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Clinical Applications of Shear Wave Elastography to Achilles Tendon Imaging and Monitoring of a Rehabilitation Protocol for Achilles Tendinopathy.

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Abstract

This thesis applied the imaging technology of shear wave elastography (SWE) to the Achilles tendon (AT) and monitoring of a rehabilitation protocol for Achilles tendinopathy (ATY). Study one assessed the reproducibility of SWE, demonstrating coefficient of variations between 2.9% - 6.3%, medium correlations between measures (r = 0.4-0.7), good to excellent Intra-class correlation coefficients (ICC = 0.54-0.85) and interclass correlations of ICC = 0.70 for transverse and ICC = 0.80 for longitudinal scans. Longitudinal scans and keeping the foot relaxed resulted in the least variable results. The second and third studies demonstrated that leg dominance, time of day or previous daily exercise do not impact interpretation of SWE measures, however a 30 minute acute bout of running significantly increased AT stiffness by almost 3%. Interpreting SWE results in the AT immediately following weight bearing exercise may not provide suitable baseline measures for assessment purposes.

The fourth study demonstrated that patients with symptomatic ATY had significantly lower AT stiffness (10%) compared to asymptomatic controls. A 12 week EcEx programme increased AT stiffness by 7.2% and the difference between pathological and control AT's at 12 weeks was 3.5%. The 12 week EcEx protocol significantly improved symptom measures, range of motion, muscular endurance and muscular power, with significant decreases in tendon diameter, pain measures and neovascularisation. After the 12 week EcEx programme, despite significant improvements, most measures in the pathological ATs had not reached parity with the controls, leading to the fifth study. This monitored participants post 12 weeks to examine alterations when the EcEx loading was removed and whether continuation of EcExs brought increased benefits. The removal of the EcExs caused regression in the pathological ATs and a decrease in stiffness of 1.8%. Resuming the EcExs resulted in further positive adaptations and after six months, the difference in pathological and control AT stiffness was 1.1% with a discrepancy in symptoms of 4.4%. This is important information for both clinicians and patients and suggests the commonly used programme duration of 12 weeks may need to be increased.

This thesis demonstrates SWE is a reproducible technique with which to assess the mechanical properties of the AT *in vivo*. SWE may be use easily and effectively to offer clinicians and researchers additional information regarding the internal state of tendons, tendinopathy development and AT stiffness alterations during rehabilitation.

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List of Abbreviations

Some of the following abbreviations may be commonly used in the field of musculoskeletal imaging or sport and exercise medicine, whereas others may be less common, specific to each study chapter or a key measurement. All abbreviations will be abbreviated in each chapter to ensure the reader maintains clarity and will be used in the legends and titles of figures and tables.

ANOVA	Analysis of Variance
AT	Achilles tendon
ATY	Achilles tendinopathy
B-mode	Brightness Mode
CE	Compression Elastography
CSA	Cross-Sectional Area
CV	Coefficient of Variation
E	Young's modulus
EcEx	Eccentric Exercise
ECM	Extracellular matrix
gPa	Gigapascal
HAT	Healthy Achilles Tendon
HSR	Heavy-Slow Resistance
ICC	Intra-class correlation coefficient
kPa	Kilopascal
m/s	metres per second
max AP	maximum anterior-posterior
mm	millimetre

MOS	Modified Öhberg Scale
MPa	Megapascal
MRI	Magnetic Resonance Imaging
MSK	Musculoskeletal
MTU	Musculo-tendinous units
n^2	partial eta squared
NICE	National Institute for Health and Care Excellence
PAT	Pathological Achilles Tendon
PD	Power Doppler
PRP	Platelet-Rich Plasma
QF	Quality Factor
ROI	Region of Interest
RPE	Rating of Perceived Exertion
SD	Standard Deviation
SSC	Stretch shortening cycle
SWE	Shear wave elastography
SWV	Shear wave velocity
TE	Typical Error
TE %	Typical percentage error
UK	United Kingdom
US	Ultrasound
UTC	Ultrasound tissue characterisation
VAS	Visual analogue scale

VISA-A	Victorian Institute of Sport Assessment - Achilles
VTIQ	Virtual Touch Intelligence Quotient

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Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed:

Catherine E Payne

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Publications

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1 Introduction

Soft tissues within the human body are frequently stressed by internal and external forces, and possess the ability to reversibly deform under these forces whilst maintaining normal function (McKee et al., 2011). Tendons are an example of a soft tissue and the Achilles Tendon (AT) is the longest, thickest and strongest tendon in the human body (Calleja and Connell, 2010; Maffulli et al., 2010). The AT produces movement around the ankle joint by transmitting force from the muscles of the triceps surae to the adjoining calcaneus (Magnusson et al., 2008). The AT is one of the most frequently injured tendons (van Dijk et al., 2011) with an incidence rate of developing Achilles tendinopathy (ATY) of 2.35 per 1,000 in the adult population (age 21-60 years) (de Jonge et al. 2011). ATY is one of the most common overuse injuries in athletes performing endurance events (Silbernagel et al., 2007), but ATY is not however, exclusively a concern of the athletic population (Cook, Feller and Bonar, 2004). The NICE guidelines suggest a lifetime cumulative incidence rate for ATY of 6% in inactive people in the UK (NICE, 2010).

There is little experimental and clinical data surrounding the exact pathogenesis of tendinopathy, mainly attributed to the difficulty in obtaining experimental or clinical evidence from non-operative subjects and those in the early stage of ATY development (Klauser, Faschingbauer and Jaschke, 2010). This means the primary cause of ATY remains elusive and the proposed mechanisms and theories behind its pathogenesis remain speculation. Regardless of aetiology, AT injuries can be severe and result in persistent pain and disability (Alfredson, Thorsen and Lorentzon, 1999), being termed a recalcitrant issue within the field of sports medicine (Khan et al., 1999). They are difficult to treat, have a major impact on quality of life and are potentially career-ending for all levels of athlete (Wren et al. 2001; Paavola et al. 2002; de Jonge et al. 2011).

Pathological tendons undergo changes to their mechanical properties and are ‘softer’ in structure compared to healthy tendons (Cook and Purdam, 2009; Chimenti et al., 2014). Rehabilitation programmes for injured tendons seek to create a stronger and stiffer tendon, more resistant to stresses and strains, and therefore to injury. The metabolically active nature of tendons mean they respond to load by altering their mechanical characteristics, including stiffness (Tardioli, Malliaras and Maffulli,

2012). Appropriate amounts of mechanical loading can therefore result in positive changes in an injured tendon. There is however, an optimal loading amount, which if exceeded, can result in further tendon degeneration (Maganaris & Paul 2002; Kubo et al. 2002; Mahieu et al. 2008; Magnusson et al. 2008). Herein lies the challenge presented to clinicians tasked with determining the correct amount and type of loading to initiate positive responses in an injured AT without crossing the line into excessive loading. Eccentric exercises (EcEx) have received a great amount of interest as a treatment modality for ATY due to their relative low cost, ease of implementation and the measurable short and long term improvements they produce (Mahieu et al., 2008). EcEx programmes are the most commonly prescribed regime for ATY (Ohberg, Lorentzon and Alfredson, 2004; Shalabi et al., 2004) as they help regain the structural integrity of the AT (Docking and Cook, 2015). The effect of EcExs on AT stiffness measured with SWE is not widely reported.

Calculations of tendon stiffness previously required the use of cadaver tendons and tensile testing to hold and stretch the tendon to measure the change in length and the corresponding force being put through the tendon (Ravary et al., 2004). It is impossible to use this method in the *in vivo*, clinical environment as you cannot remove the tendon, meaning objective and quantitative measures of the stiffness of the human AT *in vivo* have been difficult to obtain. The problems encountered obtaining data through a reliable, non-invasive and cost effective method have resulted in little being known about the adaptation of human tendon (Brandenburg et al., 2014). The technology of elastography and some of its subdivisions are discussed in detail in this thesis, however in basic premise, elastography can measure the stiffness of different structures and assess soft tissue stiffness *in vivo* (Klauser et al., 2014).

Changes to tendon stiffness may occur earlier than morphological changes, therefore elastography has the potential to indicate changes in the tendon matrix and detect pathology earlier than B-mode imaging (Wu et al., 2012; Horton, 2013). Elastography quantifies differences in the mechanical properties of different structures by measuring the deformation of a tissue in response to a given force (Muraki et al., 2015). As elastography provides an estimate of tissue stiffness (De Zordo et al, 2008), its use in the assessment of *in vivo* human tendons has received increased attention in recent years. The stiffness of asymptomatic and ruptured ATs obtained using elastography have been reported (Arda et al., 2011; Chen et al., 2013; Fu et al., 2016;

Siu et al., 2016). Studies have also recently been reporting the usefulness of elastography in the diagnosis of ATY (Dirrichs et al., 2016), with the results suggesting it can detect the degeneration associated with tendinopathy at earlier stages than traditional ultrasound (US) (Horton, 2013). Elastography may measure disease induced changes in the mechanical properties of tissue (Itoigawa et al. 2015), and therefore could also measure positive adaptations in tendon stiffness occurring throughout rehabilitation from injury.

There are varying elastography techniques available (Sarvazyan et al., 2011; Jeong et al., 2014), including compression elastography (CE) and shear wave elastography (SWE), both discussed in this thesis. SWE has been described as a non-invasive, convenient method to obtain real-time, quantitative measures of tissue stiffness (Chen et al., 2013; Brandenburg et al., 2014). SWE achieves this by measuring the velocity of generated shear waves as they pass through a tissue to estimate its stiffness (Bercoff, Tanter and Fink, 2004). SWE functions independently of the operator and offers quantitative, real-time measures whilst being easy to use by different operators in differing settings at a relative low cost (Shinohara et al., 2010; Youk et al., 2014).

As a technique, SWE is still an emerging technology in the musculoskeletal (MSK) imaging setting. Research so far has shown SWE is a valid way to obtain stiffness measures in skeletal muscle (Eby et al., 2013) and a significant correlation has been shown between stiffness values in cadaveric AT's when measured with traditional tensile testing and SWE (Haen et al., 2017). The work of Leung et al. (2017) demonstrates that SWE can identify acute increases in AT stiffness following exercise and Zhang et al. (2016) utilised SWE to evaluate the stiffness of healing ATs following surgical rupture repair. Despite these positive findings, many gaps remain in the literature which this thesis has tried to address. To date, no research has assessed the reproducibility of SWE for the imaging of the AT over more than two testing sessions or over consecutive days, both of which may impact the future use of SWE in the clinical setting. No research to date has assessed the influence of foot position on the results obtained from the AT with SWE and there is no available research examining the impact of time of day, leg dominance or a prior acute bout of running on AT stiffness using SWE. Finally, no research has utilised SWE in a clinical setting to monitor AT stiffness throughout a rehabilitation protocol, which again may impact its future clinical use.

1.1 Research Overview

This thesis aims to add to the growing body of research surrounding SWE and AT imaging by assessing the methodology of SWE to obtain values of AT stiffness and to apply SWE to the monitoring of AT stiffness throughout a rehabilitation protocol for ATY.

The proposed research questions and hypothesis for this thesis are presented at the end of the literature review (section 2.7) and the aims of this thesis are presented in the following chapters:

- Chapter 2 reviews the literature surrounding the mechanical properties of the AT, the development and treatment of ATY, available imaging techniques for AT assessment and describes elastography and its sub divisions in detail.
- Chapter 3 describes the general methods used throughout all experimental chapters.
- Chapter 4 presents the first experimental chapter (study 1) that assessed the reproducibility of compression and shear wave elastography. It provided evidence of the reproducibility of the techniques over consecutive measures, consecutive days and different foot positions whilst controlling for prior activity.
- Chapter 5 (study 2) outlines whether the extraneous variables of time of day or leg dominance had an impact on AT stiffness measures obtained using SWE.
- Chapter 6 (study 3) examined whether prior exercise should be controlled for when obtaining measures of AT stiffness with SWE by assessing the impact of an acute 30 minute bout of running on AT stiffness.
- The findings from chapters 4, 5 & 6 were carried forward and utilised in chapter 7 (study 4) which applied SWE to the clinical setting and assessed AT stiffness in symptomatic, pathological ATs. This chapter then utilised SWE to assess the alterations and associated timeline of AT stiffness and clinical outcome measures when a pathological AT completed an eccentric exercise (EcEx) programme as treatment for ATY.
- In a continuation from the previous chapter, chapter 8 (study 5) measured alterations in AT stiffness, symptom and clinical outcome measures that occur

when the regular loading of an EcEx programme is removed at 12 weeks. This chapter also continued monitoring participants as they resumed the EcEx programme and provided measures for up to 6 months to offer insight into whether the measured variables in a pathological AT can reach full parity with a healthy AT.

- Chapter 9 discusses the overall findings from the thesis as a whole and summarises the new contributions it has made to the literature. This chapter also proposes a database of SWE values obtained from both healthy, asymptomatic ATs and pathological ATs symptomatic of ATY.

2 Literature Review

2.1 Anatomy and Physiology of the Achilles tendon

2.1.1 Function, location and structure

Soft tissues within the human body are frequently stressed by varying internal and external forces, and possess the ability to reversibly deform under pressure whilst maintaining normal function (McKee et al., 2011). Tendons are one example of soft tissue within the body and have two main functions; firstly, to transmit large tensile forces whilst undergoing minimal deformation and secondly, to use the process of elastic recoil to release stored energy during locomotion (Ker, Alexander and Bennett, 1988; Maganaris and Paul, 2002; Witvrouw et al., 2007; Milgrom et al., 2014). Tendons act as biological springs that stretch elastically, store and then release energy during locomotion (Alexander, 2002), providing 52 - 60% of the total work during locomotion (Voigt et al., 1995). Located between muscles and bones, tendons transmit force created by the muscles, to the bones to produce movement (Jozsa and Kannus, 1997; Campbell and Grainger, 2001; Hodgson, O'Connor and Grainger, 2012). The Achilles Tendon (AT) transmits force from the muscles of the triceps surae to the adjoining calcaneus, producing movement around the ankle joint (Magnusson et al., 2008). The anatomy of the triceps surae includes three separate muscle compartments; i) medial gastrocnemius, ii) lateral gastrocnemius and iii) soleus, that merge aponeuroses into the common AT (Magnusson et al., 2008) (see Figure 2.1, taken from (Mazzone and McCue, 2002)). The unique anatomy of the triceps surae, make it possible for different muscles to contribute to the load experienced by the free AT (Magnusson et al., 2008), with the relative contribution of load from the gastrocnemii and soleus muscles attributed to the relative physiological cross-sectional area of each muscle. The AT comprises distinct fascicles from the lateral and medial gastrocnemius muscles as well as the soleus, with superficial fibres originating from the medial head of the gastrocnemius and deep fibres originating from the lateral head of gastrocnemius (Szaro et al., 2009). The fascicles twist helically along the length of the tendon, causing the relative positioning of the fascicles to vary (Szaro et al., 2009) and the structure to be non-homogenous (Slane and Thelen, 2014). To enable and support movement, musculo-tendinous units (MTU's) produce force, store energy and stabilise joints (Sikdar, Wei and Cortes, 2014) and in the case of the ankle, the MTU includes the AT as the common tendon of the gastrocnemius-soleus complex (triceps

surae) (Milgrom et al., 2003). When these muscles contract concentrically, plantar flexion of the ankle occurs providing force against the ground for locomotion (Leung, Chu and Lai, 2017)

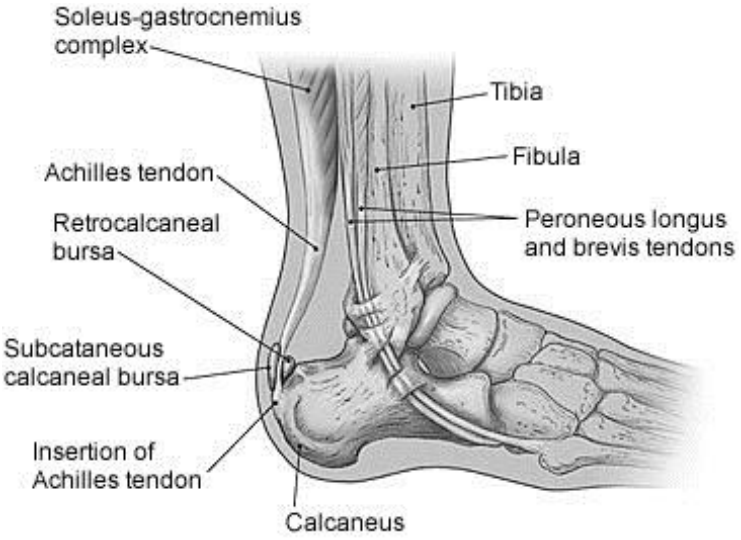


Figure 2.1: Lateral view of the anatomy of the Achilles tendon.

***Figure 2.1 taken from (Mazzone and McCue, 2002)*

The AT is located superficially along the majority of its length (Szaro et al., 2009), incidentally making it very well suited to sonographic examination. The depth of the tendon increases consistently from its insertion at the calcaneus to the musculo-tendinous junction (DeWall et al. 2014). The AT inserts into bone at the posterior calcaneus, known as the osteotendinous junction (Szaro et al., 2009). A subcutaneous bursa between the skin and the AT acts to reduce friction (Khan et al., 1999) and in some, but not all individuals, a subtendinous (retro calcaneal) bursa sits between the calcaneus and the tendon to reduce friction (Hodgson, O'Connor and Grainger, 2012). The length of the 'free' human AT, with no other attachment, extends from the calcaneus insertion to the most distal part of the soleus (Maffulli, 1999), with the tendon carrying on past that point, alongside muscular attachments. This makes the AT the longest tendon in the human body, as well as the thickest (Calleja and Connell, 2010) and strongest (Maffulli et al., 2010). Tendons such as the AT are made up of densely packed fibres of collagen and the hierarchical structure of tendons dictates that the collagen macromolecules are grouped into fibrils, bundled into fibres and fascicles and multiple fascicles make up collagen bundles surrounded by vascularised connective tissue and bound together to form tendon (Pang and Ying, 2006). The AT

is surrounded by a thin, loose connective tissue, sheath type membrane called the epitenon (Figure 2.2, taken from (Aslan et al., 2008)) which contains the vascular, lymphatic and nerve supply. More superficially, the epitenon is encased along its entire length by a firm, fibrous sheath called the paratenon and the epitenon and tendon move longitudinally within the paratenon which contains many layers rich in mucopolysaccharides. There is a lack of synovial fluid within the AT (Kvist et al., 1985; Williams, 1986), so the mucopolysaccharide layers act as the main lubricant to allow easy movement.

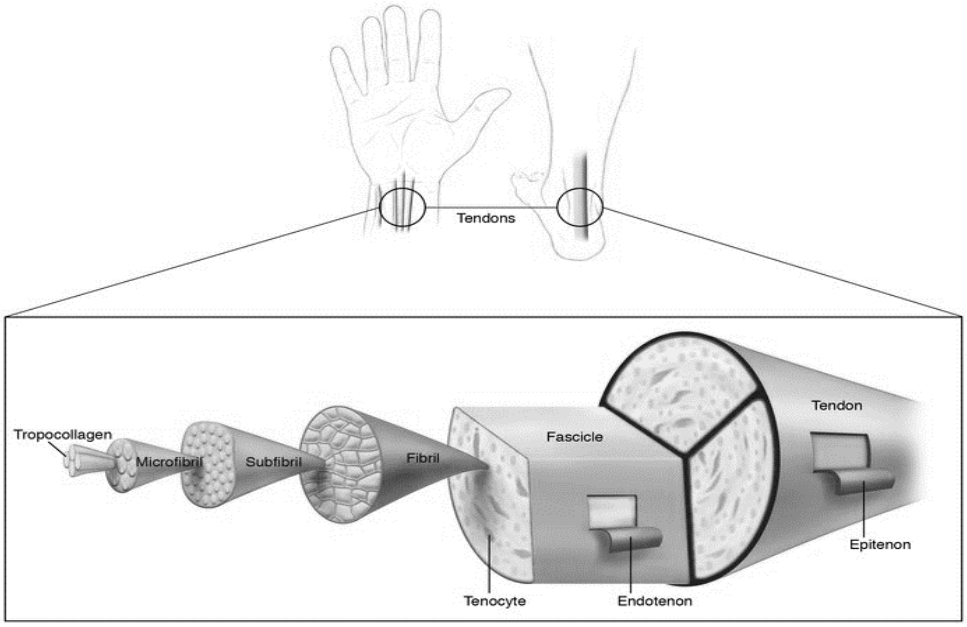


Figure 2.2: Achilles tendon structure.

* *Figure 2.2 taken from* (Aslan et al., 2008)

The collagen bundles within the AT contain bundles of type I collagen composed of 30% collagen and 2% elastin (Klauser, Faschingbauer and Jaschke, 2010), embedded in a viscous substance known as ground substance or extracellular matrix (ECM) (Khan et al., 1999). The ECM comprises 68% water, and cell-produced proteins including proteoglycans, fibroblasts and tenocytes (Wang, 2006; Klauser, Faschingbauer and Jaschke, 2010; Ghorayeb et al., 2012). Tenocytes are the main fibroblast responsible for collagen, elastin, proteoglycan and matrix production (Klauser, Faschingbauer and Jaschke, 2010). Proteoglycans are involved in binding and lubrication and comprise approximately 1-20% of the dry weight of a tendon, dependent on age, site and mechanical loading history (Kirkendall and Garrett, 1997). The protein elastin is responsible for tendon flexibility and present in structures that

experience large changes in length without undergoing permanent structural changes (Kirkendall and Garrett, 1997). Elastin can undergo up to 200% strain before it fails (Kirkendall and Garrett, 1997; Tuite, Renström and O'Brien, 2007) making it resistant to stresses, yet it only accounts for a small fraction (less than 1%) of tendon dry weight (Robins, 1988). Healthy tendons predominantly contain type I collagen, accounting for 95% of total collagen and approximately 60% of tendon dry mass (Kirkendall and Garrett, 1997; Wang, 2006). Type I collagen is fibrillar, tightly packed and determines the mechanical strength and structural integrity of a tendon (O'Brien, 1992; Wang, 2006).

2.1.2 Mechanical properties and normative data

The mechanical properties of a tendon are determined by its structural and physical composition as well as the size and orientation of the fibres and fascicles that make up the collagen bundles and hence the tendon (Kirkendall and Garrett, 1997). The average structural and anatomical characteristics of the AT are presented in Table 2.1 (taken from (Stenroth et al., 2012; Mogi et al., 2018). The values reported for stiffness and Young's modulus in the work of Mogi et al. (2018) were estimated by calculating the stress/strain relationship using US to measure change in length and an isokinetic dynamometer to measure the applied forces. The authors (Mogi et al., 2018) suggest that stiffness in the AT of adults is significantly higher than in high school age children and as the cross-sectional area of the AT was shown to remain unchanged from childhood through to adulthood, they concluded that the increase in AT stiffness is caused by a change in the material properties of the tendon (Mogi et al., 2018). An increase in AT stiffness with advancing age to adulthood has been postulated as a response to maturation and changing load such as increases in body weight, weight bearing tasks and muscle strength (O'Brien et al., 2010; Waugh et al., 2012). Other research has suggested that AT thickness in adults (approx. 24 years old) normally averages 4.5 ± 0.5 mm with thickness being greatest at the mid-point of the tendon between calcaneus insertion and soleus attachment (Slane and Thelen, 2014). Also noted in related research is that the cross-sectional area (CSA) of the AT as well as its stiffness is higher in males compared to females of similar age (Stenroth et al., 2012). Stenroth et al. (2012) also noted that older adult men and women (75 years) have a significantly larger AT CSA, but lower stiffness compared to younger (24 years) adult

individuals (Stenroth et al., 2012), suggestive of an increase in CSA to account for the decrease in stiffness to reduce strain.

Table 2.1: Achilles tendon properties

	Age (years)	Length (mm)	CSA (mm^2)	Stiffness (N/mm)	Young's modulus (GPa)
[a] **High School Age Boys**	17.1 ± 88	185 ± 20	59 ± 6	547 ± 138	1.72 ± 0.39
[a] **Adults**	25.7 ± 2.2	182 ± 27	62 ± 7	665 ± 340	1.94 ± 0.92
[b] **Young Men**	23.7 ± 2.0	197 ± 26	57 ± 10	186 ± 37	0.86 ± 0.20
[b] **Old Men**	74.8 ± 3.6	190 ± 22	69 ± 12	164 ± 47	0.59 ± 0.17
[b] **Young Women**	24.5 ± 2.8	174 ± 19	50 ± 9	151 ± 29	0.71 ± 0.18
[b] **Old Women**	74.3 ± 3.3	159 ± 19	55 ± 9	120 ± 39	0.48 ± 0.18

[a] – Data taken from (Mogi et al., 2018). Young's modulus from [a] reported in MPa and converted to GPa

[b] – Data taken from (Stenroth et al., 2012). Length in [b] reported in cm and converted to mm

The mechanical properties of a tendon can influence the amount of damage and subsequent impairment it undergoes following injury (Stenroth et al., 2012). Many conditions can affect the mechanical properties of a tendon including age, medication, exercise history and immobilization (Wu et al., 2012). Information about the mechanical properties of *in vivo* tendon is not comprehensive (Fusini et al., 2018) with much tendon research being conducted on *ex vivo* animal tendon (Maganaris and Paul, 1999). Although some animal tendons have anatomical similarities to human tendon (van Schie et al., 2010), they do remain only an analogy for human tendon (Rosengarten et al., 2015) and no research using animal models has been successful in producing the changes associated with Achilles tendinopathy (ATY) through exercise (Webborn, 2008). The inherent difficulties in extrapolating data obtained through *ex vivo* animal testing to humans, coupled with the possibility of material changes due to the use of clamping, storage and preservation, raises questions

concerning the application of the results to human *in vivo* tendon (Cook and Purdam, 2009).

The safety factor (difference between tendon stress and failure) for the AT, measured at approximately 1.5, is low relative to other tested tendons that have a safety factor around 8 (Ker, Alexander and Bennett, 1988; Kongsgaard and Aagaard, 2005). This relatively low safety factor may account for its high injury rate when compared with other tendons (Kongsgaard and Aagaard, 2005). The AT is highly stressed, even during low level physical activity and if the tendon fails to adapt to this stress by developing correspondingly high material properties, injury risk increases (Ker, Alexander and Bennett, 1988). Interestingly, the maximum isometric force a muscle can produce is approximately one-third of that required for tendon failure meaning normal healthy tendons do not normally fail in response to normal deformation (Kirkendall and Garrett, 1997). Eccentric contractions however have been shown to produce a maximal force 5.33 times higher than isometric force which can impact on failure rates (Huxley, 1957; Herzog, 2014). Degenerative changes that can soften or weaken the tendon, may also decrease the safety factor further, resulting in higher injury and rupture risk (Kirkendall and Garrett, 1997). Interestingly, during early descriptions of the cross-bridge theory of muscle contraction, it was noted that the maximal force production during eccentric contractions was 5.33 times greater than the maximal isometric force (Huxley, 1957; Herzog, 2014) and therefore shows the increased loading that can be imposed through the use of differing muscular contractions. Although based on an *in vitro* model, it has been shown that a tendon loaded to 20% of its failure stress will fail after approximately 300,000 cycles which is equivalent to approximately 4 months of walking (Schechtman and Bader, 1997). This does not consider the process of remodelling and healing that occurs *in* vivo and implies that healthy tendons *in vivo* must constantly remodel themselves to prevent failure occurring at such a relatively low number of cycles. Tendons appear to develop different material properties over time as a result of varying physiological load as they alter their structure and mechanical characteristics in response to a mechanical force in a process described as tissue mechanical adaptation or mechanotransduction (Kirkendall and Garrett, 1997; Maganaris and Paul, 2002).

The mechanical properties of the AT ensure the efficiency of human locomotion (Klauser, Faschingbauer and Jaschke, 2010) with properties such as stiffness affecting

the elastic energy, storage-recoil process and subsequent transmission of muscular tension (Houghton, Dawson and Rubenson, 2013). Research regarding the mechanical properties and stiffness of the *in vivo* human AT is still limited (Fouré, Nordez and Cornu, 2010; Fusini et al., 2018), therefore expanding our knowledge and understanding in this area will aid in the future prevention, management and rehabilitation of AT injury.

2.2 Achilles tendon injury

2.2.1 Nomenclature, Pathophysiology and aetiology

Research into AT injuries notes the aetiology of tendon disorders as being multi-factorial (Wren et al., 2001). A lack of studies look directly at human tissue (Klauser, Faschingbauer and Jaschke, 2010) and many different terms and names are used within the literature for tendon disorders (Maffulli, 1999). There is a lack of standardisation in the nomenclature surrounding the pathophysiology of tendon disease makes comparison between studies more difficult (van Dijk et al., 2011). Campbell & Grainger (2001) attempted to summarise the differences in commonly used terminology with a summary of their findings shown in Table 2.2 (adapted from (Campbell and Grainger, 2001)). The terms below are often used by differing authors when describing the same entity (Fahlström and Jonsson, 2003), and although some may use them interchangeably, their meanings are subtlety different. The term tendinopathy will be used from now on within this thesis as this term is the clinical description for a variety of both chronic and acute tendon abnormalities and can encompass any abnormal tendon condition (van Dijk et al., 2011). The term tendinopathy was updated in 1998 to characterise a specific clinical syndrome that included a combination of pain, impaired performance and either diffuse or localised swelling (Khan et al., 1999). Since then, much as the term fracture has come to incorporate many varying types of bone damage, the term tendinopathy is used repeatedly in research as it can describe all pathologies that become apparent in and around the tendon (Campbell and Grainger, 2001; Drew et al., 2014).

Table 2.2: Differing terms describing tendon disease

Term	Explanation
Tendinopathy	Clinical description for a variety of both chronic and acute tendon abnormalities
Tendinosis	Non-inflammatory degeneration of collagen
Paratenonitis	Inflammation of the paratenon
Tendinitis	Degeneration is localized within the tendon, but, this term is a misnomer as inflammatory cells are rarely seen in histologic assessment

** Table 2.2 adapted from (Campbell and Grainger, 2001)*

Mid portion ATY will be the focus of this thesis, as 80% of the alterations in the structure of the AT have been shown to occur in the mid portion of the tendon and most AT disorders occur 3-5 cm proximal to the calcaneus (Järvinen et al., 2005; Kujala, Sarna and Kapiro, 2005; Klauser et al., 2014). Insertional problems of the AT are not as common, accounting for approximately 20-25% of clinical diagnoses (Järvinen et al., 2005) and may relate to systemic disorders such as seronegative arthropathies, rather than just mechanical load. The definition of tendinopathy outlined above is still be accepted for mid-portion ATY, with the addition that typically, any nodular swelling will be located between 2-7cm from the insertion of the AT the calcaneus (De Zordo et al. 2009; De Zordo et al. 2010). Of all the tendons in the body including the patellar and supraspinatus tendons, the AT is one of the most frequently injured (van Dijk et al., 2011) with an injury rate of approximately 12 in every 100,000 people (Hess, 2009). Ruptures of the AT are more common in men than women (Leppilahti, Puranen and Orava, 1996) and more likely to occur on the left hand side due to a higher incidence of right handed individuals using the left side to 'push off' with (Maffulli et al., 1999). There are two main noted peaks of incidence of AT rupture, the first at age 39 years and the second at 80 years of age with a rise in incidence from the age of 60 (Webborn, 2008). In a study of 891 apparently spontaneously ruptured tendons, it was found through tendon biopsy during repair surgery, that 97% contained histopathological patterns characteristic of degeneration and no ruptured tendons contained a 'healthy' structure (Kannus and Józsa, 1991). Age and sex-matched control tendons obtained from previously healthy cadavers were

used as a control group and 34% of these tendons also demonstrated these degenerative changes leading the authors to conclude that degenerative changes are common in those over the age of 35 and in many cases, rupture is the final stage of tendon degeneration, and not always a spontaneous event with no previous degeneration (Kannus and Józsa, 1991).

Both the acute and chronic forms of ATY are regarded as one of the most common overuse injuries in athletes performing endurance events whether that be running, jumping or triathlon (Silbernagel et al., 2007). When considering sports-related injuries as a whole, tendinopathies account for approximately 30-50% and the lifetime risk of developing ATY can be as high as 52% in sports such as top level running (Kujala, Sarna and Kapiro, 2005). Kvist (1994) suggested that 11-24% of runners will suffer from ATY at some point in their lives (Kvist, 1994). The prevalence of AT injuries, in particular ruptures, is said to have an increasing incidence (Holm, Kjaer and Eliasson, 2014). AT ruptures occurred six times more frequently during an 8 year period ending in 1994 than in the 8 years preceding (Leppilahti, Puranen and Orava, 1996). This was attributed to an increase in the numbers of both men and women participating in sport and/or health related activities (Järvinen et al., 2005). In Great Britain specifically, between the years of 1987 and 1990, participation in sport and physical activity was increasing reaching a peak in 1990 when two thirds of adults were regularly participating (Sport England, 2002). Since 1990 this growth slowed reducing from 48% in 1990 to 43% by 2002 (Sport England, 2002), however the increase in AT injury prevalence could be due to other factors including better understanding, knowledge, awareness and diagnosis. When an athlete develops a tendinopathy, micro-tears may occur in the structure of the tendon that may start a degenerative cascade impacting and altering the loading capacity of that tendon (Kannus, 1997; van Dijk et al., 2011; Klauser et al., 2014).

2.1.1 Proposed mechanisms and theories of Achilles tendon injury

There is little experimental and clinical data surrounding the exact pathogenesis of tendinopathy, attributed to the difficulty in obtaining experimental or clinical evidence from non-operative subjects and those in the early stage of the condition (Klauser, Faschingbauer and Jaschke, 2010). Therefore, the primary cause of ATY remains elusive and the proposed mechanisms and theories behind the pathogenesis remains

speculation. Despite the precise aetiology not being well understood (Joseph et al., 2012), research suggests a continuum of degeneration involving three main stages; reactive tendinopathy, tendon disrepair and degenerative tendinopathy (Cook and Purdam, 2009). The addition or removal of a mechanical load on the tendon acts as the primary stimulus to move it forwards or backwards along this continuum. Reactive tendinopathy is the first stage of the proposed continuum, comprising a short-term response to overload. This may result in a short-term thickening of a portion of the tendon in an attempt to reduce stress or to allow adaptation to compression (Cook and Purdam, 2009). This thickening has been proposed to also have a secondary role in ensuring structural homeostasis by maintaining an aligned fibrillar structure to support load bearing (Cook and Purdam, 2009). This could explain why some clinically degenerative, but asymptomatic tendons are still able to withstand high levels of loading. Tendon thickening experienced after injury may therefore be the tendon attempting to adapt to the pathology (Docking and Cook, 2015). This stage of reactive tendinopathy usually derives from an unaccustomed burst of physical activity providing a rationale for the increase in prevalence of the AT injuries in middle aged males who suddenly participate in an irregular sporting activity (Webborn, 2008; Docking and Cook, 2015). Also reported is a change in shape of the tendon in the reactive stage due to an increase in bound water associated with the increase in large proteoglycans experienced with tendinopathy (Cook and Purdam, 2009). The tendon can revert back to normal during the reactive tendinopathy stage upon removal of the original overload potentially due to the fast upregulation of these proteoglycans as seen from analysis of in vitro bovine samples (Samiric, Ilic and Handley, 2004).

The magnitude of AT loading during activity is an influential factor in the degeneration process (Kannus, 1997), therefore, if overload is not addressed or removed, the tendon moves into the next stage in the continuum, tendon disrepair. This stage results in an increase in the number of cells, mainly chondrocytes and myofibroblasts which increase the production of proteins such as collagen as the tendon attempts to repair (Cook and Purdam, 2009). The tendon appears swollen with small focal areas of hypo echogenicity visible on US as collagen becomes more disorganised, and an increase in vascularity, accompanied by neural in-growth due to an increased blood supply to the inflamed tissue may be imaged with power or colour Doppler. The final stage of the continuum is degenerative tendinopathy, where areas

of cell death are apparent (Cook and Purdam, 2009) as noted by Lian et al. (2007) who demonstrated using biopsy, the presence of increased tendon cell apoptosis in subjects with patellar tendinopathy (Lian et al., 2007). The classic presentation of this stage includes focal AT swelling and pain. There seems to be little capacity for the pathology to be reversed once it reaches this stage. The increase in vascularisation is easily imaged with Doppler and if this stage is extensive enough, or if a tendon in this stage is placed under sufficient load, the tendon may rupture (Lian et al., 2007). Once in this stage, a tendon is unable to recover fully, although function may be improved, the tendon does not appear to regain its original size, morphology or structural integrity(Cook and Purdam, 2009). Before reaching the latter stages of degeneration, abnormal tendons can move either further away or closer to repair. Approximately 10-30% of abnormal tendons in the earlier reactive tendinopathy stage, when followed up at a later stage can seemingly appear to be normal again (Cook and Purdam, 2009). As well as tendons being able to move between stages, it may also be likely that there can be areas within the tendon at different stages of the tendinopathy model at any one time. Tendons in the latter stages of tendinopathy usually have heterogeneous pathology within the same area (Malliaras and Cook, 2006). There are many theories proposed to account for tendinopathy development, some of which are noted below.

2.1.1.1 Overuse

A single impact macro-trauma can result in tendon rupture, however complete ruptures of the AT can also be associated with multiple recurrent micro-traumas, where tendon damage reaches a critical point, leading to failure (Kannus and Józsa, 1991; Cawston, Riley and Hazleman, 1996). Healthy tendons have the ability to repair low level micro-failures (Kannus, 1997), and can adapt dynamically to mechanical stresses (Campbell and Grainger, 2001). A study using rat tendon demonstrated that over a period of 4 weeks following transection and surgical repair, regrowth of the tendon extra-cellular matrix brought the matrix content to around 94% of the contralateral healthy tendon (Ghorayeb et al., 2012). This regrowth in ECM content was associated with a 78% recovery in stiffness over the same time and it can be assumed that repair of a micro-trauma would be less than that required from transection. Cook & Purdam (2014) suggest that the type I collagen response within a normal tendon to high levels of loading peaks around 3 days after loading and removal of the high load should improve tendon cell response (Cook and Purdam,

2014). Other studies suggest that acute exercise results in an increase of both collagen synthesis and degradation which results in a net collagen loss over 24-36 hours followed by a new synthesis 36-72 hours after exercise (Magnusson, Langberg and Kjaer, 2010). The work of Magnusson et al. (2010) suggests that if a tendon is not given sufficient time (24h) to rest after exercise, further loading can result in net collagen degradation, leaving the tendon vulnerable to overuse injury. Repair of microtrauma will continue to happen to a certain point, but beyond this, repetition of high forces is implicated in tendinopathy pathogenesis. Overuse, repetitive strain and mechanical over load are all suggested as primary triggers for symptomatic ATY (Cook and Purdam, 2009; Aubry et al., 2013). The term 'overuse' in this context therefore refers to repetitive strain of an aligned tendon, from the same direction, above the normal physiological load until such time as the tendon cannot take any more tension (Campbell and Grainger, 2001). Tendinopathy in frequently overloaded tendons can result in a decreased tolerance to exercise as the tendon is less capable of maintaining a repeated tensile load due to pain (Cook and Purdam, 2009). The exact amount of load that induces tendinopathy development is unclear, however not leaving sufficient time between heavy loads to allow the tendon to respond to that load appears to be important (Joseph et al., 2014). Both the type and frequency of tendon loading is an important determinant of potential injury (Cook and Purdam, 2009), therefore it is somewhat paradoxical that repetitive eccentric loading of an injured tendon is the favoured treatment for mid-portion ATY (Slane and Thelen, 2014).

Tendon injuries are significantly higher in sports and activities that include stretch-shortening cycles (SSC's), where the storage and release of energy is mainly seen (Witvrouw et al., 2007). During the SSC, AT forces may potentially reach as high as 9000N (equivalent to 12.5times body weight) (Komi, Fukashiro and Järvinen, 1992). During plantar flexion, the weight going through the AT has been estimated in the region of 600kg with this mass being repeated 1000-1200 times per mile during running, with tensile loads equivalent of up to eight times body weight (Ljungqvist, 2015), providing a rationale for why the AT is a common site for overuse injuries. Tendons have a characteristic elastic range within which they operate efficiently, however working beyond this range increases risk of injury (Onambele-Pearson and Pearson, 2007), therefore explosive sporting events or exercises, where tendons are subjected to especially high loads, can increase injury risk (Hess et al., 1989). As

noted earlier, most human tendons, are not usually at risk of injury as the physiological load they experience is less than their failure stress (Maganaris and Paul, 1999), however specific sites of the body have a high vulnerability of developing tendinopathy and rupture and are classed as 'vulnerable zones' (Buchanan and Marsh, 2002). Specific to the AT, the most common rupture site is located 4-6cm proximal to the tendons insertion (Kainberger et al., 1997).

A tendon subjected to a strain of less than 4% (within the usual limits of the majority of physiological loading), the tendon will be likely to regain its original configuration once that load is removed (Milgrom et al., 2003), similar in principle to the reactive stage of the continuum of tendon degeneration described by Cook & Purdam (2009). Should the strain placed on the tendon be increased to between 4-8%, micro-failures begin to occur as the collagen bundles slide beyond the range where cross-links between them can be sustained (O'Brien, 1992). The work of O'Brien (1992) suggests that once strain levels reach over 8%, this can exceed the tensile failure of the fibres and as it matches the fracture stress of the tendon, rupture is likely (Ker, Alexander and Bennett, 1988). Studies using roosters have shown that exercise results in increases in collagen turnover (Curwin, Vailas and Wood, 1985) using the ability of the tenoblasts and tenocytes to repair fibre damage, resulting in tendon adaptation (Kannus, 1997). Past this point however, is likely to result in tenocyte death and a decrease in collagen matrix production, predisposing the tendon to further damage (Campbell and Grainger, 2001). Figure 2.3 illustrates how repetitive strain on the tendon of roughly 3-8% may result in cumulative fibre micro trauma, which, if not given sufficient time to recover and repair, can lead to tendon overuse injuries (Kannus, 1997). The amount, frequency, duration and rate of stresses being applied to a tendon potentially all play a part in the pathogenesis of tendon injuries, and although tendons can adapt to a change in stress, when the ability of the tendon to repair is exceeded by the amount of stress experienced, overuse injuries will occur (Kannus, 1997).

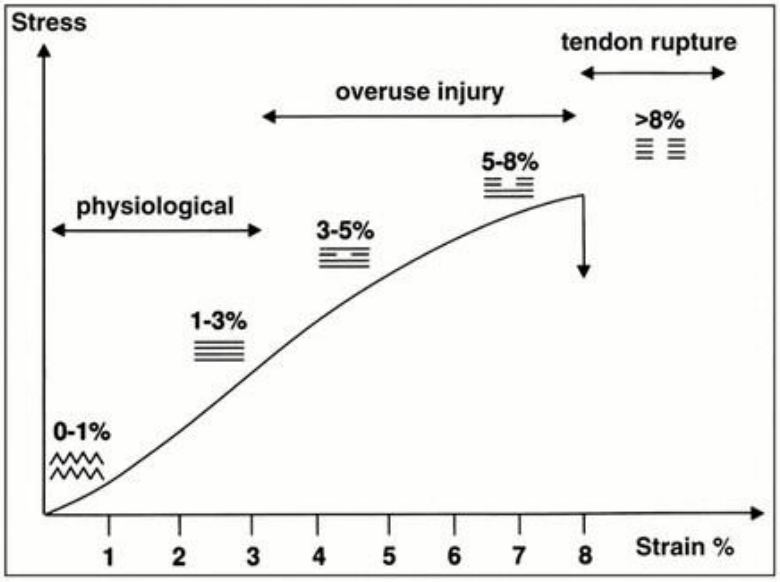


Figure 2.3: Schematic of the development of chronic tendon disorders

**Figure 2.3 taken from ((Kannus, 1997)*

Rupture of collagen fibrils normally occurs prior to a full tendon tear, with these collagen rupture sites merging to become an intra-substance tear (Campbell and Grainger, 2001). These lesions and tears can continue to the surface before progressing to a full thickness tear (Calleja and Connell, 2010), with degenerative tendon changes being visible on US imaging before a macroscopic tendon tear occurs (Calleja and Connell, 2010). This provides further support for the theory that ATY is a degenerative condition that can lead to rupture (Buchanan and Marsh, 2002). When small lesions or tears are present within a tendon, a load placed on that tendon will be distributed differently as the more intact parts take more of the load (Hodgson, O'Connor and Grainger, 2012), resulting in the strain being unequal through the tendon, and further increasing risk of rupture in certain areas.

AT injuries are not exclusively a concern of the athletic population and can also affect relatively inactive individuals in the workplace and general population (Cook, Feller and Bonar, 2004) having consequences on the economy given time taken off work in sick days. The incidence rate for ATY in the Dutch General Practice setting is 1.85 per 1000 registered patients, rising to 2.35 per 1,000 in the adult population (age 21-60 years) (de Jonge et al. 2011). De Jonge et al. (2011) note that there are no other studies available on the incidence rate of ATY in the general population for their

results to be compared against, but they do demonstrate that only 35% of the cases of ATY recorded in their study was related to a sporting activity, leaving a further 65% un-related to sporting activity (de Jonge et al. 2011). The NICE guidelines for ATY suggest a lifetime cumulative incidence rate of 6% in inactive people in the UK (NICE, 2010). AT injuries are especially common in individuals who are usually sedentary, but engage in intermittent strenuous activity, or those who abruptly increase either the duration or intensity of activity resulting in the tendon being unable to withstand the resulting mechanical load (de Jonge et al. 2011). Although injury is likely to be involved in ruptures of the AT, the patient is not necessarily involved in regular physical activity (Webborn, 2008). There are bimodal peaks of incidence for rupture, the first peak is at 39 years old and the second at 80 years of age, not a group always considered regularly physically active. Therefore AT injuries may be a consequence of the possible involvement of other risk factors including symptomatic and/or asymptomatic tendon degeneration, body misalignment, age, gender and insufficient muscle strength (Kvist, 1994; Webborn, 2008).

2.1.1.2 Cellular response

The exact pathophysiological processes occurring within a tendon during tendinopathy development are unclear (Arya and Kulig, 2010), meaning most theories remain speculation. Tendon injury resulting from loading activity was previously thought to be coupled with the release and subsequent accumulation of inflammatory mediators (Jozsa et al., 1989). However histological findings were inconsistent, finding no inflammatory cells in tendinopathic tendons (Leadbetter, 1992). In comparison with a healthy tendon that contains mainly type I collagen fibres, injured tendons contain a higher percentage of type III collagen which is less resistant to tensile forces and can weaken the tendon, predisposing it to further injury and potential rupture (Khan et al., 1996; Khan and Cook, 2000; Cook, Feller and Bonar, 2004). In injured tendons, there is therefore a mismatch between the mechanical loading and the adaptation of collagen, however it is suggested that eccentric exercises in particular may stimulate collagen formation and therefore repair of the tendon (Verrall, Schofield and Brustad, 2011).

Certain stages are reported in tendinopathy development, starting with the acute phase consisting of ischemia, cell membrane damage and metabolic disturbances within the

first five days (Kannus, 1997). Oedema may occur due to the fluid changes initiated by these processes and as it increases, it can impair circulation, increasing the risk of local tissue hypoxia in the paratenon, which may produce an audible and palpable crepitation sound on motion (Järvinen et al., 1997). Following the acute phase, is the proliferative phase lasting from day 5 - day 21 and characterised by a proliferation of fibroblasts, synovial cells, capillaries and fibrin clotting (Kannus, 1997). Lastly is the maturation and reorganisation phase (typically over 21 days), where the paratenon and tenosynovium may thicken and fibrotic adhesions occur which can increase friction within the tendon (Jozsa and Kannus, 1997). If the excessive loading that resulted in tendon injury is not removed, an acute tendon injury is left untreated, or treatment is unsuccessful, the fibrin within the tendon may start to organise itself and form adhesions on the tendon and the paratenon which can result in a chronic form of the injury (Jozsa and Kannus, 1997). A chronic injury of the tendon will occur when the tendon cells (i.e. tenocytes or tenoblasts) are unable to repair the fibre damage in time before the next repetitive micro traumatic process occurs (Cook, Feller and Bonar, 2004). This stage is when the basal reparative ability of the tendon is fatigued and further repetitive activity can result in collagen cross-linking weakening, and disruptions in the matrix and vascular environment of the tendon (Jozsa and Kannus, 1997). When breakdown in the tissue exceeds the rate of repair, the continuous cycle of load and irritation begins, stimulating the release of cytokines, promoting further cell activity (Kannus, 1997). It is highly likely that tissue damage begins and accumulates long before any symptoms manifest themselves and treatment is initiated (Leadbetter, 1992). Some suggest that tissue micro trauma and dysfunction will precede even the first perception of stiffness, tenderness, discomfort or pain highlighting the difficulties in diagnosing thee injuries at an early stage (Kannus, 1997).

2.1.1.3 Vascularisation

Many theories postulate the interaction of vascularisation and pain or symptom development in tendinopathy as symptomatic tendons are known to demonstrate increased vascularity (Cook, Feller and Bonar, 2004). One study examined 163 patients presenting with the classic symptoms of ATY for at least 3 months and demonstrated the presence of neovascularisation in all of them (Aström and Rausing, 1995). Nociceptive nerves are commonly found close to vessels, therefore this could

influence the pain that correlates with the presence of neovascularisation (Öhberg, Lorentzon and Alfredson, 2001). Vessel ingrowth into the tendon from the surrounding peritendinous tissue has been seen on 65% of biopsies taken during surgical intervention on patients suffering achillodynia (Movin et al., 1997). This formation and ingrowth of vessels is a widely recognised hallmark seen during the progression of tendinopathic disease (Watson et al., 2018) and may cause structural changes or alterations in tendon stiffness if it impacts the alignment of collagen fibres. Cook et al. (2004) propose that mechanical loading results in rounding of tenocytes which promotes production of proteoglycans and glycosaminoglycans rather than collagen which can result in separation of the collagen fibres, affecting vascular supply and stimulating neovascularisation (Cook, Feller and Bonar, 2004).

Neovascularisation is considered a normal physiological response for a tendon in early stages of repair, however its continuing presence is likely a result of pathology that will delay further healing (Watson et al., 2018). Neovascularisation has been associated with tendon thickening and shown to correlate with pain (Kannus, 1997). One study assessed 28 tendons from symptomatic patients with chronic ATY and compared them with 20 normal, asymptomatic tendons examined with grey scale ultrasonography and colour Doppler. Neovascularisation was present in all of the painful tendons but not in the normal, healthy pain free tendons (Öhberg, Lorentzon and Alfredson, 2001). The authors concluded that neovascularisation in the symptomatic tendons was in some way involved in the mechanisms responsible for pain. This theory was given further support when it was shown that injecting a sclerosing agent against the neovessels, cured pain in 73% (8 out of 11) of patients (Ohberg and Alfredson, 2003), allowing them to return to pain free loading activity. The authors were clear to point out that the exact background to the positive results in tendon pain after this treatment were unknown. It should however be noted that sclerosing of the vessels may also have negative consequences as without the pain to inhibit activity, there is a possibility that an individual may subsequently overload the tendon, increasing injury risk. Other studies have demonstrated that neovascularisation occurs in anywhere between 63 – 88% of symptomatic tendons (Peers et al. 2003; Reiter et al. 2004; de Vos et al. 2007; Sengkerij et al. 2009). These figures interestingly imply that 12-37% of tendons demonstrate neovascularisation but are asymptomatic. Therefore, although all symptomatic tendons have increased

neovascularisation, not all asymptomatic tendons show the absence of neovascularisation and as such, not all pain can be attributed simply to neovascularisation.

Some authors show the presence of neovascularisation to be associated with increased pain measured using the VISA-A score (Reiter et al., 2004), whilst other research found no correlation between symptoms and neovascularisation (Sengkerij et al. 2009). This lack of association between symptoms and neovascularisation was not expected and attributed to differences in machine settings and the lack of standardisation for prior activity (Sengkerij et al. 2009). Some authors found that the blood flow at rest in a healthy AT will be evenly distributed, however in a pathological tendon, increased blood flow exists in areas with increased pain (Aström and Rausing, 1995), in agreement with other studies (Astrom and Westlin, 1994) demonstrating neovascularization is present in painful tendons, but not in pain-free tendons. Other authors suggest that neovessels are not always solely due to a pathological condition as physical activity can result in the presence of neovessels (Boesen et al., 2006). Increased vascularisation has also been postulated to be part of the tendons reparative process (Aström and Rausing, 1995; Movin et al., 1997), which may offer a rationale for the increase in neovessels seen post exercise.

The onset of pain can commence at any point in ATY development (Ooi et al., 2013). Therefore, when some tendons are imaged, they can be apparently normal, yet painful, and conversely, approximately two thirds of tendons at a stage of degeneration advanced enough to rupture, were pain free (Cook and Purdam, 2009). Pain is a clinical feature of tendinopathy that is very important to clinicians and one they seek to change with treatment as pain response is a major indicator of the success of treatment (Kannus and Józsa, 1991). Traditionally, there have been two main theories to explain why pain arises during tendinopathy development, the first being inflammation and the second being collagen fibre separation (Cook and Purdam, 2009). The theory that inflammation causes the pain experienced in tendinopathy has long been unable to withstand scientific scrutiny (Alfredson, Thorsen and Lorentzon, 1999), particularly when specimens obtained from patients with chronic tendon pain were found not to contain inflammatory cells (Khan and Cook, 2000). Despite this,

the alternative theory regarding a separation of the collagen fibres resulting in pain appears to not fully explain the feelings of pain either (Khan et al., 2000). Therefore, our knowledge of the development of pain in a tendinopathic tendon is still unclear and warrants further research.

Different methods have been used in previous research to assess the degree of neovascularisation present in tendons, with some simply noting the presence or absence of vessels (Öhberg, Lorentzon and Alfredson, 2001). The Modified Öhberg Score (MOS) to rate neovascularisation was developed by Öhberg & Alfredson (2002) which has been more commonly used in recent literature, with this scoring system assessing neovascularisation according to the appearance of vessels within the tendons. A score of 0 was given when no vessels were visible, 1+ was given when one or two small vessels were present, mainly in the anterior part of the tendon. Several irregular vessels present throughout the tendon represents a score between 2+ and 4+ with 2+ denoting 2 vessels, 3+ denoting 3 vessels and 4+ denoting more than 3 vessels (Ohberg and Alfredson, 2002). A cohort of sport and exercise medicine consultants used the MOS to rate neovascularisation in the AT and patella tendon with the results showing excellent inter-rater and intra-rater reliability (ICC = 0.86 & ICC = 0.95 respectively) (Watson et al., 2018). Watson et al. (2018) concluded that the results demonstrated the MOS could be reliably utilised in the clinical setting.

Vascular supply in the AT decreases significantly after just the 3rd decade of life (Kannus and Józsa, 1991), and hypo-vascularisation of the mid-portion area in particular has been postulated as a mechanism for the increased vulnerability of this specific area within the tendon (Campbell and Grainger, 2001). Blood flow at rest has been shown to be approximately 50% (and significantly) lower in older (74 yrs.) people (1.6 ml.100g tissue^{-1}.min^{-1} in comparison to younger (26 yrs.) people (2.7 ml.100g tissue^{-1}.min^{-1}) (Langberg et al., 2001). These decreases in blood flow can also potentially result in hypoxic conditions which may be a factor involved in the development of degenerative changes preceding ruptures (Järvinen et al., 1997). This may offer a rationale for the increased rupture rate seen in older individuals (Webborn, 2008).

Peritendinous blood flow increases significantly during intermittent static calf muscle exercise, regardless of age (Langberg et al., 2001) and it may be speculated that an appropriate level of exercise could help increase blood flow in the peritendinous space around the tendon during exercise, aiding in the clearance of metabolites (Langberg and Skovgaard, 1999). If the level of exercise is too high however, the vasculature both at the micro and macro-vasculature level can be damaged through overuse, disturbing normal oxygen transport and impairing metabolic activity which has further implications for tissue repair (Boushel et al., 2000). Finding a level of exercise and training following injury that can stress the tendon without causing damage to the vasculature is important to long term rehabilitation.

2.3 Anatomical and physical changes with Achilles tendinopathy

To establish what constitutes a tendinopathic tendon, comparison with a healthy tendon is most helpful. When using US to view a healthy tendon, the brightly reflective fascicles that make up the tendon can be easily seen, with the parallel bundles of type 1 collagen being viewed as a hard and firm structure (Magnusson et al., 2008; Hodgson, O'Connor and Grainger, 2012). A healthy AT will exhibit linear hyperechoic fibres, uniform thickness in the sagittal plane, a tightly packed fibrillar structure with dense and clearly defined parallel and/or slightly wavy bundles of collagen (Hodgson et al. 2012; Wu et al. 2012; Tan et al. 2012).

Following acute injury, fluid content around the injury site increases, reducing brightness on the US image (Kainberger et al., 1997). With tendinopathy, the tendon demonstrates collagen disorganisation caused by a change in the structure of the collagen fibres as they lose their parallel bundling (Ghorayeb et al., 2012). When imaged with US, tendinopathic tendons show hypoechoic regions corresponding to the areas of disorganised collagen and an increase of hydrophilic extracellular matrix and/or neovascularisation (Aström and Rausing, 1995; Joseph et al., 2012). Pathological ATs can show an increased cross-sectional area of aligned fibrillar structure (as measured by Ultrasound Tissue Characterisation (UTC)), despite there also being a high prevalence of disorganisation. The authors suggest this increase in cross-sectional area ensures the tendon retains a sufficient amount of aligned fibrillar structure and therefore load bearing capability, resulting in the total amount of aligned fibrillar structure being above that of a tendon classed as structurally normal (Docking and Cook, 2015).

