%)	1.2 (0.5 - 2.6)	0.9 (0.2 - 1.9)*	1.4 (0.6 - 2.8)	1.0 (0.8 - 7.2)

Median and min-max reported

*p≤0.05 compared to the start of pre-season within group

#Difference is greater than the MDC

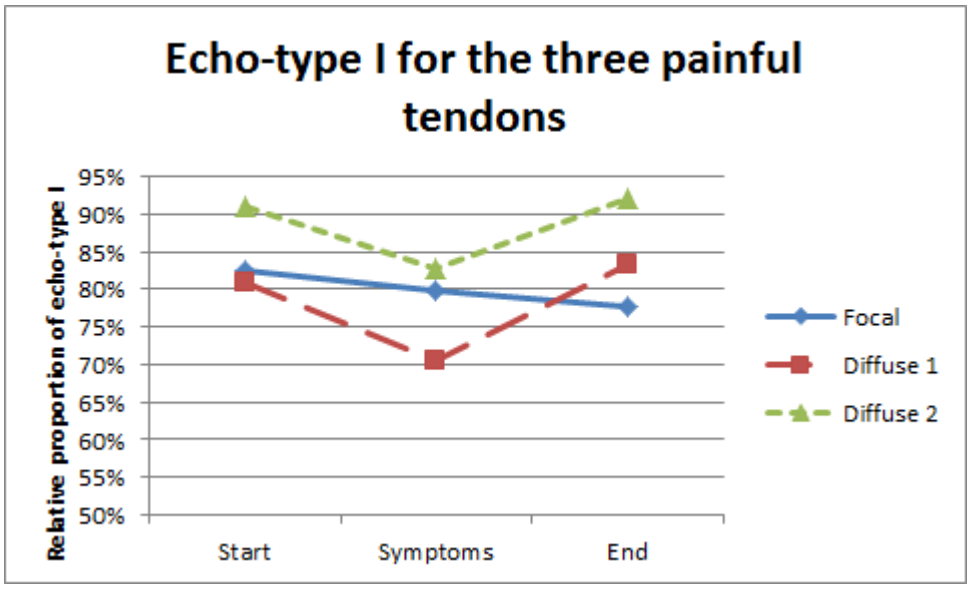


Figure 5.1- The relative percentage of echo-type I, indicating intact and aligned tendon bundles, for the three participants that developed Achilles tendon symptoms. Both participants with diffuse changes (red and green dotted lines) showed a decrease in echo-type I greater than the MDD at the time of symptoms, which resolved by the end of the pre-season. The participant with a focal area of disorganisation (blue solid line) progressively worsened from the start to the end of the pre-season.

5.6- Discussion

This study showed that the UTC echopattern of the pain-free Achilles tendon improved over a pre-season in elite male Australian football players. This result was contrary to our hypothesis that the UTC echopattern would deteriorate due to the high loads on the tendon during the pre-season after a period of relative unloading in the off-season. However, three out of 18 participants developed symptoms during the pre-season that coincided with a compromised UTC echopattern consistent with disorganised tendon structure. This study demonstrates the paradoxical nature of load, where loading of the tendon during pre-season training can elicit differing responses and changes in the UTC echopattern.

The significant increase in echo-type I coinciding with a decrease in the other echo-types in the asymptomatic players suggests that an improvement in tendon structure occurred. Echo-type I indicates high stability in the grey scale pixels over multiple transverse scans representing intact and aligned tendon bundles (van Schie et al, 2010). The increase in echo-type I over the pre-season suggests an increase in the proportion of the tendon that contains intact and aligned tendon. Whether this is due to collagen deposition and remodelling or changes in the non-collagenous matrix is unknown.

Increases in collagen synthesis markers have been reported within the tendon proper and the peritendinous space suggesting an increase in collagen turnover and metabolic activity (Langberg et al, 2001; Miller et al, 2005). However, recent evidence measuring ^{14}C content within the tendon demonstrated that collagen turnover is minimal after puberty (Heinemeier et al, 2013). The increase in collagen synthesis markers after load in previous studies may reflect increased metabolic activity of the tenocytes with increases

in procollagen occurring without the incorporation of new tendon fibrils into the matrix (Kjaer, 2004). In the current study, improvements in the UTC echopattern may not be due collagen remodelling or deposition of new tendon fibrils. Potential changes in the non-collagenous proteins, such as proteoglycans, and water content may effect the alignment of tendon fascicles (Screen et al, 2005b; Thorpe et al, 2013).

To date, UTC has been used to characterise a surgically-induced tendinopathy in the horse (Cadby et al, 2013), assess the diagnostic accuracy in detecting Achilles tendinopathy in humans (van Schie et al, 2010), assess the efficacy of various treatments (platelet-rich plasma and eccentric loading)(de Jonge et al, 2011a; de Vos et al, 2012; de Vos et al, 2011) and demonstrate that the asymptomatic Achilles tendon is structurally compromised in patients with unilateral tendon pain (Docking et al, 2014). Recently, short-term transient changes in the UTC echopattern have been demonstrated in the equine and human athlete (Docking et al, 2012; Rosengarten et al, 2015). These studies have shown an altered UTC echopattern, consistent with a slightly disorganised tissue structure, two days post-maximal exercise that returned to baseline 72-96 hours post-exercise. From these studies it is unclear whether the observed change in UTC echopattern was a positive/adaptive response or a negative/early pathological response from the tendon. The current study focused on changes in the UTC echopattern over the course of five months during pre-season training. While an improvement in the UTC echopattern was observed, the data cannot definitively state that these tendons have adapted and have increased their load tolerance. Further research is needed to determine if these changes in the UTC echopattern effect the long-term health of the tendon.

The continuum of tendon pathology was developed to explain the pathoaetiology of tendon pathology with a focus on how tendons respond to load (Cook & Purdam, 2009). The continuum model highlights that tendons adapt positively to optimal load (fig 5.2). From this study, it is unclear whether the improved UTC echopattern in the athletes who remained pain-free fall into the adaptive pathway of the continuum as there is little evidence that the tendon has increased its load tolerance. As these athletes were undertaking high training loads and remained asymptomatic over the course of the entire season, further research may show that these tendons are more load tolerant and have adapted positively.

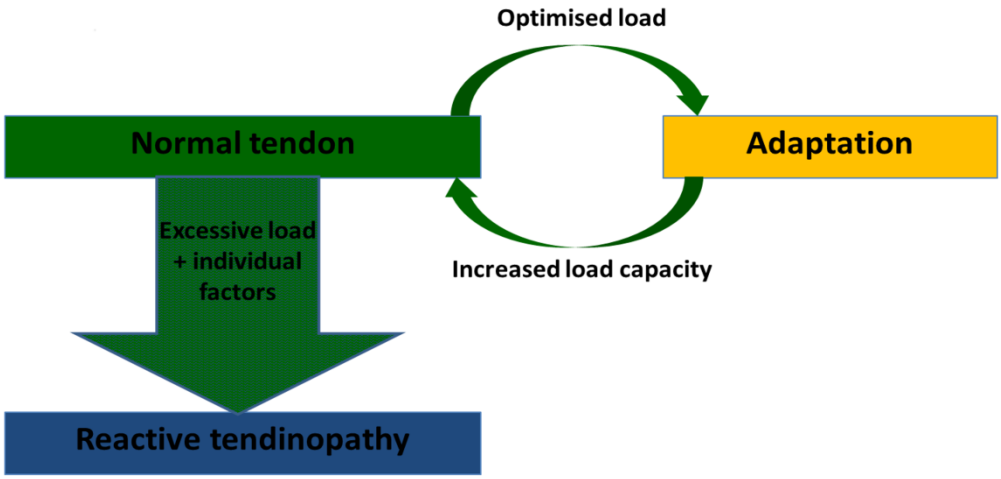


Figure 5.2- Aspect of the continuum of tendon pathology by Cook & Purdam (2009). This highlights how load can effect the tendon in a positive or negative way. With optimised load, tendon adaptation may occur resulting in a stronger more load tolerant tendon. Excessively loading the tendon may result in a negative response where the tendon transitions into a reactive tendinopathy. This aspect of the continuum appears to be supported by the current study

While athletes who remained pain-free over the course of the pre-season exhibited improved tendon structure, three participants became painful and moved to the reactive / dysrepair phase of pathology. An alteration in the UTC echopattern was observed in all three participants at the onset of symptoms. The distribution of echo-types representing alterations in tendon bundle alignment (echo-type II and III) were diffusely spread throughout the tendon in two of the participants, potentially representing reactive pathology (fig 5.3). The remaining participant exhibited a focal area of echo-type III and IV at the midsubstance of the tendon suggesting the tendon had progressed to tendon dysrepair (fig 5.3). Interestingly, the UTC echopattern of the participant with focal pathology deteriorated further over the pre-season and continued to have pain during the season while the two participants with diffuse pathology returned to baseline by the end of the pre-season and had no pain during the season (fig 5.1). Understanding the factors that contribute to these differing pathological responses is critical, with the application of excessive or appropriately modified load likely to be the key driver.

Despite an abundance of research demonstrating changes in the tendon in response to load, we are still unclear whether tendon responses observed in previous studies are positive/adaptive or negative and implicated in the development of tendinopathy. This is partly due to the paradoxical response of tendon structure to load. Heinemeier et al (2012) attempted to induce pathology in rats using a previously published protocol (Glazebrook et al, 2008). Interestingly, instead of developing pathological changes, the tendon improved in mechanical properties and gene expression differed to that seen in tendinopathy. While the loading protocols where similar between the two studies, the two differing responses

suggest that similar loads can elicit differing responses from the tendon and that a number of other intrinsic factors may have a role.

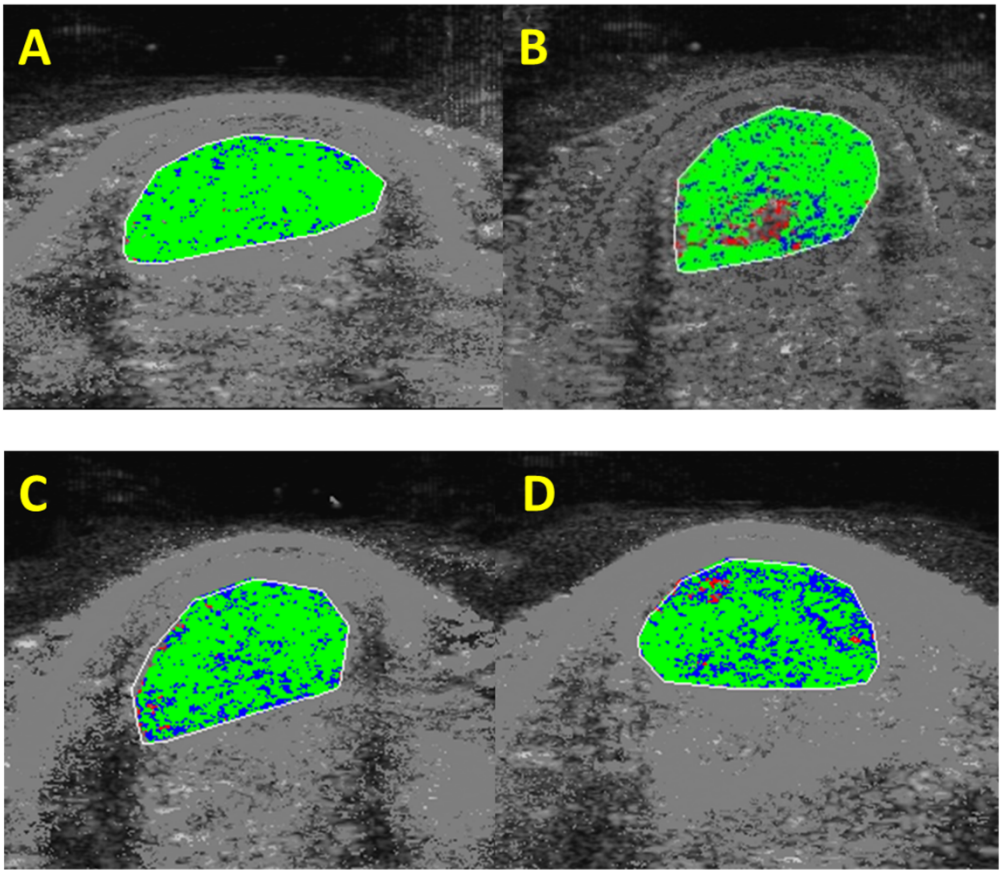


Figure 5.3- – Transverse UTC images of (A) a normal Achilles tendon, characterised by a high proportion of echo-type I, and the (B-D) three participants that developed Achilles tendon pain. (B) A focal area of echo-type III and IV was observed within the midsubstance of the Achilles of one participant, where (C and D) diffuse increases in echo-type II were observed indicating reactive changes within the remaining painful participants. Echo-type I = Green, Echo-type II = Blue, Echo-type III = Red, Echo-type IV = Black

Determining whether a tendon response will be positive or negative may be largely dependent on loading parameters (amount of high load, frequency of loading)(Visnes & Bahr, 2013), but a number of other factors may also be implicated. Factors such as baseline tendon structure (Comin et al, 2013), person factors (older age (de Jonge et al, 2011b), other tendon injuries) and systemic factors (genetics (September et al, 2009), adiposity

(Gaida et al, 2009; Malliaras et al, 2007)) have been shown to increase the risk of developing Achilles tendinopathy. Many of these factors are absent in an elite sporting group. The findings of the current study possibly suggest that baseline tendon structure, age and seasons played at AFL level are not risk factors. However, these findings are limited due to the small sample size.

Tendons have been shown to lose mechanical strength and even structure with prolonged periods of unloading (Frizziero et al, 2011; Kubo et al, 2012), with the length and intensity of unloading possibly determining how much a tendon is compromised. The off-season in Australian football varies from eight to twelve weeks. Despite the reduced formal loading within this time, the players are required to maintain a minimum standard of conditioning that could maintain the tendon capacity. Longer periods of unloading or complete rest may be more detrimental, illustrated by a significant increase in pre-season Achilles tendon ruptures (12 ruptures compared to 1-3 in previous years) in American football players on return to training after the NFL lock-out (extended off-season with a shortened pre-season)(Myer et al, 2011). The re-loading parameters may be critical in determining tendon response. Elite teams should re-introduce load gradually allowing time for the tendon to respond positively and avoid movement along the pathology continuum.

A limitation of this study was that load was not quantified, however it is a cornerstone of conditioning that there would be a structured increase in load over the course of the pre-season with average training loads greater in the pre-season than in-season (Colby et al, 2014; Jeong et al, 2011). While the pre-season in elite Australian football players consists of high training loads, conclusions are limited as to how and what loads directly affect the UTC echopattern. This study is a real-world pragmatic study and as a

result is limited by the small sample size in both groups. While there are increasing publications on the use of UTC, the findings of this study and inferences from the UTC echopattern to other studies is limited. These studies have used different UTC components (manual tracking unit vs automatic), differing scanning positions (lying prone vs a standing lunge position) and differences in the region of interest quantified (2cm region at the midsubstance vs whole tendon). While comparison between other studies are limited, it can be concluded that the UTC echopattern has improved as this is a prospective study and the changes are greater than the reported minimum detectable difference.

5.7- Perspectives

This study showed that the UTC echopattern of the Achilles tendon changed, indicative of improved tendon structure, in the pain-free Australian football player over the course of a pre-season. While load was not quantified as part of this study, the pre-season of elite Australian football players has been shown to consist of higher average loads than in-season. Further research is needed to ascertain whether this alteration in tendon structure results in increased load tolerance and injury prevention. The alteration in tendon structure provides further evidence that UTC may be able to detect changes in the tendon in response to load with further studies to focus on what extrinsic and intrinsic factors may effect this response to load.

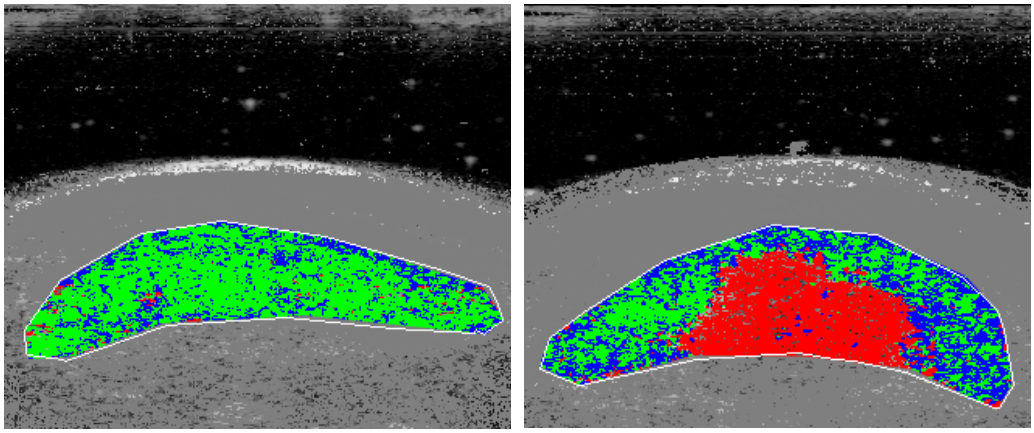
5.8- Acknowledgements

This paper was supported by the Australian Centre for Research into Sports Injury and its Prevention, which is one of the International Research Centres for Prevention of Injury and Protection of Athlete Health supported by the International Olympic Committee.

Chapter six
Pathological tendons maintain sufficient aligned fibrillar structure on ultrasound tissue characterisation (UTC)

Docking SI and Cook JL.

Submitted to *the Scandinavian Journal of Medicine and Science in Sports*.



"It's all about the doughnut, not the hole"

Purdam (personal communication)

6.1- Declaration for Thesis Chapter

In the case of Chapter six, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Pathological tendons maintain sufficient aligned fibrillar structure on ultrasound tissue characterisation	75%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Cook, J	Senior supervision of study design, statistical analysis and preparation of manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature		**Date**

Main Supervisor's Signature		**Date**

6.2- Abstract

Structural disorganisation in the tendon is associated with tendinopathy, with little research investigating whether disorganisation overwhelms the overall structural integrity of the tendon. This study investigated the mean cross-sectional area (CSA) of aligned fibrillar structure as detected by ultrasound tissue characterisation (UTC) in the pathological and normal Achilles and patellar tendon

Ninety-one participants had their Achilles and/or patellar tendon scanned using UTC to capture a 3-dimensional image of the tendon and allow semi-quantification of the echopattern. The mean CSA of aligned fibrillar structure (echo-type I+II) and disorganised structure (echo-type III+IV) were calculated based on UTC algorithms. Each tendon was classified as either pathological or normal based solely on grey scale ultrasound.

The mean CSA of aligned fibrillar structure was significantly greater ($p \leq 0.001$) in the pathological tendon compared to the normal tendon, despite the pathological tendon containing greater amounts of disorganised structure ($p \leq 0.001$). A significant relationship was observed between the mean CSA of disorganised structure and anteroposterior diameter of the Achilles ($R^2=0.587$) and patellar ($R^2=0.559$) tendon.

This study is the first to show that pathological tendons have sufficient levels of aligned fibrillar structure. Pathological tendons may compensate for areas of disorganisation by increasing in tendon thickness.

6.3- Introduction

Tendon pathology observed on ultrasound (US) imaging has been extensively described in the literature. Pathology is represented as thickening of the tendon, areas of hypoechogenecity, and increased Doppler signal representing increased blood flow within the tendon (Astrom et al, 1996; Rasmussen, 2000). While these changes on US can assist in the diagnosis of tendinopathy, tendon pathology is often subjectively graded with limited ability to quantify tendon structure and integrity.

Recently there have been a number of new US imaging techniques that attempt to remove the reliance on subjective interpretation, and to use varying imaging techniques to quantify parameters in the tendon (ie echo-textual features, amount of bound water, micro-vascular volume)(Bashford et al, 2008; Grosse et al, 2013; Pingel et al, 2013a). One such imaging technique, ultrasound tissue characterisation (UTC), utilises conventional US to semi-quantify tendon structure (van Schie et al, 2010). A 3-dimensional image is rendered from 600 transverse US images that are captured every 0.2mm over the length of the tendon. From this, the stability of pixel brightness over 25 consecutive transverse images (4.8mm) is calculated and categorised into four echo-types correlating to the degree of structural homogeneity, with relative proportions for each echo-type being quantified in relation to the tendon volume.

Compromised tendon structure in pathology has driven interventions designed to 'heal' tendons. Regenerative therapies, such as platelet rich plasma (PRP), autologous blood injections and stem cells are introduced into areas of tendon pathology to try to improve structure (Chaudhury, 2012; Mei-Dan et al, 2010). However, evidence suggests these treatments are no more effective in improving structure and returning sufferers of Achilles

and patellar tendinopathy (pain and dysfunction) back to pain-free function than placebo treatments (de Jonge et al, 2011a; Dragoo et al, 2014).

In addition, there is little evidence that improvement in tendon structure is linked to recovery. Drew et al (2014) performed a systematic review to determine the relationship between structural changes in the tendon and clinical outcomes following therapeutic exercise. There was strong evidence that improvements in tendon pain and function after eccentric exercise were not mediated by changes in tendon structure (ie tendon diameter, structural abnormalities and neovascularisation). These findings suggest that tendon thickness and areas of disorganisation are not the limiting factor in returning tendons back to pain-free function. Interestingly, treatments outside the tendon targeting the region with high blood flow and nerves on the ventral side of the Achilles and dorsal side of the patellar tendon have shown benefit in regards to return to pain free loading (Alfredson, 2011; Lind et al, 2006; Willberg et al, 2011). Critically, despite pathology being associated with tendinopathy (Comin et al, 2013; Khan et al, 2003) there is no evidence that a pathological tendon has a reduced volume of normal tendon structure. Tendon thickening, a hallmark of a pathological tendon, may be an adaptive response to compensate for areas of disorganisation.

As there is a disconnect between tendon pain and structure (Scott et al, 2013), this study focuses solely on tendon structure as quantified by UTC independent of pain. This study aims to investigate the mean cross-sectional area (CSA) of aligned fibrillar structure (echo-types I and II) in pathological and normal Achilles and patellar tendons. As there are no previous data, the null hypothesis that there is no significant difference in the mean CSA

of aligned tissue structure in the pathological and normal Achilles and patellar tendons was taken.

6.4- Methods

People who had a UTC scan of their Achilles and/or patellar tendon as part of their clinical management or previous research project were retrospectively invited to participate in this study. Those with a history of use of fluroquinolones in the last six months or previous tendon rupture at any site were excluded. The study was approved by the university ethics committee and participants provided informed consent (Appendix K).

6.4.1- UTC imaging

Imaging of the Achilles and/or patellar tendon was performed using UTC. Participants' Achilles tendons were scanned standing on an elevated platform, with their toes and knee against a wall (Rosengarten et al, 2015). For the patellar tendon, participants lay supine with their knee flexed and the tibia at ~60°. A linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000, Teratech) mounted within a customised tracking device with motor-drive and built-in acoustic stand-off pad (UTC Tracker, UTC Imaging) was placed on the skin, perpendicular to the long axis of the tendon (Achilles or patellar), ensuring that the calcaneus or inferior pole of the patella was visible. Coupling gel was applied between the transducer, stand-off pad and skin to ensure maximum contact.

Once a clear transverse image of the tendon and bony landmark was established, the motor-drive automatically moved the transducer over the length of the tendon capturing a transverse grey scale US image every 0.2mm over a distance of 12cm. By compounding these images, a grey scale US image data block is rendered that allows the tendon to be

viewed in three planes; transverse, sagittal and coronal. From this data block, each tendon was classified as either normal or pathological.

An algorithm consisting of three key imaging criteria was used to classify the tendon as normal or pathological (fig 6.1)(Astrom et al, 1996; Rasmussen, 2000; Schmid et al, 2002). The presence of tendon pain was not assessed as this study focuses solely on structure. A single investigator with five years' experience with tendon imaging and UTC was blinded at the time of analysis to participant, limb, and UTC echopattern of the tendon. Tendon structure was quantified with dedicated UTC algorithms that assess the echopattern by means of relative intensity and distribution of grey levels of corresponding pixels over 25 images (4.8mm). Based on their stability/degree of persistence, four echo-types were discriminated (van Schie et al, 2010). Echo-type I and II are generated by ultrasound reflection due to a single interface structure and are indicative of aligned fibrillar structure. Echo-type III and IV are generated by multiple reflections that interfere as a consequence of multiple interfaces. These echo-types represent disorganised amorphous fibrillar structure that has non-parallel fibre arrangement. For this study echo-type I and II were grouped together and classified as having aligned fibrillar structure (AFS), whereas echo-type III and IV were termed disorganised tissue structure (DIS). These echo-types have previously been matched with equine histopathological specimens (van Schie et al, 2003).

Quantification of the UTC echopattern was performed by the same investigator who remained blind to participant, limb, clinical history and tendon classification. For each tendon, regions of interest (ROI's) were placed around the border of the tendon in the transverse view. For the Achilles, multiple ROI's were selected at intervals no greater than 5mm from the disappearance of the calcaneus to the musculotendinous junction. A similar

method was used for the patellar tendon; ROI's were determined over a distance of 3cm starting from the disappearance of the inferior pole of the patella.

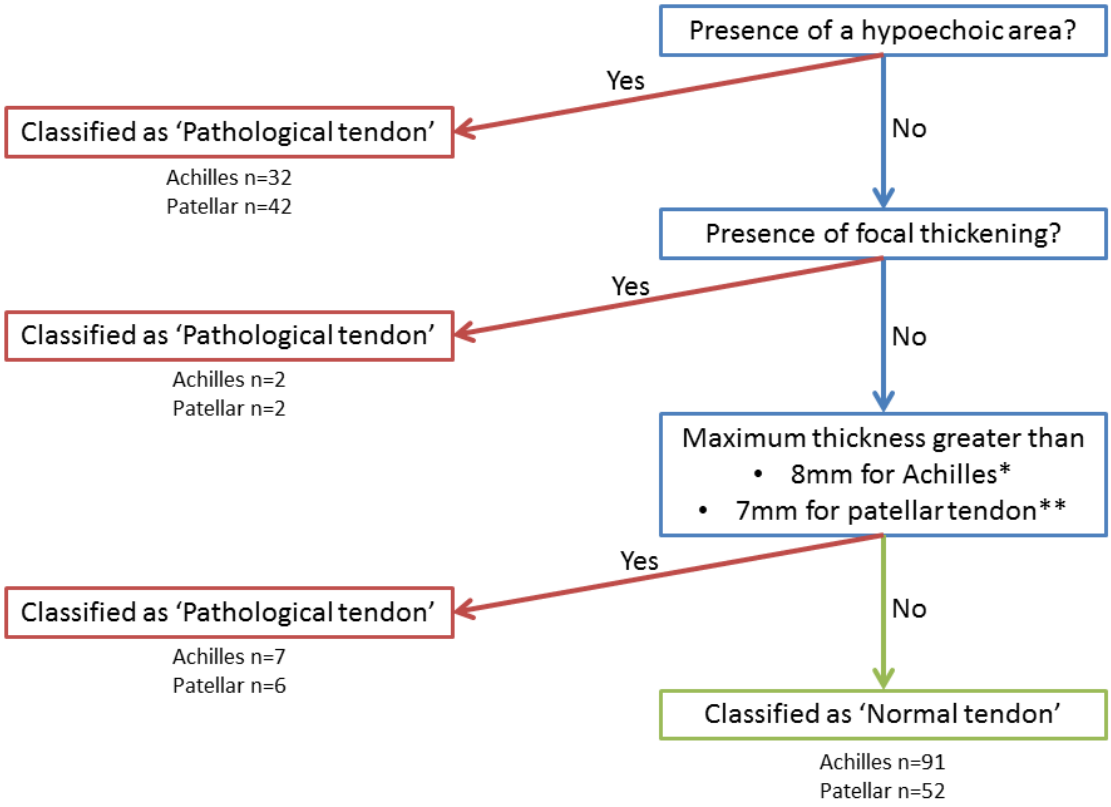


Figure 6.1- Algorithm for the classification of the Achilles and patellar tendon based on grey scale imaging

6.4.2- Calculation of proportions and mean CSA

The UTC software interpolated contiguous ROI's within the defined ROI's creating a tendon volume where the proportions of the each echo-type and the total number of pixels in the ROI was calculated for each transverse scan. The CSA for each transverse ROI was calculated by multiplying the number of pixels within the defined ROI by the area of the pixel (0.011mm^2). The area of an individual pixel did not differ between scans as the US settings (gain, focus and depth) were standardised.

The CSA of AFS and DIS structure were calculated for each transverse scan by multiplying the total CSA by the proportion of echo-type I+II or echo-type III+IV. The volume of AFS, DIS and total tendon volume (mm^3) were calculated by plotting the CSA of each variable over the length of the scan and determining the area under the curve. As tendon length differed between individuals, the mean CSA (mCSA = absolute volume divided by the length of the tendon) were calculated for each variable.

