MEASUREMENT OF TENDON TRANSVERSE STIFFNESS IN PEOPLE WITH ACHILLES TENDINOPATHY- A CROSS SECTIONAL STUDY

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis/dissertation entitled:

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Abstract

The Achilles tendon is the largest and strongest tendon in the human body and is vital for locomotion. One of the most important indicators of tendon function is tendon stiffness. Tendinopathic Achilles tendon displays reduced stiffness compared to healthy tissue, meaning that it experiences more strain for a given load, putting the tendon at a higher risk of damage.

Recently, in vivo measures of Achilles tendon stiffness have become more common, although they are limited to the research setting due to low reliability values. In order to address these limitations, we have begun validating a newly available technology, the MyotonPRO. This is a handheld, digital palpation device that sends out a small impulse into the tendon through a probe which houses an accelerometer. The measured deformation and acceleration determined by the device, are then used to derive the transverse stiffness of the tendon. The aim of this cross-sectional pilot study is to assess whether the transverse stiffness of tendinopathic Achilles tendon (the mid-portion tendinopathy group) is lower than those who are free from symptoms (the control group). We hypothesized that the injured tendons will be significantly less stiff than the tendons of the control group.

To test this hypothesis, we used the MyotonPRO to measure the Achilles tendons of 25 individuals who either had midportion tendinopathy (n=10) or who were healthy controls (n=15), and compared their transverse stiffness values taken at the same average location on the tendon (3.7cm from the insertion).

We found that there was a significantly lower transverse stiffness in tendinopathic subjects compared to controls ($p=0.006$). These findings suggest that the MyotonPRO can provide information about the tendon mechanical properties that may be useful in understanding how tendinopathy affects tendon function.

This study opens the door to the continued investigation of a relatively inexpensive, accessible and easy-to-interpret device. We believe that this device could be used to monitor the healing in tendinopathy patients as well as predicting and preventing injuries and monitoring adaptive changes in tendons in response to exercise.

Lay Summary

The main goal of this study was to assess differences in the Achilles tendons in people with a chronic Achilles tendon injury. The results of this study showed that the injured Achilles tendon was softer (less stiff) than healthy tendons. We also found that participants with advanced age had significantly different tendon properties than their younger counterparts. We measured tendon stiffness using a hand-held device that could be used in a clinical setting, and the measurements were highly reliable, but the reliability was worse when the tendon was placed on stretch. For this reason we recommended that stiffness measurements be taken with the tendon in a resting position.

Preface

This thesis is original work by the author, E. Finnamore, under the supervision of Dr. Alex Scott with guidance from Dr. Michael Ryan and Dr. Christopher West. The study protocol and procedures were performed according to the University of British Columbia Clinical Research Ethics Board. The Clinical Research Ethics Board certificate number is H16-03381, which was written by Dr. Charlotte Waugh**.** Development of study protocol and documentation were developed by Dr. Scott and me with assistance from Doctors Ryan and West. Statistical analysis was performed by me, E. Finnamore, under the supervision of Dr. Scott. To date, the experimental data in this study has not yet been published.

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List of Abbreviations

AT	Achilles tendon
ATRA	Achilles tendon resting angle
BMI	Body mass index
CSA	Cross-Sectional Area
CT	Computed Tomography
EF	Evan Finnamore (Investigator)
FAS	Foot and Ankle Stabilization device
GAG	Glycosaminoglycans
GL	Gastrocnemius Lateralis
GM	Gastrocnemius Medialis
ICC	Intraclass Correlation Coefficient
K	The spring constant as seen in Hooke's law
MDC	Minimum Detectable Change
MRI	Magnetic Resonance Imaging
N/m	Newtons per meter
N/mm	Newtons per millimeter
PG	Proteoglycans
PGE_2	Prostaglandin E_2
RSA	Radiosteriophotogrammetric analysis
SD	Standard Deviation

SEM	Standard Error of Measurement
SPSS	Statistical Package for the Social Sciences
UTC	Ultrasonic Tissue Characterization
VISA-A	Victorian Institute of Sports Assessment - Achilles

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Dedication

I would like to dedicate this thesis to my parents Hugh and Joanne, and my wonderful girlfriend Caitlin for their love and support. You have instilled in me of the value of education and of never giving up.

Chapter 1: Introduction

Achilles tendinopathy is an injury that is described as a dysfunctional, painful and swollen section of tissue within the tendon proper (Maffulli, Khan, & Puddu, 1998). Achilles tendinopathy is an injury which affects both athletic and non-athletic populations (de Jonge et al., 2011) and is most commonly found in middle-aged males (Maffulli, Wong, & Almekinders, 2003; Jarvinen, Kannus, Maffulli, & Khan, 2005).

Achilles tendinopathy is thought to occur primarily as a result of overuse. These high repetitive loads put onto the tendon result in an accumulation of subclinical micro-damage (Selvanetti & Cipolla, 1997). Although Achilles tendinopathy is an injury which is long lasting, is associated with a reduction in quality of life and commonly requires extensive rehabilitation in order to heal, it has been postulated that the same accumulation of damage sustained in tendinopathy may also contribute to spontaneous tendon rupture (Kannus & Jozsa, 1991; Tallon, Maffulli, & Ewen, 2001). The morphological changes which accompany Achilles tendinopathy, including; increased cross-sectional area, thickness and collagen fibre disruption, are believed to be related to the load-bearing capacity of tendinopathic tissue, which is decreased in those suffering from this injury (Sano et al., 1997).

Early, well-informed medical interventions are paramount in preventing worsening of Achilles tendinopathy and to prevent a further tendon degeneration which may lead to a tendon rupture. Measuring the mechanical properties of tendon can reveal important information about how the tendon reacts to forces applied to it. This knowledge can then provide researchers with a deeper

understanding of how tendon responds to various pathologies. One such measure that can give researchers insight into how various treatment modalities and interventions affect tendon properties is tendon stiffness.

Stiffness is crucial to the load bearing capacity (Lacroix, Duenwald-Kuehl, Lakes, & Vanderby, 2013) and functionality (Fletcher, Esau, & MacIntosh, 2010) of the Achilles tendon and has been shown to be reduced in those with Achilles tendinopathy (Arya & Kulig, 2010). The fact that tendon stiffness is reduced in tendinopathic subjects is also reason to investigate this property as a potential therapeutic target of rehabilitation programs.

Tendon stiffness is most commonly measured in the longitudinal direction (e.g. tensile stiffness, k_{TE}), in accordance with its line of pull (Bohm, Mersmann, & Arampatzis, 2015). However recently, there have been several novel methods of stiffness assessment which emit oscillations transversely to the Achilles tendon aponeurosis in order to provide measurements of tendon stiffness (Dirrichs et al., 2016; Ooi, Malliaras, Schneider, & Connell, 2014; Ooi, Schneider, Malliaras, Chadwick, & Connell, 2015; Orner, Kratzer, & Schmidberger, 2017; Sohirad, Wilson, Waugh, Finnamore, & Scott, 2017; Zhang et al., 2016). The main benefit of these methods is that a maximal force through the tendon is not required and therefore individual variability (in the form of physical effort) is eliminated.

The main issue with many of these assessment methods, regardless of what type of stiffness is being investigated (longitudinal or transverse) is that they are expensive, they can require extensive analysis, are not always readily available, and can be time consuming in their data

collection process. The fact that many of these studies are conducted on small sample sizes may be a result of an accumulation of the aforementioned drawbacks (Morrison, Dick, & Wakeling, 2015).

One recently investigated tool for the assessment of transverse stiffness assessment in humans is called the MyotonPRO. The main benefits of this tool are that it is objective, easy to use/ interpret, relatively inexpensive, and preliminary studies using the device are highlighting the potential for larger studies on tendon in real-world situations.

The feasibility of measuring transverse stiffness on Achilles tendons in recreational runners has been previously assessed (Sohirad et al., 2017). A commercially available unit (MyotonPRO, Myoton AS, Estonia) has been reported to accurately measure transverse stiffness, and in adult tendon tissue the presence of skin beneath the probe tip had a negligible influence on transverse stiffness (Sohirad et al., 2017). In a field study of 66 recreational runners, men displayed stiffer tendons than women, and more highly trained individuals had increased Achilles tendon transverse stiffness compared to those who were less highly trained (Sohirad et al., 2017). These results encouraged us to explore the potential utility of measuring Achilles tendon transverse stiffness in people with symptomatic tendinopathy.

The current, cross-sectional study is designed to inform us of the MyotonPRO's ability to assess differences between the material properties of tendinopathic tissue compared to healthy controls and also to assess the reliability of the device on this population.

1.1 Overview

The Achilles tendon (AT) is a common site of injury as it has prolonged, extreme, functional demands placed on it by the muscular architecture of the lower leg during locomotive and jumping activities. The AT can undergo almost four times more stress than the peak stress experienced by most tendons (Kongsgaard, Aagaard, Kjaer, & Magnusson, 2005) and can reach peak stress up to 12 times body weight or 9kN (Komi, Fukashiro, & Järvinen, 1992). Due to the repetitive nature of loading through the AT, repetitive (overuse) injury is the most common form of damage to this structure in humans (Järvinen, Kannus, Maffulli, & Khan, 2005).

In 1998, Khan et al argued that a combination of pain, swelling and impaired performance should be called tendinopathy (Khan & Maffulli, 1998). Tendinopathy can either be insertional (pain and abnormalities adjacent to the calcaneal tuberosity) or mid-portion (pain and abnormalities 2-6 cm proximal to the calcaneal tuberosity). The AT is hypovascular impaired healing of this structure (Arverud et al., 2016) and the mid-portion of the AT is also the most common site of total rupture in the tendon (Gillespie & George, 1969; Shields, Kerlan, Jobe, compared to other tissues, which may contribute to the Carter, & Lombardo, 1978).

Current methods of assessment typically include, but are not limited to, assessment of tenderness upon palpation along the AT mid-portion, presence of a nodular thickening of the AT mid-portion, and pain upon loading of the AT (Alfredson & Cook, 2007; de Jonge, 2015; Scott, Huisman, & Khan, 2011). Questionnaires such as the VISA-A are also commonly used to help evaluate the presence and severity of injury. Doppler Ultrasound has been used to differentiate

between tendinopathic and healthy AT, with limited results (Leung & Griffith, 2008; Reiter, Ulreich, Dirisamer, Tscholakoff, & Bucek, 2004). Other studies have successfully quantified the tendon biomechanical property of stiffness using radiosteriophotogrammetric analysis (RSA) and computed tomography (CT) (Schepull, 2013; Schepull, Kvist, Andersson, & Aspenberg, 2007). However RSA involves implanting tantalum beads inside the tendon. Tendon biomechanical properties have been successfully examined using methods such as the tendon oscillation method, and ultrasonography methods (Kubo, Yata, Kanehisa, & Fukunaga, 2006; Wilson, Wood, & Elliott, 1991), however these techniques require bulky, expensive equipment that requires extensive training (Orner et al., 2017).

1.2 Tendon Structure

1.2.1 Tendon Macrostructure

The AT is the largest, strongest and thickest tendon in the human body with a mean average length in adults of 18 cm (O'Brien, 2005). The muscles of the lower leg (gastrocnemius mediallis (GM), gastrocnemius laterallis (GL) and soleus) along with the Achilles tendon, make up the musculotendinous unit, which is responsible for plantarflexion (Doral et al., 2010). The primary purpose of the Achilles tendon is to transmit forces from the plantar flexors of the lower leg to the calcaneus, producing and controlling joint movement (i.e., rotation of the talo-crural joint) (Robert, Moczar, & Robert, 1974). The Achilles tendon can handle high compressive and tensile forces, and can store and release energy during everyday movements in humans (Alexander & Bennet-Clark, 1977).

The AT is formed by fibrous, dense connective tissue, which forms bundles of bright, white collagen in healthy specimens. The AT is covered by a structure called the peritenon (Jozsa & Kannus, 1997). In contrast, smaller tendons in the human body are surrounded by a dense connective tissue called a sheath (Kannus, 2000). The peritenon is made up of the paratenon (the outermost layer), the epitenon (which is closest to the tissue proper) (Tuite, Renström, & O'Brien, 1997) and the mesotenon. Together these three layers of connective tissue along with blood vessels, nerves and lymphatic vessels form the peritenon (Gould & Korson, 1980). These tissue layers allow the tendon to stretch and slide during movement (Jozsa & Kannus, 1997). The fibers of the AT rotate 90 degrees when descending towards the calcaneal insertion. This leads the fibers arising from the soleus to insert medially where the fibers from the gastrocnemius muscles insert laterally (Barfred, 1973). This twisting results in increased stress and shearing, especially around the midportion of the tendon (2-6 cm proximal to insertion to the calcaneal tuberosity) (Jozsa & Kannus, 1997), which may contribute to increased risk of injury in this area.

Also involved in the tendon's ability to store and release energy is the interfascicular matrix. The interfascicular matrix (also called the endotenon) is hypothesized to facilitate smooth sliding between fascicles, aiding in a greater capacity for extension. High strain capacity for energy-storing tendons such as the Achilles is aided by the interfascicular matrix, as its properties allow the tendon to store energy and efficiently elongate in humans (Thorpe et al, 2015). This smooth gliding is essential as excessive friction between fascicles is hypothesized to be one contributing factor to tendinopathy (Screen et al, 2015). Although sharing in general may contribute to the development of tendon injury, the fact that the Achilles tendon has differential shear

displacement and direction of shear during various knee angles may be another contributing mechanism (Bojsen-Moller et al, 2004). In a 2004 study by Bojsen-Moller et al, displacement of the medial gastrocnemius was 36% greater than the displacement of the soleus aponeurosis during ramped contractions of the plantar-flexor muscles (Bojsen-Moller et al, 2004). The differences in aponeurosis displacement are hypothesized to cause a shearing effect of the tendon, which is governed by knee position (Bojsen-Moller et al, 2004). This shearing may be particularly evident in runners as this movement involves varying degrees of plantarflexion when the knee is both in an extended and flexed position. It has also been noted that in middle-aged adults there is a reduced inter-fascicular gliding of the tendon, contributing to an increased shearing of fibres (Slane & Thelen, 2015). In the 2015 study by Slane & Thelen, they found that the middle and deep portions of the Achilles tendons in the middle aged group showed more uniform displacement patterns when compared to the younger group (Slane & Thelen, 2015). This lead researchers to believe that the more uniform displacement within the tendon may be a result of an age-related reduction in inter-fascular gliding (Slane & Thelen, 2015). Shear between tendon fibrils during stretching and loading of crimped collagen fibrils may put individual fibres at risk for damage (Arnoczky, Lavangnino & Egerbacher, 2007). Therefore, the changes in the tendon that occur with shearing of tendon fibrils may be one of the reasons why middle-aged people and runners are at higher risk of tendon injury.

