

**A CROSS-SECTIONAL STUDY TO EVALUATE THE EFFECT OF STATINS ON
ACHILLES TENDON MORPHOLOGY USING ULTRASOUND TISSUE
CHARACTERIZATION.**

by

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Abstract

There has been growing interest in studying the possible effects of statins on tendons due to case studies and retrospective chart reviews that reported a potential relationship between statin use and tendon pathology, as well as laboratory studies demonstrating that statins directly influence tenocyte metabolism. However, a recent epidemiological study found no association between statin use and the risk of tendon rupture. Since statins are widely used cholesterol-lowering medications, it is important to understand the potential relationship between statin use and tendon health. With the use of Ultrasound Tissue Characterization (UTC), the aims of this pilot cross-sectional study were to see if there were signs of reduced collagen organization or increased cross-sectional area (CSA) of the Achilles tendon in individuals who had been taking statins for at least a year (the Statin group), compared to those who had never taken a statin (the Control group). We hypothesized that individuals in the Statin group would demonstrate a greater cross-sectional area and reduced collagen organization (determined by the percent of echo-type I) of their Achilles tendons compared to individuals in the Control group.

To test this hypothesis, we analyzed the UTC scans of 66 individuals who were either taking Statins (n=33) or who had never taken statins (n=33, control group) and compared their resulting Achilles tendon CSA and echo-type I values. There were no significant differences in Achilles tendon CSA or proportion of echo-type I patterns between the two groups, implying that statins do not negatively impact the health of the Achilles tendon. In the entire cohort (n=66), there were significant effects of age ($r = -0.31$, $p = 0.012$) and BMI ($r = -0.31$, $p = 0.012$) on echo-type I values. These findings support previous work which demonstrated a lack of association between

statin use and tendon pathology, but demonstrate the negative effects of aging and elevated BMI on tendon health.

Lay Summary

The main goal of this study was to assess the relationship between the use of statins and Achilles tendon structure using Ultrasound Tissue Characterization (UTC). Based on our results, we did not find an association between statin and Achilles tendon structure, as visualized UTC.

However, we did find some expected relationships between different variables. We found that advancing age and increasing body mass index were associated with worse tendon structure.

These findings support previous work done by Spoenlin et al. (2016) and add to the field by providing information where a gap in knowledge exists. Specifically, by focusing on Achilles tendon structure, our findings suggest that statins do not impact tendon cross-sectional area or collagen organization.

Preface

All study procedures were performed according to the guidelines for human experimentation approved by the Clinical Research Ethics Board of the University of British Columbia. The Clinical Research Ethics Board Certificate number is H14-01808.

This study was conducted by me, Agnetha de Sá, under the supervision of Dr. Alex Scott.

Development of the study protocol and documents was done by Dr. Scott and I, with the assistance of Dr. Saul Isserow and Taira Birnie from the Centre for Cardiovascular Health at Vancouver General Hospital. For UTC analysis, Dr. Scott blinded all UTC scans so that I could conduct echo-typing and cross-sectional area analysis. Statistical analysis was done by me, under the supervision of Dr. Scott. To date, the research included in this thesis has not been published.

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List of Abbreviations

2-D	Two-dimensional
3-D	Three-dimensional
Ad	Agnetha de Sá
ATR	Achilles tendon rupture
A-20	Atorvastatin 20 mg
A-80	Atorvastatin 80 mg
BTR	Biceps tendon rupture
BMI	Body mass index
CHHM	Centre for Hip Health and Mobility
CSA	Cross-sectional area
CTGF	Connective tissue growth factor
DNA	Deoxyribonucleic acid
ECM	Extra cellular matrix
FAS	Foot and ankle stabilizer
HR	Hazard ratio
HDLs	High-density lipoproteins
HE	hematoxylin-eosin
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HMGCR	HMG Co-A reductase
ICC	Intraclass correlation coefficient

ICTP	Type I carboxyterminal peptide
ID	Identification
IPAQ	International physical activity questionnaires
LDLs	Low-density lipoproteins
MDC	Minimum detectable change
MMPs	Matrix metalloproteinases
mRNA	Messenger ribonucleic acid
PAR	Pixel aspect ratio
PICP	Collagen propeptide
PRIMO	Prediction of muscular risk in observational conditions
PS	Propensity score
SD	Standard deviation
SDFT	Superficial digital flexor tendon
S-20	Simvastatin 20 mg
S-80	Simvastatin 80 mg
TGF- β -1	Transforming growth factor- β 1
UTC	Ultrasound tissue characterization
VGH	Vancouver General Hospital
VLDLs	Very low-density lipoproteins

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Last but not least, I would like to thank my parents for encouraging me to pursue further education.