6.4.3- Validation of total mean CSA calculations

An ultrasound phantom was created (Bude & Adler, 1995) and a hollow plastic cylinder of known dimensions (length=62mm, radius=4mm, total volume=3117.7 mm^3, mCSA=50.3mm^2) was included (Appendix L). The phantom was scanned using UTC five times using the same technique described above. Multiple ROI's were defined around the border of the cylinder along the length of the scan with the investigator blinded as to the size of the defined ROI's. Total mCSA was calculated for each UTC scan and used to calculate the accuracy and precision. The coefficient of variation (CoV=SD/mean) was used as a measure of precision, with the accuracy determined by comparing the calculated volume on UTC to the measured volume.

6.4.4- Intratester reliability

Eight Achilles and eight patellar tendons in a sample population of similar demographics were scanned twice. A two-way mixed single measures intra-class correlation (ICC) for absolute agreement was performed to calculate the standard error of the measurement (SEM = SD of population x √(1 – ICC)). The minimum detectable difference (MDC = 1.96 x SEM x √2) was calculated for all parameters.

6.4.5- Statistical analysis

Statistical analysis was performed separately for the Achilles or patellar tendon. Tests of normality demonstrated that the data were not normally distributed, hence non-parametric tests were used. A Bonferroni adjustment was made due to the high number of statistical tests and the alpha level was set at 0.01.

Each participant was classified as having normal, unilateral or bilateral tendon pathology in either the Achilles or patellar. A Mann-Whitney U Test was performed to compare differences in all UTC parameters between the normal and pathological tendon between participants. In participants with bilateral normal or pathological tendons, the left or right tendon was randomly selected for statistical analysis by a coin toss. The pathological tendon was selected in participants with unilateral pathology. To test differences in the all UTC parameters between the normal and pathological tendon within subjects, participants with unilateral tendon pathology were selected. A related-samples Wilcoxon signed rank test was used to compare medians. All analyses were conducted using a statistical package (SPSS V.20.0 for Windows, SPSS Inc, Chicago, Illinois, USA).

A linear regression between AP diameter, total mean CSA and the mean CSA of disorganised tissue was performed in pathological tendons. In bilateral pathology, the left or right tendon was randomly selected for statistical analysis by a coin toss.

6.5- Results

6.5.1- Demographics

Of the 186 participants who were invited to participate in this study, 91 participants provided informed consent (66 Achilles and 50 patellar tendons, table 6.1). The participants ranged from sedentary individuals to elite athletes. The presence of symptoms was not noted as part of this study.

A high percentage of the Achilles (31.1%) and patellar (48%) tendons scanned were classified as pathological (fig 6.1). The majority of these tendons were classified as pathological due to the presence of a hypoechoic lesion (78% and 87.5% of pathological Achilles and patellar tendons, respectively). Bilateral pathology was observed in the patellar tendon in 36% of all participants, compared to 16.7% of all participants in the Achilles tendon.

Table 6.1- Demographics of participants.

	Age, mean±SD (y)	Gender	
		Male, n (%)	Female, n (%)
Achilles tendon			
Normal bilateral (n=36)	24.5 ± 6.6	36 (100)	0 (0)
Unilateral pathology (n=19)	32.1 ± 14.4	17 (89)	2 (11)
Bilateral pathology (n=11)	33.5 ± 12	10 (91)	1 (9)
Patellar tendon			
Normal bilateral (n=20)	24.7 ± 11.8	19 (95)	1 (5)
Unilateral pathology (n=12)	24.2 ± 4.7	12 (100)	0 (0)
Bilateral pathology (n=18)	23.2 ± 4	18 (100)	0 (0)

6.5.2- Validation and reliability of UTC

The mean total CSA from five successive scans of the phantom was 50.6mm^2 (known mCSA= 50.3mm^2) with the measurements over-estimating by 0.7%. The measurements ranged from 49.3 to 52.5 mm^2, a standard deviation of ±1.05 mm^2 and a coefficient of variation of 2.1.

6.5.3- Between participant analysis

There was a significant difference in echopattern between the pathological tendon and normal tendon in both the Achilles and patellar tendon (table 6.2). Pathological tendons were significantly larger in AP diameter ($p< 0.001$ for both tendons) and total mCSA ($p< 0.001$ for both tendons). The relative percentage of AFS (echo-type I and II) was significantly lower in the pathological tendon in comparison to normal tendons ($p< 0.001$ for both tendons), coinciding with a significant increase in DIS (echo-type III and IV)($p= 0.001$ for both tendons).

Despite lower percentages of AFS, the pathological tendon contained a significantly larger mCSA of AFS in comparison to the normal tendon ($p< 0.001$, $p= 0.004$ for the Achilles and patellar tendon, respectively). As expected, significantly larger mCSA of DIS were observed in the pathological tendon ($p< 0.001$ for both tendons). All significant differences were greater than the MDC.

Table 6.2- Relative proportions (%) and mean cross-sectional area (mCSA) of aligned fibrillar structure (AFS) and disorganised structure (DIS) for both the Achilles and patellar tendon between and within participants. All values are median ± interquartile range.

	Minimum detectable change (MDC)	Between participant analysis		Within participant analysis	
		Normal	Pathological	Normal	Pathological
Achilles tendon		**n = 36**	**n = 30**	**n = 19**	**n = 19**
AP diameter (mm)	0.5	6.5 ± 0.5	8.4 ± 1.5*#	6.9 ± 0.9	8.4 ± 1.1*#
Total mCSA (mm^2)	6.4	83.6 ± 16.7	99.1 ± 26.0*#	82 ± 9.5	103.1 ± 26.6*#
% of AFS (%)	1.0	98.2 ± 1.4	95.0 ± 6.3*#	97.8 ± 1.0	96.6 ± 5.5*#
% of DIS (%)	0.9	1.8 ± 1.4	5.0 ± 6.3*#	2.2 ± 0.9	3.4 ± 5.5*#
mCSA of AFS (mm^2)	6.5	80.8 ± 15.8	94.8 ± 26.5*#	80.5 ± 9.4	96.0 ± 23.1*#
mCSA of DIS (mm^2)	0.7	1.4 ± 1.4	4.7 ± 8.3*#	1.7 ± 1.3	3.0 ± 4.7*#
Patellar tendon		**n = 20**	**n = 30**	**n = 12**	**n = 12**
AP diameter (mm)	0.4	6.0 ± 0.6	7.8 ± 2.6*#	6.4 ± 0.4	7.1 ± 2.3#
Total mCSA (mm^2)	13.4	130.8 ± 11.0	154.9 ± 23.6*#	131.1 ±26.2	144.3 ± 24.0
% of AFS (%)	1.7	96.5 ± 2.2	88.9 ± 9.6*#	96.4 ± 2.4	94.5 ± 12.5#
% of DIS (%)	1.7	3.5 ± 2.2	11.1 ± 9.6*#	3.6 ± 2.4	5.5 ± 12.5#
mCSA of AFS (mm^2)	12.1	125.9 ± 11.7	139.9 ± 23.1*#	126.1 ± 23.7	127 ± 18.7
mCSA of DIS (mm^2)	2.2	4.5 ± 3.4	17.1 ± 22.3*#	5.2 ± 4.3	8.6 ± 21.2#

*p≤0.01, #Difference is greater than the MDC

6.5.4- Within participant analysis

19 participants had unilateral Achilles tendon pathology. The pathological tendon was significantly larger in tendon dimensions (AP diameter and total mCSA, $p< 0.001$ for both variables). The relative proportion of AFS was significantly lower in the pathological tendon ($p= 0.002$), corresponding with an increase in the percentage of DIS ($p= 0.002$). Mean CSA of AFS ($p< 0.001$) and DIS ($p= 0.001$) were significantly greater in the pathological tendon. All changes were greater than the MDC.

Unilateral patellar tendon pathology was observed in 12 participants. No significant differences were observed in AP diameter or total mCSA ($p= 0.021$ and 0.060, respectively). A non-significant decrease in percentage of AFS ($p= 0.012$) was observed in the pathological tendon that was greater than the MDC, coinciding with an increase in percentage of DIS greater than the MDC ($p= 0.012$). No significant difference was observed in the mCSA of AFS ($p= 0. 695$), with the difference in mCSA of DIS between the pathological and normal tendon greater than the MDC, yet not significant ($p= 0.012$).

6.5.5- Relationship between AP diameter and mean CSA of disorganised tissue

A significant linear relationship ($p< 0.001$) was observed between the tendon dimensions (AP diameter and total mCSA) and mCSA of DIS for both the pathological Achilles ($R^2= 0.587$ and 0.441 for AP and total mCSA, respectively) and patellar tendon ($R^2= 0.559$ and 0.518 for AP and total mCSA, respectively).

6.5.6- mCSA of aligned fibrillar structure in the pathological tendon

For all pathological tendons, the mCSA of AFS was plotted in relation to the median and interquartile range of the mCSA of AFS in normal tendons (fig 6.2). The majority of pathological tendons fell within (53.7% & 37.5% for the Achilles and patellar, respectively) or above (43.9 & 60.4% for the Achilles and patellar, respectively) the range for aligned fibrillar structure in normal tendons (fig 6.2). Only one Achilles (2.4%) and one patellar tendon (2.1%) contained less aligned tendon structure than the IQR for normal tendons.

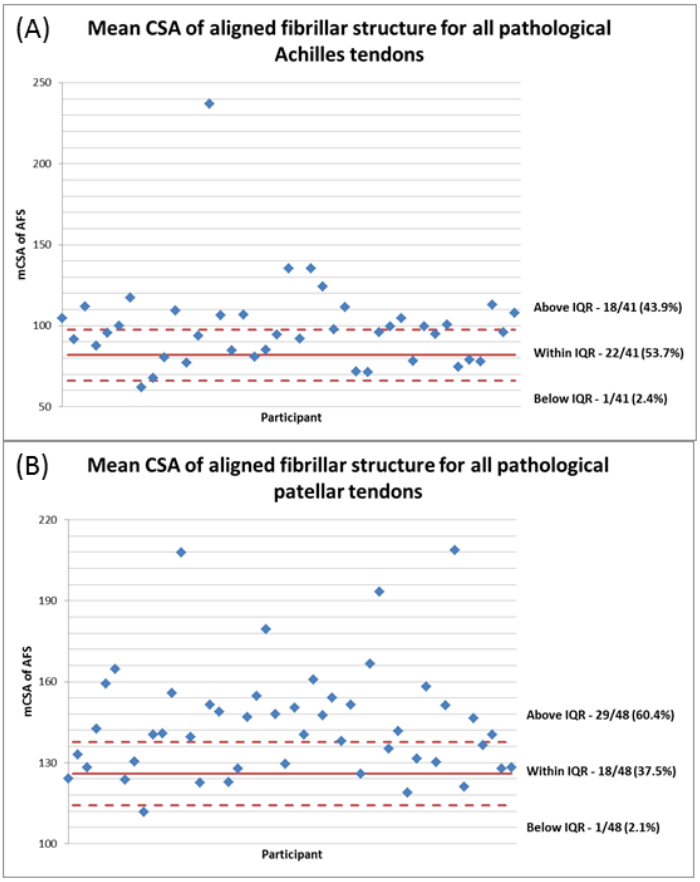


Figure 6.2- Scatter-plots of mean cross-sectional area of aligned fibrillar structure in the pathological Achilles (a) and patellar (b) tendon in relation to the mean cross-sectional area of aligned fibrillar structure in normal tendons. The number of pathological that fell below, within or above the interquartile range is presented within the graphs. Bold line = Median for normal tendons, dotted line = upper and lower border of the IQR for normal tendons, blue diamonds = pathological tendon

6.6- Discussion

This study showed demonstrates that the pathological Achilles and patellar tendon contain increased mCSA of aligned fibrillar structure as quantified by UTC despite significant areas of disorganisation. The significant relationship observed between the mCSA of disorganised structure and tendon dimensions (AP diameter and total tendon mCSA) may suggest that the pathological tendon compensates to maintain sufficient amounts of aligned fibrillar structure and tissue homeostasis. These results question previous models of tendon pathology where the accumulation of micro-trauma or failed healing is suggested to be the predominate feature of tendon pathology.

Several models suggest tendon pathology accumulates within the tendon to a point where the tendon can no longer remodel to compensate for the area of disorganisation. Abate et al (2009) described the 'Iceberg theory' of tendon pathology where relative overload induces micro-ruptures within the tendon that sets of a cascade of changes in the extracellular matrix. The increased expression of cytokines (VEGF, PDGF) and digestive extracellular matrix enzymes (MMP's and ADAMTS) result in degeneration, neovascularisation and weakening of the normal tendon structure (Abate et al, 2009). A similar model was proposed where tendons would enter a cycle of micro-tears and degeneration that could lead to structural deterioration and tissue failure (Leadbetter, 1992). The findings from the current study appears to conflict with these models. While significant areas of disorganisation are apparent within the Achilles and patellar tendon, these tendons maintain a level of load bearing aligned fibrillar structure greater than is present within structurally normal tendons. Insufficient amounts of aligned fibrillar

structure, or critically a lack of remodelling, does not appear to be a feature of tendon pathology within the pathological Achilles and patellar tendon.

The continuum of tendon pathology (Cook & Purdam, 2009) differs from the previous models as it places activation of tenocytes as the primary histopathological event, rather than disruption in the fibrillar matrix. The early cellular and ground substance changes, termed reactive tendon pathology, were proposed to be a pathological adaptation resulting in the tendon increasing in size that in turn reduces stress (force/CSA)(Cook et al, 2004a). However, the current study suggests that the thickening of the tendon may have a secondary role in mandating structural homeostasis and ensuring that there are sufficient amounts of aligned fibrillar structure to tolerate load without failure. This appears to be the case in the degenerative tendon where substantial areas of disorganised tissue are present.

The results of this study might explain how degenerative tendons can still tolerate high tensile load and remain asymptomatic. Malliaras et al (2010) investigated changes in patellar tendon structure on US monthly over the course of a volleyball season and reported that tendons could vary in appearance from normal to diffusely thickened and hypoechoic. The patellar tendons with a hypoechoic area were likely to remain unchanged, with 81% of the hypoechoic tendons scanned continuing to exhibit a hypoechoic area. This supports the current study that the degenerative tendon adapts, and despite areas of pathology, the degenerative tendon reaches tissue homeostasis and does not need to remodel back to a normal tendon. This might explain why therapies targeted at remodelling tendon structure have had limited efficacy to date.

Regenerative therapies for tendon pathology, such as platelet-rich plasma (PRP) and stem cells, aim to stimulate healing within the disorganised tissue in tendons by facilitating

the regeneration of tendon tissue that is able to withstand and transmit tensile load (Chaudhury, 2012). De Jonge et al (2011a) performed a double-blinded randomised control trial comparing PRP and saline injection in chronic (>2 months) Achilles tendinopathy. Despite improvements at one-year in tendon pain, function and structure on UTC after a single PRP injection, there was no difference in structure compared to the placebo group. While regenerative medicine may not be appropriate in the majority of the tendinopathic population, these interventions may be appropriate in certain individuals. Scatter-plots of all pathological tendons revealed that only one Achilles and one patellar tendon contained less aligned tendon structure than the IQR for normal tendons and here regenerative medicine may be appropriate, despite little evidence for the efficacy of these treatments in improving tendon structure (Gross et al, 2013; van Ark et al, 2011). Future research is needed to better identify and sub-group tendons that might benefit from regenerative medicine and assess whether these tendons have superior outcomes in comparison to progressive loading.

Eccentrics are a commonly prescribed exercise for tendinopathy (Alfredson et al, 1998; Fahlstrom et al, 2003; Kongsgaard et al, 2009). Not only have eccentrics shown to be beneficial in the management of tendon pain but increases in biomarkers of collagen synthesis within the peritendinous space have also been demonstrated in both normal and pathological tendons (Langberg et al, 2007). However, de Vos et al (2012) demonstrated that clinical improvements in chronic midportion Achilles tendinopathy after a 16-week eccentric exercise program did not correlate with changes in the UTC echopattern, specifically the relative percentage of echo-type I and II (AFS). In the context of the current study, eccentrics, or potentially any other form of controlled exercise (heavy-slow resistance exercise), may not be effective in remodelling the pathological lesion; rather these exercises

may cause adaptation and load tolerance in the surrounding aligned fibrillar structure and musculature in series. As the pathological tendon adapts by thickening to maintain a sufficient volume of aligned fibrillar structure, treatment strategies may be better focused at increasing the load capacity of the aligned fibrillar structure.

Interestingly, no significant difference was observed in any UTC parameter between the normal and pathological patellar tendon in participants with unilateral pathology, which differs to the findings in the Achilles tendon. However, the percentage of aligned fibrillar structure, disorganised tissue and mCSA of disorganised tissue are near significance and greater than the MDC. A lack of significant difference between patellar tendons in individuals with unilateral pathology may be due to subtle changes within the normal tendon. The algorithm developed to classify whether a tendon is pathological was based on the ultrasound features of the pathological tendon. These features are a subjective interpretation where subtle changes in hypoechogenecity might be overlooked. Previous research using UTC has reported that the asymptomatic Achilles tendon in patients with unilateral pain is structurally compromised (Docking et al, 2014). Animal studies also support these findings and suggest that the central nervous system or systemic cytokines, such as Substance P, may drive pathological changes bilaterally (Andersson et al, 2011; Williams et al, 1984).

These results have important implications for the clinical interpretation of not only UTC images, but also conventional ultrasound and magnetic resonance imaging. Tendon thickening is indicative of pathological changes; this study has shown that thickening may well be a positive finding. In addition, visualisation of the tendon in 2-dimensions may be limited as it is unable to determine the volume of the pathology in relation to the

surrounding tissue. This can lead to the pathological area being over-emphasised and not accounting for possible remodelling and adaption peripherally to the pathological area.

The UTC algorithms have been compared to normal and pathological histopathology specimens in horses (van Schie et al, 2000; van Schie et al, 2003). Similar data are not available in humans. However, structural similarities between horses and human have been described in the literature (Kristoffersen et al, 2005; Patterson-Kane et al, 2012; Patterson-Kane & Rich, 2014). It is also important to note that the UTC method used in this study differs from the original articles (eg transducer moving automatically compared with manually, transducer captures over 12cm compared with 9.6cm, the region of tendon selected for quantification)(Rosengarten et al, 2015). It cannot be stated that the aligned fibrillar structure quantified by UTC is normal tendon (ie there may be changes in cell number and phenotype, alterations in water and proteoglycan content). However, as echo-type I and II are generated by one ultrasound reflection that is produced by one interface structure, in conjunction with the previous validation studies, it can be assumed that it represents organised tendon bundles.

As this study is cross-sectional, we are unable to definitively state whether the findings of this study represent that the pathological tendon compensates for the development of tendon pathology. Future longitudinal studies are required to investigate whether the pathological Achilles and patellar tendon changes in dimensions in relation to the mCSA of disorganised structure. Another potential limitation of this study is a lack of variability in age and sex within the sample population, however the young age and high proportion of men reflects the prevalence of Achilles and patellar tendinopathy (Clayton & Court-Brown, 2008; Lian et al, 2005).

6.7- Perspective

This is the first study to characterise the overall structural integrity of the pathological Achilles and patellar tendon. The pathological Achilles and patellar tendon contains increased amounts of aligned fibrillar structure. While increases in tendon thickness have previously been described as negative, the findings of this study suggest that tendon thickening might be the tendons method of adapting to pathology. Treatment strategies may be better served in building load capacity within the already present aligned tendon structure, rather than attempting to regenerate the area of pathology with intra-tendinous injections or surgery.

6.8- Acknowledgements

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Chapter seven
Structural integrity is decreased in both Achilles tendons in people with unilateral Achilles tendinopathy

Docking SI, Rosengarten S, Daffy J & Cook J

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"This clearly demonstrates that the contralateral Achilles tendon is an unsuitable control—a fact of great importance to establish before any further studies can be made."

Andersson et al (2011)

7.1- Declaration for Thesis Chapter

In the case of Chapter seven, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Structural integrity is decreased in both Achilles tendons in people with unilateral Achilles tendinopathy	70%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Rosengarten, S	Data collection and preparation of manuscript
Daffy, J	Study design and preparation of manuscript
Cook, J	Senior supervision of study design, statistical analysis and preparation of manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature		**Date**

Main Supervisor's Signature		**Date**

7.1- Abstract

A high proportion of people suffering with Achilles tendinopathy develop bilateral symptoms with human and animal studies showing bilateral histological changes associated with overuse/pathology in one tendon. The current study examined changes in tendon structure, assessed semi-quantitatively using ultrasound tissue characterisation (UTC), in both the symptomatic and asymptomatic tendon in unilateral Achilles tendinopathy patients in comparison to individuals with no history of tendinopathy.

Participants with Achilles tendinopathy (n=21), with varying severity and length of clinical symptoms, and six participants with no history of tendinopathy were recruited. Tendons were scanned using UTC, which captures contiguous transverse ultrasound images every 0.2 mm and renders a 3-dimensional image. UTC quantifies tendon structure by measuring the stability of echopattern over contiguous transverse images. Four echo-types were discriminated and expressed as a percentage. Antero-posterior diameter of all tendons was measured.

Significant differences were observed in the proportion of normal tendon structure between all three groups ($p< 0.01$), with the symptomatic tendon containing the least amount of normal tendon structure (symptomatic – 79.5%, asymptomatic – 81.8%, control – 86.4%). The asymptomatic tendon contained significantly less normal tendon in comparison to the control tendon ($p= 0.008$), suggesting the asymptomatic tendon is structurally compromised despite the absence of symptoms.

Both Achilles tendons are structurally compromised in patients with unilateral Achilles tendinopathy. Future studies need to investigate whether these changes increase the risk of developing symptoms.

7.2- Introduction

Achilles tendinopathy is a prevalent and debilitating musculoskeletal injury affecting anyone from elite athletes to older sedentary people. Clinical symptoms associated with Achilles tendinopathy include thickening of the tendon and pain during exercise that often leads to decreased participation in physical activity (Cook et al, 2002). A high proportion of these patients present with, or develop, symptoms in both Achilles tendons (Aroen et al, 2004; Fahlstrom et al, 2002).

Cross-sectional studies in chronic Achilles tendinopathy patients and elite badminton players reported bilateral symptoms occurring in ~35% of all cases (Fahlstrom et al, 2002). Paavola et al (2000) reported that 41% of patients developed symptoms in the previously unaffected Achilles tendon. Patients treated surgically for Achilles tendon rupture had an increased risk of contralateral tendon rupture (odds ratio = 186) with 6% of these patients rupturing the contralateral tendon (Aroen et al, 2004). Degenerative changes within the contralateral tendon have been suggested to account for the increased risk of bilateral tendon disease/symptoms. However, research into bilateral structural changes in Achilles tendinopathy has been limited to animal studies or Achilles tendon rupture patients, which may not be representative of the painful non-ruptured tendon.

Histological changes in tendon structure in the contralateral tendon have been described in a number of animal studies. Andersson et al (2011) showed increases in cell number and neovascularisation in the unloaded tendon when mechanically inducing tendinopathy in one tendon. Similar changes were observed after unilateral collagenase injection in horses (Williams et al, 1984). One of the few studies that have investigated changes in the human was performed in Achilles tendon rupture patients. Tissue biopsies

were taken from the unaffected contralateral tendon, with tissue degeneration observed in 47 out of 50 cases (Cetti et al, 2003). To date, these bilateral changes have not been investigated in humans using conventional imaging modalities.

Conventional imaging modalities, such as ultrasound (US) imaging, use subjective grading of pathology or quantitative measures, such as cross-sectional area (CSA), antero-posterior (AP) diameter or %CSA of hypoechoic area. These measures have limited reliability and are subject to imaging artefacts (Rasmussen, 2000). Due to the limited reliability of these measures and the inability to detect subtle intra-tendinous changes, investigators are developing methods to quantify US images and draw associations with tendon structure (Bashford et al, 2008; Meghoufel et al, 2010). One of these recent imaging developments, Ultrasound Tissue Characterisation (UTC), captures contiguous transverse grey scale US images and renders a 3-dimensional image of 12 cm of the tendon. In addition, UTC analyses the stability of pixel brightness along the length of the tendon and allows for quantification of tendon structure. Differences in tendon structure have been reported using UTC between symptomatic Achilles tendons and asymptomatic controls, making UTC suitable to quantify changes in tendon structure (van Schie et al, 2010). Previous research has also reported that UTC is highly reproducible and able to detect subtle changes in tendon structure in response to maximal exercise in human and equine athletes not possible utilising conventional imaging modalities(Docking et al, 2012; Rosengarten et al, 2015).

The aim of this study was to examine tendon structure and a control group of normal tendons using UTC. It was hypothesised that tendon structure will be poorer in the symptomatic tendon than the contralateral tendon, with normal tendons having better structure than symptomatic and asymptomatic tendons.

7.3- Methods

Twenty-one participants with unilateral Achilles tendinopathy (insertional or midportion), diagnosed by an experienced physiotherapist, who presented to a sports medicine clinic over 18 months, were retrospectively recruited for this study. Participants with a previous or current history of tendinopathy in the contralateral Achilles tendon or any other lower limb tendon (ie patellar, hamstring, etc) were excluded. This was based on the treating clinicians medical records, which included a brief clinical history, including length of symptoms, and the presence/absence of pain during various clinical tests such as single-leg heel raise and/or single-leg hop. Each participant was referred to the investigator (SID) for a UTC scan as part of their clinical diagnosis and management. Six participants were recruited from the general population and used as controls (CON) as they had no history of tendinopathy at any site of the body.

This study was approved by a university ethics committee, with all participants providing written informed consent (Appendix M).

Structural integrity of both Achilles tendons was quantified using UTC. Participants stood on an elevated platform, with their toes and knee against a wall. A linear-array ultrasound transducer (SmartProbe 10L5, Terason 2000, Teratech) was mounted within a customised tracking device with motor-drive and built-in acoustic stand-off pad (UTC Tracker, UTC Imaging). Acoustic coupling gel was applied to the stand-off pad to ensure maximum contact between the skin and transducer. The tracking device was placed on the posterior aspect of the calcaneal region perpendicular to the long axis of the Achilles tendon, ensuring that the calcaneus was observed on the ultrasound as this bony landmark served as a reference point for the analysis of tendon structure.

Once a clear US image of the tendon was established and with transducer tilt angle, rotation, gain, focus and depth standardised, the ultrasound transducer moved automatically over the length of the tendon capturing 600 transverse grey scale US images at intervals of 0.2 mm (creating a scanning window of 12 cm in length). By compounding these images, a 3-D data block was created and UTC algorithms quantified the dynamism of the echopattern by means of relative intensity and distribution of grey levels of corresponding pixels in contiguous images over 25 images (4.8 mm). Based on their stability/degree of persistence, four different echo-types were discriminated. Echo-type I is generated by intact and correctly aligned secondary tendon bundles, echo-type II by increased separation and/or more waving fasciculi, echo-type III represents decreased fibrillar integrity and echo-type IV indicative of an absence of fibrillar organisation, mainly cellular matrix and/or free fluid. The echo-types generated by UTC algorithms have previously been validated against pathological equine tendons that were matched with the generated UTC-processed image (van Schie & Bakker, 2000), with these algorithms also able to discriminate against symptomatic and asymptomatic Achilles tendons in humans (van Schie et al, 2010). The following UTC settings were used; Corr Norm = Matched to the Pre-Proc Mean, Entr Norm = 8 and Window Size = 25.

Based on the presence of clinical symptoms (eg pain, swelling, loss of function) each tendon was assigned to the symptomatic (SYM), asymptomatic (ASYM) or control (CON) group. All scans and UTC analysis were performed by a single trained researcher (SID) who was blinded at the time of analysis to participant, scan, limb and clinical history with scans identified as randomly generated numbers prior to analysis. The ultrastructure information was visualised topographically in three planes of view and in 3-D. Structure of the tendon

was semi-quantified by means of calculation of relative percentages of total pixels in the region of interest (ROI) that was selected around the border of the Achilles. Each scan was checked for imaging artefacts, due to poor contact or movement, with ROI's selected from the insertion (defined at the point that the calcaneus disappeared) to the musculotendinous junction. Contours were then interpolated between the defined ROI's allowing for semi-quantitative analysis of the echopattern over the entire length of the tendon. Antero-posterior (AP) diameter was calculated 2 cm proximal from the insertion of the tendon were recorded for all tendons.

Test-retest reliability was performed in eight Achilles tendons of similar demographics to the sample population. Tendon structure was quantified using the same methods as described above. A two-way mixed single measures intra-class correlation was performed to calculate the standard error of the measurement (SEM = Standard deviation x √(1-ICC)). The minimum detectable change was calculated using the following formula: MDC = 1.96 x SEM x √2.

As the data were not normally distributed more conservative non-parametric tests were used. A Bonferroni adjustment was made due to the high number of statistical tests and the alpha level was set at 0.01. Differences between the SYM and ASYM tendon for all four echo-types and AP diameter were analysed using a related-samples Wilcoxon signed rank test. An independent-samples Mann-Whitney U test was used to analyse differences in the median between either the SYM or ASYM tendon compared to the CON group and any differences between elite athletes and recreational participants for all four echo-types and AP diameter between the SYM and ASYM tendon.