A clinical diagnosis of Achilles tendinopathy (ATY) will usually include progressive and gradual pain over the AT area lasting longer than 6 months together with the presence of one or more of the following factors: 1) History of swelling, 2) Tenderness to palpation, 3) Palpable nodular thickening, 4) Movement of the painful area with plantar- and dorsi-flexion, 5) Early morning pain and 6) early morning stiffness (Mokone et al., 2006). Although diagnosis is becoming standardised, it is apparent that the cause and pathogenesis of AT injuries are still not well understood, and the condition remains difficult to treat (Kirkendall and Garrett, 1997). Despite this, the changes noted in an abnormal tendon will usually appear similar regardless of the aetiology of the injury or damage (Ohberg, Lorentzon and Alfredson, 2004). Tendinopathic tendons usually appear thickened (Hodgson, O'Connor and Grainger, 2012), and can include a hypoechoic area, presence of focal thickening or a maximum thickness greater than 8mm (Aström et al., 1996). This thickening can result in tendinopathic tendons demonstrating a loss of normal fibre integrity and fatty infiltration (Aström et al., 1996; Docking and Cook, 2015).

Pathological tendons are also associated with changes to their mechanical properties (Cook and Purdam, 2009). Assessed using US and an isokinetic dynamometer, decreased levels of tendon stiffness (12 N/mm) coupled with increased levels of tendon strain (1.2%) have been demonstrated in individuals suffering with ATY compared to healthy control tendons (Chimenti et al., 2014). Other research has demonstrated that during a passive task, a 27.8% decrease in tendon stiffness was noted between those with ATY in comparison to healthy matched controls (Wu et al., 2012). Elastography technology and some of its subdivisions are discussed in detail in section 1.6, however in premise, elastography measures the elasticity of differing structures and can be used to assess soft tissues (Klauser et al., 2014). When viewed with compression elastography (CE), healthy tendons will usually be seen as a harder tissue associated with less deformability (Szaro et al. 2009; De Zordo et al. 2009). When assessed using shear wave elastography (SWE), the mean elasticity value of an asymptomatic, normal AT has been reported as 291.91 ± 4.38 kPa (Chen et al., 2013) with others reporting similar values 289.6 ± 23.4 kPa approximately 4 months after surgical repair (Zhang et al., 2016). There is some discrepancy in the literature with

an earlier study from Arda et al. (2001), reporting AT stiffness values of 98.8 ± 47.1 kPa in men and 62.5 ± 40.1 in women, which appears low in comparison to other research and potentially attributed to the early stage of research with SWE and that Arda et al. (2011) did not explicitly mention using a transducer orientation parallel to the tendon. When considering the actual measurement of shear wave velocity (SWV) in a healthy AT, the values obtained from longitudinal and transverse data from 326 healthy volunteers (165 men and 161 women) were 8.20 ± 1.07 and 4.08 ± 0.54 m/s, respectively (Fu et al., 2016). Consistent within the literature is that the ATs of healthy volunteers are seen as hard and stiff in structure (Chen et al., 2013).

Pathological ATs are 'softer' in structure compared to healthy tendons (Chimenti et al., 2014) and a ruptured tendon would be expected to be softer still. The mean stiffness values for a ruptured tendon, measured with SWE was 56.48 ± 68.59 kPa, significantly lower than the asymptomatic healthy tendon (291.91 ± 4.38 kPa) (Chen et al., 2013). Zhang et al. (2016) utilised SWE to assess the change in stiffness of AT's recovering from surgical repair and although pre-operative stiffness values were not noted, 12 weeks post-surgery, the values were 187.7 ± 23.8 kPa rising to 289.6 ± 23.4 kPa after 48 weeks (Zhang et al., 2016). It is possible to identify the predicted changes in a pathological tendon such as being softer using elastography (Wu et al., 2012) potentially prior to symptoms emerging. In many cases of 'spontaneous rupture', biopsies taken during surgical repair demonstrate histological signs of underlying degeneration (Kannus and Józsa, 1991). The implication is therefore that these degenerative changes could have been visible through imaging well before the actual rupture occurred. Wu et al. (2012) suggests changes to tendon stiffness may occur earlier than morphological changes, and Horton (2013) demonstrated that elastography can demonstrate changes in the tendon matrix earlier than B-mode imaging. There is therefore a potential for elastography to be used clinically to detect pathological tendons at an earlier stage. With SWE, tendinopathic tendons show a predominantly soft consistency, representative of a reduced stiffness (Chen et al., 2013) with this visible softening most frequently involved with the middle third of the tendon (Palle et al. 2011). This softening visualised with elastography is postulated as being associated with the loss of fibre integrity in a damaged AT (Klauser et al., 2013). Changes in fibre integrity may impact the mechanical properties of the tendon due to fibre separation and a loss of type I collagen which imparts greater mechanical

strength to the tendon (Holmes and Lin, 2006; Arya and Kulig, 2010). Coupled with a decrease in type I collagen are increased synthesis of type III collagen which is mechanically weaker and further reduces the stiffness and mechanical strength of the tendon (Holmes and Lin, 2006; Arya and Kulig, 2010). Degenerative changes in collagen structure resulting in a softer and weaker tendon is of high clinical importance as these changes may result in partial tears or spontaneous rupture (Klauser et al., 2013). Information on *in vivo* tendon stiffness can demonstrate subclinical changes which are more evident and can be visualized earlier with elastography than B-mode US alone, making elastography a very useful tool for prognosis (De Zordo et al. 2009).

The work of Kannus & Józsa (1991) examined biopsies taken during repair surgery of 891 spontaneously ruptured AT's and concluded that every third person in a healthy urban population who is over the age of 35 will show degenerative changes in their tendons (Kannus and Józsa, 1991). Not every person over the age of 35 has a symptomatic AT, but Kannus & Józsa (1991) also demonstrated that 34% (151 out of 445 tendons) from reportedly healthy cadavers showed degenerative changes. This could imply that early stages of tendon degeneration are potentially asymptomatic. To further this argument, a cohort study of 36 patients with Achillodynia (pain due to inflammation of the AT) had sonograms taken and at a follow up of approximately 48 months (±8 months) from the sonograms, seven tendons (28%) had spontaneously ruptured (Nehrer et al., 1997). When the original sonograms were analysed, all the ruptured tendons showed signs of tendinitis, implying that tracing changes in the AT over time may hold the potential to identifying early stages of degeneration and potentially help clinicians manage tendinopathy better and potentially avoid some ruptures. Previous studies have shown that tendons experiencing overuse tendinopathy will typically exhibit a failed healing response (Webborn, 2008; van Dijk et al., 2011) which can occur for some time before eventual rupture. Also noted is that at least two thirds of rupture patients will experience no symptoms until the time of rupture (Webborn, 2008). Therefore, regular assessment of tendon stiffness could be vital in detecting early stages of degeneration and/or the signs of failed healing which can alert a clinician to the possible development of symptomatic ATY. An assessment mode such as SWE could be used to identify the degenerative changes that pre-empt this condition at an earlier stage, so clinicians can prevent the further development of symptomatic ATY to a stage typically resilient to treatment (de Jonge

et al. 2011). Not only could SWE be utilized to identify degenerative changes earlier, but as it also has the potential to differentiate the mechanical properties of an injured tendon and a healing tendon suggesting it also has a practical application in the monitoring of recovering tendons (Ooi et al., 2013).

2.4 Treatment Options for Achilles Tendinopathy

Regardless of location or aetiology, AT injuries can be severe and result in persistent pain and disability (Alfredson, Thorsen and Lorentzon, 1999). Tendinopathies are called a recalcitrant issue within the field of sports medicine (Khan et al., 1999). They are clinically difficult to treat, have a major impact on the quality of life of those with the condition and are potentially career-ending for all levels of athlete, particularly runners and walkers who are more susceptible to the condition (Wren et al. 2001; Paavola et al. 2002; de Jonge et al. 2011). The frequent incidence and severe consequence of ATY within the athletic population has been highlighted, as only 37% of those suffering with ATY demonstrating definitive improvement after two years (Fredberg and Bolvig, 2002). If the remaining 63% of athletes do not experience definitive improvement in this time, tendon injuries in athletes are associated with a high level of morbidity and it is clear that current treatment regimens for these conditions remain unsatisfactory (Verrall, Schofield and Brustad, 2011).

Tendons are metabolically active, responding to load by altering their mechanical characteristics such as stiffness (Tardioli, Malliaras and Maffulli, 2012). Although appropriate amounts of mechanical loading placed on an injured tendon can result in positive changes, there is an optimal loading amount, which if exceeded, can result in further tendon degeneration (Maganaris & Paul 2002; Kubo et al. 2002; Mahieu et al. 2008; Magnusson et al. 2008). Herein lies the challenge presented to clinicians and coaches, tasked with determining the correct amount and type of load to initiate positive responses without crossing the line into excessive loading. Eccentric exercise (EcEx) has received a great amount of interest due to its relative low cost, ease of implementation and the measurable improvements in tendon structure it can produce in both the short and long term (Mahieu et al., 2008). EcEx programmes remain a commonly prescribed regime for tendinopathy (Ohberg, Lorentzon and Alfredson, 2004; Shalabi et al., 2004) as they provide a stimulus for cell activity and restructuring of the matrix, helping regain the structural integrity of the tendon (Docking and Cook,

2015). As increasing load on a tendon can increase pain, exercise based treatment programmes need to be appropriate to the stage of pathology (Cook and Purdam, 2009). At an early stage of injury, a reduction in load may be sufficient to allow the tendon time to adapt and the reactive state of the cells to settle down, potentially reducing pain (Cook and Purdam, 2009). Type I collagen response to a high load in a normal tendon are suggested to peak approximately 3 days after loading and therefore if the injury is within an early stage of injury, days of removing the abusive load may be enough to allow the tendon to repair (Cook and Purdam, 2014). If the stage of injury is later, or the presentation more severe, then authors have suggested that most tendons will heal in stages including the inflammatory stage (1-7 days), proliferative stage (7-21 days) and remodelling phase (3 weeks to 1 year) (Kader et al., 2002). This highlights how the treatment of AT injuries can be both difficult and lengthy. During the healing process, cells that produced type I collagen at the repair site now produce type III collagen instead (Cook and Purdam, 2009). The replacement of type I collagen with type III collagen may lead to incomplete healing and a reduced level of strength and elasticity when compared to pre-injury levels (Ghorayeb et al., 2012) as type III collagen is smaller, shown by an inverse relationship noted between fibril diameter and amount of type III collagen resulting in the formation of smaller collagen fibres (Magnusson et al., 2002; Rees, Stride and Scott, 2014). Smaller, weaker fibres that are less organised all contribute to the stiffness and tensile strength of a pathological tendon being lower than a healthy tendon (Riley, Harrall and Constant, 1994; Ghorayeb et al., 2012), leaving it vulnerable to injury if applied mechanical load is too high. An increase in type III collagen diminishes mechanical strength but type III collagen is capable of rapidly forming crosslinks which can at least attempt to maintain tendon stiffness (Ghorayeb et al., 2012), and help to maintain structural homeostasis as discussed in section 1.2.2.

Regenerative therapies, designed to heal injured tendons such as platelet-rich plasma (PRP) injections and stem cell therapy, aim to improve the repair process within the tissue to enhance tendon structure (de Vos et al., 2010). De Vos et al. (2010) examined patients presenting with chronic mid-portion ATY and treated with an EcEx programme together with either an injection of PRP or a saline placebo and concluded that PRP did not result in any further improvements than just EcEx and should not be recommended (de Vos et al., 2010). A link between regenerative treatments and an

improvement in tendon structure has not yet been shown (de Jonge et al., 2011; Chaudhury, 2012). The use of friction, ultrasound, extracorporeal shock wave therapy (ESWT), sclerosing therapy and various types of therapeutic exercises have all been proposed as treatments for tendinopathy, all having their own benefits and drawbacks (Cook and Purdam, 2009). Patients not finding relief from ATY symptoms following conservative care, such as those noted above, can often consider operative care as their next step (Cook and Purdam, 2009). Surgery brings significant financial considerations, time off work as well as a known risk of complications present with any surgical procedure (Chimenti et al., 2014). Recent research supports the notion that treatment protocols should focus on improving the load capacity of the tendon through the use of conservative exercise-based treatment protocols (Docking and Cook, 2015).

2.4.1 Therapeutic Exercise Regimes for Achilles Tendinopathy

There is conflicting evidence surrounding the effectiveness of differing therapeutic exercises such as EcEx programmes and heavy-slow resistance (HSR) programmes (Docking and Cook, 2015). Kongsgaard et al. (2009) examined the effect of a heavy-slow resistance training (HSR) training programme for patellar tendinopathy against peritendinous corticosteroid injections and eccentric decline squat training (EcEx). The HSR and EcEx programmes produced similar short term results, but at a 6 month follow up, the group treated by HSR were recorded as being more 'satisfied' by the treatment than the EcEx group (Kongsgaard et al., 2009). There is moderate support for the use of HSR programmes in the treatment of tendinopathy (Drew et al., 2014) as HSR programmes can promote higher compliance rates and higher patient satisfaction than EcEx due to the lower time required to complete HSR (Beyer et al., 2015). It is suggested that more studies should examine HSR as an alternative to EcEx (Drew et al., 2014), however the current evidence base for HSR programmes predominantly surrounds its use with the patellar tendon (Malliaras et al., 2013) with the exercises it recommends designed to target this area, reducing its potential effectiveness for the treatment of ATY. Another therapeutic exercise regime using elements of EcEx, with the addition of stretching and concentric movements has also been developed to treat tendinopathy (Silbernagel et al., 2001). The treatment has been refined and modified since its inception and now contains 4 distinct phases (Silbernagel et al., 2007)). The exercises are carried out once a day with the number

of repetitions and exercise intensity determined by the participants status (Silbernagel et al., 2007). The repetitions start from 3 sets of as many exercises as can be tolerated and increase to a maximum of 3 sets of 15 repetitions. The intensity is increased by increasing range of motion of the exercises from performing the exercises on the floor to performing them standing on a stair. The load is also increased by using a backpack to increase weight and increasing the speed of the movement before the final phase which sees the addition of plyometric exercises (Silbernagel, Brorsson and Lundberg, 2011). The protocol is effective at recovering patients in both symptoms and function from ATY (Silbernagel, Brorsson and Lundberg, 2011), producing significant improvements in plantar flexion, reductions in pain when walking and fewer patients experiencing swelling (Silbernagel et al., 2001). This rehabilitation protocol may be a valuable option for ATY (Silbernagel et al., 2007), however more research is needed before it becomes common place in clinical settings. The treatment protocol of choice by many clinicians when treating ATY remains EcExs, which are explained in detail in section 1.4.2.

2.4.2 Eccentric Exercise

Stanish et al. (1986) were among the first to study EcExs as a treatment modality for ATY. EcExs involve the lengthening of a muscle-tendon unit as a load is applied to it (Murtaugh and Ihm, 2013) with the main aim of prescribing EcEx programmes to injured tendons being to create a stronger and stiffer tendon, more resistant to stresses and strains, and therefore to injury. Stanish et al. (1986) demonstrated that in 200 patients with ATY, a 6 week EcEx program led to complete relief in 44% of patients (Stanish, Rubinovich and Curwin, 1986). The first controlled study into the effect of EcEx in patients with mid portion ATY was conducted by Alfredson et al. (1998), who proposed the now well utilised protocol, entailing lowering body weight through one foot off a step with the knee fully extended for 3 sets of 15 repetitions and repeating again with the knee partially flexed to target different areas of the triceps surae (Alfredson et al., 1998). The mode of progression for this method is the addition of a weighted backpack to increase the weight and therefore increase the load through the tendon throughout the eccentric movement. This exercise regime should be carried out twice a day, every day, for 12 weeks and has resulted in a high success recovery rate for the treatment of mid portion ATY (Ohberg and Alfredson, 2004).

As the effects of tendinopathy on tendon stiffness have not been specifically studied (Morrissey et al., 2011), the impact of treatment modalities on tendon stiffness also remains poorly understood. Six weeks of EcEx training has been shown to decrease tendon stiffness measured using US and isokinetic dynamometry (Morrissey et al., 2011), however there are also many documented benefits brought about by completing EcEx programmes as a treatment modality for ATY (Alfredson et al. 1998). Compliance is high with EcEx programme and the associated benefits include a reduced tendon thickness, normalised tendon structure and decreased pain (Ohberg and Alfredson, 2004; Langberg et al., 2007; Beyer et al., 2015). A particularly high success rate (82 – 100%) is found when using EcEx programs as a treatment intervention for athletes (Alfredson et al., 1998; Mafi, Lorentzon and Alfredson, 2001; Fahlström and Jonsson, 2003; Ohberg, Lorentzon and Alfredson, 2004), helping make EcEx programmes the mainstay of conservative treatment for ATY (Webborn, 2008). Despite these positive findings, when an EcEx programme was applied to a non-athletic patient population, the results were less successful, with less than 60% (19 out of 34) of patients experiencing beneficial outcomes (Sayana and Maffulli, 2007). This implies that EcEx programmes are less effective for non-athletic populations. This lack of efficacy could potentially be due to a non-athlete having a reduced calf strength and/or an increased weight: calf strength ratio compared to an athlete meaning that the EcEx programme imposed a relatively greater load on the sedentary AT (Sayana and Maffulli, 2007). Other explanations include a potentially later presentation with non-athletes as athletes are more likely to seek medical help earlier. There is also a possible decreased motivation to comply to the EcEx programme potentially present in non-athletes (Sayana and Maffulli, 2007).

The clinical benefits gained with EcEx have not yet been shown to correlate with any specific quantitatively measured changes in tendon structure including tendon diameter or neovascularisation (Drew et al., 2014). Some authors suggest this indicates that controlled exercise (including EcEx) may not actually remodel a pathological tendon, but cause adaptation in the musculature and fibrillar structures surrounding the tendon to improve the load tolerance of the tendon (Docking and Cook, 2015). This implies that alterations in tendon structure are not a sufficient explanation of the beneficial response to therapeutic exercise regimes (Drew et al., 2014). Taking into account the theory of the continuum of pathology (Cook and

Purdam, 2009), it may be possible that those receiving EcEx treatment may have been at varying stages of pathology, thereby affecting the effectiveness of the treatment regime (Cook and Purdam, 2014). Initiating an EcEx programme during certain stages of tendinopathy may be more or less beneficial for the tendon and simply applying the same intervention to all tendinopathy presentations is unlikely to lead to success in each person (Cook and Purdam, 2009). Cook & Purdam (2009) propose that the beneficial outcomes of older and pre-surgical patients generally used in the majority of EcEx research (Alfredson et al., 1998) are apparent due to the participants all being in the same stage of tendinopathy. Little research has been conducted using early-stage tendinopathy, potentially due to the ability of tendons in this group to spontaneously recover and that the majority of people in this early stage may not even have symptoms prompting them to seek help (Cook and Purdam, 2009). It is likely that removal of the abusive load on the tendon will result in positive adaptation of the tendon and a return to function, however it is likely that high levels of eccentric loading, particularly on successive days with little time for recovery will aggravate tendons that are in this stage (Cook and Purdam, 2009). The authors go on to suggest that individual factors (i.e. age, sex, body composition, body biomechanics etc.) may influence the progression between the stages of the tendinopathy continuum and suggest that clinicians should stage the pathology and apply load according, however more research is needed to define 'appropriate' loading (Cook and Purdam, 2009, 2014).

Due to EcEx regimes being low cost and low risk, it is often considered a primary line of treatment for ATY (Magnussen, 2009). Despite this, authors have been unable to explain why alterations to the tendon occur, or at what point these changes occur (Ohberg, Lorentzon and Alfredson, 2004). The mechanisms by which EcEx training produces positive effects for tendinopathy treatment remain unclear and poorly understood (Rees et al. 2008; Mahieu et al. 2008) and whether the effectiveness of EcEx regimes is attributed to the load or the duration of the stretch is also still unclear (Verrall, Schofield and Brustad, 2011). The majority of studies assessing the effectiveness of an EcEx protocol as a rehabilitation method have referred to return of function, as reported by the patient, to estimate the success rate of the intervention with few actually focusing on the quantitative changes that occur in the mechanical properties of the tendon (Chimenti et al., 2014). This may be important in clinical practice as de Vos et al. (2012) noted that despite changes in symptoms, normalisation

of tendon structure may require a much longer time frame (de Vos et al., 2012). The implications of this are that should clinicians, researchers or patients interpret subjective improvement for structural change and increase load at too early a stage, it may cause further regression for the tendon. As such, no consensus has yet been reached as to the optimum dose of exercise or the recommended progression of EcEx regimes (Meyer, Tumilty and Baxter, 2009) required for effective treatment of ATY.

At present, there is little evidence as to why and how therapeutic exercise programmes are effective in the treatment of ATY, and the mechanisms behind recovery remain poorly understood (Drew et al., 2014). Understanding the recovery processes occurring in the AT during treatment for ATY will enhance the current level of understanding and improve ability to return tendons, and hence individuals, to their pre-injury functioning. Emerging technologies such as elastography may allow for studies to obtain measures of tendon mechanical properties, including stiffness. Changes in the mechanical properties of a tissue such as tendon can be a result of the internal health of the tissue as an injured or degenerative tendon is noticeably softer (Wu et al., 2011), therefore a healing tissue should exhibit increases in stiffness. Research suggests that treatment interventions should be tailored to the specific stage of tendinopathy (Cook and Purdam, 2009), therefore uncovering a time course for alterations in tendon mechanical properties could potentially allow tendons to be classified into stage easier, allow more appropriate treatment to be advised and improve monitoring of the progression of rehabilitation.

A non-invasive, readily available, simple to use and reliable method for quantifying the mechanical properties of tendons would aid in early diagnosis (Yoshitake et al., 2014) and the monitoring of treatment protocols (Khan et al., 1999). Evidence surrounding the structural changes in the AT during rehabilitation is scarce, together with a lack of objective, evidence based rehabilitation programmes (Geremia et al., 2015). Research has called for future longitudinal studies to focus more on the post treatment follow up of tendon disease and for tendon stiffness following an EcEx programme to be examined (Witvrouw et al., 2007; Wu et al., 2012).

2.5 Tendon Imaging

Obtaining measures of the mechanical properties of a tendon would enable an objective and quantitative assessment of tendon stiffness to be made. Previous

calculations of tendon stiffness have required the use of cadaver tendons and tensile testing to hold the tendon and stretch it to measure the change in length of the tendon and the corresponding force being put through the tendon (Ravary et al., 2004). Using the recorded data, a graph of the change in length divided by the original tendon length (strain), can be plotted against the force applied per unit area (stress), see Figure 2.4 (taken from (Rosskopf et al. 2016)). By assessing the stress/strain relationship of the tendon, an estimate of tendon stiffness can be calculated (Onambele-Pearson and Pearson, 2007).

In tendon, many crimped collagen fibres make up the fibrils and when the tendon is stretched, these fibres straighten leading to a curve of tendon strain which is the first section (1) in Figure 2.4, (from (Rosskopf et al. 2016)), referred to as the toe region. Section 2 represents the linear section, where increased load leads to a proportional change in length, here there is an increase in the number of fibres tightening until they are all straight (Rosskopf et al. 2016) and it is in this section that elastic stiffness can be calculated as the slope of this section represents the Young's modulus (Hoskins, 2012). Young's (sometimes referred to as elastic) modulus of a material is a quantification of its stiffness (Shinohara et al., 2010; Chen et al., 2013). At the end of section 3 of Figure 2.4, unpredictable failure begins which can result in rupture (Kirkendall and Garrett, 1997). The amount of tendon deformation experienced will be affected by the stiffness of the tendon (Kirkendall and Garrett, 1997), the steeper the slope of the linear section of the graph, the harder/stiffer the tissue.

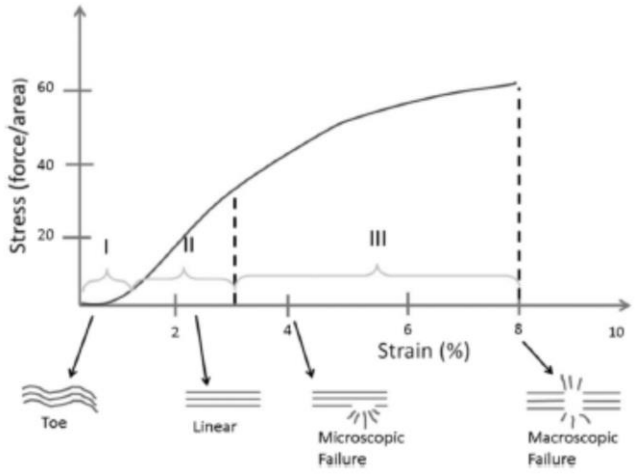


Figure 2.4: Stress/Strain curve

It is impossible to use this method in the *in vivo* and clinical environment to measure stiffness as you cannot remove the tendon. As such, objective and quantitative measures of the *in vivo* stiffness of the human AT have been difficult to obtain. Extrapolating the data obtained from cadaver studies using the above method to the *in vivo* environment is fraught with issues including differences in temperature, hydration and vascular supply (Klauser et al., 2013), therefore our precise understanding of the stiffness properties of *in vivo* human tendons remains limited. Little is known about the adaptation of human tendon, due to the difficulty in isolating the tendon from the associated musculature and the problems encountered obtaining data through a reliable, non-invasive and cost effective method (Brandenburg et al., 2014).

Early research assessing tissue mechanical properties started with the physics of vibration in soft tissue, dating back to the 1950's, where the mechanical impedance of tissue was shown to increase with frequencies between 500 – 5000 Hz (Oestreicher, 1951). Since then, many methods for assessing the mechanical properties of human *in vivo* tissues have been proposed, developed and evolved. The introduction of a pulsed Doppler ultrasonic system to measure the stiffness of soft tissues was proposed in the 1980's (Krouskop, Dougherty and Vinson, 1987) and Magnetic Resonance Imaging (MRI) was initially developed in the early 1970's (Damadian, 1971), with the technique being refined over the years and capable of providing a measure of tissue stiffness. MRI has a high spatial resolution and can image through all bodily tissues, but is expensive and time consuming to deliver and lacks the ability to image whilst the tissue undergoes varying stresses (Hodgson, O'Connor and Grainger, 2012). More recent developments, specifically in the diagnosis of tendon disease have focused on B-mode (brightness mode) US imaging (Yoshitake et al., 2014), which has good specificity and sensitivity for detecting tendon tears and has been useful in examining the structural organization of the fibrils within tendons (Brandenburg et al., 2014). US is low cost, safe, readily available and highly accurate in assessing suspected AT injuries (DeWall et al. 2014) and provides a precise picture of both the peritendinous soft tissue and the internal tendon structure (Maffulli et al., 1987; Brushøj et al., 2006). The test-to-test reliability for ultrasonography when used to assess the same tendon over 10 examinations produced a coefficient of variation of just 1.1% (Ohberg and Alfredson, 2004).

Despite the benefits of US as a modality for tendon assessment, it is not without its own issues and limitations. US lacks the spatial resolution of other technologies such as MRI (Ghorayeb et al., 2012) and it is not able to image through bone, making the assessment of deep lying tendons difficult (Hodgson, O'Connor and Grainger, 2012). The intra-observer reliability of US when used to assess the cross-sectional area of the AT is higher than the inter-observer reliability of the modality (Ohberg, Lorentzon and Alfredson, 2004), indicating a potential effect of more than one operator obtaining images. US is a very operator dependant technique with both the settings of the machine and the handling of the transducer being potential causes of varying results (van Schie et al., 2010). Another potential issue with US imaging is the impact of anisotropy. The term anisotropy can be used to describe the variability of properties when measured in different directions (Seynnes et al., 2015). When examining tendon the term refers to images that may appear to vary due to the angle of the US beam (Ghorayeb et al., 2012). When viewing an US image, obliqueness in the angle of the US wave will result in some of the energy being reflected at a greater angle resulting in a greater loss of energy (Connolly, Berman and McNally, 2001), leading to anisotropy. With anisotropy, the brightness of some features can appear less structured with a loss of reflectivity, giving a false positive, or the impression of underlying pathology when in reality none exists (Connolly, Berman and McNally, 2001). Increasing the angle of an US probe longitudinally by 7 to 10° can result in normal and healthy tendons appearing seemingly hypoechoic, a common feature with pathology (Lehtinen, Bondestam and Taavitsainen, 1994) with these noted changes in results following small changes in transducer positioning highlighting the highly operator dependent nature of US as an imaging technique.

US and MRI have been extensively used in human *in vivo* tendon studies to measure anatomical properties (Bashford and Tomsen, 2008) but not mechanical properties (Hodgson, O'Connor and Grainger, 2012). MRI has a higher resolution but greater cost than US, leading to US being a more common tool for imaging, although it is not able to detect degenerative tendon changes at an early stage nor accurately measure the degree or stage of pathology (Ghorayeb et al., 2012). The use of US coupled with measurements of force to establish *in vivo* tendon mechanical properties has increased

over the past two decades, however the mechanical properties reported in various studies differ considerably, with these inconsistencies further limiting knowledge and understanding of *in vivo* tendon function (Seynnes et al., 2015). An example of this discrepancy in reported stiffness values is demonstrated by reported measures patellar tendon stiffness. One study reports patellar tendon stiffness as 2277 ± 145 N/mm obtained using ultrasonography and an isokinetic dynamometer (Seynnes et al., 2013), whereas Couppe et al. (2009) report stiffness as 5546 ± 1871 (N/mm) obtained using a strain gauge and US (Couppe et al., 2009). The difference in reported values is over 2-fold, despite them both using US to measure changes in length and commonly used methods to measure force. Both studies used male participants of a similar age (29 and 27 respectively). The reported values from these two studies do seem to be two ends of the range of reported stiffness values for the patellar tendon which were all obtained using similarly aged, male participants. These values include 2924N/mm (age 27) (Carroll et al., 2008), 3487 N/mm (age 31.5) (Kongsgaard et al., 2010), 3684 N/mm (age 21) (Helland et al., 2013) and 3716 N/mm (age 25) (Kongsgaard et al., 2007). All the above studies obtained stiffness values using US and either a strain gauge or isokinetic dynamometer from male participants of a similar age, implying a large range of measures are available in the literature with errors associated with each method of obtaining the data.

Various methodological limitations in the available research using US-based methods has recently been discussed by Seynnes et al. (2015), with the authors noting many potential factors causing inconsistency. These include incomplete scanning of tendons due to narrow fields of view, overestimation of tendon elongation through the difficulty in tracking anatomical features during muscular contraction, slight movement of the transducer and the calculation of force exerted by a tendon in the *in vivo* environment only being estimated (Seynnes et al., 2015). Calculations of tendon force are based on joint moments produced by the muscle the tendon inserts into (Seynnes et al., 2015), however Maganaris et al. (2000) note that there is no gold standard way to measure moment arms, adding further difficulty in obtaining comparable data. The reported values of Young's Modulus for tendons within the body including the tibialis anterior tendon, AT and patellar tendon have included values of 0.16GPa, 0.60 GPa, 0.71 GPa, 0.74 GPa, 0.91 GPa, 1.20 GPa, 1.53 GPa and

1.94 GPa (Maganaris and Paul, 1999; Maganaris et al., 2006; Burgess, Graham-smith and Pearson, 2009; O'Brien et al., 2010; Stenroth et al., 2012; Helland et al., 2013; Bohm et al., 2014; Mogi et al., 2018). This variation in results influences our current knowledge and understanding (McKee et al., 2011) and have been attributed to methodological differences including calculation of tendon force and transducer movement etc. (Seynnes et al., 2015). The presence of these differences make direct comparison of the results within the literature difficult (McKee et al., 2011) and being able to accurately measure tendon stiffness would be vital to improving understanding of the behaviour of *in vivo* tendon (Ianculescu et al., 2014).

For many years, alterations in the palpable stiffness of a human soft tissue has been associated with some form of disease or injury (Milgrom et al., 2014), with stiffness being the main parameter used to examine tissue quality (McKee et al., 2011). A non-invasive, readily available, simple to use and reliable method that can quantify tendon stiffness would aid in improving our mechanistic understanding of tendons as well as the diagnosis and treatment of varying disorders and injuries (Onambele-Pearson and Pearson, 2007). Being able to quickly and accurately determine the mechanical properties of soft tissues could help in understanding how these properties relate to normal functioning. The need for a technology that can offer a direct assessment of the stiffness of *in vivo* biological tissues, has spurred the introduction of elastography. Elastography may provide the opportunity for earlier diagnosis and therefore allow for the implementation of an earlier management plan of injury which can prevent tendon injuries progressing to a stage where treatment is ineffective, time consuming and chronic (McKee et al., 2011). It could also provide an objective measure to be used in conjunction with commonly used clinical objective and subjective measures to help establish recovery during rehabilitation regimes helping to assess their effectiveness (Ooi et al., 2013).

2.6 Elastography

Being able to quantify the differences in stiffness between healthy, degenerating and pathological tendons objectively and to monitor responses in tendon stiffness to current treatment interventions would improve our current understanding of tendons, tendinopathy development and current treatment practices. If changes to stiffness occur in rehabilitation following improvements in symptoms, it would be beneficial

for clinicians, researchers and individuals to understand exactly when changes to the mechanical properties of a tendon occur that enable the tendon to be physically able to withstand a greater loading element. Any technique for measuring mechanical properties includes an application of a stress and a measurement of the subsequent deformation caused by that stress (Brandenburg et al., 2014). Elastography quantifies differences in mechanical properties of different structures by measuring the deformation of tissue in response to a given force (Muraki et al., 2015). Elastography functions on the knowledge that compression of a tissue results in a measurable displacement, and by measuring this displacement, objective information on tissue stiffness can be calculated (Itoh et al., 2006). The amount of displacement occurring within a tissue is higher in softer tissue and lower in harder tissue, and this information can be displayed in real time, over the top of a standard US image to provide clinicians with a view of the stiffness of the tissue being examined (Palle et al. 2011). As elastography provides an assessment of tissue stiffness (De Zordo et al, 2008), its use in the assessment of *in vivo* human tendons has received attention. Studies have demonstrated the usefulness of elastography in the diagnosis of ATY (Dirrichs et al., 2016), with the results suggesting it can detect degeneration associated with tendinopathy at earlier stages than traditional US (Horton, 2013). Despite being a relatively new clinical technique, authors have noted that it can provide quantitative mapping of muscle hardness reveal patterns of change after exercise (De Zordo et al, 2008, Debernard et al, 2013) and show changes in tendon matrix earlier than traditional B-mode imaging alone (Horton, 2013).

Elastography may be able to measure disease induced changes in the mechanical properties of tissue (Itoigawa et al. 2015), taking away the subjective measures of manual palpation (Hoskins, 2012). Palpation is still used by most clinicians to establish the hardness of one tissue in relation to the tissue surrounding it (Brandenburg et al., 2014) as abnormal mechanical properties are often a result of dysfunctional or diseased tissue (Hoskins, 2012), Palpation is therefore used to assess the location and range of abnormal, dysfunctional or diseased tissues (Ooi et al., 2013; Ianculescu et al., 2014). Elastography was first described in the literature in the early 1990's (Ophir et al., 1991) and is currently used to differentiate malignant and benign tumours in varying body organs. There are review articles on the work of elastography

in differentiating tumours in the breast (Goddi, Bonardi and Alessi, 2012), liver (Frulio and Trillaud, 2013) & thyroid (Kwak and Kim, 2014a) demonstrating the extent of research and use in these areas. For breast imaging, elastography can provide additional objective information that clinicians can use to diagnose lesions with a diameter of <10 mm. Even if the tumour is not palpable or expressing serum tumour markers and the US findings are inconclusive, elastography can provide an additional indication for the need for surgical intervention (Itoh et al., 2006). For application to tendon assessment, an injury to the tendon will be expected to result in changes to its mechanical properties and elastography can help improve the diagnostic capability of conventional imaging by differentiating a softer injured tendon from the surrounding stiffer, more healthy tendon (Brandenburg et al., 2014).

There are varying elastography techniques available (Sarvazyan et al., 2011; Jeong et al., 2014), however there is disagreement and/or uncertainty about the exact terminology used to describe each distinct type. The literature surrounding elastography contains a confusing mix of terminology providing difficulty in examining the differences between methods. The description of "tissue displacement as a result of compression" has previously been used to describe real-time sonoelastography (De Zordo et al. 2009; Wu et al. 2011), real-time ultrasound elastography (Drakonaki, Allen and Wilson, 2009), Quasi-static elastography (Sarvazyan et al., 2011; Treece et al., 2011) and real-time tissue elastography (RTE) (Gheorghe et al., 2009) alike. Furthermore, the terms static strain elastography and sonoelastography have been used as a synonym for compression elastography (Botar Jid et al., 2012; Bamber et al., 2013; Klauser et al., 2014; Pastore et al., 2014). A review article outlined 10 differing elasticity imaging methods (Sarvazyan et al., 2011), however, to ensure clarity and effective cross referencing within the literature, there is a distinct requirement for consistency in the terminology used. To simplify, the varying types of elastography can be categorized according to how the stress is applied to the tissue and how its subsequent deformation is measured (Palle et al. 2011). The stress can be applied either internally through internal physiological motions, or externally through mechanical compressions or US push pulses (Treece et al., 2011). The measurement of displacement can use magnetic resonance imaging (MRI) or US. Using MRI to assess displacement resulted in Magnetic Resonance

Elastography (MRE), however this carries with it the limitations of normal MRI imaging (i.e. space, availability, expense, subject positioning and operator experience), as such, this technique is unlikely to be routinely incorporated to clinical examination (Sarvazyan et al., 2011). Elastography is a quick, easy and non-invasive measure that may increase our understanding of tendon mechanical properties and the changes that occur in disease, damage and repair. Elastography measures the elasticity of differing structures and can be used to assess soft tissues including tendons, where stiffness is determined by the composition and structure of the tendon (Klauser et al., 2014). Preliminary findings have shown that under pathological conditions, the elastic properties of normal tendons are altered and softening can be detected (Brandenburg et al., 2014), and using elastography techniques, the stiffness of healthy and pathological AT's have been estimated. Table 2.3 outlines the young's modulus values obtained using elastography in both healthy and pathological ATs.

Table 2.3: List of reported young's modulus values (kPa) in Achilles tendons obtained using elastography

Tendon	Reported Stiffness	Technique used	Authors
Achilles tendon longitudinal	74.4 ± 45.7 kPa (range 6-242 kPa)	SWE	Arda et al. (2011)
Achilles tendon transverse	51.5 ± 25.1 kPa (range 10-111 kPa)	SWE	Arda et al. (2011)
Healthy Achilles tendon	291.9 ± 4.4 kPa (range 261.0 – 300 kPa)	SWE	Chen et al. (2013)
Healthy Achilles tendon	154.2 ± 28.3 kPa	SWE	Dirrichs et al. (2016)
Healthy Achilles tendon	254.6 ± 59.8 kPa	SWE	Leung et al. (2017)
Healthy Achilles tendon	289.6 ± 23.4 kPa (range 268.6 – 295.7 kPa)	SWE	Zhang et al. (2016)
Symptomatic Achilles tendon	53.4 ± 23.2 kPa	SWE	Dirrichs et al. (2016)
Ruptured Achilles tendon	56.5 ± 68.6 kPa (range 3.5 – 228.3 kPa)	SWE	Chen et al. (2013)

The benefits of elastography can span different populations, as shown by Dirrichs et al. (2016) who demonstrated that symptomatic tendinopathies demonstrate a lower stiffness level using elastography (Dirrichs et al., 2016). Elastography can therefore be utilised to help determine stage of tendinopathy to help establish the best options for treatment (Cook and Purdam, 2009). Healthy tendons show a higher stiffness level in comparison to pathological tendons and rehabilitation protocols aim to remodel abnormal tendon structure (Drew et al., 2014; Dirrichs et al., 2016), therefore rehabilitation may also impact mechanical properties. It is therefore possible that elastography could be used to identify alterations in mechanical properties brought about by therapeutic rehabilitation protocols. Elastography could potentially monitor and optimise rehabilitation protocols as it offers additional information to clinicians (Klauser, Faschingbauer and Jaschke, 2010). Within a sporting context, athletes, coaches and managers could also benefit from using elastography to track training regimes and load, as the subtle alterations in mechanical properties that occur in the early stages of injury or disease could be detected earlier, improving the management of training loads by alerting clinicians to a decline in function, enabling them to prescribe interventions to prevent further tendon damage (De Zordo et al. 2009; De Zordo et al. 2010; Klauser et al. 2010; Brandenburg et al. 2014).

It would be prudent to note at this time that few studies have examined inter- or intra-reliability of elastography (Wu et al, 2012). At present, the main body of research into the reliability and validity of varying types of elastography as modalities to assess tissue properties has been conducted in muscles or organs (Motosugi et al., 2010; Chino et al., 2012; Eby et al., 2013) with limited work examining its reliability and validity for assessing tendon properties. Klauser et al. (2013) compared conventional B-mode US and elastography of the AT with histological assessment of biopsies taken from cadavers. Elastography was able to depict loss of fibre integrity in the AT with greater sensitivity than US alone and could also detect loss of collagen structure, fatty infiltration and capillary proliferation in the AT. A limitation of this research was that it was conducted using cadavers, with the authors noting that future research using elastography should include *in vivo* studies. The differing studies that have so far employed any method of elastography to assess human *in vivo* tendon appear to use differing techniques, they lack standardisation of transducer placement and use different outcome measures (i.e. Strain ratio, Young's modulus and Shear wave

velocity) (De Zordo et al. 2009; De Zordo, Lill, et al. 2009; De Zordo et al. 2010). This makes effective comparison of the studies and analysis of overall results difficult, adding to the confusion surrounding this research area.

The two types of elastography discussed further in this research include the two commercially available methods, which can be split into two types, compression (strain) elastography (CE) and shear wave elastography (SWE) (Hoskins, 2012). Both methods are relatively new, have the ability to measure in real-time, and are proposed to non-invasively offer a measure of mechanical properties (Brandenburg et al., 2014). Despite the type, elastography methods have been noted as being very useful as a diagnostic tool and in particular for the assessment of tendon (Fusini et al., 2018). Both techniques carry their own advantages and disadvantages which are outlined in sections 1.6.1 and 1.6.2.

2.6.1 Compression Elastography

Compression (strain) elastography (CE) uses special software alongside a conventional US machine (Chino et al., 2012) and functions on the principle that compressing a tissue will cause displacement in that tissue which can be measured in real-time (Klauser, Faschingbauer and Jaschke, 2010; Muraki et al., 2015). The displacement and deformation experienced within a tissue is caused by compression, hence the name, and is represented as strain (Brandenburg et al., 2014). These compressions can be applied by the operator exerting pressure on the US transducer, or as a result of the participants natural motion (i.e. heartbeat or respiration), however neither of these methods are quantified or standardised (Benson and Fan, 2012; Ianculescu et al., 2014). Regardless of the delivery method, compression results in measurable tissue displacement (strain), and as the technology is extremely sensitive, even very small movements can provide strain data (Hoskins, 2012).

The basic premise of CE is based on the knowledge of how soft tissues behave under compression. When placed under equal amounts of stress, compressing a soft tissue results in it being squashed and expanding in a different direction, whereas a hard tissue (like a stiff, hard lesion for example) will not change its shape (Hoskins, 2012). It can therefore be possible to differentiate areas of harder and softer tissue, as the

softer tissues deform more easily. Softer tissues have higher strain values, as the areas closest to the point of compression displace more than those further away from it, resulting in greater levels of overall strain. In firmer and harder tissues, all points within the tissue move together and displace to approximately the same amount when compressed, reducing overall strain. Displacement is therefore lower in harder tissue and higher in softer tissue, with CE software allowing a clinician to identify differing grades of strain and estimate tissue stiffness (Klauser, Faschingbauer and Jaschke, 2010). The weakest point in a tissue (i.e. that which experiences the greatest strain and is therefore the softest) can be identified, corresponding to the point at increased risk of damage and injury (Wu et al, 2012). As alterations in tissue stiffness occur with pathological change (Horton, 2013), the advent of CE presents the opportunity to assess the relative stiffness of *in vivo* tissues in real-time. Due to elastography software being used in conjunction with a standard US machine, CE can provide both a traditional B-mode US image as well as a colour coded map of the strain, called an elastogram, over the top of, or next to the US image to indicate relative stiffness (Klauser, Faschingbauer and Jaschke, 2010; Chino et al., 2012; Wu et al., 2012; Klauser et al., 2014). To ensure the best possible images are used for analysis, the elastograms also provide an indication of the quality of the image (Lalitha et al, 2011). The quality of the image is determined by the software, with each image given a quality factor (QF). A QF above 60 has been used in other studies to represent an accurate image (Wu et al, 2011). This thesis used a QF of at least 75, seen for at least 5 consecutive frames, to ensure a high level of image quality.

To add a semi-quantitative approach to CE, strain ratios can be used to compare two areas within the same elastogram (Brandenburg et al., 2014) and compare the strain experienced in one tissue to that experienced in another (Chino et al., 2012). The calculation is performed by the elastography software and is calculated in an operator selected region of interest (ROI). A higher strain ratio has been used to indicate a diseased tissue (malignant tumour), due to the tumours higher stiffness value (due to the hard nature of the tumour) being measured against the normal, softer surrounding tissue (Brandenburg et al., 2014). The technique of CE is dependent on the positioning of the reference region used to calculate the strain ratio and compare stiffness in one area to another. The applied stress (measured in force per unit area) will vary depending on the depth within the tissue being imaged. Figure 2.5 (taken from

Hoskins, 2012) demonstrates the differing outcomes of a strain ratio calculation when the reference region is either superficial or deep in relation to the ROI. In Figure 2.5, (taken from Hoskins, 2012), the letters A, B and C show the strain % experienced within the reference region at different depths. The lesion is represented by L and the strain experienced within the lesion is measured at 3%. If the reference region is positioned at the same depth (B) as the Lesion (L), the strain ratio is calculated as a value of 5. If the reference region is either superficial (A) or deep (C) in relation, then the calculated strain ratio value varies from 2.7 (deep) to 8.3 (superficial) (Hoskins, 2012). Knowledge of the impact depth has on strain ratio calculations is critical as strain ratios should be calculated from the same depth as the ROI.

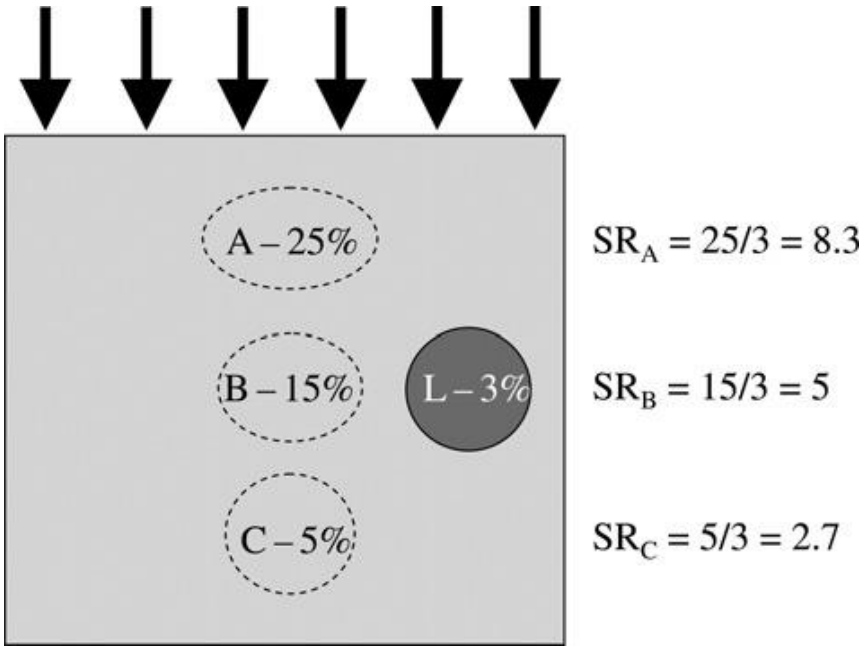


Figure 2.5: The effect of the positioning of the reference region on the strain ratio calculation

A limitation of CE is that any external compression exerted by the operator will be varied, with the distribution of stress onto the tissue unknown and not uniform. Therefore, measures provided are not quantitative in nature, rendering any attempt to convert or calculate Young's modulus from the results unreliable (Brandenburg et al., 2014). A further limitation is a phenomenon termed the 'egg shell effect' which has been described as when areas of soft tissue are located within areas of incompressible and hard background tissue, as the software will be unable to pick these up as the hard outer tissue limits the generation of internal readings (Ophir et al., 2002; Klauser et

al., 2014). Other limitations include the angle of the US transducer, which should always be kept perpendicular to the tissue being assessed to avoid non-linear compression which can lead to inaccurate strain readings (Klauser et al., 2014).

Figure 2.6 demonstrates a typical CE elastogram obtained from an AT in the longitudinal plane using a Siemens ACUSON S2000™ (Siemens Medical Solutions, USA). The elastogram (colour map) in Figure 2.6 is overlaid on the B-mode image, highlighting the areas of harder and stiffer tissue. The colour coding of CE can alter depending on the software used. The software used to produce the elastogram in Figure 2.6, uses a scale where red indicates a harder tissue and blue/purple indicating a softer tissue as seen in the key. There is at present, no standardisation of colour coding used between different elastography machines, suggesting this needs to be taken in to account when analysing elastograms and comparing results of different studies. In Figure 2.6, the elastogram overlies the B-mode image showing the calcaneus in the right-hand side of the image and the AT running between the white lines as anatomical points of reference.

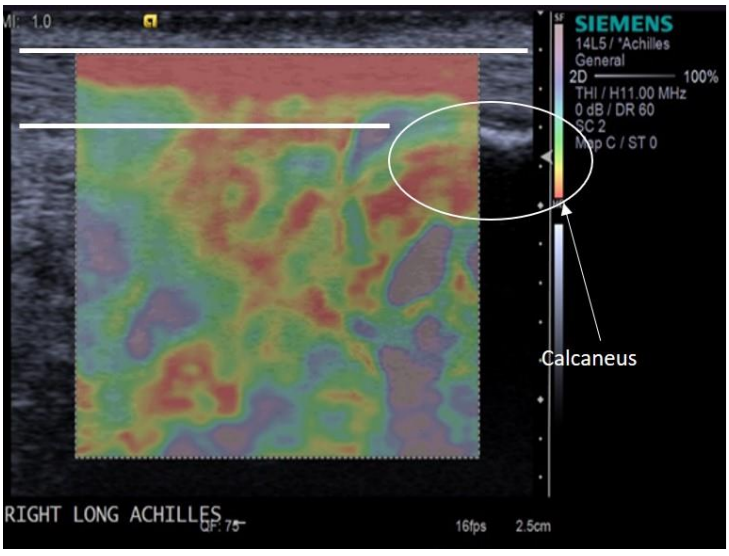


Figure 2.6: Compression Elastography elastogram of Achilles tendon insertion

Figure 2.7 demonstrates the elastogram positioned adjacent to the traditional B-mode image. This time, the attachment of the soleus muscle (shown on the left of the image) was used as the anatomical point of reference. Both images show a clearly defined red structure at the top of the image, representative of a non-pathological, healthy, stiff tendon (De Zordo et al. 2009).

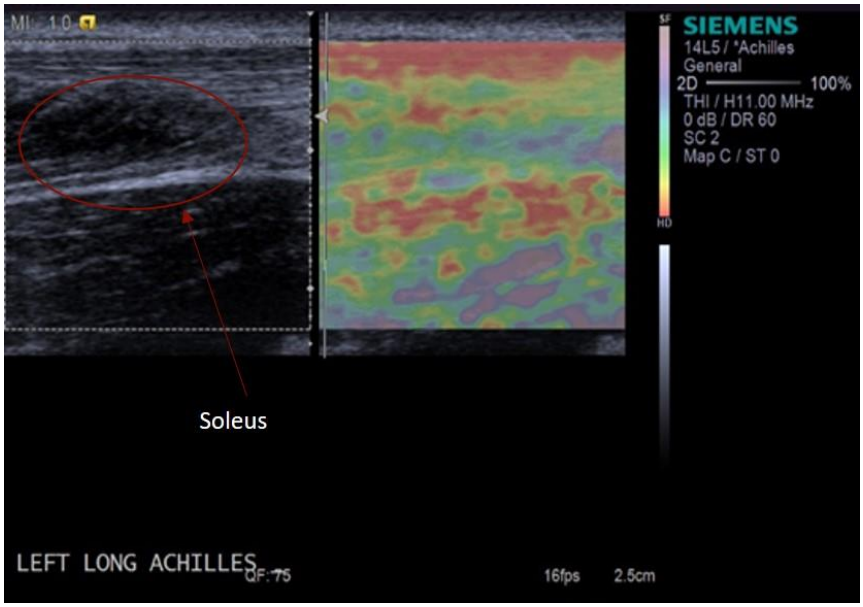


Figure 2.7: Compression Elastography elastogram at musculo-tendinous junction

The term 'sonoelastography' is considered by some to be a separate elastography technique due to its use of different tracking methods to asses tissue deformation (Sarvazyan et al., 2011; Ahn et al., 2014), however in practice the term sonoelastography is often used as an analogy or synonym for CE (Smajlovic and Licanin, 2010; Wu et al., 2011; Klauser et al., 2013; Pastore et al., 2014). Sonoelastography has received increasing attention over the last decade, yet it is hard to identify the exact technique used in the varying literature, as sonoelastography has been described purely as a combination of B-mode US and elastography (Buck, Verstraete and Li, 2012; Wu et al., 2012). Ahn et al. (2014) suggest that all US elasticity imaging can be classified according to the method of tissue excitation and the method used to track the tissue deformation. They recognise three main categories including CE, SWE and sonoelastography. They define the three categories as CE tracking tissue movements during compression and providing estimates of strain. Sonoelastography uses colour Doppler to measure tissue movement in response to external vibrations and lastly SWE tracks shear wave propagation to obtain measure of stiffness (Ahn et al., 2014). It appears that the most agreed upon description for sonoelastography is that which explains it as using some form of external vibrations to cause tissue movement which is then imaged through the use of Doppler (Li and Snedeker, 2011; Wu et al., 2012). Some authors however also suggest that sonoelastography can use both Doppler or US to image displacement (Sarvazyan et al., 2011) which can add confusion to the exact methodology being used. A case in

point is demonstrated by Wu et al. (2011) that examined the application of CE to the plantar fascia, but documented the technique as sonoelastography (Wu et al., 2011). This paper concluded that sonoelastography demonstrated excellent reliability but noted as a limitation that the study did not assess the reliability of performing sonoelastography itself (Wu et al, 2011).

The work of De Zordo et al. (2010) compared sonoelastography to a clinical examination by US of ATY, however, the actual technique used fell under the definition of CE. De Zordo et al. (2010) compared the colours shown by elastography to represent differing levels of stiffness to 3-tiered grading system from US examination. They reported that sonoelastography has a sensitivity of 94%, specificity of 99% and accuracy of 97% indicative of a very promising technique with which to image tendon stiffness. More recent work (Klauser et al, 2013) sought to compare sonoelastography and conventional B-mode US findings with histological findings, with the results indicating that sonoelastography can depict histopathological degenerative changes and a loss of fibre integrity associated with tendinopathy of this region with more sensitivity than B-mode US. The research using CE in human *in vivo* tendons has all noted several methodological limitations including; no histopathological comparison for pathologies, no data taken on reproducibility or inter- and intra-observer variability and small sample sizes (De Zordo et al. 2009; Drakonaki et al. 2009; Tan et al. 2012). Other methodological limitations include observers not being blinded during examinations, subjects not being matched for age and sex and a lack of quantitative analysis (Wu et al., 2011; Sconfienza et al., 2013).

There is limited data available on the validity and reliability of the use of CE for assessing the mechanical properties of *in vivo* human tendons, and specifically the AT. The reliability and validity of CE when used to assess muscle hardness has previously been confirmed (Chino et al, 2012) using 2 separate experiments. The first used a commercially produced tissue mimicking material with a known Young's modulus, whilst the second used two separate investigators to assess the medial gastrocnemius muscle to enable assessment of both intra and inter-investigator reliability. For the first experiment, the coefficient of variation (CV) was measured at 6.9% with a value less than 12% being considered acceptable for a biological measure (Chino et al.,

2012). The intra-class correlation coefficient (ICC) was calculated as ICC = 0.99 with a value > 0.75 being considered excellent (Drakonaki, Allen and Wilson, 2009; Chino et al., 2012). In the measurement of human muscle, the CV values for intra-investigator reliability were 3.6% and 5.2% with inter-investigator reliability calculated at 3.1%. The ICC values for intra-investigator reliability were ICC = 0.77 and ICC = 0.89 with the figure for inter-investigator standing at ICC = 0.89. The authors reported that CE was able to measure absolute muscle hardness with good validity. Despite these encouraging findings, CE is unable to provide a direct measure of Young's modulus (Hoskins, 2012), and instead this can only be calculated using regression analysis based on previously examined reference material (Chino et al., 2012). CE can therefore only provide qualitative, or at best, semi-quantitative measures due to the variable amount of initial stress placed on the tissue, depending on both initial transducer pressure and underlying tissue composition (Ianculescu et al., 2014). Others report contradictory findings with CE, such as Horton (2013) who concludes that CE is an operator dependent technique, limited in its use by low reproducibility and that the result can be easily influenced by technique and transducer position (Horton, 2013) if the procedure is not properly standardised. This variable amount of initial stress leaves CE vulnerable to both intra-and inter-operator variability which should be minimised before the technique can be utilised in the mainstream clinical setting (Ooi et al, 2014). An example of this low reliability is demonstrated by Nordez et al. (2008) who reported CV's of 60.4 % with CE. Treece et al. (2011) describe CE as a purely qualitative technique, unsuitable for measuring absolute tissue stiffness and Klauser et al. (2010) note that an additional advantage in imaging mechanical properties might be the use of shear wave propagation (Klauser, Faschingbauer and Jaschke, 2010) which is discussed further in section 1.6.2

2.6.2 Shear Wave Elastography

Shear wave elastography (SWE) has been described as a non-invasive, convenient method that can obtain real-time, quantitative measures of tissue stiffness (Chen et al., 2013; Brandenburg et al., 2014). Compared to the results obtained using CE by Nordez et al. (2008), SWE greatly improves measurement reliability to CV = 7.5% and ICC = 0.652 (Maïsetti et al., 2012), attributed to SWE not having to rely on knowledge of the applied stress (Brandenburg et al., 2014). SWE technology can also use Virtual Touch IQ (VTIQ) technology, that uses an acquisition sequence including

reference, excitation and tracking pulses (Ianculescu et al., 2014). The VTIQ software uses acoustic radiation force impulses (ARFI) to generate shear waves (Chang et al., 2013), and therefore does not rely on the operator to apply the correct amount of pressure on the transducer (Chen et al., 2013). The VTIQ software creates information on tissue elasticity by collating information from up to 256 sequential acquisition beam lines within the user defined two-dimensional ROI and is able to display a qualitative and quantitative map of SWV ranging from 0.5 - 10.0 m/s (Benson and Fan, 2012). SWE provides quantitative information on tissue mechanical properties whilst relying less on the operator (Chen et al, 2013), meaning it can be used easily by different operators with differing levels of experience.