A popular model of tendon pathophysiology is known as the continuum model. This model proposes that tendinopathy has three stages: reactive tendinopathy, tendon disrepair and degenerative tendinopathy (Cook & Purdam, 2009). This model indicates that there are three stages of tendinopathy, however they are on a continuum, thus there is continuity between these

stages (Cook & Purdam, 2009). It is believed that during reactive tendinopathy, there is primarily a non-inflammatory response of the tendon to excessive load. However this model also acknowledges the influence of inflammation during the injury process which can negatively affect tendon cell structures and the tendon matrix (Cook & Purdam, 2009; Dakin et al, 2018).

1.2.2 Tendon Internal Architecture and Ultrastructure

Tendons are composed of predominantly type I collagen and elastin, which lie in a proteoglycan-water solution. Type I collagen is the main component of healthy tendon tissue and composes 65-80% of the dry weight of tendon (Kannus, 2000; Moira O'Brien, 2005) while elastin accounts for 1-2% (Kannus, 2000; O'Brien, 1997). The nearly 90% proportion of type I collagen that makes up the AT is important as it is responsible for the mechanical strength of the tendon (Jozsa & Kannus, 1997; O'Brien, 2005). However in tendinopathic and ruptured Achilles tendon, tenocytes produce a higher proportion of type III collagen fibers, which are less resistant to tensile forces than type I fibres (Maffulli, Ewen, Waterston, Reaper, & Barrass, 2000).

The AT is made up of bundles of collagen fibrils categorized by their size. From the smallest to largest in cross sectional area, a group of collagen fibrils compose a collagen fiber, which in larger numbers form collagen fiber bundles. The collagen fiber bundles then form secondary bundles, and in larger numbers compose tertiary bundles separated by endotendon, also known as interfascicular matrix (Kannus, 2000). Finally, it is the largest unit of fiber bundles (tertiary fiber bundles) that compose the tendon proper.

Between the collagen fibers is something called ground substance, which mostly consists of glucosaminoglycans (GAG) and proteoglycans (PG). The GAG and PGs that are more common in tendinopathic tendon are more hydrophilic and therefore the water content of the tendon increases. Inflammatory mediators such as substance P and prostaglandin E_2 (PGE_2) may also play a role in the structural abnormalities and deficits present in tendinopathic tendon. In painful Achilles tendon (such as in a tendinopathy), peripheral nerve cells release substance P, which activate mast cells and may contribute to fibrosis of the tendon (Scott & Ludvig J. Backman, Speed, 2015). Tendon cells of tendinopathic tissue also produce more PGE_2 than healthy cells, which is important as PGE_2 mediates the inflammatory response in the human body (Zhang, Shaffer, Portanova, Seibert, & Isakson, 1997), and also depressed collagen synthesis in tendon fibroblasts. The overexpression of PGE_2 could lead to a chronic increase in cellular response to the pain (Fu et al., 2002). The body's negative response to PGE2 (which can induce pain) with tendon injury is one of the main reasons why pain should be monitored during rehabilitation. Continuous inflammation may cause the tendon more pathological harm if it is not dealt with during rehabilitation.

1.3 Prevalence of Achilles Tendinopathy

The AT is the largest tendon in the body, as well as one of the most commonly injured and ruptured tendons in humans (Jozsa et al., 1989; Lopes et al., 2012). Injury rates for this tissue are very high among athletes (Clement, Taunton, & Smart, 1984; Lysholm & Wiklander, 1987; Maughan & Miller, 1983) and sedentary individuals (Astrom & Rausing, 1995). The lifetime cumulative incidence of Achilles tendinopathy in former long distance runners is 52% (Kujala,

Sarna, & Kaprio, 2005). The annual incidence has been reported as being 7% to 10.9% (Johannsen & Gam, 2010; Lopes et al., 2012; Lysholm & Wiklander, 1987). In a systematic review by Lopes et al. (2012) it was found that the tendinopathy in the running population is predominantly restricted to the AT (Lopes et al., 2012).

Achilles tendinopathy is a common injury among athletes as well as the general public (Jozsa & Kannus, 1997; Maffulli & Kader, 2002). De Jong et al. (2011) state that although it seems plausible that athletes have a higher incidence rate of midportion Achilles tendinopathy than members of the general public, only 35% of tendinopathy cases in their study had a documented relationship with sports activities (de Jonge et al., 2011). These findings are similar to those in a 1998 study which found that 25% to 30% of those affected by Achilles tendinopathy are non-athletes (Aström, 1998). This is an important injury to examine as people who are affected by tendinopathy may become less motivated to exercise and may lose a substantial amount of work days per year, increasing the financial impact of injury on our society.

1.4 Factors Affecting Achilles Tendon Structure

1.4.1 Achilles Tendon and Adiposity Measurements

It has been known for many years that high adiposity is a risk factor for many chronic diseases. Recently, researchers have begun to question the relationship between higher levels of adiposity and musculoskeletal injury. Injuries that have been suggested to be impacted by the presence of increased adiposity have in the past been attributed to higher loads on the body due to a higher body mass of the individual. The idea that the increased body mass of obese individuals is the sole reason behind their increased injury risk has been questioned by researchers in the medical field (Gaida, Ashe, Bass, & Cook, 2009; Gaida, 2009; Scott et al., 2015). Increased adiposity also promotes microvascular abnormalities that have associations with chronic diseases such as cardiovascular disease and type 2 diabetes (Park, Park, & Yu, 2005; Yudkin, Kumari, Humphries, & Mohamed-Ali, 2000).

In a large frequency-matched case-control study conducted in 2004 by Wendelboe et al, it was concluded that there is an association between a high BMI and an increased risk of rotator cuff tendinopathy (Wendelboe et al., 2004). In this study, 311 patients whose ages ranged from 53-77 years of age were age- and frequency-matched to 933 randomly selected control participants. They found that the highest odds ratios for men and women were 3:13; 95% confidence interval = 1.29 to 7.61 and 3.51; 95% confidence interval = 1.80 to 6.85, respectively indicating obese individuals were upwards of three times more likely to require shoulder tendon repair surgery (Wendelboe et al., 2004). Wendelboe and colleagues also conducted a multiple linear regression

that indicated an association between an increasing body-mass index (BMI) and shoulder surgery (beta = 1.57; 95% confidence interval = 0.97 to 2.17; $p \leq 0.001$) (Wendelboe et al., 2004).

In two studies of anthropometric risk factors for jumping athletes, both studies found that a higher abdominal fat distribution was a risk factor for developing a tendinopathy (Gaida, Cook, Bass, Austen, 2004; Malliaras, Cook & Kent, 2007). These findings were consistent with research done by Scott et al. (2013) where they found that patients with Achilles tendinopathy had higher BMIs than control participants when age was accounted for (Scott, Hyer, & Granata, 2013).

One suggested mechanism for why higher abdominal adipose levels are a risk factor for tendon injury lies with the low-level inflammation with which higher adiposity is associated. Elevated adiposity has been associated with high cytokine levels such as IL-1, IL6, MMP1, MMP3, VEGF and PGE_2 (Gaida, Ashe, Bass, & Cook, 2009; Park et al., 2005; Yudkin et al., 2000). Over the past few years new evidence has emerged that highlights an inflammatory component of tendon pathogenesis (Dean, Gettings, Georgina Dakin, & Jonathan Carr, 2015; Millar & Dean, 2016; Rees, Stride, & Scott, 2014). Indeed, cytokine levels which are elevated in people with high adiposity may directly or indirectly contribute to tendon structure abnormalities (Jain, Nanchahal, Troeberg, Green, & Brennan, 2001).

Because anthropometric values have such a profound impact on tendon structure, we will attempt to account for adiposity in this study to avoid this possible confounding variable impacting our findings. Measurements such as adiposity estimation in the form of waist circumference and

BMI measurements will be taken of all participants, and the groups will be balanced to ensure similar BMI distributions.

1.4.2 Achilles Tendon and Age

Age is another factor that can affect the mechanical properties of tendon. During the aging process there are loading-induced changes that affect the properties of tendon. For example, as humans age they produce less muscular force and may be less active than when they were younger, however aging itself can also alter tendon properties. The aging process and its effect on tendon is multifactorial and various muscular, neural, hormonal and biomechanical changes all play their part in the change in aging tendon's mechanical properties. Size and stiffness of tendons increases from age 9 to adulthood (Kubo, Kanehisa, Kawakami, & Fukanaga, 2001). Age-related increase in body mass and force production during the transition from childhood to adolescence have been thought to be the main contributing factors to the changes in mechanical properties of tendon (O'Brien, Reeves, Baltzopoulos, Jones, & Maganaris, 2010). The increase in stiffness may be due to an increase in tendon size caused by increased fibril diameter (Diamant, Keller, Baer, Litt, & Arridge, 1972; Parry, Barnes, & Craig, 1978) and collagen cross-linking (Bailey, Paul, & Knott, 1998). Past studies have reported that children's tendons demonstrate more tendon elongation and higher strain for a given load during isometric contractions (Kubo et al., 2001; O'Brien et al., 2010). In a study by Waugh et al, no differences were found in the maximal strain in the AT between children aged 5-12 (n=52) and adults aged 22-29 (n=19) (Waugh, Blazevich, Fath, & Korff, 2012) even though in previous studies structural dimensions

such as cross-sectional area, thickness and length were found to be greater in adults than children (Kubo et al., 2001; O'Brien et al., 2010).

When we examine the effect of aging on tendon in older participants we start to see mechanical changes that are similar to those seen in immobilized subjects, and which may put aged individuals at greater risk for traumatic or chronic tendon injury. In a study by Onambele et al, the researchers compared mechanical properties of Achilles tendons between younger (24±1 yr, n=24), middle-aged (46±1 yr, n=10) and older (68±1 yr, n=36) participants (Onambele, Narici, & Maganaris, 2006). The researchers found that tendon stiffness and Young's Modulus decreased gradually with age (Onambele et al., 2006). They found that the stiffness and young's modulus decreased with age by 36 and 48% respectively ($p<0.05$) (Eriksen et al., 2018; Onambele et al., 2006).

In an attempt to better understand the increased rate of tendon injury and rupture in the middle-aged and older populations, a study by Pardez et al in 2017 used in-vivo analysis of tendon tissue in rats to compare the effects of age on tendon properties (Pardes et al., 2017). The authors found that inferior material properties such as a decreased maximum stress and decreased modulus occurred with increasing age (Pardes et al., 2017). Additionally they found that fibre size distribution was not affected by age so they concluded that other factors must be responsible for the changes in tendon material properties due to age (Pardes et al., 2017).

Despite all of the negative tendon adaptations to aging, which put older people at risk for injury, there is a light at the end of the tunnel as it is suggested that older tendon has been demonstrated

to be mechanosensitive to long-term mechanical loading (Epro et al., 2017). In response to a 14-week loading program in 21 female adults (60-75 yrs) AT stiffness and Young's modulus increased from 488.4±136.9 Nmm at baseline to 598.2±141.2 Nmm and the tendon had a 6% hypertrophy along the whole free-tendon (Epro et al., 2017).

In summary, there appears to be a reduction in mechanical properties of tendon (modulus and strength) with aging, however the mechanisms for exactly why this occurs are largely unknown. These factors are important as tendon stiffness has a relationship with tendon failure (LaCroix et al, 2013). Tendon failure is known to be highly strain dependent, and tendon stiffness is related to the maximal amount of stress a tendon can withstand before rupturing, therefore it is beneficial to have a non-invasive tool that could measure tendon stiffness in vivo to predict ultimate tensile stress (LaCroix et al, 2013).

1.4.3 Achilles Tendon and Sex

Males typically have larger, stiffer and longer tendons than females. Despite having seemingly "stronger" tendons, tendon injury is more common in males. In a study by Vosseller et al, it was found that there was a male:female sex ratio of 5.39:1 and 1.56:1 for Achilles tendon rupture and tendinosis respectively (Vosseller et al., 2013). In another study authors also found that men had a three times the risk of developing Achilles tendinopathy, even when sport participation was accounted for (Astrom & Rausing, 1995). Although other authors have found that there is a lower tendon stiffness in females (Kubo, Kanehisa, & Fukunaga, 2003) in a study conducted by Morrison et al they found that tendon stiffness varied more so by muscle strength and not

necessarily by sex (Morrison et al., 2015). When looking at the tendon stiffness in 20 trained cyclists (m=10, f=10), they found that there was no significant effect of sex on AT stiffness (Morrison et al., 2015). What also may help us explain why there is a differing tendon injury rate in males when compared to females is a study by Fryhofer et al conducted at the University of Pennsylvania (Fryhofer et al., 2016). The authors aim was to see if biomechanics of healing tendon was different between males and females. To test this, authors studied the material properties of transected tendon between male and female rats; theyalso included a group of female rats with ovaries removed to test for the effect of female sex hormones. They found that female tendons were stronger and more elastic, however they were smaller on average than the male tendons (Fryhofer et al., 2016). Post-injury muscle size recovered more quickly in females, and their tendons demonstrated superior mechanical properties during healing (Fryhofer et al., 2016). The female rats who were deprived of sex hormones also had inferior tendon mechanics and tendon composition compared to non-operated females (Fryhofer et al., 2016).