7.4- Results

Participant demographics are presented in table 7.1. The age and level of activity were similar between the control and tendinopathy group. Of the 11 elite participants with Achilles tendinopathy who participated in this study, seven were elite Australian football players and the rest included elite athletes participating in cricket, basketball, tennis and decathlon. All participants with symptoms had undergone a wide range of treatments prior to participation in this study including conservative management, shock wave therapy, corticosteroid and platelet-rich plasma injection.

The MDC was calculated as 2.5%, 2.7%, 0.3% and 0.8% for the four echo-types, respectively.

The SYM tendon showed significant differences in the echopattern in comparison to the ASYM tendon (fig 7.1, 7.2)(Appendix N). A significant decrease in echo-type I ($p= 0.005$) was observed in the SYM tendons with a concomitant increase in echo-type III and IV ($p\leq0.001$ & $p\leq0.001$, respectively) representing worse tendon structure. However, the difference in echo-type I was not greater than the MDC. Interestingly, the echopattern of the ASYM tendon differed in comparison to the CON group with echo-types I and III exhibiting significance ($p= 0.008$, $p= 0.020$, $p\leq 0.001$ & $p= 0.258$ for all four echo-types, respectively). All these significant differences were greater than the MDC. As expected, significant differences in the echopattern were observed between the SYM tendon and CON group, with the SYM tendon exhibiting a lower percentage of echo-type I, with elevations in echo-type II and III ($p\leq 0.001$, $p= 0.002$, $p\leq 0.001$, $p= 0.011$ for all four echo-types, respectively). All these significant findings were greater than the minimal detectable change.

Table 7.1- Demographic of participant with and without Achilles tendinopathy

	Participants with Achilles tendinopathy (n=21)	Participants without Achilles tendinopathy (n=6)
Age, mean ± SD (y)	30.3 ± 9.6	26.8 ± 7.3
Gender		
Male, n (%)	20 (95)	5 (83)
Female, n (%)	1 (5)	1 (17)
Professional athlete		
Yes, n (%)	11 (52)	3 (50)
No, n (%)	10 (48)	3 (50)
Symptoms present in		
Right, n (%)	10 (48)	N/A
Left, n (%)	11 (52)	N/A
Duration of symptoms, months, mean (min-max)	31.8 (0.5 – 120)	N/A

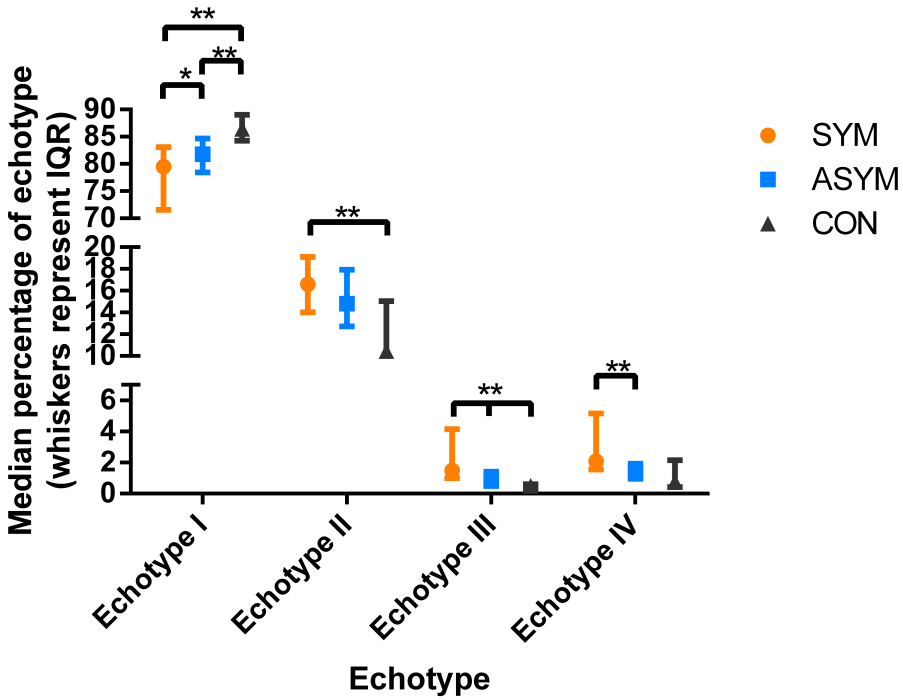

*Figure 7.1- Median ± interquartile range for all echo-types in the symptomatic (SYM), asymptomatic (ASYM) and control (CON) tendons. * indicates p< 0.01 **indicates p< 0.01 and the difference is greater than the MDC*

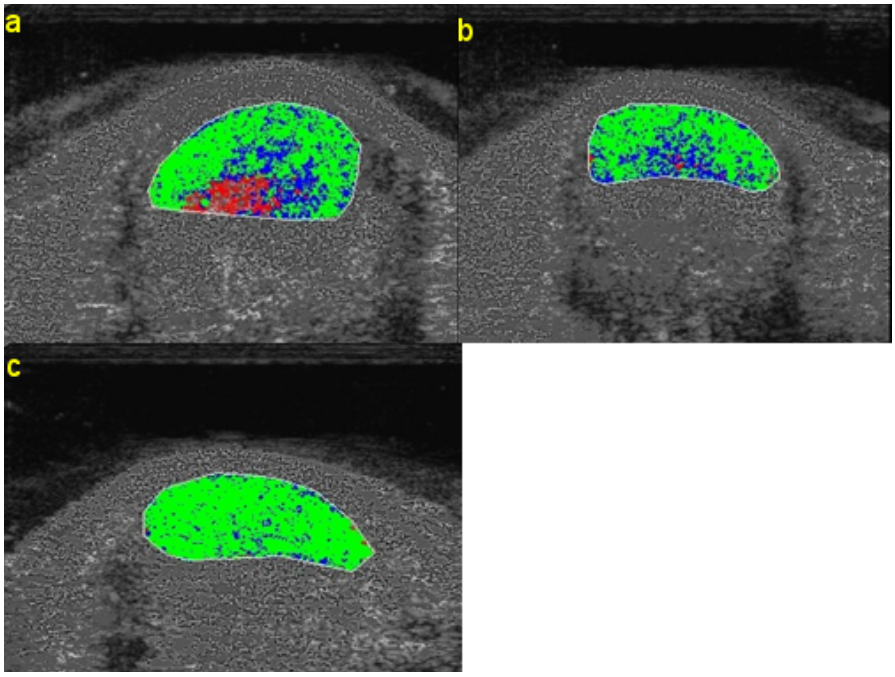

Figure 7.2- Example of a transverse UTC image of symptomatic (a), asymptomatic (b) and control (c) tendons. Green = echo-type I, blue = echo-type II, red = echo-type III, black = echo-type IV.

AP diameter was significantly larger in the SYM (median±IQR, 8.3±2.4mm) tendon in comparison to both the ASYM (median±IQR, 7.1±1.3mm) and CON (median±IQR, 5.9±0.7mm) group (p= 0.002 & p≤ 0.001, respectively), with the AP diameter also significantly larger in the ASYM tendon in comparison to the CON group (p= 0.001).

The dimension and structure of the SYM and ASYM tendon were compared between elite athletes and the recreational participants. No difference was observed in AP diameter or echopattern for either the SYM (p= 0.654, p= 0.468, p= 0.314, p= 0.756, p= 0.918, AP and the four echo-types, respectively) or ASYM tendon (p= 0.863, p= 0.387, p= 0.756, p= 0.349, p= 0.756, AP and the four echo-types, respectively) between the professional athletes and recreational participants.

7.5- Discussion

This study showed that both Achilles tendons in patients with unilateral tendinopathy were structurally compromised compared to normal tendons. This supports previous studies that have shown changes in tendon structure histologically in the unaffected contralateral tendon, providing further evidence for the bilateral nature of tendinopathy (Andersson et al, 2011; Cetti et al, 2003; Williams et al, 1984). This study confirms there is no direct correlation between structural disorganisation and the presence of symptoms.

A number of studies using conventional ultrasound have previously described the poor relationship between structure and pain with structural disorganisation being present in the absence of symptoms (Cook et al, 1998). Previous studies using UTC observed significant differences in the amount of normal tendon structure between symptomatic and asymptomatic Achilles tendons demonstrating good accuracy that is comparable to conventional imaging (Khan et al, 2003; van Schie et al, 2010). In this study, van Schie et al (van Schie et al, 2010) assigned an arbitrarily determined threshold (75% echo-type I and II combined) for normal tendon. However, three symptomatic tendons contained more normal tendon structure than this threshold and six asymptomatic tendons contained less. These findings suggest that there is a spectrum of tendon pathology that is not directly linked to symptoms. de Vos et al (de Vos et al, 2012) described a lack of correlation between improvements in tendon symptoms (quantified using a VISA-A questionnaire) and tendon structure, which remained the same after a 16-week eccentric exercise program. In the current study, differences in tendon structure were observed in the ASYM tendon in comparison to the CON group. The findings of this study and previous studies utilising UTC

provide further evidence that structural pathology within the Achilles tendon is heterogeneous and that there is no direct relationship between pathology and pain.

UTC has previously been compared to histopathological samples in the horse, while validation with human tendon samples has not been performed due to the limitations associated with collecting tissue. As the UTC scanning technique is different to that described in the original articles, making direct inferences on structure from the echopatterns in this study is not possible. Based on the continuum of pathology, these changes may be indicative of early pathological changes, such as tenocyte proliferation and increases in large proteoglycans (aggrecan and versican) that bind water within the extracellular matrix and separate tendon fibrils (Cook & Purdam, 2009; Samiric et al, 2004a).

The mechanism accounting for the compromise in tendon structure in the contralateral asymptomatic tendon remains unclear. Three potential mechanisms are hypothesised based on the literature. Firstly, biomechanical changes (eg altered gait) associated with the development of Achilles tendinopathy may overload other structures within the lower limbs. A number of studies have shown widespread biomechanical changes in Achilles tendinopathy patients, which may overload the contralateral tendon and induce changes in structure (Azevedo et al, 2009). Interestingly, changes in the ASYM tendon were no different between elite athletes and recreational participants, nor was the severity of changes different in the SYM tendon. As elite athletes are exposed to higher loads in comparison to the normal population, the finding that changes in the ASYM tendon are no different suggests that load and altered biomechanics may not be the sole driver of contralateral changes.

Secondly, degenerative changes within the contralateral tendon may be centrally or systemically driven. Andersson et al (2011) showed increases in tenocyte number and blood vessels in a unilaterally overloaded animal model of tendinopathy. These changes were associated with increases in the neurotransmitter substance P, which has been suggested to have a role in tenocyte proliferation and collagen reorganisation (Backman et al, 2011; Fong et al, 2013). Thirdly, these changes may be due to intrinsic factors, such as genetics, adiposity and waist circumference. These factors have been shown to be deleterious to tendon structure and increase the presence of symptoms and structural disorganisation within the tendon (Gaida et al, 2008). Understanding whether the changes observed in this study predispose the tendon to symptoms needs to be ascertained in future studies.

A number of studies have observed changes in tendon structure on imaging in individuals with no history of symptoms (Cook et al, 2000; Khan et al, 2003; Malliaras et al, 2012). In a cohort of volleyball players, patellar tendon abnormalities were observed in 23% of individuals with no history of anterior knee pain, with the majority remaining asymptomatic over a 12-month period despite ongoing imaging abnormalities (Malliaras & Cook, 2006). This phenomenon has also been reported for the Achilles (Giombini et al, 2013). Despite these findings, the presence of structural abnormalities on imaging has been shown to increase the risk of developing pain (Comin et al, 2013; Malliaras & Cook, 2006). These contradictory findings suggest that the relationship between structure and the development of symptoms is complex and not fully understood. In the current study it is difficult to suggest whether the subtle change in tendon structure observed within the ASYM tendon predisposes the tendon to symptoms in the future. Consideration and further

research is needed to ascertain whether contralateral changes in tendon structure require treatment to minimise the risk of developing symptoms.

Despite structural changes being present within the ASYM tendon, differences in the proportion and distribution of the echo-types representing disorganisation were observed in comparison to the SYM tendon. As expected, the ASYM tendon contained higher levels of normal tendon structure, represented by echo-type I, and less disorganised tissue (echo-types III and IV). Subjectively, the high proportion of echo-type III and IV within the SYM tendon was often localised to a focal area within the tendon, while these echo-types were diffusely spread throughout the ASYM tendon suggesting the presence of subtle disorganisation only observed on UTC. However, caution is advised in extrapolating these results to suggest that significant disorganisation and a focal lesion are required for the presence/development of symptoms.

There are a number of limitations associated with this study. Firstly, this study is limited by a lack of variability in the sample population in regards to the age and gender. The high proportion of men in the Achilles tendinopathy group may be due the young age of the sample population, with higher incidences of Achilles tendon injury in males in their 2nd to 4th decade of life (Clayton & Court-Brown, 2008; Maffulli et al, 1999). Future investigation is warranted to determine whether this bilateral phenomena occurs in the entire population. Secondly, as this study was a pragmatic study within the clinical setting, the patients were heterogeneous with varying severity of clinical symptoms and UTC scans taken at various time points in relation to their clinical presentation, with some participants already undertaking various rehabilitation programs (eg platelet-rich plasma, corticosteroid and conservative management) with varying time intervals. These treatments may have had

a positive/negative effect on tendon structure, yet as the majority of these treatments would not have been performed on the ASYM tendon it is unlikely to have an effect on the findings in comparison to the CON tendon.

It is important to state that the UTC technique in this study is different to that described in the original articles (de Vos et al, 2012; van Schie et al, 2010), with the critical differences between UTC scanning techniques being outlined previously (Rosengarten et al, 2015). It is suggested that this data should not be used as reference data unless the scanning techniques and protocols are identical.

7.6- Conclusion

Changes in tendon structure are present within the contralateral tendon in unilateral Achilles tendinopathy patients. UTC allows for the detection and quantification of this subtle disorganisation, where conventional imaging modalities may not be able to detect these changes in the internal architecture of the tendon. Long-term follow-up of these participants will identify whether these subtle changes in tendon structure increase the risk of developing tendinopathy and whether future clinical assessment and treatment protocols need to account for structural change in the contralateral tendon, in the hope of reducing the high proportion of patients developing bilateral symptoms.

7.7- Acknowledgements

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Chapter eight
General discussion

8.1- The normal tendon and its response to load

Chapters 3 to 5 focused on the response of the structurally normal tendon to load. The concept that normal tendon responds to load is not a new one and has been investigated in *in vitro* and *ex vivo* studies using animal and human tissue. *In vitro* studies have used cell (Backman et al, 2011; Li et al, 2004; Wang et al, 2003) and tissue (Arnoczky et al, 2008; Bayer et al, 2014; Lavagnino & Arnoczky, 2005) cultures to examine alterations in gene expression and protein production in response to the addition or removal of load. The changes observed include cell shape and activation with changes in signalling cytokines (VEGF, COX-2, TGF-α and β, prostaglandin-2, IL-1)(Devkota & Weinhold, 2010; Li et al, 2004; Maeda et al, 2011; Mousavizadeh et al, 2014; Thorpe et al, 2014), ECM proteins (collagen I & III, GAG's, PGs, digestive proteins such as matrix metalloproteinases and collagenases)(Arnoczky et al, 2008; Arnoczky et al, 2004; Asundi & Rempel, 2008; Lavagnino & Arnoczky, 2005; Li et al, 2004; Maeda et al, 2009; Robbins et al, 1997; Screen et al, 2005a), cell death (Devkota & Weinhold, 2010) and cell properties (cilia length and deflection angle, cytoskeleton)(Gardner et al, 2011; Lavagnino et

al, 2011; Ralphs et al, 2002). The majority of these studies have shown that the resident tenocyte can detect mechanical stimuli and respond within 24 hours. The advantages of using *in vitro* and *ex vivo* studies are that they allow for investigations at the tissue and molecular level and standardisation of mechanical stimuli. However, the relevance of these studies to clinical tendinopathy has been questioned due to the application of non-physiological loads that focus on a single aspect of the tendon (ie gene expression of certain cytokines)(Arnoczky et al, 2007), resulting in difficulty in extrapolating these findings to the whole tendon organ. Investigation of the entire tendon using more clinical outcomes, such as imaging, may allow for improved clinical translation.

The increased use of US and MR imaging for tendons and improvement in the technology supporting these imaging modalities have allowed investigation of the short-term load response of tendons. Chapters 3 and 4 found that the normal tendon responds to maximal load within 24-48hrs and returns to baseline 72-96hrs post-exercise. However, the temporal nature of these changes observed in the current studies differs to previous studies. Changes in AP diameter (Grigg et al, 2010; Wearing et al, 2013b), blood flow (Boesen et al, 2011; Pingel et al, 2013a; Pingel et al, 2013b) and water content (Syha et al, 2014) were observed immediately after various types of exercise that tended to return to baseline within 24 hours (section 2.6.4). For example, Grigg et al (2009) reported decreases in AP diameter of the Achilles tendon after eccentric exercise, which recovered within ~2.5hrs post-exercise. In pilot testing for Chapter 3, UTC images of the SDFT were taken within 2 hours of the competitive race. No change in the overall echopattern was observed compared to baseline so this time point was not examined further. The differences in the temporal-response between Chapters 3 and 4 and previous studies may be due to the

differing tendon features investigated between studies (ie AP diameter and blood flow compared to stability in the echopattern quantified by UTC).

Ultrasound tissue characterisation differs to previous imaging modalities that have investigated tendon response to load as it quantifies the stability of the echopattern allowing for inferences to be made about the structural integrity of the tendon. Research performed on equine histopathological samples found that UTC echo-types were able to distinguish between the different tissue types (normal, granulation and fibrotic tissue) where basic grey level statistics could not (van Schie et al, 2000; van Schie et al, 2003). However, it is not possible to suggest that one UTC echo-type solely represents one structural or composition component. For example, changes in tendon fibre alignment is not solely dependent on the collagen fibrils but can be influenced by changes in the ground substance (ie water, glycoproteins and PGs)(Screen et al, 2005b). To better understand the changes seen in UTC echopattern comparison with *ex vivo* and *in vitro* studies is needed to put in context the possible structural and compositional changes observed within the tendon.

Based on molecular biology investigations, the alteration in the UTC echopattern and temporal nature of these changes observed in Chapter 3 and 4 are unlikely to be due to an increased turnover or alterations in collagen fibrils. A number of studies have shown increases in markers of collagen synthesis (carboxy-terminal propeptide of type I collagen; PICP) within the tendon and peritendinous space 24 hours post-exercise that remains elevated for at least 72 hours (Langberg et al, 1999b; Miller et al, 2005; Olesen et al, 2007). However, while this indicates an increase in the amount of synthesised type I collagen it does not necessarily suggest that the newly synthesised collagen is integrated into the

matrix (Kjaer et al, 2009). In various tissues, newly synthesised collagen can be degraded both by intra- and extra-cellular pathways (Laurent, 1987). While intra-cellular degradation has not been quantified in tendon, this process has been shown to degrade up to 90% of newly synthesised collagen in other tissues. Collagen may be more susceptible to degradation prior to the development of covalent cross-linking, with the development of these cross-links in mature fibrils protecting the fibrils from degradation (Kjaer et al, 2009; Laurent, 1987).

While collagen synthesis markers have been shown to be elevated in response to exercise, concomitant changes in markers for collagen degradation (carboxy-terminal telopeptide region of type I collagen; ICTP) and digestive enzymes have been shown (Koskinen et al, 2004). Langberg et al (1999b) observed a decrease in ICTP immediately post-exercise in the peritendinous space that returned to normal 72 hrs post-exercise. Olesen et al (2007) repeated the exercise protocol of the Langberg et al (1999b) study yet sampled at more time points (baseline and immediately post-exercise, 24, 48, 72 and 96 hrs post-exercise). Similarly, the mean ICTP level within the peritendinous space was increased in the exercise group compared to a control group. Frustratingly, changes in ICTP were not described at each time period so little is known about the temporal nature of this response. Magnusson et al (2010) suggested that the balance between collagen synthesis and degradation in response to exercise is to maintain tissue homeostasis. As the temporal nature of collagen synthesis and degradation may be different, insufficient recovery may lead to an imbalance in the synthesis and degradation of collagen and an alteration in collagen content (fig 8.1).

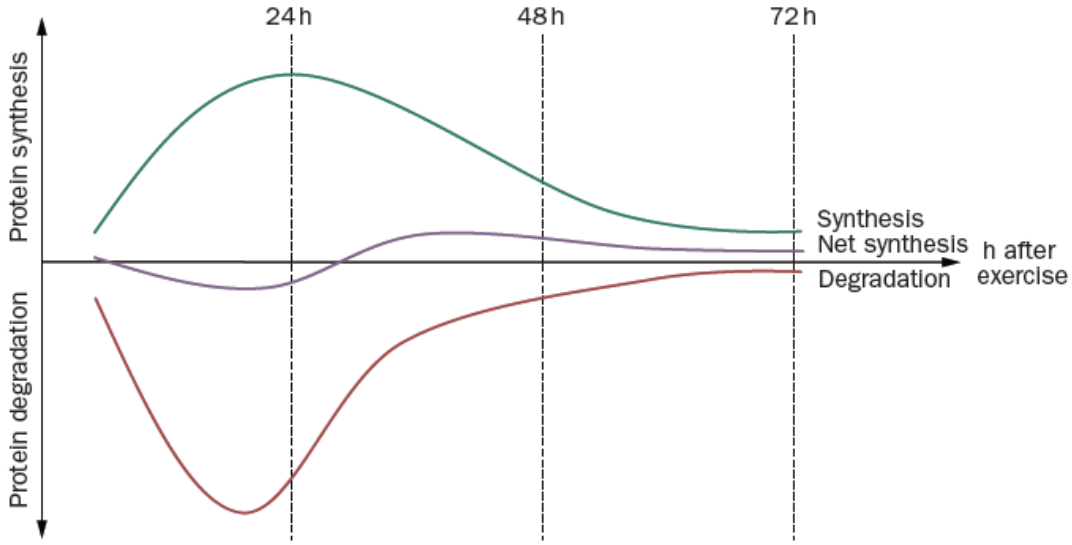

Figure 8.1- Schematic representation of collagen synthesis and degradation to acute exercise, with an increase in both collagen synthesis and degradation. A net loss of collagen is suggested within the first 24-36hrs, which is followed by a net synthesis 36-72hrs after exercise. Repeated training with rest periods to short may result in a net degradation of the matrix (adapted from Magnusson et al (2010) with permission).

It is important to iterate that changes in collagen synthesis and degradation markers discussed above may not necessarily result in a change in collagen content or collagen turnover. The half-life of collagen within equine tendon has been suggested to be as high as 200 years, with little collagen turnover after skeletal development in the human Achilles (Heinemeier et al, 2013; Thorpe et al, 2010). In this context, authors reporting elevations in collagen synthesis markers suggested that the synthesis of large collagen proteins post-exercise may be broken down relatively quickly and not integrated into the tendon matrix, especially in the mature tendon.

The lack of collagen turnover after skeletal maturity may explain the conflicting results seen in changes in tendon dimensions in response to exercise. Several cross-sectional imaging studies have found that high habitual tendon loading results in greater tendon dimensions (Couppe et al, 2008; Kongsgaard et al, 2005; Magnusson & Kjaer, 2003).

Similarly, experimental studies that have investigated changes in tendon dimension in response to cumulative exercise (ie weeks) have reported conflicting results, with both increases and no change in tendon dimensions (table 2.4)(Carroll et al, 2011; Kongsgaard et al, 2007; Reeves et al, 2003a; Reeves et al, 2003b; Seynnes et al, 2009; Standley et al, 2013). These conflicting results may be due to the different age of participants. Studies that recruited young people (20-24 years old) consistently reported increases in tendon dimensions in response to heavy resistance training (Kongsgaard et al, 2007; Seynnes et al, 2009), studies with older participants showed no change in tendon cross-sectional area (67-74 years old) (Carroll et al, 2011; Reeves et al, 2003a; Reeves et al, 2003b; Standley et al, 2013).

Studies investigating short-term changes (≤72 hours) in tendon dimensions have reported either no change or a decrease in AP diameter in response to loading suggesting that collagen content does not alter appreciably immediately post-exercise (Freund et al, 2011; Grigg et al, 2009). Chapters 3 and 4 did not investigate tendon dimensions, rather they investigated tendon response in terms of the stability of the echopattern. It appears unlikely that the changes in the UTC echopattern are due to remodelling of collagen fibres. However, the non-collagenous component of tendon is turned-over rapidly with a half-life of ~2 years (Thorpe et al, 2010). This rapid turnover is highlighted particularly by the large PGs, such as aggrecan and versican, which are synthesised within days of loading (Koob et al, 1992; Robbins et al, 1997) and catabolised rapidly (half-life ~2 days)(Samiric et al, 2004b). The temporal-response of these large PGs are similar and may be responsible for the changes in UTC echopattern observed in Chapters 3 and 4. As large PGs attract water in to the tendon, this transient response may reduce stress within the tendon.

While tendon load is primarily discussed as a tensile force, some authors have suggested that as a result of tensile load there is an internal shear stress due the movement of water perpendicular to the long axis of the tendon (Helmer et al, 2006; Lavagnino et al, 2008). This perpendicular shear stress may exert a force on the tenocyte that is dependent on the load amplitude not the amount of tensile strain (Appendix O)(Lavagnino et al, 2008). Critically, increases in large PGs at the enthesis have been shown to slow the dissipation of fluid, reduce shear stress and protect the ECM (Wren et al, 2000a). Not only may this increase in large PGs protect the ECM matrix from mechanical load, but also the tenocyte. Research in cartilage has shown that hyaluronan, which links large PGs, is bound to the cell via the binding receptor CD44 (Knudson & Knudson, 2001). The deposition of aggrecan around the cell may be an attempt to protect the cell and slow the dissipation of fluid around the cell, and as a result stiffen the ECM (Wren et al, 2000b).

There has been considerable evidence, both *in vivo* and *in vitro*, showing that tendon are responsive to load in the short-term (section 2.4 and 2.6.4). While changes in various components within the tendon have been observed, few investigators have been able to establish the role of these responses and its implications for the health of the tendon. In the preparation of Chapters 3 and 4, considered evaluation was undertaken for the terms used for this response. From the data, we were unable to term this as a positive or adaptive response as there was no long-term evidence to suggest that the tendon was stronger or more load tolerant. Conversely, we could not state that the response was pathological or negative to the health of the tendon for the same reason. This response appears to occur when the tendon is maximally loaded. The control group in Chapter 3 was a pragmatic control, with these horses still loading but well within the capacity. Additionally, preliminary

data was unable to detect a tendon response using UTC in recreational runners that partake in a non-competitive run within their capacity, as well as in the patellar tendon in adolescent volleyball players (van Ark et al, 2015; Wong et al, 2014). As such, this response from the tendon may need to be considered in the development of loading programmes. Future studies are required to determine whether this short-term response effects to long-term health of the tendon.

Magnusson et al (2010) published a review on the pathogenesis of tendinopathy and the importance of balancing the response to loading (fig 8.1). The authors discussed the role of cumulative load on the tendon from the perspective of collagen synthesis and degradation and suggested that insufficient rest would give preference to catabolic processes within the tendon (fig 8.1). While this may not be relevant for the mature tendon due to the focus on collagen synthesis and degradation, it is worth considering as it highlights the importance of tendon recovery and the return to homeostasis after loading. Ristolainen et al (2014) showed that endurance athletes (cross-country skiing, swimming, long-distance running) who had less than 2 days rest per week were 5.2 times more likely to develop an overuse injury. Interestingly, total volume of training was less of a risk factor for the development of overuse injuries (training over 700 hours per year increased risk 2.1 fold). While this was not specific for tendons, it illustrates the concept that connective tissues require a period of rest to respond positively to load. Future studies are needed to understand the effect of repeat loading (both within and after the temporal tendon response) on the structure of the tendon and the development of clinical symptoms.

Chapter five focused on understanding how the Achilles tendon responds to cumulative high load over a five month pre-season training programme. It potentially highlights the paradoxical nature of load (positive/adaptive versus negative/maladaptive/pathological) and its effect on tendon structure. The decision to investigate Achilles tendon structure over the course of the pre-season was chosen due to the high prevalence of Achilles tendon injuries in this period and the possibility that normal tendons can transition into a pathological state (Woods et al, 2002). A few clinical studies have shown that repeated controlled loading can elicit a positive response from the pathological tendon in the long-term (ie years)(Ohberg et al, 2004; van der Plas et al, 2012). However, little research has investigated the response of the normal tendon to cumulative exercise due to limitations associated with conventional imaging modalities. The improvement in the overall UTC echopattern may be due to changes in the more metabolically active interfascicular matrix (Screen et al, 2005b; Thorpe et al, 2013)(the histological features that may have been responsible for this change in UTC echopattern are outlined in section 5.6).