Dedication

To my parents, for their endless love, support, and encouragement.

Chapter 1: Introduction

Over the past several years there has been some interest surrounding the potential relationship between the use of statins (a class of cholesterol-lowering drugs) and the risk of tendinopathy or tendon rupture (Marie et al., 2008, Pullatt et al., 2007, Carmont et al., 2009, Ganta et al., 2011, Celik et al., 2012, de Oliveira et al., 2013, 2015; Kuzma-Kuzniarska et al., 2015, Deren et al., 2016). While cholesterol plays important physiological roles in cell membranes and steroid hormone synthesis, blood cholesterol levels elevated beyond ideal levels (> 200 mg/dL) are associated with increased risk of cardiovascular disease (Schaiff et al., 2008; Lecerf and Lorgeril, 2011). Cholesterol homeostasis involves a highly regulated network of feedback control involving diet, medications, genetics, bile acid metabolism, hormones, and body weight (Alphonse and Jones, 2016). One point of feedback involves the HMG Co-A reductase (HMGCR) enzyme which is both the committed and rate-limiting step of cholesterol synthesis (Cerqueira et al., 2016). HMGCR also serves as the target of statins. Through competitive inhibition, statins prevent the substrate (HMG-CoA) from binding to the HMGCR active site, thus preventing the production of cholesterol (Cerqueira et al., 2016). Statins have also been postulated to impact tenocyte metabolism, with potential mechanisms involving increased activity of specific matrix metalloproteinases (MMPs), decreased tenocyte mobility, changes in the cytoskeleton such as cell rounding, and altered mRNA levels of matrix proteins (de Oliveira et al., 2013; Kuzma-Kuzniarska et al., 2015). Subsequently, based on the results of a large retrospective chart review, using the French Pharmacovigilance database to specifically evaluate tendon complications in patients with statin use, a potential relationship between statins and tendon injury was identified (Marie et al., 2008). However, a recent large epidemiological study

conducted by Spoendlin et al. (2016) provided conclusive evidence that there is no association between the use of statins and the risk of Achilles or biceps tendon rupture.

1.1 Overview

Cholesterol, a type of lipid molecule, plays many important roles in human physiology.

Accounting for 30% of the content of mammalian cell membranes, cholesterol is important for maintaining cell membrane fluidity and structure (Alberts et al., 2002; Krause & Regen, 2014).

Additionally, cholesterol serves as the precursor for steroid hormones, bile acid, and vitamin D, making it vital for human physiology (Lecerf and Lorgeril, 2011). The amount of total

cholesterol in the blood depends on a variety of factors such as dietary intake, synthesis, cellular metabolism and biliary excretion (Schaiff et al., 2008; Lecerf and Lorgeril, 2011). With the

involvement of over 15 enzymes and over 30 reactions, cholesterol synthesis is a complex

biochemical process (Alphonse and Jones, 2016). To begin cholesterol synthesis, mevalonate is produced from acetate through a series of biochemical steps (Cerqueira et al., 2016). One of

these steps is the rate-limiting and committed step of the entire synthesis pathway; the production

of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) from acetoacetyl-CoA and acetyl-

CoA by HMG Co-A reductase (HMGCR) (Cerqueira et al., 2016). Since mammalian cells are

able to synthesize cholesterol, dietary intake is not mandatory, and synthesis also influences

cholesterol levels (Russell, 1992; Lecerf and Lorgeril, 2011). If blood cholesterol levels are too

low, synthesis and absorption are upregulated, and if levels are too high, biliary and intestinal

excretion will increase while negative-feedback control will be exerted on cholesterol synthesis

(Quintao et al, 1971; Lecerf and Lorgeril, 2011).