Although Achilles tendons have been shown to have higher stiffness in female animal models, there have been different findings in studies involving human tendons. In our own study we found that male tendons were slightly stiffer on average than the tendons of female runners although the difference was not significant (M=611.23N/m, F=585.76N/m, p=0.067, n=132) (Sohirad, Wilson, Waugh, Finnamore, & Scott, 2017). Other studies have also supported the findings that there are not sex differences in tendon stiffness between sexes in both elderly (Burgess, Pearson, Breen & Onambele, 2009) and younger (aged 20-25) tendons (Caglar & Oz, 2017).

Lastly, other studies have found that male subjects have stiffer tendons than those of females (Burgess, Graham-Smith & Pearson, 2008; Joseph et al, 2014; Kubo, Kanehisa & Fukunaga, 2003). Although all three studies came to similar conclusions about sex and stiffness, the stiffness values greatly varried between the studies (M=76.7N/mm, F=49.6N/mm, $p<0.05$, n=18males17females) (Burgess et al, 2008), (M=835.4N/mm, F=530.6N/mm, $p<0.01$, n=17men14 women) (Joseph et al, 2014), (M=25.9N/mm, F=16.5N/mm, $p<0.05$, n=16males13females) (Kubo et al, 2003).

As stiffness is such an important measure of tendon health and function, and findings vary between studies so greatly, it would be invaluable to have a consistent measurement of tendon stiffness. This measurement could then be compared easily between studies to give researchers a broader view of how various sex differences affect tendon structure.

1.4.4 The Achilles Tendon and Running

Like many other tissues in the human body, tendons are responsive to mechanical loading. The structural adaptations that take place during loading activities cause the tendon to become stiffer and therefore more resistant to increased load (Buchanan & Marsh, 2002; Kjaer et al., 2006). When the loads applied to the tendon are too high, overload of the tendon can occur, causing structural damage (Magnusson, Langberg, & Kjaer, 2010). After exercise, collagen synthesis is enhanced (Kongsgaard et al., 2005), however degradation of collagen also increases after exercise (Kubo & Tabata, 2010). The degradation of collagen after exercise is so great that it actually initially outweighs the collagen synthesis (Kubo & Tabata, 2010). As collagen

breakdown is enhanced 18-36 hrs after exercise and collagen generation increases 37-72 hrs after exercise (Magnusson et al., 2010). This tells us that there needs to be adequate time in between training sessions to avoid excessive tendon breakdown which may lead to tendon injury.

It had been shown in experimental groups that either took part in weight-bearing exercise greater than 6 hours per day (defined in this study as "force-generating activities which generates loading to the skeletal regions with intensity stronger than daily activities") (Siu, Chan, Lam, Lee, & Ying, 2016), took part in a plyometric training program (Fouré, Nordez, & Cornu, 2010), or performed heel drop exercises (Leung, Chu, & Lai, 2017) that tendon stiffness increases with activity levels. However, there are fewer findings looking at the effect of running on the Achilles tendon stiffness. Arampatzis et al found that there was no difference in the stiffness of tendons between endurance runners and controls, although there was a higher stiffness of sprinters when compared to endurance runners and controls (Arampatzis et al, 2007). When differences in transverse tendon stiffness were examined between three different racing groups (10km, half marathon and full marathon), there was a significant difference between groups ($p<0.001$) (Sohirad et al, 2017). Thus, variability in stiffness between runners engaged in various magnitudes and intensities of running should be further studied, and accounted for in future studies of tendon biomechanics (Morrison et al., 2015).

1.5 Tendinopathy Assessment Methods

1.5.1 Achilles Tendinopathy Severity Assessment

Achilles tendinopathy has a substantial impact on pain, function and activity level of the individual (Maffulli et al., 1998). In 2001, a disease-specific outcome questionnaire for Achilles tendinopathy, the Victorian Institute of Sports Assessment- Achilles (VISA-A), was developed by Robinson and colleagues (Robinson et al., 2001). This questionnaire has been shown to be responsive to clinical change over time (Silbernagel, Thomeé, Eriksson, Karlsson, & Silbernagel, 2007). It has also been shown to have good validity and reliability in multiple languages (Kaux et al., 2016; Maffulli et al., 2008; Robinson et al., 2001; Silbernagel, Thomeé, & Karlsson, 2005). In one case series, individuals with Achilles tendinopathy undergoing exercise-based rehabilitation demonstrated an average starting VISA-A score of 38.5 +/- 18.1 – after 12 weeks, this improved to 72.3 +/- 19.9 (McCormack, Underwood, Slaven, & Cappaert, 2015). Changes of 6.5 (McCormack et al., 2015) and 10 points (Beyer et al., 2015) have been considered to be clinically important. In the current study each participant completed a VISA-A questionnaire to both assess the level of dysfunction in the tendon and to see if this measure would be correlated to transverse stiffness measurements taken with the MyotonPRO.

1.5.2 Imaging and Tendon Integrity

A painful, localized thickening of the AT is consistent with the diagnosis of Achilles tendinopathy (Alfredson & Lorentzon, 2003; Arya & Kulig, 2010; de Jonge, 2015; Ohberg, Lorentzon, & Alfredson, 2004; Syha et al., 2007). Other studies that use ultrasonography have shown localized thickening and irregular tendon structure which corresponds to the patient's area of tendon fibre disorganization and damage within the tendon (Alfredson & Lorentzon, 2003; Ohberg et al., 2004; Syha et al., 2007). In essence, perhaps the body attempts to compensate for the pathological tendon tissue that is less stiff, by increasing the volume of the tissue. Using ultra-sonographic imaging, studies have shown a gradual decrease in thickness of the tendinopathic Achilles tendon following rehabilitation (Grigg, Wearing, Smeathers, Nicole, & Grigg, 2008; Ohberg et al., 2004). We will use ultrasound in this study to confirm the presence of changes in the Achilles tendon that are consistent with tendinopathy.

1.6 Clinical Measures of Achilles Tendon Function

Functional measures are useful for medical professionals in evaluating disability associated with Achilles tendinopathy. Achilles tendinopathy causes pain in people with this condition, but it is equally important to mention that impairments of Achilles tendon function are also present in these patients. It is important to include objective functional measures of lower leg function in order to help guide treatment and rehabilitation of tendon injuries and to give clinicians insight into how Achilles tendinopathy affects the functionality of this important, loadbearing tendon.

Currently the main methods of objective, functional assessment fall under the categories of muscular strength, muscular endurance, lower leg flexibility and motor control. These factors are important in assessment of function as rehabilitation protocols often assess these factors as targets of rehabilitation (Cook & Purdam, 2003). Functional assessment measurements take into account the functionality of the whole Achilles muscle-tendon unit, and not the properties of the tendon proper in isolation. Although these functional assessment methods differ from other methods that are only interested in examining the tendon proper and its relationship to loading, these functional tests can provide additional, important information. The Achilles tendon works in such close conjunction with the plantar-flexors of the lower leg therefore it is important to understand the interaction of all lower-leg structures.

Muscle strength is important in Achilles tendinopathy research as these measures are related to tendon function and functional ability in humans. Strength is typically measured either concentrically, eccentrically or isometrically with the use of dynamometry. The ankle position

used in the research study is dependent upon the research goals of the investigators. Although these measurements typically involve a dynamometer, measuring muscle strength on a weight-machine has also been found to be reliable and valid (Paavola et al., 2000). In a study by Silbernagel et al, 2006 it was also found that patients with tendinopathy had impaired strength when compared to control subjects (Silbernagel, Gustavsson, Thomeé, & Karlsson, 2006). This is an important finding because this reduced strength may lead to physiological imbalances during activity or indicate that there are important changes to the muscle-tendon unit that should be explored further.

Related to muscle strength, the measurement of muscle power (in the form of jump-height tests) also relay useful information on how the Achilles tendon functionality may be influenced by various pathologies (Silbernagel, Thomeé, Thomeé, & Karlsson, 2001; Westin et al., 2018). Jump tests are a commonly used tool to assess functional performance of the lower leg muscle-tendon unit in athletes and individuals with Achilles tendon injuries (Silbernagel et al, 2001; Westin et al, 2018). As stretch-shortening cycles are brief, and high loads are placed through the tendon during many functional, daily activities, jump tests can provide useful information on how injury effects tendon biomechanics. Additionally, as most activities involving the lower leg involve a large eccentric force followed by a concentric one, jump tests can provide additional information about how the Achilles tendon reacts to loading which other tests cannot.

Another functional measure used in tendinopathic populations is muscular endurance. Also called the heel raise test, endurance is typically investigated through repetitive heel raise exercises performed while standing on one leg, until fatigue is reached. This test is useful in

monitoring the effect of various interventions on plantarflexion musculotendinous function (Habets, Cingel, Backx, & Huisstede, 2017; Schepull & Aspenberg, 2013; Schepull et al., 2007; Silbernagel et al., 2001).

The combined compliance of the Achilles tendon and muscle has also been used as a valid and reliable functional assessment measure of tendon health. Tendon/ muscle-unit compliance has been measured by way of the Achilles Tendon Resting Angle (ATRA). This test measures the angle of the ankle either with the knee flexed at 90° or the knee straight with the leg in a resting position. This measurement has been shown to be important as it is related to tendon elongation as well as negatively correlated with work done on a heel raise test. The ATRA is valid, reliable and is sensitive to changes over time (Carmont et al, 2015; Zellers, Carmont, & Silbernagel, 2017), illuminating its ability to be used as a clinically useful tool to monitor how the Achilles tendon is impacted by injury.

All of these measures are useful in providing some important, functional information about the muscle-tendon unit, however it could also be of value to examine Achilles tendon properties in isolation.

1.7 Measurement of Mechanical Properties in Tendon

Although tendons are strong, they are not completely unyielding, i.e. they elongate when subjected to muscle and ground-reaction forces (Reeves, 2006). When examining the mechanical properties of the Achilles tendon, traditionally the musculotendinous junction is used as a

reference point in ultrasound images. The displacement relative to the calcaneus of the musculotendinous junction during contraction (seen on ultrasound images) represents the tendon's elongation (Kongsgaard, Nielsen, Hegnsvad, Aagaard, & Magnusson, 2011; Kubo et al., 2007; Kubo & Tabata, 2010; Peltonen, 2014). The forces eliciting the change in strain of the tendon are then measured with a dynamometer based on the joint torque, which usually requires some form of 3D image analysis of the ankle. The amount that a tendon deforms for a given load is dependent on the dimensions of the tendon in healthy individuals. For example, a tendon with a larger cross-sectional area (CSA) would be expected to have a smaller elongation (less compliant) than one that has a lower CSA.

1.7.1 Tendon Elasticity

Tendons react much like springs do when they undergo stretching or compressive forces. Ideal springs follow Hooke's law ($F=-kx$), where F is the force that causes the stretch or compression, k is the spring constant and x is the amount of deformation (Peltonen, 2014). Human tendons are elastic structures as they roughly follow Hooke's law, assuming their relative original shape after undergoing compressive or strenuous forces. Mammalian tendons are comparable to springs as they are around 90-95% efficient (Bennett, Ker, Dimery, & Alexander, 1986; Pollock, Shadwick, & Ri, 1994).

The spring constant, as seen in Hooke's law (k), is called stiffness. In our current study we will be examining stiffness transversely as the MyotonPRO assesses transverse stiffness as a function of $k= am/\Delta d$, where a is the acceleration of the probe upon perturbation, m is the mass of the

probe on the device and d is the change in displacement of the probe throughout its movement (Orner et al., 2017; Pożarowszczyk et al., 2017; Pruyn, Watsford, & Murphy, 2015; Sakalauskaite & Edukologijos, 2012; Sohirad et al., 2017).

1.7.2 The Importance of Tendon Tensile (Longitudinal) Stiffness During Healing

Several biomechanical properties such as Young's modulus, creep, and ultimate stress are useful in determining tendon function. Of these properties, tendon tensile stiffness is the most commonly investigated, being linked to functional activity performance and tendon health (Cook, 1996; Fletcher, Esau, & MacIntosh, 2010; Pruyn et al., 2015). The AT must withstand high tensile loads, requiring the tendon to display stiffness (or the resistance to being deformed). This is a property that tendon possesses through maintaining an extracellular matrix of predominantly Type I collagen. Tensile stiffness is a potentially important functional measure for health professionals as it is related to performance in stretch-shorten cycle activities (i.e., walking and running) (Pruyn, Watsford, & Murphy, 2014). AT tensile stiffness (i.e., resistance to longitudinal deformation under tensile load, N/m) is the relationship between longitudinal forces put through a tendon (i.e., contraction of the calf muscle on an AT) and the elongation of the tissue (McMahon & Cheng, 1990). AT tensile stiffness is greatly decreased after injury (from 300.37 $\pm$ 37.60 N/mm in the tendinopathy group compared to 375.25 $\pm$ 61.88 N/mm in the control group ($p<0.05$) after injury (Arya & Kulig, 2010). The tendon can take up to 12 months to regain its normal stiffness, and there is great variability in the speed/outcome of healing (Schepull & Aspenberg, 2013). As well as tendon stiffness being a measure of recovery after injury (Zhang et al., 2016), tendon ultimate stress has been found to be highly correlated with

tendon stiffness (R^2=0.785) in a meta-analysis including both human and animal studies (Lacroix et al., 2013).