SWE achieves its goal by measuring the velocity of generated shear waves as they pass through a tissue to estimate tissue stiffness (Bercoff, Tanter and Fink, 2004). The relatively recent introduction of SWE to imaging was intended to address the limitations of CE (Klauser, Faschingbauer and Jaschke, 2010; Yoshitake et al., 2014) such as its reproducibility and qualitative measures (Ooi et al., 2013). In contrast, SWE functions independently of the operator and offers quantitative, real-time measures whilst being easy to use by different operators in differing settings at a relative low cost (Shinohara et al., 2010; Youk et al., 2014). Due to the relationship between the pathological state of a tissue and its stiffness, changes in tissue stiffness can indicate a change in pathology status meaning SWE can offer an additional diagnosis on the health of soft tissues (Yeong et al., 2015). SWE generates shear waves within a tissue using an acoustic push pulse, delivered through a traditional US transducer (Youk et al., 2014). The push pulse can be thought of in a similar fashion to a stone being dropped into a pond, with the shear waves acting in a similar way to ripples, travelling perpendicularly to the push pulse through the tissue (Hoskins, 2012). The software can both produce and detect the shear waves using the same US transducer allowing shear wave velocity (SWV) to be measured as they travel through the tissue (Hoskins, 2012). SWE allows the estimation of Young's modulus from SWV, as the faster the SWV, the harder / stiffer the tissue (Lazebnik, 2008). Shear waves travel approximately a thousand times slower than longitudinal waves in a soft tissue environment, therefore US images can be used to track the propagation of the shear waves (Klauser, Faschingbauer and Jaschke, 2010; Brandenburg et al., 2014). Small alterations in stiffness cause subtle changes in SWV (Yeong et al., 2015),

therefore, tissue stiffness is directly related to the speed of shear wave propagation through it (Klauser, Faschingbauer and Jaschke, 2010; Itoigawa et al., 2015). Some commercially available US systems using SWE, report Young's modulus (kPa), whereas others report SWV in m/sec (Youk et al., 2014). Some authors have noted that shear wave propagation speeds of 1-10 m/sec correspond to a tissue elasticity of 1-300 kPa (Youk et al., 2014). Young's modulus can be calculated from SWV and tissue density (Sarvazyan et al., 1998) using the equation: Young's modulus (E) = 3 x density x (shear wave velocity)^2 (Hoskins 2012; Yeong et al. 2015), however in this instance, tissue density is assumed to remain consistent at 1000 kg/m^3 (Hoskins, 2012; Eby et al., 2013) which may not always be the case in viscoelastic tendon. This assumption of constant tissue density may limit the accuracy of stiffness measured by SWE and reported in kPa, as the exact values for tissue densities can be affected by differing physiological conditions. One study reports no significant differences between the results obtained for kPa and for m/sec in breast masses, therefore either can be used to differentiate differences in stiffness and any differences in diagnostic performances have a minimal significance in clinical practice (Youk et al., 2014). Previous work in skeletal muscle also demonstrates that SWV has good agreement with Young's modulus throughout a normal physiological range of tension (Eby et al., 2013).

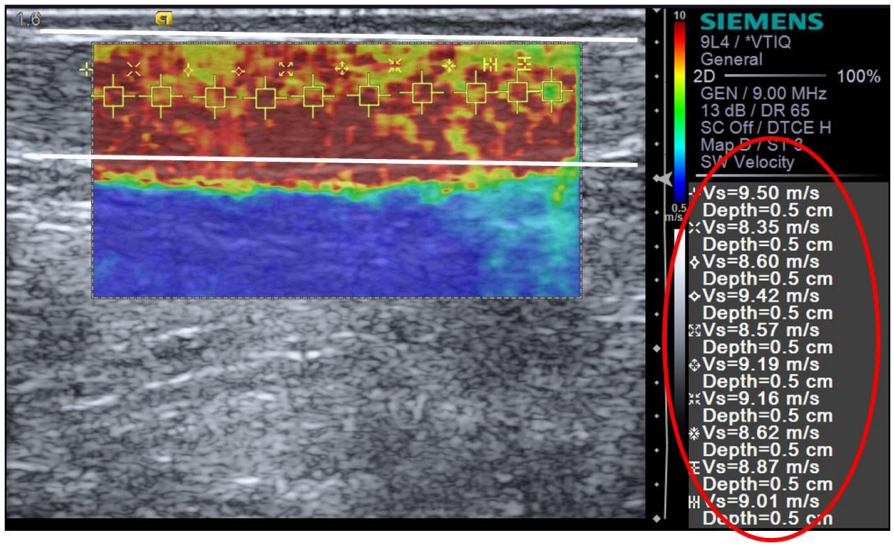


Figure 2.8: Longitudinal shear wave elastogram of the mid portion of a right Achilles tendon.

With SWE, the amount of displacement is assessed by tracking deformation in user defined ROI's with SWV calculated at multiple lateral locations relative to the ROI's

(Ianculescu et al., 2014). SWV is proportional to the square root of tissue elasticity, therefore higher tissue stiffness is related to higher SWV (Palmeri and Nightingale, 2011). SWE produces a shear wave elastogram (colour map, see Figure 2.8) which is overlaid on the B-mode image, and highlights areas of harder and stiffer tissue. Figure 2.8 was taken from the mid portion of a participant's right AT and therefore no anatomical landmarks are present in the image, however the mid-portion of the AT can be seen between the two white lines running along the top of the image. The values highlighted in the red circle down the right-hand side of Figure 2.8 represent SWV at set points along the AT, with the data obtained being displayed in SWV (m/s). There are 10 velocities taken at a depth of 0.5cm running along the centre of the tendon (along the black line) starting proximally and working distally. Again, the software, used to produce the elastogram in Figure 2.8 uses a red colour to indicate harder tissue and a blue colour to indicate softer tissue, represented in the key at the far-left hand side of the image.

SWE has been shown to be reproducible for assessing breast masses (Cosgrove et al, 2012) and improved the diagnostic performance of B-mode US in distinguishing benign and malignant breast masses during screening (Lee et al., 2014). With the addition of SWE to US, specificity increased from 17.4% to 62.1%, without a loss in sensitivity (Lee et al., 2014). In the musculoskeletal field, SWE is reported as a valuable tool for the assessment of muscle (Maïsetti et al., 2012), yet the application of SWE to tendon assessment has been less extensively studied (DeWall et al. 2014). SWE has the ability to clearly delineate different structures to provide functional information that is additional to the traditional B-mode image (Chen et al., 2013). Due to the ability of SWE to differentiate the varying stiffness of tissues, small partial tears in tendon, potentially isoechoic with the healthy tissue surrounding it and therefore overlooked with traditional US imaging, may now be detected (Klauser, Faschingbauer and Jaschke, 2010).

SWE has been validated in previous research by comparing the results obtained with SWE to those obtained with traditional tensile testing. This was carried out on swine skeletal muscle using a materials testing machine (Eby et al., 2013). It is noted that the whole-muscle samples utilised in this testing were obtained immediately post-

mortem from swine, and therefore the results should be extrapolated to the *in vivo* human tendon with caution as previously mentioned (Klauser et al., 2013). Other studies using human *in vivo* participants show support for SWE by suggesting it has an acceptable reliability when assessing both muscle and tendon (Kot et al., 2012). Other SWE studies report high intra- and inter-observer reliability together with a high level of reproducibility (Cosgrove et al., 2012) and for the assessment of tendon stiffness after exercise, one recent paper calculated inter-operator reliability for stiffness measures of the AT as ICC = 0.94 and intra-operator reliability between ICC 0.92-0.98 (Leung, Chu and Lai, 2017). Haen et al. (2017) demonstrated a significant correlation between stiffness values in cadaveric AT's when measured with traditional tensile testing and SWE (Haen et al., 2017). This study by Haen et al. (2017) demonstrated for the first time the validity of SWE in assessing the biomechanical properties of human AT *in vivo,* however the difficulties in extrapolating cadaveric data must be considered. Yoshitake et al. (2014) studied the repeatability of SWE to assess the biceps brachii and concluded SWE produces highly repeatable measures of shear modulus both between trials and between days.

Although the literature appears to support the use of SWE in the field of MSK imaging, there are, as with any technology, limitations to the modality that need to be considered. For example, variability in the measurements taken from a wide range of the population will likely exist (Sikdar, Wei and Cortes, 2014) and a database of normal stiffness ranges should be created to assist in clinical decision making and application. The effect of age, temperature, sex, hydration level and/or prior exercise may all affect the stiffness measurements obtained with SWE. SWE has limitations on the depth of measurement possible (Klauser et al., 2014) and tissue elasticity may alter during the process of a muscular contraction, therefore, the contractile state of muscles surrounding the tendon of interest must also be controlled (Sarvazyan et al., 2011).

With traditional ultrasound, the angle of the transducer to the skin is crucial to obtaining an accurate image, as an angle higher or lower than 83-88 degrees between the tendon and the emitted waves of the transducer, results in most of the reflected waves not being received and the tendon appearing hypoechoic (Kainberger et al, 1997). Transducer angle is also important to SWE accuracy as a transducer orientation

parallel to the tissue being imaged obtains the most reliable measures of SWV, given that shear waves propagate more readily along longitudinal fibres than they do across them (Eby et al, 2013). The transducer used for SWE should be orientated parallel to and along muscle fascicle direction (Maïsetti et al., 2012; Itoigawa et al., 2015) as when determining the mechanical properties of a muscle, obtaining measures along the long axis of the muscle allows for a better understanding of the function of the muscle (Miyamoto et al., 2015). A change in probe orientation of 10˚ can reduce the measured shear elastic modulus in muscles (Maïsetti et al., 2012) and the angle of the probe from the muscle fibre direction should not be over 20° (Miyamoto et al., 2015). The transfer of this to tendon assessment is that transducers should be placed longitudinal to the fibres of the structure being assessed to ensure the most accurate results (Brandenburg et al., 2014).

Machines utilising SWE technology may provide measures of stiffness using different values (i.e. Young's modulus or SWV), however as the assumptions of tissue density required to convert SWV to Young's modulus (Hoskins, 2012; Eby et al., 2013) may not always be correct, raw values of SWV (m/s) will be obtained in this research. The typical velocity of shear waves through a soft tissue is between 1-10 m/s (Benson and Fan, 2012) with many authors recently reporting stiffness using SWV in m/s (Kwak & Kim 2014; Youk et al. 2014; Aubry et al. 2015; Dirrichs et al. 2016; Rosskopf et al. 2016). It would be beneficial for stiffness measures within the literature to be standardised to enable clearer results and easier comparison between studies as SWE could potentially be used for many clinical and research related benefits in many different scenarios. In conclusion, SWE can non-invasively, easily and quickly provide a real-time assessment of the mechanical properties of a tendon alongside conventional US imaging and therefore could become a preferred imaging technique. The clinical applications of SWE could include the diagnosis of injury or tendinopathy development as well as being used to manage appropriate rehabilitation (Klauser et al., 2014).

2.2 Aim of Thesis

The aim of this thesis is to add to the growing body of research surrounding SWE and AT imaging by establishing the most appropriate methodology to be used with SWE when imaging the AT and developing a set of guidelines for best practice. To apply

SWE in the clinical setting, SWE will be utilised alongside other commonly used clinical outcome measures to monitor the effectiveness of an EcEx rehabilitation protocol being used as a treatment modality for ATY.

2.2.1 Research questions arising from literature review

The literature review forming this thesis has identified a number of areas for investigation that remain outstanding. It is understood that SWE is still an emerging technique in the musculoskeletal (MSK) imaging setting and therefore, there are a lack of studies establishing the impact of extraneous variable on the measures SWE obtains in the AT *in vivo.* Developments have been made in the research that has shown that SWE is a valid (Eby et al., 2013) and shows significant correlation with traditional tensile testing (Haen et al., 2017). SWE has been utilised to assess the impact of an acute bout of eccentric exercise and to monitor the stiffness of healing ATs following surgical repair of a rupture (Zhang et al., 2016; Leung, Chu and Lai, 2017). Despite these promising recent additions, many gaps still remain in the literature which this thesis has tried to address. Little progression has been made with regards to confirming the reproducibility of SWE measures taken on the AT *in vivo* over time and assessing the impact of certain extraneous variable to the measure SWE obtains. Refinements in the methodology of SWE may augment its use in the clinical setting. Studies are also required to assess the effectiveness of SWE in the clinical setting as a method to monitor a pathological AT over the course of a rehabilitation programme and trace alterations in AT stiffness over time.

2.2.2 Proposed research studies and hypotheses

The following research questions and hypotheses are proposed for this thesis.

Title: Reproducibility of compression and shear wave elastography measures of Achilles tendon stiffness *in vivo*.

Aim: To measure the intra- and inter-rater reliability of two types of elastography technology, Compression Elastography (CE) and Shear Wave Elastography (SWE).

Hypothesis: Stiffness measures obtained in the AT *in vivo* using CE and SWE would remain consistent and be reproducible over different measures and days as well as when used by different operators.

Title: Shear Wave elastography measures of the Achilles tendon: Influence of time of day and leg dominance.
Aim: To assess whether AT stiffness is dependent on time of day different between dominant and non-dominant standing leg.
Hypothesis: Stiffness measures obtained in the AT *in vivo* using SWE will alter throughout the course of a normal day.

Title: The impact of an acute 30 minute bout of running on measures of Achilles tendon stiffness using shear wave elastography.
Aim: To assess whether a 30-minute acute bout of running led to significant alterations in the stiffness of the Achilles tendon (AT) *in vivo* as measured using SWE.
Hypothesis: On a smaller scale to the known adaptations of long-term running, an acute bout of running was hypothesised to increase the measured shear wave velocity (SWV) in the AT.

Title: The effects of a 12 week eccentric exercise programme on clinical outcome measures and Achilles tendon stiffness measured by shear wave elastography.
Aim: To examine any alterations in AT stiffness and clinical outcome measures in a symptomatic, pathological AT as well as the healthy contra-lateral AT (HATs) and assess the timeline over which changes occurred.
Hypothesis: The 12-week eccentric exercise programme will result in improvements in clinical outcome measures and an increase in AT stiffness as measured with SWE.

Title: Alterations within the Achilles tendon following cessation and resumption of an eccentric exercise programme: Impact on clinical outcome measures and tendon stiffness measured with shear wave elastography.
Aim: To trace any alterations in AT stiffness (measured using SWE), measures of symptoms (VISA-A) and clinical outcome measures including tendon max AP diameter and Doppler score occur when the regular loading of a 12 week EcEx programme is removed and once participants restarted the EcEx programme. The

study will also have monitored progress for up to 6 months to see if the recorded values in pathological tendons became closer to those achieved in the contralateral healthy ATs.

Hypothesis: AT stiffness, symptom measures and clinical outcome measures would not alter significantly at the end of the 12 week period but would continue to improve with further involvement in the EcEx programme.

3 General Methods

This chapter describes the materials and common methods used in the series of studies described in the subsequent experimental chapters. All experiments were conducted within the physiotherapy gymnasium of Sportswise, Sussex Centre for Sport and Exercise Medicine based at the University of Brighton.

Where experimental chapters used additional or alternative methods, appropriate descriptions of those methods are detailed within the corresponding methods section of that chapter.

3.1. Health and Safety

All studies were approved by the University of Brighton Ethics and Governance committee (tier 2) and conducted in accordance with all guidelines of the revised declaration of Helsinki 1974.

For each study, participants were provided with an information sheet which detailed the study design, requirements of the participants, and any risks or benefits to participation. Participants were invited and encouraged to ask any questions regarding the study, before they consented to undertaking the research. All participants were informed that they could withdraw from any study at any time without providing justification or explanation. Both verbal and written informed consent, together with a recent medical history questionnaire were obtained from each participant prior to the start of any testing. An example of an informed consent and medical questionnaire used throughout the experimental chapters of this thesis can be found in Appendix A & Appendix B.

All studies were carried out in line with the University of Brighton risk assessment guidelines. All apparatus used was cleaned before and after use and any biological material and waste were handled and disposed of in line with relevant University and/or Sportswise guidelines.

3.2. Participants and recruitment

For each study, the participants' height (cm), weight (kg), age (years), sex (m/f), Achilles tendon (AT) length (mm) and maximum anterior-posterior (max AP) diameter (mm) was recorded prior to testing, with the results recorded on a data

collection sheet. Any other measurements taken pertaining to and specific to each study, for example shear wave velocity (SWV) were also documented on the data collection sheets. All participants filled out a Victorian Institute of Sports Assessment-Achilles (VISA-A) questionnaire (Robinson et al., 2001). Questionnaires and data collection sheets were kept in a locked filing cabinet in a key coded room to maintain confidentiality.

Recruitment occurred through word of mouth, email contact and poster display. Potential participants were asked to contact the researcher for further information if they were interested in being a participant in the study. When contact was made, the researcher gave each participant the participant information sheet and collected the initial information outlined above if they wished to be considered for inclusion in a study. At this point, all potential participants were asked to fill out a VISA-A questionnaire to see if they met the initial inclusion criteria. As many of the investigations in this thesis were aimed at establishing the methodology to be used with shear wave elastography (SWE), it was deemed appropriate to begin with using asymptomatic, healthy participants to establish normative values. For this reason, the inclusion criteria for the initial studies of the thesis (Chapters 4, 5 & 6) was set as both males and females over the age of 18 years old who achieved a minimum score of 96/100 on the VISA-A to rule out subjectively symptomatic tendons. For the last two experimental chapters (chapters 7 & 8), participants with symptomatic Achilles tendinopathy (ATY) were required and recruitment of these participants was achieved using the Sportswise Centre for Sport and Exercise Medicine clinic. Participants were contacted if they had previously presented to the clinic with ATY and those presenting at clinic during the course of the study were also invited to be part of the study.

Exclusion criteria for chapters 4, 5 & 6 included previous diagnosis of ATY, history of pain in the AT area lasting for more than 24 hours, pregnancy, previous medical or surgical intervention on the AT or abnormal features consistent with ATY on conventional B-mode US. The B-mode images were independently reviewed by an experienced sports medicine doctor, with more than 15 years' experience in musculoskeletal US, to exclude any abnormal tendons. For chapters 7 & 8, exclusion criteria were pregnancy, recent (within the last 3 months) surgical intervention of the AT and those taking fluoroquinolone antibiotics.

To control for any possible influence of prior or additional exercise, participants were asked to avoid any exercise above and beyond that required for their day to day actions during the 48hrs preceding testing and throughout the duration of the study. This excluded any exercise required as part of the study. This was based on the recommendations of previous research suggesting a rest period is required before imaging to minimise the effect of activity related variation in elastography measures (Ooi et al., 2015).

Participants were also asked to not consume alcohol or caffeine in the 24 hours prior to the studies to reduce the impact of these as extraneous variables.

3.3. Anthropometry

Anthropometric data were collected from each participant. The height of each participant was recorded using a SECA 220 stadiometer (SECA UK, Birmingham, UK) and the result recorded in centimetres (cm) to the nearest 0.5cm. Body mass was measured using AE Adam GFK150 scales, precise to 0.01 kg (AE Adam, Milton Keynes, UK) and recorded in kilograms (kg).

3.4. Self-reported data

All participants were asked to provide their date of birth and to record their sex as either male or female. Participants were also asked to fill out questionnaires, including VISA-A and VAS.

3.4.1. VISA-A (Victorian Institute of Sports Assessment - Achilles)

Inclusion criteria for the studies required participants to complete a VISA-A questionnaire. The first time the participants completed the questionnaire, the questions were all explained to them and the form was completed in the presence of the investigator. At subsequent sessions, the participants were asked if they remembered the form and to ask the lead investigator if they had any questions.

The VISA-A questionnaire was developed in 2001 to measure the severity of ATY symptoms and allows a participant to subjectively rate their symptoms (Iversen, Bartels and Langberg, 2012). It is quick to administer, requires no specialist equipment and has been shown to be both valid and reliable (Robinson et al., 2001). The questionnaire contains a total of eight questions that cover the aspects of pain (questions 1-3), function (questions 4-6), and activity (questions 7 & 8). The first

seven questions are scored out of 10, question 8 carries a maximum score of 30 and the points awarded for the answers given to the specific questions combine to give a total out of 100. A score of 0 denotes no activity and maximum pain and a score of 100 denotes the highest and a subjectively perfect score of maximum activity and no pain (de Vos et al., 2012). The VISA-A allows patients to subjectively rate their symptoms, providing the clinician with a measure of symptom severity. A study of over 1300 individuals indicated that overall mean VISA-A scores in a population ranged from 24 (severe AT) to 100 (completely asymptomatic) with 'healthy' subjects scoring a minimum of 96 (Iversen, Bartels and Langberg, 2012). An improvement on the VISA-A score of 10 points has been shown to be considered clinically significant (Roos et al., 2004; Beyer et al., 2015).

A full copy of a VISA-A questionnaire used during the experimental chapters of this thesis can be found in Appendix C.

3.4.2. Visual Analogue Scale

A pain Visual Analogue Scale (VAS) as shown in Figure 3.1 was used in chapters 7 & 8 for participants to subjectively rate and monitor the pain in their AT in different conditions on a scale of 0 to 10. A score of 0 represented no pain and a score of 10 represented the worst possible, unbearable, excruciating pain. The participants were asked to rate their pain on the VAS score under four separate conditions which included sitting down, when walking, first thing in the morning and when they are performing their eccentric exercise programme. As each question was marked out of 10 points and four questions were used, the VAS score used in the final studies was measured out of a total of 40 points. The VAS scale has been used in similar research regarding the AT (Boesen et al., 2006; Chester et al., 2008; Jonsson et al., 2008; Verrall, Schofield and Brustad, 2011).

The first time any participant completed a VAS scale, the scale was fully explained to them by the lead investigator. At subsequent sessions, the participants were asked if they remembered the form and were asked to talk to the main investigator if they had any questions regarding the VAS.

A copy of the full VAS scale used in the experimental chapters of this thesis can be found in Appendix D.

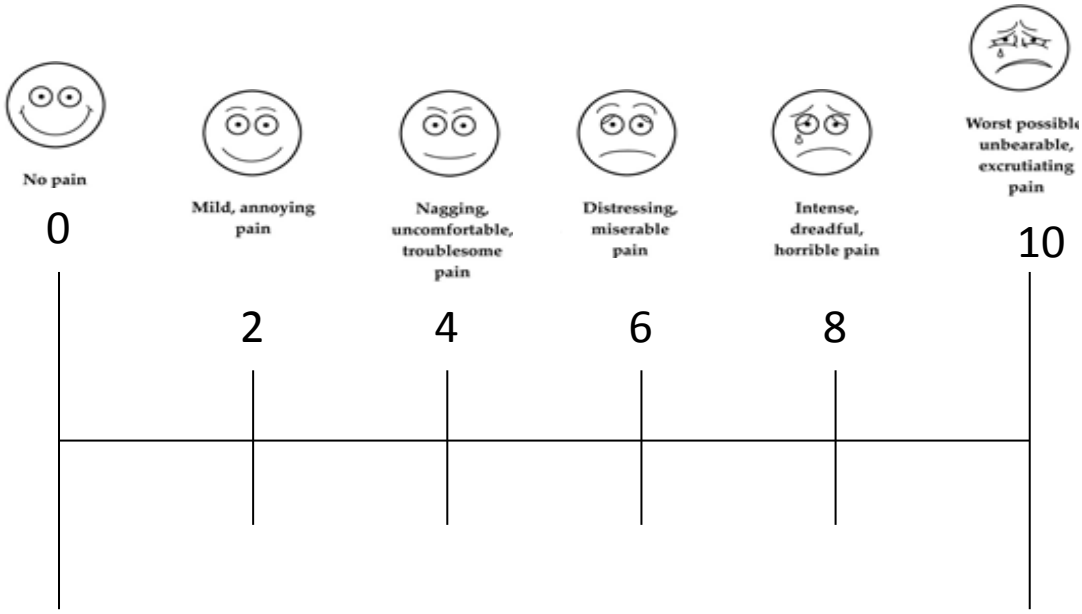


Figure 3.1: VAS scale

** Figure 3.1 as used within rehabilitation experimental studies (chapters 7 & 8)*

3.5. Ultrasound Scanning Protocol

For all US scanning sessions, measures were taken with a Siemens ACUSON S3000™ HELX EVOLUTION US System (Siemens Medical Solutions, Mountain View, CA, USA). Participants were asked to lay prone on an examination table with both feet hanging over the end of the table, as used in other published research examining ATs with elastography (Arda et al. 2011; Aubry et al. 2013; Chen et al. 2013; Brandenburg et al. 2014; DeWall et al. 2014). An appropriate amount of US gel was applied to the AT to be examined, sufficient to maintain contact between the probe and the participant and to ensure good US imaging.

Care was taken when obtaining a scan that the probe was held perpendicular to the tendon so as to avoid anisotropy and tissue shifting (Klauser, Faschingbauer and Jaschke, 2010) and any downwards pressure onto the skin by the probe was kept to a minimum and used only to maintain contact with the skin (Klauser, Faschingbauer and Jaschke, 2010).

3.5.1. Free Achilles tendon length

B-mode US was used to image an extended field of view image, termed a 'SieScape' image by the manufacturer, an example of which is shown in Figure 3.2. This 'Siescape' image was obtained using a 14L5SP probe to allow visualization of the entire ‘free’ AT length, which was recorded in millimetres (mm). The AT (A) is shown in Figure 3.2 between the solid white lines with length measured between the insertion of the AT at the calcaneus (B), to the lowest fibres of soleus (C), as shown by the dotted white lines on the B-mode US image**.** Three consecutive SieScape images were taken to measure length, and a mean average of the three measures taken to represent ‘free’ AT length. The ‘mid-point’ of the tendon (D) was calculated as half of free AT length, located and marked on participants skin following calcaneus palpation. This mark was used for all subsequent measures in both longitudinal and transverse planes, to ensure all measures were taken at the relative mid-point of the tendon for each participant.

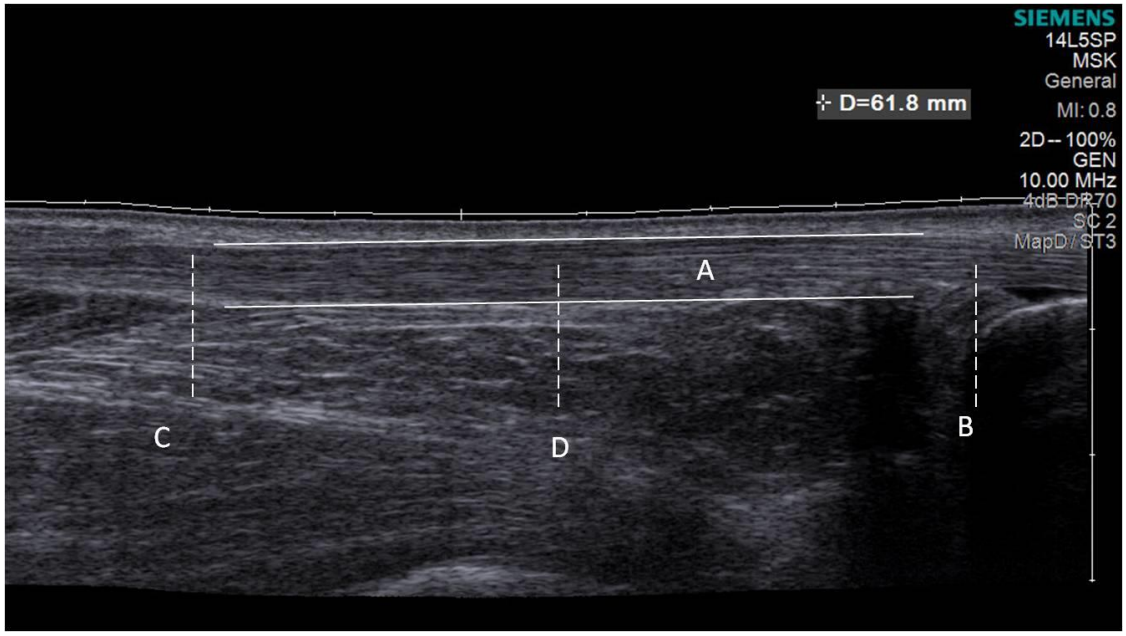


Figure 3.2: B-Mode Ultrasound Extended field of view image

** Figure 3.2 is taken from the right AT of a 26-year-old female participant. The Achilles tendon (A) is shown between the solid white lines. AT length (shown by the dotted white line) was measured as the length between the insertion of the AT at the calcaneus (B) and the lowest fibres of the soleus muscle (C). The mid-point of the tendon (D) is also shown.*

3.5.2. Maximum tendon anterior-posterior diameter

B-mode US imaging was used to image maximum anterior-posterior (max AP) diameter of the AT using a 14L5 probe. A transverse image of the AT at the tendon mid-point (and the widest point of the tendon in studies 7 and 8) was captured and the US software used to measure the maximum distance in millimetres (mm) from the anterior border of the tendon to the posterior border of the tendon as shown in Figure 3.3. As used in previous research (Beyer et al., 2015), three consecutive measures were taken, ensuring all measures fell within 0.5mm of each other, with the mean of the three measurements taken to represent max AP diameter.

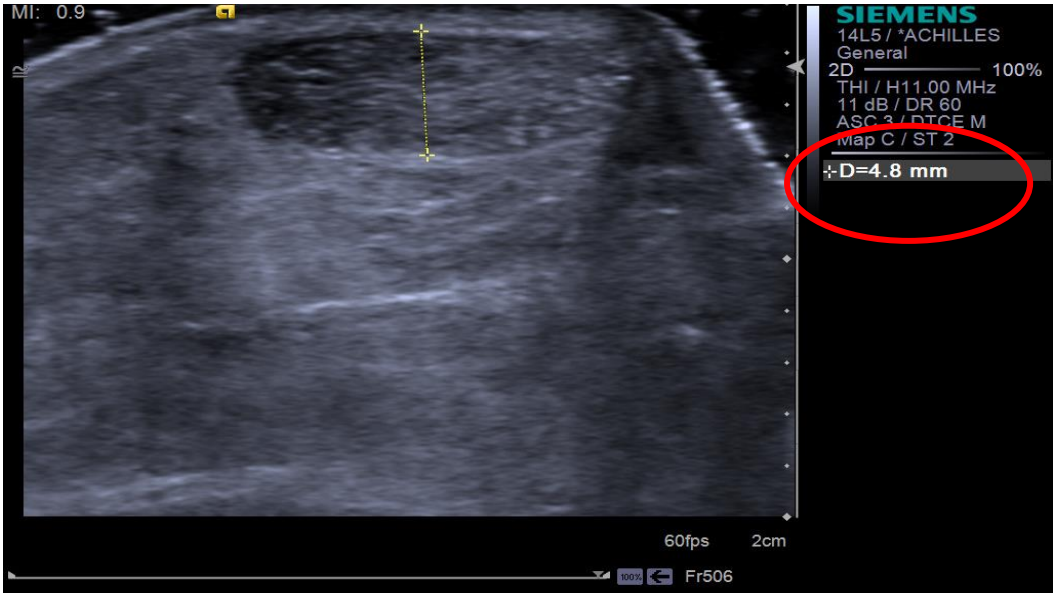


Figure 3.3: Transverse B-Mode ultrasound image of a left Achilles tendon

** Figure 3.3 taken from a 68-year-old female participant. The dotted yellow line denotes the maximum anterior-posterior diameter of the AT with the actual recorded diameter in millimetres (mm) appearing to the right-hand side of the image in the red circle.*

3.5.3. Power Doppler Scan

A Power Doppler (PD) signal measure was taken from each participant at each testing session in chapter 7 and 8 to identify hyperaemic neovasculature (Watson et al., 2018). Previous research suggests that with regards to quantifying neovascularisation, PD is more sensitive than colour Doppler as PD can identify all flow, regardless of velocity (Drew et al., 2014). PD readings are designed to detect orthogonal blood flow in human tissue and as the blood supply to the AT is small, in an asymptomatic, healthy

AT, no blood flow is seen (Rees, Stride and Scott, 2014). The in-growth of neo-vessels within the tendon is a widely recognised hallmark of pathology (Watson et al., 2018) and will therefore be assessed along with other measures of pathology. PD assessment was quantified and scored using the Modified Ohberg Score (MOS) which has been shown to have excellent inter- and intra-rater reliability in the Sport and Exercise Medicine setting (Sengkerij et al. 2009; Watson et al. 2018). A quantification of neovascularization score determined with PD is a widely used method of assessing the severity of ATY (Sengkerij et al. 2009) and each scan will be given a rating based on a previously established rating MOS (Ohberg and Alfredson, 2002) as shown in Table 3.1.

Table 3.1: 5-point Modified Ohberg Score

Modified Ohberg Score	
Score	Description
0	No Vessels
1 +	1 vessel anterior to the Achilles
2 +	1 – 2 vessels throughout the tendon
3 +	3 vessels throughout the tendon
4 +	4 or more vessels throughout the tendon

During PD assessment, care was taken to assess tendon when relaxed with as little transducer pressure as possible to avoid artificially obliterating small vessels (Reiter et al., 2004) and affecting visible vessels. A PD output is shown in Figure 3.4.

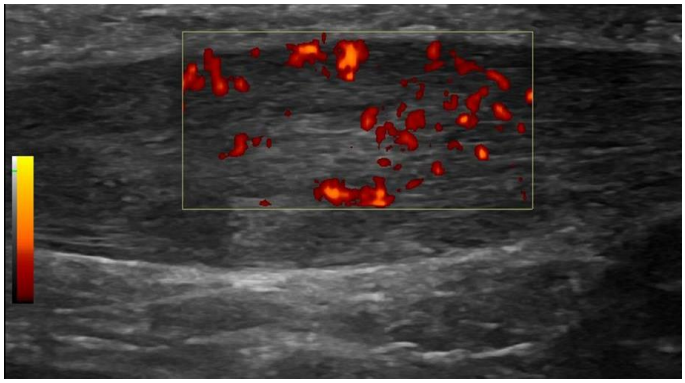


Figure 3.4: Example output from Doppler signal measure

**Figure 3.4 was scored using the MOS as 4+.*

3.5.4. Compression Elastography Scanning

During each compression (strain) elastography (CE) measure, elastograms were taken at the mid-point of the tendon and strain information was calculated at the same Region of Interest's (ROI's) every scan, placed in the same order. Ten ROI's were placed along the length of the tendon, starting proximally and working distally in the longitudinal plane as shown in Figure 3.5.

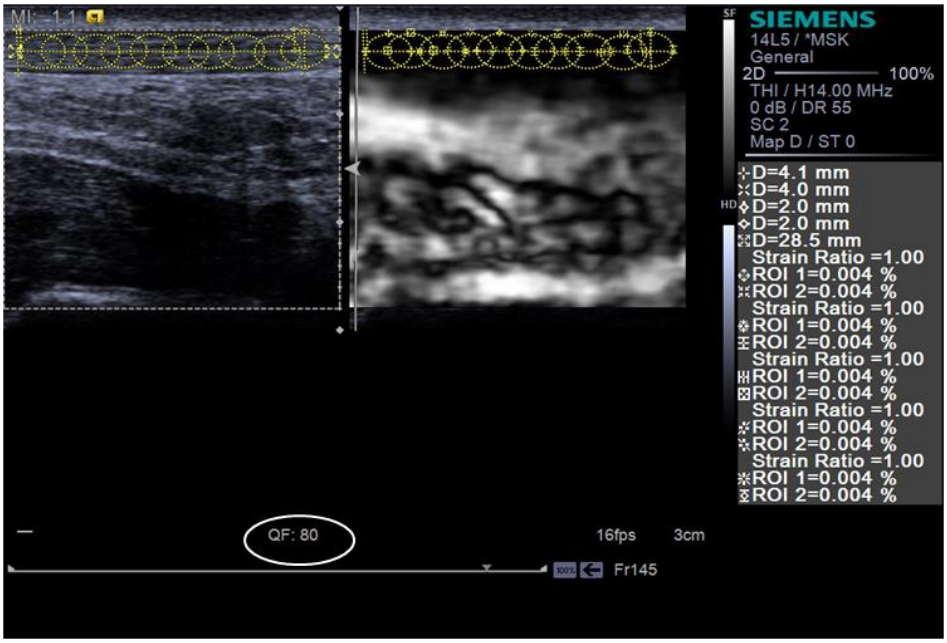


Figure 3.5: Longitudinal elastogram of a right Achilles tendon

**Figure 3.5 was taken from a 30-year-old male participant. The 10 Regions of Interest (ROI's) used to collect CE data in the longitudinal plane are shown by the yellow dotted circles. The corresponding values for strain are shown in the box at the right-hand side of the image.*

Four ROI's were used within transverse measures, placed at the same location and in the same order each time as shown in Figure 3.6.

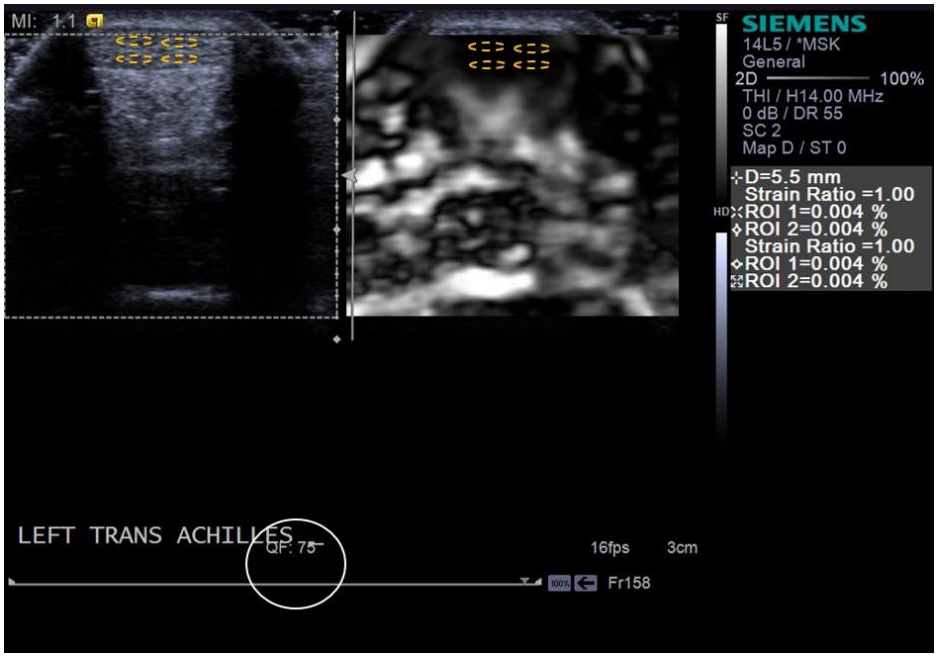


Figure 3.6: Transverse elastogram of the right Achilles tendon

***** *Figure 3.6 taken from a 30-year-old male participant. The 4 Regions of Interest (ROI's) used to collect CE data when imaging in the transverse plane are shown by the yellow dotted circles. The corresponding values for strain are shown in the box on the right-hand side of the image.*

The term 'strain ratio' is a ratio calculated from the percentage (%) displacement values for each ROI. As the ratio figure was derived from the raw data, it was deemed inappropriate to use the ratio value for statistical analysis and instead, the raw data scores for % displacement were used for all statistical analysis. This figure represents a measurement of the % value of displacement (true strain) of the pixels within the given ROI.

Image quality was closely monitored throughout CE scanning; tissue compression was avoided during examination and quality factors (QF) noted. QF's can be seen in Figure 3.5 and 3.6 circled in white at the bottom of the image. To ensure the best possible images were used for analysis, CE software provides information on the quality of the image with each image given a QF (Palle et al. 2011) indicating the amount of motion artefact compared to a reference frame (Calvete et al., 2013). A QF above 60 has been used to indicate an image of good quality (Wu et al., 2011), with some suggesting a quality factor between 50-100 represents minimal motion artefact (Calvete et al., 2013). To ensure a consistent standard of image, a minimum QF of at

least 75 was set for studies, observed for at least 5 consecutive frames (Calvete et al., 2013) for image quality standardisation.

3.5.5. Shear Wave Elastography Scanning

Following initial measures, the system was placed into Virtual Touch Imaging Quantification ® (Siemens Medical Solution, Mountain View, CA, USA) (VTIQ) mode. The VTIQ mode used in the Siemens ACUSON S3000™ HELX EVOLUTION US System (Siemens Medical Solutions, Mountain View, CA, USA) model is an acoustic radiation force based method that produces both qualitative and quantitative maps of shear wave velocities ranging between 0.5 and 10.0 m/s (Doherty et al., 2013; Ianculescu et al., 2014). There is a saturation limit associated with the S3000 which means any SWV above 10 m/s cannot be accurately measured by the technology. Therefore, any ROI's where the SWV is above 10 m/s will simply provide a reading of 'High'. As the exact SWV in this ROI cannot be measured, the SWV from that ROI will be discounted from analysis.

Unless stated otherwise in the specific methodology section of each study, 10 set size and shape Regions of Interest (ROI) were placed in the same order on longitudinal elastograms, at a standardised depth of 0.5cm running along the length of the tendon, starting proximally and working distally as shown in Figure 3.7. The ROI's were placed in locations that are fully within the boundaries of the tendon.

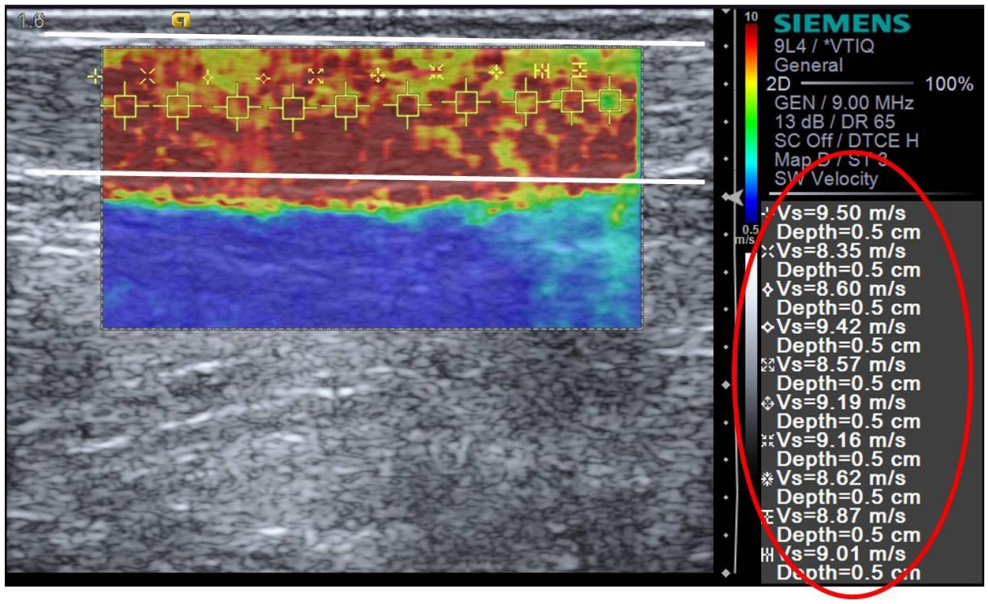


Figure 3.7: Longitudinal shear wave elastogram of a right Achilles tendon

***** *Figure 3.7 was taken from a 21-year-old male participant. This image shows 10 Regions of Interest (ROI's) used to collect SWE data with the corresponding SWE values in m/s shown to the right of the image and highlighted in the red circle.*

Four ROI's were used for transverse scans, placed in the same order, at the same locations using standardized depths of 0.3cm and 0.4 cm as shown in Figure 3.8.

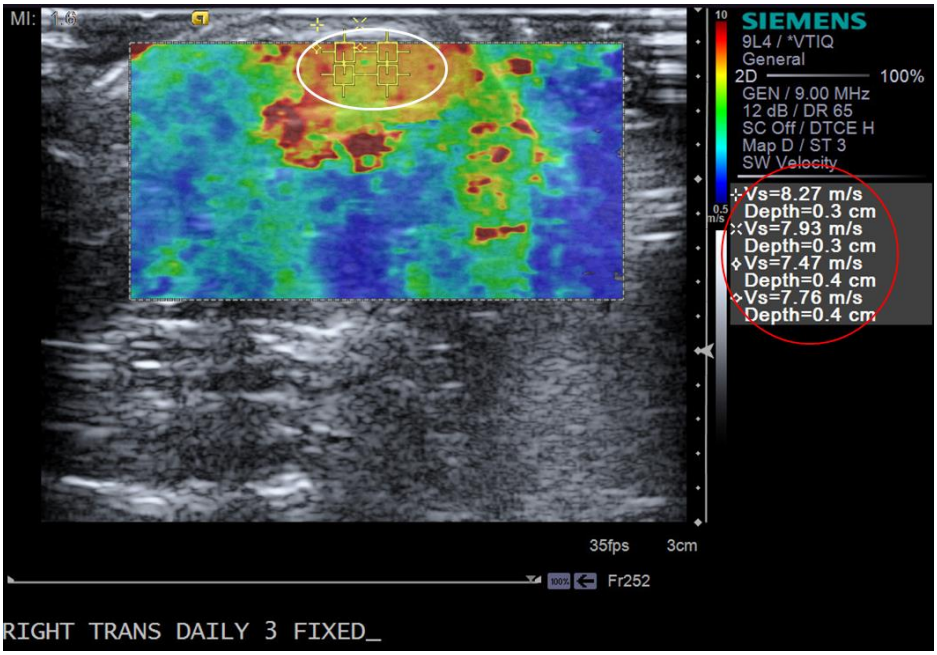


Figure 3.8: Transverse elastogram of a right Achilles tendon

***** *Figure 3.8 was taken from a 30-year-old male participant. The boundary of the Achilles tendon is denoted by the white circle. The 4 ROI's used to collect SWE data when imaging in the transverse plane image are shown by yellow squares with the corresponding SWV values in m/s highlighted in the red circle.*

Image quality was closely monitored throughout examination, and as in previous research, during examination, the transducer was placed over US gel and effort was made to avoid any tissue compression by the transducer (Wu et al., 2012; Siu et al., 2016). Information on the technical aspect of SWE is still lacking, and as tissue compression is postulated to affect the measure (Kot et al., 2012), tissue compression is simply avoided during measurements. The technical reference information in the user manual supplied by the manufacturer state that minimal-to-mild compression (of approximately 5-15%) will not cause significant bias on the measurements as measured using elasticity phantoms. The user manual states that appropriate amount of scanning pressure is an important factor to consider in achieving reliable and repeatable results, therefore minimal-to-mild compression should be used when using SWE.

To aid definition of image quality, quality maps in VTIQ mode were assessed to ensure images conformed to a high level of quality. The technical information in the user manual supplied by the manufacturer note that the quality maps confirm shear

wave formation was adequate and therefore a reliable SWV measure can be obtained. The quality maps are therefore determined by the elastography software and are useful in determining if the magnitude of the generated shear wave was sufficient and with an appropriate signal:noise ratio to enable the software to estimate SWV (Benson and Fan, 2012). The quality maps therefore reflect the level of displacement caused by the shear waves and the amount of 'noise' present in the shear wave signal. A quality map noted as good is a representation that the technology was able to detect the peak displacement within the tissue caused by the shear waves. The quality maps are colour coded for easy visual definition with green representing a good quality, a high signal:noise ratio and represent locations in the image where SWV estimations are reliable. When compared to the QF used in CE, SWE requires a QF of at least 75 to even register an image, so an all green quality map would indicate a QF of approximately 95. Marginal quality will be shown in yellow and poor quality shown in red (Ianculescu et al., 2014) (See key in Figure 3.9). The quality level in the images used in this study were standardized to use only images whose map were all green in colour to show a high level of quality indicating reliable SWV measurements can be obtained as shown in Figure 3.9.

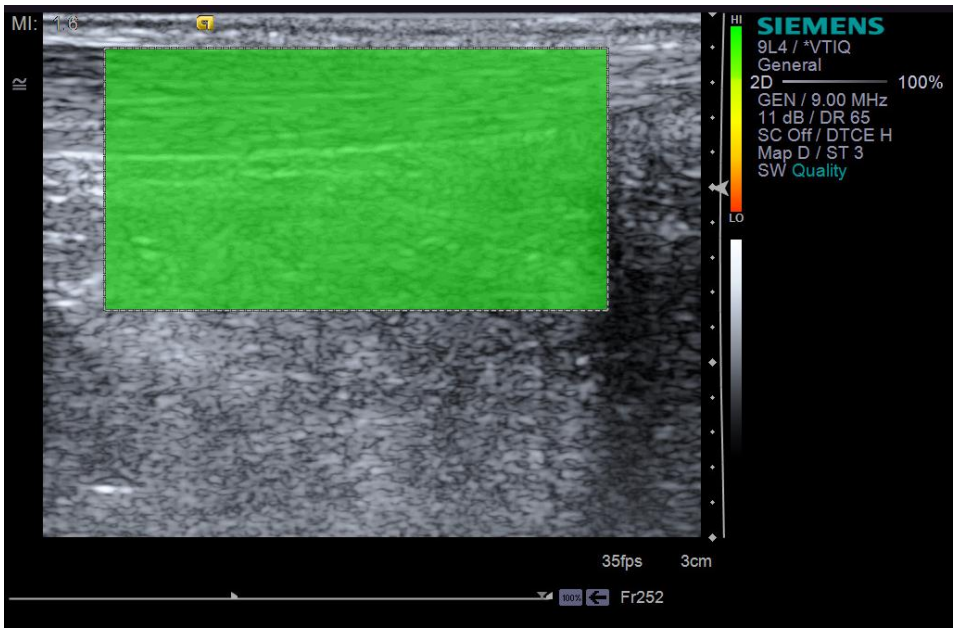


Figure 3.9: SWE Quality Map

** Figure 3.9 was taken from a 68-year-old female participant. Note the all green colour map and the key indicating a high level of quality on the right-hand side of the image*

3.6. Statistical Analysis

All data was analysed using the latest accessible version of SPSS version 20.0-22.0 (International Business Machines Corp., Armonk, New York), and reported as mean and standard deviations. Statistical significance was accepted at the α level of $p \leq 0.05$.

Prior to data collection commencing, the number of participants required to successfully complete the study was calculated using G*Power v3.1 based on conventional α and β levels of (0.05) and (0.20) respectively.

All data were first checked for normality and sphericity and adjusted if necessary using the Huynh-Feldt method. Should data be found to fit the assumptions of normality, appropriate difference testing was carried out using t-tests or ANOVA's. Appropriate non-parametric tests to assess difference were carried out on any data that did not meet the assumptions of normality.

To provide a robust analysis of reliability, it is recommended that both relative and absolute reliability statistics be calculated (Atkinson and Nevill, 1998). As such, a range of statistics were included within the first experimental chapter (chapter 4) discussing reliability. The tests for absolute reliability included typical error of the measurement (TE) calculated as TE = SD(diff)/√2 with SD referring to standard deviation (Hopkins, 2000). Standard error of the measurement (SEM) was calculated as SD * $\sqrt{1 - ICC}$ and minimal detectable change (MDC) was calculated as SEM * 1.96 * $\sqrt{2}$ (Hopkins, 2000). Pearson's correlation coefficient (r) was used to show relative reliability and ANOVA used to assess differences. Examining the results of these tests made it possible to demonstrate whether a technique has good reliability, which would be shown by a low TE, high r scores and no statistically significant differences between the measures.

Further detail of the specific analysis used in each study can be found in the statistical analysis section of each experimental chapter.

4 Reproducibility of compression and shear wave elastography measures of Achilles tendon stiffness in vivo.

4.1 Abstract

Measuring the mechanical properties of a tendon *in vivo* non-invasively is now possible due to the introduction of US elastography. As this tool remains in its infancy in musculoskeletal assessment, the effects of anatomical variables such as foot position during scanning, or the reproducibility of the technology and its methodology are not yet known. Therefore, the aim of this study was to measure the intra- and inter-rater reliability of two types of this technology, Compression Elastography (CE) and Shear Wave Elastography (SWE). This study will also measure the effects of foot position on the values obtained. Eight participants (4 males, 4 females; mean age 25.5 ± 2.5 years, height 174 ± 12 cm) underwent CE measures and 14 participants (7 males, 7 females; mean age 26.5 ± 3.8 years, height 172 ± 11cm) underwent SWE measures to determine AT stiffness. Data were collected with the foot relaxed and fixed at 90°. For both CE and SWE, five consecutive measures were taken in a 1hr period and 1 measure was taken at the same time of day for 5 consecutive days. Data obtained from CE had a coefficient of variation (CV) of >53%, weak correlations between the CE measures (r; range 0.01-0.25) and poor intraclass correlations (ICC; range 0.00-0.11). In contrast, measures of stiffness obtained with SWE had a CV ranging from 2.9% - 6.3%, medium correlations (range 0.4-0.7) and good to excellent ICC (range 0.54-0.85). Significant differences in shear wave velocity (SWV) values were noted between foot positions for longitudinal scanning ($p = <0.05$), with a relaxed foot position providing faster SWV values than a fixed position in both 1hr measures (0.42 m/s = 4.61%) and daily measures (0.52 m/s = 5.75%). ICC between operators was 0.70 for transverse and 0.80 for longitudinal scanning. In summary, CE had a low level of reproducibility and appears unsuitable for measuring the mechanical properties of the AT. In contrast, SWE produced reproducible measures over a 1hr period as well as a period of 5 consecutive days. Less variable measures were obtained from longitudinal scans in comparison to transverse and results were less variable when taken with a relaxed foot position as opposed to a fixed position. SWE also has a high level of agreement between operators implying that different operators can obtain similar measures, important for clinical usage. In conclusion, SWE is a reproducible technique for quantitatively assessing the mechanical properties of the human AT *in vivo*.

4.2 Introduction

The Achilles tendon (AT) is the strongest tendon in the human body (Pang and Ying, 2006), however, as primary plantar flexor of the ankle it experiences high levels of repeated stress and can frequently suffer debilitating and difficult to treat injuries (Wren et al., 2001; Milgrom et al., 2003). Despite the frequency and severity of AT injuries, primary cause and optimal rehabilitation regimes to treat them remain unclear (Murtaugh and Ihm, 2013). The most commonly used method to assess the AT is B-mode US imaging, however, to the detriment of both diagnosis and treatment it is unable to assess tendon mechanical properties. Tendon mechanical properties, including stiffness, alter alongside pathological change and healing (Rasmussen, 2000; Horton, 2013) and elastography technology may quickly and non-invasively assess tissue stiffness (Ophir et al., 1991) *in vivo.* B-mode US is capable of measuring alterations within the tendon of clinical significance such as hypoechoic areas and fibre misalignment, but elastography may be useful in identifying alterations in tendon matrix before they are seen with B-mode imaging (Horton, 2013; Sconfienza et al., 2013).

Elastography is similar in principle to palpation, which manually and subjectively assesses tissue stiffness. A force is applied to the tissue (stress) and the movement as a result of the stress (strain) is measured (Sarvazyan et al., 2011). There are differing types of US elastography (Sarvazyan et al., 2011), with compression (strain) elastography (CE) and shear wave elastography (SWE) being the most common commercially available types (Hoskins, 2012). Timely imaging of tendon stiffness via elastography, could highlight areas of degeneration at an early stage, informing injury prevention and treatment (Horton, 2013; Ooi et al., 2013). Elastography could also monitor positive changes during rehabilitation as a tendon heals, allowing improved assessment of rehabilitation protocols (Brum et al., 2014). Lastly, with further studies required to assess real time measures during load bearing, it could potentially provide clinicians with additional information regarding the internal health and quality of the tendon and its ability to resist deformation and withstand load when considering return to work, activity or sport.

Despite elastography software becoming more widespread across US systems in recent years (Sarvazyan et al., 2011), its application within the field of musculoskeletal imaging remains in its relative infancy. Many of the feasibility studies assessing elastography were completed within the last five years (Ooi et al., 2013; Ahn et al., 2014; Porta et al., 2014; Itoigawa et al., 2015; Yamamoto et al., 2015), and it is not yet commonly used in the clinical setting. Most of the available literature has been conducted with CE, which provides a measure of the strain within the tissue from which an indication of tissue stiffness can be subsequently derived. Inter-class correlation coefficients have been reported of ICC = 0.49 (Ahn et al., 2014), ICC = 0.66 (Porta et al., 2014), ICC = 0.61 (Muraki et al., 2015) and higher intraclass correlation coefficients of between ICC = 0.61 – 0.93 (Porta et al., 2014; Muraki et al., 2015). Despite these positive findings, the above data were obtained from the patellar tendon, lateral epicondyle and supraspinatus and not the AT. CE has so far been more widely used than other elastography techniques such as SWE to measure the mechanical properties of the AT *in vivo*, yet of these studies, some report no reliability data (De Zordo et al. 2009; Palle et al. 2011; Tan et al. 2012), some use cadavers (Klauser et al., 2013) and others report ICCs ranging between ICC = 0.41 – 78 (Drakonaki, Allen and Wilson, 2009), but these values were obtained using retrocalcaneal fat as a reference for strain ratio calculations. However, it was shown that Kager's fat pad in this instance was in-homogenously displaced against the rigid calcaneal bone, therefore under light pressure, the deeper fat pad was displaced less than the more superficial tendon giving rise to variable and inconsistent elastography patterns (Drakonaki, Allen and Wilson, 2009). Better measures of intra-observer reliability (ICC = 0.87 & 0.91) with CE has more recently been shown for AT assessment when external reference materials of a known Young's modulus are used as a reference standard (Yamamoto et al., 2015; Schneebeli et al., 2016), but large gaps remain in the reliability literature regarding reproducibility and the impact of activity and lifestyle on the data.