The regulation of cholesterol synthesis utilizes a network of feedback inhibition that controls cholesterol homeostasis (Alphonse and Jones, 2016). One target of feedback inhibition is HMGCR (Cerqueira et al., 2016). Feedback inhibition on HMGCR involves a variety of mechanisms that affect its activity and availability (Cerqueira et al., 2016). For example, dietary cholesterol creates a negative feedback on the translation of the HMGCR enzyme. Additionally, feedback control for the transcription, enzyme degradation and activity also work to control the production of cholesterol in the liver and cells in every mammalian tissue (Russell, 1992; Cerqueira et al., 2016).

Since cholesterol is insoluble in blood, it is transported with the use of lipoproteins consisting of a hydrophobic core surrounded by phospholipids and apoproteins (Berg et al., 2002).

Subsequently, the protein components allow the lipids to be soluble and targeted to specific destinations with the use of cell-targeting signals (Berg et al., 2002). Classification of lipoproteins is based on density and includes high-density lipoproteins (HDLs), low-density lipoproteins (LDLs), very low-density lipoproteins (VLDLs) and chylomicrons with the primary protein in these particles being apolipoprotein B (Stalenhoef et al., 1984; Schaiff et al., 2008).

Hyperlipidemia is characterized by elevated levels of lipids (cholesterol and adipose tissue) in the blood and is the most common form of dyslipidemia. Hyperlipidemia may result from genetic factors (primary) or from other systemic conditions (secondary or acquired), such as diabetes or excessive intake of dietary fat (Lecerf & Lorgeril, 2011). When cholesterol levels increase, LDL levels also increase resulting in accumulation and oxidation of LDL-Cholesterol

in the endothelium of arteries. If continued, the effects of hypercholesterolemia can lead to heart attack or stroke (Nirosha et al., 2014).

As stated earlier, regulation of cholesterol synthesis involves a complex system of feedback inhibition that includes regulation of HMGCR (Cerqueira et al., 2016). In addition to the biochemical processes that target this enzyme, HMGCR is also a target of pharmaceutical drugs. The statins target HMGCR by competing with HMG-CoA for the active site through competitive inhibition for which HMGCR has a slightly higher affinity for statins than HMG-CoA (Cerqueira et al., 2016). Within the statin drug class, there are two types: type I and type II (Cerqueira et al., 2016). Type I statins include lovastatin, pravastatin, and simvastatin and are fungal products while type II statins are synthetic and include atorvastatin, rosuvastatin, and fluvastatin (Cerqueira et al., 2016). Through their action, statins lower concentrations of serum cholesterol levels by 20-30 % thereby reducing the risk of cardiovascular events (Alphonse and Jones, 2016). While statins are well tolerated by the majority of individuals taking them, there are individuals who experience side effects (which range from constipation, headaches, and sleep disturbances to myopathy and rhabdomyolysis) (de Oliveira et al., 2013).

Since the number of statin prescriptions is increasing (Pasternak et al., 2002; Hoffman et al., 2012), more individuals will be exposed and potentially develop adverse side effects from their statin therapy. Research into the side effects of statins has been focused on muscles, however, landmark publications by Marie et al. (2008) and Pullatt et al. (2007) have increased awareness of the potential effects of statins on tendons. Since then, case-reports and prospective studies examining the effects of statins on tendons have increasingly been published.

A mechanism thought to underpin the detrimental effects of statins on tendons is their influence on the activity of MMPs, which are key players in maintaining homeostasis of the tendon extracellular matrix. A study conducted on rats demonstrated that chronic statin treatment resulted in significant changes in the tendon extracellular matrix (reductions in collagen I – an important collagen that makes up tendon - and augmented activity of MMPs 2 and 9 (de Oliveira et al., 2013)).

Tendon injuries are prevalent across the population, affecting both active and sedentary individuals (Gaida et al., 2008). Tendinopathy is defined as pain and swelling in and around a tendon resulting in impaired function (Jarvinen et al., 2005). Tendons affected by tendinopathy include: the Achilles, patellar, medial/lateral elbow and rotator cuff tendons (Gaida et al., 2008). The number of individuals suffering from Achilles tendinopathy has increased over the past decade, and this condition is present in athletes and the general population (Jarvinen et al., 2005).