1.7.3 Transverse Stiffness vs Longitudinal Stiffness

Stiffness is the load that is required to cause a unit deformation of a material. Based on the type and direction of load applied, different types of stiffness can be produced. Typically, when we think of Achilles tendon stiffness we think of longitudinal stiffness, which is lengthening through applied tension. This type of stiffness can be thought of as spring stiffness, as springs produce stiffness values when they are both stretched or shortened due to external forces either pulling on or pushing on them in the axial direction respectively. The equation for longitudinal stiffness is $k= F/\Delta L$ where k is the stiffness, F is the force applied to the material and ΔL is the deflection due to load on the material. A common method for assessing the longitudinal stiffness of the Achilles tendon is to use b-mode ultrasound to track relative changes in length of the tendon when the muscles of the lower leg are in contracted and relaxed states which estimates elastic values of the tendon such as average tendon strain of the tendon and muscle, with the use of tracking software overlaid upon the ultrasound image (Kurokawa, Fukunaga, Nagano, & Fukashiro, 2003; Maganaris, Baltzopoulos, & Sargeant, 2000; Maganaris & Paul, 1999; Peltonen, Cronin, Stenroth, Finni, & Avela, 2012; Theis, Mohagheghi, & Korff, 2012). One drawback of the 2D ultrasonic method of stiffness assessment is that localized elasticity values cannot be determined. Additionally an external force has to be applied longitudinally through the tissue (typically involving a graded isometric contraction from resting to maximal force production) in order to determine deformation information. Due to the high stresses of these

methods this may cause pain to the patient and may be risky for some patients, e.g., in the early stages of post-rupture healing.

Stiffness can also be calculated via perturbations in the transverse plane. Although there are no current studies evaluating the relationship between longitudinally deformed and transversely deformed tendon in relation to stiffness, transversely propagated stiffness has been assessed in several studies as a quantification of tendon collagen integrity and tendon hardness (Dirrichs et al., 2016; Haen, Roux, Soubeyrand, & Laporte, 2017; Michel et al., 2015; Ooi, Malliaras, Schneider, & Connell, 2014; Orner et al., 2017; Pożarowszczyk et al., 2017; Yeh et al., 2016). The most prevalent method for assessing tendon stiffness in the transverse direction is with the use of various elastography methods. The two most commonly used methods are strain elastography and shear wave elastography. Elastography applies a superficial stress perpendicular to the tissues being assessed. The strain elastography method uses manual compression of the probe by the operator, while shear wave elastography emits shear ultrasonic waves through the tissue. Elastography was first used to assess liver fibrosis and cancer tumors as tumorous growths are of a different stiffness than surrounding healthy tissue.

In a 2017 study by Payne and Webborn, strain elastography has been found to have a low level of reproducibility for the measurement of Achilles tendon young's modulus over multiple days (Payne, Webborn, Watt, & Cercignani, 2017). This study used strain elastography to measure healthy participant's Achilles tendons five times in one hour, and five times over given days (Payne et al., 2017). The authors found that compression elastography measures CV was above 53% (where 12% or less was acceptable), R values indicated no-to-weak correlations between

measures at best (range 0.01–0.25), and ICC values were all classified as poor (range 0.00–0.11) (Payne et al., 2017).

Chen et al, 2013 published a study that examined the ability of shear-wave elastography to assess the differences in elastic modulus (normalized stiffness) between ruptured Achilles tendons and asymptomatic controls (Chen et al., 2013). This study found that there was a significantly ($p=0.006$) lower elastic modulus in recently ruptured tendons compared to the control group (Chen et al., 2013). There was also another recent study using shear wave elastography to assess the impact of long-term weight-bearing exercise on Achilles tendon stiffness, by Sui et al, in 2016. This study found that although intra-operator reliability of the device ranged between 0.803 and 0.845, the inter-operator measurement reliability for young's modulus was 0.585 (Siu et al., 2016). Additionally, in a paper by Lin et al, published in 2011, they reported an artifact of three longitudinal bands, 1.3cm apart which they believe could influence the stiffness readings of the machine (Lin, Chen, Shau, & Wang, 2017). The authors proposed avoiding the artifact areas during analysis (Lin et al., 2017), however this limits the utility of the device if several areas of the scan are to be disregarded.

1.8 Handheld Dynamometry

Recently, we have begun testing a new, handheld dynamometer that measures the transverse biomechanical properties of soft tissues (MyotonPRO, Myoton AS, Tallinn, Estonia). It does this by measuring the oscillation of tissue following a small perturbation (tap perpendicular to the skin's surface) initiated by the device (Pruyn et al., 2015). This external, transverse force results

in a deformation of the tendon which is measured by the device and interpreted as transverse stiffness (N/m).

Figure 1.1 Illustrated Undulations of Tissue Oscillation and the Relative Displacement (S), Velocity (V) and Acceleration (a).

We have found, through the examination of materials with viscoelastic properties measured il-125through an external materials testing system, that the transverse stiffness measurements provided by the MyotonPRO are highly accurate and repeatable, with an intraclass correlation coefficient of 0.96 (Sohirad et al., 2017). Despite its reliability in healthy individuals, we do not yet know if the MyotonPRO is reliable in tendinopathic Achilles tendon. We also do not know if the MyotonPRO can differentiate between the low transverse stiffness of injured Achilles tendon and relatively high transverse stiffness of uninjured tendon.

Although there have been several studies that have used the MyotonPRO and previous models of the Myoton device (Myoton-2 and Myoton-3), there have been few studies which have examined tendon properties. The Myoton devices were originally developed for the use on muscle, however manufacturers also stated that these devices could be used to assess the mechanical properties of any superficial soft tissue in the body. This included superficial muscles, skin, tendon and other connective tissues.

The Myoton device has been shown to be reliable for assessing muscle tone and elasticity in healthy populations (Bizzini & Mannion, 2003; Viir, Laiho, Kramarenko, & Mikkelsson, 2006). Construct validity against maximum voluntary contraction has also been established in muscle tissue of healthy individuals and those with neurological disorders (Gubler-Hanna, Laskin, Marx, & Leonard, 2007; Leonard, Stephens, & Stroppel, 2001; Rydahl & Brouwer, 2004). In a 2012 study by Aird et al, they also found that within-day ICC >.90 and >.7 when the mean of two measurement sets were analyzed (Aird, Samuel, & Stokes, 2012).

A majority of the literature regarding the Myoton has been on the mechanical properties of muscle, however a few studies have used the Myoton on human tendon. The first study that looked at tendon transverse stiffness in humans using the Myoton was conducted by Marusiak et al in 2011(Marusiak, Jaskólska, Budrewicz, Koszewicz, & Jaskólski, 2011). They used the Myoton-3 device (precursor to the MyotonPRO) to assess passive transverse stiffness in the muscle bellies and tendons of the biceps brachii and triceps brachii of patients with Parkinson's disease (Marusiak et al., 2011). These researchers found that the transverse stiffness in both the

biceps brachii and triceps brachii tendons were significantly ($p<0.05$) stiffer than those of control participants (Marusiak et al., 2011).

In 2015 Pruyn et al examined the validity and reliability of three different methods of lower leg stiffness assessment, one of which was the MyotonPRO (Pruyn et al., 2015). This study used one cohort of 15 female netball players who were assessed for tendon stiffness twice within one week using the unilateral hopping, free oscillation, and myometry methods (Pruyn et al., 2015). The conclusions of this study were that there was high reliability of both the unilateral hopping and myometry methods and that myometer transverse stiffness measurements correlated with isometric rate of force development (Pruyn et al., 2015).

A study by Pożarowszczyk et al (Pożarowszczyk et al., 2017), showed that Achilles tendon transverse stiffness significantly increased (751.57 ± 123.493 N/m; 809.43 ± 160.425 N/m) after eight, two minute bouts of karate fights. This increase in transverse stiffness after repeated tendon loading is similar to our lab's findings in marathon runners (Sohirad et al, 2017), however the changes in this study were noticed much sooner. This may potentially be because of the higher forces going through the Achilles tendon during karate sparring matches.

Regarding the studies that have used the Myoton in the past, we can see that it is reliable and sensitive to structural changes within the tissue it is measuring. Although this device's measurements of tendon transverse stiffness are not what is typically thought of in regards to longitudinal stiffness, the transverse stiffness recorded by this device can give clinicians

information about tendon structural changes. This information may be relevant in understanding the changes in Achilles tendon structure during the healing process.

1.9 Study Aim

Our primary study aim is to determine whether Achilles tendon transverse stiffness is decreased in tendinopathic participants compared to those with healthy Achilles tendons.

1.10 Justification

As our population has become increasingly active in recreational activities, tendon injuries have subsequently increased (Lantto, Heikkinen, Flinkkila, Ohtonen, & Leppilahti, 2015) and there are currently few reliable, practical methods for health practitioners to assess tendon healing and stiffness between time points (Lantto et al., 2015; Moller, Astrom, & Westlin, 1996). Through the use of manual palpation only superficial structural changes such as sclerosis, fibrous formations, and other drastic abnormalities can be detected. We hope to use a handheld dynamometer to assess transverse tendon transverse stiffness in a population of patients with tendinopathy. If successful, this study will support the further development of a lower cost and accessible tool that can estimate tendon structure. This research can lead to further understanding of the role of tendon function with clinical status such as risk of re-injury or pain levels.

1.11 Objectives

There are two objectives of this research study:

Objective 1: Assess differences in transverse stiffness in tendinopathic Achilles tendons compared to healthy controls.

Objective 2: Assess the reliability of transverse stiffness measurements taken by the MyotonPRO among patients with mid-portion Achilles tendinopathy.

1.12 Hypotheses

Based on previous literature and pilot studies, our hypotheses for our cross-sectional study are that:

1) Tendinopathic Achilles tendons will have a significantly ($p<0.05$) lower transverse stiffness when compared to that of control participants.
2) The MyotonPRO transverse stiffness measurements will demonstrate high reliability (ICC>0.90) among Achilles tendinopathy patients.
3) Transverse Stiffness measurements will be positively correlated with VISA-A and waist circumference and negatively correlated with age.

Chapter 2: Methods

2.1 MyotonPRO Reliability

A test-retest reliability assessment of the MyotonPRO was conducted to establish the consistency of transverse stiffness measurements of injured tendon between weeks. We tested participants in a prone position on a plinth with one week between testing sessions. We felt it was important to test under these conditions as this protocol closely replicates the intended examination conventions for clinical settings.

2.2 Participant Data

25 recreational runners who had been diagnosed with mid-portion Achilles tendinopathy or who had non-symptomatic Achilles tendons (10 tendinopathic and 15 control participants) were recruited for this study. Participants were all recruited either by word of mouth or through poster ads on social media. All symptomatic participants must have had symptoms $\geq$3 months. All participants must have had running experience of more than one year. Control participants were tested using the MyotonPRO and ultrasound protocols under the same conditions as the tendinopathic participants.

We did not plan to conduct a matched analysis (e.g., paired t-tests), so once our patient group was known we recruited control participants until a control group with equivalent average values for running volume, age, sex, body mass, height and waist circumference, to the patient group

was achieved (see inclusion criteria, below). In our statistical analysis we determined that the assumptions of homogeneity and equal variances were met to ensure comparable groups.

Written informed consent was obtained from all participants in this study before their participation. The written consent form was approved by the University of British Columbia clinical research ethics board under certificate number H16-03381. A table depicting the analysis of participant characteristics is included in the results section (**Table 3.1**).

2.2.1 Inclusion Criteria

The inclusion criteria for both groups were age (18 – 50), fluency in English, and running frequency (≥ 1 run per week for the past year). We included a broad definition of what it meant to be a recreational runner as in a previous study also conducted in Vancouver, 5.8% of runners enrolled in a running clinic ran only once per week (Taunton et al., 2003). A systematic review by Videbaek et al, 2015 has also previously defined regular running as at least 1 day per week (Videbæk, Bueno, Nielsen, & Rasmussen, 2015).

Achilles tendinopathy participants must also have been diagnosed by a healthcare professional with mid-portion Achilles tendinopathy, have been symptomatic ≥ 3 months, and demonstrate tenderness on palpation, pain with tendon loading, and typical ultrasound findings. Controls had to be symptom-free, with no history of Achilles pain or injury. Exclusion criteria for both groups were pregnancy, major surgery in the past 3 months, previous corticosteroid injections or recent fluoroquinolone use, insertional Achilles pain, diabetes, and medical conditions that could affect tendon properties.

2.3 Participant Recruitment

All participants were recruited from the Lower Mainland with the use of flyers and posters (both electronic and paper copies) placed in areas where runners are commonly found (e.g., running-goods stores, community centers, running groups, races and events). Additionally posters were placed on Facebook running forums (Vancouver Marathon Runners, VanRun, etc.).

2.4 Study Visits

Each subject attended two data collection appointments separated by one week, at the Centre for Hip Health and Mobility at Vancouver General Hospital, Vancouver BC, Canada. Participant anthropometric data such as weight, height and waist circumference were collected on the first of the two appointments by the investigator (EF). Height and weight were both measured with the use of the Seca digital measurement system (Seca 284, Seca, Hamburg, Germany). Waist measurements were taken for each participant with a measurement tape just above the superior aspect of the iliac crest. Other relevant information such as age, sex assigned at birth, amount of running per week, number of running days per week, leg dominance and years of running experience were all self-reported by participants. One investigator (EF) collected ultrasound scans, a customized activity questionnaire **(Appendix A)** and a VISA-A questionnaire for each subject on the first appointment. One the first and second appointment, the MyotonPRO was used on the Achilles tendon at four different ankle angles resulting in eight transverse stiffness measurements being taken over the course of the two weeks on each participant.

2.5 Transverse Stiffness Measurement

Subjects were lying on an examination bed with their foot (the foot being examined; variable between subjects) placed on a foot and ankle stabilizer (FAS) which can be adjusted from 90° degrees in 10° increments in order to achieve various ankle angles.