The improvement in the UTC echopattern supports the concept of an adaptive pathway in the continuum of tendon pathology (Cook & Purdam, 2009). Similarly, the improvement in UTC echopattern is contrary to the collagen-tearing models that suggest microtears occur at physiological loads. From this study, we are unable to definitively state that an improved load tolerance and decrease in the risk of developing pain existed. However, these 15 players remained asymptomatic over the course of the season based on the clubs medical records possibly suggesting that these players' tendons have adapted to deal with the high maximal load associated with elite Australian football.

While the majority of athletes remained asymptomatic and their UTC echopattern improved, 3 out of the 18 players developed Achilles tendon pain and a UTC echopattern consistent with tendon pathology. It can be suggested that these previously normal tendons have progressed along the continuum model to the reactive or tendon dysrepair state (section 2.3.3). This transition along the continuum model suggests that there may be a tipping point where load is deleterious to tendon health. *In vitro* research has shown that the amount of cyclic tensile strain applied to tissue is critical. Wang et al (2013) showed that a cyclic strain of 6% maintained the structural integrity and cellular function of tendon tissue within a bioreactor system, where 3% and 9% resulted in matrix deterioration and altered expression levels. There appears to be loading parameters that are beneficial to the tendon where under or overload of the tendon results in a disruption of the homeostatic mechanisms within the tendon. However, the application of load may not be the sole factor in the determining whether a positive or negative response is elicited.

While the continuum model states that load is critical and essential to the development of tendon pathology, it is not the only factor that influences the risk of moving negatively through the continuum model. This was demonstrated by two studies that used similar loading protocols in rat Achilles tendons and reported differing response from the tendon: pathological changes by Glazebrook et al (2008) where improvement was shown by Heinemeier et al (2012). Chapter 5 was unable to determine other factors that may have contributed to these differing responses due to the homogenous nature of the participants and the small sample size. Factors such as baseline tendon structure (Comin et al, 2013), person factors (older age (de Jonge et al, 2011b), other tendon injuries) and systemic factors (genetics (September et al, 2009), adiposity (Gaida et al, 2009; Malliaras et al, 2007)) have

been shown to increase the risk of developing Achilles tendinopathy. Larger prospective studies are required to better understand the concomitant risk factors that result in differing responses to load.

8.2- Understanding the features of the pathological tendon on UTC

The imaging features of the pathological Achilles and patellar tendon have been described extensively (Adams et al, 2010; Kainberger et al, 1990; Khan et al, 1997; Khan et al, 2003; Nehrer et al, 1997; Warden et al, 2007). On US these include the presence of focal or diffuse thickening, hypoechogenecity, Doppler signal and calcification. As UTC utilises conventional US, many of the same features can be observed in the conventional grey scale US mode of UTC (ie changes in tendon dimensions, hypoechogenecity and calcification). However, UTC allows for the capture of a three-dimensional grey scale image and the semi-quantification of the stability of the echopattern that allows further investigation of the features of tendon pathology.

The continuum of tendon pathology describes the progression of the pathological tendon through three states; reactive, dysrepair and degenerative. While it is a continuum, these three stages are differentiated on imaging primarily by the type and extent of pathological changes within the tendon. Reactive tendon is described as exhibiting an increase in tendon thickness and sometimes slight diffuse hypoechoic changes within the tendon. The tendon in dysrepair also appears thickened, and small focal areas of hypoechogenecity become apparent. Degenerative tendon pathology has well circumscribed hypoechoic areas and increased Doppler signal. While the continuum also

allows for the classification of differing pathological states, it also provides a framework on the likelihood of tendon normalisation over time in response to treatment.

Evidence for the continuum has been provided from prospective imaging studies using US have characterised the both the transition and stability of the pathological tendon over time. Malliaras et al (2010) demonstrated the limited capacity of the degenerative tendon to return to normal. Previous research utilising UTC supports the concept that improvements in tendon structure are limited in the degenerative tendon. de Vos et al (2012) reported that a 16-week eccentric loading program did not alter tendon structure despite improvements in pain and function. The inability of the degenerative area to transmit and detect mechanical stimuli may be responsible for this lack of normalisation over time.

Arnoczky et al (2007) discussed the role of mechanobiology (the understanding of how mechanical stimuli regulate biological processes) in the aetiopathogenesis of tendon pathology and questioned whether over-stimulation or under-stimulation of the tenocyte with mechanical stimuli is critical. *In vitro* studies have shown that over-stimulation of the cell can lead to the release of signalling cytokines and degenerative changes (Almekinders et al, 1993; Archambault et al, 2002; Archambault et al, 2001; Banes et al, 1999; Skutek et al, 2001b; Tsuzaki et al, 2003; Wang et al, 2003). This mechanical over-stimulation and response of the cell may correspond to the early reactive changes proposed in the continuum. Of interest, under-stimulation of the tenocyte can also produce a negative response from the tendon (Arnoczky et al, 2004; Egerbacher et al, 2007; Lavagnino & Arnoczky, 2005; Lavagnino et al, 2006; Lavagnino et al, 2005). The early reactive response may be the tenocyte's attempt to reduce the mechanical stimulation experienced by the cell

by depositing large PGs (resulting in a decrease in water permeability and reduction in shear stress). Continuing overload and response from the tenocyte could lead to a situation where the cell has protected itself from mechanical stimuli and the fibrillar matrix is sufficiently disrupted. This environment could result in the under-stimulation of the cell, initiating a catabolic response and the progression of the pathological lesion. Critically, in this area of fibrillar disorganisation the inability of the disorganised fibrils to transmit the mechanical stimuli to the cells may lead to a 'mechanically-silent' area of the tendon and be responsible for the inability of the pathological area to remodel.

Not only does mechanical stimuli initiate a tendon cell response, it may also be critical in our understanding of the progression of tendon pathology. The initial over-stimulation of the cell, response and resulting matrix disorganisation may result in the under-stimulation of the cell and the progression of tendon pathology. The lack of mechanical stimuli on the cell continues the response from the cell and leads to the propagation of the pathological lesion. This concept would be supported by experimental evidence in the SDFT of horses where the US and histological appearance of tendon pathology (increased deposition of non-collagenous matrix and immature collagen, increased separation and less densely packed fibroblast nuclei) was observed to worsen up to six weeks post-injury despite little mechanical load (confined to box rest)(Schramme et al, 2010). Inferences from this study may be limited as it was a surgical model of injury, however little research on the natural history of tendon pathology exists due to methodological and ethical issues. However, it may support the concept that once there is disruption of the homeostatic mechanisms and tendon integrity, continuing a negative response from the tendon independent of continuing load.

The loss of normal tendon architecture within the pathological area can lead to this area being stress-deprived. With little tensile load being placed on this area, the cell may not receive the necessary mechanical stimuli to remodel this pathological area. The concept that the area of pathology may be under-stimulated, or 'mechanically-silent', may also help explain the limited capacity of the pathological tendon to normalise. Thornton & Hart (2011) proposed that non-resolving chronic pathology can be present within the tendon with considerable matrix turnover without the formation of mature tendon that is associated with acute wound healing. Clinical evidence supports this concept as the degenerative tendon (presence of hypoechoic area on US) is unlikely to return to normal (Malliaras et al, 2010) and controlled exercise had little effect on the UTC echopattern of the pathological tendon (de Vos et al, 2012). With the pathological area unable to transmit normal tensile load, the tendon needs to compensate in the surrounding tendon.

Chapter 6 provides evidence for how the pathological Achilles and patellar tendon may compensate for the 'mechanically-silent' area of pathology. It provides evidence that is contrary to models of tendon pathology that suggest the limited ability of the tendon to remodel is responsible for the failure of the tendon. The critical finding of this study was that the pathological Achilles and patellar tendon exhibited significantly greater mean cross-sectional area (mCSA) of aligned fibrillar structure, as quantified by UTC, compared to the structurally normal tendon. These cross-sectional data also showed that the pathological tendon exhibited a greater mCSA of disorganised structure compared to normal tendon. The amount of pathology was variable but a significant linear relationship was observed between tendon dimensions (AP diameter and total mCSA) and pathology for both the pathological Achilles and patellar tendon. Although limited by the design of this study, the

pathological tendon compensates for areas of disorganisation by increasing in tendon dimensions to ensure there is a sufficient mean CSA of aligned fibrillar structure.

While the findings of Chapter 6 are novel, increases in tendon dimension have been shown in the older, structurally and mechanically altered tendon. Magnusson et al (2003) observed an increase in Achilles tendon CSA at the midsubstance of elderly women (mean=79 years). This increase in tendon dimensions was hypothesised to counteract the reduced tendon quality in the older tendon and result in reducing stress within the tendon and thereby increasing the safety margin (point where mechanical stress results in tendon rupture) (Kjaer et al, 2005; Magnusson et al, 2003). Frustratingly, the MR images were not described and information on whether the older tendon exhibited an altered appearance is unknown.

An understanding of the pathological tendons potential to compensate for an area of pathology may be critical. Various theories of tendon pathology suggest that the accumulation of micro-trauma within the tendon overwhelms the reparative/remodelling capacity of the tendon and can lead to tissue failure (Abate et al, 2009; Leadbetter, 1992). The progressive loss of aligned fibrillar structure, or critically a lack of remodelling, does not appear to be a feature of tendon pathology. The pathological tendon appears to abandon attempts to normalise the pathological area and compensates with an increase in tendon dimensions with amounts of aligned fibrillar structure allowing the pathological tendon to still tolerate tensile load.

Structurally, the pathological, degenerative tendon appears to have compensated for the area of pathology, but what factors limit clinical improvement in pain and function remain unclear. Reduced capacity in associated muscles, altered biomechanics of the lower

kinetic chain and alterations in the central nervous system have been suggested to contribute to the clinical presentation of tendinopathy. However, alteration in tendon structure and cell activity is still a critical factor in the clinical presentation of tendinopathy. Thornton & Hart (2011) suggest that reduced load in the degenerative portion may result in overload in the remaining normal portion of the tendon. The continuum of tendon pathology accounts for this possibility by suggesting that a reactive response may be present adjacent to the degenerative portion of the tendon. As the pathological tendon has sufficient aligned fibrillar structure and a cellular reaction within the normal portion of the tendon being a potential contributor to pain, treatments may be better suited at focusing on the normal portion of the tendon.

The limited ability of the tendon to remodel and the lack of evidence that tendon remodelling is required for clinical improvement (de Vos et al, 2012; Drew et al, 2014) suggests that solely focusing on the pathological lesion is unwarranted. Controlled exercise programs, such as eccentrics and isometrics, have consistently been shown to have a positive effect on tendon pain and function (Alfredson et al, 1998; Frohm et al, 2007; Rio et al, 2013). These interventions may be responsible for reducing over-stimulation and building capacity in the increased amounts of aligned fibrillar structure. In this context, stability in tendon structure accompanied by improvements in pain and function can be considered a positive outcome. While improvements in tendon structure may be observed (Shalabi et al, 2004), the expectations of the clinician and patient should be that structure of the degenerative tendon may never return to normal. Future research is needed to investigate differences in clinical outcome when tendon pathology remains stable, improves or worsens.

It is unclear when and how this possible compensation and adaptation occurs, as the study in Chapter 6 was cross-sectional. Also, we were unable to determine the compositional and structural changes that were responsible for this adaptation. Adaptation to load is fundamental to tissue biology, yet our understanding of how this is orchestrated in the matrix of pathological tendons requires further research. Heinemeier et al (2013) demonstrated that collagen within normal tendon is relatively inert, although whether this is the case in tendon pathology is yet to be determined. There is considerable evidence that the content and composition of collagen differs significantly in the pathological tendon (de Mos et al, 2007; Eriksen et al, 2002; Jarvinen et al, 1997; Maffulli et al, 2000b; Paavola et al, 2002; Riley, 2005; Riley et al, 1994). Potentially this alteration in tendon collagen may result in the formation of new aligned fibrillar collagen adjacent to the area of disorganisation. However, while the area adjacent to pathology may appear to have normal fibrillar alignment, the composition of the tendon may be altered.

Changes in cellularity (increased number and cell rounding) and PG content have been observed adjacent to areas of pathology, despite the appearance of normal echogenicity on US or fibrillar alignment histologically (Movin et al, 1998; Smith et al, 2008). These increases in PG content can alter the tendon and collagen morphology. Screen et al (2005b) showed the associated GAG side chains of aggrecan and other PGs can have a significant effect on the appearance of tendon fascicles. Incubating tendon explant in phosphate-buffered saline resulted in an increase in the proportion of large diameter fascicles (greater than 400 μm) and interfascicular space with no change in collagen content. Treating the explant with chondroitinase, which digested 90% of GAG's, resulted in the reversal of this finding suggesting that the hydrophilic nature of GAG's can bind water and

alter the dimensions of the tendon fascicle and interfascicular matrix. In Chapter 6, the increases in aligned fibrillar structure may be mediated by alterations in GAG content rather than the integration of new collagen fibres. Further research is needed to ascertain the structural and compositional changes that are responsible for this adaptation. Whatever the potential changes, it is likely that the influx of tendon cells, which is a feature of early tendon pathology (Cook et al, 2004a), is responsible for mediating this response.

8.3- UTC as a research and clinical tool compared to other imaging modalities

Ultrasound tissue characterisation was the primary outcome used in this PhD. Improvement in imaging and computing technology has allowed for the advent of high resolution imaging of musculoskeletal structures and the development of semi-quantifiable imaging techniques. A number of new techniques have been developed for the imaging of tendons.

US elastography is a technique that evaluates the mechanical properties of tissues. Tendon pathology alters mechanical properties and US elastography may improve the diagnostic capability of conventional US. US elastography is based on the principle that tissue displacement in response to external compression produces “strain” within the tissue (Klauser et al, 2010). The strain is higher in softer tissue than harder tissue. A visual strain map overlaid on the conventional B-mode image provides a visual representation of the relative stiffness of tissues (De Zordo et al, 2010; De Zordo et al, 2009; Ooi et al, 2014; Tan et al, 2012). While in its infancy in terms of research and clinical use, preliminary research has shown improved sensitivity (0.96), specificity (0.95) and accuracy (0.98) when combining B-mode US and US elastography (Ooi et al, 2014). However, similar to conventional grey

scale US, the repeatability and reliability of this imaging modality has been questioned (2-dimensional image and user-dependency).

Various techniques associated with MR imaging have been used to quantify intratendinous signal and improve correlation with histopathological features. Shalabi et al (2002) compared dynamic gadolinium contrast-enhanced MR imaging against histopathological specimens from 15 Achilles tendons with tendinosis. Intratendinous signal was found to correlate significantly with the severity of tendon pathology. Interestingly, an increased number of rounded cells also correlated with intratendinous signal. These findings suggest that despite the resolution of MR being too low to detect cell morphology, it is able to detect the consequences of this altered cell phenotype. Magnetisation transfer contrast imaging using ultrashort echo time MR imaging have been proposed to allow for the indirect quantification of the hydration level of the tendon and has been shown to be able to reliably differentiate between healthy and degenerated tendons (Grosse et al, 2013; Syha et al, 2011). While these techniques have merit and good-to-excellent reliability and repeatability, MR imaging is costly, of limited availability, time consuming and a laborious technique (especially when using contrast agents).

The technique of UTC accounts partly for some of the imaging limitations stated above. Ultrasound's superior spatial resolution of fibrillar alignment allows for better imaging of the internal architecture of the tendon. However, the user-dependency of US, especially relating to transducer tilt and rotational angle, has been shown to create imaging artefacts that negatively affect repeatability. The use of the UTC tracking unit standardises these parameters by holding the transducer in a fixed position perpendicular to the long axis of the tendon and improves the repeatability. All Chapters within this PhD have included

repeatability measures and have included minimum detectable differences for the UTC quantified parameters where appropriate. These minimum detectable differences have highlighted the good-to-excellent repeatability of UTC.

The four UTC echo-types correspond to the stability of pixel brightness over contiguous transverse images. Echo-type I and II, sometimes termed structure-related echoes, are generated when the ultrasound wave hits one interface and is reflected at a consistent angle, resulting in one reflection that is stable over contiguous transverse images. Interfering echo-types (echoes III and IV) that exhibit a lack of stability over contiguous images result from inconsistencies in the angle of reflection of the ultrasound wave due to multiple interfaces. The standardisation of gain, depth and focus point and the use of relative measures of pixel brightness (ie stability over multiple transverse US images) also contribute to the high repeatability of UTC and allow for inferences to be made about tendon structure and fibre alignment.

As mentioned throughout, the four UTC echo-types have been validated and compared to histopathological specimens from normal and naturally-occurring tendon pathology in horses (van Schie et al, 2000; van Schie et al, 2003). Precise superficial digital flexor tendon sections corresponding to the transverse UTC image were compared. Each tendon was subjectively classified as normal, necrotic, early granulation, late granulation, early fibrotic, late fibrotic and scar tendon tissue based on previously published classification (van Schie et al, 2000; van Schie et al, 2001). These classifications were histopathological criteria based on the extent of pathological changes relating to cellularity, fibrosis, oedema and vascular proliferation. It was found that the UTC-generated echopattern could differentiate these tissue types where basic grey level statistics could not

(van Schie et al, 2000; van Schie et al, 2003). While these data are promising and show the UTC has the ability to differentiate between different tissue types, making inferences on the UTC echopattern about tendon structure needs to be considered.

While normal tendon appears to have a relatively simple ultrastructure, a number of components (collagen, PGs, water, infiltration of blood vessels) can affect tendon architecture. The four UTC echo-types cannot be a directly correlated to individual structural changes as many variations in composition may be responsible for changes in tendon architecture. While in a different imaging modality, the histopathological validation work by Shalabi et al (2002) supports this concept. While signal intensity on gadolinium contrast-enhanced MR imaging was significantly correlated with the semi-quantitative score of total tendon pathology, none of the other parameters showed a significant correlation (fibre structure and arrangement, vascularity, GAG stainability). Inferences can be made on the specific histopathological changes responsible for changes in UTC echopattern based on previous research. However, caution is advised making definitive statements in relation to the compositional changes.

What can be stated with some confidence from how the UTC classifies the echopattern and the validation work in horses, is that echo-types I and II are generated by tendon bundles that are aligned. While the area containing echo-type I and II may contain alterations in the cellularity (both number and cell morphology) and ground substance, the stability of the echopattern being generated from one US reflection suggests that this area contains relatively aligned tendon bundles. Echo-types I and II are generally confined to areas of normal echogenicity and aligned fibres can be ascertained on the matching transverse grey scale image (fig 8.2). Conversely, echo-types III and IV are more likely in areas of hypoechogenecity where previous research using histopathological specimens have shown disorganised altered fibrillar structure (Movin et al, 1998). The validation work of van Schie et al (2003) supports this with high levels of echo-type I and II in normal tendon with

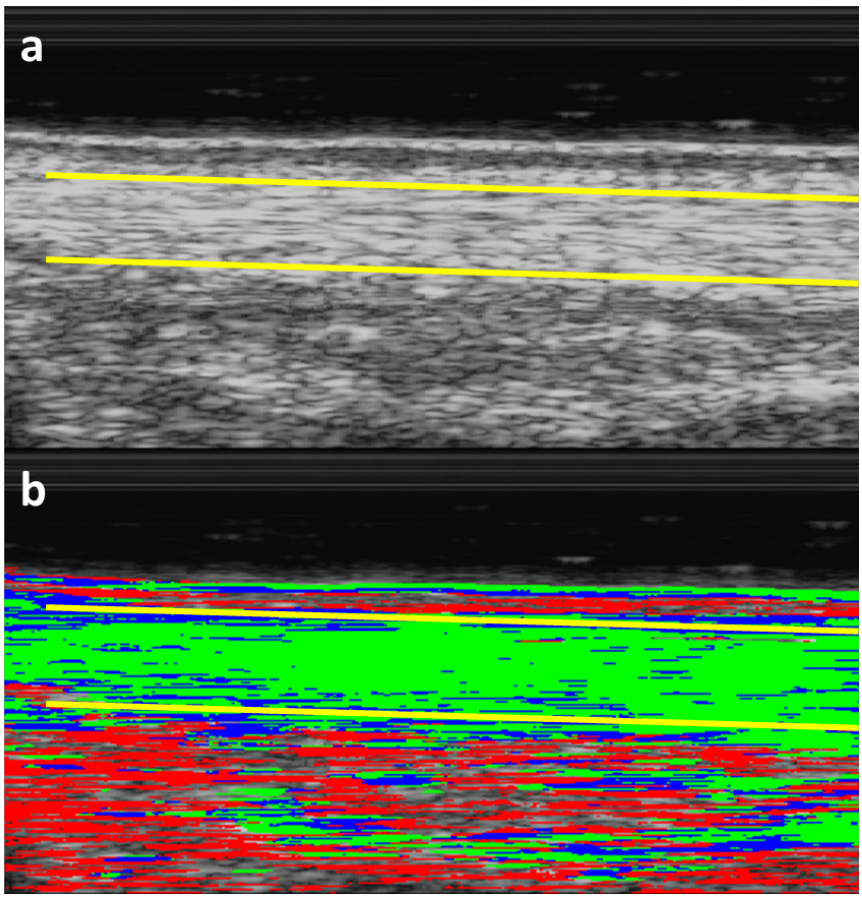

Figure 8.2- Corresponding sagittal a) grey scale and b) UTC echopattern images of a normal Achilles tendon. Aligned fibrillar structure can be observed in areas that correspond with high proportions of echo-type I (green) and echo-type II (blue). Yellow lines indicate the dorsal and ventral borders of the tendon.

increases in the remaining echoes observed in pathological tissues in horses.

A criticism of UTC is that the histopathological validation work has only been performed in horses. While an acknowledged limitation of UTC, this limitation does not warrant the dismissal of UTC as an imaging modality for tendons. Work comparing human tendon tissue to the UTC echopattern is limited due to ethical issues and difficulties in collecting both normal and pathological tissue of varying degrees of disorganisation. The structural similarities between horse and human tendons (both normal and pathological) have been described in the literature (Kristoffersen et al, 2005; Patterson-Kane et al, 2012). Future research looking into this relationship, while not impossible, will facilitate interpretation of research using UTC. Similarly, classification of tissue structure should be based on quantifiable measures (ie the semi-quantitative Bonar score that quantifies extent of pathology (Cook et al, 2004a)) rather than subjective classifications (ie necrotic, granulation tissue etc).

Currently, the utilisation of UTC has been confined to the Achilles and patellar tendon. The reasons for this limitation are multiple. Firstly, the Achilles tendon was initially selected as it is analogous to the SDFT in structure, composition and function. Secondly, the Achilles and patellar tendon are large tendons that are not impeded by bone, such as the rotator cuff of the shoulder. Lastly, these tendons are linear and do not bend around a bony landmark, such as the hamstring or gluteus medius tendon. As UTC algorithms quantify the alignment of tendon bundles and transducer tilt changes can create imaging artefact, it is essential that the transducer remains perpendicular to the long axis of the tendon for the entire length of the scan. This is why the Achilles tendon was scanned in a standing position

with standardised amount of dorsiflexion to ensure that there was little sag in the tendon due to the flexor retinaculum.

The window size relates to the segment of the tendon over which the stability of the echopattern was quantified. In the studies of this PhD, a window size of 25 transverse scans was selected, which relates to the stability of the echopattern quantified over a distance of 4.8 mm. The window size can be reduced to 3.4 mm and 1.8 mm using the UTC software, which results in an alteration of the UTC echopattern (generally a reduction in echo-type I). Criticisms of some of the studies in this PhD, especially those in relation to the detection of load responses, were that a smaller window size was not used to detect subtle changes. However, this is less important as the window size is standardised across all groups. A sample population from Chapter 6 was quantified using different window sizes and found that while window sizes resulted in an altered UTC echopattern it did not affect the statistical findings of this study (Appendix P). It appears less important what window size is used, rather that window size is consistent.

8.4- The horse as a human model for tendon pathology and tendinopathy

The similarity in the short-term temporal response to maximal load observed in horses and humans (Chapters 3 and 4) raises the questions of the horse as a suitable animal model for tendon pathology and tendinopathy in humans. Research investigating the pathogenesis and/or the efficacy of treatments of tendinopathy in humans has been limited to clinical outcome measures (return to activity, VISA-A etc) and imaging (MRI and US) with any *ex vivo* research performed on chronic, end-stage pathology. Research of the early stages of tendon pathology at the tissue, cell and molecular level are often unable to be

performed in the human as biopsies impact the tendon negatively (normal control tissue is unable to be collected for this same reason). The need for suitable, reproducible animal model of tendon pathology is needed to help understand the progression of normal tissue to pathological and develop rational based treatments.

8.4.1- What makes a good model?

Experimental models of tendon pathology in animals allows for the study of the underlying pathological process evaluation of regenerative therapies and rehabilitation protocols and a better understanding of the factors that trigger cell and matrix stress responses, all at a tissue, cell and molecular level (Warden, 2007). When considering the use of experimental models of tendon pathology, whether it be cell/tissue cultures or animal models, a number of criteria need to be considered as to whether the model is suitable and translates to tendinopathy in the human.

- The model must represent the clinical presentation (pain, loss of function and swelling) and progression of tendinopathy. Tendinopathy in humans is often a long standing injury with an insidious onset. A period of inactivity can lead to the removal of any pain, yet returns when the tendon is reloaded.
- The model needs to represent key tissue, cellular and molecular changes associated with naturally-occurring tendon pathology.
- Selection of the species needs to be considered. Preferably, experimental models should be developed in those species that develop tendinopathy naturally.
- The induced pathology/lesion needs to be standardised, with respect to age, stage and extent (length and %CSA) of the pathology.

- Lastly, having the induced lesion easily accessible to imaging and other non-invasive monitoring methods to assess the progression of the tissue pathology and/or evaluating the efficacy of treatments, rather than a reliance on sacrifice of animals and post-mortem evaluation (an important ethical consideration).

Small mammals, such as mouse, rats and rabbits, are the preferred species used in studies of the pathogenesis of tendon pathology and the efficacy of treatments. These models have a number of benefits due to their relative ease of handling, similarity to human tissue and cell physiology, and cost-effectiveness (relatively low costs associated with purchase of animals, housing and maintenance). Experimental models vary in their mechanism of inducing tendon pathology with forced-treadmill running (Soslowsky et al, 2000), electrical stimulation against resistance (Backman et al, 1990) and the introduction of exogenous substances resulting in pathology of the tendon (Watts et al, 2012). These models have been shown to mimic aspects of naturally-occurring tendon pathology yet the translation of results from studies using these species has been questioned. A negative of these models is that the pathological changes induced using these methods are reversed after 2 weeks of rest and these species do not succumb to tendon injuries naturally (Lui et al, 2011; Warden, 2007). Despite the apparent low cost of these models, the relevance and translation of this research has been questioned. Limited translation of treatments based on these models of injury, the cost-effectiveness of these animal models may not be as good as previously thought.

8.4.2- Why not the horse, of course

Tendinopathy naturally occurs in the performance horse, affecting the SDFT of the forelimb. Epidemiology studies have reported that equine tendon injury accounts for 11% of all injuries, while post-mortem studies have reported 26% of horses demonstrated abnormal tendon lesions (Kasashima et al, 2004; Webbon, 1977). The SDFT is an energy storage tendon, functioning similar to the Achilles tendon in humans by acting as a spring reducing the metabolic energy expenditure of high-speed locomotion (Butcher et al, 2009). Along with its high prevalence, equine tendinopathy is associated with poor performance, prolonged periods of recovery (up to 12 months) and reinjury rates of up to 65% (Dyson, 2004; Guest et al, 2008; Lacitignola et al, 2008). Similarities in the risk factors (loading history, genetics and track conditions)(Bailey et al, 1998; Bailey et al, 1997; Oki et al, 2008), pathological changes (Kristoffersen et al, 2005), acute responses to load as detected by UTC (Chapters 3 and 4) and the clinical presentation/outcomes of tendinopathy in the human and equine athlete suggests the horse may be a suitable model for translational research, specifically for Achilles tendinopathy in the human (Patterson-Kane & Firth, 2009; Smith et al, 2000).

8.5- Conclusion

The contents of this thesis provide a new insight into the features of tendon pathology, and it's pathogenesis, specifically on how the normal tendon responds to load. Chapters 3 and 4 provide new insight on the temporal sequence of changes in the UTC echopattern in response to an acute bout of exercise. The transient change in tendon structure that returned to baseline suggests a cell-driven response, rather than collagen tearing. Furthermore, prolonged periods of loading appear to have a positive effect on the UTC echopattern (Chapter 5). Future research is needed to ascertain whether the results of these studies affect the health of the tendon (ie pain and dysfunction) and relate to the continuum of tendon pathology.