1.2 Tendon Structure, Function, and Composition

1.2.1 Tendon Structure and Function

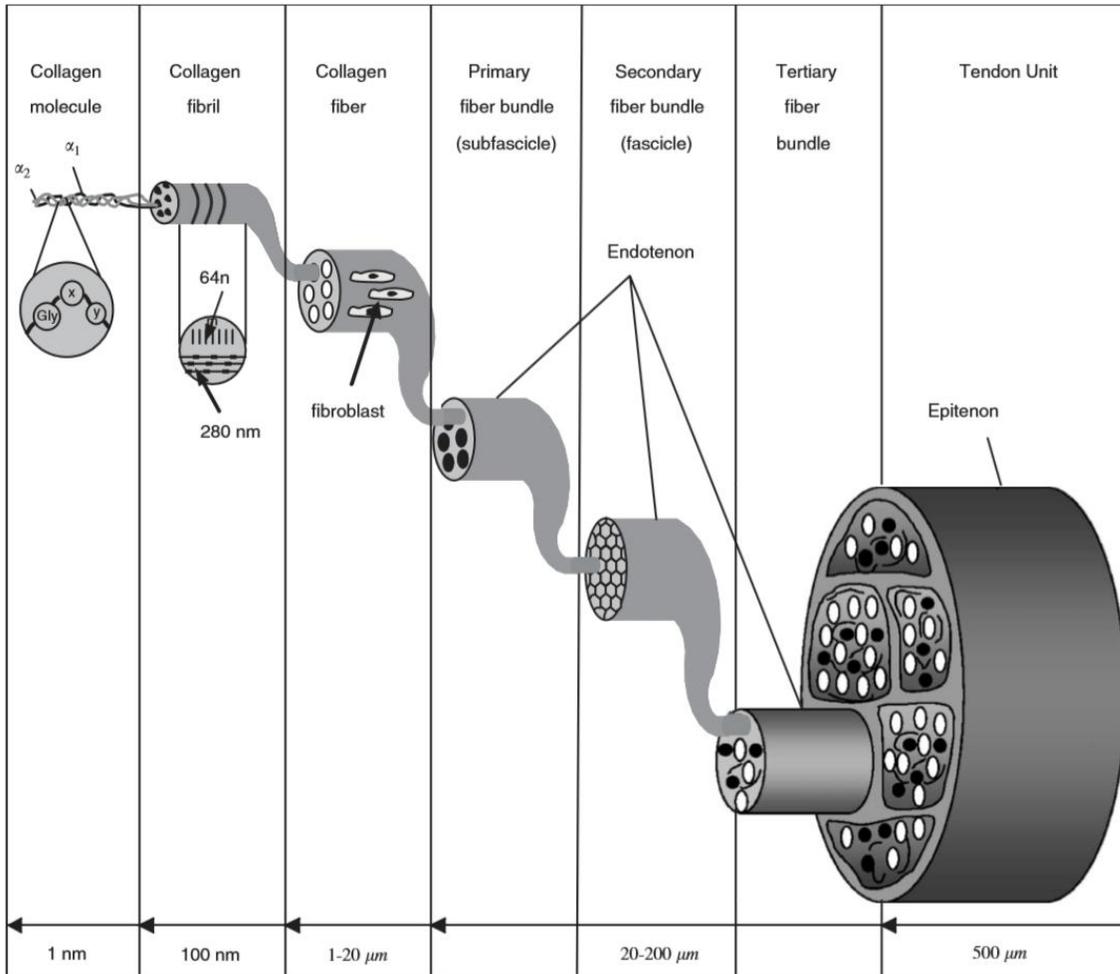


Figure 1.1 Diagram of the multi-unit hierarchical structure of tendon (Wang, 2006, reproduced with permission from Elsevier).