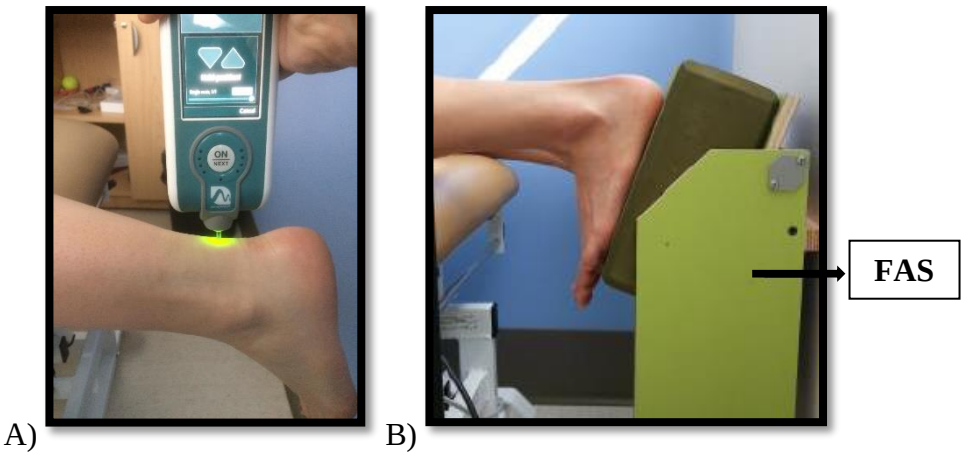


Figure 2.1 Orientation of Subject's Leg.
This image is an example of ankle being tested A) with the MyotonPRO at the participant's anatomical resting position and B) at 100° with the use of the FAS.

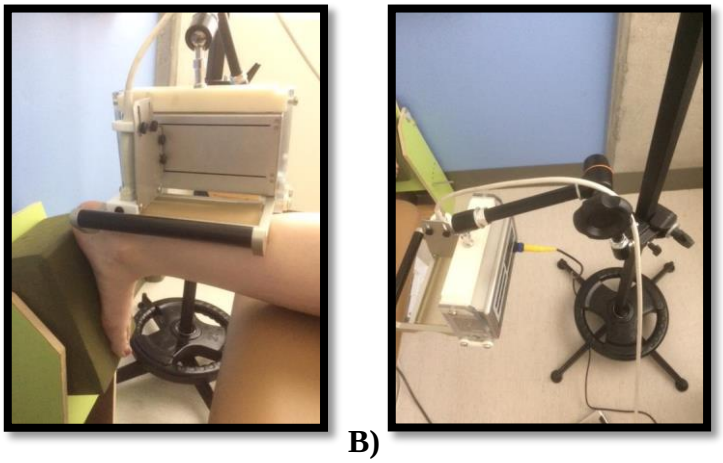
The MyotonPRO manufacturer recommends that transverse stiffness measures be made with tissue in a relaxed state, therefore the Achilles tendons were assessed with participants lying prone on a padded surface (treatment plinth), the calf muscles relaxed, and the foot hanging freely. The transverse stiffness measurements were taken at the mid-point site of dysfunction. The site of the MyotonPRO assessment was determined using ultrasonography as well as identification of nodular swelling or pain upon palpation. The distance of this point from the top of the calcaneus (located via palpation) was recorded so that control participants could be tested

the same distance superior to the calcaneus. The device was placed vertically at 90° on the selected portion of tendon to compress any subcutaneous tissue at a force of 0.18N. After the pre-compression phase, the device was programmed to emit five impulses of 0.4N at an impulse time of 15ms. The transverse stiffness was measured as the maximum acceleration multiplied by the mass of the probe divided by the change in length of the probe during its initial impulse. After this measure was calculated by the device, the average of the five measures was presented on-screen. If the coefficient of variation of the five measurements was over 3%, the measurements were retaken, as recommended by the manufacturer.

As a secondary question (to address the possibility that a participant's Achilles tendon slack length may affect the tendon transverse stiffness readings), we also measured Achilles tendon transverse stiffness as above with the ankle at increasing degrees of dorsiflexion (90°, 100°, and 110°).

2.5.1 Acquisition of Tendon Thickness

The ultrasound unit was equipped with a 10-MHz linear-array transducer (Smartprobe 10L5; Terason 2000, Teratech, USA) secured in a robotic movement tracker with dedicated image capture and analysis software (UTC Technologies). The movement tracker scanned the probe in a straight line in order to capture 600 transverse images over the tendon's length. This method means that the probe is not manipulated by the operator, removing risk of human error (such as variable pressure of the probe on the tendon during operation).



A) **B)**

Figure 2.2 Ultrasound scanning orientation.
A) During ultrasonic measurement the leg was secured against the FAS so that the Achilles was flat an on stretch (110° of plantarflexion). B) The scanner and housing were mounted on a stationary arm to prevent any movement of the device during scanning. The arm was equipped with a ball and socket joint with 360° of movement. This device allowed the researcher to maneuver the device into the preferred position (to obtain a clear ultrasound image) and then lock the arm in place to avoid movements of the probe during the scanning process.

It has been noted in a previous study that in order for the ultrasound machine to produce consistent images, (free from errors where the probe is not flush against the tendon) the Achilles tendon and the probe should be perpendicular to one another. For this reason the FAS was used to standardize the ankle angle, ensuring that the Achilles tendon was parallel to the ultrasound

probe and free of depressions. The FAS degree for each participant during their ultrasound scans was recorded.

Transverse ultrasound images were collected every 0.2mm over a total length of 12cm. The 2-D images were collected and stored on a computer where they were combined to produce a 3-D image using the proprietary algorithms accompanying the ultrasound scanner (UTC2010, UTC imaging). This 3-D image was used for the discrimination of tendon thickness and cross-sectional area. For calibration, ultrasound scans of a Near Field Ultrasound Phantom (Model 050, Computerized Imaging Reference Systems, INC, Virginia, USA) were taken to determine the parameters needed to calculate cross sectional and thickness measurements of the tendon. After ultrasound scans of the Ultrasound Phantom were taken, the calibration scale was determined using six pairs of hyper-echoic nylon wires 0.1mm in diameter (also embedded in the phantom). A straight line tool in the software was used to determine the distances between the wires (Figure 2.4A).

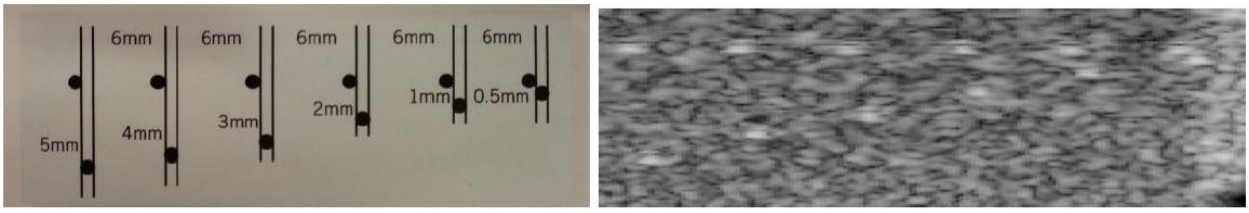
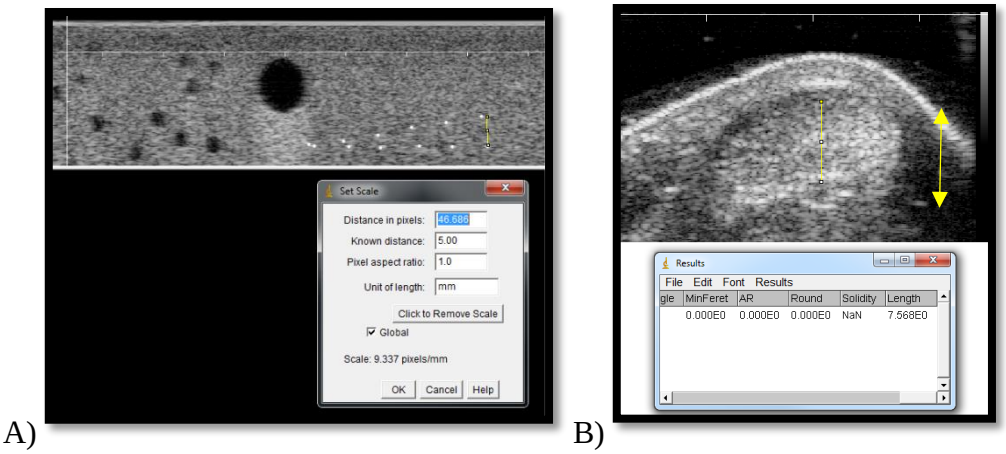
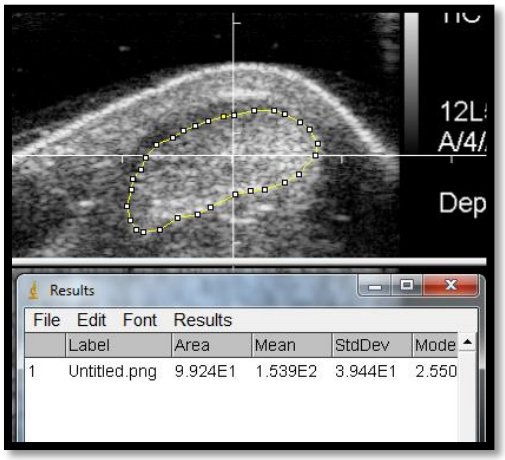


Figure 2.3 Ultrasound Distance Calibration.
Left: This is the Axial Resolution Target found in the user manual provided by CIRS, Inc. along with the Ultrasound Phantom with known measurements provided. Right: The ultrasound image of the Ultrasound Phantom is 0.5mm which is the highest resolution typically used for ultrasound images. These known distances can also be used to determine the scale of the image when determining CSA and thickness.

2.5.2 Tendon Thickness and Cross-Sectional Area Measurements

Using the calibration scale developed according to the methods used above, one thickness and CSA measurement of each participant was recorded on the first visit and analyzed with the use of ImageJ. The thickness was determined as the maximum anterior-posterior distance of the area of tendon being examined (i.e., the mid-point of the most swollen portion of tendon). The area of greatest tendon thickness was determined as the largest AP distance between three measurements of the callus.





C)

Figure 2.4 Tendon Thickness and CSA Determination.
This figure shows A) how the scale was set for the measurements using the UTC calibration phantom, and (B,C) how the measurements were determined for tendon thickness and cross- sectional area using the ImageJ program.

2.6 Statistical Methods

Statistical analysis was performed by the investigator (EF) using the commercial software Statistical Package for the Social Sciences (SPSS) (SPSS Software, Version 25.0, IMB Corporation, Armonk, New York).

Skewness of the data and outliers were examined by dividing the skewness value by the standard error of the skewness. If the resulting ratio of this calculation was greater than +2 or less than -2, we reject the null hypothesis that there is no skew in the data. In the current study, all the values were normally distributed excluding weekly running distance and running experience of control subjects. With the outliers removed, the data was normal, however we decided to leave in the outliers to fully represent our testing population and compared the groups using Mann-Whitney U tests for the weekly running distance and running experience data. To compare the transverse stiffness values of injured participants (n=10) and controls (n=15), a student's independent t-test was performed.

To assess the reliability of transverse stiffness measures of the Achilles tendon in people with Achilles tendinopathy, we conducted between-week reliability tests on the same group of subjects. Reliability was assessed by determining the test-retest reliability coefficients between the two measurements, the standard error of measurement, the intra-class correlation coefficient, and minimum detectable change at 95% confidence. A scatterplot on the z-scale (between the two transverse stiffness measurements) and a Bland-Altman plot was also conducted to assess the agreement between the measurements taken at the two time points.

We also conducted a Pearson correlation to determine if there was an association between transverse stiffness values and Achilles tendon thickness or VISA-A questionnaire scores. All tests were performed by Evan Finnamore (EF) under the supervision of Dr. Alexander Scott.

2.7 Sample Size Calculation

At the start of this study, we did not have any previous data on which to base a convincing sample size calculation for group differences in transverse stiffness. Based on the results in this thesis (see below), for a power level of 0.80 and an alpha level of 0.05 we can now calculate a sample size for a larger study on injured tendon using the MyotonPRO. With a mean transverse stiffness of the injured and control groups being 776.8N/m and 873.33N/m respectively, and a common standard deviation of 90.32, a sample size of 14 was calculated. This sample size would be the recommended size of a future research study using the MyotonPRO to assess differences between tendinopathic and healthy tendon.

Chapter 3: Results

3.1 Recruitment

Participants were recruited from March 2018 to July 2018. A total of 15 tendinopathic subjects were contacted for eligibility purposes and 12 participants passed the initial phone screening. Two tendinopathic participants were excluded after the first visit according to the following criteria: one participant was not physically active enough to be included in the study and the second participant had pain upon palpation that was <2cm (1.4cm) from insertion. A total of 20 controls contacted the investigator in hopes of participating after seeing advertisements regarding the current study. Five participants were not included in this study as two voluntarily withdrew before the study began (due to a busy schedule), two were excluded based on not meeting running volume requirements and one was excluded based on not fitting the age requirements. All participants (tendinopathy n=10, controls n=15) who were included in the study attended both required appointments.

3.1.1 Participant Characteristics

The participant variables for the 25 subjects can be found in **Table 3.1**. 12 males and 13 females were enrolled in this study. The most symptomatic ("worst") leg was the non-dominant leg in 90% of Achilles tendinopathy patients. Although both legs were tested in control participants, we decided to use just the non-dominant leg for comparison to the tendinopathic group, to avoid any confounding influence related to leg dominance. Incidence of bilateral tendinopathy and duration

of symptoms was not recorded and this is mentioned in the limitations section of this thesis. Although the duration of symptoms was not recorded, all participants had tendinopathy symptoms for over three months, which is the diagnostic criteria for what is titled chronic tendinopathy. The average age of all participants was 49.1 (26-71yrs) and the average BMI was 24.7 (19.1-30.9). All tendinopathic participants displayed ultrasound abnormalities typical of tendinopathy (**Figure 3.1**), received a physician's diagnosis, presented with pain/ discomfort upon palpation, and had localized swelling that was readily observable upon observation of the patient when in a prone lying position.