An important finding of this thesis was that the pathological tendon contained increased amounts of aligned fibrillar structure compared to the structurally normal tendon (Chapter 6). Despite the design of the study (cross-sectional), it appears that the pathological tendon increases in tendon dimensions to compensate for an area of disorganisation. These findings may have major implications in our understanding of the critical features of tendon pathology, how we monitor tendon pathology and develop treatment strategies that address the limiting features of tendinopathy. As the tendon appears to adapt to areas of pathology, previous models of pathogenesis suggesting that tendons fail due to the accumulation of micro-trauma or failed healing are not supported by these data.

Further research is needed to better understand the relationship between changes in the UTC echopattern and the presence/development of tendon pain and dysfunction. While a compromised UTC echopattern was observed in the asymptomatic tendon in

participants with unilateral Achilles tendon pain, it is unclear whether these changes predispose the tendon to symptoms (Chapter 7). Similarly, it is unclear whether improvements in the UTC echopattern over time reduce the susceptibility of the tendon to symptoms and increases load tolerance. As there is a disconnect between structure and pain, it is suggested that future studies investigate the risk of developing symptoms rather than making direct inferences.

Chapter nine
Appendices

9.1- Appendix A

Original article

Relationship between compressive loading and ECM changes in tendons

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Summary

Tendons are designed to absorb and transfer large amounts of tensile load. The well organised, strong yet flexible, extracellular matrix allows for this function. Many tendons are also subject to compressive loads, such as at the entheses, as the tendon wraps around bony protuberances or from internal compression during tensile loading or twisting. Tendinopathy, the clinical syndrome of pain and dysfunction in a tendon is usually the result of overload. However, it is not only the tensile overload that should be considered, as it has been shown that compressive loads change tendon structure and that combination loads can induce tendon pathology. This review summarises how load is detected by the tenocytes, how they respond to compressive load and the resulting extracellular matrix changes that occur. Understanding the effect of compression on tendon structure and function may provide directions for future matrix based interventions.

KEY WORDS: compression, extracellular matrix, tendon, tendinopathy.

Tendon ECM changes and compression

Tendons are exposed to different types of load during normal function. Tensile load is the prime load that tendons endure, often functioning as elastic tissue to decrease the metabolic costs of high level function. In addition to tensile loads, compressive load is high at the enthesis and at points where the tendon has bony contact[1]. Pathology at these points of compression is common and some features of pathology in tensile and compressed regions are similar to fibrocartilage, which is a normal response to compressive load. To fully understand this response to compressive load, it is important to briefly review the structure of normal tendon.

Normal tendons are a three dimensional network of tendon cells (tenocytes), interspersed between tightly packed collagen fibres that are orientated along the line of tensile loading[2]. Also present in the extracellular matrix (ECM) are proteoglycans, glycoproteins and water as well as a range of enzymes, growth factors and cytokines.

Tenocytes synthesise all ECM proteins of tendon tissue[3] and are capable of expressing different phenotypes in response to differing mechanical stimuli[4]. The synthesis of the ECM components is based on load applied, therefore there is variation in tendon architecture and composition along the length of tendons and in tendons with different functions (e.g. elastic storage compared to positional tendons)[5]. The difference in structure in tendons that are subjected to differing mechanical stimuli clearly demonstrates a capacity of the tendon and its cell to detect and respond to load.

Cook and Purdam[1] examined the evidence for compression in the development of overuse tendinopathy and highlighted the many tendons subject to compression, particularly close to their insertion into bone. Tendons that develop tendinopathy where compression is an important factor include the Achilles insertion, proximal hamstring, tibialis posterior, biceps long head, supraspinatus, gluteus medius and minimus, adductor longus/rectus abdominus, peroneal tendons, quadriceps and pectorals. Given that most tendons are affected by compression, it is timely to review the cellular and matrix response to compressive loads. To understand the response, it is important to first appreciate how tendons detect, respond and transduce mechanical stimuli.

Overview of mechanotransduction in tendons

Mechanotransduction describes the conversion of a mechanical stimulus to a biochemical response and is important in tendon remodelling. The exact mechanisms by which tenocytes detect mechanical stimuli and thereby alter the ECM remains poorly understood[2]. It has been shown that the composition and tendon mi-

cro-architecture is continually adapting to the loads applied or removed, and that this adaptive process is driven by the tenocyte.

Tenocytes are sparsely distributed spindle shaped cells located end to end in rows in channels between collagen fibres. Tenocytes possess numerous cell processes extending between the cells in the rows and between rows of cells allowing communication between cells via the cell processes and gap junctions[6]. These gap junctions allow rapid exchange of ions and signalling molecules between cells, which can induce stimulatory and inhibitory responses to tensile load[7]. Whether these gap junctions play a role in compressive load is yet to be determined.

Tenocytes not only respond to mechanical loads[8] but to local stimuli (e.g., hydrostatic pressure changes, cytokines and growth factors)[9]. Different types of mechanical load may activate tenocytes differently (e.g. shear stress compared with substrate strain)[10]. That is, both the type and magnitude of the load may elicit a different cellular response.

The tenocyte has a number of mechanisms for detecting the mechanical environment such as their internal cytoskeleton, cellular projections (cilia), cell-cell communication and local/circulating chemical messengers. In the 1990's, Ingber described the 'tensegrity' model in an attempt to understand mechanotransduction and the role of cell deformation and the cytoskeleton[11-13]. The internal cytoskeletal structure forms a network of struts and cables that are placed in a state of isometric tension, due to the forces applied on the cell by the surrounding ECM, allowing the cell to be responsive to mechanical stresses. When under tensile strain, Type I collagen production is up-regulated in cultured tenocytes, however when the cytoskeleton is disrupted with cytochalasin D, collagenase mRNA expression is shown to be upregulated indicating increased catabolism[14]. This three dimensional internal network can detect and respond to tensile strain; however the ability of the cytoskeleton to detect compressive stresses has not been investigated.

Primary cilia, solitary finger-like immotile projections that extend from the cell surface into the extracellular environment, have been observed in almost two-thirds of all tenocytes[2] and have a role in detecting tensile load. Cilia are microtubule based sensory organelles and shown to be aligned parallel to the collagen fibres forming a cantilevered beam with adhesions to the fibrillar matrix[2]. The cilia have been shown to deflect in response to tensile loading and lengthen when deprived of stress suggesting these cellular projections play an important role in detecting load[15, 16]. Although, it is not known how cilia react to compressive load in the tendon. In other tissues such as bone, cilia has been shown to detect fluid flow[17, 18], and this may be increased with compressive load.

How does tendon react to compression?

Pauwels[19] described connective tissue differentiation (fibrous, fibrocartilage, cartilage and bone) in response to differing mechanical stimuli, which as a result determines the expression of an appropriate form of connective tissue. Tissues respond to different loading parameters by altering their matrix structure to be suitable to transmit and absorb the applied loads[20]. Tendon, designed primarily to withstand tensile load, demonstrates several adaptive responses when subjected to compression. A substantial change in tendon composition and structure adjacent to a bony prominence has been described where the tendon is subjected to compressive forces. Gillard et al.[21] demonstrated fibrocartilage at the compressive region (where the tendon wraps around the calcaneus and talus) within the normal flexor digitorum profundus tendon of the rabbit, with a return to normal fibrous tissue upon removal of compression through surgical intervention. Milz et al.[22] also showed in the Achilles tendon that areas of fibrocartilage at, and proximal to, the insertion were normal adaptive changes.

What is the structure of compressed tendon (fibrocartilage)?

Tenocytes alter their phenotype as a result of compressive forces by becoming more rounded (chondrocytic) and express cartilage-like matrix proteins such as large proteoglycans and Type II collagen. They protect themselves by their position in lacunae and also by releasing large proteoglycans that slow the dissipation of fluid and reduce fluid shear stress (CJ Handley, personal communication[23]). Large proteoglycans such as aggrecan and versican are found in higher concentrations in both compressed and pathological tendon. This suggests that compression may be critical in the overload that drives the onset of pathology. These large proteoglycans may help with cell and/or tendon protection by limiting loads on the cell and decreasing stress on the tendon. Small proteoglycans are still synthesised by the tenocytes, but there is a greater predominance of large proteoglycans. In addition to the ongoing slow production of Type I collagen, there is some production of Type II collagen in the areas subject to compression within tendons[24]. Whether this production also exists in tendon pathology where compressive overload is a factor, is not yet known.

When does adaptation to compression become pathological?

A naturally occurring response to compressive load resulting in fibrocartilage occurs when tendon sustains compressive loads near a bony prominence that are not excessive but due to the normal positional and functional demands. The fibrocartilage is essential to allow the tendon to both tolerate the compressive load and maintain capacity to act in conjunction with the tensile load bearing part of the tendon.

When the compressive loads are excessive and/or suddenly increased in magnitude or volume, then tendinopathic changes occur. The cell and matrix changes in tendon pathology are described extensively. These

Table 1. Differences between fibrocartilage and tendon pathology (Reproduced with permission from Cook and Purdam: Is compressive load a factor in the development of tendinopathy?. British Journal of Sport Medicine 2012; 46:53).

	Normal tendon	Fibrocartilage	Pathological tendon
Cells	Few spindle shaped cells	No cell proliferation Cells rounder	Cell proliferation Cells rounder, more endoplasmic reticulum
Proteoglycans	Minimal mostly decorin and biglycan	5-10-fold higher than in tensile tissue, mostly aggrecan	3-fold higher than tensile tissue, 25-fold higher metabolic rate of normal tendon[32] Biglycan and aggrecan increase, decorin maintained[33]
Collagen	Predominately Type I	Type I & II	Type I collagen, some Type II, substantial increase in Type III collagen
Collagen structure	Ordered collagen network	Ordered collagen network	Disorganised collagen network
Vascularity	Minimal	None to minimal	Variable but can be abundant

changes include cell activation and proliferation, which leads to substantial matrix changes[25]. The cell proliferation drives a rapid increase in the production and degradation of large proteoglycans, with a half-life of around 2-3 days[26, 27]. The cells preferentially synthesise Type III collagen (some Type I and II is produced also) leading to increased collagen turnover. As Type III collagen is thinner and less capable of fibril formation, collagen disorganisation and neurovascular ingrowth results[28].

These changes associated with pathology are sometimes referred to as fibrocartilaginous metaplasia[29, 30] due to the similarity to fibrocartilage[31]. However, despite the role of compressive stresses in pathology and the obvious similarities between tendon pathology and fibrocartilage, the term fibrocartilaginous metaplasia in reference to pathology is incorrect due to key differences as listed in Table 1.

Compressive loads, in isolation and in combination with tensile load have been investigated for their ability to induce tendon pathology. Soslowsky et al.[34] investigated the effect of different loads on rat supraspinatus tendon and examined the effect of compressive load, tensile load and the combination of both. They showed that compressive load (by interposing tissue between the tendon and acromion) in itself had minimal effect in the tendon, tensile load (running downhill) was clearly detrimental, but the combination of loads was especially damaging to the tendon[35]. Increased cross-sectional area and decreased mechanical properties were maximal in tendons exposed to both compressive and tensile loads. This has immediate clinical relevance as many tendons are subject to an environment of both tensile and compressive loads in relative combinations.

How does this relate to tendinopathy?

Normal adaptation to compression is present within the tibialis posterior tendon as it passes posterior to the medial malleolus and presents as an appropriate model for understanding compression in the development of tendinopathy[36, 37]. Within these areas of fibrocartilage the presence of aggrecan binds with water, slowing the permeability of fluid and protecting the fibrillar and cellular components of the tendon from lateral forces[23]. In contrast, the tensile region of the tendon is proposed to have higher fluid permeability due to low concentrations of aggrecan, allowing the tendon to withstand high tensile load. However, this zone of fibrocartilage is not well demarcated and a zone of transitional tissue exists between the two mechanical distinct regions. As this transitional zone is unsuited to compressive loads, this area of tendon may be implicated in the development of tendinopathy. If excessive loading (tensile, compressive or more likely combination load) is placed upon tendon, this may lead to the flow of fluid and the depletion of bound water within the high fluid permeability areas (tensile and transitional zones). Grigg et al.[38] reported a reduction in the Achilles tendon AP diameter at these high fluid permeability areas (mid-substance of the tendon) as a result of repeated eccentric load. In pathological tendons, which have been shown to contain high levels of aggrecan, this alteration in AP diameter was not observed[38].

The movement and loss of water through the tendon may expose the tenocyte to compressive load. In response to the loss of water from the tendon, the tendon may synthesise and release large water binding proteoglycans in an attempt to maintain homeostasis. This process has been shown to occur in a pathological state and occurs within days[39]. As previously discussed, this would bind water to the matrix and protect the cellular and fibrillar components of the tendon against future insult by reducing the permeability of water through the matrix. Further loading to the tendon may perpetuate the response and result in extensive disorganisation of structure[40]. Pathological features similar to fibrocartilage (cell rounding, aggrecan deposition) have been induced in the supraspinatus tendon in the rat within the transitional zones normally occupied by normal spindle shaped tenocytes[41].

Compression may not only occur as a result of the tendon being adjacent to a bony prominence, but occur

during tensile loading such as in the midsubstance of the Achilles tendon. Lavagnino et al.[42] developed a finite computational model to measure mechanical stresses placed on the cell during tensile strain. Cellular tensile strain was suggested to be similar to the strain on the tendon yet shear stress (perpendicular to the long axis of the tendon) was significantly increased when strain rate was increased. The reason for this lateral shear stress was suggested to be due to fluid flow perpendicular to tensile strain. This increase in lateral compression placed on to the tenocyte may help explain why high elastic storage (high strain rate) movements are deleterious to the tendon and implicated in tendinopathy[34, 43] yet heavy slow resistance loads (low strain rate) are more beneficial[44].

In summary, tendons adapt to the loads placed on them either with normal adaptive responses or with a pathological response. The exact mechanisms that lead to adaptation versus pathological change are not completely understood but are likely to be related to the frequency and type of load (with a combination of tensile and compression load being the most provocative). Characterisation studies of the clinical and imaging presentation of tendinopathy at various tendons[45, 46] identify the site of compression adjacent to the tendon insertion as a predominant site of pathology, strongly suggesting that compression is an important consideration in the development and management of tendinopathy. Compression to the tendon is not solely isolated to the insertion and can occur due to normal anatomical bony prominences away from the insertion, due to alterations in biomechanics that induce compression from an adjacent bony prominence or changes to fluid flow and matrix structure. Compression is not responsible for all tendinopathies as some tendons lack a nearby bony prominence (e.g. flexor tendons of the forearm, proximal insertion of the patellar tendon). However, clearly compression appears to be implicated in pathology and results in substantial changes to the structure and function of the ECM and therefore of the tendon. Opportunities to reduce compressive loads on the tendon, especially when in combination with tensile loads may prevent a deleterious tendon response.

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9.2- Appendix B

TENDINOPATHY: IS IMAGING TELLING US THE ENTIRE STORY?

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Abstract

The clinical use of ultrasound and magnetic resonance imaging for tendinopathy has been the focus of numerous academic studies and clinical discussions. However, as there is no direct link between structural disorganisation and clinical symptoms, imaging can frequently create a confusing clinical picture. This review will summarise the accuracy and sensitivity of imaging in its detection of clinical tendinopathy and the role of imaging in the clinical setting from both a clinical and research perspective. The advent of new imaging modalities, such as ultrasound tissue characterisation and sonoelastorgaphy, will be introduced and possible future applications discussed.

Keywords

Tendon, ultrasound, magnetic resonance imaging, tendinosis

Introduction

The old saying goes 'a picture is worth a thousand words'. Imaging of tendons with ultrasound (US) and magnetic resonance (MR) imaging have been utilised in the clinical setting to assist in the diagnosis of tendinopathy, monitor the efficacy of treatments and assess the risk of developing symptoms.[43, 72, 79] However, while imaging shows the presence and extent of structural changes within the tendon, the clinical interpretation of the images requires context in regards to the features of pain (location and distribution) and the aggravating factors (loads that are related to increases in pain). This is due to the limited relationship between structural disorganisation and pain; similar to other musculoskeletal conditions such as osteoarthritis and intervertebral disc degeneration.[79] Clinically, tendon abnormalities on imaging do not confirm that pain and dysfunction is generated by the tendon.[75] Conversely, a relatively normal tendon does not rule out the tendon as the source of pain and dysfunction (this phenomemon is rare). In the case of tendons, we may need to make an addition to the old saying where 'a picture is worth a thousand words *but only as an adjunct to the clinical picture'.* This narrative review discusses the imaging features of the pathological tendon, accuracy and sensitivity of US and MR imaging, new and novel imaging modalities, the role of imaging in the clinical and research setting (prediction of symptoms and monitoring) and future directions.

Tendinopathy is the clinical condition that describes pain and dysfunction of the tendon, which is independent of pathology within the tendon.[52] It is frequently described as localised pain that increases with greater load (ie single leg hopping is more painful for the Achilles tendon than calf raises).[15, 75] Degenerative changes in structure observed histologically or on imaging, independent of clinical symptoms, is termed tendinosis.[52, 71] These terms have been used interchangeably in the literature, however it is important to clarify that they represent differing aspects of the same condition and highlight the disconnect between structure and pain.

Features of normal and pathological tendons on imaging

Imaging allows for the visualisation of the internal architecture of the tendon. Normal tendon primarily contains type I collagen that is hierarchically arranged into parallel aligned fibrils, fibres and fascicles.[31, 63, 74, 78] This uniform alignment of fibres can be visualised in normal tendons using US imaging at the fascicle level (Figure 1). Water and non-collagenous proteins, such as proteoglycans, are present between the fibres and fascicles, which allow tendon lengthening through interfascicular sliding rather than fibre extension.[80, 90] This relationship between water and collagen results in a strong dipole interaction and little signal being observed on MR imaging in the

normal tendon (Figure 2).[9, 97] Finally, normal tendons are relatively avascular especially when observed with Doppler US (Figure 3a,b).[64]

Four main histological changes are observed in tendinosis; the primary change being increases in cell number that exhibit an altered more metabolically active phenotype.[10, 46] Rather than flattened cells that are arranged parallel between fibres, increased numbers of rounded chondrocytic-like cells are interspersed throughout the pathological tendon.[46] While changes in cell number and phenotype are beyond the resolution of clinical imaging modalities, the consequence of these active metabolic cells can be detected. A shift in the proteoglycan (PG) content, from the small leucine-rich PG's (eg. decorin) to the larger hydrophilic PG's (eg. aggrecan), results in an increase in bound water and tendon thickening.[20, 78] These changes have been described on US as increases in tendon dimensions and heterogenous or diffuse changes in echogenicity,[19, 56] as well as ultrashort echo time MR imaging shown alterations in the hydration state of the tendon.[40] Previous work in asymptomatic patellar tendons suggest that abnormal tenocyte morphology and changes in proteoglycan content are the primary changes in tendinosis.[14]

Fibrillar disorganisation is another feature of tendinosis where fibres are present in a haphazard arrangement, somewhat due to the change from type I collagen to type II and III collagen.[53] The parallel arrangement of normal tendon fibres generates a single US reflection when the US probe is perpendicular to the long axis of the tendon. Multiple reflections and shadowing are generated by fibrillar disorganisation and lack of parallel aligned fibres, which is represented by an area of hypoechogenecity on US (Figure 4).[72] On MR imaging, the alteration in fibrillar alignment and increased water content results in an increase in intratendinous signal (Figure 5).[9, 97]

Neovascularisation within the pathological tendon can be imaged with colour and power Doppler US imaging (Figure 3c,d).[6, 33] Weinberg et al[96] reported that Doppler flow was only observed in tendons that contained an area of hypoechogenecity and not in abnormally thickened tendons with normal echogenicity. This association between the presence of blood vessels and areas of matrix disorganisation suggest that the infiltration of blood vessels may be opportunistic.[42] The infiltration of blood vessels and accompanying nerves have previously been implicated as a source of pain, with moderate associations reported between Doppler signal and the presence and location of pain[22, 27, 38]; with Doppler signal also being associated with poorer clinical outcomes.[48] However, increased Doppler signal is present in asymptomatic tendons suggesting that blood vessels and accompanying nerves are not the primary source of pain.[18, 38] It is also important to note that the reliability of detecting Doppler signal is variable,[17] with exercise effecting the presence/absence of Doppler signal.[16]

The pathological features outlined above are not the only indication of pathology within the tendon. Partial tears, especially in the rotator cuff of the shoulder, are frequently described. However, there is no consensus on the features of partial tears and a number of studies have reported difficulty in differentiating partial tears from tendinosis.[67, 73] Bony spurs and areas of calcification, frequently near the bony insertion, have previously been described as a 'tombstone of tendon pathology' yet recent investigations have shown that they have little impact on the development of symptoms.[12] Extra-tendinous tissues, such as paratendinous sheath, associated bursa and fat pad, can also display changes on imaging yet have a different pathogenesis to tendinosis.[72]

Paratendinitis is described as an inflammatory response of the tendon sheath, which clinically can present as crepitus and pain through range of movement. Thickening of the sheath and the presence of fluid surrounding the tendon are frequently observed on ultrasound (FIG), with adhesions of the sheath to the tendon apparent in the chronic cases that can be observed on dynamic ultrasound [66, 99]. Imaging can be useful in the diagnosis of paratendinitis as it can be present in conjuction with tendinosis and needs to be considered during rehabilitation (ie reducing movement through range and friction between the sheath and tendon).

Accuracy, sensitivity and validity of imaging

The diagnosis of tendinopathy can be complex due to a confusing clinical picture and few clinical diagnostic tests for tendinopathy (please refer to the difficult patellar tendon article in this JOSPT special issue on tendinopathy). Numerous studies have investigated the accuracy (number of correct imaging diagnoses, both abnormal and normal imaging, divided by the total number of cases) and sensitivity (number of correct abnormal imaging diagnoses divided by the total number of symptomatic cases) of a number of imaging modalities in detecting clinical tendinopathy. These findings have consistently shown that both US and MR imaging have good-to-excellent accuracy (0.63-0.83 and 0.68-0.70, for US and MR imaging respectively), with varying sensitivity (0.68-0.87 and 0.50-0.57, for US and MR imaging respectively) in detecting clinical tendinopathy.[1, 44, 47, 48, 61, 95] Critically, caution is required when interpreting the results of these studies as asymptomatic participants are frequently included and may over-estimate the accuracy and sensitivity of imaging in detecting clinical tendinopathy. Participant selection in these studies is critical and future studies need to reflect the use of imaging in the clinical setting (ie use of imaging to differentiate tendinopathy from other pain conditions in the region).

Most studies have compared imaging to a clinical diagnosis[48], with few studies comparing findings on imaging to surgical or histological findings due to ethical issues in collecting tissue from appropriate controls. A number of studies have suggested variable sensitivity (0.33-1.00) and good-to-excellent accuracy (0.91-0.95) of imaging compared to surgical findings.[45, 60, 86, 94, 98] Interestingly, Adams et al[1] reported subscapularis tears on MR imaging in 16 patients that were confirmed by arthroscopy, yet 28 patients with subscapularis tears identified arthroscopically were normal on MR imaging. Similar findings have been demonstrated in the Achilles tendon where US diagnosis correlated with surgical findings in ~80% of cases[3, 67], with US imaging limited in its ability to differentiate between partial Achilles rupture and local degenerative lesions.[67] Caution is advised extrapolating the accuracy and sensitivity findings for tendinopathy as the majority of studies investigated partial tears due to this condition frequently requiring surgery. Shalabi et al[83] found that intratendinous signal observed on dynamic contrast-enhanced MR imaging correlated with the severity of histopathological changes. Histopathological comparison to ultrasound has shown that areas of hypoechogenecity contained significant tendon pathology, with areas adjacent subjectively described as 'normoechoic' also exhibiting pathological changes yet to a lesser extent.[60]

Which imaging modality is the gold standard?

Radiographs and computed tomography scanning have been used to image tendons[7, 58, 87], however US and MR imaging are the preferred imaging modalities. MR imaging has excellent soft tissue contrast detail and multi-planar imaging capabilities with excellent reproducibility.[5] However, MR imaging is costly and of limited availability. The advancement in US transducer technology and improvement in the sensitivity of Doppler imaging have increased the utility of US. However, it is somewhat user-dependent as slight changes in the US transducer tilt generate imaging artefacts that are similar to tendon pathology.[72] US imaging can focus on an area of pain or clinical suspicion of pathology, whereas MR imaging provides a global assessment of the region of concern.

Few studies have directly compared MR and US imaging. Westacott et al[98] performed a systematic review and reported that the sensitivity of MR imaging for detecting gluteal tendon tears ranged from 0.33-1.00, whereas specificity remained high (0.92-1.00). US was found to be consistently sensitive (0.79-1.00) compared to MR imaging, suggesting that US may be used as first-line imaging modality for evaluating gluteal tendon tears. Similarly, previous investigations have revealed that US demonstrates higher sensitivity but lower specificity compared to MR imaging in detecting clinically symptomatic rotator cuff tendinopathy.[97] However, MR imaging has been reported to be superior in the evaluation of various degenerative changes in the Achilles tendon.[60, 62]

There is value in both MR and US imaging, with the imaging modality used based on patient presentation. MR imaging may be suitable where differential diagnosis is required (eg. visualisation of patellofemoral joint chondral changes mimicking aspects of patellar tendon pain). However, due to the superior spatial resolution of US in the visualisation of fibrillar alignment and vascularity[72], US may better image the internal architecture of the tendon. Ultrasounds ability to provide a dynamic image during active and passive movements may provide further information, especially in differential diagnosis (eg fat pad or sheath adhesion to the tendon, sciatic nerve tethering to the proximal hamstring tendon).

New imaging modalities

Conventional imaging modalities are criticised for their reliance on subjective interpretation of images. Research has been limited to classifying the tendon as abnormal or normal or a subjective grading score based on a myriad of pathological features and their severity. Quantification of tendon structure has been limited to measurements relating to tendon dimensions (antero-posterior diameter or cross-sectional area) and the percentage cross-sectional area of the hypoechoic lesion. Tendon research may be improved with new imaging techniques that address these limitations and to begin to provide information of the mechanical properties of the tendon.

Ultrasound Tissue Characterisation (UTC)

The aligned fibrillar structure of tendon results in a homogenous US echotexture, where the stability in echotexture can be quantified.[4, 59] Ultrasound tissue characterisation (UTC) captures contiguous transverse US images over the length of the tendon and semi-quantifies the stability of the echotexture over the length of the tendon into four echotypes (see van Schie et al[92] for further explanation of the four echo-types). Research performed on equine histopathological samples found that UTC echo-types were able to distinguish between the different tissue types (normal, granuation and fibrotic tissue) where basic grey level statistics could not.[91, 93] The ability to capture a 3-dimensional US image of the tendon, which standardises parameters that affect the repeatability of conventional US (ie tilt transducer angle, depth and gain setttings), and semi-quantification of tendon structure attempts to address the limitations of conventional US imaging.

While UTC echo-types have not been compared to tendon biopsies in humans due to ethical considerations, the structure of equine and human tendons are similar.[68] Van Schie et al[92] scanned a cross-section of individuals with and without Achilles tendon pain and reported that the percentage of intact and aligned tendon bundles (echo-type I) were reduced in the symptomatic tendon with increases in echo-types that represent disorganised structure (echo-type III and IV). The ability to

semi-quantify the structural integrity of the tendon has allowed for classification of the tendon pathology based on a continuum of tendon pathology (Figure 6), treatment efficacy to be investigated[21, 23, 24], detection of subtle changes in response to load[29, 77] and demonstrate that the asymptomatic Achilles tendon is structurally compromised in patients with unilateral tendon pain.[30]

Sonoelastography

US elastography is a technique that evaluates the mechanical properties of tissues. Tendon pathology alters mechanical properties, and US elastography may be of value in improving the diagnostic capability of conventional US. US elastography is based on the principle that tissue displacement in response to external compression produces "strain" within the tissue.[49] The strain is higher in softer tissue than harder tissue. A visual strain map overlaid on the conventional B-mode image provides a visual representation of the relative stiffness of tissues (Figure 7).[25, 26, 65, 89]

Ooi et al[65] imaged 120 patients with Achilles tendinopathy and 120 gender- and age-matched controls. The authors found that combined B-mode US and US elastography demonstrated improved sensitivity (0.96), specificity (0.95) and accuracy (0.98), with better clinical correlation ($k=0.9$, $p<0.001$) compared to B-mode and colour Doppler US (sensitivity 0.67, specificity 0.94, accuracy 0.83).[65] Adding US elastography to conventional US may improve correlation with clinical symptoms. Sonoelastography has previously been used to monitor post-rupture Achilles tendon[89], investigate tissue displacement patterns under varying loading conditions[85] and has been compared to histological specimens.[50] Future studies are needed to investigate its effectiveness in management and prevention of tendinopathy.

Can imaging predict the onset of pain or clinical outcome?