Many of the studies using CE to assess the AT have only tested participants on one occasion (Wu et al., 2011; Chino et al., 2012; Porta et al., 2014), therefore reproducibility over consecutive measures and days remains unknown. In a clinical and sports medicine research setting, reliability refers to the absence of error and reliable data collection is critically important (Atkinson and Nevill, 1998) with a high

level of both inter- and intra-observer reliability are prerequisites for diagnostically relevant measures. The ability of CE to assess tendon properties robustly has been questioned as CE software only offers a qualitative or semi-quantitative value (strain ratio) and no absolute values are obtained. The technique of CE appears to be operator dependant and qualitative, therefore potentially unsuitable for objectively measuring tissue stiffness or comparing to data from other laboratories (Arda et al., 2011; Treece et al., 2011). More recently, articles have started to assess the impact of various alterations to methodology including assessing subjects on the same day with the lower limb muscles either relaxed or under contraction (Schneebeli et al., 2016). Schneebli et al. (2016) reported reliability of the strain ratio as ICC = 0.87 in the relaxed condition and ICC = 0.94 in the contracted state. Others have assessed intra-rater reliability over four consecutive measures and inter-rater reliability from two examiners (ICC = 0.61) (Yamamoto et al., 2015). It is worthy to note that these positive results from both studies were obtained using an external reference material.

The method of SWE may prove more useful for assessing mechanical properties as it is independent of user skill (Chen et al., 2013), and validated against traditional tensile testing for both muscle (Eby et al., 2013) and tendon (Haen et al., 2017). SWE provides quantitative measures of shear wave velocity (SWV) by tracking the velocity of shear wave propagation which is used as a surrogate for stiffness (Siemens, 2008; Hoskins, 2012). SWV is faster through harder in comparison to softer tissue (Hoskins, 2012), and shear wave propagation rate is dependent on the shear modulus of the tissue, which is linearly proportional to its Young's modulus (Garra, 2011). Therefore, quantification of tissue stiffness should be possible by measuring SWV. Little has been reported on the reliability of SWE and of the available studies, many assess one single measure (Arda et al. 2011; Cosgrove et al. 2012; Kot et al. 2012; Aubry et al. 2013; Chang et al. 2013; Chen et al. 2013; DeWall et al. 2014), or two testing sessions (Ferraioli et al. 2012; Peltz et al. 2013). No research has assessed reproducibility over more than two sessions, a prerequisite to obtaining a clinically reliable measure and a factor important to assess when considering progression of disease or response to therapy and prognosis.

Tendon stiffness may be affected by factors such as exercise, as there may be physiological changes to the tendon (Boesen et al., 2006), as any elastic structure will alter its measured stiffness with use. Some studies have suggested that exercise results in a significant reduction in normal tendon structure (Rosengarten et al., 2015), however other studies using SWE have not controlled for activity to date. Low levels of repeatability when using both CE and SWE have been attributed to potential tendon and transducer movement during examination (Drakonaki et al. 2009; Peltz et al. 2013), suggestive of the fact that fixing the tendon or foot in some way may increase repeatability. SWE studies assessing the AT have utilised a relaxed foot position when scanning so as to avoid tendon stress (Drakonaki, Allen and Wilson, 2009; Chen et al., 2013) with no studies comparing this to a fixed foot position. No studies have compared CE and SWE, whilst controlling for previous activity or foot position. It is important to assess individual day to day variation in elastography results gained from the AT of a healthy population, before assessing pathological change, as distinction between normal daily variation and pathological change should be well defined prior to either technology being utilised in clinical settings.

The aim of this study was to assess the reproducibility of CE to measure strain and SWE to depict stiffness in the human AT *in vivo*. The results from CE and SWE over consecutive measures, consecutive days and different foot positions will be compared whilst controlling for prior activity. It was hypothesised that stiffness measures obtained in the AT *in vivo* using CE and SWE would remain consistent and be reproducible over different measures and days as well as when used by different operators.

4.3 Materials and Methods

4.3.1 Participants

Eight healthy volunteers (4 males, 4 females; mean age 25.5 ± 2.5 years, height 174 ± 12cm) underwent CE measures and fourteen healthy volunteers (7 males, 7 females; mean age 26.5 ± 3.8 years, mean height 172 ± 11cm) underwent SWE measures. All participants were recruited from the University of Brighton where the research was conducted. All participants provided verbal and written informed consent to participate in the study and all procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national

research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, as detailed in the General Methods (section 3.1). Inclusion criteria were set out in accordance with those detailed in General Methods (section 3.2). No recruited volunteers were excluded from the study.

4.3.2 Materials and Methods

Participants were asked to identify their dominant leg by indicating which foot they would kick a ball with (Pang and Ying, 2006). Data was collected in two testing blocks in a randomised order. In one testing block, participants attended once and during that visit, had five consecutive CE measurements taken over a one-hour period (1hr Measures). During this 1hr period, participants continued lying prone on the examination couch keeping movement to a minimum. In the other testing block, the same participants attended at the same time of day, every day for five consecutive days. On each day, one measure of CE and SWE was taken (Daily Measures). Participants were asked to maintain typical daily walking activity but to refrain from any additional exercise above walking for 48 hours before the first visit and throughout the whole testing period.

4.3.3 Scanning techniques

All measures were taken with a Siemens ACUSON S2000™ HELX EVOLUTION US System (Siemens Medical Solutions, USA) in both longitudinal and transverse planes on the dominant AT following the procedures outlined in the General Methods (section 3.5). For both CE and SWE measures elastograms were obtained with the foot in two different positions, i) a relaxed position, and ii) both feet fixed at a 90° position to minimize movement. The participant's feet were fixed using a custom-made strap wrapped around the back of both feet and secured to the examination table. The strap was tightened until a 90° ankle joint position was achieved in the dominant ankle, measured by goniometry with the fulcrum of the goniometer placed on the lateral malleolus, one arm along the longitudinal axis of the fibula and the other arm parallel to the fifth metatarsal bone (Mahieu et al. 2008; DeWall et al. 2014). Once the correct ankle position was achieved the strap was fixed.

4.3.4 Conventional Ultrasound Technique

Measures of AT length were made using B-mode US using a 14L5SP probe and maximum anterior-posterior (Max AP) diameter measures were made using a 14L5 probe, following the procedures outlined in General Methods (sections 3.5.1 & 3.5.2).

4.3.5 Compression elastography technique

During each measure both longitudinal and transverse plane elastograms were taken using a 9L4 probe at the mid-point of the tendon and strain information calculated at the same Region of Interest (ROI's) each scan, following the procedures outlined in the General Methods (section 3.5.4).

4.3.6 Shear wave elastography technique

Following conventional US, the system was placed into Virtual Touch IQ (VTIQ) mode, an acoustic radiation force based method that produces both qualitative and quantitative maps of SWV ranging between 0.5 and 10.0 m/s (Doherty et al., 2013; Ianculescu et al., 2014). Images were obtained by the same operator (CP) using a linear-array 9L4 transducer probe using ten set size and shape ROI's following the procedure outlined in the General Methods (section 3.5.5). Image quality was closely monitored throughout examination as outlined in section 3.5.5 of the General Methods.

4.3.7 Statistical Analysis

All statistical analysis was performed using the latest available version of SPSS version 20 - 22 (SPSS, Chicago, Illinois). Data are presented as means ± SD. Distribution of groups was analysed using the Shapiro-Wilk test. A battery of reliability measures were employed to assess the SWE data due to them all examining slightly different areas of the data, providing a more comprehensive assessment than one measure alone. Coefficient of Variation (CV) and Pearson correlation analysis (r) were calculated for each combination of testing protocol (Daily or 1hr), foot position (fixed or relaxed) and averaged over time (Measure 1, 2, 3, 4, & 5). Intra-class correlation coefficient (ICC) was calculated to determine inter- and intra-rater reliability, ICC was calculated for each combination of measures and averaged. A threshold of ≤12% for CV measures was used as an acceptable level of reproducibility (Chino et al., 2012). The following scale was used for correlations: 0 = no correlation, 0.1-0.3 = weak, 0.4 – 0.6 = moderate, >0.7 = strong and 1 = perfect (Dancey and

Reidy, 2004). The following scale was used to quantify ICC results: 0.00 – 0.20 = Poor, 0.20 – 0.40 = Fair, 0.40 – 0.75 = Good, >0.75 = Excellent (Drakonaki, Allen and Wilson, 2009; Chino et al., 2012). CV was measured as SD/Mean, TE was calculated as SD Diff / $\sqrt{2}$, standard error of measurement (SEM) was calculated as SD * $\sqrt{1}$ − ICC and minimal detectable change (MDC) was calculated as SEM * 1.96 * $\sqrt{2}$ (Hopkins, 2000). Differences between foot position, time and protocol were assessed by ANOVA, data was checked for sphericity and the Huynh-Feldt Correction applied if necessary. Statistical significance was defined as an alpha level of $p<0.05$. Actioned

4.4 Results

4.4.1 Compression Elastography

SD and CV scores for measurements of AT length and max AP diameter obtained using the CE software are shown in Table 4.1.

Table 4.1: Analysis of AT length and diameter measurements (mm)

AT Length (mm)						
	1hr			Daily		
Participant	Ave	SD	CV	Ave	SD	CV
1	35.0	1.6	4.5%	33.6	0.8	2.5%
2	58.4	1.0	1.7%	58.5	0.6	1.1%
3	39.6	1.2	3.1%	39.6	1.2	2.9%
4	45.4	0.7	1.6%	45.3	0.5	1.2%
5	55.6	0.5	0.9%	55.5	0.6	1.0%
6	68.8	0.8	1.2%	68.6	0.7	1.0%
7	30.4	0.7	2.3%	30.8	0.6	1.9%
8	54.0	0.5	0.9%	54.6	0.8	1.4%
Max AP Diameter (mm)						
	1hr			Daily		
Participant	Ave	SD	CV	Ave	SD	CV
1	5.0	0.1	1.7%	4.9	0.1	1.1%
2	4.4	0.1	1.2%	4.4	0.1	1.2%
3	4.8	0.1	1.8%	4.8	0.1	1.2%
4	4.6	0.1	1.3%	4.6	0.0	1.0%
5	5.7	0.1	1.0%	5.7	0.0	0.8%
6	5.4	0.1	1.9%	5.4	0.1	1.0%
7	4.1	0.1	2.4%	4.1	0.1	1.3%
8	4.6	0.0	1.0%	4.6	0.0	1.0%

** Table 4.1 includes averages, standard deviations and coefficient of variation taken from each participant over each differing testing protocol.*

For each participant, CE data was collected from 10 ROI's in the longitudinal plane and four ROI's in the transverse plane. The data from each participant was averaged to provide one value from each measurement session. Scores for Mean ± SD, CV, R and ICC for the various CE testing procedures are shown in Table 4.2. The CV's for the CE variables were all above 53%, indicating large differences between measurements. Correlation values indicate no correlation to weak correlations between the measures ($r = 0.01 – 0.25$; $p = 0.38 - 0.60$), and ICC values are all classified in the 'poor' category (ICC = 0.00 – 0.11), again indicating poor reliability.

Some negative ICC values were observed, however as it has been said that negative ICC's have no theoretical legitimacy and should not be quoted (Giraudeau, 1996) these values were treated as zero.

Table 4.2: Analysis of CE data from each testing protocol.

	Mean± SD	CV	R	ICC
Longitudinal Fixed 1hr	0.010 ± 0.011%	111.5%	0.25 (p = 0.49)	0.11
Longitudinal Fixed Daily	0.014 ± 0.013%	92.8%	0.04 (p = 0.60)	0.01
Longitudinal Relaxed 1hr	0.007 ± 0.008%	105.7%	0.13 (p = 0.53)	0.06
Longitudinal Relaxed Daily	0.009 ± 0.007%	80.8%	0.07 (p = 0.38)	0.10
Transverse Fixed 1hr	0.010 ± 0.011%	112.4%	-0.06 (p = 0.49)	0.00
Transverse Fixed Daily	0.010 ± 0.008%	80.4%	0.03 (p = 0.38)	0.02
Transverse Relaxed 1hr	0.007 ± 0.004%	53.6%	-0.04 (p = 0.50)	0.00
Transverse Relaxed Daily	0.008 ± 0.005%	60.9%	0.01 (p = 0.42)	0.11

4.4.2 Shear Wave Elastography

The results obtained from the various SWE testing procedures are shown in Table 4.3. The CV's for all measured variables were considered acceptably low (<12%) ranging from 2.9% - 6.3%. Correlations between measures ranged from r = 0.4 - 0.7 (p = 0.005 - 0.19), all considered moderate to strong. ICC's were all considered good or excellent (range; ICC = 0.54 - 0.85). Full results are shown in Table 4.3.

Differences were noted between the data collected from transverse and longitudinal scans with longitudinal scans revealing faster mean SWV values than transverse scans as seen in Table 4.3. Further difference testing was completed on longitudinal and transverse data separately with the results discussed in section 4.5.

Table 4.3: Full analysis on SWE results

	Daily Measures				1hr Measures			
	Longitudinal		Transverse		Longitudinal		Transverse	
	Fixed	Relaxed	Fixed	Relaxed	Fixed	Relaxed	Fixed	Relaxed
Mean SWV (m/s) ± SD	9.04 ± 0.44	9.56 ± 0.27	7.96 ± 0.31	7.93 ± 0.48	9.12 ± 0.47	9.54 ± 0.29	7.98 ± 0.35	7.91 ± 0.50
SEMean	0.12	0.07	0.08	0.13	0.13	0.08	0.09	0.13
CV	4.9%	2.9%	3.9%	6.0%	5.2%	3.1%	4.4%	6.3%
R	0.6	0.4	0.5	0.6	0.5	0.4	0.6	0.7
ICC	0.71	0.54	0.62	0.71	0.67	0.55	0.78	0.85
TE	0.31	0.20	0.14	0.25	0.33	0.23	0.15	0.19
SEMeasure	0.23	0.19	0.19	0.26	0.27	0.20	0.17	0.19
MDC	0.65	0.51	0.53	0.71	0.76	0.55	0.46	0.53

** Table 4.3 shows results for all participants over all protocol and foot position combinations.*

No significant differences for either the longitudinal data or the transverse data with respect to protocol or time were found. No significant differences were apparent in SWV between measures collected over 1hour and those collected over 5 days. Results did not differ significantly over time (Measure 1, 2, 3, 4 and 5). No significant differences were found for transverse data between a fixed and relaxed foot position, indicating foot position during transverse scanning does not significantly influence results. A significant difference was shown to exist between a fixed and relaxed foot position for longitudinal scanning ($p = <0.05$) resulting in $\eta_p^2 = 0.75$ ($p = 0.003$), defined as a moderate effect size (Cohen, 1988). With a relaxed foot position, longitudinal scans produce less variable results than transverse scans concluded from the data in Table 4.3. This indicates lower values of SD, SEmean, CV, TE, SEmeasure & MDC values.

Additional analysis on the CE data was carried out on the data with regards to the CV's obtained for all conditions. The CV's in the longitudinal scans were re-calculated for each participant using all 10 ROI's and just the middle four (i.e. ROI 4, 5, 6 & 7). The difference in CV between using 10 ROI's and four ROI's ranged from 0.77 – 2.74%, therefore whether using 10 ROI's in longitudinal scans, or just four ROI's, they are likely to provide similar results. The data was also revisited and after

selecting one random day for each participant, the same number of data points were compared from longitudinal and transverse scans. In longitudinal scans, data points from four ROI's (ROI 4, 5, 6, & 7) were compared against the four ROI's taken in the transverse scans. The average CV for the transverse data was calculated at 42% vs 20% in the longitudinal data when just four data points were analysed, indicating the increased amount of ROI's in the longitudinal scans may result in an increased CV result.

A second operator scanned three participants and ICC assessed agreement in the measures between the two separate operators. The result for transverse scanning was ICC=0.70 (95% confidence intervals (CI) 0.03 - 0.91), classed as good. ICC = 0.80 (95% CI 0.62 – 0.89) for longitudinal scanning, classed as excellent.

4.5 Discussion

In clinical practice, the ability to form a real-time, objective and quantitative assessment of AT stiffness *in vivo* using a fast, easily available and inexpensive system may prove useful for diagnosis and treatment. Obtaining quantitative data, to be used as a clinical reference point, and providing objective assessment will ensure robust diagnosis. This chapter assessed the reproducibility of both CE and SWE in assessing measures of stiffness within the human AT *in vivo* using two different foot positions. Our hypotheses were that stiffness measures obtained with CE and SWE from the AT *in vivo* should remain consistent across measures and days and be reproducible between operators. The data revealed that CE measures had a low level of reproducibility across all measures, however in contrast, SWE measures were robust and consistent showing good levels of reproducibility over measures and days as well as between operators.

Measures of AT length and max-AP diameter calculated from the conventional US aspect of the machine indicated a high level of reliability (CV = 0.8-4.5%). This was expected given the high reliability of US imaging when used to assess the anatomical properties of the AT (Kharate and Chance-Larsen, 2012) and a previously noted high ICC for AT diameter measures (ICC = 0.68) (Ying et al., 2003). If it were possible to have the benefits of B-mode US (speed & ease of measure) linked to a reliable measure of stiffness, the possibilities to increase our current level of understanding of tendons and tendinopathy also increase.

The calculated CV's for the CE variables were all over 53%, and a threshold of ≤12% for CV measures was used as an acceptable level of reproducibility (Chino et al., 2012). The correlation and ICC values were all in the weak or poor categories, all demonstrating very low levels of reproducibility when CE is used to assess strain data (and hence measures of stiffness) of the human AT *in vivo*. In contrast, for SWE the CV's were all acceptably low (2.9% - 6.3%), correlations (between the averaged raw SWV data points obtained from each scan) were considered moderate to strong and ICC's were good or excellent. This demonstrates that SWE is more suitable for clinical assessment due to the lower level of error associated with its obtained measures. There were no significant differences in either longitudinal or transverse data in relation to protocol, suggesting no differences between measures taken over 1hr and those taken over five days. Therefore, SWE not only has good repeatability, but also good reproducibility as well. The implication of this is that similar SWE measures can be obtained from healthy, asymptomatic AT's whether taken consecutively or over a period of five days when activity and foot position has been carefully controlled for. When using SWE in the clinical setting with patients, similar standardised protocols should be implemented.

CE scanning took place using two different foot positions, feet relaxed, and feet fixed at 90°. Although both foot positions produced poor data as seen in the results section, the results were less variable when the foot was relaxed (see table 4.1). Scanning with CE using a relaxed foot position is common, as it avoids tendon stress (De Zordo et al. 2009; Chen et al. 2013), however Peltz et al. (2013) attribute low levels of repeatability with CE to movement of both the tendon and participant during scanning. This current study suggests that fixing the foot with the ankle at 90° does not improve reproducibility. Peltz et al. (2013) reported an ICC value which was averaged over right and left sides and considered "moderate" at ICC=0.42. It is worthy to note that the ICC obtained from Peltz et al. (2013) purely from the right AT (as used in this current study) was reported as poor (ICC = 0.17), a finding not dissimilar to that found in this study. Maffulli et al. (1999) reported a discrepancy in AT ruptures between right and left AT's which was attributed to a higher prevalence of right-handed individuals using the left leg to 'push off' from. This may potentially have had an impact on the discrepancy in ICC findings reported by Peltz et al. (2013).

A difference was noted in SWV values between longitudinal and transverse scans, with transverse scans returning significantly slower SWV values than longitudinal scans as seen in table 4.3 in the results section. The results of this study suggest that longitudinal scans provided a lower level of variance in the SWE results gained, shown by longitudinal scans having marginally higher r and ICC scores. This is a finding in agreement with previous research that has shown CE reproducibility to be higher in longitudinal planes (Drakonaki, Allen and Wilson, 2009). This increased reproducibility has been attributable to transverse scans being more prone to inhomogeneous compression towards the edges of the probe (Klauser et al., 2014) and waves propagating along fibres more easily than they do across them (Eby et al., 2013). When assessing CV values for CE data however, CV results across all conditions for transverse scans (CV = 76.8%) were lower than longitudinal scans (CV = 97.7%). A potential reason for this lower CV in transverse scans may be an inherent problem with sampling error between longitudinal and transverse scans as the amount of tissue included per measurement is less with transverse scans. When using 10 ROI's in a longitudinal scan, it is possible that although the subjects are asymptomatic, they have within their AT, an asymptomatic area of lower stiffness. This theory was supported by the further analysis of CV data that indicated a much lower CV in longitudinal scans when just four ROI's were used and could imply an increase in the likelihood of finding an area of differing stiffness by using more ROI's.

Other CE research reports high inter- and intra-rater reliability and a confirmed ability to measure absolute muscle hardness (Wu et al., 2011; Chino et al., 2012). Inter-rater reliability was reported as ICC = 0.77 and intra-rater reliability as ICC = 0.82 by Wu et al. (2011), whereas Chino et al. (2012) reported inter-rater reliability as ICC = 0.89 and intra-rater reliability as ICC = 0.89 & ICC = 0.77 for 2 operators. Despite these findings, Wu et al. (2011) and Chino et al. (2012) assessed muscle and plantar fascia and not tendon. They also did not assess the reproducibility of measures obtained over consecutive days. Sconfienza et al. (2010) also demonstrated high levels of intra-observer (ICC = 1) and inter-observer (ICC = 0.89) reliability. Despite these findings, reproducibility over consecutive days or measures was not assessed and reliability was assessed using actual strain data, but by the prevailing colour of the elastograms. Previous research into the use of CE specifically on the AT, has shown low reproducibility and that the technique is heavily operator dependant (Arda et al.,

2011). Some describe CE as a purely qualitative technique and unsuitable for measuring tissue stiffness (Treece et al., 2011). The results of this study agree with the conclusions of Arda et al. (2011) and Treece et al. (2011), suggesting CE has a low level of reproducibility for assessing the stiffness properties of the AT and is not suitable for producing measures over time to assess change. The magnitude of variation in the CE data from this study was too large to infer statistically, or clinically significant results to the wider population.

SWE research often reports results in Young's modulus (E), however for this study to do so would rely on conversion of SWV to E using the equation $E = 3pv^2$ where v is SWV and p is tissue density (Hoskins, 2012). This equation assumes tissue isotropy based on a constant tissue density of 1000 kg/m^3 which may not always be true for anisotropic tendon (Tanter and Pernot, 2010). Increased density can be a result of dehydration and decreased density a potential result of oedema, therefore reporting the values provided by the equipment and its software of SWV removes any problems in the clinical setting and does not require the measurement of factors that may potentially affect the conversion of SWV to E. Values in this study (and others (DeWall et al. 2014)), were therefore reported as SWV (m/s).

The effect of foot position was assessed to examine whether fixing the foot at 90° or allowing a relaxed foot position during SWE examination provided less variable results. No significant differences were shown for SWV in transverse scans between the two different foot positions. A significant difference was shown to exist between the two-foot positions for longitudinal scans with a fixed foot position providing slower SWV values of approximately 0.45m/s. As slower velocities relate to softer tissue, it is recommended that foot position remains consistent when comparing scans. Previous research has also shown a lower SWV (6.61 m/s) in maximal plantar flexion in comparison to a neutral ankle position (SWV = 15.75) in healthy, asymptomatic ATs (Aubry et al., 2015), however the authors do not offer any explanation as to the mechanisms behind this. In the longitudinal plane, the results obtained from a relaxed foot position were less variable, concluded from the data shown in table 4.3 in the results section. More repeatable results obtained from a relaxed foot position agrees with previous research (De Zordo et al. 2009) and provides support for imaging of the AT to be conducted in a resting position (DeWall et al. 2014). The correlation between SWE and tensile testing for reporting the biomechanical properties of the human AT

in vivo was also only demonstrated in the neutral position (Haen et al., 2017) and therefore the suggestion from the results of this study must be for SWE assessment to be carried out with the foot in a relaxed position.

The finding of transverse scans measuring significantly slower SWV values than longitudinal scans is a finding also in line with similar research (Arda et al., 2011; Aubry et al., 2015). With a relaxed foot position, transverse scans provided SWV values 1.63 ± 0.54m/s slower than longitudinal scans ($p = 0.000$) and with a fixed foot position transverse scans were 1.11 ± 0.62m/s slower than longitudinal ($p = 0.000$). The differences in SWV between transverse and longitudinal scans were likely due to shear waves propagating along the length of fibres more easily than across them (Eby et al., 2013; Aubry et al., 2015), resulting in faster SWV's from longitudinal scans. This should be interpreted with caution however, as there is an upper saturation limit associated with this system where SWV's above 10 m/s cannot be measured. Out of all the measures obtained in this current study, only two measures (2.5%) were returned as 'High', indicating that with a healthy, asymptomatic sample of AT's, reported values of 'High' are not common. Due to the significant differences between longitudinal and transverse scans, further examination was conducted and revealed less variable data (concluded from lower SD, SEMean, CV, SEMeasure and MDC) were obtained from longitudinal scans. Therefore, longitudinal scans of the free AT with the foot relaxed are recommended over transverse scans or fixing the foot.

Previous research has shown that SWE measures are not dependent on operator skill (Chen et al., 2013), however there is a lack of research into consistency of measures obtained by different operators using SWE during AT assessment. The ICC results in this study were good and excellent, in agreement with previous research (Chen et al., 2013). For intra-rater results across both 1hr and daily measures, for both longitudinal and transverse scanning, there were no significant differences noted in time, suggesting the five separate measures taken were not significantly different from one another. This low level of variance is suggestive that the measures obtained are stable and provides a high level of confidence that the measures obtained with SWE are consistent. This indicates there may be no significant differences in the mechanical properties of the free AT between days and agrees with the findings of previous research (Kongsgaard et al., 2011). The results of this study suggest that longitudinal measures of the free AT taken with the foot relaxed, provide an average MDC (of 1hr

and daily measures) of 0.53 m/s, implying that with this sample, a change in SWV should be above 0.53 m/s to be the minimal change detected as potentially clinically relevant and not just error. Whenever a measurement is taken, there is an element of error associated with it, with the total error of the measure being comprised of systematic bias (general trend for measurements to vary between repeated measures in a particular direction) and random error (i.e. inherent biological differences or a lack of control in the measures). The proposal of an MDC provides a benchmark for where change in SWV can be referred to as clinically meaningful and not just a product of the total error whether that be systematic bias or random error.

This chapter is the first study to directly compare CE and SWE findings in the AT of the same participants. It is also the first to assess the reproducibility of both techniques over this many measures and days as well as the first to assess the impact of changing foot position. As with all studies, it also carries some limitations, including the use of a small, homogenous population limiting the extrapolation of the results. Due to the extremely high level of variability in the results obtained for CE from only eight subjects, further study was not deemed justifiable. Despite this, a number of separate CE measures were made on each participant in this study, providing a total of 80 measures for analysis, equating to more or similar amounts of measures analysed in similar CE research (De Zordo et al. 2009; Drakonaki et al. 2009; Tan et al. 2012; Klauser et al. 2013). For SWE assessment each participant had 20 measures taken, totaling 280 measures included for analysis, a number much higher than comparable SWE research (Arda et al., 2011; Chen et al., 2013; Peltz et al., 2013). Other limitations include the use of a single operator for CE data (Obuchowski and Lieber, 2008). Although this study showed results for CE scanning were considered too variable, the results for SWE were very promising. This is a novel examination and provides an initial step in the assessment of SWE in the tendon imaging setting, therefore future research with larger cohorts should be undertaken.

SWE affords real-time measurements of SWV within the AT *in vivo*. When used as a surrogate measure of stiffness, SWE can inform tendinopathy development and patterns of rehabilitation from injury. In comparison to CE, SWE shows a much higher level of reliability for obtaining measurements of stiffness measures from the AT. Using SWE could lead to earlier diagnosis of degeneration and a screening tool for use with 'at risk' athletes on a regular basis to prevent tendon injuries progressing

to more chronic and potentially irreversible stage (Ooi et al., 2013). The application of SWE for detecting subtle positive changes in tendon stiffness associated with rehabilitation protocols should also be assessed in future studies, as being able to optimise current rehabilitation protocols by assessing their effectiveness on tendon stiffness would be of the upmost importance to clinicians and athletes when tasked with difficult decisions surrounding return to play.

In conclusion, this study is the first to control for previous activity and examine the reproducibility of both CE and SWE over five consecutive measures and days as well as assessing the impact of foot position. The results demonstrate the specific CE system utilised in this study can provide measures of AT length and diameter with a high level of reproducibility, but given the wide variation obtained in CE strain data it was concluded that CE has a low level of reproducibility and poor quantitative value for measuring the stiffness of the human AT *in vivo*. In contrast, SWE is a repeatable and reproducible technique for depicting and quantitatively assessing the mechanical properties of the human AT, *in vivo* and consistent results can be gained over different measures and days with high levels of agreement between different operators. This study offers novel findings in the use of SWE for AT assessment and suggests that longitudinal scans and a relaxed foot position offer the least variable results.

5 Shear Wave elastography measures of the Achilles tendon: Influence of time of day and leg dominance

5.1 Abstract

Shear wave elastography (SWE) is a reliable and valid imaging tool that can provide an estimate of tissue stiffness. It remains unknown however, whether stiffness values obtained with SWE from the Achilles tendon (AT) are dependent upon the time of day they are made and whether leg dominance affects results. This study assessed AT stiffness obtained with SWE taken in the morning compared to those taken in the afternoon and evening to assess whether time of day has a significant impact on SWE measures. The study also measured AT stiffness in the dominant and non-dominant legs to assess whether this property influenced measures in healthy asymptomatic AT's. Fifteen participants (9 males and 6 females; mean age 28 ± 4 years, height 176 ± 8cm and weight 71 ± 7 kg) with no reported symptoms of Achilles tendinopathy (ATY) had SWE measures taken on their dominant and non-dominant AT's at three separate time points in a day; 08:00h, 12:30h & 17:00h. There were no significant differences apparent in the SWE measures obtained from asymptomatic AT's over the three measured time points. There were no significant differences in AT length or maximum anterior-posterior diameter over the measured time points and no differences in AT stiffness between the dominant and non-dominant leg. Leg dominance does not affect AT stiffness obtained with SWE from asymptomatic ATs and AT stiffness does not change throughout a day. Leg dominance and timing of clinical appointments are unlikely to affect the interpretation of the results obtained from SWE scans.

5.2 Introduction

The mechanical properties of human tendons are likely to be influenced by factors known to affect elastic structures, including patterns of loading and unloading during the day. The scale and relevance of these variables to tendon structure and function remain relatively unclear (Joseph et al., 2012). In particular, there are few systematic reports on how the stiffness of a human tendon alters throughout the course of a day (Pearson and Onambele, 2006; Onambele-Pearson and Pearson, 2007). Periods of sleep, rest and activity over a day will cause altered loading and unloading at differing frequencies and intensities on tendons, which will impact their stiffness. This property of elastic structures becomes important when considering the phenomenon of morning stiffness in the Achilles tendon (AT). This is a common presentation of those suffering from symptomatic Achilles tendinopathy (ATY) and one that correlates well with the severity of the tendinopathy (Alfredson et al., 1998; Kader et al., 2002; Roos et al., 2004; Asplund and Best, 2013). When considering clinical diagnosis, it is most likely a clinician would measure tendon stiffness within normal clinic hours. Some research suggests tendon stiffness may decrease throughout the day (Onambele-Pearson and Pearson, 2007). It is important to understand whether alterations in tendon stiffness occur over the course of these hours and whether this may impact clinical measures or diagnostic decisions.

Despite the AT being very thick and strong (Maffulli, 1999; Pang and Ying, 2006), it is also one of the most frequently injured tendons in the body (Wren et al., 2001; Hess, 2009; Joseph et al., 2012). Although the precise mechanisms behind the aetiology of AT injury remain unclear (Morrissey et al., 2011), tendons operate efficiently within a characteristic elastic range, with work completed outside this range increasing injury risk (Onambele-Pearson and Pearson, 2007). *Ex vivo* work suggests the AT fails at stresses of approximately 100MPa (megapascal) (Kongsgaard and Aagaard, 2005) with most tendons experiencing peak stresses below 30 MPa. The AT however experiences peak stresses of nearer 70MPa, the increased injury rate seen in the AT attributed to these higher forces experienced within the AT (Schechtman and Bader, 1997; Kongsgaard and Aagaard, 2005). Direct measures of tendon stiffness involve measuring the original tendon length, applying a force to the tendon, measuring the corresponding force being put through it and the subsequent change in tendon length

as a direct result of this force (Hoskins, 2012). This data is used to plot a graph of the change in length divided by original length (strain) against the force applied per unit area (stress), the slope of the resulting graph provides a measure of elasticity or Young's modulus (E). In tendons, E describes the amount of longitudinal deformation (strain) occurring in response to the applied longitudinal force (stress) (Manduca et al., 2001) with the a steeper slope gradient representing a harder/stiffer tissue. The research of Pearson & Onambele. (2006) examined the effect of time of day on the patella tendon *in vivo*, with tendon stiffness estimated using an isokinetic dynamometer to measure force and B-mode US to measure length (Pearson and Onambele, 2006). This research measured a decrease in tendon stiffness of 20.2 ± 9.5% between the testing times of 08:00h and 18:00 (Pearson and Onambele, 2006). No research has yet assessed the impact of time of day on tendon stiffness measures obtained using shear wave elastography (SWE).

The relatively recent introduction of SWE offers a novel way to quantitatively assess tendon stiffness repeatedly and non-invasively. SWE measures the velocity of shear waves travelling through a tissue, providing information on the mechanical properties of the tissue and an estimate of its stiffness (Treece et al., 2011; Cosgrove et al., 2013). Shear waves travel faster through harder tissue and slower through softer tissue, therefore SWE measures provide information on tissue stiffness by initiating shear waves within the tissue and tracing their velocity (Hoskins, 2012). Although the traditional methods of calculating tendon stiffness can produce accurate results, they are time consuming, require complex procedures, a lot of equipment and space to run and are therefore not usually integrated into a clinical assessment. SWE can be used to provide quick, real-time, quantitative measures of the stiffness of a human tissue *in vivo* including tendons (Siemens, 2008; Hoskins, 2012) whilst avoiding the issues with other testing methods. This means it can be implemented alongside usual clinical US to provide real-time measures of AT stiffness.

Reported reliability and validity of SWE (Muraki et al., 2010; Eby et al., 2013; Miyamoto et al., 2015) appears to be good, but information on the scale of effects that many variables may influence SWE measurements remain unclear (Kot et al.,

2012). Research into structure and function of limbs indicates that it is necessary to understand the effects of limb dominance on AT stiffness (Siu et al., 2016), and clinicians need to know if time of day will cause significant differences in obtained results. Assessing the impact of these variables will be of importance to the clinical usefulness of SWE for diagnosis and prognosis. It would provide evidence on the size of effects of time of day (hence appointment time) and leg dominance and whether they should be considered when tracing the AT stiffness with SWE. The AT cross-sectional area of an individual's dominant leg may be larger by 4.9mm^2 than their non-dominant leg (Pang and Ying, 2006) and alterations in cross-sectional area may impact stiffness. One study found evidence of different mechanical properties in the AT between legs, attributed to different loading profiles of both legs during daily activity due to foot dominance (Bohm et al., 2015). Despite this, differences in AT stiffness with leg dominance has not yet been measured with SWE.

The aim of this study was twofold. Firstly, to measure AT stiffness in the morning (08:00h), afternoon (12:30h) and evening (17:00h) to assess any measurable differences dependent on time of day. Secondly, it examined SWE measures obtained in bi-lateral AT's, *in vivo*, to assess any measurable differences in AT stiffness between dominant and non-dominant standing leg AT's. The hypothesis is that based on previous research, the AT stiffness will alter throughout the day, and the results of this study will provide evidence towards best practice for use of SWE for assessing AT properties.

5.3 Materials and Methods

5.3.1 Participants

All participants provided written informed consent to participate in the study and all procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, as detailed in the General Methods (section 3.1). Fifteen healthy participants, (9 males, 6 females; mean age 28 ± 4 years, height 176 ± 8cm and weight 71 ± 7 kg) took part in the study. Inclusion and exclusion criteria were set in accordance with those outlined in the General Methods (section 3.2). Participants were asked to identify

which foot they would kick a ball with to determine foot dominance (Purcell et al., 2009; Wu et al., 2010; Keles et al., 2014). Measures were taken on both the dominant and contra-lateral leg to assess any differences.

5.3.2 Methods

Participants were measured by SWE three times throughout the course of a day. Firstly, between 08:00h - 08:30h, secondly between 12:30h - 13:00h and lastly between 17:00h - 17:30h. Participants were asked to maintain any daily walking activity they deemed normal but refrain from any exercise above that required for normal daily walking for 48hours before testing and during the testing period.

5.3.3 Scanning techniques

Participants lay prone on examination table and an appropriate amount of US gel to maintain constant contact between the probe and the leg was applied to both legs

5.3.4 Conventional Ultrasound technique

Measures of AT length (mm) were taken with a 14L5SP probe and measures of maximum anterior-posterior diameter (Max AP) were made using a 14L5 probe, as detailed in General Methods (sections 3.5.2 & 3.5.3).

5.3.5 Shear wave elastography technique

The Siemens ACUSON S3000™ was placed into Virtual Touch IQ (VTIQ) mode, capable of producing both qualitative and quantitative maps of shear wave velocity (SWV) (Doherty et al., 2013; Ianculescu et al., 2014). A 9L4 probe was then used to obtain shear wave elastograms in accordance with the procedure outlined in General Methods (section 3.5.5). Due to the saturation limit of the SWE technology as discussed in General Methods (section 3.5.5), a SWV value measured above 10m/s was labelled by the software as 'High'. Only 4 of these were observed throughout the course of the study and when they did occur, they were discounted from the study. Image quality was closely monitored as outlined in section 3.5.5 of General Methods. All elastograms were taken in a longitudinal plane with the foot in a relaxed foot position, as this provides the most reproducible measures with SWE (Payne et al. 2017).

5.3.6 Statistical Analysis

All statistical analysis was performed using SPSS version 22 (SPSS, Chicago, Illinois). Measurements of the participants' data were expressed as mean ± standard deviation. Distribution of groups was analysed using the Shapiro-Wilk test. A 1 Way RM ANOVA assessed whether any changes to SWV occurred over the three measured time points. A paired sample t-tests was used to examine whether measures taken on the dominant and non-dominant sides of the participants were significantly different from each other and a 2 sample t-test analysed whether any significant differences were apparent in the SWV obtained in male and female participants. Standard error of measurement (SEM) was calculated as SD * √1 – ICC and the minimal detectable change (MDC) was calculated as SEM * 1.96 * √2 (Hopkins, 2000). Data was checked for sphericity with the Huynh-Feldt Correction applied if necessary and alpha level was set at $p<0.05$ throughout.

5.4 Results

5.4.1 Achilles tendon length and maximum anterior-posterior diameter

The mean, SD and range of the measurements obtained for AT length and AT max AP diameter in both the dominant and non-dominant AT's over the three measured time points are shown in Table 5.1.

Table 5.1: Mean, SD's and range of AT length and AT max AP diameter

Time measured	Dominant or Non-Dominant AT	AT Length (mm)					AT max AP diameter (mm)			
		Mean	SD	Min	Max		Mean	SD	Min	Max
08:00h	**Dominant AT**	50.1	12.6	29.6	69.9		4.8	0.4	4.0	5.7
12:30h		50.2	12.1	30.0	70.1		4.9	0.4	4.1	5.7
17:00h		50.7	12.2	32.1	72.9		4.9	0.4	4.1	5.7
	Non- Dominant AT	Mean	SD	Min	Max		Mean	SD	Min	Max
08:00h		51.3	11.7	31.9	71.0		4.9	0.4	4.1	5.8
12:30h		51.8	11.4	32.4	72.4		5.0	0.4	4.2	5.8
17:00h		52.3	11.7	32.1	73.6		4.9	0.4	4.1	5.7

** Table 5.1 is calculated from measurements taken over the three measured time points for both dominant and non-dominant AT's.*

There were no significant differences shown to exist over the three measured time points for either AT length (p = 0.411) or max AP diameter (p = 0.286) in the dominant AT's or in the non-dominant AT's (p = 0.062 and p = 0.322 respectively).

5.4.2 Shear wave elastography measures

In relation to the effect of time of day on SWE measures, there were no significant differences (p > 0.05) over the three measured time points in the SWV within either the participants dominant AT (p = 0.094) or non-dominant AT (p = 0.143). The mean SWV measured in the dominant AT of both male (Mean = 9.61 ± 0.21; range 9.24 – 9.88 m/s) and female (Mean = 9.76 ± 0.23; range 9.31 – 9.92 m/s) participants were very similar (p = 0.203) indicating no significant differences between the SWE measures within the AT of male and female participants.

The means, SD and range of SWV measured in both dominant and non-dominant AT's are outlined in Table 5.2.

Table 5.2: Mean, SD's and range of SWV (m/s) measurements

		08:00h	12:30h	17:00h
Dominant AT	**Mean (m/s)**	9.67	9.46	9.53
	SD (m/s)	0.22	0.29	0.23
	Min (m/s)	9.24	8.93	8.93
	Max (m/s)	9.92	9.80	9.89
Non-Dominant AT	**Mean (m/s)**	9.57	9.38	9.53
	SD (m/s)	0.21	0.31	0.37
	Min (m/s)	9.25	8.83	8.88
	Max (m/s)	9.80	9.8	9.97

** Table 5.2 shows SWE measures obtained over the three measured time points.*

There were no significant differences in SWV measures within the dominant AT throughout the day, and any changes were small and not significantly different and are shown in Figure 5.1. There was a small reduction in mean SWV throughout the morning, between 08:00h and 12:30h of 0.20 m/s (-2.07%). Between the hours of 12:30h and 17:00h there was a small increase in measured SWV of 0.06 m/s (0.63%)

from the value recorded at 12:30h. Between 08:00h - 17:00h, the decrease in SWV was -0.14 m/s (-1.45%). None of these alterations were significant and all were within the calculated MDC value which was 1.03 m/s.

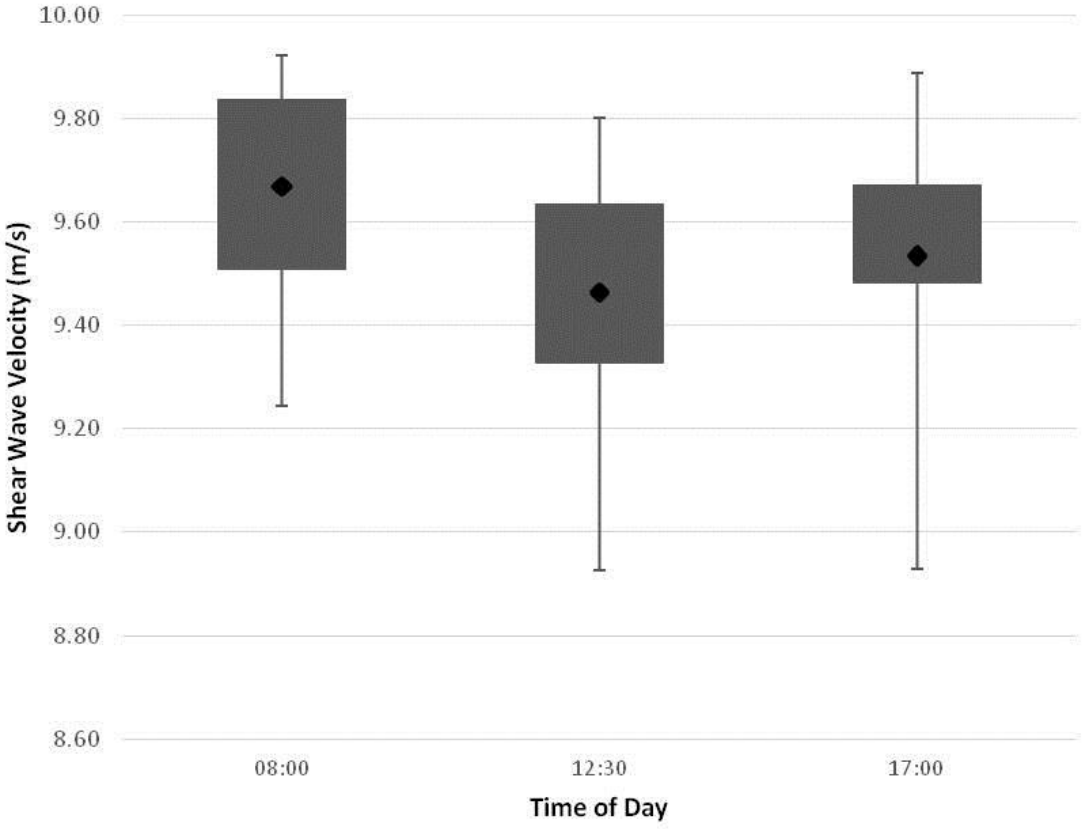


Figure 5.1: Group mean SWV (m/s) in the dominant AT measured at 08:00h, 12:30h and 17:00h.

The change over the time points of all individuals was also assessed by an individual trend analysis shown in Figure 5.2. The SWV in ten participants (66.6%) decreased between 08:00h and 12:30h by an average of -3.9%, but five participants (33.3%) experienced an increase in SWV although the scale of this was less at only 1.7%. When looking at the data between 08:00h and 17:00h, twelve participants experienced an overall decrease in SWV, by an average of -2.6%, yet three participants experienced an increase in SWV between these time points, by an average of 3.6%.

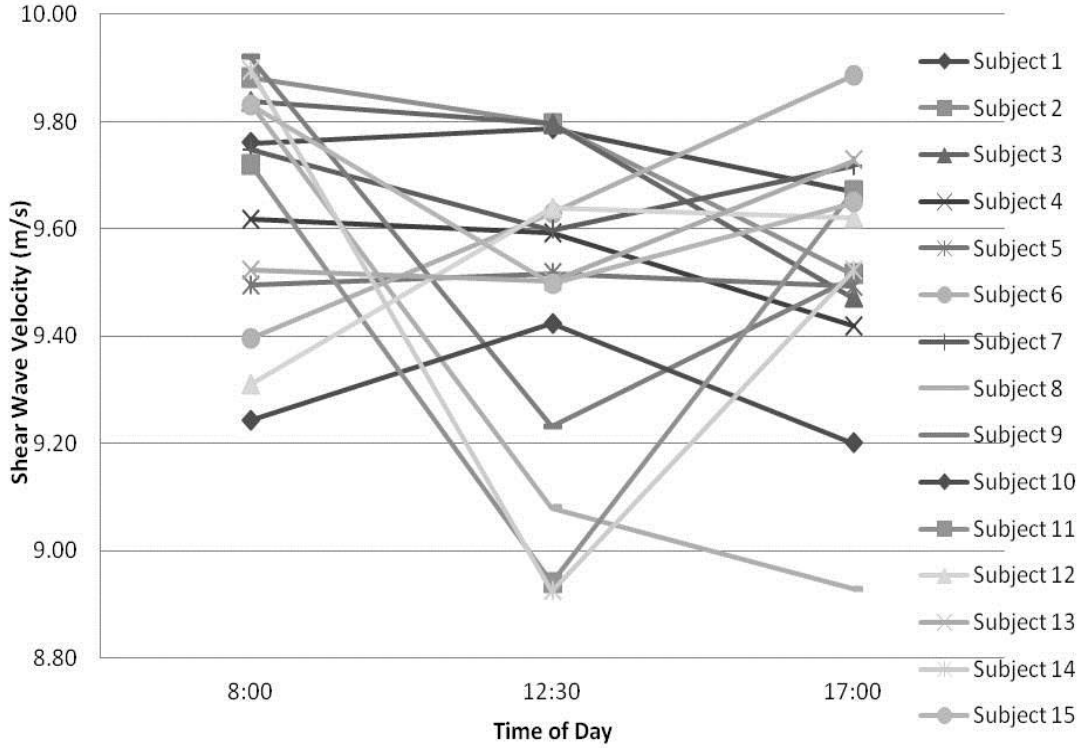


Figure 5.2: Individual SWV trend analysis

**Figure 5.2 shows individual SWV (m/s) values over the three measured time points of 08:00h, 12:30h & 17:00h for each participant.*

5.4.3 Leg dominance

When compared to each other, there were no significant differences between dominant and non-dominant AT's with regards to AT length at 08:00h ($p = 0.789$), 12:30h ($p = 0.718$) or 17:00h ($p = 0.727$). There were no significant differences between dominant and non-dominant AT's with regards to AT max AP diameter at 08:00h ($p = 0.608$), 12:30h ($p = 0.681$) or 17:00h ($p = 0.714$). There were no significant differences in the SWV measures in the dominant AT compared to the Non-dominant AT ($p > 0.05$) at 08:00h ($p = 0.176$), 12:30h ($p = 0.402$) and 17:00h ($p = 0.915$).

5.5 Discussion

This is the first study to trace the stiffness of the human AT *in vivo* over the course of a normal working day using SWE and to compare the results between dominant and non-dominant standing leg AT's. This study also assessed differences in AT length and max AP diameter between dominant and non-dominant standing leg AT's and changes over the course of the day. The main findings of this study suggest there were no significant differences in AT length, max AP diameter or SWV over the course of

a day and that there were no significant differences in length, diameter or stiffness between dominant and non-dominant standing leg AT's. The findings of this study advance our understanding of SWE by showing that measures taken on healthy asymptomatic AT's do not show measurably significant alterations in SWV (and hence stiffness) throughout the day. The largest changes in SWV by any individual in this study were in the order of 10% and it can be suggested that time of day therefore does not need to be considered when performing repeated scans of the AT in varying clinic appointment times. Our data also provides clear evidence that no significant alterations were found in the SWV (and hence stiffness) measured in either the dominant or non-dominant AT of participants over the measured time points as shown in Table 5.2 and Figure 5.1.

The results of this current study indicate no significant alterations in AT length or max AP diameter occurred over the three measured time points of 08:00h, 12:30h or 17:00h for either the dominant or non-dominant AT's. This suggests that no significant alterations in either length or max AP diameter of the AT occur over the course of a day. As shown in Table 5.1, the means of AT length and max AP diameter were slightly higher in the non-dominant AT in comparison to the dominant leg. None of these differences between dominant and non-dominant AT's for AT length or max AP diameter were significant. This current study found differences in AT length between the dominant and non-dominant AT's of 2.4%, 3.2% and 3.2% at the three measured time points of 08:00h, 12:30h and 17:00h, none of which were significant. Pang and Ying (2006) has also shown no significant variation in the length of the AT between dominant and non-dominant ankles using US, however in contrast, other authors have found the length of the free AT of the dominant leg to be significantly greater (by 8.23%) than the non-dominant leg (Bohm et al., 2015). There are differences in the way the studies attribute leg dominance which may account for the difference in their findings. The Pang & Ying (2006) study assessed leg dominance by asking participants which leg they would use to kick a ball. The elected side for ball kicking was then considered the non-dominant side due to the planted foot being used for push-off resulting in it being exposed to increased forces (Maffulli et al., 1999; Pang and Ying, 2006). In contrast, the Bohm et al. (2015) study utilised an 11-item behavioural inventory to classify foot preference and assigned dominance based on the results of the inventory (Chapman, Chapman and Allen, 1987; Bohm et al., 2015).

The Pang & Ying. (2006) study also differs from the Bohm et al. (2015) study in their findings in relation to cross-sectional area of the AT, as one study found no significant difference in the average cross-sectional area of the free AT between legs (Bohm et al., 2015) whereas the other demonstrated cross-sectional area to be significantly larger (4.9mm^2 or 8.4%) in dominant ankles (Pang and Ying, 2006), which may impact stiffness. This current study did not measure cross-sectional area but did measure max AP diameter in the same way that Pang & Ying. (2006) also measured max AP. The differences in max AP diameter between dominant and non-dominant AT's in this current study were calculated as 2.08%, 2.04% and 0.00% at the three measured time points of 08:00h, 12:30h and 17:00h, none of which were significant. This current study therefore would agree with published research that states that the leg dominance of an individual does not affect either the length or the thickness of their AT (Pang and Ying, 2006).

The main rationale behind the study was to examine whether time of day should be considered when performing repeated scans of the AT for diagnosis or during treatment. Although the study does not encompass a whole 24-hour period, it relates directly to times where most clinical assessment is likely to occur, providing direct significance to the clinical usage of SWE. The results of this study provide evidence towards best practice when considering the use of SWE in the clinical setting, as the variables measures may have had a clinical impact on the values obtained. Previous research reported decreases in tendon stiffness throughout the course of the day by 20.2% to 21% (Pearson and Onambele, 2006; Onambele-Pearson and Pearson, 2007). The authors note that the physiological mechanisms underlying the decreases found in tendon stiffness in the evening in comparison to the morning were not clear and were not measured by their study (Pearson and Onambele, 2006). When discussing potential mechanisms, they disregard alterations in body temperature and therefore attribute the decrease in stiffness to either hormonal changes or the action of general mobilisation throughout the day (Pearson and Onambele, 2006). Of note, is that the decrease in tendon stiffness over the day noted in the Pearson & Onambele (2006) paper was only shown to occur when tendon stiffness was calculated at low force levels (calculated from the gradient of the tangent over force levels corresponding to 1205N (Pearson and Onambele, 2006). When tendon stiffness was calculated at high force levels (100% MVC), there was no significant change noted between morning

and evening (p=0.10). This finding was attributed to tendon stiffness being associated with the rate of force development which is increased during an MVC and the fact that in this position, the tendon is already stretched to a position on its force-elongation curve where stiffness is highest (Pearson and Onambele, 2006).

The results of this present study were markedly different from previous research (Pearson and Onambele, 2006), with individual changes showing no systematic change in stiffness and the mean magnitude of change in tendon stiffness was only approximately 2%. These changes were not statistically significant changes in tendon stiffness and none of them were above the MDC. Methodological differences between the Pearson & Onambele (2006) paper and this present study, may provide reasoning behind the different findings. These differences include the tendon being examined (patella vs AT), the method of obtaining stiffness measures (ultrasonography & isokinetic dynamometer vs SWE) and differences in samples (purely male cohort vs mixed sex cohort). The results of this present study showed no significant differences between the SWV of males and females, so the different cohorts used should not have impacted results. A direct comparison between SWE and direct measures of tendon stiffness *in vivo* has not yet been completed, however SWE as a methodology has previously been validated against traditional tensile testing in the *ex vivo* environment (Eby et al., 2013). Eby et al. (2013) do note that the study should be repeated in the *in vivo* setting, therefore the use of different methods of calculating stiffness, together with the use of a differing tendon may provide a rationale for the different findings in tendon stiffness over the day between this current study and previous research (Pearson and Onambele, 2006).

There were no differences in SWE based on leg dominance when the dominant and non-dominant AT's of each participant were identified. The Bohm et al. (2015) study also reported no significant differences between the dominant (339 ± 114 N/mm) or non-dominant (320 ± 113 N/mm) sides in tendon stiffness (N/mm) (Bohm et al., 2015). The differences in AT stiffness between dominant and non-dominant AT's in the Bohm et al. (2015) study was calculated at 5.9%, and the results of this current study demonstrate that the difference in AT stiffness between dominant and non-dominant AT's was 1.03% at 08:00h, 0.85% at 12:30h and 0% at 17:00h. The findings between the Bohm et al. (2015) study and this current study regarding AT stiffness

between dominant and non-dominant sides were similar, despite the two studies using differing methodologies. This current study utilised SWE, whereas Bohm et al. (2015) used US and an isokinetic dynamometer. This study also attributed foot dominance based on which foot an individual would kick a ball with whereas Bohm et al. (2015) employed a behavioural inventory. Despite these differences, the results obtained by Bohm et al. (2015) and the results of the current study both note there are no significant differences in stiffness measures obtained between dominant and non-dominant AT's.

This study is the first to use SWE to trace alterations in AT stiffness *in vivo* over the course of a day and to compare AT stiffness between dominant and non-dominant standing leg AT's. As with all studies, it does carry some limitations, including the saturation limit to the measures of the Siemens ACUSON S3000™ HELX EVOLUTION US System (Siemens Medical Solutions, USA). Velocities above 10 m/s are returned with a label of 'High', therefore any measures returned as 'High' were discounted from the study. This study examined variation in the AT of 15 healthy, asymptomatic participants, using similar numbers to those measured in published studies (Pearson and Onambele, 2006; Onambele-Pearson and Pearson, 2007; Chino et al., 2012). A limitation of the study however is the use of a relatively small homogenous sample. Despite this, 10 ROI's were taken from each elastogram at each testing session. There were 3 testing sessions (08:00h, 12:00h & 17:00h), therefore the total numbers of measures taken from each participant over the course of the testing, totalled 30 measures in the dominant AT and 30 in the non-dominant AT. A total of 60 measures per participant equals 900 measures included in the analysis, a number higher than in similar research. All subjects were asymptomatic, healthy and free of lower limb injury and therefore it is not possible to generalize the results to symptomatic individuals with pathology or who fall outside the tested age range. Future research should define other populations based on age, sex or other covariates expected to influence stiffness as well as examining the results obtained from pathological samples.