A tendon is a type of musculoskeletal tissue that stores and transmits energy from muscle to bone allowing for the movement of joints and limbs (Wang, 2006; James et al., 2008; Killian et al., 2012). The tendon is structured in a hierarchical manner ranging from the molecular to the tissue levels as can be observed in Figure 1.1 above (Wang, 2006; Killian et al., 2012). Type I pro-collagen molecules, produced by tenocytes, align to form a triple helix whose helical structure is

stabilized by the action of prolyl hydroxylase in the endoplasmic reticulum (Killian et al., 2012). These pro-collagen molecules are then secreted by the tenocyte, and assembled extracellularly into collagen molecules following cleavage of their pro-domains, resulting in the longitudinal assembly of linked units. Bundles of around five collagen molecules associate laterally to create microfibrils, the next level of structure (Killian et al., 2012). Next, neighbouring microfibrils are cross-linked together, creating the fibril (Killian et al., 2012). Multiple bundles of collagen fibrils further associate to form collagen fibres. Bundles of collagen fibres are enclosed by endotenons, comprising the primary fibre bundle (Wang, 2006; James et al., 2008). Endotenon is defined as loose connective tissue between primary fibre bundles, and contains blood vessels, lymphatics and nerves (Wang, 2006). Fibre bundles may also be grouped to comprise fascicles (also referred to as the secondary fibre bundle) and multiple bundles of fascicles are enclosed by the epitenon that encloses the tendon unit. Epitenon, like endotenon, supplies the tendon with circulatory, lymphatic and neural tissue. A final layer of connective tissue called the paratenon (referred to as the synovial sheath for some tendons) surrounds the epitenon, allowing for reduced friction with adjacent tissue (Wang, 2006). The longitudinally oriented, cross-linked organization of the collagen fibres gives tendons their strength and allows them to function in efficient energy transfer (Wang, 2006). Like epitenon and endotenon, paratenon is rich in blood vessels and neuronal innervation whereas the tendon proper is not (Ackermann and Hart, 2016). The implications of this are that the possible effects of statins on tendons could be localized to the connective tissue surrounding the tendon proper since the concentration of statins could be higher in the vascular regions of tendons such as the connective tissue layers, the muscle-tendon junction, and the enthesis (bone-tendon junction) (Kirchgesner et al., 2014).

1.2.2 Tendon Composition

Throughout the body, tendons transfer forces generated by muscles to the skeleton (Thrope et al., 2015). However, some tendons also have an additional function by stretching and recoiling and subsequently storing and returning energy with each stride (Thrope et al., 2015). Therefore, tendon composition is influenced by function with energy storing tendons (such as the Achilles and patellar tendons) having less collagen type I and more collagen type III than positional tendons (Ackermann and Hart, 2016). Healthy tendons consist of tenocytes and their surrounding extracellular matrix of type I collagen fibres and elastin embedded in a proteoglycan-water matrix (Kannus, 2000; Wang, 2006). Type I collagen accounts for around 70-80% of the dry mass of a tendon and up to 95% of the total collagen (Kannus, 2000; Wang, 2006; James et al., 2008; Deren et al., 2016). Proteoglycan, glycosaminoglycans, glycoproteins, elastin, water and other types of collagen (e.g. types III and V) comprise the remaining 30% dry mass of a tendon (Wang, 2006; Killian et al., 2012). While the majority of the tendon is comprised of type I collagen fibres, the epitenon and endotenon are where type III collagen fibres can be found (James et al., 2008). Type III collagen forms less organized fibrils than type I collagen, accounts for up to 10 percent of collagen in tendons, making it the second most abundant collagen, after type I and is thought to have a role in regulating the size of type I fibrils (Kadler et al., 1996; Wang, 2006; Ackermann and Hart 2016). Additionally, type III collagen is found in greater quantities in the ageing tendon and forms smaller fibrils than type I collagen; which is thought to result in a tendon that has decreased tensile strength (James et al., 2008; Wang, 2006; Calleja and Connell, 2010). Additional types of collagen (e.g. types II, VI, IX, X

and XI) are found in trace amounts in the tendon and function mainly at the connections to bone and muscle (Wang, 2006).