A feature and criticism of the use of imaging in tendinopathy is the poor correlation with pain and pain severity. Abnormal imaging has been reported in various tendons in up to 59% of asymptomatic individuals.[8, 13, 34, 37, 41, 47, 51] This reflects the complex nature, and our limited understanding, of tendon pain. While abnormal structure is associated with tendon pain it is not driven solely by local tissue changes, but is likely to be an interaction between the local tissue and the peripheral and central nervous system.[75]

Despite the poor relationship between pathological changes and pain, local tissue changes and the use of imaging in visualising these changes is important. Fredberg et al[35] followed 54 asymptomatic Danish elite soccer players for development of Achilles tendon pain over 12 months. The authors reported that players with substantial imaging changes (thickening and hypoechoic region greater than 2mm in the transverse planes) at baseline had a three-fold increase in the relative risk of developing symptoms (95% CI, 1.6-4.9). Similarly, Malliaras and Cook[55] reported that

abnormal US imaging of the patellar tendon increased the relative risk of developing pain 15-fold in volleyball players (95% CI, 1.9-111.4). Conversely, Giombini et al[37] who investigated the Achilles, patellar and quadriceps tendons in 37 asymptomatic elite fencers reported that hypoechogenicity predicted future symptoms only in the patellar tendon, not the quadriceps or Achilles tendons.

Sonoelastography has been utilised to investigate the development of Achilles tendon pain in elite Australian football players. Structural and mechanical property changes of the Achilles tendon such as intratendinous delaminations, hypoechogenicity, neovascularization as well as soft tendon properties were present in asymptomatic elite Australian football players (52.4%)(Ooi et al, unpublished data). Increased mid Achilles tendon thickness and cross-sectional area, intratendinous delaminations and soft tendon texture were significant predictors for future symptoms. This suggests that conventional US supplemented with US elastography may identify Australian football players at risk of developing Achilles tendon injuries.

Structural disorganisation observed on imaging should be considered as part of the risk factor profile for tendinopathy similar to that of load, anthropometric factors and genetics, rather than solely as a diagnostic feature. There is a significant association between polymorphisms within the gene that encodes type V collagen, COL5A1, and the presence of Achilles tendon pain.[81] Collins[11] states that gene variances are not predictive, but rather increase the risk and susceptibility of developing the injury, similar to the increased risk of developing lung cancer in those with a history of smoking. Men who have smoked cigarettes for up to 20 years have an odds ratio of 7.4 (95% CI, 5.5-9.8) for developing lung cancer.[69] Based on previous cross-sectional studies, abnormal Achilles tendons have a similar odds ratio ranging from 3.9 (95% CI, 1.5-10.2)[48] to 16.2 (95% CI, 6.0-43.3)[51] for the presence of tendon pain. While imaging and the presence of structural abnormalities should never be the sole or predominant test in the diagnosis of tendinopathy, local tissue structure is a risk factor for the development of symptoms.

Monitoring of tendon structure – What can we expect from the tendon?

When assessing the efficacy of treatments, outcome measures that assess pain and function (VISA-A for the Achilles[76]), return to activity and structure on imaging have been used. As mentioned, assessment of tendon structure has been limited to subjective grading or measurements of tendon dimensions.

Recent studies that have semi-quantified aspects of tendon structure have provided information on how tendons respond structurally to various treatments. Shalabi et al[84] reported a significant decrease in Achilles tendon volume and intratendinous signal following a three month

eccentric loading program. While improvements in tendon structure were observed, tendon volume and intratendinous signal did not return to normal. Similarly, long-term follow-up (mean of 4.2 years) of the same cohort reported no significant difference in tendon volume compared to baseline measures, despite improvements in pain and function [36]. Furthermore, previous research has found that Achilles tendon structure on UTC is no different after a 16-week eccentric loading program despite improvements in the VISA-A score.[23] The findings of these studies suggest improvements in the tendon are not needed to facilitate clinical improvement after an eccentric exercise programme. A systematic review by Drew et al[32] reported that improvements in pain and function with eccentric exercise were not mediated by changes in structure. In this context, while improvement in, or normalisation of, tendon structure is a positive result, it is not necessary for improvement in pain and function, suggesting that the pathological tendon may have adapted to remain load tolerant.

While some interventions aim to regenerate and remodel structure within the tendon (eg. PRP, stem cells, autologous tenocyte implantation) this may not be necessary. The pathological Achilles and patellar tendon demonstrated increases in mean CSA (mCSA) of aligned fibrillar structure compared to structurally normal tendons.[28] It appeared that the pathological tendon maintains sufficient amounts of aligned fibrillar structure by increasing tendon dimensions (AP diameter and total mCSA) in parallel with the mCSA of disorganisation (ie more disorganisation the bigger the tendon). The increase in tendon dimensions may be a mechanism by which the pathological tendon maintains sufficient mCSA aligned fibrillar structure to still tolerate load. Interventions such as eccentric exercise may not be efficacious in remodelling the area of pathology, rather these loading protocols may cause adaptation and increase the loading capacity of the surrounding aligned fibrillar structure.

In the context of previous research, stability in tendon structure accompanied by improvements in pain and function can be considered a positive outcome. Interestingly, progression of rotator cuff tears was found to be related to the increase risk of symptom manifestation compared to those that remained stable over time.[54] Future research is needed to investigate whether tendons that remain stable, in terms of extent of pathology, have a better clinical outcome to those that worsen over time.

Future directions for research

Load is a critical factor in the development of tendinopathy, and numerous investigators are using imaging to improve our understanding of how tendons respond to load. Grigg et al[39] reported an acute response in the Achilles tendon where AP diameter reduced immediately after eccentric

exercise and was restored after 24 hours. UTC has been used to detect a short-term temporal response in the Achilles tendon[77] and superficial digital flexor tendon of the horse[29] where a loss of aligned fibrillar structure was observed 48hrs post-maximal load, which returned to baseline at 3-4 days. This is consistent with other studies that have reported a short term response in hydration level using off-resonance saturation MRI[88], tendon volume and intratendinous signal on MR imaging[82], and microvascular volume using real-time harmonic contrast-enhance ultrasound[70] in response to load. Of interest, these studies reported an altered response in tendinopathic tendons.[39, 70, 77]

These new and novel imaging modalities may provide insight in how tendons respond to load. There is overwhelming evidence that structural disorganisation predates the development of symptoms and tendon rupture.[47, 57] These new imaging modalities may help to define loading parameters that result in structural disorganisation and identify the point where tendon load exceeds the tendon capacity.

Proposed clinical role of imaging in tendinopathy

The role of imaging in the clinical setting will be somewhat limited as it does not directly relate to symptoms. Imaging allows for the visualisation of structure, it does not represent the entire clinical picture and should not be used as the sole diagnostic criteria in determining whether the clinical presentation is generated by the tendon. While not discussed as part of this review, imaging may be useful in differential diagnosis (eg. detection of an invaginated plantaris tendon[2], paratenonitis etc.) where treatment may need to be altered to address the specific condition.

As has been consistently stated throughout this narrative review, imaging needs to be placed in the context of the clinical image and this may be enhanced by the clinician performing the imaging themselves (specifically US). However, caution is advised with the potential for imaging in the clinician's hands to confuse the clinical picture and lead to poor outcomes due to technical insufficiencies. As stated above, US is highly user-dependent with specifically trained musculoskeletal radiologists able to produce high quality images that may provide more clinically relevant information than in the hands of a clinician with less experience in imaging. The converse to this discussion would be that in an attempt to improve the clinical utility of imaging, the radiologist may need to concern themselves further with the unique clinical presentation of tendinopathy. There is definitely scope for the specialised musculoskeletal radiologist with a special interest in tendon imaging to take their own detailed history beyond the referral note. Whether it is the clinician having a role in imaging or the radiologist taking detailed clinical history themselves, there is

a need for the sharing of skills and clear dialogue between the two fields to enhance the clinical utility of imaging.

In testing the efficacy of treatments or monitoring the improvement of clinical symptoms using imaging, a potential shift away from focusing on improving tendon structure may be needed. As clinical improvements have been shown not to be mediated by structural changes, stability in tendon structure may be a positive outcome in the clinical context of reduced pain and dysfunction. Currently, we are limited by conventional imaging modalities reliance of subjective interpretation. The development of new imaging techniques that utilise more quantifiable parameters, such as UTC or sonoelastography, will hopefully enhance our ability to diagnosis, predict the development of symptoms and monitor the efficacy of treatments.

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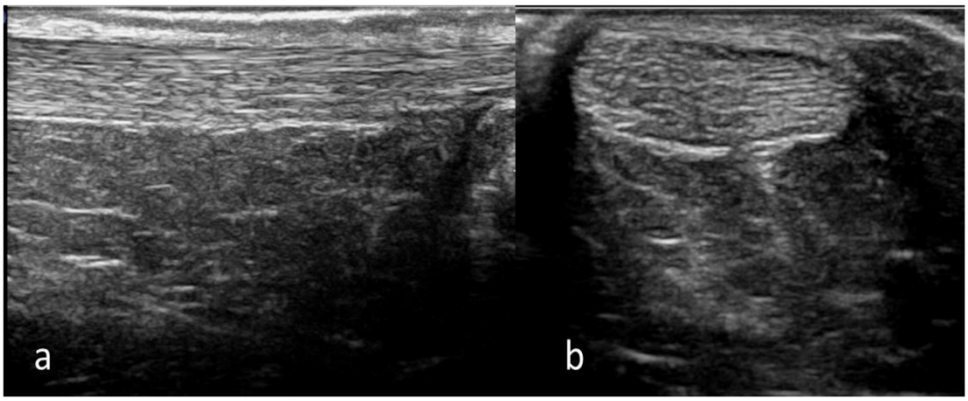

FIGURE 1. Ultrasound of a healthy Achilles tendon. (**a**) Longitudinal and (**b**) transverse ultrasound images of the mid Achilles tendon. The normal tendon texture appears homogeneous with parallel echogenic lines reflecting the internal fibrillar structure.

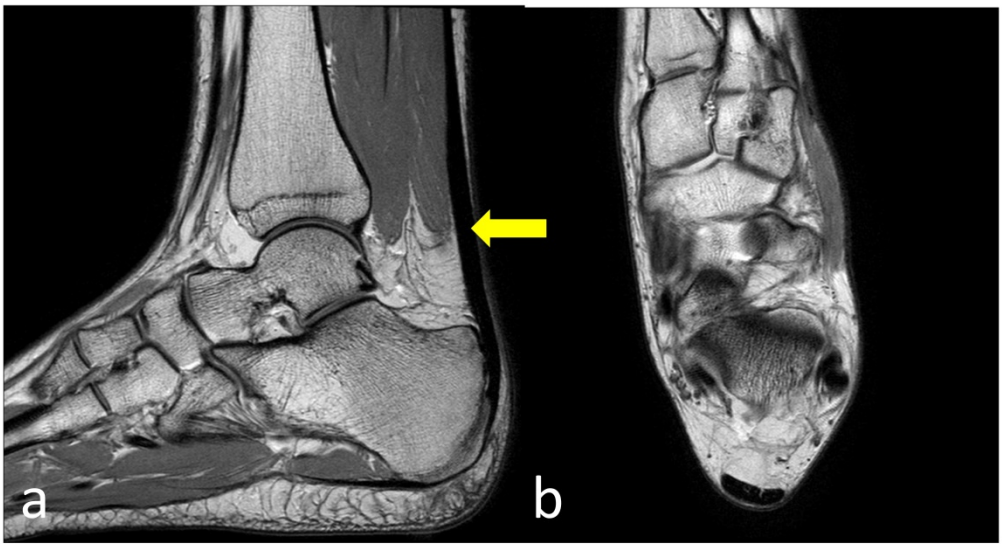

FIGURE 2. Magnetic Resonance (MR) imaging of the normal Achilles tendon. (a) Sagittal and (b) Axial Proton-denstiy sequence MR image showing a normal Achilles tendon (arrow) with uniform thickness and predominantly low signal intensity

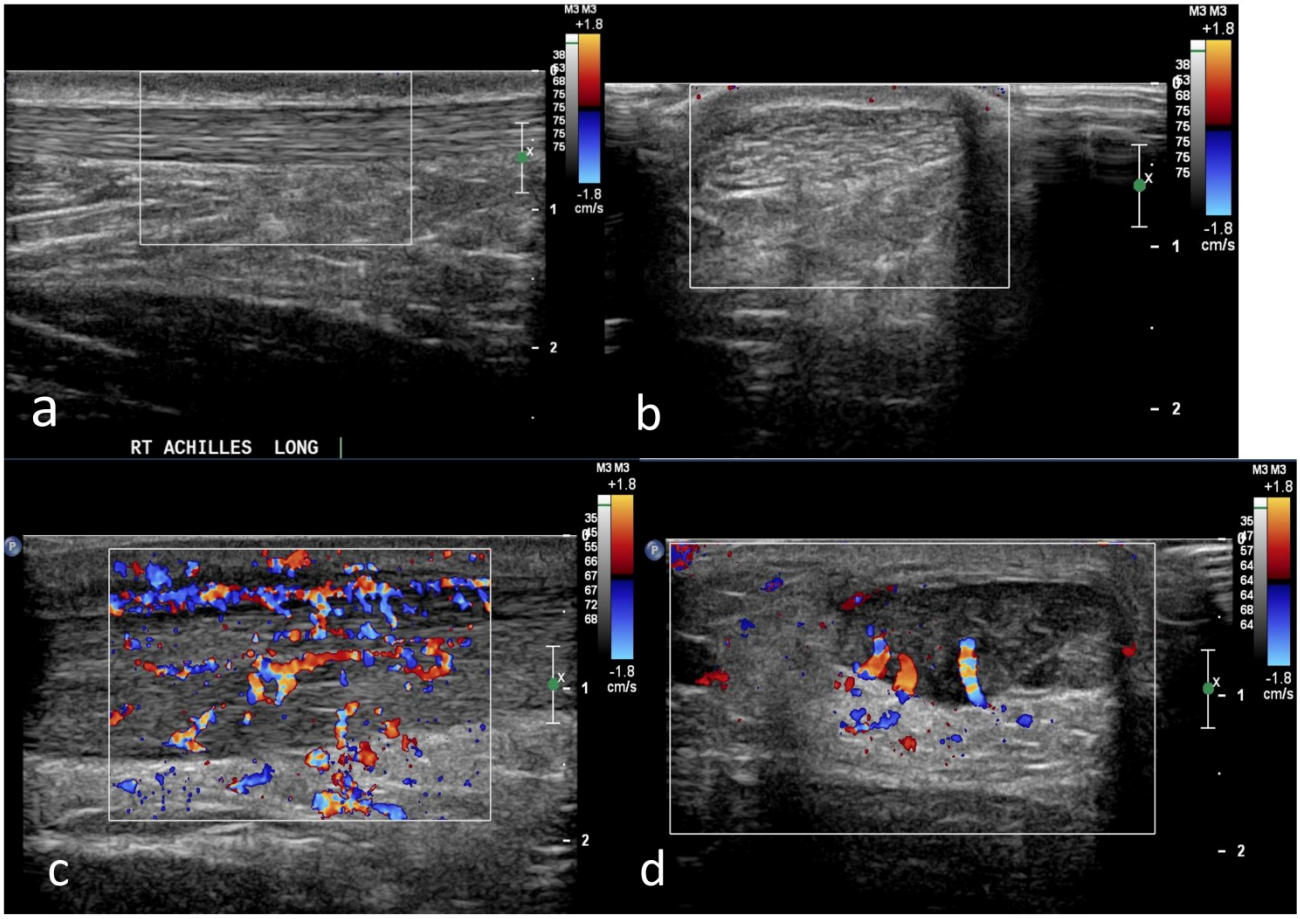

FIGURE 3. Ultrasound image of the Achilles tendon with Colour Doppler. **(a)** Longitudinal and **(b)** transverse image of an normal, asymptomatic Achilles tendon. Normal tendon echotexture with no Doppler signal. **(c)** Longitudinal and **(d)** transverse image of a symptomatic Achilles tendon. Significant thickening of the tendon with the presence of a hypoechoic changes. Significant Doppler signal is apparent within the tendon.

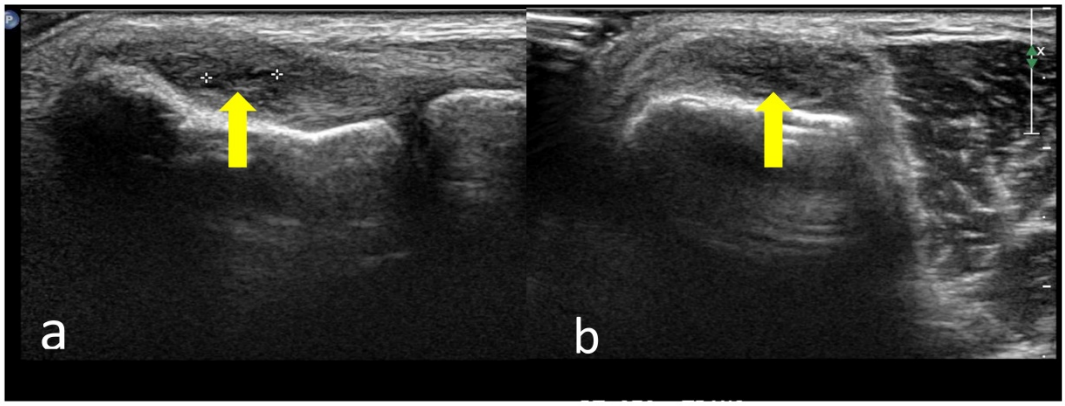

FIGURE 4. Ultrasound image of a pathological common extensor tendon of the elbow. **(a)** Longitudinal and **(b)** transverse image reveal a focal intratendinous hypoechoic lesion (yellow arrow)

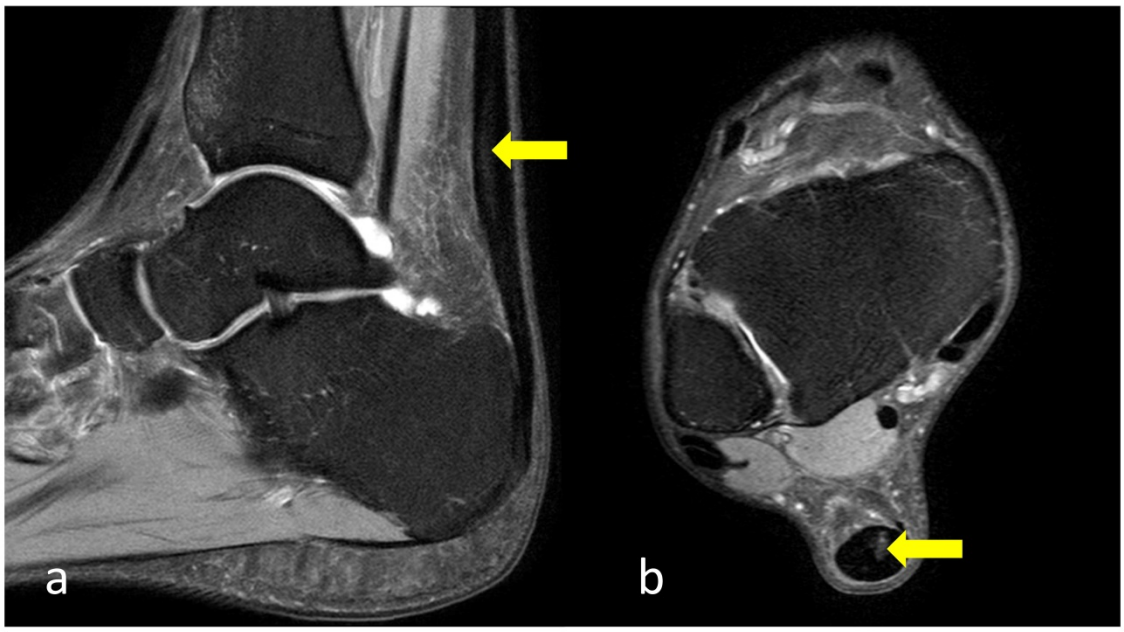

FIGURE 5. Magnetic Resonance (MR) imaging of an abnormal Achilles tendon. **(a)** Longitudinal and **(b)** transverse image revealing increased signal intensity (yellow arrow) within the midsubstance of the tendon

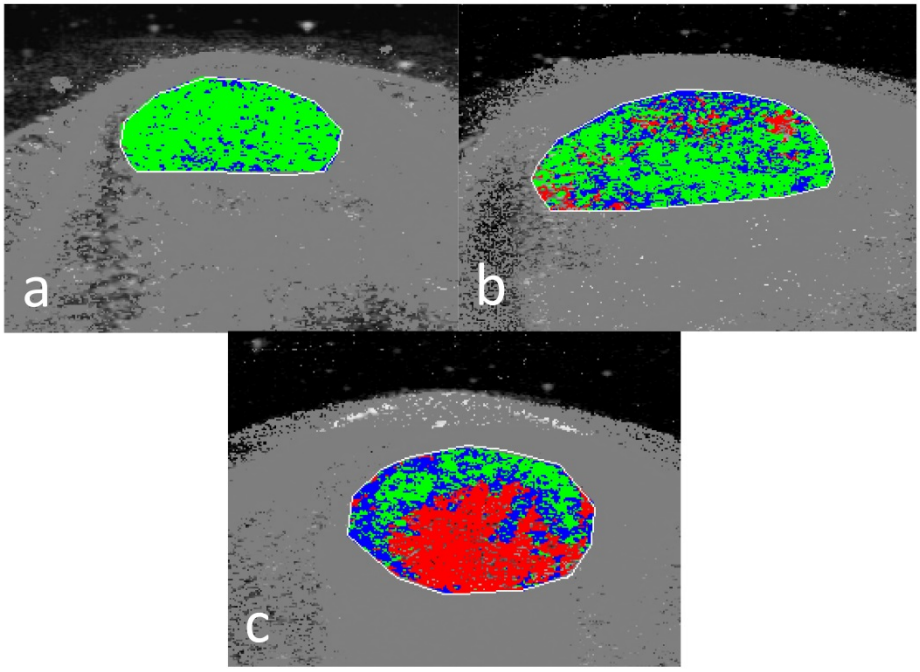

FIGURE 6. Ultrasound tissue characterisation (UTC) of the Achilles tendon midsubstance. **(a)** Normal Achilles tendon characterised by a high proportion of echo-type I (green pixels) representing aligned tendon bundles. **(b)** Reactive tendinopathy due to tendon thickening and the presence of diffuse speckling of echo-type II (blue pixels) and echo-type III (red pixels). **(c)** Degenerative tendinopathy indicated by significant focal area of echo-type III (red pixels).

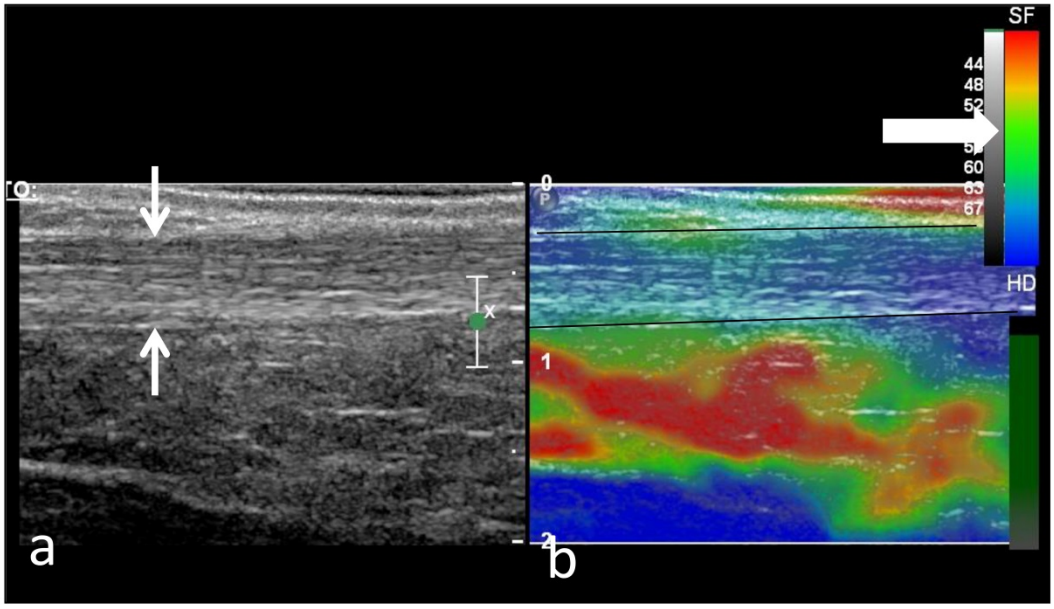

FIGURE 7. Dual mode display of B-mode US (**a**) and sonoelastogram (**b**) of a 25 year-old male volunteer. (**a**) Longitudinal B-mode US displays the normal homogeneous, linear fibrillar echo pattern of the mid Achilles tendon (arrows). (**b**) Corresponding sonoelastogram shows blue-coloured Achilles tendon (between lines, b) overlaid on the conventional B-mode image which represents normal, stiff tendon texture. Elastography colour map bar is shown on the right upper corner of the image (arrow).

9.3- Appendix C

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OPEN ACCESS

Sports and exercise-related tendinopathies: a review of selected topical issues by participants of the second International Scientific Tendinopathy Symposium (ISTS) Vancouver 2012

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ABSTRACT
In September 2010, the first International Scientific Tendinopathy Symposium (ISTS) was held in Umeå, Sweden, to establish a forum for original scientific and clinical insights in this growing field of clinical research and practice. The second ISTS was organised by the same group and held in Vancouver, Canada, in September 2012. This symposium was preceded by a round-table meeting in which the participants engaged in focused discussions, resulting in the following overview of tendinopathy clinical and research issues. This paper is a narrative review and summary developed during and after the second ISTS. The document is designed to highlight some key issues raised at ISTS 2012, and to integrate them into a shared conceptual framework. It should be considered an update and a signposting document rather than a comprehensive review. The document is developed for use by physiotherapists, physicians, athletic trainers, massage therapists and other health professionals as well as team coaches and strength/conditioning managers involved in care of sportspeople or workers with tendinopathy.

ANATOMICAL AND BIOLOGICAL BACKGROUND

As mechanical loading plays such a key role in the development, and rehabilitation, of many cases of sports-related tendinopathy, the distinct structural and functional adaptations and loading environments of tendons at different anatomical locations are important.[1] Lower extremity tendons, such as the Achilles, store and release substantial amounts of tensile energy,[2 3] whereas gliding tendons such as those at the wrist demonstrate specific adaptations to resist primarily frictional loading, such as retinaculae or synovial sheaths, whose function can be affected by injury.[4] Thus, tendons are distinct and varied in their loading requirements.

The structure and function of tendons have been well described elsewhere.[5–7] We alert the reader to recent findings regarding the scleraxis gene.[8] Tendon cells (tenocytes) are characterised by their expression of scleraxis, both in developing tendon and ligament, as well as in adult human tenocytes. As expected, scleraxis expression is mechanically regulated, showing a reduction following tendon transection[9] and exhibiting a dose-response with increasing strains or repetitions of movement.[10] Scleraxis expression is increased during the repair and remodelling stages of tissue healing, as the tendon attempts to restore its phenotype—this attempt to restore normal tendon phenotype following injury is frequently imperfect, leading to metaplastic or fibrotic change in injured tendon.

Dynamic tissues like tendon shift their anabolic/catabolic balance according to their mechanical loading history.[11] Emerging evidence suggests that local production of classically neuronal modulators, like neuropeptides, by tenocytes in response to load may regulate local tissue remodelling,[12–14] in addition to their role in nociception. The tendon's surroundings are richly innervated by mechanoreceptors, including Ruffini corpuscles, Pacinian corpuscles and free nerve endings, all of which may contribute both to proprioception and to nociception.[15] The nerve supply of tendon also includes many autonomic fibres, likely involved in regulating tendon blood flow as well as local tenocyte metabolism and pain signalling.[16 17]

PAIN AND PATHOGENESIS: INTERNATIONAL SCIENTIFIC TENDINOPATHY SYMPOSIUM 2012 UPDATE

The past decade has seen impressive strides in our knowledge of pathological processes which underpin the development of chronic tendon pain. The pathology of tendinosis—in which the structural degeneration of the load-bearing matrix is a key feature, with an absence or minimal presence of inflammatory cells—has been confirmed in several recent studies. The opening session of the International Scientific Tendinopathy Symposium (ISTS) 2012 focused on advances in knowledge of chronic tendon pain.

In tendinopathies, tenocytes produce nociceptive as well as inflammatory/catabolic substances.[18 19] These are induced through repetitive mechanical loading as seen both in vitro and in vivo.[13 20] Whether the release of these substances is associated with the perception of pain is unknown. Nerve fascicles containing sensory afferents are present in tendons and especially in the peritendinous tissue; these nerves express receptors for the nociceptive substances which could thereby sensitise the nerves and augment pain signalling.[18]

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Relatively little is known about the pathogenesis of tendon pain in the early stages; tendinopathic changes in tendon are typically progressive, yet frequently asymptomatic. Many patients present themselves to physicians and physiotherapists when they are symptomatic; this presentation may be precipitated by a temporary increase in tendon loading. In a proportion of patients, the symptoms will inevitably settle spontaneously, only to recur later; thus, a cyclical pattern of symptoms and remission is not uncommon. This natural history of the condition needs to be factored into randomised clinical trials and laboratory studies as otherwise there might be a bias in the interpretation of findings (eg, if the trials require a change in usual activity levels, particularly a reduction of load).