In conclusion, this is the first study to utilise SWE to trace the stiffness of the human AT *in vivo* during a normal working day. The time of day a SWE measure is taken does not significantly alter AT stiffness and does not influence the measured values

by more than 2.07%. Therefore, clinical appointments can be scheduled between 08:00h and 17:00h without affecting the measures taken, improving appointment accessibility when using SWE. The other results of this study show that SWE results do not differ significantly between males and females and leg dominance does not affect SWE results in asymptomatic, healthy tendons, therefore the contra-lateral tendon may be used as a comparator for clinical investigation for this population group as there was no influence of leg dominance on the values measured.

6 The impact of an acute 30-minute bout of running on measures of Achilles tendon stiffness using shear wave elastography.

6.1 Abstract

Mechanotransduction refers to the processes that convert mechanical loading, such as exercise, into measurable responses which, for a tendon, can include increases in cross sectional area and net collagen synthesis. Most alterations in the anatomical properties of tendons are results of long term exercise, however, the effects of acute bouts of exercise on the physical properties of a tendon remain unclear. Understanding the scale and direction of any changes may have important implications for diagnosis and interpreting data collected using novel imaging methods such as shear wave elastography (SWE), should prior exercise occur before assessment. This study assessed whether a 30-minute acute bout of running led to significant alterations in the stiffness of the Achilles tendon (AT) *in vivo* as measured using SWE, with the hypothesis being that on a smaller scale to the known adaptations of long-term running, an acute bout of running may induce increases in the measured shear wave velocity (SWV). Twenty-four tendons from 12 participants (7 females, 5 males; mean age 27 ± 4 year, mean VISA-A Score 99.1 ± 1.1) were analysed. Participants were randomly assigned to either a control (CONT) group which remained sitting for 30-minutes or a running (RUN) group who performed a 30-minute run at a subjective intensity of 13-15 on the rating of perceived exertion (RPE) score. SWE measures were taken before, immediately after, 6hr, 24hr, 48hr and 72hr following the intervention. Significant increases were noted in SWV measures for the RUN group between pre-post run (0.27 m/s, 2.95%, $p = 0.037$) indicating a significant increase in tendon SWV immediately after exercise. Significant decreases were also noted in the data between post – 6hr (-0.43 m/s, -4.56%, $p=0.019$), post – 48hr (-0.39 m/s, -4.13%, $p=0.015$) and post – 72hr (-0.38 m/s, -4.03%, $p=0.013$). In contrast, no significant differences were found in either the acute or chronic measures of SWE for the CONT group ($p = 0.614$). The results indicate that a 30-minute run significantly increases SWV values in the AT. The implications of these results are that a prior bout of physical activity may cause changes within the physical properties of the AT that could result in a significantly different SWE measure when compared with resting values. A clinician or researcher should therefore be cautious of interpreting any SWE results obtained from the AT if weight bearing exercise has been performed beforehand.

6.2 Introduction

The term mechanotransduction refers to the processes that convert mechanical loading, such as exercise, into measurable cellular responses that may result in structural or functional change (Khan and Scott, 2009). Exercise has been shown to be capable of altering both the structural and chemical makeup of human tendon by inducing increases in its cross sectional area and increasing the concentration of metabolic enzymes to increase collagen turnover and prostaglandin production (Curwin, 2005; Tuite, Renström and O'Brien, 2007). Mechanical loading, such as that experienced during exercise, can increase the turnover and net synthesis of tendon collagen which may, over a period of time, potentially alter tendon size (Kjaer, 2004), structure (Magnusson et al., 2002) and function. Mechanical stimulus is also postulated to initiate changes within the extracellular matrix of tendon that results in a more damage resistant tissue with optimal force transmission properties (Kjaer, 2004). The cellular responses to mechanical loading, such as exercise, underpin the rationale behind the prescription of therapeutic exercises in the treatment of many injuries including tendinopathies (Khan and Scott, 2009). It has been suggested that the early adaptive responses to mechanotransduction can initiate longer term alterations in the mechanical properties of a tendon (Wang, 2006). Most of the alterations brought about by mechanotransduction are noted as results of repeated exercise (loading) programmes. This could be because the adaptation in the mechanical properties of tendons takes a long time, or that acute bouts of exercise have not yet been as extensively studied. This means that the exact influence of acute bouts of specific forms of exercise on the mechanical properties of healthy human tendon remains relatively unclear in comparison (Magnusson et al., 2008; Joseph et al., 2012). Long term exercise training such as running has been shown to increase the mass, collagen content, ultimate tensile strength and load to failure of a tendon (Tuite, Renström and O'Brien, 2007), but changes in relation to acute exercise bouts are less well understood.

A systematic review of the immediate effect of exercise on AT properties was conducted by Obst et al. (2013). The conclusion of this paper was that the impact of acute bouts of exercise on the mechanical properties of the AT occur in a manner dependent on both the mode and dose of the exercise. This is due to the differences

in tendon stress-strain characteristics (e.g. rate, duration and frequency) between different types of exercise all cause varying changes to tendon mechanical properties over time (Obst, Barrett and Newsham-West, 2013). Of the papers included in the review by Obst et al. (2013), 14 articles assessed AT stiffness, 12 of these did so using data obtained from ultrasonography and dynamometry (Kubo et al. 2001; Kubo et al. 2002; Mademli et al. 2006; Mademli & Arampatzis 2008; Morse et al. 2008; Burgess et al. 2009; Kay & Blazevich 2009; Kato et al. 2010; Kay & Blazevich 2010; Nakamura et al. 2011; Park et al. 2011). Whilst the remaining two studies also utilised ultrasonography, they used either a force platform or a force transducer to obtain measures of force (Peltonen et al., 2010; Farris, Trewartha and McGuigan, 2012) Six of those papers reported a decrease in AT stiffness following exercise, one reported a significant increase and the remaining seven reported no difference (Obst, Barrett and Newsham-West, 2013). Of the articles that found a decrease in AT stiffness, four studies assessed the effect of stretching, whilst some assessed isometric or concentric muscle contractions and not an acute bout of running. The study that found a significant increase in tendon stiffness post exercise, did so after 10 minutes of static stretching with stiffness calculated using ultrasonography and isokinetic dynamometry (Nakamura et al., 2011). Of those articles included in the review that assessed tendon stiffness after running, one study found no difference in AT stiffness post running (Farris, Trewartha and McGuigan, 2012) and the other study also found no significant differences in stiffness, however only looked at changes after a 6 minute warm up jog and stretching (Park et al., 2011). Also noted in the Obst et al. (2013) paper was the lack of studies evaluating the mechanical properties of the 'free' AT (Obst, Barrett and Newsham-West, 2013), with 'free' AT referring to the part of the tendon without any other attachment to either bony or muscular structures. This current study therefore aims to focus on the 'free' portion of the AT to address this gap in the literature.

Habitual running of long distances (> 80 km/week for >3 years) has been shown to result in a marked increase in the cross-sectional area of the AT by approximately 22% in comparison with a non-running control group (Kongsgaard and Aagaard, 2005). This increase in cross-sectional area may be a compensatory mechanism as it has been shown that pathological tendons can compensate for significant areas of disorganised fibres by increasing their thickness to reduce stress and maintain structural

homeostasis to ensure adequate load bearing (Cook and Purdam, 2009; Docking and Cook, 2015). The stiffness in the muscle-tendon complex (tendon and aponeurosis) of the vastus lateralis of long distance runners has also been measured as approximately 20% stiffer when compared to non-running controls (Kubo et al., 2000). Stiffness in this case entailed the Young's modulus of the tendon being calculated, which is the ability of a material to resist deformation to an applied stress (McKee et al., 2011) by describing a linear relationship between stress and strain and therefore regarded as a measure for the stiffness of a given material (Bogaerts et al., 2016). The stress-strain data obtained using US measures of length change and an isokinetic dynamometer to measure applied force (Kubo et al., 2000). An increase in tendon stiffness as a result of training could provide an increased capacity to store elastic energy, but only if the force applied to the tendon by the muscle is also increased (Buchanan and Marsh, 2002). However, increased tendon stiffness may not necessarily be associated with either an increase in muscular strength or an increased storage or elastic energy (Kubo et al., 2000). Another rationale for tendon remodelling and an increase in stiffness from loading could be to increase the load at which failure may occur and improve safety factors. However, the maximum isometric force produced by muscles is only approximately one-third required for tendon failure indicating that healthy tendons should not normally fail in response to normal loading patterns (Kirkendall and Garrett, 1997). Lastly, increases in tendon stiffness may occur in order to provide some protection to resist tendon damage caused by mechanical fatigue (Buchanan and Marsh, 2002), and reduce injury risk. If placed under the same load, stiffer tendon should allow for less extension which could potentially reduce the amount of damage caused (Buchanan and Marsh, 2002). *In vitro* studies demonstrate that a tendon loaded to 20% of its failure rate was shown to fail after approximately 300,000 cycles which equates to approximately 4 months of normal walking (Schechtman and Bader, 1997). Therefore, regular repair and remodelling of the tendon has to occur in accordance with the rate of damage (Buchanan and Marsh, 2002). Despite a large body of work surrounding the effects of habitual running, studies exploring the impact of a single acute bout of running on stiffness measures of normal, healthy human ATs *in vivo* remain scarce (Park et al., 2011; Obst, Barrett and Newsham-West, 2013).

Although not directly assessing stiffness, research using Ultrasound Tissue Characterisation (UTC) has assessed the structure of the human *in vivo* AT before and after exercise. The results of these studies with UTC suggest there were changes in tendon structure and integrity following a bout of exercise, including a decrease in aligned tendon fibrils and an increase in the separation and waviness of fibrils, both of which returned to baseline over the following 72-hours (Rosengarten et al., 2015). A decrease in the alignment of the tendon fibres may decrease tendon stiffness, and this current study proposes to measure shear wave velocity (SWV), a surrogate measure of stiffness, in the AT *in vivo* using shear wave elastography (SWE). In recent years, there have been several studies published using SWE to assess tendon stiffness (Arda et al., 2011; Kot et al., 2012; Aubry et al., 2013; Chen et al., 2013; Peltz et al., 2013), however an examination into the alterations in SWE measurements experienced within a healthy human AT *in vivo,* in response to an acute bout of running has not yet been reported. This study used SWE to assess measures of stiffness taken before, immediately after, 6hr, 24hr, 48hr and 72hr after an acute 30-minute bout of running to trace the time course of SWV in the AT *in vivo.* This study will follow similar time frames utilised in related research by Rosengarten et al. (2015). Long term mechanical loading or weight-bearing exercise can affect the mechanical properties of the AT, including increasing the stiffness of the tendon (Siu et al., 2016). Therefore, it is important to understand the normal variation in SWV measures within the *in vivo* AT and the influence of external load on these values. Without such information, abnormal or clinically relevant values cannot be decided upon and accurate interpretation is not possible. This study will help to inform the future usage of SWE and provide informed decisions, protocols to use and interpretation of the data, as it will provide quantitative values for the effects of previous activity. It will also provide information on the time course of any alterations and how long following activity this needs to be considered. SWE has been postulated as a reliable and useful modality (Buck, Verstraete and Li, 2012) by which to assess tissue stiffness, however there are a lack of studies that have controlled for previous activity.

The aim of this study is to assess whether an acute bout of running leads to significant alterations in the SWV experienced in the healthy, asymptomatic AT *in vivo* as measured using SWE and to trace the subsequent time course of any alterations over

the subsequent 72hr period following exercise. The hypothesis was that, in a similar fashion to longer term tendon adaptations to mechanical loading, an acute bout of running may result in an increase in tendon stiffness.

6.3 Materials and Methods

6.3.1 Participants

A total of 24 tendons from 12 participants were analysed for the study (7 females, 5 males; mean age 27 ± 4 year, mean Victorian Institute of Sport Assessment – Achilles (VISA-A) Score 99.1 ± 1.1, mean free AT length 37.1 ± 11.4 mm and mean AT maximum anterior-posterior (max AP) diameter 4.5 ± 0.5mm). Participants were recruited by word of mouth from the University of Brighton. The study received ethical approval from the University of Brighton tier 2 ethics committee and experiments were carried out in accordance with the 1964 Helsinki declaration and its later amendments as detailed in the General Methods (section 3.1).

The cohort in this study were a group of asymptomatic, healthy volunteers who self-reported as being moderately physically active on a regular basis in activities that included running. Inclusion and exclusion criteria for this study are detailed in the General Methods (section 3.2). Both written and verbal information about the study was given to each participant after they expressed initial interest in participating and both verbal and written informed consent was obtained from all participants before proceeding with testing.

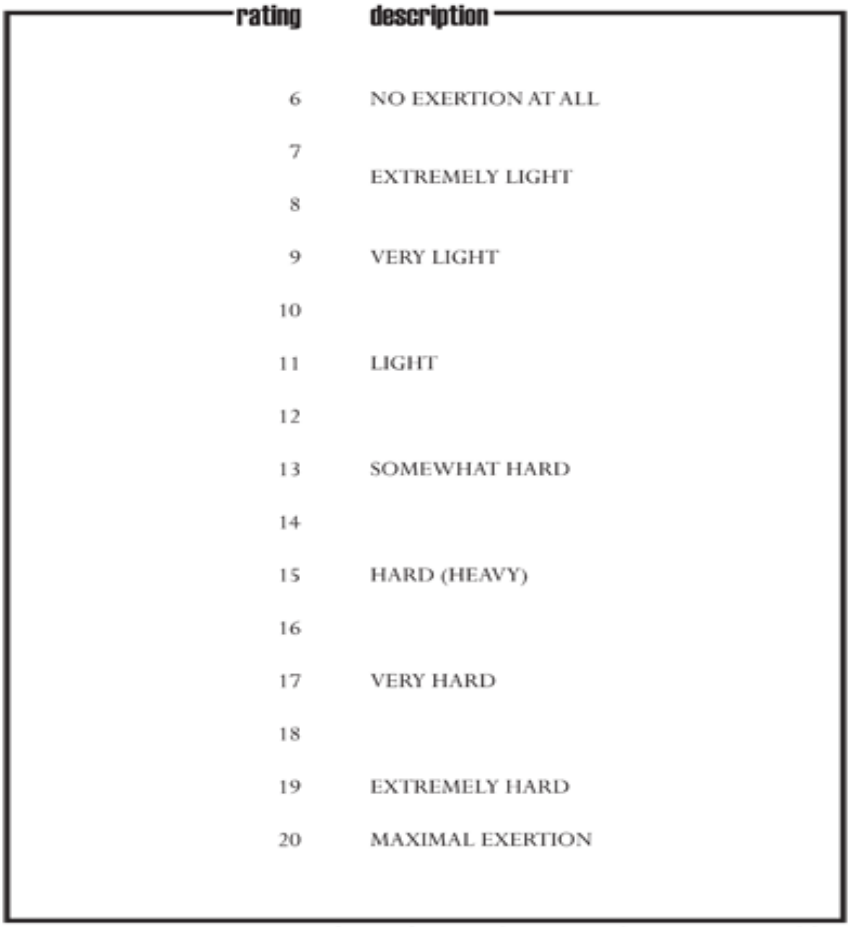
6.3.2 Methods

The protocol for scanning is outlined in section 3.5 of the General Methods. Baseline measures were taken from the AT's of all participants before they were randomly placed into the intervention group of running (RUN) or the control (CONT) group. If assigned to the CONT group, participants were asked to remain seated in the examination room for a period of at least 30 minutes, and no more than 40 minutes during their testing session. The participants were asked to remain seated, keeping their legs still, during this period and not move around. The experimenter was always present to ensure this occurred. If selected in the RUN group, participants were instructed to follow the exercise protocol detailed below.

Following assignment to each intervention group, the measures outlined below were taken from each participant immediately before and immediately after their 30 minute sitting or running intervention period. Subsequent follow up measures were also taken at 6hr post intervention, 24hr post intervention, 48hr post intervention and 72hr post intervention.

6.3.3 Exercise protocol

Participants were randomly selected into either the RUN or CONT group. If selected in the RUN group during their testing session, the participants were asked to change into running kit and were then given the possibility to warm up on a treadmill for no more than 5-minutes. Following the warm up, they were asked to keep the incline on the treadmill at 1% and increase the speed of the treadmill to a pace they felt comfortable with, and to run for a period of 30 minutes. A Rating of Perceived Exertion (RPE) scale, as shown in Figure 6.1 was placed in the direct eye line of the participants and they were asked to maintain an RPE of at least 13 (representing an intensity of somewhat hard), but below 15 (hard). The participants were asked every 5 minutes to rate their current RPE value and were encouraged by the experimenter to maintain an RPE of between 13 and 15 if it was outside of these values. The participants were able to alter their speed during their 30 minute run to maintain their RPE between these boundaries. Following a period of 30 minutes of running, the participants could complete a cool down at a walking speed of their choice for a period of no more than 5-minutes.



rating	description
6	NO EXERTION AT ALL
7	
	EXTREMELY LIGHT
8	
9	VERY LIGHT
10	
11	LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD (HEAVY)
16	
17	VERY HARD
18	
19	EXTREMELY HARD
20	MAXIMAL EXERTION

Figure 6.1: Copy of the Borg RPE scale

** Figure 6.1 is taken from* (Borg, 1982)*, ranging from 6-20, used during the exercise protocol to assess the intensity of the exercise.*

6.3.4 Scanning techniques

All measures were taken with a Siemens ACUSON S3000™ HELX EVOLUTION Ultrasound System (Siemens Medical Solutions, USA) in the longitudinal plane and followed the procedures outlined in the General Methods (section 3.5). Measures obtained were shear wave velocity (SWV) which was used for analysis without converting to Young's modulus. Testing sessions measured both the left and right legs of all participants.

6.3.5 Conventional Ultrasound technique

Firstly, AT free length was measured using a 14L5 probe on grey scale US as the length from tendon insertion at the calcaneus to lowest visible fibres of soleus, as outlined in section 3.5.1 of General Methods. Max AP diameter was calculated at the tendon mid-point relative to each participant as outlined in section 3.5.2 of General Methods.

6.3.6 Shear wave elastography technique

AT SWV was calculated using a 9L4 probe following the procedure outline in General Methods (section 3.5.5).

6.3.7 Statistical Analysis

All statistical analysis was performed using SPSS version 22 (SPSS, Chicago, Illinois). Measurements of age, VISA-A score, AT length, mid-point and maximum anterior-posterior thickness measures of all the participants are expressed as mean ± standard deviation. Distribution of groups was analysed using the Shapiro-Wilk test and mean values for all subjects will be used as a summary measure for each variable. A 2 Way RM ANOVA (Time (6) & Group (2)) was conducted to assess differences, and to establish where the differences lay, separate One-Way RM ANOVA's were completed on the data for the RUN group and the data for the CONT group with the findings followed up using Bonferroni post hoc test. Data was checked for sphericity with the Huynh-Feldt Correction applied if necessary and alpha level was set at $p < 0.05$ throughout.

6.4 Results

The summary variables for both the RUN and CONT group taken at baseline before the exercise (Pre) are shown in Table 6.1.

Table 6.1: Summary variables for the RUN and CONT group.

Intervention Group	Mean Age (years)	Mean Height (cm)	Mean VISA-A	Mean Tendon Length (mm)	Tendon Mid-Point (mm)	Mean max AP diameter (mm)
RUN	28.1 ± 4.9	173.0 ± 2.65	99.3 ± 0.8	44.3 ± 11.2	22.1 ± 5.6	4.4 ± 0.3
CONTROL	25.1 ± 3.0	163.8 ± 8.06	98.8 ± 1.4	29.9 ± 5.7	14.9 ± 2.9	4.7 ± 0.6

There were significant differences between the groups for mean height ($p = 0.005$) and mean AT length ($p = 0.001$) with a significant correlation ($r = 0.596$, $p = 0.002$) shown between the two variables. When considering the effect of the exercise bout, measurements of AT length for both the RUN and the CONT group remain stable over the six measured time points with only minimal alterations in mean measures and standard deviation and no significant differences apparent over time ($p > 0.05$). When considering maximum anterior-posterior diameter (mm) and SWV values, the CONT group experienced no significant differences between the time points. In contrast, the RUN group did experience significant changes ($p < 0.05$) in both max AP diameter and SWV. The values obtained at the measured time points are shown in Table 6.2.

Table 6.2: AT Length (mm), AT maximum anterior-posterior (Max A-P) diameter (mm) and SWV at all measured time points for both RUN and CONT group.

	PRE	POST	6hr POST	24hr POST	48hr POST	72hr POST
RUN AT Length (mm)	44.3 ± 11.2	45.0 ± 11.2	44.8 ± 11.3	44.1 ± 11.1	44.3 ± 11.1	44.1 ± 10.8
CONT AT Length (mm)	29.9 ± 5.7	29.6 ± 6.2	30.0 ± 5.9	30.0 ± 5.6	30.2 ± 5.6	30.0 ± 5.2
RUN Max A-P (mm)	4.37 ± 0.27	4.08 ± 0.19	4.58 ± 0.29	4.38 ± 0.26	4.45 ± 0.31	4.34 ± 0.28
CONT Max A-P (mm)	4.66 ± 0.64	4.69 ± 0.63	4.63 ± 0.64	4.71 ± 0.61	4.63 ± 0.69	4.66 ± 0.63
RUN SWV (m/s)	9.16 ± 0.39	9.43 ± 0.39	9.00 ± 0.42	9.19 ± 0.31	9.04 ± 0.36	9.05 ± 0.28
CONT SWV (m/s)	9.11 ± 0.23	9.08 ± 0.22	9.04 ± 0.26	9.05 ± 0.22	9.06 ± 0.21	9.09 ± 0.22

There were no significant differences noted in the max AP diameter score between the RUN and CONT group in the PRE-measure ($p = 0.410$). The results demonstrated significant differences in the data for the RUN group in relation to the max AP diameter. The absolute difference, % difference and the significance of the significant differences in max AP diameter are shown in Table 6.3.

Table 6.3: Significant differences in max AP of the RUN group

	Absolute difference	% difference	Significance
Pre - Post	- 0.29 mm	- 6.64%	P = 0.000 **
Pre – 6hr	0.21 mm	4.81%	P = 0.042 *
Post – 6hr	0.50 mm	12.25%	P = 0.000 **
Post – 24hr	0.30 mm	7.35%	P = 0.000 **
Post – 48hr	0.37 mm	9.07%	P = 0.000 **
Post – 72hr	0.26 mm	6.37%	P = 0.000 **
6hr – 24hr	- 0.20 mm	- 4.37%	P = 0.028 *

*Table 6.3 shows Absolute difference, % difference and p value for the significant differences shown in max AP diameter between the RUN and CONT groups. * = $p < 0.05$, ** = $p < 0.01$.*

With regards to measures of SWV, the measurement for the RUN and CONT groups were not significantly different from one another at the PRE-measure (p = 0.319). The results demonstrated a significant main effect of Time (p = 0.001), no significant differences between the groups (p > 0.05) but a significant interaction effect of time x group (p = 0.003), implying a significant difference between the measured time points depending on which group a participant was in. There were no significant differences in the time points for the CONT group (p = 0.614) implying that the SWV data collected for all the participants in the CONT group did not vary significantly over the measured time points and therefore remained stable.

Significant differences (p = 0.001, η_p^2 = 0.399) were shown in SWV between the measured time points for the RUN group, suggesting that the results obtained from the RUN group altered significantly POST run in comparison to all other measures taken. Significant differences in SWV were shown to exist between the measured time points in the RUN group and these are shown in Table 6.4.

Table 6.4: Significant differences in SWV for the RUN group

	Absolute difference	% difference	Significance
Pre - Post	0.27 m/s	2.95 %	P = 0.037 *
Post – 6hr	- 0.43 m/s	- 4.56 %	P = 0.019 *
Post – 48hr	- 0.39 m/s	- 4.14 %	P = 0.015 *
Post – 72hr	- 0.38 m/s	- 4.03 %	P = 0.013 *

Table 6.4 shows absolute difference, % difference and p value for the significant differences shown in SWV for the RUN group.
** = $p < 0.05$*

The largest changes in the SWV values were in the RUN group where SWV increased pre-post by 0.27 m/s (2.95%). After this, the SWV decreased by approximately -4.5%, before once again increasing by just over 2%. In contrast to the above changes noted in the RUN group, the largest percentage change experienced in the CONT group was just 0.44%. If considering all the measured time points in relation to the PRE-values, the largest change again was an almost 3% increase measured in the RUN group between pre and post as seen in Figure 6.2. However, as the POST measure is the only measure to be significantly different from PRE, it would suggest that the SWV measures return to normal after a period of 6 hours following an acute 30 minute bout of running.

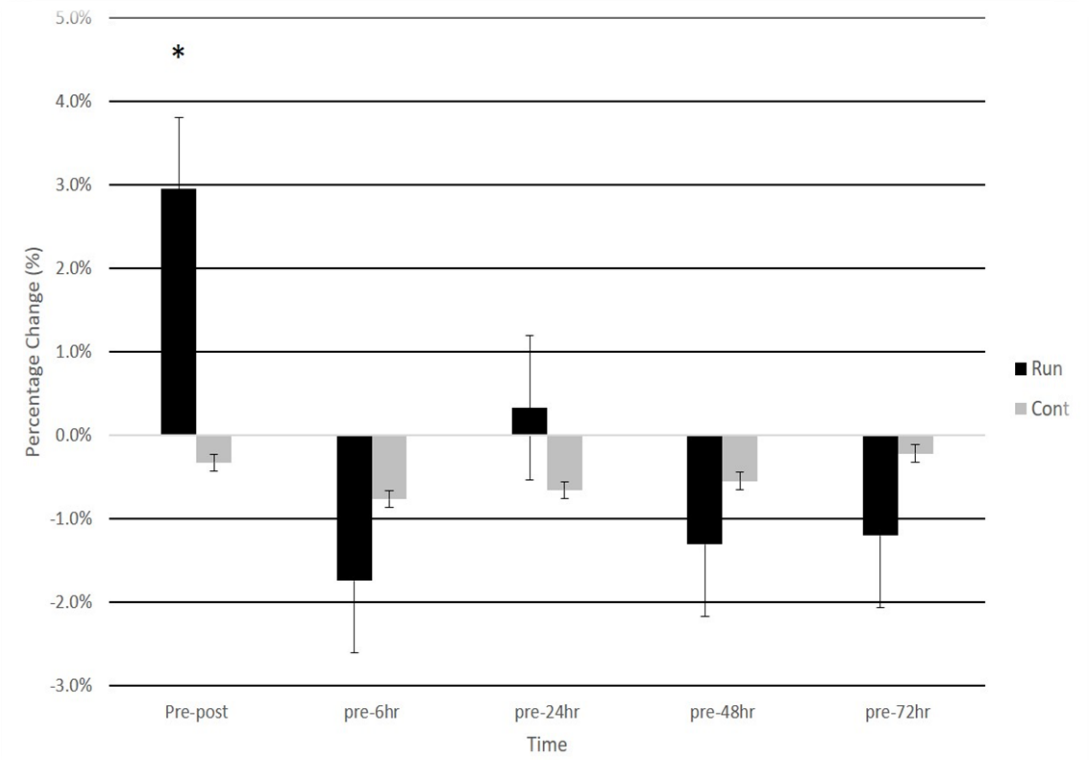


Figure 6.2: % Change in SWV results

*Figure 6.2 shows % change of initial SWV in both RUN and CONT groups. * = p<0.05.*

The mean increase in SWV following the exercise bout was 0.27 m/s, followed by a decrease between the post measure and 6hr measure of -0.43 m/s with the range of increase being between 0.05 – 0.81 m/s. These alterations were shown to be statistically significant in the analysis and chapter 4 of this thesis proposed a typical error of SWE of 0.22 m/s and minimal detectable change (MDC) of 0.53 m/s. Figure 6.3 A & B, both demonstrate that all AT's in the RUN group, both left and right (and hence dominant and non-dominant AT's) experienced an increase in SWV (m/s) post run of between 0.05 – 0.81 m/s. The average increase pre-post in the left AT of the RUN group was 0.33 m/s (3.7%) which compares to an average increase of 0.21 m/s (2.3%) in the right AT

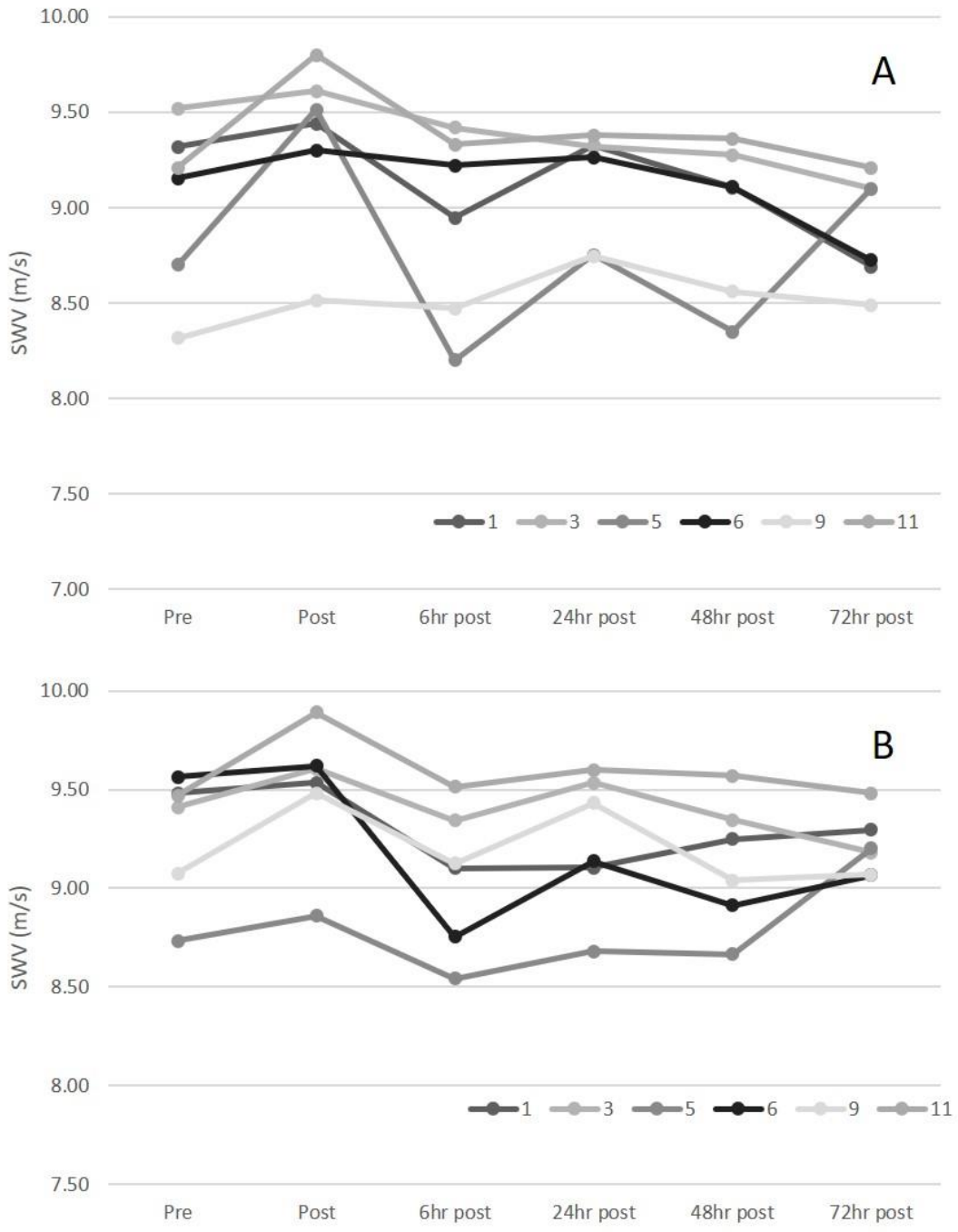


Figure 6.3: Individual trend analysis for RUN group in A) Left AT and B) Right AT

** Figure 6.3 shows individual changes for participants 1, 3, 5, 6, 9 & 11 in the RUN group in SWV (m/s) in A) Left AT and B) Right AT*

6.5 Discussion

The aim of this study was to examine whether an acute bout of running for a period of 30-minutes lead to any significant alterations in the SWV values experienced in the AT *in vivo* as measured using SWE. The main results of this study indicate that a 30-minute bout of running resulted in a significant increase in SWV (and hence AT stiffness) immediately after a 30-minute run. In contrast, the control group experienced no significant changes in SWV over the course of a 72-hour period. The mean increase in SWV following a 30-minute bout of running (0.27 m/s) was above the typical error (shown in chapter 4) with some participants experiencing increases in SWV above the MDC (also shown in chapter 4). This demonstrates that both statically and clinically significant increases in SWV (and therefore AT stiffness) were measured with SWE following exercise. The implications of these results are that a prior bout of physical activity may initiate changes within the physical properties of the AT that could result in a significantly different SWV measure, therefore a clinician should be aware of the possibility of obtaining a significantly increased SWV measure from the AT using SWE if 30 minutes of weight bearing exercise has been recently performed. The responses to longer periods of tendon loading on SWV, and its time course have not been investigated and may merit further enquiry.

Studies using UTC have shown that exercise leads to a change in tendon structure in both race horses and humans (Docking et al., 2012; Rosengarten et al., 2015) when tendons were measured prior to the bout of exercise, then again one, two and four days after the exercise bout. Changes in the structure of the tendon, may be expected to affect its mechanical properties, e.g. Tendon stiffness. This study adds some support to that notion as exercise resulted in significantly increased measure of SWV, even after an acute bout of exercise lasting 30-minutes. In the UTC study involving human participants, the authors concluded that exercise resulted in a short-duration (72-hour period) and fully reversible response of the tendon which occurred with no loss of integrity of the collagen matrix (Rosengarten et al., 2015). The Rosengarten et al. (2015) paper may offer further support for the work of Cook and Purdam. (2009) who proposed the idea of placing tendon pathology on a continuum containing three stages; reactive tendinopathy, tendon dysrepair and degenerative tendinopathy (Cook and Purdam, 2009). Due to the continuum nature of the model, it is possible for a tendon to move between the stages of the model with the primary stimulus moving a tendon

forward or back on the continuum being the addition or removal of loading (Cook and Purdam, 2009). In the first stage of reactive tendinopathy, acute overload can result in short term adaptations within the tendon that predominantly result in tendon thickening. Assuming the increase in cross-sectional area is not due to fluid accumulation, but due to structural adaptations, it could reduce the stress experienced within the tendon and increase stiffness. Given the short time frame involved in the findings of this study (i.e. tendon SWV returning to similar to PRE measures after 6 hrs), it is unlikely to be structural adaptations occurring within the tendon, but alterations in the extra-cellular matrix of the tendon including an increase in cytoplasmic organelles for increased protein production, in particular proteoglycans, which are associated with an increase in bound water and hence can result in an increase in cross-sectional area (Cook and Purdam, 2009).

All changes in the tendon are expected to revert to normal should the load be removed or sufficient time be made available for recovery between loading sessions (Cook and Purdam, 2009). This study would agree with aspects of this continuum by finding increases in stiffness following an acute bout of loading which returned to normal once the load was removed. This study however, did not show that exercise results in an increase in max AP diameter. Instead, the results demonstrated that a 30-minute acute bout of running exercise resulted in a significant decrease in max AP diameter following an acute bout of exercise. This decrease in tendon thickness has also been shown in other research and is hypothesised to be attributed to a loss of fluid from the tendon to the peritendinous space caused by the mechanical load inducing increased hydrostatic pressure (Obst, Barrett and Newsham-West, 2013). This temporary 'dehydration' within the tendon could potentially attribute for differences found in SWV, by affecting the tissue density which is assumed to be constant in the equations leading to the production of a velocity reading.

Previous research demonstrated that in young healthy ATs, an acute bout of eccentric exercise resulted in a significant increase in AT stiffness as measured with SWE (Leung, Chu and Lai, 2017). Other studies show that a single 30-min bout of running do not impact upon the stiffness of the AT and concluded that the mechanical properties of tendon remain constant throughout locomotion (Farris, Trewartha and McGuigan, 2012). The research of Farris et al. (2012) utilised the same number of participants as in this study, but required participants to complete a 30-min run at a set

pace of 12 km/ph. This speed was selected as being representative of a recreational run, as all participants said they were recreational runners, therefore the speed of the run between the two studies varied and may account for some of the differences in findings. In contrast to the Farris et al. (2012) paper, this study utilised a self-paced run, asking participants to maintain an RPE level between 13-15 to ensure the run was the same subjective intensity for all participants. The mechanical properties of the ATs of each subject in the Farris et al. (2012) study were recorded both before and after the run during a series of hops, with tendon stiffness estimated using AT length data obtained using an US probe secured to the participants leg, using bandaging tape, and AT force measured using force plates (Farris, Trewartha and McGuigan, 2012). In contrast, this study utilised SWE to assess SWV. Previous research suggests that reductions in tendon stiffness following unaccustomed acute bouts of exercise may increase injury risk (Obst, Barrett and Newsham-West, 2013). The participants in this study all self-reported as being moderately active on a regular basis in activities that involved running, and who therefore should have been accustomed to this dose and manner of exercise used. The results of this study also demonstrate that an acute bout of exercise resulted in an increase in tendon stiffness. Alterations in tendon mechanical properties including increases in stiffness in response to exercise is the bodies response to a new level of loading and this increase can potentially aid in reducing tendon damage caused by mechanical fatigue (Buchanan and Marsh, 2002), which may provide a rationale for the results found in this study. An increased level of stiffness detected immediately after an acute mechanical load, may also allow for less extension of the tendon (Buchanan and Marsh, 2002) which potentially may help reduce macro-trauma risk.

The tendon stiffness of trained individuals is known to be higher than that of un-trained controls (Kubo et al., 2000) and higher levels of tendon stiffness have been shown to be associated with a lower energy cost of running (Fletcher, Esau and MacIntosh, 2010). There are suggestions that there is an optimal level of tendon stiffness that reduces the energy cost of running, shown using modelling data to be approximately 180 Nmm^{-1}. Working above this optimal level of stiffness however, the energy cost of running increases again (Lichtwark and Wilson, 2007), Others have noted that an optimal level of stiffness within the AT is likely to be activity dependant (Joseph et al., 2014). These findings potentially suggest that tendon stiffness can be amended

and adapted for optimal efficiency in a given activity through the process of tendon remodelling as a result of mechanotransduction through regular physical training (Lichtwark and Wilson, 2007). A highly compliant tendon may allow more tendon lengthening and optimise the power-velocity relationship, however high levels of compliance within a tendon may also interfere with the direct transmission of muscle shortening to joint movement affecting power (Fletcher, Esau and MacIntosh, 2010). The muscle-tendon complex of long distance runners, (assessed by measuring the elongation of the tendon and aponeurosis of vastus lateralis muscle) have been shown to be 20% stiffer than non-running controls (Kubo et al., 2000), so it would be interesting to see a comparison of the impact of acute bouts of exercise between these two groups using SWE and whether any differences in SWV are apparent. Also of interest would be to look at a longer bout of exercise as the stiffness of the ATs of marathon runners has been shown decreased following a race, with this change resolved by 6 weeks post-race (Ooi et al., 2015).

The lengthening of an elastic structure such as tendon, is a phenomenon known as creep. It occurs when the tendon is held under consistent tension or when it is loaded repeatedly in a cyclical fashion (Ker, Wang and Pike, 2000). This tendon lengthening can allow for an increased energy storage which can be released upon shortening, increasing the available power (Fletcher, Esau and MacIntosh, 2010). The occurrence of creep during the process of running has been suggested to result in micro-damage of the tendon increasing injury risk, and with the increased lengthening of the tendon corresponding to any given force that occurs during creep, would be indicative of a reduction in tissue stiffness (Farris, Trewartha and McGuigan, 2012). This study revealed no significant differences ($p > 0.05$) in AT length for the RUN group over the six measured time points, suggesting that creep was not a significant factor over the time course of this intervention. It is worthy of note that there was a large and significant discrepancy between the RUN and CONT group in AT length at baseline. There was also a significant difference in height between the two groups which may provide a rationale for the difference in AT length found between them. A significant correlation ($r = 0.614$, $p = 0.001$) has been previously shown between AT length and an individual's height (Patel and Labib, 2018) with a similar strength correlation between the two variables being shown in this study. The participants were randomly selected for either the RUN or CONT group, therefore the discrepancy in height and

AT length between the two groups could not have been pre-empted. Due to these differences, the main focus during the study has been on the changes experienced within each group rather than between them and the analysis has been conducted on the groups separately. The SWE measure between the groups was not shown to be significantly different at the PRE-measure and therefore the height and AT length differences did not appear to impact stiffness measures. As the study analysed within group changes, it can still be said that the RUN group experienced a significant increase in stiffness after their run, whereas the CONT group experienced no such alteration.

This study carries its own limitations including a saturation limit associated with the Siemens ACUSON S3000™ HELX EVOLUTION Ultrasound System (Siemens Medical Solutions, USA). Any SWV above 10 m/s was identified as a value of 'High'. As it was not possible to quantify the exact SWV of these measures, a value of 'High' was discounted from the study. This occurred 7 times in the study and therefore only accounted for 0.97% of the SWV measures obtained. This study used 12 participants, matching that used in very similar research (Farris, Trewartha and McGuigan, 2012). All participants in this study were considered healthy and free of lower limb injury. This was considered important as until the normal variations within healthy tendons are established and some baseline clinical values recorded, it will be impossible to establish what normal and acceptable change is, versus pathological change. Future research should examine results obtained from pathological samples. The exercise mode utilised in this study was running, however other modes of exercise such as stretching have been shown to also significantly alter tendon stiffness over short time periods. Tendon stiffness immediately after static stretching has been measured at 53.2 ± 2.4 Nm/cm which was significantly higher than prior to (32.5 ± 2.4 Nm/cm)and 10 min after (32.8 ± 2.7 Nm/cm) stretching, however the stiffness of the muscle and the muscle tendon unit as a whole decreased (Nakamura et al., 2011). It would be of interest to look at the impact other types of exercise will have on the SWV in the AT in comparison to those found with running in this study. Lastly, this study only assessed the impact of running on the AT, however the impact of different types of exercise on other injury prone tendons in the body such as the patella tendon would also be useful. As noted in previous research, to tailor appropriate mechanical loads of a particular training regime to a specific tendon, the immediate effects of that

exercise mode on a given tendon should be further explored (Leung, Chu and Lai, 2017).

In conclusion, this study has shown that a 30- minute run has no impact on AT length, however it does result in significant decreases in max AP diameter and significant increases in SWV measures, a surrogate measure of stiffness. The measured max AP diameter and SWV measures return to PRE-like values when measured 6hrs after the exercise. These results suggest that measures of AP are more useful in the measurement of tendon properties than those of AT length. The knowledge of the impact of running on SWE measures is vital to the ongoing studies within this thesis, and also to clinicians and researchers who need to be aware of these results when using SWE and consider the presence of previous exercise when obtaining SWE measures as activity may potentially lead to misleading results.

7 The effects of a 12 week eccentric exercise programme on clinical outcome measures and Achilles tendon stiffness measured by shear wave elastography.

7.1 Abstract

Treatment options for Achilles tendinopathy (ATY) are numerous, however eccentric exercises (EcExs) have the most evidence of effectiveness in treatment. This study examined the effect of a commonly prescribed 12 week EcEx programme on symptoms and clinical outcome measures and measures of Achilles tendon (AT) stiffness properties, throughout the programme, using shear wave elastography (SWE). This study examined alterations to symptomatic, pathological ATs (PATs) as well as the healthy contra-lateral AT (HATs) and assessed the timeline over which changes occurred. A total of 21 participants presented with symptoms of mid-portion ATY (12 males, 9 females; mean age 58 ± 10.68 years, range 35 – 76 years; height 172.2 ± 8.5cm, range 158 – 183cm) and of these 16 were examined throughout the 12 week EcEx programme. One AT from each participant was identified as pathological resulting in 16 tendons in the pathological AT (PAT) group with the remaining 16 identified as a healthy AT (HAT). Participants completed a 12 week EcEx rehabilitation programme on their symptomatic PAT, and measures of SWE, Victoria Institute of Sport Assessment – Achilles (VISA-A) score, Visual Analogue Scale (VAS), AT length (mm), AT maximum anterior-posterior (max AP) diameter (mm), Doppler score, Range of motion (ROM), muscular endurance and muscular power were taken at week 0, 4, 8 and 12 from starting the EcEx programme. Over the 12 week EcEx programme, the PAT group experienced a significant increase in stiffness as measured by SWE. Other significant increases were shown in VISA-A, SWE, ROM, muscular endurance & muscular power coupled with significant decreases in VAS, max AP diameter and Doppler. The HAT group experienced significant increases in VISA-A, ROM, muscular endurance and power despite no direct intervention being carried out on this side. At baseline, scores between the groups were significantly different for VISA-A, VAS, max AP diameter, Doppler score and SWE. The data for muscular power and endurance was not significant between the groups at week 0. At 12 weeks, the scores of VAS and SWE were no longer significantly different between the groups. Many improvements in clinical outcomes were obtained in the PATs by completing a 12 week EcEx programme including

reductions in AT diameter, Doppler score and VAS together with increases in VISA-A score, ROM, muscular endurance and muscular power. This study also demonstrated that a 12 week EcEx programme increases SWE measures (and hence stiffness) and results in improvements in the contralateral HAT as well. However, by week 12, VISA-A score, max AP diameter and Doppler score remained significantly different in the PATs in comparison to the HATs. It appears that the measured variables in a PAT do not reach parity with those in the non-affected tendon in this typically prescribed period.

7.2 Introduction

Achilles tendon (AT) pain, manifested as a localised and painful thickening of the AT has an incidence rate of 2.35 per 1,000 in the general adult population (de Jonge et al., 2011). It is a relatively common presentation among middle aged recreational athletes (Ohberg, Lorentzon and Alfredson, 2004) and of all sports injuries, approximately 30-50% of them will be classed as a tendon injury (Beyer et al., 2015). Of all reported tendon injuries, 55-65% will be a clinical diagnosis of Achilles tendinopathy (ATY) (Järvinen et al., 2005). The morphological characteristics of symptomatic ATY include an area within the AT that has a high concentration of glycosaminoglycans, irregular tendon fibre structure and arrangement, but without the presence of inflammatory cell infiltrates (Ohberg, Lorentzon and Alfredson, 2004). Historically, tendon pain was usually referred to as tendinitis, implying the presence of inflammation was integrally responsible for the symptoms and pathology accompanying the condition (Rees, Stride and Scott, 2014). More recently, there has been shift in thinking which hypothesises that ATY is a result of a failed healing process that initiates degenerative changes within the tendon including structural changes, neovascularisation and nerve in-growth (Beyer et al., 2015). Despite a wealth of knowledge on what now constitutes a tendinopathic tendon, the pathogenesis and primary cause of ATY remains unclear, rendering the condition as one notoriously difficult to treat (Ohberg, Lorentzon and Alfredson, 2004; Beyer et al., 2015)

Treatment options proposed for ATY are numerous and include surgery, sclerosing injection techniques, shock wave therapy, night splints, heel braces, laser therapy, microcurrent therapy and exercise therapies such as heavy slow resistance training

(Beyer et al., 2015), concentric-eccentric programmes such as the Silbernagel protocol (Silbernagel et al., 2007) and eccentric exercises (EcEx) (Sussmilch-leitch et al., 2012). More recently, the use of isometric exercises has been discussed as a useful addition to rehabilitation programmes for tendons as they have the capability to induce substantial adaptive responses (Waugh et al., 2018). Despite the growth of recent evidence for isometric exercise in ATY treatment, EcEx programmes still carry the most evidence for effectiveness in the literature for the treatment of mid-portion ATY (Silbernagel, Brorsson and Lundberg, 2011) with recorded success rates between 60% to 90% using follow up times from 12 weeks to 1 year (Alfredson et al., 1998; Silbernagel et al., 2001; Roos et al., 2004; Sayana and Maffulli, 2007; Magnussen, 2009). Some studies show significantly decreased pain measured with the Victorian Institute of Sport Assessment – Achilles (VISA-A) score and decreased tendon thickness as far as 5 years after completing an EcEx (Gärdin et al., 2010; Van Der Plas et al., 2012).

Eccentric muscle contraction occurs when the force produced by the muscle is less than the applied external load, leading to muscle fibre lengthening (Morrissey et al., 2011). EcExs involve the lengthening of a muscle-tendon unit as a load is applied to it and the main aims of EcEx programmes are to create stronger and stiffer tendons, more resistant to stresses and strains, and therefore injury (Murtaugh and Ihm, 2013). EcExs subject a tendon to greater loading and stimulates structural adaptations, such as hypertrophy (O'Neill, Watson and Barry, 2015). Eccentric loading has also been shown to reduce tendon volume and diameter, presumably by a reduction in intra-tendon fluid content (O'Neill, Watson and Barry, 2015). The literature suggests EcExs are effective at restoring function and reducing pain associated with ATY, however there are many potential explanations as to how this is achieved and many of these have not been adequately examined (O'Neill, Watson and Barry, 2015). Research surrounding the potential benefits of EcEx as a treatment modality for tendons, and specifically the AT can be dated back to the late 1980's (Stanish, Rubinovich and Curwin, 1986). The theory as to how this works is that EcExs produce microtrauma to the tendon and it is the repair to these microtears that strengthen the tendon. The study did not include a placebo control group, however, the 6 week training period resulted in complete relief in 44% of patients and a marked improvement in an additional 43% (Stanish, Rubinovich and Curwin, 1986). A 12

week EcEx programme for ATY was first proposed in 1998 (Alfredson et al., 1998), which involved the loading of the AT into full dorsiflexion. Patients with tendinopathy performed this exercise for 12 weeks and at the end of the treatment period, all 15 were able to return to their pre-injury running activity and all had improved their calf strength (Alfredson et al., 1998). This controlled study into EcEx as a treatment modality for those with mid-portion ATY set a principle for this treatment as a rehabilitation tool. The results helped the approach of EcEx (Alfredson et al., 1998) to become the mainstay of conservative treatment for ATY (Webborn, 2008). The method has been routinely used since its initial application in the late 1990's and has been shown to be effective at decreasing pain, normalising the structure of the tendon as assessed with US (Murtaugh & Ihm, 2013), improving function (Chester et al, 2008) and decreasing tendon thickness (Knobloch, 2007). Recently, an acute bout of EcEx was shown to significantly increase tendon stiffness as measured with SWE (Leung, Chu and Lai, 2017).

The positive outcomes associated with an EcEx programme as a treatment modality for ATY are well documented (Alfredson et al. 1998), however the mechanisms by which these clinical results occur are not fully understood. A high success rate (82 – 100%) for return of pre-injury activity or improvements in tendon structure has been noted when using EcEx programs as an intervention for athletes (Alfredson et al., 1998; Mafi, Lorentzon and Alfredson, 2001; Fahlström and Jonsson, 2003; Ohberg and Alfredson, 2004), helping to make them popular for tendon treatment. When applied to a non-athletic patient population the results were less successful, with less than 60% (19 out of 34) of patients benefiting from an intensive, heavy load EcEx programme (Sayana and Maffulli, 2007). This may be attributed to athletes and younger people being more likely to have a reactive tendon (Cook and Purdam, 2009) that responds quickly to changes in load. Papers questioning the effectiveness of EcExs include that by Chester et al. (2008) who compared the Alfredson model of EcEx to therapeutic US and reported no significant difference in outcomes, suggesting the two treatments were equally as effective and EcExs were not superior in treatment of ATY. However, a systematic review of randomised controlled trials looking into various physical therapies for the rehabilitation of symptomatic ATY noted that EcEx programmes remain an integral component of treatment (Sussmilch-leitch et al.,

2012). Despite extensive research conducted into the application of EcEx, there is little concerning the time frame over which changes occur.

Response to mechanical loading at the cellular level is termed mechanotransduction. Mechanotherapy is the application of mechanotransduction for the purposes of repairing and remodelling tissue (Khan and Scott, 2009). It is therefore integral to rehabilitation protocols that induce load to elicit a cellular response and encourage tendon repair and remodelling. Encouraging tendon remodelling has a subsequent impact on the mechanical properties of tendon, including that of stiffness, which alters alongside pathological change as well as during healing (Rasmussen, 2000; Horton, 2013). The literature suggests that there remains a distinct lack of available information about the impact of EcEx programmes on tendon mechanical properties including AT stiffness (Witvrouw et al. 2007) and the structural and mechanical recovery of a tendon during EcEx programmes (Geremia et al. 2015), providing a rationale for this current study. To improve our current understanding of the impact of EcExs, it will be important to analyse whether, alongside the clinical changes previously documented, any alterations in AT stiffness occur during an EcEx programme. One reason for this gap in the literature is the lack of available technology capable of accurately measuring changes in the mechanical properties of a tendon *in vivo*.

Shear wave elastography (SWE) is a novel method that assesses the stiffness of *in vivo* tissues. SWE provides quantitative measures of shear wave velocity (SWV), a commonly used surrogate for stiffness, by tracking the velocity of shear waves as they travel through a given tissue (Siemens, 2008; Hoskins, 2012). SWV is proportional to tissue stiffness (Garra, 2011), meaning the generated shear waves travel slower through softer tissue than they do through a stiffer tissue (Hoskins, 2012), therefore quantifying SWV allows stiffness to be calculated. Research using SWE has concluded that it is independent of user skill (Chen et al., 2013), validated against traditional tensile testing (Eby et al., 2013) and a reproducible technique when used to assess the AT (Payne et al. 2017). To date, SWE has not been utilised to study the impact of an EcEx programme, therefore this study will be the first to do so.

This study had two main aims. Firstly, examine the effect of a 12 week EcEx programme on AT stiffness as measured with SWE alongside effects on symptoms and clinical outcome measures. This study aimed to trace changes in the PAT as well as the contra-lateral HAT of patients presenting with ATY. Secondly, this study assessed the timeline at which any changes in the stiffness of the AT occur, by taking regular measurements throughout the 12 week EcEx programme. The hypothesis is that the EcEx programme will result in improvements in the measured clinical outcome measures but also an increase in AT stiffness. Addressing these research aims will contribute greatly to the current body of knowledge in this area and the application of SWE to the monitoring of rehabilitation protocols. It may also help to optimise rehabilitation programmes and improve treatment of ATY and be applicable for many individuals who currently or may potentially suffer with symptomatic ATY.

7.3 Materials & Methods

7.3.1 Participants

A total of 21 participants, who presented with symptoms of mid-portion ATY (12 males, 9 females; mean age 58 ± 10.68 years, range 35 – 76 years; height 172.2 ± 8.5cm, range 158 – 183cm) were recruited from a sports medicine clinic located within the University of Brighton. Five participants had to be discontinued from the study during the 12 week EcEx programme due to either sustaining unrelated injuries or through unrelated hospital admissions, leaving a total of 16 participants (32 tendons) who finished the 12 week rehabilitation programme. These 16 participants were measured at weeks 0, 4, 8 and 12 from starting the EcEx programme.

The study received ethical approval from the University of Brighton tier 2 ethics committee. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards as outlined in the General Methods (section 3.1).

Before testing commenced, self-reported data was collected from the participants as outlined in General Methods (section 3.4). All participants completed a separate VISA-A questionnaire (section 3.4.1) for each AT to assess ATY symptoms bi-laterally. All participants also filled in a VAS (Visual Analogue Scale) (section 3.4.2) containing 4 questions to assess subjective levels of pain within both ATs. Each

question on the VAS is marked out of a maximum of 10, therefore the total VAS score in this study was a maximum of 40. All participants also underwent an initial B-mode US scan and to assess tendon abnormalities associated with ATY, the B-mode images were independently reviewed by an experienced sports medicine doctor (NW), with more than 15 years' experience in musculoskeletal US.

ATs were considered to be symptomatic if they met at least 3 of the following criteria, exhibiting anomalies consistent with ATY on US, exhibiting a maximum anterior-posterior (max AP) diameter greater than 6mm (Nunley, 2008), a VISA-A score <96 (Iversen, Bartels and Langberg, 2012) and/or a VAS score ≥3. After applying these criteria, 16 of the 32 tendons examined were considered symptomatic and in the PAT group and 16 ATs were considered to be in the HAT group. The application of the above criteria resulted in all participants having one AT in the HAT group (which did not meet 3 of the above criteria) and one AT in the PAT group as it met at least 3 of the above noted criteria.

Exclusion criteria for the study included pregnancy, recent surgical intervention (within the last 3 months) of the AT, participants already receiving additional treatment for their symptoms and any participants taking fluoroquinolone antibiotics. No participants were excluded from the study based on these grounds.

7.3.2 Methods

Prior to commencement of the main testing, participants attended an initial session at the research facility to meet the lead researcher (CP) and have an initial B-mode US scan taken which was used for review by an experienced sports medicine doctor (NW) to assess the AT for abnormalities consistent with ATY. This initial session was also utilised to fill out the VISA-A and VAS questionnaires, obtain written informed consent, and for participants to ask any further questions relating to the study. Following the initial meeting, participants attended the research facility for four testing sessions which took place at week 0, week 4, week 8 & week 12 during the EcEx programme, relative to when they first started the EcEx programme. The first visit at week 0 provided all the values subsequently used as baseline measures.

Within one week of attending the first visit at week 0, all participants attended an individual session with a senior Physiotherapist (ML) who demonstrated and provided instruction on the EcEx programme. The senior physiotherapist had complete

autonomy and clinical control of the starting level of the EcEx programme which is detailed in section 7.3.3. Participants were provided with written instructions and a link to a video demonstration of the exercises. No other interventions were used by the physiotherapist during the period of testing apart from the EcEx programme. The physiotherapist (ML) contacted the participants every 2 weeks to monitor progress. Adherence to the EcEx programme was monitored via the use of a 'google form' which participants logged into every time they completed their exercises. It recorded the number of exercises completed (straight leg, bent leg, sets and repetitions), rating of pain they experienced in their AT during the exercises and noted any other exercise they had been completing that day. These answers auto populated a Microsoft Excel (2010) spreadsheet for each participant which both the main investigator (CP) and Senior Physiotherapist (ML) had access to. These forms were checked at least once a week and each participant was contacted by either the main investigator or the senior physiotherapist if anything looked incorrect. Two participants chose not to fill in their exercise log this way, one filled it in by hand, providing this to the main investigator on a weekly basis, and the other filled in a Microsoft Excel (2010) spreadsheet which was then emailed to both the main investigator and senior physiotherapist weekly.