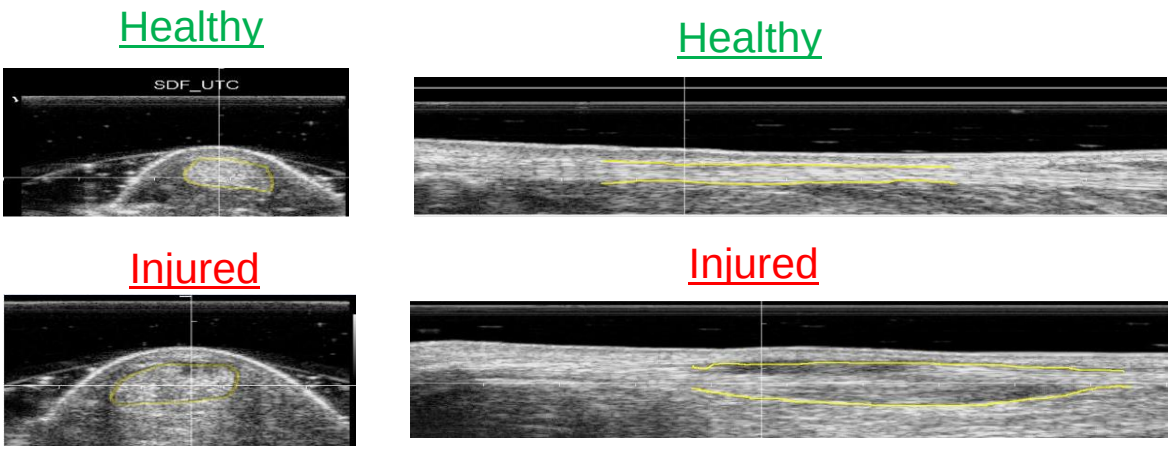


Figure 3.1 Ultrasound Image of Healthy and Injured Tendon.
Representative comparison of a tendinopathic participant and a healthy control in transverse (left) or sagittal (right) planes.

The average tendon thickness of patients was 6.83±0.93, vs 5.48±0.88 in the controls ($p < 0.001$). The average VISA-A score was 68.9±8.1 in the tendinopathic group and 99.9±0.5 in the control group, where only one control participant had a score of less than 100. All participants had chronic tendinopathy (symptoms > 3 months) with a gradual onset and no one underwent physiotherapy treatments during the one week of testing. No participants had any reported

changes to their running volume or footwear within 48 hours of testing on both appointments. All participants were recreational runners with an average weekly running distance of 37.7±24.7 km. Sex assigned at birth, body mass, height, BMI, waist circumference, weekly running distance and number of running days per week were all balanced between groups (**Table 3.1**).

Parameter	**Achilles Tendinopathy Group (n=10)**	**Control Group (n=15)**	**P Value**
Sex Assigned at Birth (male/female)	(5/5)	(7/8)	-
Age in years (mean±SD)	48.35±8.93	49.53±14.73	.823
Body Mass Index (mean±SD)	24.35±3.41	24.85±2.79	.694
Waist Circumference in cm (mean±SD)	78.94±11.99	82.73±8.46	.363
Weekly Running Distance in km (median (IQR))	45.0±29.38	30±20.69	.129
Average Number of Running Days Per Week (median (IQR))	3.75±2.25	3.0±1.0	.603

Table 3.1 Participant Characteristics.
Abbreviations: SD, standard deviations from the mean; IQR, interquartile range. P values are derived from independent t-tests for all variables excluding weekly running distance, with which a Mann-Whitney U test was used due to non-normality of the data.

3.2 Reliability of Transverse Stiffness Measurements in People with Achilles Tendinopathy

The second objective of this thesis was to test the hypothesis that the MyotonPRO transverse stiffness measurements are reliable (ICC>0.90) among Achilles tendinopathy patients. The

manufacturers of the MyotonPRO recommend that tissue be tested in a relaxed state, and this method was the primary one used in the current study. When allowing the Achilles tendon to adopt a natural resting position (participant lying prone with foot hanging freely over the end of the bed), the ICC was 0.92 (**Table 3.2**). The minimum detectable change based on a 95% confidence interval (MDC95) was calculated using the formula MDC95=SEM*1.96* √2. The minimum detectable change for the MyotonPRO at the resting ankle position on the tendinopathic subjects (n=10) was 51 N/m. The MDC for the control group was 36 N/m and the MDC for the group over all was 30 N/m (**Table 3.2**).

Level of Measurement	**Minimum Detectable Change at 95% Confidence Interval**	**Intra Correlation Coefficient**	**SEM**
Injured	51	0.90	19
Healthy	36	0.90	13
All Participants Combined	30	0.92	11

Table 3.2 Comparison of Minimum Detectable Change (MDC95), Intraclass Correlation Coefficient (ICC) Between Groups and Standard Error of the Mean.

As a secondary question, we evaluated the potential impact of ankle joint angle on tendon transverse stiffness reliability. With higher joint angles, the reliability (ICC) was lower than that achieved at the resting state (**Table 3.3**). With increasing angles, the average tendon transverse stiffness increased, but the magnitude of transverse stiffness increase was variable (**Figure 3.3**), indicating a variable amount of load may be placed through the Achilles tendons for the same joint angle. We also noted that at higher angles, it became increasingly difficult to obtain a reading from the MyotonPRO, resulting in an increasing frequency of missing data with

increasing joint angles (**Table 3.3).** Interestingly, the ability of the MyotonPRO to detect a lower transverse stiffness in the tendinopathy group disappeared when the ankle was dorsiflexed (**Table 3.4**). Although transverse stiffness increased with ankle angle, the the rate of transverse stiffness increase with progressive dorsiflexion was not uniform, the ability to detect deficits in tendinopathic tissue disappeared, and measurements became less reliable.

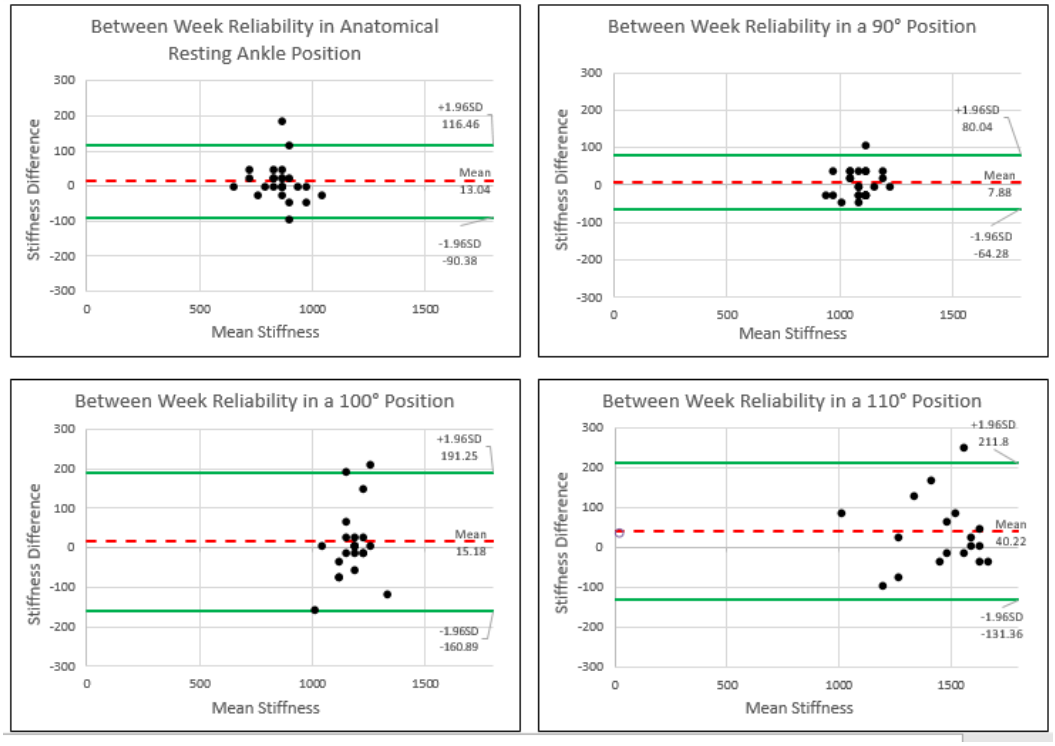


Figure 3.2 Bland-Altman Plots of Increasing Dorsiflexion Ankle Angles.
This four-panel Bland-Altman plot depicts mean transverse stiffness (N/m) vs transverse stiffness difference (N/m) in four different ankle positions. The four ankle positions are A) anatomical resting position (n=25), B) 90° fixed position (n=25), C) 100° fixed position (n=24) and D) 110° fixed position (n=20).

	Valid Measurements	
Ankle Angle	Data Collected (N)	Percent (%)
Resting	50	100%
90°	50	100%
100°	47	94%
110°	38	76%

Table 3.3 Ankle Angle and Valid Measurements.
This table displays the transverse stiffness data collected in all participants (n=25) at the two time points for a total of 50 measurements (n=50).

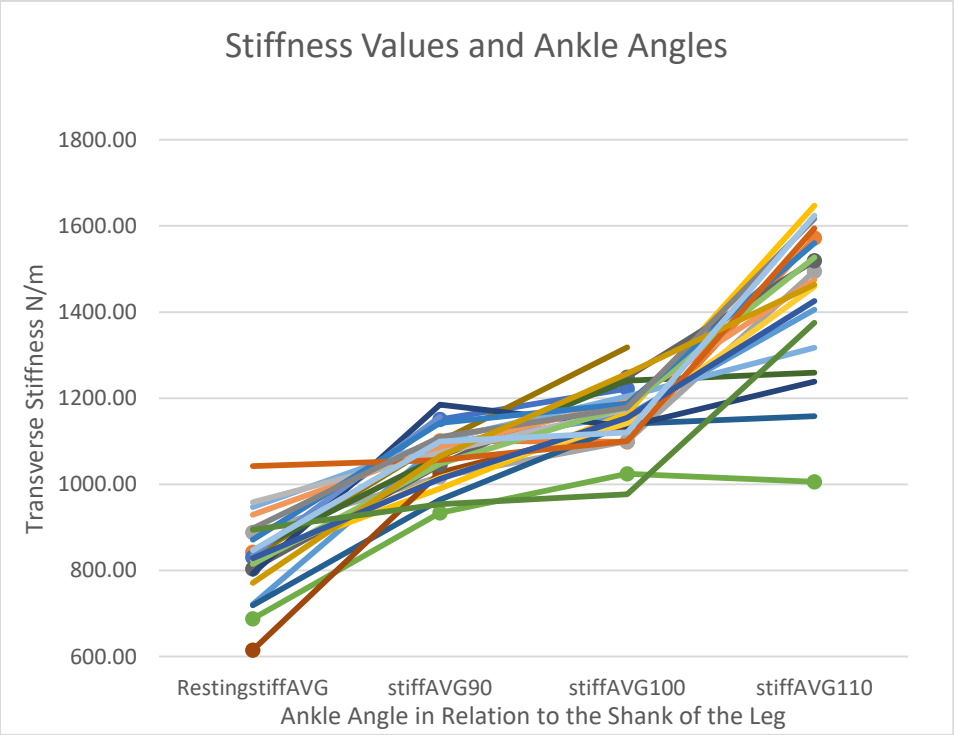


Figure 3.3 Transverse Stiffness Values in Relation to Ankle Position.
The relation between transverse stiffness and ankle ankle indicates a trend for transverse stiffness to increase as ankle angles increases.

Ankle Angles	Tendinopathic Transverse Stiffness (n=10)	Control Transverse Stiffness (n=15)	P value
Resting	776 (614.5-888.5) ±86.14	873.33 (771-1042.5) ±72.27	**.006***
90°	1042 (933-1151) ±68.8	1077.97 (954-1185) ±59.28	.179
100°	1161.45 (1024.5-1318) ±83.88	1162.83 (977-1257) ±66.38	.964
110°	1400.29 (1005.5-1647) ±233.81	1456.53 (1238.5-1624.5) ±129.89	.494

***significant difference**

Table 3.4 Tendon Transverse Stiffness Among Tendinopathic and Control Groups at Varying Ankle Angles. This table depicts the transverse stiffness in N/m, (range) ± SD. Independent t-tests with Bonferroni adjustments were conducted to derive the p value. The only ankle angle which showed a significant difference between groups was when participants had their ankles in the anatomical resting position.

3.3 Comparison of Achilles Tendon Transverse Stiffness Between Participants With and Without Achilles Tendinopathy.

The first objective was to test the hypothesis that tendinopathic Achilles tendons have a significantly lower transverse stiffness than that of control participants' Achilles tendons. Transverse stiffness scores at resting position were lower in the tendinopathy group (776.8N/m±86.14) than the control group (873.33N/m±72.27, p=0.006), however there was considerable overlap in the values. Levine's test indicated that there were equal transverse stiffness variances (F=0.909, p=0.35) between the injured and non-injured groups and we accept that there is homogeneity of variance. There is also an R^2 of 0.286 (p=0.006), which indicates that 28.6% of the variability in transverse stiffness is accounted for by the difference in diagnosis.