In the following section, we briefly summarise recent findings regarding the pathogenesis of chronic tendinopathy at various anatomic locations.

Achilles

Sweden's Professor Alfredson presented data suggesting that unilateral treatment of patients with bilateral Achilles tendinopathy (using a minimally invasive scraping procedure) can lead to reduction or resolution of symptoms both on the operated and non-operated side.[21] This is in keeping with the results cited below for other tendons, highlighting a potentially underappreciated role of the central nervous system in generating tendon pain and, potentially, tissue pathology and abnormal movement patterns. Alternately, it may be that when one tendon is treated, both tendons undergo a period of relative rest during the recovery period leading to bilateral improvement.

Patellar

In an observational study of athletes with patellar tendinopathy, van Wilgen *et al*[22] reported the occurrence of reduced mechanical pain thresholds and pinprick allodynia in patellar tendinopathy patients, which was interpreted as reflecting the involvement of central sensitisation of myelinated (Ad-fibre) input. In a separate study, the Victorian Institute of Sport Assessment (VISA)-P score correlated inversely with the presence of sulfated glycosaminoglycans, demonstrating that, although the nervous system plays a modulatory role, tissue pathology is clearly related to the pain and functional deficits of tendinopathy (ISTS abstract, in press).

Rotator cuff

A recent systematic review summarised the main findings from both human studies and animal models of rotator cuff overuse.[23] The paper concluded that 'intrinsic, extrinsic and environmental factors all have an important role to play in the disordered tendon homeostasis of rotator cuff disease, which can lead to progressive mechanical failure'. The paper described a number of degenerative mechanisms, some related to classic inflammatory pathways (eg, interleukin-1, substance P (SP)) and others related to altered loading conditions (eg, impingement) or systemic influences (eg, ageing). The paper noted a disconnection between the amount of degenerative change and the extent of symptoms.

Elbow

Vicenzino presented data from a series of sensory motor system studies that implicated the role of the central nervous system in lateral epicondylalgia (LE). Our knowledge of LE pathology has advanced little since the seminal work by Kraushaar and Nirschl;[24] however, Coombes *et al*[25] have presented an integrated model in which tissue pathology interacts with the nervous system to cause widespread mechanical hyperalgesia and motor control deficits. The model is proposed to identify subsets of patients who may have more substantial pain or motor system deficits, as these patients may respond differently to clinical intervention.

DIAGNOSIS AND IMAGING

Tendinopathy is a syndrome of tendon pain and thickening—the diagnosis is based primarily on patient history and physical examination. However, Fredberg *et al*[26] reported a high level of misdiagnosis for Achilles and patellar tendinopathy, despite receiving referrals from orthopaedic departments. The role of clinical tests (eg, palpation tenderness for patellar tendinopathy) has been questioned as palpation is highly sensitive at reproducing symptoms, yet not specific in accurately determining the painful/pathological structure.[27] Given the new findings reported above, mechanical hyperalgesia may be reducing the presumed specificity of many commonly used clinical tests. Ultrasound (US) and MRI are used in the clinical setting to confirm the presence and location of tendon thickening or other structural change and associated findings.

The choice of US or MRI has traditionally been determined by the clinician based on personal preference and experience rather than on evidence-based guidelines. A number of studies have evaluated the accuracy and sensitivity of US (0.63–0.83 and 0.68–0.87, respectively)[28–32] and MRI (0.68–0.70 and 0.50–0.57, respectively)[29 31 33] in detecting tendinopathy in different tendons. Direct comparison between the two modalities has shown US, in trained hands, to be more accurate than MRI due to the superior spatial resolution of US imaging.[29 31 34 35] These studies used clinical diagnosis as the yardstick in determining the accuracy and sensitivity. Paavola *et al*[36] reported that US imaging had correctly diagnosed 83% of surgically confirmed Achilles peritendinitis/tendinopathy cases. US with Doppler also provide the advantage of being able to visualise the areas of increased blood flow.

At ISTS 2012, the value of US imaging in achieving a more specific diagnosis was apparent in a retrospective study of 143 US scans (ISTS 2012 abstract, in press). Although the proximal pole of the patella was, as expected, the most common location of pathology (present in 71% of tendons), pathology was also frequently observed at the distal pole (38%), sometimes in conjunction with other locations, but more commonly, as the sole location. Mid-substance pathology always occurred in association with involvement of either the proximal or the distal insertion. This level of anatomic specificity in diagnosis is clearly of value when tendon-directed treatments (eg, injections or exercise) are being considered.

However, the phenomenon that imaging abnormalities do not necessarily signal the presence of clinically significant symptoms has been well established. Cook *et al*[37] reported that 22% of elite athletes demonstrated pathological lesions within the patellar tendon, despite the absence of anterior knee pain. Similar cross-sectional studies using MRI in rotator cuff injury have shown structural abnormalities at similar frequencies in symptomatic and age-matched controls.[38] Importantly, the presence of asymptomatic structural abnormalities within tendons identified using imaging has been shown to increase the risk of developing pain.[39 40]

With regard to the rotator cuff, early studies indicated a progressive course of rotator cuff tendinopathy.[41 42] However, a recent study reported a low risk for tear progression in small, symptomatic supraspinatus tears (25% in 3.5 years).[43] The

progression of rotator cuff tendinopathy is associated with increased symptom manifestation.[44 45]

Despite their clinical utility, both US and MRI are subject to artefacts and typically yield poor interobserver and intraobserver reliability.[28 34] Investigators are developing more reliable and sensitive methods of quantitative tendon imaging, but these methods are, at this point in time, more relevant for research than for clinical practice. At ISTS 2012, two emerging methods for analysing and quantifying grey-scale US images were presented. De-Groot-Ferrando and colleagues (ISTS 2012 abstract, in press) described a reliable method to quantify morphometric parameters as well as textual features such as contrast and entropy. Van Schie *et al* (ISTS 2012 abstract, in press) reviewed Ultrasound Tissue Characterisation (UTC), which captures contiguous transverse images over the length of the tendon. These methods may have the potential to detect subtle changes in tendon structure not seen previously; however, further research is required. Sonoelastography is another developing technique which needs further evaluation within the field of tendinopathy.

For a full discussion of outcome measures see Measuring outcomes section. The use of imaging as a primary outcome measure in assessing the efficacy of treatments of tendinopathy is not recommended. This is due in part to the issues outlined above with regard to not only the limited psychometrics of US and MRI but also a lack of imaging improvement (despite clinical improvement or resolution) which has been reported with a variety of treatments including sclerosing injections, eccentric exercise, tenotomy and platelet-rich plasma (PRP) injections.[46–50] Imaging may serve as a useful secondary outcome measure, particularly at longer time points. Ohberg *et al*[51] reported an improvement in the fibrillar architecture on US in chronic Achilles tendinopathy patients after an eccentric loading programme (average follow-up of 3.8 years), and Sunding *et al* (ISTS 2012 abstract) demonstrated a reduced anterior-posterior diameter of the patellar tendon treated by minimally invasive arthroscopic shaving 3–5 years earlier.

TREATMENT

Rehabilitation

Rehabilitation planning

The development of a rehabilitation plan for an individual presenting with confirmed symptomatic tendinopathy requires complex clinical reasoning, with reference to the pathoanatomical diagnosis. Tendon pathology and subsequent rehabilitation will vary considerably depending on the site of pathology; stage of the tendinopathy; functional assessment; activity status of the person; contributing issues throughout the kinetic chain; comorbidities; and concurrent presentations.

While the critical assessment of rehabilitation programmes in the literature[52–55] is welcomed and advances knowledge in the field, the application of the evidence base is likely to be enhanced by the practitioner's clinical reasoning relating to the individual presentation. While a number of clinical trials have investigated the efficacy of unimodal rehabilitation interventions, generic rehabilitation prescriptions based solely on evidence-based medicine are unlikely to be optimal in the rehabilitation of tendinopathy, particularly in athletes.

Recent literature concerning the rehabilitation of tendinopathy confirms that the most important treatment modality is appropriate loading.[56] The continuum model of tendinopathy[57] provides a reasoned basis for considering targeted rehabilitation dependent on current clinical presentation.

Influence of rehabilitation on pathology and pain

Each component of the rehabilitation programme, in particular loading, must be manipulated in relation to the nature, speed and magnitude of the forces applied to the muscle/tendon/bone unit in order to achieve the goals of the particular management phase without causing an exacerbation of the pathological state or pain. Exercise prescription can target matrix reorganisation and collagen synthesis,[11 58] reduce tenocyte activity,[57] effect tendon compliance[59 60] or have an analgesic effect.[61]

While matrix reorganisation and improved collagen integrity are sometimes considered goals of rehabilitation, measurable structural change does not necessarily correlate with therapeutic outcome.[62] There is reasonable evidence to refute observable structural change as an explanation for the benefit of eccentric exercise in tendinopathy.[63 64] Exercise prescription may exert positive therapeutic effects through other mechanisms, such as change in compliance, functional strength, innervation, vascularity or perception of pain.

There is recent evidence to support an increased spinal hyperexcitability in patients with chronically painful tendinopathy[65] and there are a number of studies illustrating central or spinal involvement in the aetiology of tendinopathy.[66–69] Consequently when planning the rehabilitation programme, a clinician should assess the potential contribution of spinal function and central sensitisation. In addition, targeted intervention to the contralateral limb can be considered. This may have the dual benefit of limiting the progression of contralateral pathology, while positively influencing the symptomatic limb through a crossover effect.[70]

The analgesic effects of exercise (in addition to manual therapy and medical interventions) on the central pain mechanisms contributing to tendon pathology may have an important role; however, there is also some evidence to suggest that it is appropriate to perform specific eccentric exercises into pain for maximal efficacy[53 71] in the later rehabilitation of certain tendinopathic changes (eg, in chronic patellar or Achilles tendinopathy in middle aged athletes).

Exercise-based treatment of tendinopathy

It is important to address the re-education of muscle function, as opposed to considering the tendon in isolation, when planning the rehabilitation strategy. While early stimulus of the muscle tendon unit is typically focused on isometric muscle activation, which may include muscle stimulation, most programmes advocate the progression to higher loads as guided by symptom presentation.

Many tendinopathies have concomitant muscle atrophy which may require a prolonged stimulus at moderate loads, generally repeated for 3–4 sessions per week for optimal muscle hypertrophy.[72 73] In more marked cases of atrophy, exercise performance in a state of vascular occlusion has been demonstrated to have positive hypertrophic effects.[74–76]

Progression beyond the early isolated strength and hypertrophy loading requires functional conditioning of the muscle–tendon unit, graduating tendon load through more explosive concentric work, prior to starting eccentric skill specific re-education such as landings, before finally introducing sports specific challenges such as sprinting and cutting.

In summary, the key considerations when formulating a graduated tendinopathy rehabilitation programme are attention to detail, functional loading progression and specific preparation of both the muscle and tendon components to meet the demands of the sport.

In order to measure the response to rehabilitation and guide progression, suitable outcome measures, such as VISA scores or functional tests should be used frequently throughout the process. These will facilitate and guide a progression throughout the rehabilitative process based on functional criteria, as opposed to less sensitive, time-related markers of progression, thus ensuring an individualised approach to management.

The development of UTC shows promise in providing a more objective means of imaging changes in tendonopathic presentation in comparison to more conventional US imaging.[77] While further research is needed in this field, early prospective studies by Rosengarten *et al* (ref from ISTS abstracts), conducted on a population of elite Australian Rules Football players, indicate that UTC can be useful in monitoring an individual's short-term response to load.

Compliance with rehabilitation

As with all rehabilitation programmes, it is critical to ensure that patients have a clear understanding and realistic expectations in order to achieve adherence to the programme and increase the likelihood of attaining successful outcomes.

From the outset, it is important to explain the nature of the pathology, including the slow progression of tendon healing,[78] while communicating what should be expected in relation to pain behaviour during, and in response to, loading stimulus and exercise performance. Furthermore, expectations should be managed throughout the rehabilitation process in relation to the ongoing participation in certain training and competition, which is unlikely to be recommended in the early stages of a significant tendinopathy.

In a sporting environment, the coach should be involved throughout the rehabilitation process. This time provides an opportunity to educate the athlete and coach about the provocative nature of repetitive or sudden exposure to high tendon loads. Furthermore, the multidisciplinary approach to programme planning should be continued upon return to sport, to encourage appropriate load management strategies and to reduce the risk of recurrence.

Adjunct treatments

The close interaction between therapist and physician in the progression of rehabilitation is important to ensure that medical interventions complement the rehabilitation process, specifically in relation to the loading strategy. The timing and choice of non-steroidal anti-inflammatory drugs (NSAIDs) must be considered in relation to the potential influence on tenocyte activity and glycosaminoglycan synthesis.[79–81]

While NSAIDs may be employed to good therapeutic effect in a reactive tendon, the tendon response to load may be affected, with potential inhibition of collagen synthesis,[82] while certain NSAIDs may contribute a deleterious effect on muscular adaption.[83 84] Medical intervention should, therefore, be determined in conjunction with and in support of the current goals of the rehabilitation plan.

Long-term management of the at-risk tendon

The recent literature on tendon turnover further illustrates the importance of the off-season management for the future prevention of tendinopathy in athletes.[85] The maxim that 'tendons don't like rest or change' (Jill Cook, ISTS 2012 workshop) should be instilled in athletes with a propensity for tendinopathy and be communicated to coaches who plan preseason training sessions.

Tendinopathy quite commonly presents as the result of a focused overload of a particular tendon within the kinetic chain. To this end, a failure to consider the energy absorption capabilities of related structures (including joint range and muscular function), as well as the consideration of the contralateral limb, may predispose the athlete to a recurrence.

The off-season training phase for athletes with a history of tendinopathy should include a continued tendon loading programme in order to prevent the reduction in tendon integrity and stiffness. Subsequent return to training phases should include appropriately spaced, graduated increases in load. In the absence of such strategies, the athlete will be predisposed to active tendinopathy upon resumption of full training.

While evidence-based support of this approach to long-term management may be limited, the principles suggested in this rehabilitation approach should decrease the likelihood of recurrent presentations of tendinopathy.

Injection

The relative accessibility of tendons and their insertions or associated structures (bursae), as well as the highly localised nature of many tendinopathies, make the local injection of medical therapies an attractive and logical therapeutic opportunity. In current practice, glucocorticoids remain the most widely used injectable therapy for a variety of tendinopathies. They are often given in conjunction with local anaesthetic, although other treatments including prolotherapy, sclerotherapy, protease inhibitor injections and, recently, biologics such as autologous whole blood and PRP have also been employed as injectable medical treatments. US guidance has also increased the confidence of physicians injecting intratendinously, peritendinously or into bursae. A key distinction made by round-table discussants was that the treatments may be divided into those directed *into* the tendon (with the potential for causing needle damage to tendon tissue) versus those which are targeted *outside* the tendon.

The role of glucocorticoid injection therapy has long been debated with the balance of benefit versus harm of such treatment a potentially serious concern for clinicians. Potential mechanisms of action in tendinopathy include a reduction of extrinsic or intrinsic inflammation, reduction of tenocyte proliferation or cellular activity, antiangiogenic activity, inhibition of scarring/adhesion, antinociceptive action or some combination thereof. The efficacy of glucocorticoid injections for rotator cuff tendinopathy, Achilles tendinopathy, patellar tendinopathy and tennis elbow has been investigated at length, with the sum of evidence suggesting that the majority of patients may experience short-term improvement in pain and/or function, but experience a higher risk of relapse in the medium to long term.[71 86–89] Clinical trials have been limited by the heterogeneity of the patient cohorts under investigation and the timing and type of outcome measures used in the studies. A recent meta-analysis demonstrated worse results from glucocorticoid use when compared with other treatments and placebo in the intermediate and long-term follow-up of treatment for tennis elbow.[90]

Prolotherapy and sclerotherapy represent treatments quite opposed in their proposed mechanisms of action. The original aim of prolotherapy was to promote a local inflammation-repair response. Conversely, sclerotherapy employs colour Doppler US to target peritendinous areas of increased signal which have been shown to be richly vascularised and innervated. There is also some suggestion that agents used for prolotherapy may themselves have vascular sclerosing properties. A systematic review by Coombes *et al*[90] concluded that "ultrasonography-

guided injection of lauromacrogol (polidocanol) and prolotherapy injection of hypertonic glucose and local anaesthetic are potential therapeutic techniques, based on moderate evidence of improvements in the intermediate term for patellar tendinopathy and lateral epicondylalgia, respectively."

For midportion Achilles tendinopathy, the US-guided injection of polidocanol into peritendinous regions with high blood flow and nerves has also been reported, in a small randomised controlled trial, to lead to pain relief and improved patient satisfaction.[91] At 2-year follow-up after successful polidocanol injections, the Achilles tendon demonstrated a decreased thickness and improved tendon structure, demonstrating the potential influence of these soft tissues on the tendon tissue proper.[92] Further prospective clinical trials may determine whether there are subgroups of Achilles midportion tendinopathy patients (sedentary/active, male/female) that may respond differently to polidocanol injection.[93 94]

Lesser studied injectable agents include sodium hyaluronate, botulinum toxin and protease inhibitor solutions. The mechanism of action for each of these treatments is unclear. There is little if any evidence to demonstrate the efficacy of these treatments in reducing pain or restoring function. Tennis elbow has been suggested as the most likely type of tendinopathy to respond to treatment with sodium hyaluronate or botulinum toxin.[90 95] The use of protease inhibitor agents has been questioned due to the high rate of systemic allergic reactions to aprotinin, the most commonly used agent.[96–98]

Biological therapies, such as autologous whole blood and PRP, have gathered popularity and media interest as treatments for tendinopathy in recent years. They aim to deliver a 'blunderbuss' of bioactive substances to the site of pathology and thereby stimulate a healing process. These treatments build on the theoretical principles of prolotherapy, although with the instigation of tendon healing via normal physiological pathways rather than via an inflammatory response to a noxious substance.

Several small case series which have investigated the autologous whole blood injection for lateral and medial epicondylalgia and patellar tendinopathy report improvements in pain and function in the short-to-medium term although the lack of control group and small numbers limits the applicability of these studies.[99]

The use of PRP as a treatment option has increased dramatically over the last decade with a large number of published articles investigating its value in the treatment of an array of musculoskeletal problems, including tendinopathy. Despite such activity in the literature, high quality randomised controlled trials into the use of PRP for specific tendinopathies are, at the time of publication, still limited in number. Existing research suggests an improvement in clinical symptoms of tennis elbow following PRP injection compared with either glucocorticoids or autologous whole blood injections.[100] Some positive results have been noted in its use for patellar and Achilles tendinopathy; however, these outcomes must be weighed against high quality studies suggesting that PRP offers no benefit in the treatment of Achilles tendinopathy.[101 102] The focus of PRP use in rotator cuff tendinopathy has been as an adjunct during rotator cuff repair surgery; there is insufficient evidence to support its efficacy to date.[100 103]

High volume injections for the Achilles and patellar tendons, in which large volumes (up to 40 ml) of normal saline (along with bupivacaine and hydrocortisone acetate) are injected peritendinously, have been reported in case series, but not yet subjected to a randomised controlled trial.[104]

Surgery

The focus of this section is the chronically painful, but not ruptured, tendinopathic tendon. There are few studies with optimal scientific design, and few studies with mid-term or long-term follow-ups. As is commonly noted in systematic reviews, studies with a poor scientific design show good results, and studies with a good scientific design show poor results.[105]

Achilles

For the chronically painful Achilles midportion and proximal patellar tendon, surgical treatment has been the gold standard when conservative treatments have failed. However, it is well known that the majority of traditional surgical techniques (eg, excision of macroscopically abnormal tendon tissue through a longitudinal split of the tendon) require long-postoperative rehabilitation periods (4–12 months), and the clinical short-term results are variable. An alternate method consisting of multiple longitudinal incisions inside the tendon, followed by a relatively quick rehabilitation period, has also been described,[106] but that method appears to have been more or less abandoned. Studies with longer follow-up results or new prospective studies using that technique are absent from the literature.

Recent studies on basic tendon biology and imaging led to the development of a new surgical treatment method for midportion Achilles tendinopathy—the US and Doppler-guided mini surgical scraping technique.[107] The surgery is directly related to the US and Doppler findings; the 'scraping' being performed in specific regions outside the tendon which demonstrate increased colour Doppler signal in association with structural grey-scale findings. This minimally invasive surgery allows for a quick rehabilitation. Patients begin ambulation after the first postoperative day, and achieve full tendon loading activity after 2–6 weeks. The preliminary clinical results using this method are promising and with very few complications,[107] but are observational in nature.

Patellar

A well-designed randomised study on patients with Jumper's knee/proximal patellar tendinopathy, comparing the traditional surgical treatment method with eccentric training treatment, showed no significant differences between the groups, with approximately a 50% rate of clinical success after surgery.[108] The traditional surgical method, in which macroscopically abnormal tendon tissue is excised through a longitudinal split of the tendon, was questioned in this study.

For the proximal patellar tendon, a recently developed method is based on the same basic biological rationale (extensive sensory innervation located peritendinously) and principles as the 'scraping' technique for the Achilles tendon (see previous section), namely US and Doppler-guided arthroscopic shaving.[109] This arthroscopic method (often performed under local anaesthesia, via 2 minor key holes) allows for a quick rehabilitation during which the patients begin ambulation on the first postoperative day and can achieve full tendon loading activity after 4–8 weeks. The preliminary clinical (observational) results using this method are also very promising, with good results in patients with different activity levels (sedentary to elite level activities) and very few complications.[109]

Further studies on these methods are required, including larger, randomised studies reported in accordance with the CONSORT statement,[110] with longer follow-up periods, and using validated outcome measures (detailed below).

Rotator cuff

Rotator cuff tendinopathy encompasses several characteristic changes of the rotator cuff tendons from tendinopathy without macroscopic tendon discontinuity, through partial-thickness and full-thickness tears, to rotator cuff arthropathy.

Surgical treatment of rotator cuff tendinopathy is applied after failed conservative treatment except in the case of a traumatic tendon tear where early surgical intervention is advocated. The interventions range from subacromial decompression in the case of therapy-resistant impingement and partial rotator cuff tears involving less than 50% of the tendon thickness, to tendon repair in the case of significant partial tears and rotator cuff tears, or tendon transfers when facing an irreparable tear; reverse shoulder prosthesis with or without tendon transfer may be applied in the case of rotator cuff arthropathy. In addition, the biceps tendon needs to be addressed with tenodesis or tenotomy when the biceps and its pulley system are affected.

Rotator cuff repair is a clinically very successful procedure, but healing occurs through scar tissue and often is incomplete. Despite the introduction of several innovative surgical techniques (eg, single row with several suture modifications, single row with triple loaded suture anchors, double row, triple row, suture bridge, transosseous and transosseous equivalent repair) aiming to optimise the mechanical construct and improve healing of rotator cuff tears, failure rates remain high, especially among large and massive tears.[111–114] Recent literature does not support an advantage by the use of one specific technique based on clinical outcome scores or healing rate analysed by CT arthrogram and MRI.[115–121] The tendon remains the weak link of the repair. Whereas some advocate the use of single row repair constructs in small rotator cuff tears and double-row repair in medium and large tears, others are concerned about the reports on medial failure of the repair construct following double-row techniques and increased costs associated with longer surgical time and higher implant costs ([114 116 119]{Lorbach, 2012, p.1778}[122–124]). Chronic rotator cuff tendon tears are accompanied by significant, irreversible deterioration of muscle quality which is decisive for the prognosis indicating timing of surgery to be essential.

Elbow

Well-conducted studies have failed to demonstrate a consistently successful operative treatment for epicondylalgia. The Cochrane database has not identified any surgical treatment as being effective. Current surgical treatments focus on one of two objectives: (1) removing the tendinosis tissue and/or repairing the origin[125] or (2) releasing the origin completely.[126] Controlled resisted stress during the rehabilitation period is advocated.

MEASURING OUTCOMES

Systematic reviews of clinical trials for the management of tendinopathies commonly report on the inconsistency of outcome measures which limits data synthesis and meta-analysis.[71 127–131] This lack of consistency of outcome measures is also a barrier identified by clinicians in the translation of research into clinical practice (see Knowledge translation, below).

There are few outcome measures specifically designed for tendinopathies, and those that do exist have not achieved widespread implementation. A case in point is the VISA-P which was originally developed in the late 1990s for patellar tendinopathy and later modified for use in other regions, such as the Achilles tendon (VISA-A).[132 133] It was developed through a review of the literature, consultation with experts and trialling on patients, as well having undergone clinimetric testing (eg, reliability, construct validity, discrimination and sensitivity to change).[132 133] At ISTS 2012, the correlation of VISA with extent of tissue pathology (as gauged by glycosaminoglycan content) was also reported (ISTS abstract, in press). The VISA incorporates elements of symptom ratings in various loaded states, amount of activity possible and ratings of participation. Tendinopathy presents clinically as an activity-related pain state that limits participation and as such the VISA scale would appear to be an appropriate outcome measure specific for tendinopathy. Despite the intervening decade since the inception of the VISA-A, it has only been used in 37% of the recently reviewed studies (census date September 2011) reporting the management of Achilles tendinopathy.[129] Research is required to evaluate why the VISA scales have not been widely adopted in clinical trials, as they seem to address the spectrum of pain, disability and participation. It appears that outcome measures for all tendinopathies require development and further evaluation in terms of their clinimetric properties. This might be facilitated by reference to the COSMIN checklist.[134]

A group of experts from the fields of general medical practice, rheumatology, public health, physiotherapy, occupational therapy, podiatry, health services research, chiropractic, clinical trial methods and designs, biostatistics and health economics produced a set of recommendations for research into the non-pharmacological management for common musculoskeletal conditions.[135] This group identified as the fifth most agreeable (81% agreement) recommendation the development of core outcome sets, which would enable comparisons between trials and synthesis of their data.[136] Currently, there are no agreed upon core outcome sets for the common tendinopathies. It was affirmed at the ISTS 2012 that core outcome sets should be developed and implemented for the different types of tendinopathy.

The notion of a standardised set of outcomes across a wide range of health matters was central to the formation of the Core Outcome Measurement in Effectiveness/Efficacy Trials initiative (http://www.comet-initiative.org/), which was launched in 2010 in order to bring together those interested in the development and application of core outcome sets. The COMET initiative would appear as an appropriate vehicle with which to engage in the development and implementation of core outcome sets for tendinopathy. An exemplar of core outcome domains that might be included in a core outcome set can be gleaned from the Initiative on Methods, Measurements and Pain Assessment in Clinical Trials (IMMPACT, http://www.immpact.org/), which determined that chronic pain clinical trials should assess pain, physical functioning, emotional functioning, participant ratings of improvement and satisfaction with treatment, symptoms and adverse events and participant disposition, such as treatment adherence.[137] Functioning and well-being have been recently found to be the areas to assess which are important to patients.[138]

A recent mixed methods study that integrated a systematic review with clinical reasoning for the management of Achilles tendinopathy reported that physiotherapists found it difficult to reconcile their use of evidence informed painful eccentric exercises with findings that the patients' primary concern is pain reduction.[130] The overwhelming use of measures of pain severity in clinical trials (eg, 79–89% in recent systematic reviews of Achilles tendinopathy[138 139]) add to these difficulties. Given that tendinopathies are in the main primarily activity-related pain states, it would seem reasonable that research also focuses on outcomes that measure physical activities and participation

(including sports) that are known to produce pain and disability in the different types of tendinopathy (also known as tendon and/or sport-specific functional performance tests). The incorporation of activities and participation is consistent with the International Classification of Functioning, Disability and Health, which is the WHO's framework for measuring health and disability (http://www.who.int/classifications/icf/en/). These functional performance tests might constitute part of a core outcome set.

It is our observation that few clinical trials utilise patient global rating of change scales (GROC), which essentially records the patient's determination of the global change in their condition after treatment.[140] GROCs implicitly allow the patient to weight whatever is important to them in coming to this determination. The inclusion of such patient centric outcomes was one of the recommendations for trials of non-pharmacological management of common musculoskeletal problems[136] and should be considered in further developing the outcome measures for tendinopathy.

Research is required into core outcome sets for the different tendinopathies, ensuring that as well as pain, activity and participation are also captured along with GROCs. While there might be an expected level of similarity in what constitutes these core outcome sets, it will be important to ensure that they are specific to the type of tendinopathy (eg, Achilles, gluteal, lateral elbow, biceps femoris, etc) and incorporate all users (eg, patients, payers, healthcare professions, researchers and service delivery) in their formation and ultimately implementation.

PREVENTION

Tendinopathies are common and often cause long-lasting symptoms of pain and dysfunction which negatively impact on working and/or sporting capacity. Thus, preventive strategies for tendinopathy seem warranted. However, little research on the prevention of tendinopathy has been published. In 1992, van Mechelen *et al*[141] introduced the sports injury prevention research model. This model will be used as a framework for describing what is known already, and what should be targeted in future research projects.