Participants attended for further testing when they had progressed through 4, 8 and 12 weeks of the EcEx programme. The measures taken at each time session included SWV (m/s), VISA-A, VAS, AT length (mm), AT max AP diameter (mm), Doppler score, range of motion (cm), muscular endurance and muscular power.

7.3.3 Eccentric Exercise Programme

The EcEx programme was only completed on the participants PAT. It called for the participants to complete their exercises for 12 weeks, twice a day (morning and evening) every day of the week. They were asked to complete two types of the same exercise in every session as shown in figure 7.1. They were first asked to complete the exercise whilst the knee of the injured leg remained straight (Figure 7.1: 1A, 1B & 1C) to maximise the activation in the gastrocnemius. To maximise the activation of the soleus muscle, they were also asked to complete the exercise whilst keeping the knee of the injured leg bent (as shown in Figure 7.1: 2A, 2B & 2C). Participants were advised to complete the exercises on a step which would allow their heel to drop below the level of their forefoot. From an upright body position standing on a step with all body weight on the forefoot and the ankle joint in plantar flexion, the calf muscle was

loaded by the participant lowering their heel down off the step, beneath the level of the forefoot. Participants were instructed to lower themselves down on the injured leg for a count of 3 seconds and to only load the calf muscle eccentrically, as no following concentric loading was to be completed. Instead, the non-injured leg was used to get them back to the start position again.

Initially, the loading element consisted only of the participant's body weight as they were standing with all their body weight on their injured leg. The participants were required to increase the intensity of the exercises on a regular basis, which was achieved by placing increasing amounts of weight in a backpack which they would wear on their back whilst completing the EcExs. The loading element was only increased once they could easily perform 3 sets of 15 repetitions of each exercise (knee bent and knee straight) twice a day for 3 consecutive days without experiencing any discomfort. The weight in the backpack was increased by 5kg at a time to gradually increase the load.

In the event of pain, participants were advised to remove the additional weight and return to the level they were using before the increase. They were advised to email the physiotherapist immediately should the increase cause them a level of pain on the VAS of 4/10 or more on VAS. Participants were advised that they may experience mild pain and discomfort when performing the exercises and that muscle soreness during the first 1 to 2 weeks of training is quite common. The pain they experience when completing the exercises should only rise to a level equivalent to 2-3/10 on the VAS scale, as discussed in section 3.4.2 of General Methods.

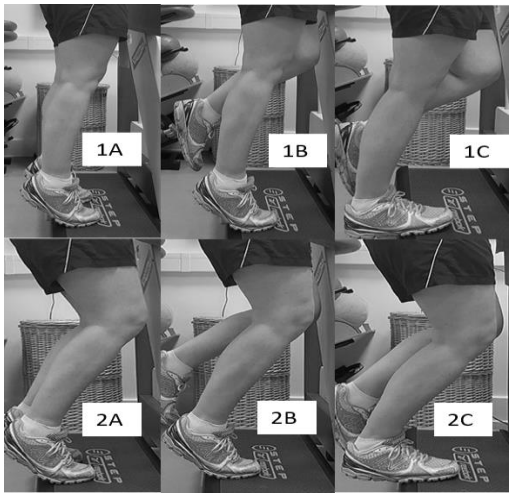


Figure 7.1 Illustration of the three positions (A, B & C) explained within the eccentric exercise programme.

** In Figure 7.1, 1 A, B & C show the leg straight whilst 2 A, B & C show the leg bent.*

For each of the two exercises shown above, the participants were asked to complete 15 repetitions of the exercise per set, to complete a total of 3 sets for each exercise in the morning and evening, and to rest for 1 - 2 minutes between sets. Two participants were initially not able to meet the requirements of 3 sets of 15 repetitions for both exercises, twice daily at the beginning of the programme due to fatigue and/or pain. Therefore the physiotherapist assessed the current level of each participant and instructed them to build up slowly from what they were able to do, working towards the 2 times 3 sets of 15 repetitions twice daily. This was to help increase compliance of the participants and help them to see progression in their exercises. The physiotherapist was able to advise the participants throughout the 12 week programme whether they should increase or decrease the sets, repetitions or weights of their exercises to ensure maximum compliance with the programme.

7.3.4 Scanning techniques

Four of the measures in this study were obtained using a Siemens ACUSON S3000™ HELX EVOLUTION Ultrasound System (Siemens Medical Solutions, USA). These measures include basic AT size measures (length and max AP diameter), Doppler signal measure and SWE. For these measures, participants lay prone on an examination table with both feet hanging clear of the end of the table and the procedures outlined in the General Methods (section 3.5) were followed. An

appropriate amount of US gel was applied to both ATs to maintain sufficient contact between the probe and the tendon before scanning took place of both ATs. All measures were taken by a single operator, the main investigator (CP).

7.3.5 Conventional Ultrasound Technique (Length and max AP diameter)

At every session, measures of AT length were made using a conventional US using a 14L5SP probe and measures of maximum AP diameter were taken with a 14L5 probe following the protocols outlined in the General Methods (section 3.5.1 & 3.5.2).

7.3.6 Power Doppler Signal Measure

Power Doppler (PD) measures were taken at every session using a 14L5 probe following the protocol outlined in the General Methods (section 3.5.3).

7.3.7 Shear Wave Elastography Technique

A shear wave elastogram enables a quantification of tendon stiffness by measuring the velocity of shear waves as they pass through the tendon, with higher velocities indicating a stiffer tendon. All SWE measures were taken in accordance with the protocol outlined in the General Methods (section 3.5.5).

7.3.8 Questionnaires (VISA-A and VAS)

Participants were asked to complete 2 questionnaires at the testing sessions regarding the symptoms in their ATs. These questionnaires are the VISA-A and VAS, chosen due to their inclusion as outcome measures in other similarly conducted research (Silbernagel et al., 2007). Both questionnaires were completed in accordance with the protocol outlined in General Methods (section 3.4).

7.3.9 Range of Motion

The range of motion (ROM) for ankle dorsiflexion motion in both participants ankles was measured using the knee to wall test, shown to be more reliable than using traditional goniometry (Konor and Morton, 2012). This test involved the participant placing their toes on a piece of tape near a wall and bending their knee against the wall whilst keeping their heel on the ground (See Figure 7.2). The tape gradually moves further back away from the wall and the distance from their toes to the wall is taken in cm to represent ROM.

Figure 7.2: Method for obtaining knee to wall Range of Motion test result

7.3.10 Muscular Endurance testing

A review article noted that calf raises as an exercise have been shown to elicit significant decreases in the free AT diameter (Obst, Barrett and Newsham-West, 2013), therefore the muscular endurance in the calf muscles of each participant was measured at each testing session, using the calf raise test. Participants were asked to raise up onto their toes from a flat surface as high as possible and return to a flat foot position and were asked to perform as many calf raises as possible in one go without stopping. The aim of this was to assess the muscular endurance of the muscles surrounding the ankle joint and attaching to the AT. The number of raises they were able to complete in one attempt without stopping was recorded as the result. Participants were asked to perform this test separately for each leg and to complete this exercise one leg at a time. The movement the participants perform during their EcExs and the calf raise test has been used in similar research (Silbernagel et al., 2001) where it showed a good level of reliability and was recommended as a functional test of muscular endurance within the calf muscles.

7.3.11 Muscular Power

This test measures how long the participants spend in the air when they complete a specific jumping action. Jumping tests are often the method of choice to provide a quantification of muscular capacity in terms of power (Meylan et al., 2009). Participants were asked to complete the test one leg at a time and commence the test standing on a 'Just jump' jump mat (Just Jump; Probotics, Alabama, USA). The participant starts in the upright position and bends the knee of the standing leg down to a squat position. Participants were verbally advised and encouraged during testing

to achieve a squat as low as comfortably possible for them, however this position was volitional, and the exact angle achieved was not measured. Following the squat, participants were instructed to immediately jump as high as possible. Similar tests have been utilised (Silbernagel et al., 2001; Morrissey et al., 2011) as an outcome measure of the effectiveness of eccentric training and the counter movement jump (CMJ) test when measured using a mat and a digital timer have been noted as the most reliable and valid field tests for the estimation of power in the lower limbs (Markovic, Izdar and Ukic, 2004). Single leg jump tests in particular are often used to determine return to sport or employment, to measure the effectiveness of rehabilitation and if there is a non-injured side, this can be used for comparison (Swearingen et al., 2011).

7.3.12 Statistical Analysis

All statistical analysis was performed using SPSS version 22 (SPSS, Chicago, Illinois). Measurements are expressed as mean ± standard deviation. Distribution of groups was analysed using the Shapiro-Wilk test and mean values for all subjects will be used as a summary measure for each variable. Repeated measures ANOVA's were conducted to assess differences if the data fulfilled the assumptions of normality. If not, appropriate non-parametric tests including Friedman's ANOVA, Wilcoxon signed paired matched-tests and Mann-Whitney Tests were utilised to assess significant differences in the data. Data was checked for sphericity with the Huynh-Feldt Correction applied if necessary and alpha level was set at $p<0.05$ throughout.

7.4 Results

Prior to any intervention, the mean combined bi-lateral VISA-A score for all participants before the EcEx intervention was 76.8 ± 17.9 (range 41 - 100) and the mean bi-lateral VAS score for all participants in the study was 3.7 ± 4.0 (range 0 - 13).

A total of 16 participants (32 tendons) finished the initial 12 week EcEx training programme, 16 tendons were classified as symptomatic PATs and 16 tendons as asymptomatic HATs. At week 0 (prior to any intervention), the PAT (n = 16) mean VISA-A score was 65.4 ± 12.0 (range 41 - 91), mean max AP diameter was 8.9 ± 2.0mm (range 5.5 - 13.3mm) and mean VAS score was 6.4 ± 2.6 (range 3 - 13). This compares with the HAT (n = 16), whose mean VISA-A score was 88.2 ± 12.8 (range 61 - 100), mean max AP diameter was 6.8 ± 1.2mm (range 5.1 - 8.13mm) and mean VAS score was 1.1 ± 2.8 (range 0 - 10).

The absolute values and ranges of values obtained for each measured time point for both the HAT and PAT groups are outlined in Table 7.1.

Table 7.1: Measured variables in HAT and PAT groups throughout EcEx programme.

	Week 0	Week 4	Week 8	Week 12
SWE HAT (m/s)	9.31 ± 0.55 (7.65 – 9.78)	9.37 ± 0.44 (8.28 – 9.80)	9.38 ± 0.42 (8.29 – 9.88)	9.39 ± 0.38 (8.25 – 9.77)
SWE PAT (m/s)	8.47 ± 1.04 (5.78 – 9.64)	8.67 ± 1.01 (6.00 – 9.70)	8.84 ± 0.93 (6.30 – 9.75)	9.07 ± 0.61 (7.74 – 9.80)
SWE Δ (m/s)	0.84	0.70	0.54	0.32
VISA-A HAT	88.19 ± 12.84 (61.00 – 100.00)	92.06 ± 7.18 (82.00 – 100.00)	97.75 ± 3.00 (92.00 – 100.00)	99.19 ± 1.83 (93.00 – 100.00)
VISA-A PAT	65.44 ± 14.94 (41.00 – 91.00)	78.63 ± 8.62 (63.00 – 93.00)	91.75 ± 10.17 (62.00 – 100.00)	95.00 ± 7.27 (73.00 – 100.00)
VISA-A Δ	22.75	13.44	6.00	4.19
VAS HAT	1.06 ± 2.82 (0.00 – 10.00)	1.06 ± 2.05 (0.00 – 6.00)	1.13 ± 1.67 (0.00 – 5.00)	0.47 ± 0.85 (0.00 – 3.00)
VAS PAT	6.41 ± 3.15 (3 – 13)	3.09 ± 2.12 (0 – 7)	2.63 ± 2.39 (0 – 7)	0.97 ± 0.92 (0 – 3)
VAS Δ	-5.34	-2.03	-1.50	-0.50
AT Length HAT (mm)	60.23 ± 11.43 (35.97 – 82.60)	60.72 ± 10.89 (40.10 – 82.20)	60.49 ± 10.90 (39.80 – 82.30)	60.45 ± 10.77 (39.80 – 81.80)
AT Length PAT (mm)	58.84 ± 9.90 (44.17 – 81.07)	58.91 ± 9.53 (45.40 – 80.30)	58.99 ± 9.73 (45.00 – 80.80)	59.13 ± 9.62 (44.70 – 80.70)
AT Length Δ (mm)	1.39	1.81	1.50	1.32
AT Max AP Diameter HAT (mm)	6.75 ± 1.19 (5.10 – 8.10)	6.74 ± 1.13 (5.10 – 8.10)	6.72 ± 1.06 (5.10 – 8.00)	6.71 ± 1.09 (5.10 – 8.10)
AT Max AP Diameter PAT (mm)	8.93 ± 2.11 (5.50 – 13.30)	8.79 ± 2.20 (5.20 – 13.60)	8.76 ± 2.78 (5.10 – 17.00)	8.68 ± 2.77 (5.10 – 16.90)
AT Max AP Diameter Δ (mm)	-2.18	-2.05	-2.04	-1.97
Doppler HAT	0.44 ± 0.96 (0.00 – 3.00)	0.44 ± 0.96 (0.00 – 3.00)	0.44 ± 0.96 (0.00 – 3.00)	0.38 ± 0.81 (0.00 – 2.00)
Doppler PAT	1.69 ± 1.40 (0.00 – 4.00)	1.69 ± 1.40 (0.00 – 4.00)	1.63 ± 1.54 (0.00 – 4.00)	1.31 ± 1.49 (0.00 – 4.00)
Doppler Δ	-1.25	-1.25	-1.19	-0.93

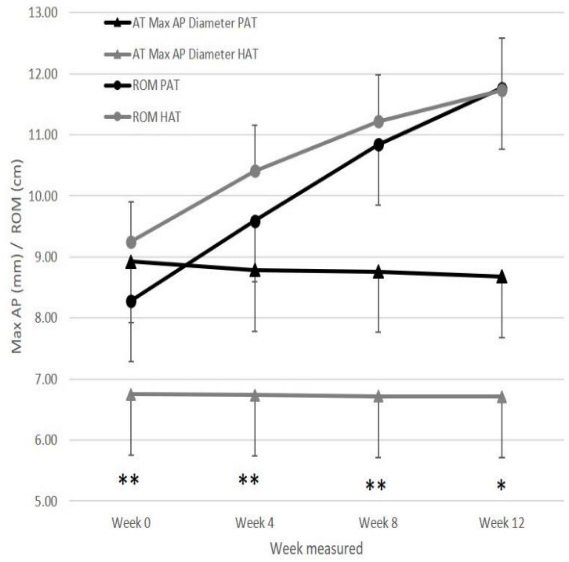
ROM HAT (cm)	9.25 ± 2.63 (6.00 – 14.00)	10.41 ± 3.01 (6.00 – 15.00)	11.22 ± 3.07 (6.50 – 16.00)	11.73 ± 3.39 (6.50 – 18.00)
ROM PAT (cm)	8.28 ± 2.47 (4.00 – 14.00)	9.59 ± 2.65 (4.00 – 14.50)	10.84 ± 3.33 (4.00 – 16.00)	11.77 ± 3.86 (4.00 – 18.00)
ROM Δ (cm)	0.97	0.82	0.38	-0.04
Calf Raise HAT	24.00 ± 9.18 (8.00 – 38.00)	24.88 ± 8.70 (10.00 – 40.00)	30.67 ± 10.86 (18.00 – 53.00)	45.60 ± 36.51 (20.00 – 171.00)
Calf Raise PAT	20.69 ± 7.36 (8.00 – 33.00)	25.44 ± 9.61 (12.00 – 45.00)	28.67 ± 11.21 (10.00 – 50.00)	46.40 ± 37.27 (18.00 – 171.00)
Calf Raise Δ	3.31	-0.56	2.00	-0.80
Vertical Jump HAT (cm)	18.60 ± 5.67 (5.70 – 28.10)	19.89 ± 5.76 (5.70 – 28.10)	20.83 ± 5.56 (8.20 – 28.10)	21.72 ± 6.07 (8.20 – 29.40)
Vertical Jump PAT (cm)	17.00 ± 6.12 (1.60 – 26.10)	19.21 ± 5.95 (4.10 – 26.50)	21.26 ± 6.24 (5.70 – 27.70)	21.85 ± 6.10 (5.70 – 28.10)
Vertical Jump Δ (cm)	1.59	0.69	-0.43	-0.13

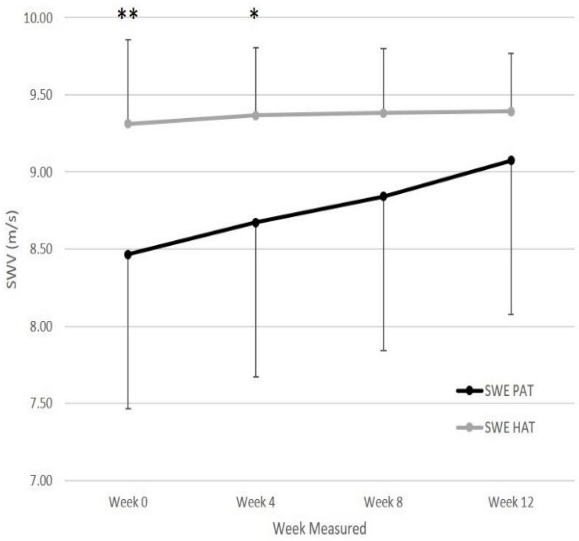
** Table 7.1 shows absolute values ± SD's and ranges with differences between the means (Δ) of all measured variables between the HAT and PAT group at the measured time points of the EcEx programme*

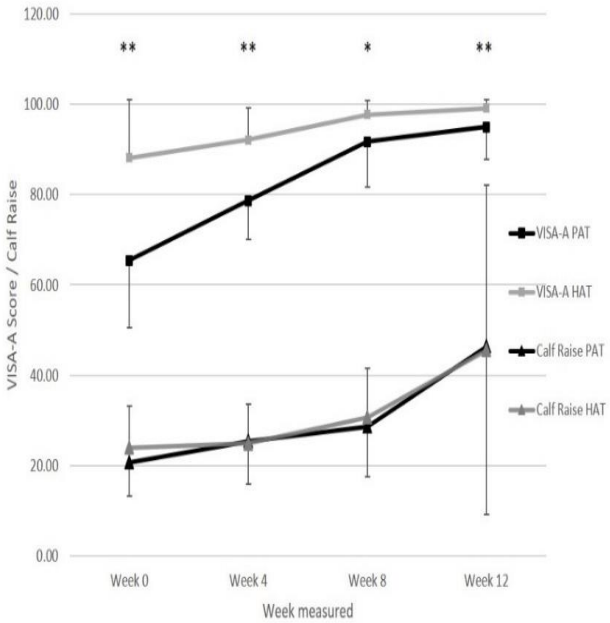
Table 7.1 shows the absolute values together with the mean differences between the measures taken from the HAT and PAT groups over the 12 weeks. A higher SWE measure and an increased VISA-A score can be seen throughout in the HAT group, although it is worthy to note that the differences in these scores between the HAT and PAT groups decrease throughout the 12 weeks. The VAS scores, max AP diameter and Doppler scores were all less in the HAT group in comparison to the PAT group, yet throughout the 12 weeks, the differences between the two groups becomes smaller.

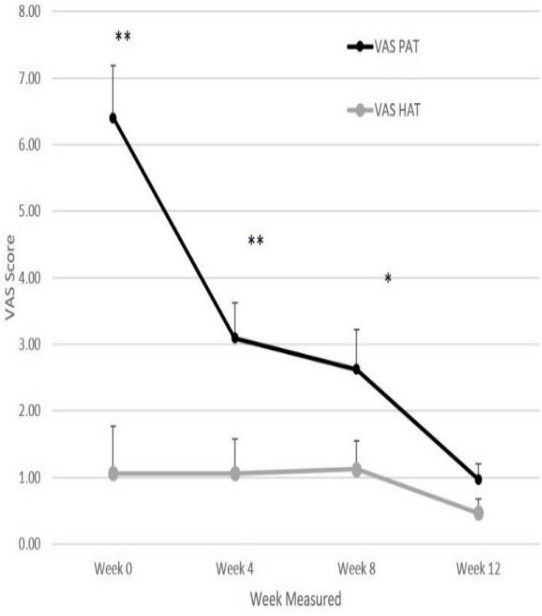
Figure 7.3 shows whether there were significant differences between the HAT and PAT groups for the measured variables at each time point. The significance values between the HAT and PAT groups decreases over time and of particular note is that the measures of SWE and VAS were significantly different between the HAT and PAT groups at week 0, but no longer had significant differences between them at week 12. This initial result agrees with previously published research suggesting that a 12 week EcEx programme is an effective treatment programme for the rehabilitation of mid-portion ATY (Silbernagel, Brorsson and Lundberg, 2011)

The results for each measured variable for both the PAT and HAT groups are outlined below.









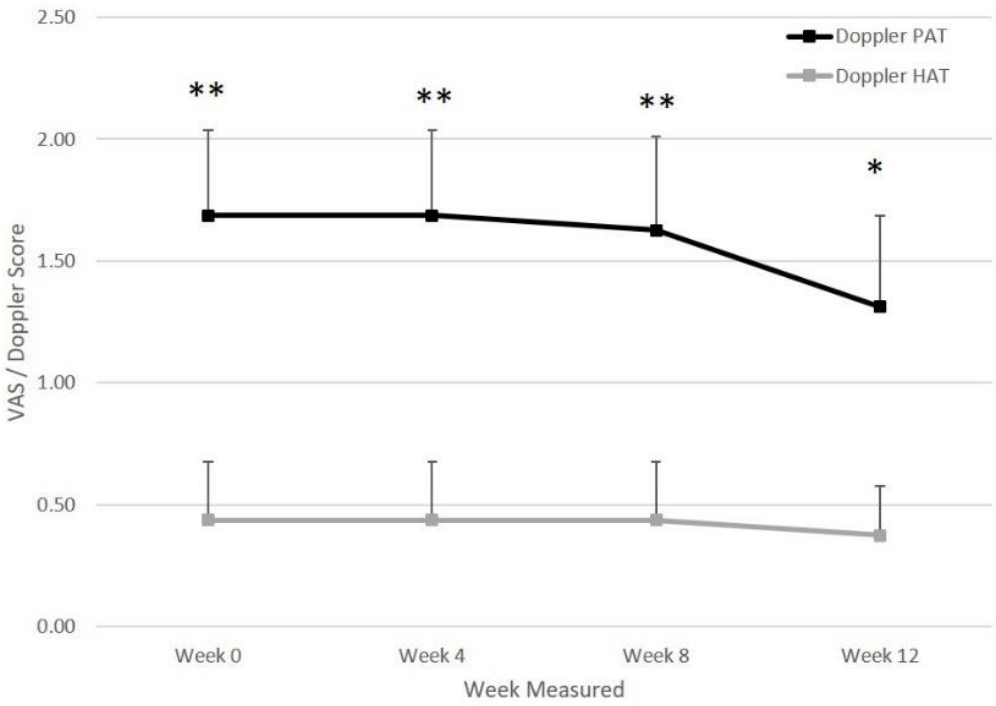


Figure 7.3: Comparison of means of measured variables for HAT and PAT groups

*Figure 7.3 shows comparison of means for each measured variable at each measured time point showing significant differences between the HAT and PAT groups. *p=<0.05, **p=<0.01.*

7.4.1 Stiffness measured with SWE

The range of SWV measures recorded for the HAT group ranged between 9.31 - 9.39 m/s over the 12 weeks, all within 0.08m/s of each other with no significant differences noted (p = 0.460). In contrast, the mean SWV measure for the PAT group increased significantly over the 12 weeks by a total of 0.6m/s (p = 0.001). The difference in SWV between the HAT and PAT groups was significantly different at week 0 at 0.84 m/s (p=0.004). The SWV for the HAT was significantly lower than PAT group at the start of the programme (p = 0.04) and up to week 4 of the treatment. Thereafter there was no significant difference in SWV between tendons as outlined in Table 7.2.

Table 7.2: Differences in SWV between PAT and HAT group

	HAT SWV (m/s)	PAT SWV (m/s)	Δ PAT vs HAT (m/s)	% Difference	P Value
Week 0	9.31	8.47	0.84	10.0%	P = 0.004 **
Week 4	9.37	8.67	0.70	8.0 %	P = 0.024 *
Week 8	9.38	8.84	0.54	6.1 %	P = 0.054
Week 12	9.39	9.07	0.32	3.5 %	P = 0.250

**=p<0.05, **=p<0.01*

Differences were noted in the SWV measurements of the PAT group over time (p=0.000), significant at $p < 0.01$ level between weeks 0-4, 0-8, 0-12 and 4-12. The difference between week 4-8 was significant at $p < 0.05$ level. The increase in SWV between weeks 8 – 12 was very close to significance (p=0.055) but fell just outside. Despite falling outside of significance, the difference of 0.54 m/s between the two groups was above both the TE and MDC of the machine as established in chapter 4. Table 7.3 suggests that by the end of the 12 weeks, the SWV for the PAT groups was significantly higher than at weeks 0 and 4.

When the alterations in the PAT group were analysed further, it was possible to trace the continuation in positive changes in SWV over the 12 weeks, despite the difference between the HAT and PAT group being non-significant. The results of this further analysis are shown in Table 7.3 and Figure 7.4 below.

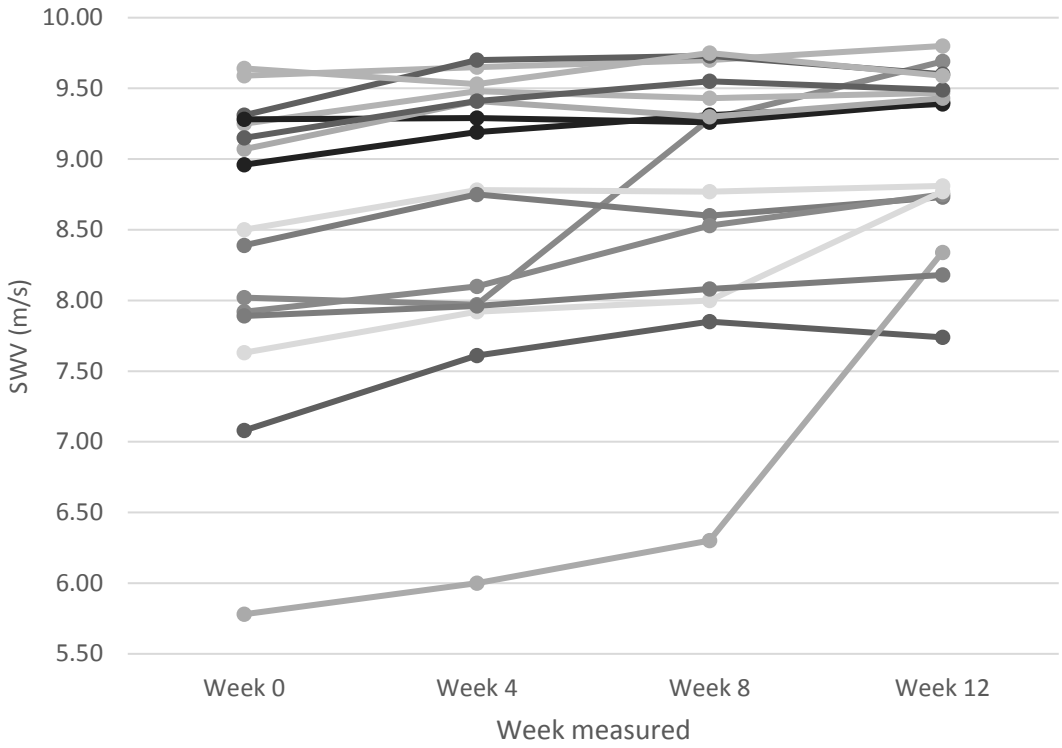


Figure 7.4: Individual SWV analysis of each PAT

** Figure 7.4 shows the individual trend analysis for SWV of each PAT at each measured time point.*

Figure 7.4 demonstrates that the magnitude of some increases in SWV were larger than others, however they also show that the SWV of the PAT group continued to increase after week 4, despite the differences between the PAT and HAT group no longer being statistically significant. The further analysis on the SWV alterations in the PAT group allowed an insight into individual changes throughout the EcEx programme as shown in Figure 7.4.

Table 7.3: Analysis of SWV alterations in the PAT group over the measured time point.

	No. of tendons increasing SWV	No. of tendons decreasing SWV	Mean increase (m/s)	Range of Increase (m/s)	Mean decrease (m/s)	Range of decrease (m/s)
Week 0 – 4	14 (87.5%)	2 (12.5%)	0.25	0.01 – 0.53	-0.08	-0.05 - -0.11
Week 4 – 8	11 (68.8%)	5 (31.2%)	0.28	0.03 – 1.31	-0.07	-0.01 - -0.15
Week 0-8	15 (93.8%)	1 (6.2%)	0.40	0.11 – 1.26	-0.02	-0.02 - -0.02
Week 8-12	12 (75.0%)	4 (25%)	0.35	0.04 – 2.04	-0.12	-0.06 - -0.16
Week 0-12	15 (93.8%)	1 (6.2%)	0.65	0.12 – 2.56	-0.05	-0.05 - -0.05

Table 7.3 indicates that the large majority of individuals experienced increases in SWV between every measured time point and the scale of these increases were much larger in comparison to the few that experienced decreases. The mean alterations in SWV in both the HAT and PAT groups are shown in Figure 7.5.

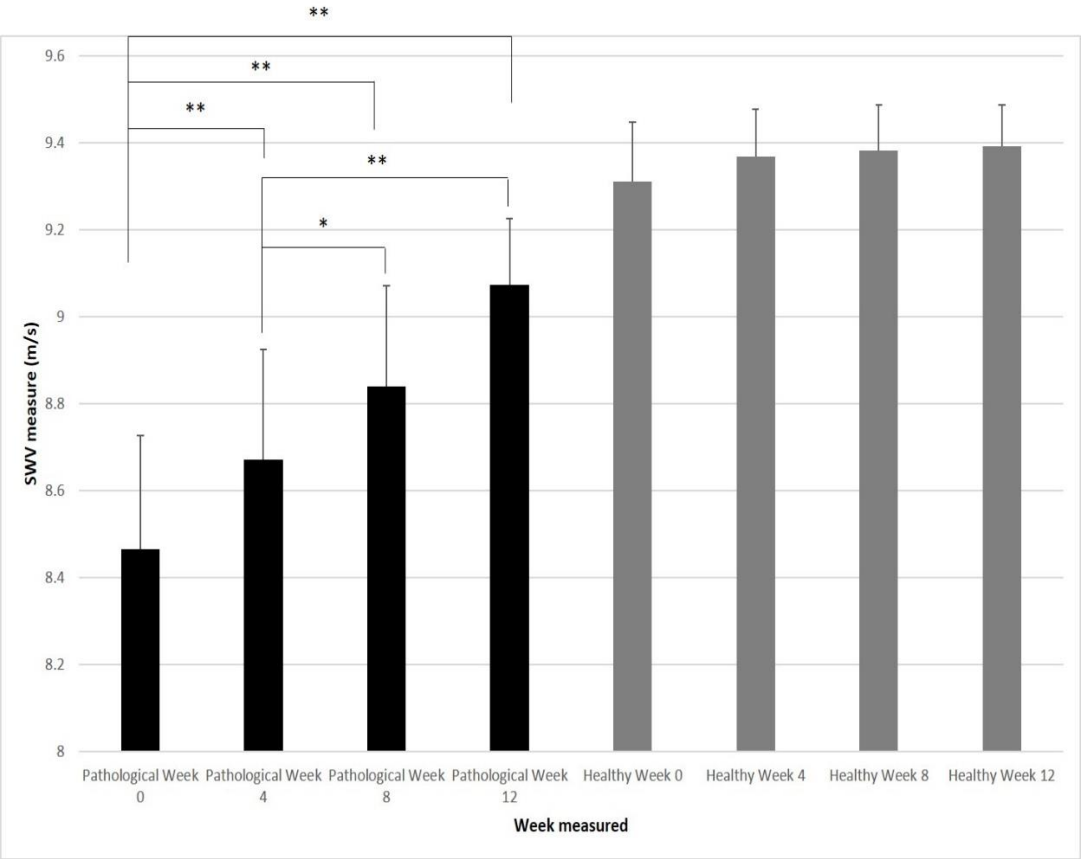


Figure 7.5: Alterations in SWV over EcEx programme

*Figure 7.5 shows AT stiffness measured by SWV (m/s) over the 12 week EcEx programme for both the HAT and PAT groups. *p=<0.05, **p=<0.01.*

7.4.2 VISA-A

The mean VISA-A score for the HAT group at week 0 was 88.19 ± 12.84 (range 61 – 100). Despite not completing the EcEx programme on this leg, as shown in Table 7.1, the VISA-A scores for the HAT group increased over the measured time points to a score deemed appropriate for a healthy AT (Iversen, Bartels and Langberg, 2012) by week 8. The VISA-A score of the HAT group reached 99.19 (out of a maximum of 100) by the end of the 12 weeks, a total improvement of 11 points, deemed clinically significant (Roos et al., 2004; Beyer et al., 2015). Table 7.1 also shows the improvement in VISA-A score for the PAT group which increased steadily over the 12 week period. By the end of the 12 weeks it had increased to 95 (out of 100 maximum), an overall improvement of 29.56 points, almost three times that experienced by the HATs with increases considered clinically significant between

each measured time point apart from week 8-12 as it was not possible for the score to increase by 10 points.

Significant differences were noted between the weeks depending on group (p=0.000) for both HAT and PAT groups. Significant differences between the HATs and PATs were found between each measured time point indicating that both the HAT and PAT groups experienced significant increases in VISA-A score over the 12 weeks. Despite the continued improvement of the VISA-A score in the PAT group, the difference between the two groups was statistically significant at week 0 (p=0.000), week 4 (p=0.000), week 8 (p=0.013) and remained significant at week 12 (p=0.007). Using the clinical significance value of 10 VISA-A points, the groups were clinically significantly different at weeks 0 and 4 but not at weeks 8 and 12 as the mean difference between the groups was less than 10 points, however a difference between the two groups of 4.19 VISA-A points remained at week 12. Despite the noted improvement in VISA-A, the score for the PAT group never reached a score achieved in other studies by healthy ATs of 96 (Iversen, Bartels and Langberg, 2012) and the mean VISA-A score of the PAT group was still lower than that of the HAT group after 12 weeks.

7.4.3 VAS

As shown in Table 7.1, the VAS score of both the HATs and the PATs reduces throughout the 12 weeks with the mean VAS score of the PATs reducing over the 12 weeks, by -5.44, a reduction over 9 times greater than that experienced in the HATs. No significant differences were found between the VAS scores at the measured weeks for the HATs (p=0.384), but significant differences were found for the PATs (p=0.000) with differences shown to be significant between week 0-week 4 (p=0.011), 0 and 8 (p=0.002), 0 and 12 (p=0.001), 4 and 12 (p=0.001) and 8 and 12 (p=0.003) as shown in Figure 7.6. The difference in VAS score between the HATs and PATs was significant at week 0 (p=0.000), week 4 (p=0.003) and week 8 (p=0.040), but by week 12, this difference was no longer significant (p=0.070) as shown in Figure 7.3.

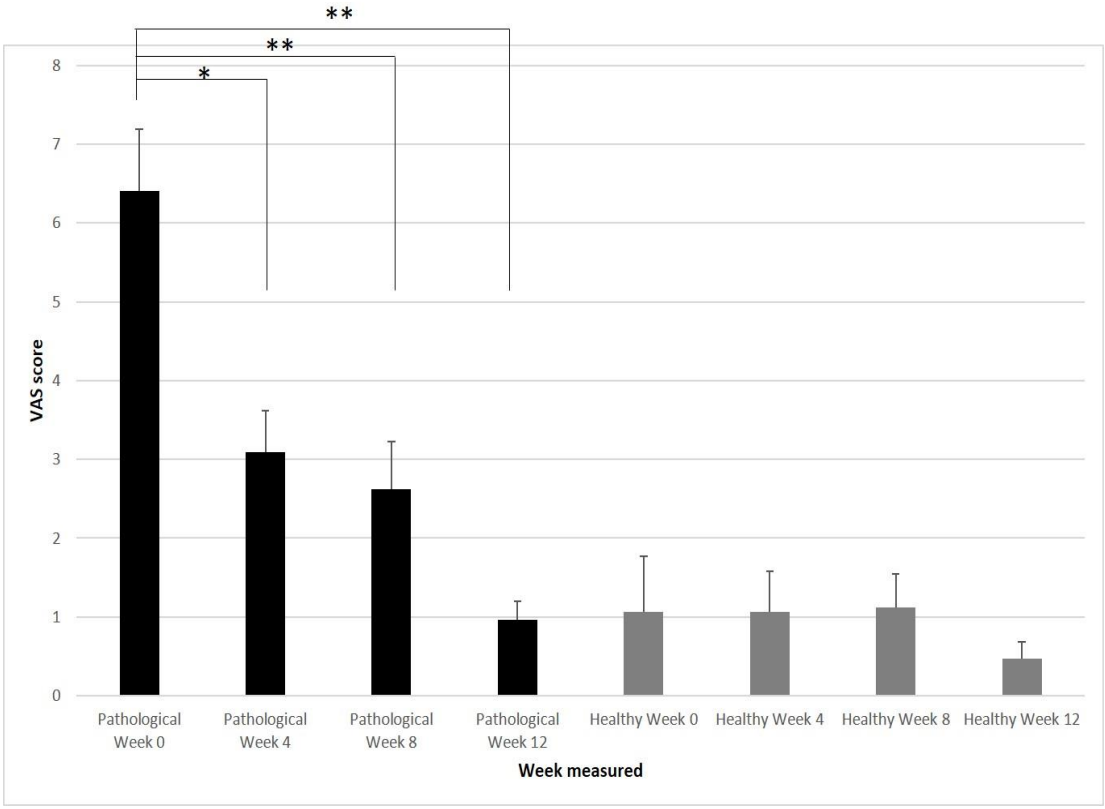


Figure 7.6: Alterations in mean VAS score for HAT and PAT groups

*Figure 7.6 shows mean VAS scores for HAT and PAT groups over the 12 week programme *= p <0.05, ** = p<0.01.*

7.4.4 Achilles tendon Length

The measurements for AT length for both the HAT and PAT groups did not show significant variations throughout the measured time points. A 2 Way RM ANOVA (Week (4) x group (2)) demonstrated no significant main effect of week ($p = 0.277$), and no significant interaction effect of time x group ($p = 0.361$), implying no significant variations in AT length occurred between the measured time points in either the HAT or PAT groups.

7.4.5 Achilles tendon maximum anterior-posterior diameter

Measurements of max AP diameter for the HATs ranged between 6.7mm and 6.8mm, all within 0.1mm of each other. In contrast, as seen in Table 7.1, the max AP diameter measurements for the PATs started at 8.9mm and decreased to 8.7mm over the 12 weeks, an overall decrease of 0.2mm. No significant differences were found over the measured time points for the HAT group ($p=0.747$), however significant differences were noted for the PAT group between every measured time point, indicative of a

steadily decreasing max AP diameter in the PAT group. The difference between the max AP diameter of the HATs and PATs was significant at week 0 (p = 0.001), week 4 (p = 0.005), week 8 (p = 0.009) and remained significant at week 12 (p = 0.017).

7.4.6 Power Doppler

The Doppler scores for the HAT group reduced over the 12 weeks (as shown in **Table 7.1**), by a score of 0.06 with the scale running between 0 (no neovascularisation) to 4 (heavy and multi-focal neovascularisation). The PAT group experienced a larger reduction in Doppler score over the 12 week period of 0.38. No significant differences were noted between the weeks for the HAT group (p=0.392), but significant differences were found for the PAT group (p = 0.025). Differences between weeks 0-12 and week 4-12 fell just outside of significance (p = 0.058), whereas the difference between week 8 – 12 was shown to be significant (p = 0.025). The difference in Doppler score between the HAT and PAT groups was significant at week 0 (p = 0.004), week 4 (p = 0.004), week 8 (p = 0.008) and remained significant at week 12 (p = 0.031).

7.4.7 Range of Motion

The ROM score of the HATs increased over the 12 weeks by 2.48cm and the ROM score of the PATs increased by 3.49cm, an increase of 1.1cm more than the increase experienced by the HATs. The measurements for ROM for both the HATs and the PATs were shown to experience significant variations throughout the measured time points with a significant main effect of week (p=0.000). The significant differences for both groups are shown in Table 7.4. The results imply that although the mean ROM for the PATs was almost 1cm lower than the HATs at week 0, by the end of the 12 weeks, the PATs had a ROM 0.03cm larger than the HATs. The differences between the two groups was not significant at week 0 (p = 0.291) and remained non-significant at week 12 (p = 0.960).

Table 7.4: Change in mean (Δ) ROM score for HAT & PAT groups and associated significance of change.

Weeks	Δ ROM HAT	P value	Significance	Δ ROM PAT	P value	Significance
0 - 4	1.16	P = 0.004	**	1.31	P = 0.002	**
0 - 8	1.97	P = 0.002	**	2.56	P = 0.002	**
0 - 12	2.47	P = 0.002	**	3.38	P = 0.001	**
4 – 8	0.81	P = 0.014	*	1.25	P = 0.020	*
4 - 12	1.31	P = 0.006	**	2.06	P = 0.008	**
8 - 12	0.50	P = 0.050		0.81	P = 0.059	

**p=<0.05 **p=<0.01*

7.4.8 Calf Raise

The calf raise scores for both the PAT and HAT groups increased as seen in Table 7.1. The score for the HAT group increased over the 12 weeks by 22 calf raises, despite the EcEx programme not being completed on this side. The PAT group also increased in the endurance of the calf muscles as the number of calf raises possible by week 12 had increased by 25 calf raises. The increase in the PAT group was higher than the HAT increase. Significant differences were shown between the weeks for both the HAT and PAT groups ($p = 0.000$). Significant differences ($p < 0.05$) for the HAT group were shown between all measured time points except week 0 and week 4 ($p = 0.377$). Significant differences were noted in the PAT group between all measured time points apart from week 4 – week 8 ($p = 0.197$). The differences between the two groups however was not significant at week 0 ($p = 0.151$) and remained non-significant at week 12 ($p = 0.940$). The above results imply that although the mean calf raise score for the PAT group was over 3 raises lower than the HAT group at the start of the programme (week 0), by the end of the 12 weeks, the PAT group were able to perform more raises than the HAT group.

7.4.9 Vertical Jump

The vertical jump data also suggests a large improvement in the strength available in the lower limb after completing the 12 week EcEx programme as shown in Table 7.1. The HAT group experienced an increase in jump height over the 12 weeks of 3.12cm and the PAT group by 4.85cm. Significant differences were shown to exist between the weeks for both the HATs ($p=0.008$) and the PATs ($p=0.000$). Significant differences were shown to exist between the weeks for the HATs between all

measured time points except for week 0 – 4 (p = 0.167) and week 4 – 8 (p = 0.050). Significant differences were shown for the PAT group between all measured time points except for week 8 – week 12 (p = 0.203). The differences between the two groups however was not significant at week 0 (p = 0.449) and remained non-significant at week 12 (p = 0.889). The above results imply that although the mean vertical jump height for the PAT group was measured as 1.59cm lower than the HAT group at week 0, by the end of the 12 weeks, the PAT group were able to jump 0.13cm higher than the HAT group.

7.4.10 Associations

During this time period (week 0 - 12), many of the outcome measures associated very well with each other. In particular, it would appear that the SWV measures correlate extremely well with the VISA-A scores and max AP diameter measures across the 12 week programme (p < 0.01). Figure 7.7 highlights the range and spread of the data between SWV measures, VISA-A and max AP.

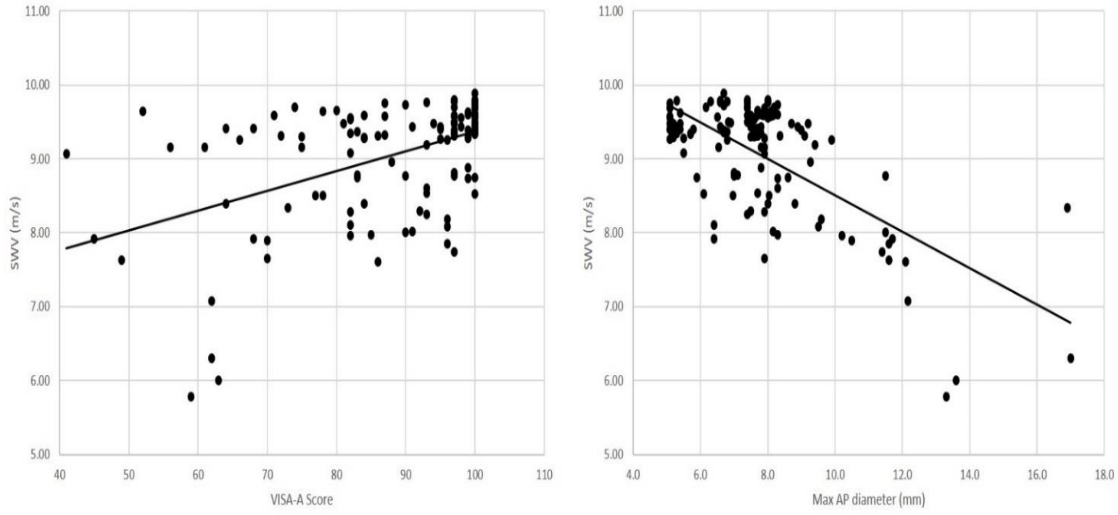


Figure 7.7: Correlations between SWE (m/s) and VISA-A score and Max AP Diameter (mm).

7.5 Discussion

The main aims of this study were to examine the effect of a 12 week EcEx programme on AT stiffness measured by SWE alongside commonly used symptom and clinical outcome measures in a symptomatic PAT. This study also traced the same measures in the asymptomatic, contra-lateral HAT for comparison purposes and examined the timeline over which any changes occurred. The primary findings from this study was

that a 12 week EcEx programme resulted in a significant increase in the PAT SWV, a measure of stiffness. After 12 weeks of EcExs, the originally symptomatic PAT group had increased measures of stiffness, felt better to the participant, had reduced max AP diameter, fewer blood vessels, an increased ROM and an increased endurance and power capacity in the surrounding muscles. The measured variables of VAS and SWV were significantly different between HAT and PAT groups at week 0, however the differences were no longer statistically significant by the end of the 12 weeks. The findings imply that the symptomatic PAT group were becoming both subjectively and objectively more like the HAT group in every measure and supports the use of EcExs as a treatment modality for ATY.

Table 7.1 in the results section provides answers to the second aim of this study which was to establish a timeline of when alterations occur over the 12 week EcEx programme. Of interest are the large changes experienced within the first 4 weeks of the programme in the subjective symptom measures of the PAT group (VISA-A and VAS). In the first 4 weeks of the EcEx, there was a significant increase in the VISA-A score of the PAT group of 20.0% coupled with a significant decrease in VAS score of 51.7%, suggesting the participants felt they had experienced a significant positive change in their PAT over this short space of time. Öhberg & Alfredson. (2004) suggest that pain can recede during the early part of an EcEx programme, but the normalisation of tendon structure may be more time consuming. Our current study agrees with subjective measures of pain subsiding early on during the programme and alterations to structure occurring later. Although various changes in structure and function were also occurring, they were to a much smaller scale and the tendon may not be at a level ready to take a large increase in load. The PAT group continued to improve in all measures over the whole 12 week period, taking them nearer to the levels found in the HAT group as time completing the EcEx programme progressed. The biggest changes in the subjective ratings of VISA-A and VAS scores occur at the start of the programme, however the largest changes in structural changes including Doppler and SWE occur between weeks 8-12 implying that in this study, structural changes were occurring at a later stage than alterations in subjective ratings of pain. Potentially this could cause issues if only subjective measures were assessed as participants might increase load at this early stage. Clinicians treating and individuals suffering ATY therefore need to be cautious when putting together a rehabilitation

programme for the AT that subjective improvement is not mistaken for positive structural change within the AT as it may not yet be able to take an increased training load without regressing.

There were significant increases in the VISA-A score, ROM, muscular endurance and muscular power of the asymptomatic contra-lateral HAT, despite the EcEx programme not being carried out on this side. The mechanisms behind this contra-lateral improvement are unknown, however speculatively, the increase could be attributed to the fact that the PATs improved in symptoms and function over the 12 weeks, as the participant was able to complete more activity, by virtue of which they experienced improvements in the HAT. Research has also shown that for patients with bilateral ATY, surgical treatment on one side resulted in pain relief bilaterally and no need for surgery on the initially non-operated side in 84.6% (n = 11 / 13) patients (Alfredson, Spang and Forsgren, 2014). The bi-lateral improvement was attributed to influences on sensory innervation in the peritendinous tissue in response to the surgery having secondary effects contralaterally (Alfredson, Spang and Forsgren, 2014). There is also a potential involvement of the central nervous system, but the authors only speculate about this (Alfredson, Spang and Forsgren, 2014). The improvements seen in the HAT group in this study may have been attributable to the fact that the participants could continue with any activities or exercises they chose to within the 12 week EcEx programme (including running), however only within the realms of mild discomfort (2/10 on VAS) and no pain. Other potential mechanisms include the fact that whilst performing the EcEx programme on their PAT, the participants were isometrically contracting their HAT which may have caused subtle improvements. It is important to note that all participants included in this current study were considered to have an active lifestyle and participated in recreational sport or activities, therefore are likely to have returned to activity quickly. This was an important aspect of the study as a previous study (Sayana and Maffulli, 2007) demonstrated that 44% of non-athletic patients (equivalent to 15 patients) did not improve with EcEx treatment.

The SWV of HAT group remained consistent over the 12 week EcEx programme, however the SWV in the PAT group increased significantly. As a tissue, tendons are metabolically active and can alter their mechanical properties in response to external

loading, however the effect of tendinopathy on tendon stiffness has not been specifically measured (Morrissey et al., 2011). Research using SWE has demonstrated the stiffness of ruptured ATs as 56.48 ± 68.59 kPa (Chen et al., 2013) as well as the stiffness of surgically repaired ATs which ranged from 187.7 ± 23.8 kPa at 12 weeks post operatively to 289.6 ± 23.4 kPa 48 weeks post operatively (Zhang et al., 2016). Only recently have figures for the stiffness of tendons symptomatic of ATY have been reported as 53.4 ± 23.3 kPa by Dirrichs et al. (2016) and 14.53 m/s (range 13.38 – 15.54 m/s) by Aubry et al. (2015). The measures obtained in this current study appear to be in between those previously reported as the stiffness measures of the PAT group were significantly lower than the HAT group at the start of the programme, by 0.84 m/s. Chapter 4 of this thesis determined a minimal detectable change (MDC) of 0.53 m/s and therefore the initial difference in the SWV of PAT and HAT groups and the increase of 0.6m/s shown by the PAT group over the 12 week EcEx programme would all be above the MDC, indicating a clinically meaningful change. By week 8, the difference between the SWV score of the PAT and HAT groups fell just outside of significance ($p = 0.054$), however the difference in absolute values between the group was 0.54 m/s, again above the MDC. By week 12, the difference was 0.32 m/s, not statistically significant or above MDC. It is possible that the total increase in tendon stiffness had improved the tendon's response to strain and may serve as a protective mechanism. Without this increase in stiffness, a given load may illicit an increase in tendon length, producing higher strain rates which may result in injury or rupture (O'Neill, Watson and Barry, 2015). A study by Mahieu et al. (2008) showed that 6 weeks of EcEx training did not significantly change AT stiffness (Mahieu et al., 2008), whereas Morrissey et al. (2011) demonstrated that 6 weeks of EcEx training decreased tendon stiffness (Morrissey et al., 2011). The differences in the findings of the impact of EcEx on tendon stiffness between this current study and that of Mahieu et al. (2008) and Morrissey et al. (2011) could therefore be attributed to the time length of the EcEx programme (12 weeks in the current study versus 6 weeks) as well as the cohort measured (pathological tendons in the current study versus healthy tendons).

Traditional approaches of measuring tendon stiffness use an isokinetic dynamometer or strain gauge to measure force and ultrasonography to measure tendon length. Studies into the impact of EcEx on tendon stiffness have employed this approach (Mahieu et al., 2008; Morrissey et al., 2011) A direct comparison between SWE and

direct measures of tendon stiffness *in vivo* has not yet been completed, though SWE as a methodology has previously been validated against traditional tensile testing in the *ex vivo* environment using swine muscle (Eby et al., 2013). Obtaining a measure of tendon stiffness using SWE is quicker, easier and requires less space and equipment in comparison to *in vivo* direct approaches, it is being increasingly utilised in research assessing tendon mechanical properties (Greenleaf and Urban, 2016). New research using SWE to assess tendon stiffness can improve our understanding of tendinopathy and rehabilitation not just in the AT specifically, but in other bodily tendons. A study examining the impact of an acute bout of heel drop EcEx specifically on the stiffness of the AT using SWE showed that just one bout produces significant increases in tendon stiffness (Leung, Chu and Lai, 2017). This suggests that SWE can register small, acute changes in stiffness and although extrapolation of this to longer term responses cannot be made with any certainty, it would be of interest to see the effect of longer term repetition of EcExs and whether they can create a stiffer, stronger tendon, more resistant to further injury. The results of this current study imply significant increases in SWV, and therefore stiffness of the AT experienced throughout a 12 week EcEx programme. Despite the significant increases, a difference of 0.32 m/s existed in the mean SWV score between PATs and HATs at the end of the 12 weeks. The mean SWV in the HAT and PAT groups at the start of the programme were 9.31m/s and 8.47 m/s respectively. Full recovery for this cohort in terms of SWV would be for the PAT group to increase their SWV by 0.84 m/s to reach parity with the HAT group. The PAT group in this study achieved a mean increase in SWV of 0.6 m/s over 12 weeks, equivalent to approximately 71% of this required recovery, or 6% per week. Assuming progress is linear, it would take a total of 16.7 weeks for the PAT score to reach parity with the HAT. It will be important for a clinician treating a PAT for ATY using EcExs to see how a typical value changes over the course of an EcEx programme. Table 7.3 indicates that 93.8% of PATs continue to increase SWV throughout the EcEx programme. Although by week 8, the values for SWV between the HATs and PATs were no longer statistically significant, the SWV increase shown between each measured time point (i.e. week 0-4, 4-8 and 8-12) by the PATs is of the same magnitude, between 0.25 – 0.35 m/s. This indicates a continual improvement in SWV throughout the 12 week EcEx programme.

The mean VISA-A score for the HAT group at week 0 was 88.19, which according to the test creators would count them as symptomatic (Iversen, Bartels and Langberg, 2012). This relatively low VISA-A score in the HATs may be due to reduced activity levels caused by their contra-lateral PAT. A study by Öhberg & Alfredson. (2004) suggested that the pain most PATs experience diminishes and mostly disappears during a 12 week EcEx training period, a result this current study would agree with. Significant improvements were noted in the subjective ratings of the PATs over the EcEx programme and by week 12, VISA-A scores had increased by 29.56 (45.2%) to 95 points and VAS scores had decreased by 5.44 (84.9%) to 0.97 points. At week 12, the scores in the PAT group for both VISA-A and the VAS were only 4.19 and 0.5 points away from the HAT scores. The results demonstrated an increase in VISA-A score of the PAT group between weeks 4-8 of 17% and over the whole 12 weeks of 45%, scores comparable to other research demonstrating an increase in VISA-A score between 4-8 weeks of 15.8% and 51.8% over 12 weeks (Herrington and McCulloch, 2007). A study assessing the impact of EcExs on a sedentary population noted an increase in VISA-A score over 12 weeks of just 11%, however the authors did note that this was unexpectedly low (Sayana and Maffulli, 2007), with the differences in population groups (active vs sedentary) likely to account for this difference. Changes in VISA-A and VAS scores have also been shown with a 12 week EcEx programme when applied to assessment of the patellar tendon (Kongsgaard et al., 2009) showing that its application is suitable for other tendons. Iversen et al. (2012) suggest that healthy subjects will score a minimum of 96 on the VISA-A score, however conclude that a score on VISA-A of above 90 points could be considered full recovery from ATY. Therefore, this study does show the PAT group reached full recovery at the end of 12 weeks. The mechanisms behind the efficacy of EcEx programmes for therapeutic treatment of tendinopathies are not clear, but one theory is that of pain habituation, which occurs as a result of completing weeks of pain provoking exercises (Rees et al., 2008). The continued practice of this pain provoking exercise results in the individual being habituated to the pain and therefore rating the pain they feel in their PAT as less. This alteration in pain perception may be beneficial in allowing an individual to complete their EcExs, however it should be noted that a reduced perception of pain may encourage an individual to increase activity and impose overload on the tendon. Despite the continued improvement of the PAT VISA-A scores over the 12 week programme, and the score reaching a figure above 90, by

week 12, a difference between the PAT and HAT groups of 4.19 points still existed. The implication of this is that although 12 weeks of EcEx can result in a significant increase in VISA-A score's, the score of the PAT group is still not as high as that achieved in the HAT group. Although the short term improvements (gained between weeks 0 – 12) that accompany EcEx programmes are known, relatively little is known about longer term responses (>3 years) (Van Der Plas et al., 2012). Van der Plas et al. (2012) evaluated the 5-year outcome of patients using EcEx and found that in the VISA-A score of all measured (n=58) had improved significantly from 49.2 at baseline to 83.6 at the 5 year follow up (Van Der Plas et al., 2012). However, in 48.3% of the treated tendons mild pain remained (Van Der Plas et al., 2012). In this current study, the largest percentage change in VISA-A scores for the PAT group occurred in the first 4 weeks of the EcEx programme, implying a successful programme could be monitored in the early stages by use of the VISA-A. In contrast, the percentage changes in the SWV scores in the PAT group in this current study measured 2.4% between weeks 0 – 4, 1.9% between weeks 4-8 and 2.6% between weeks 8 – 12. This implies that the percentage changes in SWV score, although occurring to a lesser extent, were consistent over the 12 weeks and the largest change in SWV score occurs during the last 4 weeks of the programme. Therefore, although VISA-A scores show significant improvements in the first few weeks of an EcEx programme, changes in SWV take significantly longer and should therefore be a cautionary note for clinicians to understand this relationship and avoid early overload based on improvements in symptoms. The implication of the novel finding of increases in SWE suggest that SWE could be a much more useful tool for the assessment of rehabilitation effectiveness towards the latter stages of an EcEx programme when improvements in symptoms are less. Therefore, the results of this current study have direct implications for clinical practice and use of SWE and when it may best be implemented in the monitoring of rehabilitation programmes.