Proteoglycans found in tendons vary in amount from tendon to tendon but depend, in part, on the tension- and compression-bearing conditions of the tendon with compression-bearing tendons having more proteoglycans than tension-bearing tendons (Wang, 2006). Additionally, transportation of water and soluble molecules and movement of tenocytes are facilitated by the hydrophilic nature of proteoglycans, which contribute to tendon hydration (Sharma and Maffulli, 2005). Glycoproteins (e.g. fibronectin and thrombospondin) play a role in the healing process of tendons (Sharma and Maffulli, 2005). Elastin and microfibrillar proteins are, in part, responsible for recovering a stretched tendon to the crimp configuration of the collagen fibres (Figure 2) (Wang, 2006; Killian et al., 2012). The remaining cellular components include: endothelial cells, synovial cells, chondrocytes with fibroblasts (tenoblasts and tenocytes) making up the majority of the cell population found in tendons (Wang, 2006). Fibroblasts are usually located between collagen fibres, parallel to the long axis of the tendon (Sharma and Maffulli, 2005; Wang, 2006).

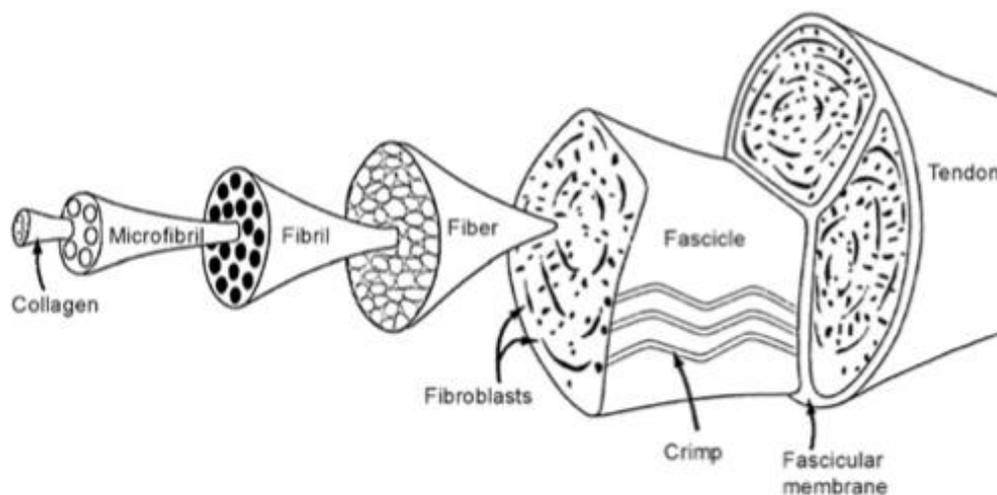


Figure 1.2 Structure of the tendon, displaying the crimp configuration of the collagen fibres (Killian et al., 2012, reproduced with permission from Elsevier).

1.3 Tendon Physiology

Due to their structure, tendons are efficient at transmitting forces from muscle to bone and thereby allowing for movement (Wang, 2006). Additionally, they have high tensile strength, are flexible and have an optimal level of elasticity, dependent on their function (positional or energy-storing tendons) (Sharma and Maffulli, 2005; Thrope et al., 2015). While positional tendons are relatively inextensible with high stiffness, energy-storing tendons have high extensibility and elasticity (Thrope et al., 2015, Ackermann and Hart, 2016). A stress-strain curve is useful in demonstrating the behaviour of tendons under tensile load (Figure 1.3) (Sharma and Maffulli, 2005; Wang, 2006). At rest, the collagen fibres are in the crimp configuration (Figure 1.2). Animal studies on rats and horses have demonstrated that larger crimp angles have been found in energy-storing tendons compared with positional tendons which could allow for increased energy storing capacities (Franchi et al., 2009; Thrope et al., 2013). Increasing the strain to 2% causes the tendon to lose its crimp pattern (Wang, 2006). As the strain increases, up to 4%, the tendon acts as an elastic band and returns to its normal length when relaxed (Sharma and Maffulli, 2005; Wang, 2006). Microscopic tearing occurs when the strain put onto the tendon is above 4% and total rupture occurs when the strain on the tendon is greater than 8% (Sharma and Maffulli, 2005; Wang, 2006).