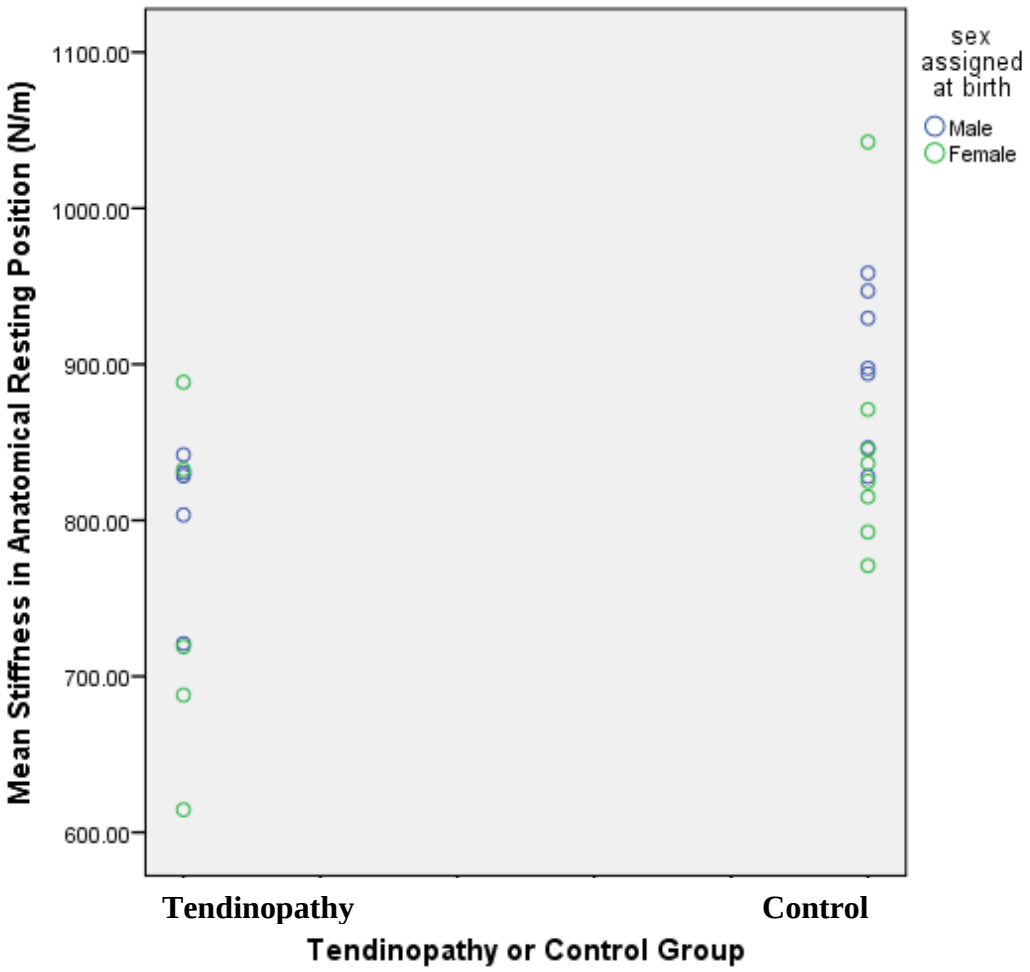


Figure 3.4 Mean Transverse Stiffness Between Groups.
This plot is comparing transverse stiffness values (in N/m) between injured (776N/m; n=10) and healthy (873.33N/m; n=15) participant tendon transverse stiffness values. The difference in transverse stiffness scores are denoted by sex (m=blue, f=green).

3.4 Correlation of Transverse Stiffness Measures With Clinical Features

At the outset of the study, we hypothesized that transverse stiffness measurements will be positively correlated with VISA-A and negatively correlated with age. As an additional analysis, we also examined the correlation of transverse stiffness with years' running experience, and with waist circumference.We found that there was a correlation between VISA-A scores and

transverse tendon stiffness, when all subjects without a perfect VISA-A score were included in the analysis (r=0.730, n=11, minimum=55, maximum=98, mean=71.54, SD=11.68, p=0.011) **(Figure 3.5)**.

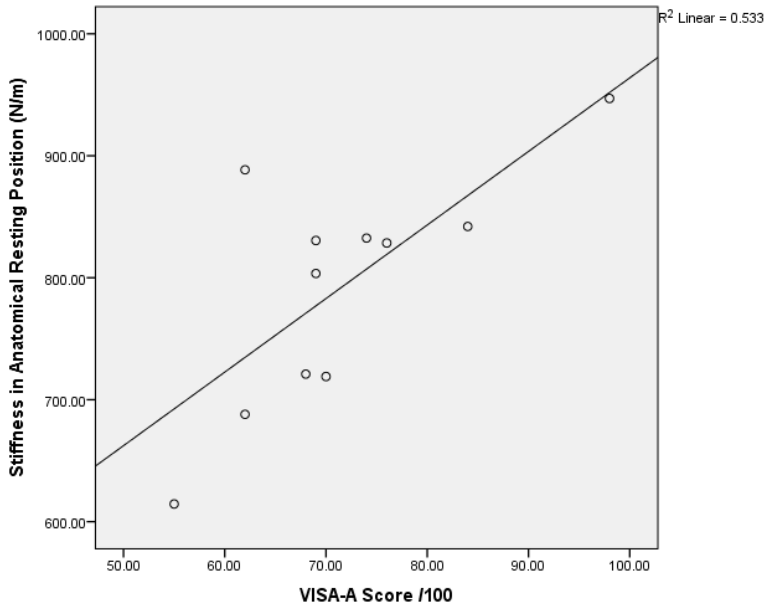


Figure 3.5 VISA-A and Transverse Tendon Stiffness Correlation.
This is a correlation between transverse stiffness and VISA-A scores in all participants with some Achilles tendon dysfunction (VISA-A score<100). There was a significant correlation between transverse stiffness and VISA-A when these participants were investigated (r=0.73, n=11, p<0.011).

We also found that age was correlated with a decreased transverse stiffness in the group as a whole (r=-0.508, n=25, p=0.009) (**Figure 3.6A**), and also when only the healthy controls were analysed (r=-0.642, p=0.01).

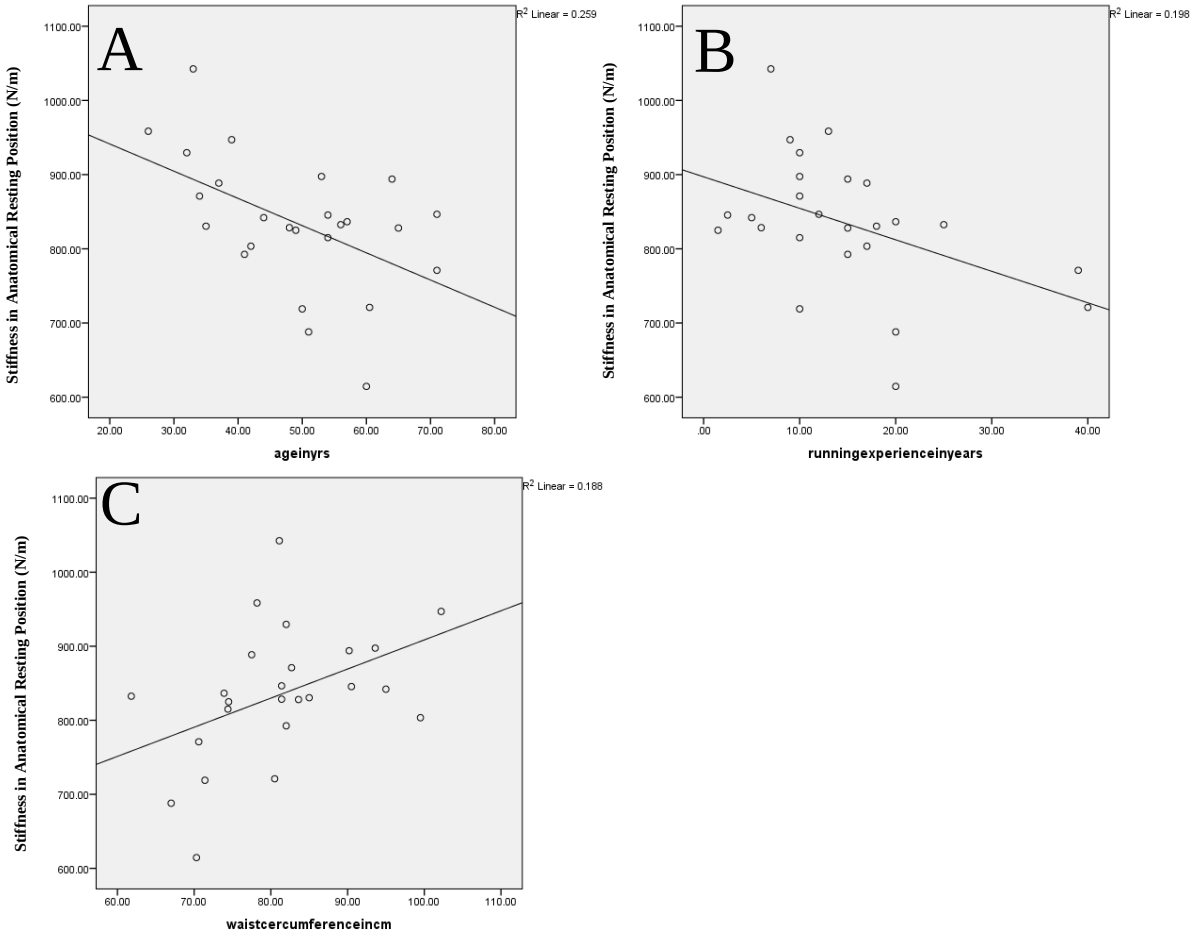


Figure 3.6 Correlation Scatterplots.
This group of scatterplots shows the correlations between transverse stiffness and A) participant age in years, B) Participant running experience in years and C) waist circumference in cm. A) There was a negative correlation between transverse stiffness values and age (r=-0.508, p=0.009; n=25). B) There was a negative correlation between transverse stiffness and running experience in years (r=-.445; p<0.05; n=25). C) There was a positive correlation between transverse stiffness and waist circumference (r=0.434; p=0.03; n=25).

The correlation between running experience and transverse tendon transverse stiffness was found to be statistically significant (r=-.445; p<0.05). We would normally expect the amount of running in years to be positively correlated with tendon transverse stiffness as has been found in other studies that have looked at physical activity in its relation to transverse stiffness (Buchanan & Marsh, 2002; Kjaer et al., 2006). However, because years of running experience is highly dependent upon the age of the individual, we conducted a partial correlation adjusting for age

and found that there was not a statistically significant correlation between transverse stiffness and running experience ($p=0.079$). We also found that waist circumference was positively correlated with tendon transverse stiffness ($r=0.434$; $p=0.03$).

Chapter 4: Discussion

Achilles tendinopathy is accompanied by material changes in the tendon proper including collagen fiber disorganization, mucoid degeneration, and neural and vascular proliferation (Khan et al, 1999). These are believed to cause mechanical changes in the tendon and therefore predispose it to further injury (Arya & Kulig, 2010a). As most tendon tears demonstrate degenerative changes, similar to those found in Achilles tendinopathy patients (such as a decreased tendon transverse stiffness), it is important to measure the mechanical properties to identify these tendon abnormalities before injury occurs, or during rehabilitation to assess the recovery of tendon function. Accurate and swift assessment of tendon functional deficits is crucial so that the success of rehabilitation measures can be evaluated.

4.1 Purpose

The purpose of this study was to determine if the MyotonPRO was able to detect differences in Achilles tendon transverse stiffness between subjects with Achilles tendinopathy and healthy controls. This study was designed to serve as a pilot study for a larger, longitudinal clinical study to examine the effect of rehabilitation on tendon function. We also hoped to describe an effective testing protocol for assessment of transverse stiffness that can be used in a clinical setting. We found that there was a lower tendon transverse stiffness (taken at the tendinopathic callus site) when compared to controls who were tested at an equivalent distance from the calcaneal insertion. We also found that the MyotonPRO provided a valid and reliable method of transverse stiffness measurement between weeks. The minimum detectable change (MDC95) in transverse

stiffness measurements was higher for tendinopathic subjects (75.5 N/m) than for the control group (51.69 N/m). The recommended clinical protocol involves testing the Achilles tendon with the ankle in an anatomical resting position. Due to the reliability of the MyotonPRO and its ability to detect differences in tendon properties between symptomatic and non-symptomatic groups, this research suggests that the MyotonPRO can be helpful as a clinical research tool.

4.2 Tendon Transverse Stiffness and Functionality

During normal gait, the Achilles tendon transmits force from the plantar flexors of the lower leg (i.e., gastrocnemius and soleus) to the calcaneus in order for humans to perform a toeing-off movement. It is important for this movement to be performed as efficiently as possible in runners to avoid injury and enhance performance. If a muscle is attached to a tendon with impaired load-bearing capacity, the time taken to stretch it could delay the transmission of forces from the calf muscle to the heel. This would in effect decrease the efficiency of the movement affecting factors such as the energy cost of running (Fletcher et al., 2010). A stiff tendon facilitates faster tension changes, therefore providing the ability to generate quicker movements. A stiff tendon is also strongly correlated with ultimate tensile stress meaning that a stiffer tendon could be protective against injury as it could withstand a higher load than less stiff tendon.

Although loading movements such as sprinting have been shown to increase tendon transverse stiffness when compared to control subjects (Arampatzis, Karamanidis, Morey-Klapsing, De Monte, & Stafilidis, 2007), Kubo et al. 2015, alternatively found that in a group of 5000m runners, runner with slower race times had stiffer plantarflexor tendons than quicker endurance

runners (Kubo, Miyazaki, Shimoju, & Tsunoda, 2015). These two studies are very interesting to examine in relationship to one another. On one hand we can infer that higher loads through the tendons (like those in sprinting) would result in stiffer tendons. On the other hand, athletes who complete a 5000m race in a faster time would also theoretically have higher loads through their tendons on average, although both of these groups' tendons seem to be adapting differently to their sport. One possible reason for these conflicting findings on how loading affects tendon may be a result of load frequency. In the Arampatzis study, they compare sprinters to control participants. In this study the sprinters may have increased number of high-loading repetitions per week compared to the tendon subjects. Similarly, in the Kubo study the athletes who had quicker race times, may have an increased amount of weekly training, leading to their better race times. Other potential confounding variables between race times and tendon transverse stiffness could be variable medication use, nutrition, hydration as well as variation in training volume and intensity, as these were not accounted for in the studies. Additionally in the Kubo study, the faster running group also had significantly longer duration of training years ($p<0.001$).