During the 12 week EcEx programme, the max AP diameter of the HATs did not vary significantly over time. In contrast, the PATs underwent a statistically significant decrease of -2.7% in max AP diameter. This may indicate the actual tendon structure is altering in response to the EcEx, although it is important to note that a decrease of 2.7% remains a minimal change. The results of this current study suggest that significant reductions in the max AP diameter of the PAT group can be achieved over

the 12 week programme. Although decreases in the PAT group were consistent throughout the 12 weeks, the max AP diameter at week 12 was still 2.0mm and significantly larger ($p = 0.017$) in the PATs in comparison to the HATs. It would be interesting to follow patients beyond the 12 week programme to see whether the tendon diameter continues to reduce. A study by Van der Plas et al. (2012) termed a decrease in tendon diameter of 1.2mm following an EcEx programme to be a minor change and questioned the clinical relevance of such a small change. Therefore, the reduction in max AP diameter found in this study of -0.24mm over the course of the 12 weeks must also be regarded as small and potentially not of real clinical significance. Previous research into the effect of EcEx programmes suggest that they may normalise glycosaminoglycans concentrations within the tendon which might be expected to enable normalisation of the fibre arrangement (Ohberg and Alfredson, 2004). The result of this would be seen by a decreased tendon thickness due to a reduction in glycosaminoglycans and the associated water glycosaminoglycans attract, coupled with an alignment in tendon fibres. The content of glycosaminoglycan in the tendon was not measured in this current study and although a reduction in max AP diameter was shown in the findings, the decrease did not bring the value for max AP in the PATs to parity with the HAT group. Normalisation in tendon structure and subsequent reductions in tendon thickness have been correlated with a decrease in associated pain (Ohberg and Alfredson, 2004), which was also shown in this current study. The max AP diameter values for the HATs and PATs at the start of the 12 week programme were 6.75mm and 8.93mm respectively, therefore full recovery in terms of max AP would be for the PAT group to decrease their max AP by 2.18mm. The PAT group in this study achieved a mean reduction in max AP of 0.24mm over 12 weeks, equivalent to approximately 11% of this required recovery, or 0.92% per week. Even if progress was linear, it would take just over 9 times the 12 week programme (approximately 108 weeks) for the PAT score to reach parity with the HAT score and achieve the decrease of 2.18mm. This is in comparison to the 16.7 weeks required for SWV in the PAT group to reach parity with the HAT group assuming linear progress. Progress is unlikely to be linear due to issues with adherence over this length of time, issues with being able to continually increase the weight to ensure the effectiveness of the programme, therefore it is unlikely max AP diameter of a PAT will recover to parity with a HAT.

A significant decrease of 22% in neovascularisation (Doppler score) was noted in the PAT group following completion of the 12 week EcEx programme. The presence of neovessels at week 12 was shown in both the PAT and the HAT group. Sengkerij et al. (2009) examined neovessels in asymptomatic tendons and found that 29% (5/17) had a degree of neovessels, a figure comparable to this current study that showed 19% (3/16) of HATs had a degree of neovessels at 12 weeks. The mechanism behind why the HATs demonstrated neovessels in the current study was not known and Sengkerij et al. (2009) also state that whether the neovessels were a physiological phenomenon or representative of early pathology was not known (Sengkerij et al. 2009). Physical activity has also been shown to result in the visible presence of neovessels (Boesen et al., 2006) and is a postulated part of tendon repair (Movin et al., 1997), therefore the presence of vessels in the HAT group, could to a certain extent be attributable to repair from prior activity.

The research of Öhberg & Alfredson (2004) demonstrated that at a mean follow up of 28 months (range 3 – 48 months) after completing a 12 week EcEx programme, a good clinical result (defined as no tendon pain during loading) was demonstrated by 36/41 tendons. Of these 36 tendons, 32 had no remaining neovascularisation (Ohberg and Alfredson, 2004). This current study demonstrated a reduction in neovascularisation for the PATs, however some vessels (average score was 1.31 vessels) were still present at the end of 12 weeks. Differences between the work of Öhberg & Alfredson (2004) and this current study include the use of power Doppler in this current study versus colour Doppler, the scoring system for vessels used and the time of follow up which may explain some of the differences in the findings. Another study showed patients with chronic painful Achilles tendinosis had vascular in-growth (neovascularisation) in the area with tendinosis, but that during eccentric loading, the blood flow in the area with neovascularisation stopped acutely (Öhberg, Lorentzon and Alfredson, 2001). Öhberg et al. (2001) postulate that the decrease in tendon thickness experienced by those completing EcEx programmes, may be due to reduced neovascularisation in the tendon induced by the EcEx, although this was only hypothesised and not actively measured. A temporary interruption of blood flow in the tendon neovessels was also shown to occur during each EcEx sequence (Rees, Wolman and Wilson, 2009) as ankle dorsiflexion occludes the blood flow to the neovessels in the area (Knobloch et al., 2007). Rees et al. (2009) suggest one possible reason for EcEx programmes being

shown to reduce neovascularisation is that the EcEx programme requires more than 15,000 repetitions of this exercise over the 12 week period causing repeated short term blood flow interruptions (Rees, Wolman and Wilson, 2009). Although the exact cause of AT pain is still unknown, research suggests that all painful tendons demonstrate the presence of neovascularisation (Van Snellenberg, Wiley and Brunet, 2007). When an individual presents with a painful AT, the internal damage to the tendon may already be quite advanced (Cook, Feller and Bonar, 2004) as the development of ATY may have already taken place. The Doppler scores in this current study remained significantly different between the HAT and PAT groups throughout the 12 week programme with a mean difference of -0.94 between the groups at week 12. This implies that although significant decreases in neovascularisation were experienced in the PATs over the 12 weeks, the reductions did not lead to the PAT scores reaching values of those of the HATs. A study by Van der Plas et al. (2012) examined patients at a five year follow up from an EcEx programme, finding that 54% of the patients who did not have any pain symptoms at the follow up still demonstrated some degree of neovascularisation and therefore this aspect of the PAT may not always return to 'normal'.

Both the HATs and PATs experienced significant increases in the scores for their ankle ROM, muscular endurance of the calf muscles and muscular power of the lower limbs (assessed with vertical jump) over the 12 weeks. The increase in ROM is in agreement with previous related research showing an increase in dorsiflexion ROM with EcEx training measured using goniometry (Mahieu et al., 2008). Silbernagel et al. (2001) did not find a significant difference in ROM between a control group and a group performing EcExs, but did show an increase in plantar flexion in patients with ATY following a 12 week eccentric overload programme measured using goniometry with the participant in a supine (non-weight bearing) position (Silbernagel et al., 2001). Research into the effects of EcEx on the *in vivo* hamstring muscles shows that eccentric training can alter the length-tension relationship of the muscle and result in increased muscle lengths due to sarcomerogenesis (Brockett, Morgan and Proske, 2001) which is the addition or increase in the number of sarcomeres in series (Williams and Goldspink, 1978; O'Neill, Watson and Barry, 2015). This can be responsible for increasing the compliance of the muscle fibres (Mahieu et al., 2008) and increasing fascicle length (Franchi et al., 2016), thus allowing the muscle fibres to operate at

longer lengths and increase the overall range within which the muscle can work (Brockett, Morgan and Proske, 2001). A 10 week EcEx programme significantly increased the fascicle length of the vastus lateralis by approximately 14% (Sharifnezhad, Marzilger and Arampatzis, 2014) and although the study reported the underlying mechanisms explaining longitudinal growth of the muscle were not well known, the length increase was again attributed to sarcomerogenesis stimulated by structural damage caused by the EcEx (Sharifnezhad, Marzilger and Arampatzis, 2014). Another study demonstrated that EcExs develop greater distal growth within the muscle rather than mid-belly changes (Franchi, Reeves and Narici, 2017), but suggest EcExs can increase muscle length. Given the lack of alterations in AT length seen in this current study, the increases in ROM are more likely attributable to sarcomerogenesis and an increase in the muscle fibre length of the gastrocnemius and soleus muscles, although muscle length was not measured in this study. The work of Kubo et al. (2001) reported that stretching of the gastrocnemius can reduce the viscosity of the muscle-tendon unit and increase ROM at the ankle joint (Kubo et al. 2001). The movement performed during the EcExs requires the individual to mobilise the ankle to the end of its ROM, thereby repeatedly stretching the muscle-tendon unit over the 12 week period. This may have decreased the viscosity of the unit as a whole and allowed for an increase in ROM as the repeated stretching and contraction of the muscle-tendon unit results in it becoming more compliant *in vivo* (Kubo et al. 2002). Whether increases in compliance are acute or chronic, it has been said that they will theoretically result in the tendon being able to absorb more energy and reduce injury risk (Witvrouw et al., 2004). The previous research in this area and the results for ROM obtained from this current study highlight the ability of a 12 week EcEx programme to increase ROM at the ankle joint. Another consequence of an increased muscle fibre length is an increase in maximal mechanical power (Sharifnezhad, Marzilger and Arampatzis, 2014) which was also shown in this current study.

It was suggested by a study in the 1980's (Stanish, Rubinovich and Curwin, 1986) that EcEx was able to provide a greater load to tendon than concentric exercises, which led to EcEx being proposed as the best way to strengthen a tendon. EcEx training is proposed to significantly increase calf muscle strength (Alfredson et al., 1998) and is an effective training option for improving muscular strength (Hoang, Herbert and Gandevia, 2007), providing greater strength increases and hypertrophy in comparison

to concentric training (Franchi, Reeves and Narici, 2017). Research suggests that it is not just the magnitude of force applied to the tendon which causes the beneficial effects of EcEx, but that it is due more to the fluctuations in force experienced and the pattern of loading and unloading which the tendon undergoes during EcEx that provides the stimulus for tendon remodelling (Rees et al., 2008). Just six weeks of EcEx training increased muscle strength measured with dynamometry (Keles et al., 2014) and 12 weeks of heavy slow resistance (HSR) training significantly increased the cross-sectional area of the quadriceps muscles (Kongsgaard et al., 2010). Increases in cross-sectional area following resistance training are attributed to the increased time spent under tension (Seynnes et al., 2013) with the increased area resulting in overall increased strength as seen in the results of this current study. This current study didn't explicitly measure muscle hypertrophy or directly measure strength; however, it did assess muscular endurance (via the calf raise test) and muscular power (using a vertical jump). Possible increases in muscle fibre length could account for power increases, and the sheer volume of training and high repetitions required by the EcEx programme are potentially responsible for the significant increases in the calf raise test. In this current study, the PATs increased the number of calf raises they were able to perform to an average of 0.80 raises above that capable from the HATs, implying that the 12 week EcEx programme was sufficient at returning muscular endurance to parity with a HAT.

The greater distal muscle growth elicited by EcEx in comparison to other training methods (Franchi, Reeves and Narici, 2017), potentially alters tendon mechanical properties. Plyometric training involves an element of EcEx, and increases in tendon stiffness together with changes in neural activity have been shown to occur following plyometric training (Hirayama et al., 2017). These adaptations improve the function of the muscle-tendon unit as a whole, improving performance in movements of the stretch-shortening cycle (Hirayama et al., 2017). Although neural activity was not assessed in this study, measures of tendon stiffness demonstrated an increase in stiffness, also shown in the work of Hirayama et al. (2017). Muscle fascicle length was not measured in this current study, however an increase in tendon stiffness coupled with the possible increases in fascicle length referred to above (Franchi et al., 2016) could increase the rate of force development within the muscle-tendon unit, leading to increases in power. This would be shown by an increase in vertical jump

height, which was shown in the PATs to significantly increase by 28% (4.85cm). Despite the starting figure for jump height in the PATs being over a cm less than the HATs, the increase in jump height experienced in the PATs resulted in them being able to jump 0.13cm higher than the HATs at week 12. The difference between the HATs and PATs was not significant throughout the testing, however the obtained values became closer throughout the 12 weeks, demonstrating the ability of the 12 week EcEx programme to increase power in the lower limb. Increases in power capacity as assessed by vertical jump height have also been shown following plyometric training which incorporates eccentric movement (Kubo et al., 2007). Mahieu et al. (2006) assessed the isokinetic ankle muscle strength of army recruits prior to their basic training. The results demonstrated that a value below 50Nm was 85% sensitive for predicting whether ATY would develop in the recruits. The authors attributed the higher AT injury risk to a lower plantar flexor strength resulting in the muscle-tendon unit being able to absorb less force (Mahieu et al., 2006). Therefore, the strength in the muscles surrounding the ankle joint can influence injury risk, with stronger muscle-tendon units at a lower injury risk.

SWE measures correlate very well with VISA-A scores over all the measured time points (as seen in figure 7.7) suggesting a potential relationship between pain and mechanical properties. This implies that as the VISA-A score increases, and pain consequently decreases (with VISA-A score moving towards the maximum possible score of 100), stiffness (measured by SWV) increases. Links between pain and tendon structure have previously been examined (Drew et al., 2014), however with conflicting results. The work of De Vos et al. (2011) suggested no improvement to tendon structure (assessed with UTC) following EcExs in chronic midportion ATY, however in a related study they also show an improvement in VISA-A score with EcEx (de Vos et al., 2010). This implies a lack of connection between pain and structure, however Öhberg et al. (2004) suggest a normalisation of tendon structure (assessed by the presence of hypoechoic areas and irregular structure seen on US) is associated with a decrease in pain following EcExs (Ohberg, Lorentzon and Alfredson, 2004). In a separate study, Öhberg & Alfredson (2004) propose an association between tendon structure and pain during AT loading, but conclude that they cannot explain the mechanisms by which tendon structure normalisation is achieved. SWE was also shown to correlate well with Doppler scores in this current study. Öhberg et al. (2004)

proposed that areas in the AT with structural changes (assessed by US) could trigger neovascularisation, but the authors do not explore any possible mechanisms for this. Evidence for optimising treatment and recovery is sparse. For example, questions remain regarding the speed of the movement during EcEx programmes and whether a faster eccentric movement may increase the force fluctuations on the tendon, resulting in an even larger tendon remodelling stimulus (Rees et al., 2008). Whether the load, frequency, number of repetitions and overall duration of the commonly used 12 weeks are optimal, also remains unknown. Some have suggested that the optimal protocol for using EcExs for mid-portion ATY has yet to be established (Murtaugh and Ihm, 2013). In this current study, after 12 weeks, significant differences remain between the HAT and PAT groups in the measured variables of max AP diameter, VISA-A and Doppler score. This indicates that the PAT group hadn't yet reached full parity with the HAT group and that completing the EcExs for longer may provide additional benefits.

This study is the first to trace alterations in AT stiffness as measured by SWE over the course of a 12 week EcEx programme in both HATs and PATs, assessing stiffness measures in conjunction with other more traditionally utilised clinical outcome measures. Improvements in measures of clinical outcomes were shown in this study including a reduction in max AP diameter, Doppler score and VAS together with increases in VISA-A score, ROM, muscular endurance and muscular power. Most of these have been shown in other studies, however this study also shows an increase in SWV (and hence AT stiffness) experienced by the PATs which was absent in the HATs. The relatively new measure of SWE is not yet commonly utilised in clinical assessment and monitoring, however as it has been shown to be a valid and reliable technique (Petrescu et al., 2016; Rosskopf et al., 2016; Zhang et al., 2016). Given how well it correlates with traditionally used clinical measures in this present study, there should be further studies conducted to show its effectiveness in clinical assessment and monitoring of symptomatic tendons.

As with all studies, this study carried some limitations. Firstly, it was completed with a small sample size, however previous research into the impact of EcEx programmes (Fahlström and Jonsson, 2003; Ohberg, Lorentzon and Alfredson, 2004; Shalabi et al.,

2004; Herrington and McCulloch, 2007; Knobloch, 2007; Langberg et al., 2007; Sayana and Maffulli, 2007; Chester et al., 2008) have used an average of approximately 34 participants per study (range 12 - 78). Therefore, the participant number utilised in this study is comparable to others. Another limitation is the lack of a control group, but as it was deemed ethically unjustifiable to leave a symptomatic individual untreated, the contra-lateral HAT of each participant acted as its own control. The primary function of this study was to assess AT stiffness throughout an EcEx programme, so measures including muscle fascicle length, neural activity or biochemistry measures were not taken which may have added additional information and rationales for the findings. This research focused purely on the AT and on EcExs, whereas the impact of differing rehabilitation protocols on differing tendons in the body should also be examined.

In summary, when a PAT completes an EcEx programme, measures of mechanical property within a PAT reach a value not considered statistically significant by 8 weeks, however other measures may take longer. After 12 weeks, the measured variables of VAS and SWE, which at the start of the programme were significantly different between the HAT and PAT groups were no longer significant. These variables had still not reached full parity with the HAT group scores and the measured variables of VISA-A, max AP diameter and Doppler score appeared to lag and remained significantly different in the PATs in comparison to the HATs. This raises several questions including whether the subject was to stop the rehabilitation at 12 weeks, would the tendon regress? Can the variables within a PAT that remain significantly different return to that shown by a HAT and if so, when?

In conclusion, a 12 week EcEx programme is very effective as a conservative treatment modality for symptomatic AT and results in an increase in AT stiffness measured using SWE.

8 Alterations within the Achilles tendon following cessation and resumption of an eccentric exercise programme: Impact on clinical outcome measures and tendon stiffness measured with shear wave elastography.

8.1 Abstract

The positive alterations that occur within a pathological Achilles tendon (PAT) after completing an Eccentric Exercise (EcEx) programme as treatment for symptomatic Achilles tendinopathy (ATY) are well documented. At the end of an EcEx programme, some subjective and objective outcome variables remain significantly different in comparison to a healthy, asymptomatic tendon (HAT). Questions were therefore raised as to whether any alterations occur when the EcEx loading is suddenly removed and whether the measured outcome variables of PATs can resemble those HATs, and if so, when. A cohort of 11 patients who had recently completed a 12 week EcEx programme as part of their treatment from symptomatic ATY were contacted after they had stopped completing the EcExs at week 12. They were asked to return and have repeat measures taken at week 16 (4 months) and to resume the EcExs at this point. Further measures were taken at week 20 (5 months) and week 28 (6 months) after initially starting the EcEx programme. Measures included AT stiffness using shear wave elastography (SWE), VISA-A score, AT length (mm), AT maximum anterior-posterior (max AP) diameter (mm) and neovascularisation. There were no significant differences shown in AT length or max AP for either the HAT or PAT group over the 6 months and the HAT group experienced no significant alterations in SWE or VISA-A. In contrast the PAT group experienced significant increases in stiffness and VISA-A. The AT stiffness in the PATs decreased when EcExs were stopped, followed by significant increases between weeks 16-28 of 0.42 m/s when further EcExs were performed. The VISA-A score of the PATs decreased (-9.41 points) after cessation of the EcEx programme but increased significantly by 11.43 points when EcExs were resumed. At the end of 28 weeks, the differences between the mean values of the HAT and PAT group remained significantly different for VISA-A score (4.25 points), max AP (-2.27mm) and neovascularisation score (-1.13). At week 28, there were no statistically significant differences between the HAT and PAT groups for measures of AT Length or SWE. The VISA-A score, max AP diameter and neovascularisation score of a PAT remain significantly different to that shown in HATs after 6 months of completing EcExs. The removal of the mechanical

loading elicited by a 12 week EcEx programme results in regression of both subjective and objective measures within a symptomatic PAT. Resumption of the EcExs re-initiated positive adaptations within the PATs and reduced differences in VISA-A and SWE between HATs and PATs to 4.3% and 1.2% respectively, suggestive of the need to increase the duration of the traditional 12 week EcEx programme duration or add in a tapering period.

8.2 Introduction

Eccentric exercise (EcEx) programmes are an integral component in the treatment of symptomatic Achilles tendinopathy (ATY) (Sussmilch-leitch et al., 2012) and have a very high reported success rate (82 – 100%) as an intervention for athletes (Alfredson et al., 1998; Mafi, Lorentzon and Alfredson, 2001; Fahlström and Jonsson, 2003; Ohberg, Lorentzon and Alfredson, 2004). Study 4 (chapter 7) assessed the impact of a 12 week EcEx programme used as rehabilitation from ATY and indicated that EcExs initiated positive changes in the symptomatic pathological Achilles tendon (PAT). This resulted in measured values becoming both subjectively and objectively more like the values achieved on the contralateral healthy asymptomatic tendon (HAT).

The initial 12 week EcEx programme initiated positive and statistically significant alterations within the PAT group including the novel finding of significant improvements in the measured variable of shear wave velocity (SWV), indicative of increased AT stiffness. Despite these positive results, the conclusion of study 4 (chapter 7) resulted in many important questions remaining unanswered. The highest mean SWV recorded in the PAT group was 9.07 m/s, recorded at week 12, however, this figure was still 0.24 m/s below the lowest mean SWV recorded for the HAT group which was 9.31 m/s recorded at week 0. By the end of the 12 week EcEx programme, measures of maximum anterior-posterior (max AP) diameter, and neovascularisation score as well as the subjective rating of VISA-A (Victorian Institute of Sport Assessment – Achilles) were all still significantly different between the PAT and HAT groups. This resulted in questions being raised as to whether it was possible for these measured variables to resemble those of the HAT group and if so, when and led to a continuation of monitoring.

The literature examining EcEx programmes highlights many questions remain concerning how EcEx protocols might be optimised. Some query whether the speed

of the movement is optimal (Rees et al., 2008), some query the frequency and duration of the programme and note that the optimal EcEx programme for mid-portion ATY is yet to be established (Murtaugh and Ihm, 2013). No available literature documents the impact on AT stiffness of completing an EcEx programme for longer than 12 weeks. The lack of available research assessing the impact of EcEx programmes on AT stiffness meant that study 4 (chapter 7) was the first to use SWE to document AT stiffness throughout an EcEx programme and that no data was available on what happens to AT stiffness when the loading of the EcEx programme is removed. This is in conjunction with the results from study 4 (chapter 7) indicating a difference of at least 0.24 m/s between the highest SWV value in the PAT group and the lowest recorded SWV in the HAT group. There was also the remaining presence of significant differences in other measured variables of VISA-A, max AP diameter and neovascularisation between the HAT and PAT groups at the end of the traditionally used 12 week EcEx programme, which produced three main questions. Firstly, would any alterations occur in the ATs when the EcExs are stopped? Secondly, is the current 12 week duration of the EcEx programme optimal? Thirdly, would continuing with the EcExs make it possible for the PAT group scores to continue to improve and replicate the scores indicative of structure and function of the HATs.

It would stand to reason that the duration of the EcEx programme is 12 weeks as this is long enough to produce and maintain positive adaptation within the tendon. The reason for extending the study and further examine participation in the EcEx programme was to produce a continuation of the rehabilitation time line and offer insight into whether the SWV of a PAT would reach the absolute values in the HAT. The aim of this study was therefore to trace any alterations in AT stiffness (measured using SWE), measures of symptoms (VISA-A) and clinical outcome measures including tendon max AP diameter and Doppler score occur when the regular loading of a 12 week EcEx programme is removed and once participants restarted the EcEx programme. A secondary aim was to monitor the participants for up to 6 months to see if the recorded values in their PAT could reach parity with the scores achieved in the contralateral HATs. The hypothesis was that AT stiffness, symptom measures and clinical outcome measures would not alter significantly at the end of the original 12 week period but would continue to improve with further involvement in the EcEx programme.

8.3 Materials & Methods

8.3.1 Participants

The sixteen participants who completed the 12 week EcEx programme detailed in study 4 (chapter 7, section 7.3.3) were contacted after their participation in study 4 (chapter 7) once they had stopped completing the 12 week EcEx programme and asked if they would like to be included in a continuation study. Of the original sixteen participants, eleven participants (7m, 4f, mean age 59 ± 7.5 years, height 173.3 ± 10.4cm and mean weight 77.9 ± 13.1 kg) agreed. The participants were asked to refrain from the EcExs and have repeat measures taken at week 16, following a 4-week break in the EcExs. At their week 16 testing session, the eleven participants were then asked to resume the EcEx programme exactly as outlined in study 4 (chapter 7, section 7.3.3) for a further 4 weeks and have further repeat measures taken at week 20. Participants continued to utilise their google forms as outlined in chapter 7 (section 7.3.2) to mark their exercises enabling programme adherence to be monitored. At week 20, three participants had to withdraw from the study due to unrelated injuries or work commitments, leaving eight participants (6m, 2f, mean age 58.5 ± 7.1 years, height 175.3 ± 9.9cm and mean weight 81.7 ± 10.1 kg) who had repeat measures taken at week 28.

Each participant had one AT in the HAT group and one AT in the PAT group in accordance with the criteria outlined in chapter 7 (section 7.3.1). All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards as outlined in General Methods (Section 3.1).

8.3.2 Methods

Eleven participants were measured at week 16 and week 20 after the initial start of the programme and 8 participants were measured at week 28 (6 months). Testing carried out on week 16, 20 & 28 was carried out by an alternative tester (PB) who assessed tendon stiffness by measuring SWV using SWE, VISA-A, AT length (mm), max AP diameter (mm) and Doppler signal score. The above testing was carried out following the same protocol as in chapter 7 (section 7.3) and as outlined in the General Methods (section 3.4 – 3.5).

8.3.3 Scanning techniques

Measures of basic AT size (AT length and max AP Diameter), Doppler signal measure and SWE were obtained using a Siemens ACUSON S3000™ HELX EVOLUTION Ultrasound System (Siemens Medical Solutions, USA). For these measures, participants lay prone on an examination table with both feet hanging clear of the end of the table following the procedures outlined in General Methods (section 3.5).

8.3.4 Conventional Ultrasound Technique (AT Length and diameter)

At every session, measures of AT length were made using a conventional US using a 14L5SP probe and measures of maximum AP diameter were taken with a 14L5 probe following the protocols outlined in General Methods (section 3.5.1 and 3.5.2).

8.3.5 Power Doppler Signal Measure

Power Doppler (PD) measures were taken at every session using a 14L5 probe following the protocol outlined in the General Methods (section 3.5.3).

8.3.6 Shear Wave Elastography Technique

A shear wave elastogram enables a quantification of tendon stiffness by measuring the velocity of shear waves as they pass through the tendon, with higher velocities indicating a stiffer tendon. All SWE measures were taken in accordance with the protocol outlined in General Methods (section 3.5.5).

8.3.7 VISA-A Questionnaire

Participants were asked to complete the VISA-A (Victorian Institute of Sport Assessment - Achilles) questionnaire at each session to subjectively rate the symptoms in their AT. Participants completed one VISA-A score for their PAT and a separate VISA-A for their HAT. The VISA-A was completed in accordance with the protocol outlined in General Methods (section 3.4.1).

8.3.8 Statistical Analysis

All statistical analysis was performed using the latest available version of SPSS (SPSS, Chicago, Illinois). Measurements of all the participants are expressed as mean ± standard deviation.

Analysis of the data obtained at week 16 and week 20 were analysed separately to that obtained in week 28 due to the lower subject number that returned in week 28. Distribution of groups was analysed using the Shapiro-Wilk test and mean values for all subjects will be used as a summary measure for each variable. T-tests and

ANOVA's were conducted to assess differences if the data fulfilled the assumptions of normality. If not, appropriate non-parametric tests including Friedman's ANOVA, Wilcoxon signed paired matched-tests and Mann-Whitney Tests were utilised to assess significant differences in the data. Data was checked for sphericity with the Huynh-Feldt Correction applied if necessary and alpha level was set at $p<0.05$ throughout.

8.4 Results

Data collection at weeks 16, 20 & 28 included AT stiffness with SWE measures (m/s), AT length (mm), AT max AP diameter (mm), VISA-A and neovascularisation score.

The results displayed in Table 8.1 outline the alterations in HAT & PAT groups when measured at week 16, 20 & 28 in comparison to week 12 at the end of the EcEx programme. The results from week 12 are different from those shown in chapter 7 (section 7.4) as measurements at week 16 included a lower subject number from that used in chapter 7, therefore, results from week 12 were reanalysed using only the 11 participants that were tested. The results shown for week 28 were calculated using the available data from the 8 participants that were measured at this point.

Table 8.1: Means of measured variables for HAT and PAT groups

	Week 12 (n=11)	Week 16 (n=11)	Week 20 (n=11)	Week 20 (n=8)	Week 28 (n=8)
SWE					
SWE HAT (m/s)	9.49	9.47	9.54	9.61	9.52
SWE PAT (m/s)	9.15	8.99	9.24	9.17	9.41
SWE Δ HAT vs PAT (m/s)	0.34	0.48	0.30	0.44	0.11
SWE % difference HAT vs PAT	3.5%	5.1%	3.1%	4.6%	1.2%
VISA-A					
VISA-A HAT	99.64	95.73	98.45	100.00	100.00
VISA-A PAT	93.73	84.32	93.73	86.88	95.75
VISA-A Δ HAT vs PAT	5.91	11.41	4.72	13.12	4.25
VISA-A % difference HAT vs PAT	5.9%	11.9%	4.8%	13.1%	4.3%
AT Length					
AT Length HAT (mm)	62.53	62.37	62.60	64.23	64.45
AT Length PAT (mm)	61.01	61.30	61.52	63.23	62.99
AT Length Δ HAT vs PAT (mm)	1.52	1.07	1.08	1.00	1.46
AT length % difference HAT vs PAT	2.4%	1.7%	1.7%	1.6%	2.3%
AT Max AP Diameter					
AT Max AP Diameter HAT (mm)	6.69	6.78	6.86	6.71	6.54
AT Max AP Diameter PAT (mm)	9.21	9.23	8.90	9.24	8.81
AT Max AP Diameter Δ HAT vs PAT (mm)	-2.52	-2.45	-2.04	-2.53	-2.27
AT Max AP Diameter % difference HAT vs PAT	-37.7%	-36.1%	-29.7%	-37.7%	-34.7%
Doppler Score					
Doppler HAT	0.18	0.64	1.00	0.63	0.50
Doppler PAT	1.45	2.27	2.27	2.13	1.63
Doppler Δ HAT vs PAT	-1.27	-1.63	-1.27	-1.50	-1.13
Doppler % difference HAT vs PAT	-705.5%	-254.7%	-127.0%	-238.1%	-226.0%

**Table 8.1 shows the absolute values and differences between the means (Δ) of the HAT and PAT groups for all measured variables at all measured time points.*

Table 8.1 demonstrates the differences between the HAT and PAT groups in the measured variables up to 6 months after starting a 12 week EcEx programme. Table 8.1 outlines the alterations in all measured variables between when the participants stopped the EcEx at week 12 and when they were measured again at week 16. The results in Table 8.1 demonstrates that all measured variable regress in the PAT group between weeks 12-16 following cessation of the EcExs. The differences between the HAT and PAT groups in VISA-A, SWV and Doppler score increase again (and therefore became more different) when measured following a 4-week break from the EcExs. Table 8.1 also highlights that further positive benefits, including an increase in SWV (m/s) and VISA-A score, coupled with a decrease in max AP and Doppler score can be achieved in the PAT group when EcExs are resumed at week 16. The data shown in Table 8.1 also demonstrates that the differences between the HAT and PAT groups continues to decrease between weeks 20 - 28. The differences in VISA-A score and SWE are particularly noticeable as the % difference between the HAT and PAT groups decreases to a level close to 0, meaning that 28 weeks (6 months) after starting an EcEx programme (albeit including a period of EcEx cessation of 4 weeks) some subjective and objective clinical outcome measures in a PAT can resemble that of a HAT.

The exact alterations experienced for all separate measured variables for both the HAT and PAT groups are outlined below:

8.4.1 Stiffness measured with SWE

The mean SWV measure in the HAT group between weeks 12 – 28 only varied between 9.49 m/s to 9.54 m/s, a fluctuation within 0.05 m/s. As such, no significant differences were shown for the HAT group between week 12 - 20 analysed with the available data from 11 participants ($p = 0.695$) or between weeks 20-28 ($p = 0.233$) analysed from the remaining 8 participants. This implies that any changes to the SWV between week 12 – 28 measured in the HAT group were not large enough to be considered statistically significant.

In contrast, the mean SWV in the PAT group showed significant differences between weeks 16-20 ($p = 0.041$) and weeks 20- 28 ($p = 0.042$). The data suggests that once the participants stopped completing the EcEx programme at week 12, measured values of SWV (and hence AT stiffness) decreased by 0.16 m/s between week 12 - 16,

although this decrease was not deemed statistically significant. When the participants resumed the EcEx programme at week 16, stiffness (SWV) measures increased by a statistically significant amount by 0.25 m/s between weeks 16 -20 and 0.24 m/s between weeks 20 – 28 as shown in Figure 8.1.

Despite the data in Table 8.1 and Figure 8.1 demonstrating that statistically significant increases in SWV can be achieved in the PAT group when EcExs are resumed, the difference in SWV (m/s) between the PAT and HAT groups at all measured time points were not considered statistically significant ($p > 0.05$) between the groups,

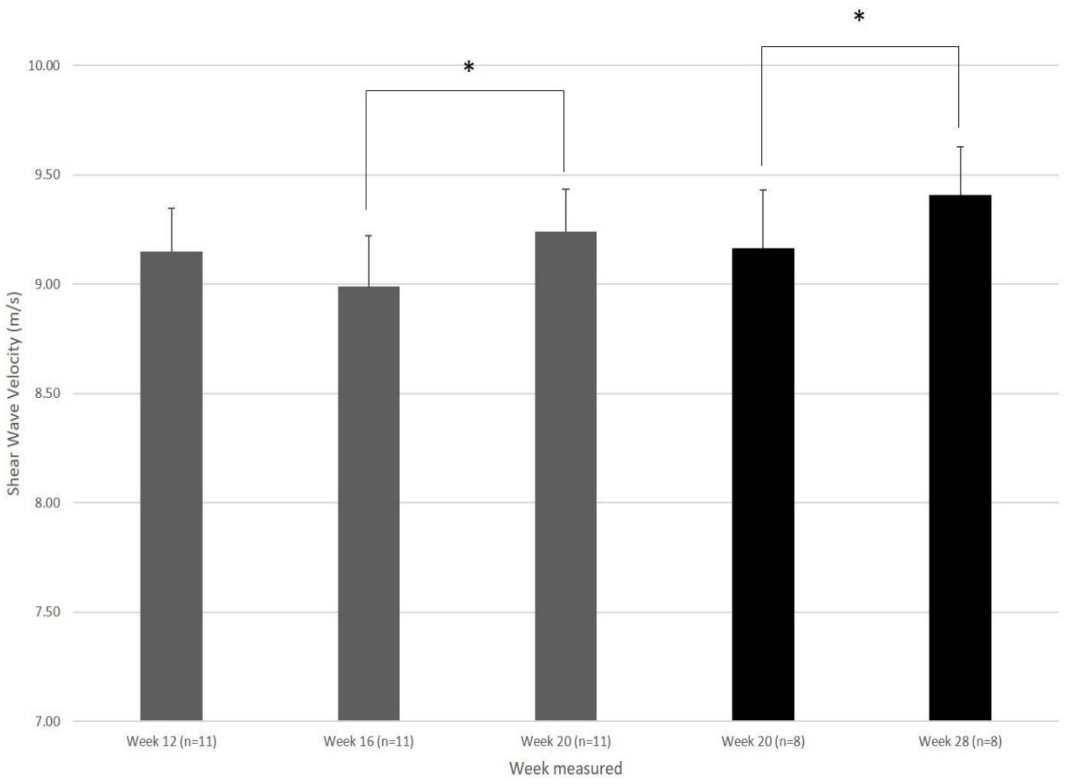


Figure 8.1: Alterations in mean SWV (m/s) for the PAT group week 12 - week 28.

** = P < 0.05*

8.4.2 VISA-A Score

The VISA-A scores for the HAT group varied between weeks 12-28 from 95.73 to 100 (maximum score possible on the test). No significant differences were noted in the HAT data either between the eleven subjects measured between weeks 12-20 ($p = 0.497$) or the eight participants measured between weeks 20-28 ($p = 1.000$). There was no change experienced in the VISA-A score in the HAT group between week 20 - 28 as their VISA-A score stayed at 100 (out of a maximum score of 100) for both

weeks. The VISA-A score of the HATs reduces by -3.91 points between week 12 and week 16, indicating the participants felt that the symptoms in their HAT were subjectively worse when the EcEx loading was removed, even though the EcEx programme wasn't being completed on this leg. When the participants restarted the EcEx programme at week 16, the subjective symptom ratings in their HAT improved again by 2.72 points between weeks 16 - week 20.

The VISA-A scores for the PAT group varied from 84.32 to 95.75 over the measured time points (week 12 – 28) as seen in Figure 8.2. The drop in VISA-A score between week 12 - week 16 was of a larger amount (-9.41 points) than that experienced in the HAT group, indicating symptoms felt worse in the PATs following removal of the EcEx loading. A change in VISA-A score of 10 points has been termed clinically significant (Beyer et al., 2015), therefore the extent of the decrease in the PAT VISA-A score between week 12-16 is just over half a point away from being considered clinically significant and fell just outside of statistical significance ($p=0.086$). Interestingly, the mean VISA-A score of the PAT group increased again by the same amount (9.41 points) between week 16 – 20 once the EcEx loading was reintroduced. When considering the data between week 20 and week 28 from the 8 remaining participants, the increase in VISA-A score between these weeks was 8.87 and with the reduced subject number did just fall into statistical significance ($p = 0.046$), although was not of clinical significance as the score was less than 10.

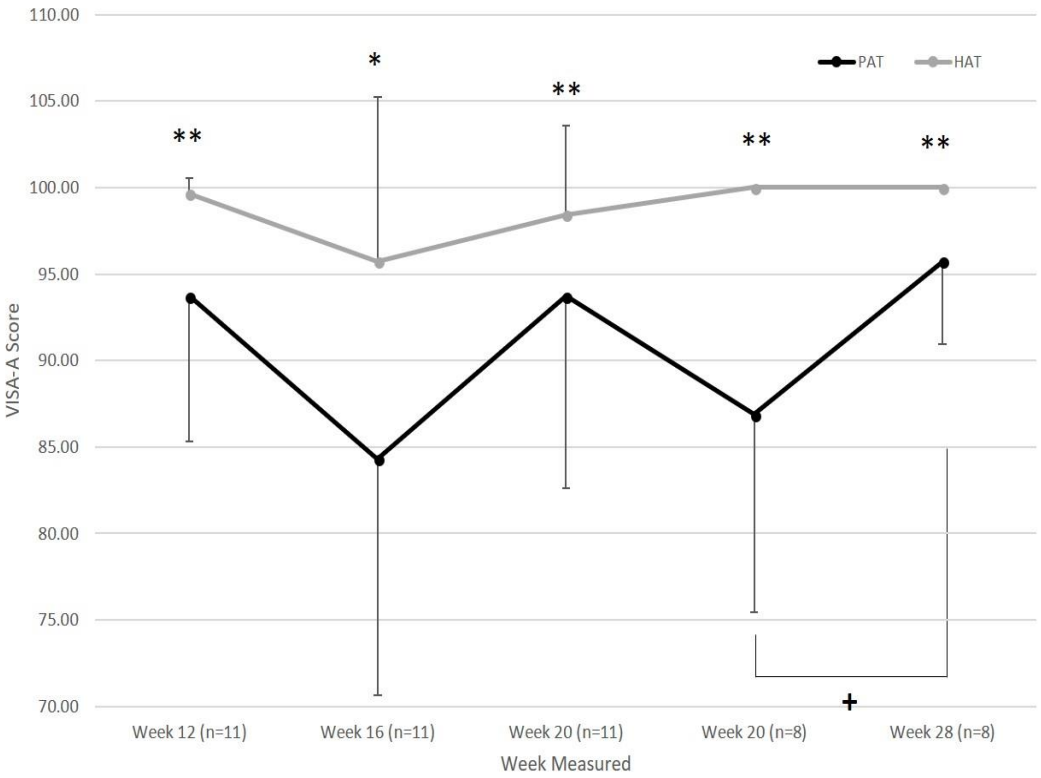


Figure 8.2: Alterations in VISA-A Score for both HAT and PAT groups over the measured time points.

** indicates significant differences between PAT and HAT group scores*
+ indicates significant increase in the PAT data shown between week 20-28 $p < 0.05$
**= $p < 0.05$, ** = $p<0.01$*

The difference in VISA-A score between the PAT and HAT groups was measured as significant over all time points as outlined in Figure 8.2 and Table 8.2. The differences between the mean VISA-A score in the PAT and HAT groups was at its lowest at week 28. At week 28, the difference in VISA-A between the HAT and PAT groups was less than it was at week 12, indicating that further involvement in the EcEx programme past week 12 can bring increased results for the PAT group, bringing them nearer in VISA-A score to parity with the HAT group.

Table 8.2: Differences in mean (∆) VISA-A scores for HAT and PAT groups

	Week 12 (n=11)	Week 16 (n=11)	Week 20 (n=11)	Week 20 (n=8)	Week 28 (n=8)
HAT	99.64	95.73	98.45	100	100
PAT	93.73	84.32	93.73	86.88	95.75
∆ VISA-A	5.91	11.41	4.72	13.12	4.25
% Difference	5.93%	11.92%	4.79%	13.12%	4.25%
P value	P = 0.002 **	P = 0.012 *	P = 0.006 **	P = 0.001 **	P = 0.004 **

Table 8.2 shows differences in absolute values between the mean (∆) VISA-A scores for HAT and PAT groups and % differences over the measured time points with associated significance of the difference.
**= p < 0.05, ** = p<0.01.*

8.4.3 AT Length

No significant interaction effect was found using a 2-way RM ANOVA (Week (3) & Group (2)) p=0.551, indicating no significant alterations in AT length for either the PAT or HAT group between weeks 12-20 (n = 11). To assess differences between weeks 20-28 with the 8 remaining participants, a paired t-test revealed no significant differences for the HAT group (p = 0.714) or the PAT group (p = 0.595), implying that measures of AT length for both groups were consistent over all measured time points.

8.4.4 Maximum anterior-posterior diameter

No significant differences were found over the measured time points between weeks 12-28 for the HAT group (p = 0.249) or PAT group (p = 0.164). This implies that no changes to max AP diameter over the course of the measured time points for either group were considered statistically significant. The difference in max AP diameter between the HAT and PAT groups for max AP diameter (mm) however were shown to be statistically significant at all measured time points (p = 0.003 – 0.016) as shown in Figure 8.3.

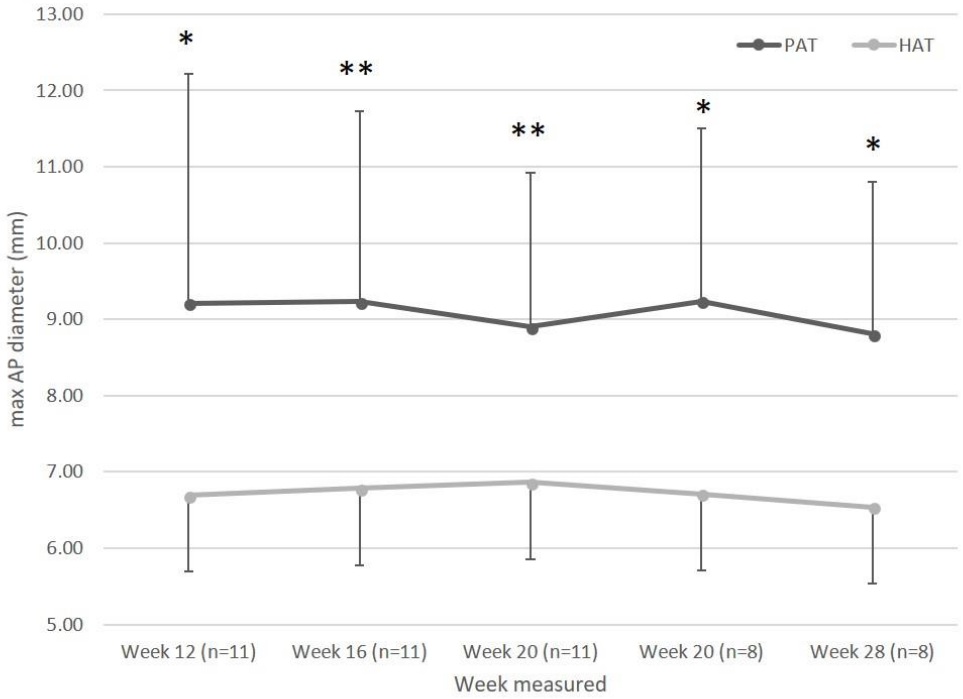


Figure 8.3: Alterations in max AP diameter (mm) for HAT and PAT groups

Figure 8.3 shows alterations in max AP diameter for both HAT and PAT groups over the total measured time points and the significance value associated with the mean differences between the HAT and PAT groups.
**= $p < 0.05$, ** = $p<0.01$*

8.4.5 Doppler score

Significant differences were shown for the neovascularisation scores between week 12 – week 28 for both the HAT ($p = 0.010$) and PAT ($p = 0.027$) groups. The significant differences in Doppler readings for the HAT & PAT groups are shown in Figure 8.4. The significant increases in Doppler score for both the HAT & PAT groups are suggestive of an increase in neovascularisation. Between weeks 12 – 28, the differences in Doppler scores between the HAT & PAT groups were significant at every measured time point. The differences in mean Doppler score between the HAT and PAT groups were highest at week 16, following cessation of the EcEx programme. The differences between the two groups was at its lowest at week 28 indicating that the difference between the groups was decreasing over time and longer-term completion of the EcEx appears beneficial for the PAT group by bringing the Doppler scores closer to parity between the groups.

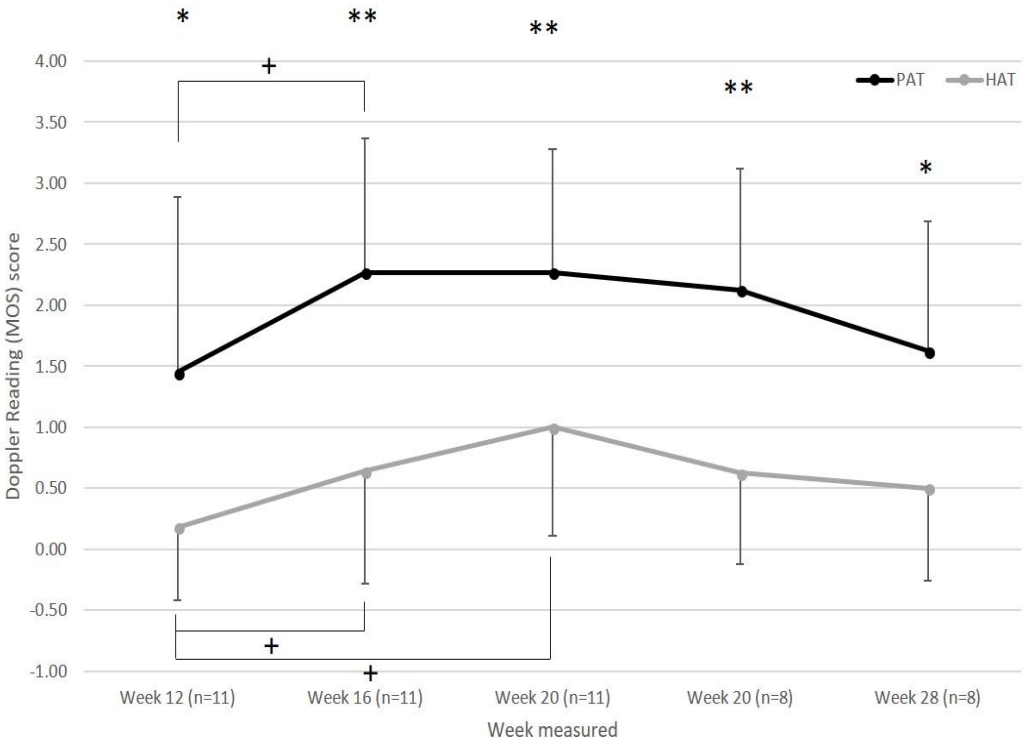


Figure 8.4: Alterations in Doppler score for HAT and PAT groups

Figure 8.4 shows Doppler score for both HAT & PAT group over the measured time points

** indicates significant differences between the mean Doppler score of the PAT and HAT groups * = p<0.05, ** = p<0.01*

+ indicates significant increase in the PAT or HAT data p < 0.05

8.4.6 Continuation of the EcEx past week 20

Eight participants had further repeat measures taken at week 28. Of these eight participants, half of them (n=4) carried on with the EcExs between week 20 – 28. The other half (n=4) stopped completing the EcEx programme at week 20. Figure 8.5 outlines the improvements that occurred between week 20 - 28 in mean VISA-A score for the four participants that continued with the EcExs and the four participants that didn't. Interestingly, the increase in VISA-A score for the PATs that did perform the EcEx between weeks 20 - 28 was 7 points, and those that did not perform the EcEx demonstrated an increase of 10.75 points.

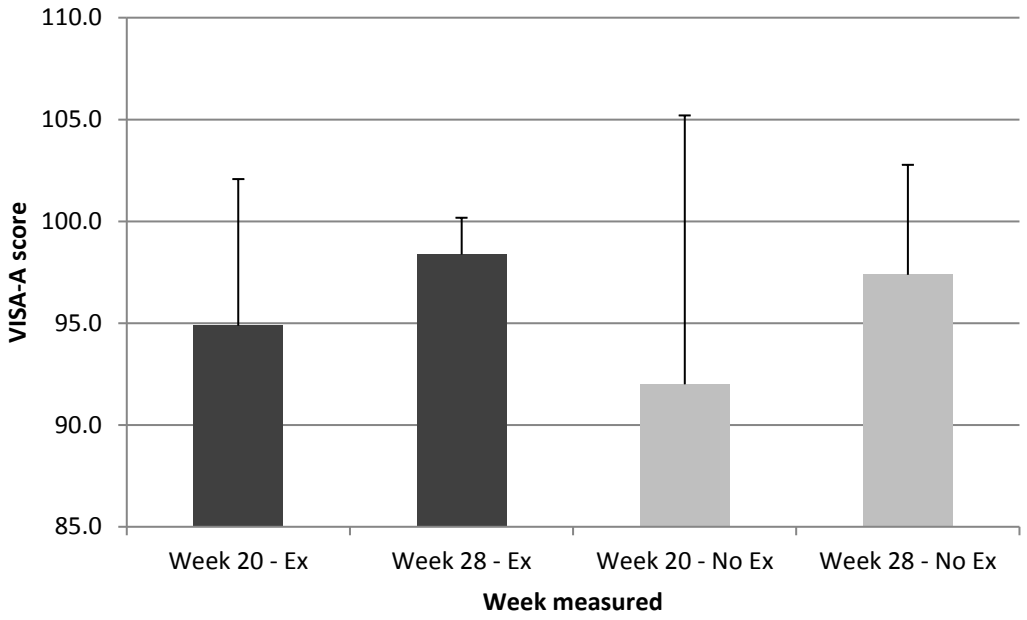


Figure 8.5: Mean alterations in VISA-A score of the PAT group between those that continued with the EcEx programme after week 20 (dark grey) and those that did not (light grey).

Figure 8.6 outlines the changes in SWV (m/s) of the PATs between week 20 and week 28 split into those that continued with the EcEx (Ex) and those that chose to stop the EcEx (No Ex).

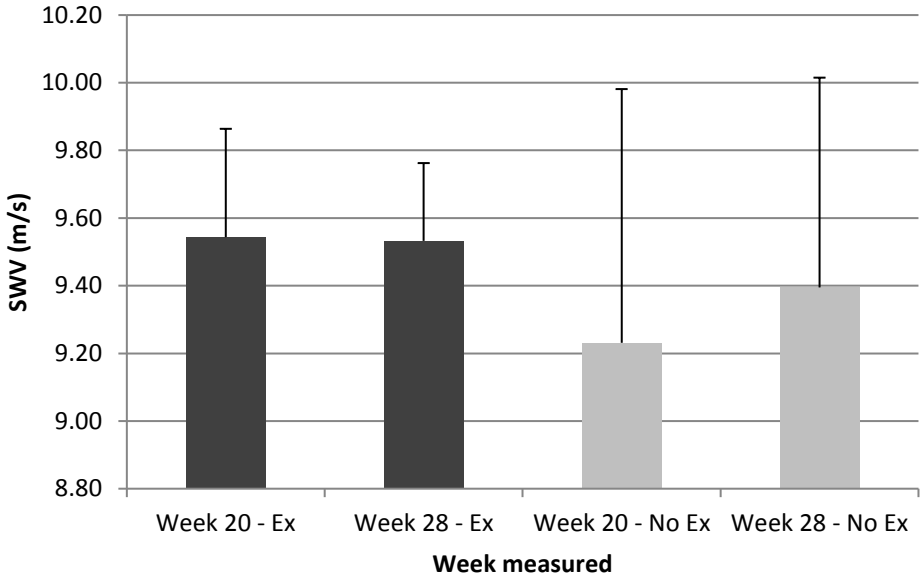


Figure 8.6: Alterations in SWV (m/s) between those that continued with the EcEx programme past week 20 (dark grey) and those that did not (light grey).

Figure 8.6 demonstrates an increase in SWV (m/s) in the PATs for those that did not continue with the EcExs, but paying reference to the axis, the increase is still relatively small. It is worthy of note that the group that did not exercise past week 20 (shown in light grey) also had much more variable results as shown by a much higher standard deviation.

To see whether there were any significant differences between the data depending on whether the participants exercised or not, the error bars (representing SD) of Figure 8.5 and Figure 8.6 were examined. As the error bars all overlap, there are unlikely to be any differences between the data. To confirm this, difference testing was conducted on the data at weeks 20 and week 28. The significance values that were obtained, compared whether any significant differences existed between those that continued with the EcEx between week 20 and week 28 and those that didn't, with the results shown in Table 8.3.

Table 8.3: Significance of differences in measured variables at week 20 and 28 between those that continued with the EcEx programme and those that did not.

	Week 20	Week 28
SWE	P = 0.599	P = 0.793
VISA-A	P = 0.954	P = 0.628
Doppler	P = 0.413	P = 0.823
AT length	P = 0.248	P = 0.345
Max AP	P = 0.293	P = 0.317

The results in Table 8.3 show p values all above p = 0.05 and therefore demonstrate that no significant differences existed in any of the measured variables between those that continued with the EcEx programme past week 20 and those that didn't. It could be concluded that continuing with an EcEx programme past week 20 does not provide any statistically significant benefits. Table 8.1 however shows that continuing with the EcEx past week 12, whether to week 20 or week 28 provides further reductions in the differences in measured variables between the PATs and the HATs, implying the scores are becoming more similar over time.

8.5 Discussion

The aim of this study was to examine alterations in AT stiffness, measured using SWE alongside subjective symptom measures (VISA-A) and clinical outcome measures (Doppler score and max AP diameter) following the removal of the loading element elicited by an EcEx programme is removed. It also monitored alterations in the variables when the EcExs were re-introduced.

The main findings demonstrate that following the completion of a 12 week EcEx programme, the difference in SWV between the HAT and PAT group was 3.5% (0.34 m/s). When the groups were re-measured at week 16 when the EcEx loading stimulus had been removed for 4 weeks, the difference in SWV between the two groups had increased to 5.1% (0.48 m/s). When the EcExs were resumed at week 16, AT stiffness in the PAT group increased, reducing the difference between the groups to just 3.1% (0.3 m/s) at week 20 and just 1.2% (0.11 m/s) at week 28. The average difference in VISA-A score between the HATs and PATs at the end of the 12 week EcEx programme was 5.9%, which increased to 11.9% when measured at week 16 once the loading of the EcExs had been removed for just 4 weeks. These data are suggestive of a subjectively worse tendon with higher pain and decreased function and these regressions of the tendon represent the ever-changing nature of the AT once a heavy training load is removed.

When the participants were re-measured at week 16, there had been negative alterations in the PAT data after the participants had stopped the EcExs at week 12. Although most changes between weeks 12 – 16 were not deemed statistically significant, the results of this study demonstrate that the mean SWV in the PAT group decreases, indicating a decrease in AT stiffness. The PAT group also showed a decrease in VISA-A score implying the participants felt the symptoms in relation to pain, function and activity were worse and a significant increase in Doppler score following cessation of the EcEx programme, suggestive of an increase in neovascularisation. Upon resumption of the EcEx programme at week 16, mean SWV of the PATs increased significantly between week 16 - 20 and between weeks 20 – 28. This was coupled with a non-significant increase in VISA-A score between week 16 - 20, and a significant increase between weeks 20 - 28. The differences between the HAT and PAT groups for SWE and VISA-A continued to show a reduction over the

measured time points and suggest that the current commonly prescribed duration of EcEx programmes (12 weeks), although long enough to produce very favourable and positive changes within a PAT, may not be of a sufficient length for the PAT to simply stop completing the EcEx programme without suffering regression. Either a longer time-period of EcEx may be required to form lasting adaptations in the AT or some sort of tapering at the end of the programme would be beneficial to ensure the positive adaptations in the PATs are sustainable and the AT is able to take the increased loading of a return to pre-injury training or a new exercise programme.