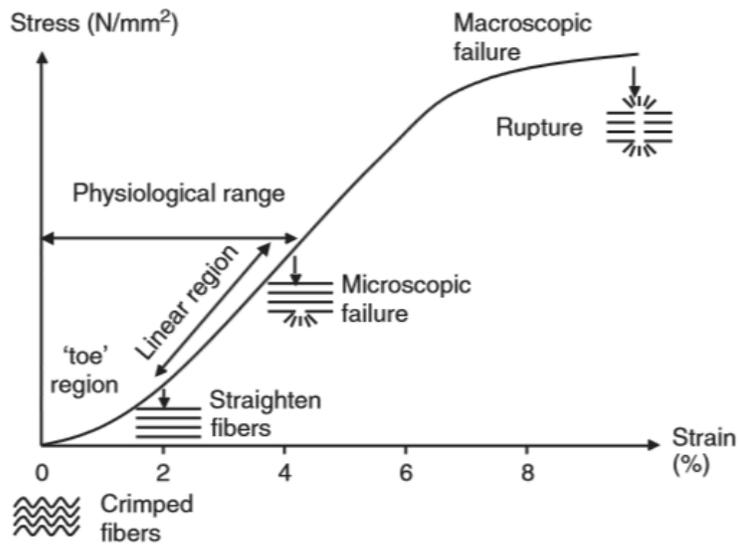


Figure 1.3 Tendon stress-strain curve (Wang, 2006, reproduced with permission from Elsevier).

1.3.1 Effects of Exercise and Immobility on Tendons

Tendons, like other tissues, are dynamic and can change their structure and functional capacity in response to exercise and immobility (Wang, 2006; Killian et al., 2012). Through the mechanical loading of a tendon during exercise, a signal cascade is initiated which results in the production of matrix proteins (Svensson et al., 2016). This increase in matrix proteins results in an increased volume of tendon tissue (a process termed mechanotransduction) that has been demonstrated *in vitro* and seen in animal studies (Svensson et al., 2016). In an *in vitro* study that looked at the responses of tendon fibroblasts to mechanical stretch, an increase in the production and secretion of growth factors was found along with increased collagen synthesis by the tendon fibroblasts (Chiquet et al., 2009). Key growth factors secreted by the tendon fibroblasts are transforming growth factor- β 1 (TGF- β -1) and connective tissue growth factor (CTGF), which have also been confirmed in animal studies (Svensson et al., 2016). In one study, the medial gastrocnemius and

Achilles tendons of female Sprague-Dawley rats were exposed to 4 days of concentric, eccentric or isometric training (Heinemeier et al., 2007). Expression of TGF- β -1 and collagen I and III were increased for all training types in the tendons (Heinemeier et al., 2007). Similarly, experienced marathon runners were studied before and after running 36 km for a duration of 3 hours (Langberg et al., 1999). With the use of microdialysis catheters placed in the peritendinous space ventral to the Achilles tendon, markers for collagen synthesis, (collagen propeptide (PICP)) and a marker of collagen resorption (type I carboxyterminal peptide (ICTP)) were measured (Langberg et al., 1999). In response to the exercise program, PICP levels increased 3-fold 72 hours after exercise in the peritendinous space (Langberg et al., 1999). ICTP levels decreased significantly immediately after exercise but returned to baseline levels 72 hours after exercise (Langberg et al., 1999). The authors concluded that in response to acute exercise, an increase in collagen I synthesis occurs (Langberg et al., 1999). However, since microdialysis was utilized, their findings might not reflect what is taking place deeper in the substance of the Achilles tendon tissue (Svensson et al., 2016).

With regard to the effect of immobilization on tendons, an animal study was conducted by Yasuda et al. (2000). Rabbit knee joints were immobilized for a period of 4 weeks which resulted in decreased stiffness of the Achilles tendons compared to control rabbits. Additionally, they found that the collagen fibres of the Achilles tendons of the immobilized rabbits were disorganized and that the blood vessels were dilated (Yasuda et al., 2000). The authors concluded that the response from being immobilized caused the effects seen in the Achilles tendons of the rabbits (Yasuda et al., 2000).