Step 1: magnitude of the problem

Several studies have been published on the frequency of sport-related and exercise-related tendinopathies.[142–147] However, comparison and interpretation of results is difficult due to a lack of consistent case definitions and inappropriate time-loss-based injury registration methods. Most studies are performed in selected populations such as elite athletes, or in participants of one specific sport. The definition of tendon problems is often unclear since tendon pain, US tendon abnormalities and sport-specific diagnoses such as jumper's knee or tennis elbow are used interchangeably. Many people continue their work or sporting activities despite their chronic overuse injury and/or tendon pain. This means that in many epidemiological studies, despite their prevalence tendinopathies are not included in the incidence rate as new injuries that cause time loss from work or sports.

Bahr[148] recommended the quantification of overuse injuries in a standardised way using prevalence (not incidence) in prospective studies with continuous or serial measurements of symptoms. Valid and sensitive scoring instruments that measure pain and functional level should be used, and severity should be determined in relation to this rather than from time lost from sport/work (as many athletes continue to compete despite pain). The VISA-A and VISA-P are examples of valid, sensitive and cross-culturally adapted questionnaires which measure pain, function and sports participation in athletes with Achilles or patellar tendon problems (discussed above). Recently, the aforementioned recommendations led to the development of a new method, including a questionnaire, for the registration of overuse problems.[149]

Step 2: establishing aetiology and mechanisms of injury

Since overuse injuries, including tendinopathies, have a multifactorial aetiology, establishing the mechanism of injury is not an easy task. Both intrinsic and extrinsic factors have been described in the literature, but there is little robust and often even conflicting evidence for these. A link between the genetic profile and tendinopathy has been reported and some people may be more susceptible to tendinopathy than others.[150] Genetic screening to identify people at risk of developing tendinopathy might play an important role in future prevention strategy. However, among all potential risk factors, the load to the tendon is considered the most important factor in the aetiology of sport-related and exercise-related tendon pathology and pain.[151] Moreover, load is a factor that can be modified by prevention strategies.

A promising development is UTC. Preliminary data show that this non-invasive, operator-independent imaging technique can visualise load-dependent changes in a very early stage, at least for the large lower extremity tendons.[77]

Another potential option is to monitor the volume of loading to which a tendon is exposed (eg, number of jumps, hours of training, distance run, etc); however, there is an inherent inaccuracy in any such measurement utilised to date. Visnes and Bahr[152] showed a significant difference between healthy and volleyball players with jumpers knee in hours dedicated to volleyball, jump training and number of sets played. In a sample population of Australian football players, no correlation was observed between the distance covered during the game and the magnitude of change in tendon structure. (ISTS 2012 abstract, in press) The major limitation of global positioning system data is that it quantifies distance and speed travelled, where the impact of load on tendon structure may be more complex (ie, jumps performed in each session, changes in surfaces, etc).

Clarification of the complex relationship between load (including an individual's own unique biomechanics) and tendon changes together with other prospective studies investigating the various (modifiable) risk factors and their influence on the tendon would certainly aid in establishing more appropriate and effective preventive measures. Currently, in most situations, the clinician is not in a position to provide guidance in terms of how much load an individual patient can safely engage in to prevent the development of tendinopathy. However, cautioning against sudden changes to tendon loading may be prudent.

Steps 3 and 4: developing, introducing and evaluating a training programme

Developing and introducing training programmes that teach coaches and athletes how to load tendons in the most appropriate way, and how to change their strength, flexibility and proprioception in the most efficient way could be important preventive measures to reduce the risk of developing tendinopathy.

So far, most studies of injury prevention in sporting populations have focused on reducing the incidence of acute injuries such as ACL rupture or ankle sprain.[153 154] To our knowledge, only a few studies have targeted the prevention of tendinopathy. A prospective study in elite female soccer players demonstrated that soccer-specific balance training can reduce the incidence of

patellar and Achilles tendinopathy.[155] A dose–effect relationship between the duration of balance training and injury incidence was found.[155] Fredberg *et al* [156] reported on a randomised controlled trial (RCT) in which elite soccer players were followed over 12 months with use of ultrasonography and injury registration. Half the teams were randomised to an intervention group with prophylactic eccentric training and stretching of the Achilles and patellar tendons during the season. This study demonstrated that the prophylactic training reduced the risk of developing US abnormalities in the patellar tendons, but had no positive effects on the risk of injury. On the contrary, in asymptomatic players with ultrasonographically abnormal patellar tendons, prophylactic eccentric training and stretching increased the injury risk.[156]

Only programmes that can and will be adopted by athletes, coaches and sporting associations will be successful in preventing injuries.[157] Hence, implementation strategies and effects of research are necessary to evaluate if preventive methods really are being adopted by the athletes. Finally, the costs and effectiveness of the introduced preventive measures should be evaluated by repeating step 1, or preferably by conducting an RCT.

KNOWLEDGE TRANSLATION: UPTAKE OF RESEARCH IN THE CLINICAL SETTING

Achieving evidence-based treatment of tendinopathies is a significant challenge. A compelling example is the persistent use of corticosteroid injections for lateral elbow tendinopathy. In general, it takes approximately 17 years to get 14% of research findings adopted into practice.[158] Moreover, only 30–50% of patients receive recommended care, 20–30% receive care that is not needed or that is potentially harmful[159 160] and 96% may receive care with the absence of evidence of effectiveness.[161] Many factors have been shown to influence the current practice patterns. The use of evidence, particularly that from primary research, ranks lower in terms of influence on practice than experience, continuing education, practice patterns of colleagues and entry level training.[162] It is therefore imperative that a concerted effort is undertaken to ensure that research is pertinent to, meaningful to and feasible for easy uptake into the clinical setting.

Common barriers to achieving evidence-based practice include: not enough time to search for the evidence; too much evidence being available; lack of relevant applicable evidence; the evidence being scattered through a number of databases/journals, that is, not collated into a single location; inadequate access to the evidence (limited availability of free text); limited training in how to access the evidence; poor presentation of the evidence; limited competence and confidence in appraising the quality of the evidence; lack of applicability of the evidence to a heterogeneous patient population; publication bias; lack of autonomy; lack of incentives; clinicians' personal attitudes/intrinsic motivation; and patients' expectations.[163–165] An interesting survey and analysis of practice patterns for subacromial pain among general practitioners (GPs) and physiotherapists found that there was only a weak association between treatments which were trusted, and treatments which had been shown to be effective for that condition. For example, GPs and physiotherapists stated that they trusted US therapy, despite the conclusion of systematic reviews that this therapy is ineffective.[166]

The struggle to successfully bridge the 'knowledge to action gap' is being informed by the efforts of 'implementation research' defined as 'the scientific study of methods to promote the systematic uptake of clinical research findings and other evidence-based practices into routine practice, and hence to improve the quality (effectiveness, reliability, safety, appropriateness, equity and efficiency) of healthcare.'.[167] Many strategies to enhance uptake of evidence into practice have been investigated including use of didactic education sessions, audit and feedback, reminders, opinion leaders, educational materials, patient mediated interventions, financial incentives and interventions tailored to identified barriers—the median improvements of these strategies is approximately 10%.[168–172] Recently, the use of multiple rather than single interventions, particularly those which are specifically tailored to identified barriers, has been associated with approximately double the improvement than those of single interventions.[173 174]

There is other evidence in the medical behaviour change literature which can inform strategies to enhance adoption of evidence into practice. Green *et al*[175] identified four general practice style of the clinician: (1) seekers—those who consider evidence and data which are systematically gathered more important in directing choice of intervention than that of personal experience; (2) receptives—although evidence oriented these individuals tend to rely more on the judgment of respected others; (3) traditionalists—regard clinical experience and authority of respected others as most important and (4) pragmatists—weigh the competing demands of day-to-day practice more heavily than the validity of the evidence. Accordingly, in order to more successfully impact practice change, one first needs to identify the relative representation of each of these practice styles. The traditional methods of knowledge translation have targeted the 'seeker'—one who hunts for the best evidence. This is easily appreciated when one considers that there have been 13 new systematic reviews pertaining to the treatment of tendinopathies published since 2012 alone (PubMed search, February 2013). However, given that seekers make up the smallest percentage of clinicians (~3%)[175] and pragmatists the greatest (~60%),[176] there is a need to address to greater degree the needs of the pragmatist. Provision of didactic education or educational materials (provision of knowledge which validates the effectiveness of the intervention) is not enough. Indeed, the prediction of a change in behaviour triggered by provision of knowledge alone is poorly substantiated.[177] Instead, there is an increased focus on shifting support to facilitate practice change to 'packaging the evidence' into evidence-based practice resources, for example, tools/toolkits which support the practical *application* of the knowledge, for example, simple algorithms and/or videos demonstrating efficient use of the intervention. This strategy is more aligned to assisting the pragmatist to change clinical practice. This approach has been employed recently with the development of the Achilles tendinopathy toolkit, which is now freely available online at the Physiopedia website.

In conclusion, the current literature dedicated to the facilitation of evidence-informed clinical practice provides some important messages for the tendinopathy research community. (1) Research should result from partnerships between researchers and clinicians. (2) Barriers to the application of evidence should be identified and specifically targeted using implementation strategies that are based on behavioural theories.[177–179] (3) Health research funders should be encouraged to create the conditions for effective knowledge translation.[180] (4) The effectiveness of these strategies should be evaluated rigorously.[181 182]

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9.4- Appendix D

Summary of the literature investigating the infiltration of inflammatory cells in naturally occurring human tendon pathology

Study	Tendon	Analysis	Normal tendon	Pathological tendon	Ruptured/torn tendon
Schubert et al (2005)	Achilles	Immunohistochemistry (CD3- T lymphocytes, CD20- B lymphocytes, CD68- macrophages) H&E staining		Compared to ruptured Achilles ↑T-lymphocytes ↑B-lymphocytes ↑ macrophages ↑iron-positive cells *Granulation tissue in 8 out of 10 samples. Granulation tissue consisted of clusters of capillaries embedded in a fibroblast-rich stroma infiltrated by macrophages, B lymphocytes, and T lymphocytes	Compared to pathological tendon ↑ granulocytes *None of the samples from spontaneously ruptured tendons contained granulation tissue or haemosiderophages
Scott et al (2008)	Patellar tendon; pathological and control group	Haematoxylin and eosin, alcian blue, immunohistochemistry (CD68- macrophages, CD3- T lymphocytes, mast cell tryptase- mast cells)		↑ mast cells Lymphocytes and macrophages were too infrequent to be quantified	

Study	Tendon	Analysis	Normal tendon	Pathological tendon	Ruptured/torn tendon
Kragsnaes et al (2014)	Achilles; non-ruptured pathological and normal	Immunohistochemistry (CD3- T lymphocytes, CD20- B lymphocytes, CD34- endothelial cells, CD56- NK cells, CD68- macrophages)	15 samples collected 14/15 exhibited T-lymphocytes 3/15 exhibited B-lymphocytes 11/15 exhibited NK cell 8/15 exhibited mast cells	↑ endothelial cells ↑ macrophages Trend ↑ mast cells	
Millar et al (2010)	Rotator cuff; Torn supraspinatus and normal subscapularis tendon from same patient; control subscapularis	Immunohistochemistry (CD68- macrophages, CD3- T lymphocytes, CD4- T helper cells, CD34- endothelial cells, CD206- M2 macrophages, mast cell tryptase- mast cells)	<10% of cells stained positive for: Macrophages Endothelial cells	Note: Normal on MRI but histologically abnormal ↑ Macrophages ↑ T-lymphocytes ↑ T helper cells ↑ endothelial cells ↑ M2 macrophages ↑ mast cells *Majority of mast cells near the vasculature	10-20% of cells stained positive for: Macrophages T-lymphocytes T helper cells Endothelial cells M2 macrophages Mast cells *Inverse relationship between amount of inflammatory cells and tear size **Inverse relationship between amount of endothelial cells and tear size

Study	Tendon	Analysis	Normal tendon	Pathological tendon	Ruptured/torn tendon
Matthews et al (2006)	Rotator cuff; full thickness tears (supraspinatus) and control (subscapularis)	Haematoxylin and eosin, toluidine blue, immunohistochemistry (CD68- macrophages, CD45- leukocytes, mast cell tryptase- mast cells, D2-40- lymphatic endothelium)			19/38 contained mast cells ↑ Mast cell ↑ macrophages ↑ leucocytes ↑ vascular endothelium with small tears *Majority of cells located around blood vessels **Inverse relationship between amount of inflammatory cells and tear size ***Inverse relationship between amount of endothelial cells and tear size
Cetti et al (2003)	Achilles; ruptured, (rupture site and adjacent sites) contralateral	Haematoxylin and eosin, Immunohistochemistry (calgranulin and neutrophilic elastase)	1/50 samples contained neutrophils		Acute inflammation was defined as infiltration of neutrophils Rupture site- All samples contained neutrophils Proximal to rupture- 49/56 samples contained neutrophils Insertion site- 35/55 samples contained neutrophils

Study	Tendon	Analysis	Normal tendon	Pathological tendon	Ruptured/torn tendon
Gotoh et al (1997)	Supraspinatus; partial and full thickness tears and control	Immunohistochemistry (UCHL-1- T lymphocytes, L-26- B lymphocytes, CD68- macrophages	1/8 normal controls contained T lymphocytes 0/8 normal controls contained B lymphocytes 2/8 normal controls contained macrophages		8/8 full thickness tears contained T lymphocytes 8/8 partial thickness tears contained T lymphocytes 0/8 full thickness tears contained B lymphocytes 0/8 partial thickness tears contained B lymphocytes 8/8 full thickness tears contained macrophages 8/8 partial thickness tears contained macrophages
Dean et al (2014)	Supraspinatus; painful and pain-free tendon	Basic histological staining, immunohistochemistry (CD45- leukocytes, CD206- macrophages)	Pain-free tendon (2 partial tears, no full thickness tears)	Painful tendon (2 partial tears, no full thickness tears) ↑ leucocytes ↑ macrophage	
Gumina et al (2006)	Supraspinatus; torn (3-5cm)	Haemotoxylin and eosin, histochemical (colloidal iron, periodic acid Schiff [PAS], Von Kossa) examination.			38/38 contained moderate inflammatory infiltrate *Lymphocytes, macrophages, plasmacytes at lip bordering the tear margin

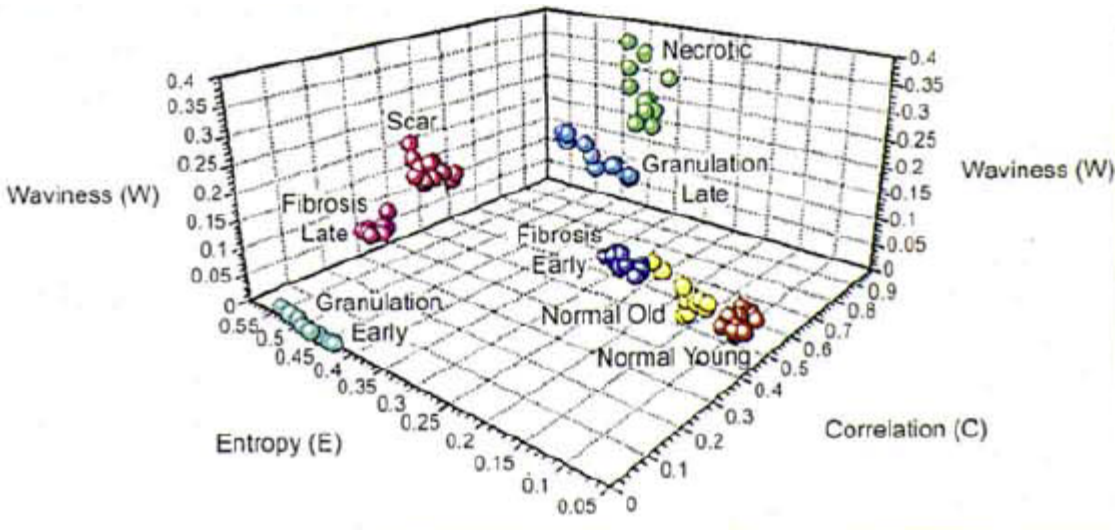
Study	Tendon	Analysis	Normal tendon	Pathological tendon	Ruptured/torn tendon
Wu et al (2011)	Rotator cuff (non-specific); ruptured	Haemotoxylin and eosin, immunohistochemistry (markers for apoptosis, autophagic cell death and myofibroblasts) *No specific staining for inflammatory cells			No inflammatory cells (neutrophils, macrophages, lymphocytes or other inflammatory cells

Greyed out area indicates tissue specimens were not collected in that category

9.5- Appendix E

Mean (±SD) values of correlation, waviness and entropy ratios of the eight tissue types from equine superficial digital flexor tendons

	Correlation ratio (ie echo-type I)	Waviness ratio (ie echo-type II)	Entropy ratio (ie echo-type III)
Normal young	0.54 ± 0.032	0.02 ± 0.005	0.10 ± 0.009
Normal old	0.52 ± 0.019	0.07 ± 0.005	0.14 ± 0.007
Necrotic	0.86 ± 0.055	0.30 ± 0.040	0.35 ± 0.029
Early granulation	0.00 ± 0.000	0.00 ± 0.000	0.47 ± 0.042
Late granulation	0.65 ± 0.016	0.21 ± 0.025	0.34 ± 0.039
Early fibrotic	0.49 ± 0.009	0.08 ± 0.008	0.24 ± 0.017
Late fibrotic	0.25 ± 0.030	0.10 ± 0.012	0.52 ± 0.007
Scar	0.41 ± 0.017	0.18 ± 0.014	0.52 ± 0.022



Three-dimensional plot of correlation (x-axis), entropy (y-axis) and waviness (z-axis) ratios for ultrasonographic images of the eight tissue types from equine superficial digital flexor tendon

9.6- Appendix F

Changes in tendon mechanical properties

The biomechanical tendon properties *in vivo* has become possible due to recent improvements in US technology and the development of new investigative techniques. Obst et al (2013) performed a systematic review on 21 articles on the immediate effect (≥30 mins post-exercise) of exercise, ranging from prolonged stretching to functional activities, on biomechanical properties of the Achilles tendon. Exercises that involved active concentric-eccentric contractions, such as running, had no significant effect on Achilles tendon stiffness. This was a consistent finding in studies that were not included in this systematic review (Farris et al, 2012; Houghton et al, 2013; Peltonen et al, 2010; Peltonen et al, 2012). Interestingly, there was consistent evidence suggesting that tendon stiffness decreased post isometric contractions and passive stretching.

It is worth noting that of the participants included in the systematic review, 77.6% were male. Joseph et al (2014) found that changes in tendon mechanics differed in response to a repeated jumping exercise (100 toe jumps in a Smith machine with 20% of body mass). Decreases in stiffness and Young's modulus were observed in women immediately after exercise, with these parameters unchanged in men. The change in tendon compliance in woman may be a protective mechanism due to the large difference in incidence between the sexes, however why this may be protective is unclear.

A major issue with quantifying biomechanical properties using US is that it is often calculated at the musculotendinous junction, with a force/elongation curve generated based on the excursion of the MTJ during a maximum voluntary isometric contraction

against a dynamometer. Measurements of tendon force, stress, stiffness and Young's Modulus are calculated based on this force/elongation curve. The assumption is that any changes in mechanical properties are solely dependent on changes within the tendon and not the triceps surae musculature. As muscle is a more mechano-responsive soft tissue than tendon, the biomechanical properties may be more aptly described as triceps surae complex force, stress and stiffness.

9.7- Appendix G

MONASH University

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(Revised September 2009)

APPLICATION FORM FOR THE USE OF ANIMALS FOR SCIENTIFIC PURPOSES IN RESEARCH AND TEACHING

School of Biomedical Sciences B — **ANIMAL ETHICS COMMITTEE**

AEC NUMBER	**SOBSB/Physio/2010/65**
Project Type	X Research ☐ Undergraduate Teaching ☐ Training in Procedural Techniques
Project Title	Understanding the clinical and ultrasound presentation of equine tendon injury
Animal Use Categories (Refer to List of Categories attached)	2.1 No anaesthesia, minor procedures used 5.3 Field/work/ Off Campus Work
Standard Operating Procedures	x **No** ☐**Yes: Title/AEC Number:**

SOPs indicated are to be read in conjunction with the application. Detail any variations from the SOP.

Proposed Start Date 01/01/2011 actual start determined at time of AEC approval	**Proposed Finish Date** 01/01/2014 Actual finish determined at time of AEC approval

	Title & Full Name	Qualifications & Position	Department / Institution
Chief Investigator	Professor Jill Cook	Principal Research Fellow PhD, PG Dip Manip Therapy, B App Sci (Phty)	Department of Physiotherapy
Person to act in Chief Investigator's absence	Dr Jamie Gaida	Assistant Lecturer PhD, B.Physio (hons)	Department of Physiotherapy
Investigator responsible for animal care	Mr Sean Docking	PhD Candidate BHSc (Hons)	Department of Physiotherapy

DECLARATION BY CHAIRPERSON OF AEC

I certify that the procedures/ personnel/ location in this project has been considered and approved by the Animal Et[redacted] **the period** ...5.../.7.../.11..... **to** ...5.../.7.../.14...

.....[redacted] MMRP - 2 5/7/14

Chairperson's signature AEC Date

[redacted]

Approval is subject to the following conditions:

1. An Annual Report must be provided each February.
2. A Final Report must be submitted within six months of the completion of the project
3. Unexpected or adverse effects which impact on the welfare of the animals must be reported to the AEC **Chair immediately**.

9.8- Appendix H

UTC echopattern of the right and left Achilles tendons (median ± IQR). Tendons were compared using a related-samples Wilcoxon signed rank test.

	Left SDFT (n=18)	Right SDFT (n=18)	p-value*
Echo-type I (%)	91.0 ± 4.0	92.5 ± 4.0	0.074
Echo-type II (%)	4.15 ± 4.0	2.5 ± 2.4	0.064
Echo-type III (%)	0.61 ± 0.41	0.46 ± 0.71	0.472
Echo-type IV (%)	3.1 ± 3.4	3.7 ± 3.8	0.931

9.9- Appendix I

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**29 July 2011**
Project Number:	**CF11/1022 - 2011000511**
Project Title:	**How do tendons respond to load?**
Chief Investigator:	**Dr Jill Cook**
Approved:	**From: 19 July 2011 to 19 July 2016**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research**.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

cc: Mr Sam Rosengarten

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Email muhrec@adm.monash.edu.au www.monash.edu/research/ethics/human/index/html
ABN 12 377 614 012 CRICOS Provider #00008C

9.10- Appendix J

UTC echopattern of the right and left Achilles tendons (median ± IQR). Tendons were compared using a related-samples Wilcoxon signed rank test.

	Left Achilles (n=18)	Right Achilles (n=18)	p-value*
Echo-type I (%)	91.5 ± 3.0	91.5 ± 4.0	0.407
Echo-type II (%)	8.1 ± 3.5	8.1 ± 3.4	0.485
Echo-type III (%)	0.2 ± 0.2	0.2 ± 0.4	0.408
Echo-type IV (%)	0.5 ± 0.4	0.4 ± 0.5	0.856

*Related samples Wilcoxon signed rank test

9.11- Appendix K

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

This is to certify that the project below was considered by the Monash University Human Research Ethics Committee. The Committee was satisfied that the proposal meets the requirements of the *National Statement on Ethical Conduct in Human Research* and has granted approval.

Project Number:	CF13/2835 - 2013001527	
Project Title:	The Volume Of Normal Tendon Structure in Achilles and Patellar Tendinopathy	
Chief Investigator:	Prof Jill Cook	
Approved:	**From:** 10 October 2013	**To:** 10 October 2018

Terms of approval - ***Failure to comply with the terms below is in breach of your approval and the Australian Code for the Responsible Conduct of Research.***

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must include your project number.
6. **Amendments to the approved project (including changes in personnel):** Require the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Nip Thomson
Chair, MUHREC

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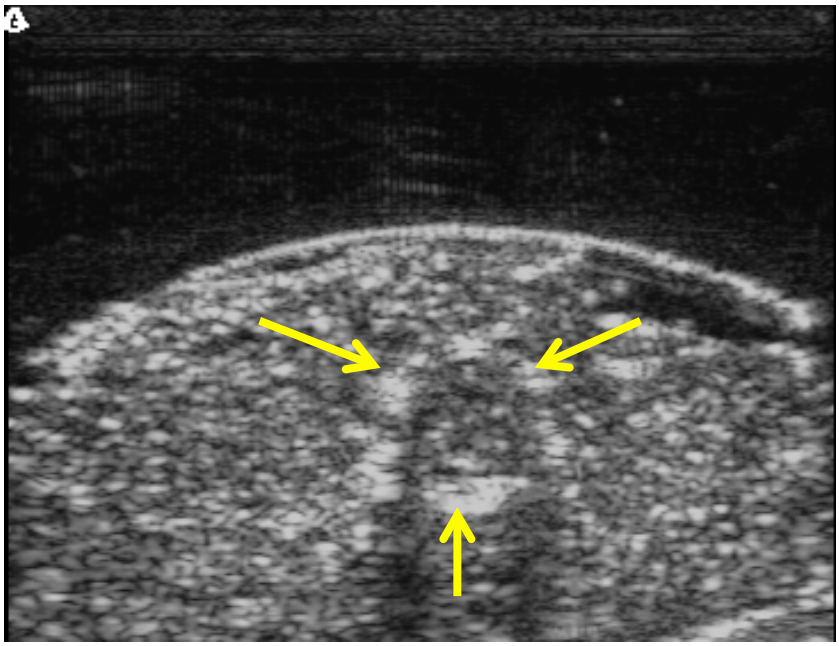
9.12- Appendix L

Procedure to make ultrasound phantom

Mix 250mL of boiling water with 20g of unflavoured gelatin and 10g of sugar-free psyllium hydrophilic mucilloid fibre (brand-name: sugar-free Metamucil). To form a phantom with an inclusion, partially fill a container with the mixture and allow to cool until slightly firm. Place the inclusion on top of the mixture and fill the rest of the container covering the inclusion. Cool until firm.



UTC image of ultrasound phantom with inclusion. Yellow arrows indicate the border of the hollow cylinder within the ultrasound phantom.

9.13- Appendix M

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**20 December 2012**
Project Number:	**CF12/4029 – 2012001925**
Project Title:	**Do bilateral changes exist in people with unilateral Achilles symptoms?**
Chief Investigator:	**Prof Jill Cook**
Approved:	**From: 20 December 2012 To: 20 December 2017**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research.**
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

Professor Ben Canny
Chair, MUHREC

cc: Mr Sean Docking

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9.14- Appendix N

UTC echopattern for the symptomatic, asymptomatic and control tendon. Data is expressed as medium ± interquartile range

	MDC	Symptomatic	Asymptomatic*	Control
Echo-type I (%)	2.5	79.5 ± 11.6	81.8 ± 6.2	86.4 ± 4.7
Echo-type II (%)	2.7	16.6 ± 5.1	14.8 ± 5.2	10.5 ± 6.1
Echo-type III (%)	0.3	1.5 ± 3.2	0.8 ± 0.7	0.5 ± 0.3
Echo-type IV (%)	0.8	2.1 ± 3.6	1.4 ± 1.0	0.8 ± 1.7

9.15- Appendix O

Computational model quantifying cell stress and strain to differing tissue strains and amplitudes (adapted from Lavagnino et al (2008) with permission)

1% @ 2%/min	0.157 (shear stress) 1.26 (cell strain)	**3% @ 2%/min**	0.179 (shear stress) 3.67 (cell strain)
1% @ 20%/min	0.346 (shear stress) 1.33 (cell strain)	**3% @ 6%/min**	0.459 (shear stress) 3.97 (cell strain)

9.16- Appendix P

UTC echopattern of a sample of 10 pathological and 10 normal tendons from Chapter six calculated at different window sizes. Changes in the echopattern were observed, however the statistical findings are similar across differing window sizes.

	Window size 25			Window size 17			Window size 9*		
	NORM	**PATH**	**p-value**	**NORM**	**PATH**	**p-value**	**NORM**	**PATH**	**p-value**
mCSA of echo-type I+II (mm^2)	80.5±20.9	93.8±36.0	0.143	80.1±25.0	92.6±30.3	0.123	78.3±24.5	90.0±30.2	0.123
mCSA of echo-type III+IV (mm^2)	1.8±1.4	5.8±11.9	0.002	2.3±1.6	7.0±8.2	0.001	4.6±2.3	10.0±8.7	0.001
Echo-type I+II (%)	98.0±1.8	94.8±10.3	0.015	97.3±1.9	93.2±8.0	0.004	95.2±2.9	90.2±8.8	0.007
Echo-type III+IV (%)	2.0±1.7	5.2±10.0	0.015	2.7±1.9	6.8±8.1	0.004	4.8±3.0	9.8±8.8	0.007

Chapter 10
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