Section 7.4 of chapter 7 demonstrated the many positive outcomes that occur in a PAT over the course of a 12 week EcEx programme. The results were the first to measure AT stiffness over the course of an EcEx programme and demonstrated an increase in AT stiffness to an extent where differences in SWV between the PAT and HAT groups were no longer deemed statistically significant. Despite not being statistically significant, over the course of the entire 12 week EcEx programme, the difference between the highest mean SWV recorded in the PAT group and the lowest mean SWV recorded in the HAT group in chapter 7 was 0.24 m/s. The results of this current chapter suggest that longer term involvement in the EcExs can provide even more beneficial results as the highest mean SWV in the PAT group was 9.41 m/s measured at week 28, and the lowest mean SWV in the HAT group was 9.47 m/s measured at week 16. The difference between these measures was only 0.06 m/s, indicative of the increased SWV in the PAT group after resumption of the EcExs. The results of this current chapter further examined the EcEx programme by tracing the alterations that occur in SWV after the 12 week point when patients usually just stop the EcEx programme and most try to return to their usual level of activity. The alterations experienced in the PATs upon cessation of the EcEx programme, when the mechanical stimulus of the EcExs are removed are shown in table 8.1 and show an impact on SWV from the removal of the EcEx load. These noted regressions in the PAT could simply be the AT adapting to the new (now reduced) level of mechanical loading placed on it. It may be possible that after a few more weeks, the AT would adapt again and start to regain some of its structure and function. It has however been shown in rats, that the matrix content regrowth and recovery in stiffness of a tendon following transection and surgical repair can reach figures relative to a contralateral healthy tendon of 94% and 78% respectively in only 4 weeks (Ghorayeb et al., 2012). Therefore, adaptation

can realistically be expected within this time frame, rendering this theory unlikely to provide all the answers. Another theory is that 12 weeks after starting the EcExs, a PAT is not yet at a stage structurally where the repetitive high loading of the EcExs can simply be stopped without suffering negative consequences as the new level of structure, function and stiffness is not yet fully developed within the AT. Once participants resumed the EcEx programme at week 16, even over a small-time period of 4 weeks, the SWV of the PAT group again increases and the differences between the HAT and PAT groups reduce, showing the reversible nature of the tendon and potentially its ability to move between the stages of the continuum of degeneration as proposed by Cook & Purdam (2009). It is interesting to note that only by week 28 does the mean SWV of the PAT group come to within 0.06 m/s of the lowest recorded mean SWV in the HAT group, which reinforces the need for an extended period of EcExs.

Tendinopathic tendons are known to have a decreased level of stiffness in relation to healthy tendons (Arya and Kulig, 2010), a result also shown in chapter 7 (section 7.4). A significant difference in SWV was noted at week 0 between the HAT and PAT groups and the PAT group demonstrated a lower SWV (and hence a lower level of AT stiffness). The impact of EcEx programmes on the stiffness of the AT measured with SWE has not been previously examined, however the results of chapter 7 (section 7.4.1) demonstrated that a 12 week EcEx programme had no significant impact on SWV of the HAT group. In contrast, it led to a significant increase in SWV values in the PAT group by an average of 0.61 m/s (7.2%), indicating a stiffer AT after the EcEx programme. No study has yet assessed the implications on AT stiffness of a PAT when the regular loading of the EcEx programme is removed at week 12. For comparison purposes, this study also examined the asymptomatic, contralateral HAT. Past the 12 week point, the largest alteration between the measured time points in mean SWV for the HATs was 0.09m/s. In contrast, the PAT group experienced a decrease in SWV of 0.16 m/s after the EcExs had been stopped and the training stimulus was removed. It is not known whether this decrease in SWV would have continued had the participants not resumed the EcEx programme again at week 16, however this data highlights that resumption of the EcEx programme at week 16 resulted in further significant increases in SWV. When the participants resumed the EcExs at week 16, the PAT group experienced significant increases in SWV between

weeks 16-20 and 20-28 as outlined in the results section (section 8.4). The data revealed no statistically significant differences between the HAT and PAT groups between the SWV measure obtained at the end of the 12 week EcEx programme or the subsequent SWV measures taken at weeks 16, 20 or 28. When looking at the actual figures of SWV as well as the percentage differences between the PAT and HAT groups (Table 8.1) at all the measured time points, continual improvements in SWV can be seen. These improvements in the SWV score of the PATs were such that at week 28, the percentage difference between the HAT and PAT groups was only 1.2%. As the differences between HAT and PATs continued to decrease up to week 28, it would imply that carrying on the EcEx programme for longer than 12 weeks, or perhaps introducing some sort of tailored portion to the EcEx programme could illicit further positive benefits to the mechanical properties of the AT that could be maintained. This increase in AT stiffness may serve a protective function to reduce tendon strain rates and protect against injury (O'Neill, Watson and Barry, 2015).

Over the course of the initial 12 week EcEx programme, the PAT group experienced a percentage decrease of -0.24mm (-2.7%) in max AP diameter, potentially suggestive of a more aligned fibrillar structure within the AT. Past the 12 week point there were no significant differences noted in max AP diameter results for either the PAT or HAT groups. The data does suggest however that further reductions in max AP diameter can be obtained through completing the EcEx programme for a longer time-period. When the mechanical loading elicited by the EcEx programme was removed, the max AP diameter score for the PAT group increased between weeks 12-16 by a negligible 0.02mm. Once the training stimulus of the EcEx was reintroduced, the max AP diameter decreased between weeks 16 - 28 by 0.42mm, indicating further benefits can be achieved by continuing with the EcEx programme. Similar studies have noted reductions in tendon thickness following an EcEx programme of 0.6mm measured at the 5-year follow up (Van Der Plas et al., 2012). The authors do note that the clinical relevance of this minor decrease in tendon diameter was questionable and therefore the same should apply to the results obtained in this current study. When the max AP diameter between the HAT and PAT groups is compared, figure 8.3 shows that significant differences are still apparent between the groups at all time points. This, together with the data reported by Van der Plas et al. (2012) imply that even 6 months after starting an EcEx programme, a PAT can expect to achieve significant reductions

in AP diameter, but it is unlikely that this aspect of a PAT will reach full parity with the HAT.

Previous literature demonstrates a significant decrease in tendon thickness ($p = 0.005$) from before an EcEx treatment (8.8 ± 3mm) to a mean of 3.8 years after treatment (7.6 ± 2.3mm), however the authors were unable to explain why tendon thickness decreased or when during or after treatment this alteration occurred (Ohberg, Lorentzon and Alfredson, 2004). Öhberg et al. (2004) hypothesised that the EcEx programme induced remodelling within the tendon that allowed for a normalisation within the tendon fibre arrangement, leading to a decrease in tendon thickness (Ohberg, Lorentzon and Alfredson, 2004). They also proposed that a decrease in thickness may be linked to a reduction in neovascularisation allowing for fibre arrangement normalisation. These theories may also explain the decreases in max AP found in this current study. A study by Docking & Cook (2015) revealed that pathological tendons are comprised of significant areas of fibre disorganisation, yet compensate for this by having an increased thickness (Docking and Cook, 2015). This thicker tendon reduces stress and ensures that tendon structure is maintained to help support load bearing (Cook and Purdam, 2009). When assessed with UTC, pathological tendons can maintain a level of load bearing aligned fibrillar structure greater than that within a healthy tendon, however the authors note that what is quantified as aligned fibrillar structure on UTC, cannot necessarily be stated as being 'normal' tendon (Docking and Cook, 2015). A conclusion of the Docking & Cook (2015) study and one shared by other studies (Cook and Purdam, 2009) was that despite including areas of distinct pathology and appearing clinically degenerative, a pathological tendon may reach homeostasis, allowing it to withstand high levels of loading without remodelling back to what is considered a healthy tendon (Docking and Cook, 2015). This finding may have direct relevance to this current study as the results demonstrated that significant reductions in max AP diameter can be achieved in the PAT group over a period of 28 weeks, however the max AP diameter of the PATs remained significantly higher than the HAT group at every measured time point. It is possible this new increased diameter may reduce the amount of strain experienced within the AT as it may include many areas of aligned fibres, and therefore it may not need to return to the diameter of a HAT, assuming it is able to withstand the loading required of it. The results of the study by Docking and Cook. (2015) suggest that

tendon diameter is not a limiting factor in returning to pain free function following a therapeutic exercise protocol, a theory supported by the findings of this current study, as despite improvements in pain and function, tendon diameter did not decrease to the same extent.

A systematic review of tendon responses to therapeutic exercise regimes showed that tendon diameter is not always associated with reductions in pain or improvements in function (Drew et al., 2014). UTC has been used to assess structural changes induced by an EcEx programme and the results demonstrated that severity of symptoms was not related to tendon structure (as assessed by UTC) at any time over a 24 week programme and the programme elicited no measured improvements in tendon structure (de Vos et al., 2012). The lack of change to tendon structure was shown by a non-significant decrease in the mean percentage of echo types I and II (representing organised tendon bundles) of 0.3% (de Vos et al., 2012). De Vos et al. (2012) showed no correlation between increases in VISA-A and decreases in tendon structure after 24 weeks. In contrast, chapter 7 (figure 7.8) shows that SWE measures correlate highly ($p<0.05$) with both measures of AP diameter and VISA-A scores, showing a high level of agreement between the SWE measures, measures of symptoms (VISA-A) and measures of structure (AP diameter). The process of UTC imaging uses an US transducer fixed in a transverse position on a motorised track that captures images at regular distances of 0.2mm over a 12cm length (van Schie et al., 2010). The stability of the echo pattern obtained from the contiguous transverse US images are analysed and quantified using custom-designed algorithms originally developed on horse tendon (van Schie et al., 2010). Although horse tendon is a well-accepted model of the AT (Patterson-Kane and Rich, 2014), the initial algorithms were based on harvested tendon and differences in the *ex vivo* and *in vivo* behaviour of tendons still exist (Klauser et al., 2013) meaning extrapolation of the results should be made with caution. Alongside this, it is said that certain alterations within the tendon matrix can be seen with elastography before they are seen with B-mode imaging (Horton, 2013; Sconfienza et al., 2013) and as UTC uses only B-mode US images, it is possible that certain alterations within the tendon may potentially not yet be visible. It was concluded by De Vos et al. (2012) that although symptoms and some element of function may improve within 6 months of starting an EcEx programme, it is unlikely that significant alterations to tendon structure occur within this time. They suggest

that tendon structure should not be utilised as a predictor of clinical outcome (de Vos et al., 2012) as normalisation of the structure of a pathological tendon may be preceded by certain molecular processes involved in the improvement of tendon symptoms, however transfer to actual alterations in tendon structure may require longer (de Vos et al., 2012). The results of this current study would agree with tendon symptoms improving before structure as the data from chapter 7 and this chapter suggested that VISA-A scores increase rapidly at the start of the EcEx programme with the largest increase in SWV occurred later in the programme (between weeks 8-12 (0.23 m/s) and week 16-20 (0.25 m/s)) together with the largest decrease in max AP diameter between week 16-20 (-0.33mm). This agrees that subjective measures of pain alter before structural changes occur in a PAT during an EcEx programme.

The VISA-A questionnaire was used to assess the severity of ATY symptoms by the subjective rating of various aspects including pain, function and activity (Robinson et al., 2001; Iversen, Bartels and Langberg, 2012). Chapter 7 (section 7.4.2) demonstrated that prior to any intervention, the VISA-A score of the PAT group was significantly lower compared to the HAT group, suggestive of a more symptomatic tendon. This lower VISA-A score in a pathological tendon has been previously reported (Iversen, Bartels and Langberg, 2012) and may be explained by tendinopathy impacting upon at least one, if not all the aspects measured by the VISA-A. The HAT group experienced a decrease in VISA-A score between weeks 12-16 of -3.91 points which is interesting because no intervention was directly carried out on this AT. For the PAT group, when they stopped performing the EcExs, mean VISA-A score decreased by 10% and subsequently increased by 11% upon resumption of the EcEx programme between weeks 16 – 20. A further increase of 10% was experienced in the PAT group between weeks 20-28. Once again, the implication of these results is that although 12 weeks of EcEx illicit very favourable results on the VISA-A score of PATs, stopping the EcExs at this point has a detrimental effect on the subjective rating of symptoms within the AT. Therefore, the recommendation should be that the length of the EcEx programme be extended or a tapering period implemented in order for the AT to achieve a higher level of VISA-A score and be able to maintain it. The mechanisms behind why EcEx programmes appear to initiate decreases in subjective ratings of pain remain unclear. Systematic reviews suggest no association behind a decrease in pain and reduced structural abnormalities including reduced

neovascularisation or tendon diameter (Drew et al., 2014). It therefore seems unlikely that structural changes impact upon a subjective rating of pain, but further research into the mechanisms behind the decreases in pain elicited by an EcEx programme would be very beneficial.

A Power Doppler assessment was used to assess neovascularisation and the Modified Öhberg Scale (MOS) used to quantify and score the results as used in other similar research (Sengkerij et al. 2009; Watson et al. 2018) and outlined in General Methods (section 3.5.3). The results of chapter 7 (section 7.4.6) showed a reduction in neovascularisation over the initial 12 week EcEx programme of -22.2%, in agreement with other similar research (Ohberg and Alfredson, 2004). No significant reductions in neovascularisation were shown in this current study past 12 weeks, however both the HAT and PAT groups demonstrated increases in Doppler score between weeks 12-16 when the EcEx stimulus was removed. The increase in the PAT group (0.82 points) was almost double that of the HAT group (0.46 points). The HAT group experienced another increase (0.36 points) between weeks 16-20 whereas the PAT group score remained the same before both groups experienced decreases in Doppler score between weeks 20-28.

Previous research notes that a painful tendon will almost always demonstrate the presence of neovascularisation (Van Snellenberg, Wiley and Brunet, 2007) and the increase in neovascularisation noted in this study coinciding with a cessation in EcEx, would add further support to the notions introduced in chapter 7 (section 7.5) that include dorsiflexion at the ankle limiting blood flow to the surrounding neovessels (Knobloch et al., 2007). During each EcEx movement, a temporary interruption to tendon blood flow occurs (Rees, Wolman and Wilson, 2009). It is postulated that the reduction in neovascularisation achieved through an EcEx programme can be attributed to the sheer number of repetitions in blood flow interruption that occurs (Rees, Wolman and Wilson, 2009). It is feasible therefore, that removing this constant blood flow interruption when the EcEx programme is stopped, the presence of the neovessels increases. Reductions in neovascularisation following a therapeutic exercise programme have been shown to not be associated with improvements in treatment satisfaction or improvements in function (Drew et al., 2014). Potential

explanations for this include a lack of standardisation within the examination of neovascularisation as studies use power and colour Doppler interchangeably as well as a lack of an available gold standard scoring system resulting in challenges when comparing studies (Drew et al., 2014). The use of a subjective scoring system may also provide a rationale for the increase in neovascularisation seen between week 12 and week 16 as two different operators obtained the Doppler score at these time points and therefore the differences may be attributed to the use of a different examiner. When the participants resumed the EcEx at week 16, no further increases in Doppler score were noted for the PAT group between weeks 16 - 20 as the score was maintained. Between weeks 20-28 there was a decrease in PAT Doppler score of 24%, although this decrease was not statistically significant.

Despite the improvements to PAT Doppler score occurring at the latter stages of the monitoring, at each measured time point in the study, there were significant differences in Doppler score apparent between the HAT and PAT groups as shown in figure 8.4. A significant difference between the HAT and PAT group remained even at week 28 ($p = 0.019$) indicating that although a PAT can demonstrate a reduction in Doppler score over a 12 week EcEx programme, in a similar fashion to max AP diameter, this aspect of the AT does not seem to reach full parity with the score of a HAT, even after 6 months. A link has been proposed that decreases in tendon thickness may be linked to reductions in neovascularisation (Ohberg, Lorentzon and Alfredson, 2004), and the results of this current study would seem to agree with that link by the results showing reductions in both. The results of this study would also agree with the work of Docking & Cook (2015) that suggest a pathological tendon may not remodel back to parity with a healthy tendon assuming the tendon has enough aligned fibrillar structure to withstand the loading required of it.

This study found no significant differences over any measured time points for AT length, indicating that no significant alterations in AT length occurred over the measured time points for either the HAT or the PAT group. A measure of AT length was taken to ensure that all other measures for each participant were taken at the relative tendon ‘mid-point’. The rationale behind this is that 80% of the alterations in the structure of the AT have been shown to occur in the mid portion with the

predominance of disorders in the AT occurring 3-5 cm proximal to the calcaneus (Järvinen et al., 2005; Kujala, Sarna and Kapiro, 2005; Klauser et al., 2014). To ensure all measurements were taken at a similar place on each participant, calculating the 'mid portion' of the tendon as half free AT length for each participant may decrease the likelihood of errors occurring simply because the measure one week was taken on a different portion of the tendon.

EcEx protocols are the most widely prescribed conservative treatment of midportion ATY due to them having the most evidence of effectiveness in the literature. Although the short term improvements of EcExs are well known, relatively little is recorded in relation to the longer term (Van Der Plas et al., 2012). One study showed that at a follow up length of 4.2 years after an EcEx programme, 65% of patients reported none or mild pain in their tendon (Gärdin et al., 2010). Another study reported patients report an increased VISA-A and had a decreased tendon thickness at a 5-year follow up (Van Der Plas et al., 2012), however only 39.7% of the measured tendons were pain free at the 5-year follow up, implying pain still remained in 60.3% of tendons (Van Der Plas et al., 2012). Also noted was that almost half (48.3%) of the studied tendons had received one or more treatment other than the EcEx, (Van Der Plas et al., 2012), therefore attribution of the positive effects being purely to the impact of EcExs is not possible. This current study only monitored participants up to 6 months, so it would be beneficial for future studies to monitor participants for a longer time frame including measures of SWE. Because no other treatment protocols were utilised on the participants in this current study however, it is easier for the positive results shown in this study to be attributed to the EcEx programme itself.

Of the eight participants who continued to be monitored from week 20 – 28, only half (n=4) carried on with the EcEx programme. There was a noted improvement in VISA-A score between weeks 20 - 28 for those in the PAT group that did not continue with the EcEx programme past week 20 shown in figure 8.5. This could potentially be attributed to the fact that by this stage, the participants felt they were simply able to do more, as the EcExs allowed their PAT to progress to a stage by week 20 where they felt able to participate in any activity. As the VISA-A covers pain, function and activity, it may be that by week 20, participant's pain and function were back to pre-

injury levels and they were able to participate in any activity without thinking of their PAT. Participating in more activity overall will affect their subjective ratings of symptoms and may also have an impact on other areas, including the SWV measurement as they are exercising more and placing an increased and varied amount of mechanical loading through the tendon as they do so. Those that continued the EcExs past week 20 had a higher VISA-A score at week 20 of 5.75 points and therefore the larger improvement in VISA-A score experienced after week 20 in those that did not continue with the EcEx programme may be due to this group having a larger capacity for improvement in their score. This study can conclude that those participants who were consistent with their EcEx between week 20 and week 28 did have a higher overall VISA-A score at week 28 than those that didn't. In terms of stiffness measures, the SWV for those that were completing the EcExs between weeks 20-28 remained the same with a change of just -0.01m/s over the 8 weeks. The group that were not performing the EcExs experienced an increase in SWV of 0.16 m/s.

The main reason given for the participants deciding not to complete the EcEx programme from week 20 was that they felt their tendon was now "completely better" and they felt they were getting no additional benefit from completing the EcExs as they had returned to pre-injury activity level and were able to perform any activity they wanted. It is important to note that the participants did not feel this way at any stage before week 20. There were no significant differences in the data depending on whether the participants chose to continue with the EcEx programme or not, and therefore it could be concluded that no additional benefits are gained from continuing with the EcEx programme past 20 weeks. However, this may only be correct for those who feel their tendons are completely better and they are able to participate in any activity they want. In a similar fashion to the VISA-A data, there was a larger increase experienced in SWV in the group that were not performing the EcExs in comparison to those that were between weeks 20-28. This finding could potentially be attributed to the fact that those continuing with the EcExs past week 20 had a higher SWV (m/s) measurement (representative of a stiffer tendon) at week 20 compared to those that chose not to continue with the EcExs, providing an increased capacity for improvement in that group. The increase in SWV in the group that were not completing the EcEx programme may also be due to the fact they were loading the tendon in other ways as they were completing more exercise. Regardless of the

mechanisms, Table 8.1 demonstrates that by week 28 (6 months), the differences in both SWV and VISA-A scores between the HAT and PAT groups had decreased to a point where they are close to parity with the HATs.

No studies have assessed the impact of performing EcEx on measures of AT stiffness for over 12 weeks. It has been noted that it may take up to 12 weeks of daily EcEx to see substantial improvements in the tendon (Scott, Huisman and Khan, 2011), but no recommendation for exact duration. A systematic review into EcExs highlighted that there is no definitive evidence on the efficacy of differing dosages of EcExs (Meyer, Tumilty and Baxter, 2009) whilst another systematic review into tendinopathy treatment states that the duration of EcEx treatment varies between studies with no mention of any studies looking at intervention periods longer than 12 weeks (Andres and Murrell, 2008). This current study is therefore unique as it is the first to utilise SWE to trace alterations in stiffness measures within the AT *in* vivo when the relatively high loading elicited by an EcEx programme is removed. It also looks at a longer time frame and traces alterations when the EcEx programme is resumed at week 16 and includes a 5 month and 6 month follow up of participants to obtain a fuller picture of the timescale of an EcEx rehabilitation programme.

As shown by the results of chapter 7 (section 7.4), very positive adaptations within the PATs were shown to occur over the traditional 12 week period of the EcEx programme. At 12 weeks, the point where EcEx programmes commonly stop, participants no longer performed the EcEx programme and as a result, most of the measured variables regressed. This may suggest that the standard length of 12 weeks might not be long enough to produce sustainable change within all ATs or that a tapered end to the EcEx programme should be introduced. Upon resumption of the EcEx programme at week 16, the differences between the HAT and PAT groups were again shown to decrease between weeks 16 - 28, suggesting a move towards parity in the scores between the groups. Changes in VISA-A score and SWE were most noticeable, as by week 28 (6 months), the differences in VISA-A score and SWV between the HAT and PAT groups had reduced to just 4.3% and 1.2% respectively.

This study also carries some limitations, including a relatively small sample size, therefore it would be beneficial for future research to include larger cohorts. The current study monitored the alterations occurring when the participants stopped the

EcEx programme at week 12, however it would have been beneficial to also see the results had the participants carried on with the EcEx programme past week 12 without stopping the EcExs. It would be beneficial for future studies to include further longer-term monitoring of patients completing EcEx programme, including SWE measures. This study also did not have a control group, as leaving those still with symptoms without treatment was not justifiable, therefore contra-lateral HATs acted as the controls.

Some measured variables within a PAT including Doppler score and max AP diameter, even 6 months after starting an EcEx programme remain significantly different from that shown in a HAT. The results of this study therefore agree with a recent systematic review into tendon responses to therapeutic exercises which demonstrated structural changes are not wholly an explanation for the favourable responses seen (Drew et al., 2014) as significant differences in max AP diameter and Doppler score still existed and the length of time required for these variables to reach non-significance can only be estimated. Despite this, the subjective and objective variables of VISA-A and SWV continue to improve, even up to week 28. Although the VISA-A score at week 28 in the PAT group remained significantly different from the HAT group, the mean VISA-A score obtained by the PAT group at week 28 can be considered full recovery from ATY (Iversen, Bartels and Langberg, 2012). The difference between the HAT and PAT groups in SWV continued to decrease over the 6 month time frame. Whether these improvements are attributable purely to the EcEx programme or a new-found ability to perform an increased level and/or the effect of applying different forms of mechanical loading to the tendon through completing more activity remains unknown. None of the participants who stopped the EcEx programme at week 20 felt their tendon was at a suitable level to stop the EcEx programme before this and therefore, there is a potential cause to increase the current duration of the 12 week EcEx programme or to add a taper to the end of the programme. It would be beneficial for further research to examine longer duration programmes and to start to examine the optimisation and potential tailoring of programmes to assist in a quicker and more efficient programme with which to treat mid-portion ATY.

In conclusion, this study has shown that upon the removal of the mechanical loading elicited by a 12 week EcEx programme, a PAT will regress in both subjective and objective measures. Performing the EcEx programme again for a further 4-8 weeks

re-initiates the positive adaptations within the PATs and reduces the differences in the measured variables of VISA-A and SWE between HATs and PATs to 4.3% and 1.2% respectively. This suggests that increasing the length of the EcEx programme to longer than 12 weeks may be beneficial in creating a more stable, stronger and stiffer tendon or that the addition of a tapering section to the programme may be of benefit in helping PATs maintained their positive adaptations.

9 General Discussion

9.1 Thesis Aims

The aim of this thesis was to apply the imaging technology of shear wave elastography (SWE) to the Achilles tendon (AT) and utilise it to monitor a rehabilitation protocol for Achilles tendinopathy (ATY). To meet the aims, this thesis assessed the parameters of SWE measures obtained from asymptomatic, healthy ATs and compared them to values obtained from pathological ATs symptomatic of ATY. Experimental chapters incorporated a review of reproducibility and investigated methodological concerns with regards to AT imaging. Once methodology and guidelines for best practice were established, SWE was utilised in the clinical setting to monitor a rehabilitation protocol. This thesis utilised a novel, holistic approach to the application of SWE to AT imaging and monitoring of rehabilitation, with the results providing additional information for the application of SWE to ATY.

This general discussion will summarise the overall findings of the work from the thesis before presenting a retrospective analysis of the obtained data, providing a database of SWE values from which to characterise asymptomatic, healthy tendons and pathological ATs symptomatic of ATY. Based on the conclusions drawn from the thesis chapters, practical recommendations surrounding the clinical application of SWE to AT imaging and as a monitoring tool for ATY rehabilitation will be provided. This general discussion will then acknowledge limitations of the thesis and discuss areas upon which future research can extend.

9.2 Summary of findings

This thesis investigated the reproducibility of two elastography techniques over consecutive measures, consecutive days and different foot positions whilst controlling for prior activity. This was the starting point for the thesis as reliability should be the first thing tested in a new measurement tool as it cannot be valid if it is not firstly consistent in its value over repeated measures (Atkinson and Nevill, 2001). The issues with compression elastography (CE) were numerous, including most importantly its lack of reproducible and/or quantitative measures which led to CE being rejected in favour of SWE. SWE is a repeatable and reproducible technique that provides quantitative measures of the mechanical properties of the human AT, *in vivo*. Longitudinal scans and a relaxed foot position were shown to offer the least variable

SWE results, consistent results were obtained over consecutive measures and consecutive days with a high level of agreement between different operators.

As SWE is a relatively new addition to the imaging market, few studies have assessed the impact of extraneous variables on its obtained results. This thesis demonstrated that neither leg dominance nor time of day are likely to affect interpretation of SWE results, however significant increases in shear wave velocity (SWV) and hence AT stiffness occur immediately after an acute 30 minute bout of running. This significantly increased SWV returned to baseline within 6hrs after the exercise bout. The application of SWE in this thesis had so far only involved asymptomatic, healthy tendons as until normal variation within healthy tendons is established and baseline clinical values recorded, it is impossible to establish what normal acceptable change is, versus pathological change. The thesis then applied SWE to the clinical setting and used it alongside commonly used symptom and clinical outcome measures to assess its effectiveness for monitoring a rehabilitation programme for ATY.

SWE was used to monitor AT stiffness throughout a 12 week Eccentric Exercise (EcEx) programme which resulted in a significant increase in the SWV recorded in pathological ATs. This was coupled with significant increases in symptom measures (Victorian Institute of Sport Assessment – Achilles (VISA-A)), ankle range of motion, muscular endurance and muscular power and significant decreases in pain (Visual Analogue Scale (VAS)), maximum anterior posterior (max AP) diameter and neovascularisation. Symptom severity improved significantly within the first 4 weeks of the EcEx programme resulting in improvements of 20% in VISA-A and 52% in VAS score. In contrast, the largest structural changes in Doppler and SWE measures occurred between weeks 8 – 12 implying structural changes occur at a later stage, providing a cautionary note for clinicians to understand this relationship and avoid early overload based on symptom improvement. At the end of the 12 week EcEx programme, VISA-A, max AP diameter and neovascularisation score in the pathological ATs remained significantly different from the control tendons. Analysis of SWV measures also demonstrated that pathological tendons had not yet reached full parity with the control tendons, prompting an extension of the study as the longer-term responses of SWV measures in response to an EcEx programme were so far unreported.

All participants stopped the EcEx programme at week 12 and were re-measured 4 weeks later, allowing the measurement of any alterations that occurred when the relatively high loading of the EcEx programme was removed. All measured variables in the pathological ATs regressed when the EcEx loading was removed, but when the EcExs were resumed, significant increases in SWV and VISA-A were shown to occur for up to 6 months. The knowledge that a pathological AT can improve symptomatically, yet still not return to parity with the stiffness of a HAT provides important information for both clinicians and patients and suggests that the traditionally used duration of a 12 week EcEx programme may not be long enough to maintain the positive adaptations the programme initially elicits. Increasing the length of the EcEx programme to longer than 12 weeks or adding a tapering section may be beneficial in creating a stronger and stiffer tendon with fewer symptoms that is able to maintain these positive benefits when the EcEx programme finishes.

9.3 Database of SWE values in the Achilles tendon *in vivo*

The data accumulated in the thesis has made it possible to conduct a retrospective analysis of the measures obtained to produce a database of SWE values. The main findings of this database are outlined in Table 9.1 and offer the first step to allowing quantification between healthy and pathological tendons to be made on the basis of SWV.

The values presented in Table 9.1 for healthy ATs are the accumulation of data taken from a total of 41 participants (14 from chapter 4, 15 from chapter 5 and 12 from chapter 6). Table 9.1 compares this data to the mean values found in the 16 symptomatic pathological ATs measured at the beginning of chapter 7.

Table 9.1: Database of AT measures

	Age (years)	Height (cm)	VISA-A score	AT Length (mm)	Max AP diameter (mm)	Mean longitudinal SWV (m/s)	SWV converted to E (kPa)
Healthy Asymptomatic Tendons							
Mean	27.4	170.9	99.1	45.1	4.7	9.4	265.1
SD	4.1	9.4	1.3	13.4	0.5	0.3	0.3
Minimum	21.0	155.0	96.0	23.6	3.9	8.7	227.1
Maximum	37.6	189.0	100.0	74.1	5.8	9.9	294.0
Symptomatic Pathological Tendons							
Mean	58.8	173.4	64.1	57.6	8.7	8.2	201.7
SD	9.7	10.5	13.6	9.8	2.4	1.1	3.7
Minimum	39.3	158.0	41	43.0	5.5	5.8	100.9
Maximum	76.9	183.0	91	81.1	13.7	9.3	259.5
Difference between means of healthy and pathological tendons							
Absolute difference	32.1	1.6	-35	12.8	4.0	-1.2	-63.4
Percentage Difference	54.6%	0.9%	-54.6%	22.2%	45.9%	-14.6%	-31.4%

**Table 9.1 shows basic measures, absolute and percentage differences obtained from both healthy, asymptomatic ATs and symptomatic pathological ATs throughout this thesis.*

The participants whose data was used to represent pathological tendons in Table 9.1 tended to be older individuals (by 32 years), with a longer (12.8mm) average AT length than those participants from chapters 4, 5 and 6 whose data represented healthy, asymptomatic ATs. It is possible that the scores of the healthy tendons of participants from chapter 7 may have been affected by their contra-lateral pathological tendon. Due to the pain caused in their symptomatic tendon, their activity level was decreased which may have impacted all measured variables in their healthy AT. Therefore, the values of the healthy, asymptomatic ATs assessed in chapter 7 were not included in the database presented in Table 9.1 but have been assessed separately in Table 9.2.

Table 9.1 demonstrates that in comparison to healthy, asymptomatic ATs, a symptomatic, pathological ATs have on average a lower (54.6%) VISA-A score, a higher (45.9%) max AP diameter and a lower (14.6%) SWV measurement of 1.2 m/s. The knowledge that pathological tendons have a lower VISA-A score has been previously shown by Iversen et al. (2012) who demonstrated those with ATY can often indicate a VISA-A score below 60. A clinical diagnosis of ATY also usually includes a history of swelling with a maximum AT thickness greater than 8mm (Aström et al., 1996; Mokone et al., 2006) as shown in Table 9.1. The results obtained from this thesis including pathological ATs having a lower VISA-A score and a higher max AP diameter would therefore agree with the previously published literature. The recording of SWE data for asymptomatic and ruptured ATs has been previously reported (Chen et al., 2013), but it has not been until more recently that the stiffness values of symptomatic ATs with ATY have been examined with SWE (Aubry et al., 2015; Dirrichs et al., 2016). The findings of this thesis demonstrate that symptomatic pathological ATs have a lower SWV than healthy, asymptomatic ATs, which agrees with the previously conducted research. The alterations of SWE measures throughout an EcEx programme however, had not previously been shown.

The majority of research into tendon stiffness using SWE has reported values in terms of Young's modulus (kPa). The reported range for healthy, asymptomatic ATs is between 154.2 – 291.9 kPa (Arda et al., 2011; Chen et al., 2013; Dirrichs et al., 2016; Zhang et al., 2016; Leung, Chu and Lai, 2017) with reported values shown in the Literature Review (

Table **2.3**). SWE values for torn ATs have been reported as 56.5 kPa (Chen et al., 2013) with others reporting a seemingly low SWE value for ATs symptomatic of ATY of 53.4 kPa (Dirrichs et al., 2016). The SWE values in this thesis were recorded in SWV (m/s) and conversion of SWV to Young's modulus (E) was not performed during the experimental chapters of this thesis due to the associated assumptions of the conversion equation as discussed in the Literature Review (section 2.6.2). However, to compare the data obtained within the experimental chapters of this thesis to previously published work, the obtained SWV (m/s) values from Table 9.1 were converted to E (kPa) for comparison purposes using the conversion factor SWV = √

E / 3. In this equation, E is the Young's modulus in kPa, with this conversion factor shown to be applicable to isotropic tissues (Ewertsen et al., 2016). The results of the conversion, as shown in Table 9.1 demonstrates an agreement between the results of this thesis and related research in terms of the stiffness values reported for asymptomatic, healthy ATs. This thesis provides a mean stiffness value for healthy asymptomatic ATs (after using the above noted conversion factor) of 265.1 ± 0.3 kPa (range 227.1 – 294.0 kPa) which would agree with the range of 154.2 – 291.9 kPa shown by related research.

When the SWV value obtained from ATs symptomatic of ATY were examined in chapter 7, the results seem to fall within the two reported values available in the literature. There will always be differing degrees of pathology within a pathological AT and therefore the range of results may be reflecting the differing degrees or stages of pathology presenting in the patients. The range of stiffness values obtained from pathological ATs, as shown in Table 9.1, was between 5.8 – 9.3 m/s (100.9 – 259.5 kPa), a figure higher than that reported by Dirrichs et al. (2016) of 53.4 ± 23.3 kPa, but lower than the SWV of 14.53 m/s reported by Aubry et al. (2015). Dirrichs et al. (2016) utilised a stand-off pad between the transducer and the skin, and took measures from the proximal, mid- and distal portions of the AT. Aubry et al. (2015) used a gel stand-off pad, placed regions of interest (ROIs) at two-thirds of the AT, 5cm from its end and passively mobilised the ankle, with no indication of how this position was maintained. In contrast to the work of Dirrichs et al. (2016) and Aubry et al. (2015), the ankle position in this thesis was maintained by a custom-made strap, no stand-off pads were used, and measures were taken at the tendon mid-point relative to each participant. The magnitude of difference in SWV (1.2 m/s) between a healthy (9.4 m/s) and pathological tendon (8.2 m/s), shown in Table 9.1 is the same magnitude of difference (1.22 m/s) between healthy (15.75 m/s) and pathological (14.53 m/s) tendons shown by Aubry et al. (2015), however the values obtained in this thesis were comparatively much lower. The methodological differences noted above may suggest a rationale for these differences, however the saturation limit of the Siemens S3000 would make obtaining values even remotely similar to the SWV values noted by Aubry et al. (2015) impossible. This highlights the need for more research to be conducted in this area to clarify the findings and for a standardisation of reported stiffness values across SWE technologies.

Sarvazyan et al. (2011) state that comparison of SWE results across different machines and manufacturers must be made with caution, however Long et al. (2018) conducted acceptance testing on ten commercial SWE machines and compared measurements from two different manufacturers, with all systems passing the acceptance testing and accepted for clinical use. Although the work of Long et al. (2018) did not include the Siemens S3000 machine utilised in this thesis, the work of Rosskopf et al. (2016) demonstrated that the absolute SWV values recorded from two different SWE systems (Siemens S3000 and a Supersonic Aixplorer) can differ significantly, attributed to differences in methods of producing shear waves and reconstruction algorithms. The authors concluded that for controlled mechanical loads, the two SWE systems yield reproducible and comparable measurements (Rosskopf et al. 2016). For validation testing of SWE, custom elasticity phantoms of a known Young's modulus can be used to assess whether the SWE technology measures the same Young's modulus value as present in the phantom (Long et al., 2018). This can provide a value of error associated with the result obtained by the SWE machine and is how the Siemens S3000 is validated.

The scope of this thesis did not extend to phantom testing, as this has already been reported on in the literature with SWE technology with regards to MSK imaging (Miyamoto et al. 2015; Rosskopf et al. 2016; Baumer et al. 2017). However, this thesis did collect SWE measures from multiple individuals over a six month time frame throughout the duration of testing for chapters 7 & 8. This allows the variability in SWE measures taken over an extended time frame to be assessed. This allows the variability of SWE measures obtained using the Siemens S3000 to be evaluated over a time frame not yet reported on in the literature. The results of this analysis are shown in Table 9.2.

Table 9.2: Variability in SWE measures taken in healthy ATs over a six month period.

	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 28
No. of participants	N = 16	N = 16	N = 16	N = 16	N = 11	N = 11	N = 8
Mean SWV (m/s)	9.31	9.37	9.38	9.39	9.47	9.54	9.52
SD (m/s)	0.55	0.44	0.42	0.38	0.29	0.23	0.24
CV (%)	5.9%	4.7%	4.5%	4.0%	3.1%	2.4%	2.5%
Typical Error (m/s)	Week 0 – 4 (n = 16) = 0.18		Week 8 – 12 (n = 16) = 0.10		Week 16 – 20 (n = 11) = 0.18		Week 20 – 28 (n = 8) = 0.16
Typical % error	2.6%		2.4%		2.6%		2.4%

The calculated coefficient of variation (CV) for the measured SWVs in healthy ATs over the course of 6 months varies between 2.4% - 5.9%, all considered acceptable for a biological measure (Chino et al., 2012). To further confirm the low variability in SWE measures, the random variability of a single individuals values on repeated testing (within-subject standard deviation) has also been represented by calculating the typical error (TE) of the measurement (Hopkins, 2000). The TE in this specific case represents the variation that can be expected between SWE measures from someone having multiple SWE measures taken, with lower scores representing a more reliable measure. This was calculated as outlined in General Methods (section 3.6) as $TE = SD(diff)/\sqrt{2}$ (Hopkins, 2000). The typical percentage error is the TE expressed as a percentage of their respective means. It is a dimensionless measure allowing direct comparison of reliability between different manufacturers of SWE machines or different populations (Hopkins, 2000). This thesis is the first to produce such statistics over this length of time, and as such can be utilised to further confirm the reliability of the Siemens S3000 with regards to AT assessment.

The reproducibility of SWE to assess healthy ATs was confirmed in Table 9.2 and chapter 4 (section 4.4.2) that demonstrated the average CV in longitudinal SWE

measures with the foot relaxed was 3.0%, with the average TE being 0.22 m/s, figures not dissimilar from those in Table 9.2. These findings only related to asymptomatic, healthy tendons, therefore it was important to assess the reliability with pathological tendons as well, which was assessed during chapter 7. At week 0 and week 4 of chapter 7, all participants had 5 separate SWE measures taken, allowing calculations of reliability to be made. At week 0, average CV was 2.4% and TE was 0.20 m/s and at week 4, CV was 1.4% and TE was 0.18 m/s. These findings were in line with the results obtained from the healthy asymptomatic tendons, and therefore the reliability of SWE to assess AT stiffness of both healthy and pathological ATs has been confirmed within this thesis.

9.4 Practical applications from results

Due to the limited amount of published studies in the area, the clinical application of SWE with regards to musculoskeletal (MSK) imaging remains in its infancy (Ewertsen et al., 2016). The results of this thesis therefore add new and vital knowledge regarding the methodology most appropriate with SWE and its suitability in the clinical setting for monitoring rehabilitation protocols. As well as producing the database of SWE values shown in Table 9.1, this thesis can also provide the below recommendations for the clinical usage of SWE in relation to AT imaging and monitoring of a rehabilitation protocol for ATY:

1) SWE has high levels of both inter- and intra-rater reliability when assessing healthy, asymptomatic ATs and pathological ATs.
2) Longitudinal scans produced less variable results than transverse scans.
3) A relaxed foot position produced less variability in SWE measures in comparison to fixing the foot at 90°.
4) Leg dominance and time of day do not impact SWE measures, valuable knowledge for clinicians and/or researchers regarding appointment time.
5) Previous weight bearing activity in the 6hrs leading up to SWE assessment should be avoided as an acute 30 minute bout of running significantly increased AT stiffness.
6) A 12 week EcEx programme significantly increased AT stiffness of a symptomatic, pathological AT, but it did not reach full parity with a control tendon.

7) Throughout an EcEx programme, symptom measures improved significantly in the early stages, however the largest alterations in AT stiffness occurred at a later stage. This provides a cautionary note for clinicians to avoid early overload based on symptom improvement
8) SWE measures correlate highly with the commonly used clinical outcome measures of VISA-A and max AP diameter during a rehabilitation programme from ATY.
9) Measures of AT stiffness, VISA-A, max AP diameter and neovascularisation regress when an EcEx programme is stopped at 12 weeks. Further positive adaptations occur in a pathological AT when EcExs are resumed, suggesting that the traditionally prescribed 12 week duration of EcEx programmes may need to be increased or a taper period introduced.

The introduction of this thesis (section 1.1) states "This thesis aims to add to the growing body of research surrounding SWE and AT imaging by assessing the methodology of SWE to obtain values of AT stiffness and to apply SWE to the monitoring of AT stiffness throughout a rehabilitation protocol for ATY". The studies conducted within this thesis were intended to inform clinicians and researchers utilising SWE of the best methodology to use to assess the AT and when tracing rehabilitation protocols for ATY. Given the data presented in the experimental chapters of this thesis and outlined above, the aim of the thesis has been achieved.

9.5 Limitations

Although the work carried out in the experimental chapters was conducted taking caution to ensure valid and robust measures were taken at all times, the findings of this thesis should be considered in light of the following limitations. The first limitation was the use of small, homogenous samples in most of the experimental chapters, limiting the extrapolation of results. The sample sizes used fell in line with related research and the number of SWE measures taken increased the amount of data points used for analysis to equal or above that used in other research. In chapter 4, for example, each participant had 20 SWE measures taken, totaling 280 measures included in the analysis, a number much higher than comparable research (Arda et al. 2011a; Chen et al. 2013; Peltz et al. 2013).

A limitation of the SWE technology is that results obtained are more variable and show a decreased SWV at increased depths (Ewertsen et al., 2016) although the impact of depth is minimal if the tissue being imaged is less than 22mm from the skin (Hatta et al., 2014). All SWE measures in this thesis were obtained in the AT from depths of 0.3 - 0.5cm (range 3-5mm) as shown in General Methods (section 3.5.5), rendering the potential impact of depth on SWE variability in this thesis as minimal. The AT is located very superficially and has a limited coverage of soft tissue, making it ideal for elastography imaging (Busilacch et al., 2016; Petrescu et al., 2016). As increasing the depth of the tissue being imaged increases the deviation and variability in SWE measures (Long et al., 2018), imaging with SWE should be limited to measurements at one depth (such as those taken in this thesis).

Another technical limitation is the saturation limit of the Siemens ACUSON S3000™ HELX EVOLUTION Ultrasound System (Siemens Medical Solutions, USA). Velocities above 10 m/s are returned with a label of 'High' and it is not possible to quantify the exact SWV in that area. For this reason, any values returned as 'High' were discounted, although this was not a common occurrence in testing. Chapter 4 had 2 ‘High’ measures retuned, chapter 5 had 7 and chapter 6 had 7 ‘High’ values, accounting for 0.97% of the SWV measures obtained in the chapter.

Throughout the experimental chapters of this thesis, certain measures were not taken which may have strengthened the data or helped provide rationales for the findings. These measures were not taken due to the additional cost, equipment and time associated with them which may have delayed testing and decreased available participant numbers. These measures included the measure of cross-sectional area which could have been useful for comparison to other related research. Other measures including muscle fascicle length, ankle strength using dynamometry, neural activity or various histological measures were also not taken which may have added additional information and rationales for the findings.

9.6 Future directions

The findings of this thesis offer a novel and valuable contribution to the knowledge surrounding the application of SWE to AT imaging and the monitoring of rehabilitation protocols from ATY. Given the grounding this thesis has provided, there are several potential future directions the research could take to build no these

results. As the aim of this thesis surrounded the application of SWE to imaging of the AT, consideration was not given to other tendons in the body and the extrapolation of the results to other tendons is not possible. Given the promising results shown in this thesis, future research should consider applying SWE and its methodology to other tendons, whilst remaining mindful of the impact of tendon depth on measurement variability.

The database of SWV values shown in Table 9.1 could be the initial step required in being able to use SWE as a diagnostic tool for ATY and AT injuries. The SWV of a pathological tendon has been shown to be approximately 1.2 m/s slower than a healthy, asymptomatic tendon. Future work should be conducted with much higher participant numbers, as continuation of the database in Table 9.1 could begin to provide limits for classifications of pathology such as those used to classify the pathological tendons in chapter 7 (section 7.3.1). This thesis only concentrated on EcEx as a rehabilitation programme for ATY, however the effectiveness of differing rehabilitation programmes should also be considered to offer a comparison to the traditionally utilised EcEx programmes.

The exercise mode utilised in chapter 6 was running, however other modes of exercise can also significantly alter tendon stiffness over short time periods (Nakamura et al., 2011; Leung, Chu and Lai, 2017). It would be of interest to examine the impact of acute bouts of different types of exercise on AT stiffness to offer comparison to the results from this thesis. Assessing the impact of differing exercise intensities and durations on AT stiffness would be of great interest as would examining the impact of long term training in certain sports on the mechanical properties of the AT. The use of SWE in a monitoring capacity over a competitive season would also prove a valuable future direction. This information could be utilised to monitor AT stiffness longitudinally and would be useful for athletes, coaches and clinicians planning training loads as well as those planning a return to activity following recovering from ATY. Being able to monitor AT stiffness over the course of a competitive season, may provide early indications of issues and injuries that can be addressed through alterations in training loads before they progress to a stage requiring additional treatment. It could also provide indications of times in the competitive season where athletes are considered more ‘at risk’ of developing injury. This would be of the

upmost importance to those involved in high-level sports as alterations in training are always preferable to longer term injury and potential rupture.

9.7 Conclusion

This thesis has investigated the imaging technology of SWE and established the most appropriate methodology and guidelines for best practice for its application to AT imaging. This thesis has shown SWE to be a simple, practical and reliable tool for assessing the mechanical properties of both healthy and pathological ATs. It has also shown the promise that SWE has in effectively monitoring long term changes in AT stiffness throughout a rehabilitation programme for the treatment of ATY. It demonstrated an increase in AT stiffness throughout an EcEx programme and highlighted when these alterations in stiffness occur in relation to other commonly used clinical measures. Few scientific studies have examined the methodology required to obtain reliable measures of AT stiffness using SWE in the clinical setting or as a monitoring tool for rehabilitation. In addition to the best practice recommendations, the results of this thesis have led to the creation of a database outlining average SWV measures in healthy and symptomatic, pathological ATs. This is the initial step in the development of classification limits and the volume of data presented in this thesis regarding best practice and normative SWE values, collectively provide an original contribution to the literature.

The thesis provides novel assessments of reproducibility and new findings regarding the impact of extraneous variables on SWE measures. This thesis provides a data set observing normative SWE values in both healthy and pathological tendons and is the first to offer insight into alterations in AT stiffness throughout and after an EcEx rehabilitation programme.

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11 Appendices

11.1 Appendix A – Informed Consent

University of Brighton

School of Sport and Service Management

Participant Informed Consent

Title of Project: Alterations in the mechanical properties of the Achilles tendon throughout the Alfredson model of Eccentric Exercise Rehabilitation Protocol.

Name of Researchers: Catherine Payne, Dr Nick Webborn, Dr Peter Watt

Please initial box

I confirm that I have read and understand the information sheet dated for the above study and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

I am satisfied that the researchers have explained the purpose, principles and procedures of the study, outlining any possible risks.

I understand how my data will be collected, that it will be kept confidential and anonymous and that the confidential information will only be seen by researchers and will not be revealed to anyone else.

I agree that if I were to withdraw from the study, the data collected up to that point may be used by the researcher for the purposes outlined in the information sheet.

I agree to take part in the above study.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

☐

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has fully informed me of, and I have understood, the purpose of the experiment and possible risks involved.

_____	_____	_____
Name of participant	***Date***	***Signature***
_____	_____	_____
Name of Person taking consent	***Date***	***Signature***

(if different from researcher)

_____	_____	_____
Researcher	***Date***	***Signature***

(1 copy for participant; 1 copy for researcher)

11.2 Appendix B – Medical Questionnaire

University of Brighton

School of Sport and Service Management

Medical Questionnaire

Name…….. Date of Birth……

Are you in good health? Yes No

(If No, give details)

Emergency contact Name:……...
Relation:……..

Number:……..

How often do you currently participate in moderate to vigorous physical activity?

< Twice a week

2 - 4 times per week

4 + time per week

What group best describes the majority of your training?

Endurance training

Strength/Power training

Team Sports

Other

How often do you currently participate in stretching exercise?

< Twice a week

2 - 4 times per week

4 + time per week

Have you suffered from a serious illness or accident?
Yes/No

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

Respiratory Problems (e.g. Asthma, Bronchitis?)
Yes/No

Diabetes?
Yes/No

Epilepsy?
Yes/No

High/Low Blood Pressure
Yes/No

Cardiovascular problems
Yes /No

Is there a history of heart disease in your family?
Yes /No

If yes, please give details:

Are you currently taking medication or dietary supplements incl. contraceptive pill?
Yes/No

If yes, please give details:

In the last 3 months, have you consulted your GP for any condition?
Yes/No

If yes, please give particulars:

Are you currently taking part (or have recently taken part) in any

other laboratory experiments?
Yes/No

Are you, or have ever been a smoker?
Yes/No

If yes, how many do/did you smoke per day?

Do you currently have any form of muscular or joint injury, or have you had

to suspend your normal training for the past two weeks prior to this test?
Yes /No

If yes, please give details:

Have you had any previous known injury to your Achilles tendon?
Yes /No

Is there anything to your knowledge to prevent you from successfully
Yes /No

completing the tests that have been outlined to you?

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to participate in the study if they:

- Have a fever, suffer from fainting spells or dizziness.
- Have a known history of medical disorders, i.e. high blood pressure, heart or lung disease.
- Are unsure of the test protocol and the possible risks and discomforts designated on the subject information sheet.
- Have been verified, or documented as having any blood carried infections (Hepatitis, HIV), are diabetic or obese (Body Mass Index>30), or have a known history of haematological, cardiac, respiratory, or renal disease.
- The answers given on the medical questionnaire or informed consent form do not meet the required criteria.

DECLARATION

Title of Study: Alterations in the mechanical properties of the Achilles tendon throughout the Alfredson model of Eccentric Exercise Rehabilitation Protocol.

I ……hereby volunteer to be a subject in experiments/investigations during the period commencing……..……201…..

Signature of Volunteer

……..……
….. Date ……..

Signature of Experimenter

……..……
….. Date ……..

11.3 Appendix C – VISA-A Questionnaire

The VISA-A questionnaire: An index of the severity of Achilles tendinopathy

IN THIS QUESTIONNAIRE, THE TERM PAIN REFERS SPECIFICALLY TO PAIN IN THE ACHILLES TENDON REGION

1: For how many minutes do you have stiffness in the Achilles region on first getting up?

100 mins
0 Mins

0	1	2	3	4	5	6	7	8	9	10

Question 1 Points:

2: Once you are warmed up for the day, do you have pain when stretching the Achilles tendon fully over the edge of a step? (keeping knee straight)

Strong
No Pain
Severe
Pain

0	1	2	3	4	5	6	7	8	9	10

Question 2 Points:

3: After walking on flat ground for 30 minutes, do you have pain within the next 2 hours? (If unable to walk on flat ground for 30 minutes because of pain, score 0 for this question)

Strong
No Pain
Severe
Pain

0	1	2	3	4	5	6	7	8	9	10

Question 3 Points:

4. Do you have pain walking downstairs with a normal gait cycle?

Strong
No Pain
Severe
Pain

0	1	2	3	4	5	6	7	8	9	10

Question 4 Points:

5. Do you have pain during or immediately after doing 10 (single leg) heel raises from a flat surface?

Strong
No Pain
Severe
Pain

0	1	2	3	4	5	6	7	8	9	10

Question 5 Points:

6. How many single leg hops can you do without pain?

0
10

0	1	2	3	4	5	6	7	8	9	10

Question 6 Points:

7. Are you currently undertaking sport or other physical activity?

0 ☐ Not at All

4 ☐ Modified training ± modified competition

7 ☐ Full training ± competition but not at same level as when symptoms began

10 ☐ Competing at the same or higher level as when symptoms began

Question 7 Points:

8. Please complete EITHER A, B or C in this question.

• If you have no pain while undertaking Achilles tendon loading sports please complete Q8a only.
• If you have pain while undertaking Achilles tendon loading sports but it does not stop you from completing the activity, please complete Q8b only.
• If you have pain that stops you from completing Achilles tendon loading sports, please complete Q8c only.

A.

If you have no pain while undertaking Achilles tendon loading sports, for how long can you train/practise?

NIL	1-10 mins	11-20 mins	21-30mins	>30 mins
☐	☐	☐	☐	☐
0	7	14	21	30

Question 8 A Points:

OR

B. If you have some pain while undertaking Achilles tendon loading sport, but it does not stop you from completing your training/practice for how long can you train/practise?

NIL	1-10 mins	11-20 mins	21-30mins	>30 mins
☐	☐	☐	☐	☐
0	4	10	14	20

Questions 8 B Points:

OR

C.

If you have pain that stops you from completing your training/practice in Achilles tendon loading sport, for how long can you train/practise?

NIL	1-10 mins	11-20 mins	21-30mins	>30 mins
☐	☐	☐	☐	☐
0	2	5	7	10

Questions 8 C Points:

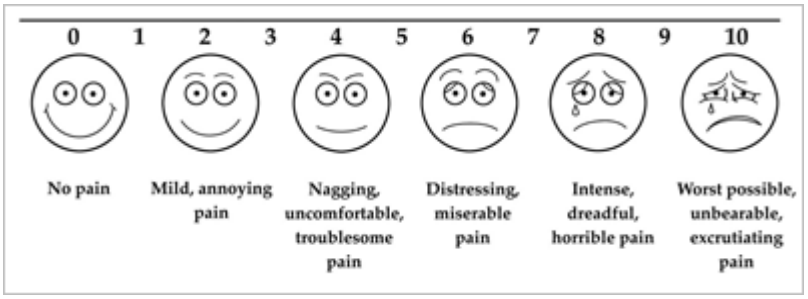
TOTAL SCORE (_____ /100) = _____ %

11.4 Appendix D – Visual Analogue Scale (VAS)

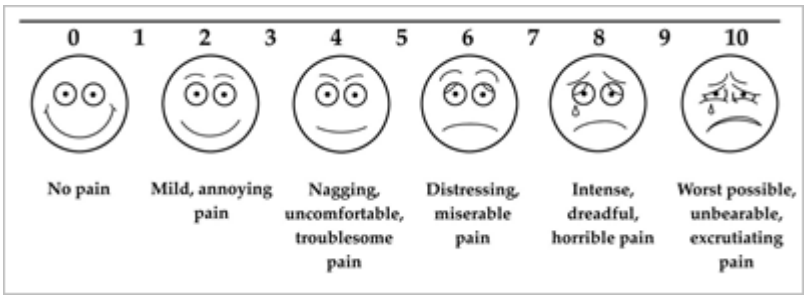
Visual Analogue Scale (VAS)

Please mark your pain on the following scales for each activity:

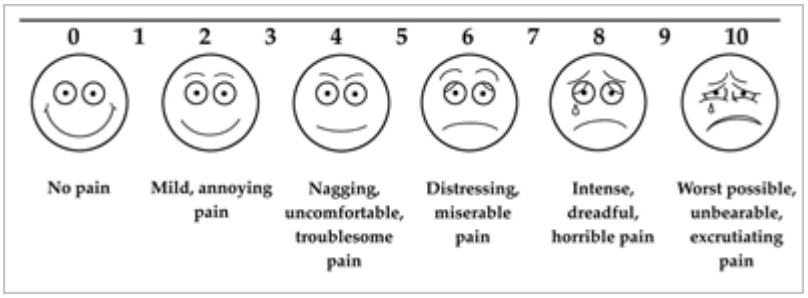
1) Sitting



2) Walking



3) First thing in the morning



4) When completing your exercises