Although this guess as to why these studies seem to be showing different findings may have some sound rationale behind it, in actuality we do not yet know how running affects tendon as there are so many variables that have not yet been extensively researched in large, randomized, longitudinal studies.

Although running has many health benefits and is generally regarded to have a positive effect on tendon health (as long as it does not exceed a training threshold), there are many unknowns about how different types of loading or various running variations affect tendon structure.

4.3 Main Findings

Achilles tendon transverse stiffness was found to be significantly lower in the tendinopathic participants (776.80N/m±86.14SD) compared to the control group (873.33N/m±72.27SD) ($p=0.006$, $n=25$). Additionally, there was a trend for subjects with more advanced changes (lower VISA-A score) to have lower tendon transverse stiffness. These findings are of interest as tendon transverse stiffness is associated with the amount of load a tendon can withstand before failure: a stiffer tendon experiences lower levels of strain and hysteresis, which are both hypothesized to increase the risk of traumatic tendon injury (Lacroix et al., 2013; Magnusson et al., 2010; Schepull, 2013). Although the method of transverse stiffness assessment used in this study is different than the more traditional methods of tensile stiffness measurement (Kongsgaard et al., 2011; Kubo et al., 2007; Kubo & Tabata, 2010; Peltonen, 2014), recently other methods such as elastography have been used to successfully determine tendon stiffness in the transverse plane (De Zordo et al., 2010; Klauser, Faschingbauer, & Jaschke, 2010.; Ooi et al., 2014.; Ooi, Schneider, Malliaras, Chadwick, & Connell, 2015). With previous methods of measuring stiffness, values greatly differ from study to study (Morrison et al., 2015) and reliability is insufficient for clinical use (Payne, Webborn, Watt, & Cercignani, 2017). Future studies could use the method of assessment described in this thesis to monitor functional recovery of tendons.

It has been found in both human and animal studies that as age increases, tendon stiffness decreases (Couppé et al., 2012; Dressler et al., 2002; Karamanidis & Arampatzis, 2006; Lacroix et al., 2013; Stenroth, Peltonen, Cronin, Sipilä, & Finni, 2012). The mechanism for this phenomenon is not fully established, but could involve reduced function of tendon cells (Tsai et

al., 2011; Yu et al., 2013), and may be confounded by an age-related reduction in physical activity. The fact that we also found a similar, negative relationship between transverse tendon transverse stiffness and age tells us that this device may be measuring important differences between groups such as older and younger participants. Also this similarity between findings in this study and others assessing the differences in transverse stiffness in aging tendon can assist us in validation of the device. The similarities between studies help exhibit construct validity as we can assume this device is testing the tendon properties that we already know are different between young and older tendon.

Tendon stiffness and obesity have also been found to be positively correlated in other studies (Biancalana, Veloso, & Gomes, 2010; Wearing, Hooper, Grigg, Nolan, & Smeathers, 2013). Although tendon stiffness (which is typically regarded to be a healthy adaptation) is increased in people who are obese, this adaptation may be due either to an impaired interstitial fluid movement (Biancalana et al., 2010) or due to increased collagen production as a result of heavier loading (Biancalana et al., 2010). We initially used waist circumference as a measure of adiposity as it indirectly reflects central fat distribution: waist circumference is often used to determine body fat distribution, and adiposity has been hypothesised to be related to increased risk of tendon injury. However, we did not detect any impairment in tendon function in those with increased waist circumference, and indeed the waist circumference was equivalent in tendinopathy and control groups, suggesting that this may not be a strong risk factor for tendon impairment.

Although both tendon CSA and anteroposterior diameter have been noted to be larger in tendinopathy patients when compared to control participants, only the tendon AP diameter was significantly different between our two groups. Tendinopathic tendons often demonstrate a pregnant appearance in the A-P diameter, with the majority of tendon thickening attributed to a change in thickness, with minimal change width. The fact that this study only included 25 participants may mean that with the collection of more subjects we may have noticed a difference in tendon CSA, as other studies have shown these differences (Arya & Kulig, 2010; Kader, Saxena, Movin, & Maffulli, 2002).

4.4 Importance

The findings in our study are important as this study opens the door to the measurement of tendon transverse stiffness in studies assessing rehabilitation interventions or factors predicting a safe return to activity. Recurrence rates of tendon injuries as high as 27% have been reported (Gajhede-Knudsen, Ekstrand, Magnusson, & Maffulli, 2013) which may be because although symptoms (such as pain) have subsided in the patient, the tendon's material properties may have not been returned to adequate levels. As full recovery of musculotendinous function after having a tendinopathy was achieved in only 25% of subjects in a study by Silbernagel et al., they suggest the importance of having a method available to objectively determine return to activity guidelines (Silbernagel et al, 2007). Although we do not currently know what a healthy transverse tendon stiffness value is, several studies report average values in the general population. In a study by Orner et al, 87 male and 120 female participants had their Achilles tendon transverse stiffness examined using the MyotonPRO (Orner et al., 2017). In the Orner

study, the researchers found that transverse stiffness was 877.62 (±88.31) N/m and 826.53±95.51 N/m for males and females respectively. All participants were asymptomatic in this study and further investigatory measures to affirm the "health" of their tendon (e.g. imaging) was never done. The methods in the Orner study and our current study were similar despite the former using a younger sample with a mean age of 33.15 (SD 13.66) compared to our sample's mean age of 48.35 (SD 8.93) (Orner et al., 2017). Additionally, Orner et al. did not specify the measurement distance from the calcaneus, whereas our distance was set at a mean of 3.7cm for control subjects (Orner et al., 2017). This study gives us some insight into what an average transverse stiffness measurement may be with the MyotonPRO, however this cannot be generalised to injured participants or recreationally active runners.

This study has highlighted the Myoton device's potential ability to differentiate between tendinopathic and healthy tendon, however there was considerable overlap of values between tendinopathy and controls, meaning that its use may lie with detecting intra-individual change as opposed to diagnosis. A possible use of this device would be to monitor adaptive changes in tendon properties in athletes who are attempting to train to improve tendon transverse stiffness, for example in order to improve running efficiency.

4.5 Other Factors Affecting Tendon Transverse Stiffness.

There are many factors that may increase the risk for the development of tendinopathy such as sex (Astrom & Rausing, 1995; Leppilahti, Puranen, & Orava, 1996; Longo, Ronga, & Maffulli, 2009; Möller, Astron, & Westlin, 1996), increasing age (Epro et al., 2017; Eriksen et al., 2018; Onambele et al., 2006; Pardes et al., 2017b), obesity (Gaida, Alfredson, Kiss, Bass, & Cook,

2010; Gaida et al., 2009; Scott et al., 2004), high cholesterol levels (Taylor, Cheema, & Soslowsky, 2017; Yudkin et al., 2000), diabetes (Gaida, 2009; S de Jonge, Rozenberg, & Vieyra, 2015; Park et al., 2005; Reddy et al., 2015b), previous tendinopathies (Saragiotto et al., 2014) and training volume (Ramskov et al., 2018; Saragiotto et al., 2014) (i.e., increasing or decreasing activity levels). In this study we attempted to account for these factors by ensuring groups were similar in sex ratio, age, obesity (measured as waist circumference and BMI) and training volume. In future studies we would have to see how various risk factors (such as diabetes or high cholesterol) would affect the tendon transverse stiffness. Also we would suggest that it would be beneficial in future studies to use this device to monitor the effect of modifiable risk factors of tendon transverse stiffness. This could be done by comparing the tendon transverse stiffness before and after weight loss, or after administration of a cholesterol lowering drug.

4.6 Study Limitations

Although the present research was a pilot study, there were a few limitations that should be mentioned and taken into consideration when a larger study is conducted.

The MyotonPRO gives information about the transverse stiffness of a tissue in a continuous, objective scale. However, it does not give health practitioners information about the dimensions of the tissue. The MyotonPRO measures the transverse stiffness of all tissues under its probe (i.e. skin, paratenon, adipose tissue or other connective tissues); the individual contributions of other tissues surrounding the tendon, including skin, adipose tissue, and paratenon, are not known. In a study by Sohirad et al, 2017 it was noted that there was minimal interference from overlying skin

and subcutaneous tissue in transverse stiffness values. Therefore, we can assume that in situations where monitoring change in transverse stiffness over time is beneficial (e.g. a patient is undergoing rehabilitation), we may be able to assume that contributions from skin and subcutaneous tissue are relatively constant. Therefore if changes in transverse stiffness values exceed the MDC, this change is likely to be due to changes in the tendon itself, rather than change in adjacent tissues.

Due to the indirect measurement of tendon elasticity (which is affected by surrounding tissues), we propose the use of the term "transverse stiffness" as opposed to "stiffness" to avoid confusion when comparing the transverse stiffness measurements taken by the MyotonPRO to other traditional methods of stiffness measurement. Tendons are primarily designed to function in tensile loading, not transverse. However, the two measurements, at least under some circumstances, appear to correlate.

We also used the MyotonPRO on the Achilles tendon with the probe tip oriented in the anteroposterior direction relative to the tendon. A suggested area of exploration in future studies to also test the tendon mediolaterally and lateromedially to the Achilles tendon. As pathology is sometimes laterally located, it would be useful to know the reliability of testing in this orientation as well.

Our sample of 25 subjects (10 tendinopathy participants and 15 controls) was quite small. We cannot generalize to elite athletes, or other clinical populations (diabetes, hypercholesterolemia, etc.). We also did not record the duration of symptoms for each individual participant and data

was not recorded on who had bilateral or unilateral tendon issues. This was done as we did not believe that bilateral vs unilateral issues would affect the tendon structure differently from one another, although we acknowledge that it may have affected things in ways we did not foresee and it would have been a useful parameter to take into account on future studies.

We found that the position in which the tendon is tested is also very important to the measurements. The ideal for assessment of tendon stiffness measurements in tendinopathic subjects as well as healthy controls in a clinical setting, as carried out in this study, is in a resting state.

Chapter 5: Conclusion

The use of the MyotonPRO in assessment of tendon transverse stiffness is a potentially useful measurement tool that can complement US findings. Unlike other measurements assessing transversely propagated measurements of stiffness/ hardness such as real time elastography, the MyotonPRO is highly reliable, its results are easily interpretable and it can be used in many different settings. It is also clear that literature on the use of this device for tendon assessment is sparse, however very promising as transverse stiffness measurements in this study correlated with other clinical parameters usually associated with stiffness. Future longitudinal studies are needed to further investigate the use of this product's ability to serve as an outcome measure. Hopefully in the future we can assess the ability of the MyotonPRO to monitor the healing in tendinopathy patients as well as predicting injury rates.

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Appendices

Appendix A Participant Questionnaire

Name:	Standing Height:
Study ID:	Body Mass:
Waist circumference (cm):	Sex: M/F:
Weekly running distance:	Age (yr/mo):
Running experience (yr/mo):	

		YES	**NO**
1	Have you ever had, or suspected an Achilles tendon strain or tear in the past year?		
2	Have you ever taken oral corticosteroids, fluoroquinolones within the past year?		
3	Have you taken any anti-inflammatory medication in the last 48 hours?		
4	Have you ever been diagnosed with diabetes?		
5	Have you ever been diagnosed you have any form of arthritis?		
6	Are you pregnant?		
7	Have you experienced any Achilles tendon, heel or foot pain in the past year?		
8	Do you have any lower extremity pain? Yes? Specify:_____		
9	Have you made any drastic changes to your activity level in the past month? Yes? Specify:_____		
10	Have you made any drastic changes to your footwear in the past month? Yes? Specify:_____		
11a	Have you run in the last 48 hours?		
11b	If yes, what did you do? E.g. 1 hour of soccer		

12	How many days a week do you run?	
13	What surface do you usually run on? (ie. Trails, road, grass, hills)	
14	What is your approximate average weekly running distance?	

Appendix B Recruitment Protocol

Letter of Initial contact to be sent to the following:

• Lions Gate Road Runners – president@lgrr.com

• Pacific Road Runners – Online Form

• Forerunners – Online Form

• Central Vancouver Cycling – info@cvcracing.com

• Vancouver Bicycle Club – Online Form

• BikeHub – vancouver@bikehub.ca

• Cycling British Columbia – Online form

• Vancouver Rowing Club – office@vancouverrowingclub.ca

We will also use the following Twitter accounts to further improve the visibility of the study to potential volunteers. The proposed tweets (below) will provide a link that will redirect potential participants to UBCs CREB approved poster, which will be hosted on CHHM's website (hiphealth.ca).

Accounts:

1. CHHM (@Mobility_Health)

2. VCHRI (@VCHResearch)

3. Vancouver Coastal Health (@VCHHealthcare) Proposed Tweets:

1. Help us test a new medical device on your ATs. Participate in our

Tendon Research study (link to shortened URL linking to approved poster)

#UBC #Research

2. Help us validate a new tendon testing device. Participate in our Tendon

Research study (link to shortened URL linking to approved poster) #Vancouver #Research

3. Help us learn more about tendon properties. Participate in our

Tendon Research study (link to shortened URL linking to approved poster)

#Vancouver #Research

4. Help us learn more about tendons & sport performance. Participate in our

Tendon Research study (link to shortened URL linking to approved poster)

#Vancouver #Research

5. Are you active? Contribute to needed research in tendon recovery. (link to shortened URL linking to approved poster) #Vancouver #Research

6. Are you active? Contribute to needed research in tendon physiology. (link to shortened URL linking to approved poster) #Vancouver #Research