1.3.2 Effects of Ageing on Tendons

With regard to ageing, the effects on the tendon relate primarily to changes in cell density and activity, as well as to age-related changes in collagen cross-linking (Svensson et al., 2016). In an effort to determine the effects of ageing on tendon properties, tail tendon fascicles from 1, 3 and 12 month-old rats were studied (Lavagnino et al., 2013). The results showed that cell number and contraction rate decreased while tensile modulus increased with age (Lavagnino et al., 2013). Additionally, the shape of the tendon cell nuclei had flattened and the tendon cells matured (Lavagnino et al., 2013). The authors speculated that the observed decrease in contraction rate (with increasing age) might prevent tendon cells from retightening lax tendons, increasing the risk of injury (Lavagnino et al., 2013). While these results are supported by findings from other animal studies, the results might not be due to ageing but maturation (Svensson et al., 2016). Support for this idea comes from a study that analyzed horse superficial digital flexor tendon (SDFT) from 3 to 30 years of age and found that there were no age-related changes in the cellularity and DNA content in the tendon with no change in the mRNA and protein levels of matrix proteins and degradative enzymes (Thorpe et al., 2015).

The finding that tendons stiffen with age has been well documented (Lavagnino et al., 2013; Wood and Brooks, 2016). The mechanism thought to underlie this process is due to the intermolecular cross-linking between collagen fibrils (Wood and Brooks, 2016). Additionally, with the slow turnover of collagen, the products of glycation accumulate over time resulting in additional intermolecular cross-linking which further stiffen the tendon (Bailey, 2000; Wood and Brooks, 2016). In a study conducted on mice tibialis anterior tendons, results showed that age-

related stiffening of the tendons occurred with no corresponding changes in collagen fibril morphology (Wood et al., 2011). The authors concluded that accumulation of intermolecular cross-linking due to glycation might influence the material properties of the fibrils during ageing (Wood et al., 2011). With the hypothesis that moderate intensity exercise would decrease tendon stiffness in old mice, tendon properties were compared between 28 month-old treatment and 28 and 8 month-old control mice (Wood and Brooks, 2016). After 10 weeks of an uphill exercise program, plantaris tendon stiffness and modulus had decreased to a point where they were similar to values found in adult (sedentary) mice (Wood and Brooks, 2016). A reduction in the amount of glycation end products was also found in the exercised (28 month-old) mice compared to the old (28 month-old) controls (Wood and Brooks, 2016). Elevated levels of mRNA for collagen type I, MMP-8 and lysyl oxidase were also found in the Achilles tendons suggesting to the authors that collagen turnover might have been increased (Wood and Brooks, 2016). These changes occurred with no corresponding changes in tendon cell density or morphology (Wood and Brooks, 2016). The authors concluded that with exercise, tendons from old mice are able to replace extracellular matrix components comparable to mechanical and structural properties found in adult mice (Wood and Brooks, 2016).

Additionally, histological studies have found that cholesterol accumulates in the tendons of elderly humans (Adams et al., 1974). In their study, Adams et al. (1974) found that the largest amount of lipid accumulation occurred after the fourth decade. Additionally, the most lipid accumulation was found at the Achilles, triceps and tibialis anterior tendons with the Achilles tendon having the most amount of lipid accumulation with the highest concentration located at the posterior border (Adams et al., 1974).

1.4 Tendon Injury and Repair

Although tendons are capable of handling large forces, the sites of attachment into bone can be a concern (Killian et al., 2012). Since there is a transition from tendon to bone, the forces being transferred can sometimes be too much for a tendon to withstand, thus making attachment sites a potential site of injury for some tendons (Killian et al., 2012). Damage to tendons can result from an acute injury (e.g. sports injury) or chronic overload (Killian et al., 2012). The capability of tendon repair mechanisms subsequently depends on the duration, location and severity of the injury (Killian et al., 2012). For example, one of the most commonly injured tendons in the lower extremities is the Achilles tendon, with an incidence rate of acute ruptures estimated at 18 per 100,000 (Pedowitz and Kirwan, 2013). Injuries to this tendon include both ruptures (typically resulting from athletic activity) and chronic overuse injuries associated with the accumulation of inferior quality tissue (occurring both in athletic and sedentary populations) (Pedowitz and Kirwan, 2013).

1.4.1 Tendon Repair

The tendon healing process is composed of three phases: inflammation, repair (or proliferative) and remodeling. In the inflammatory phase, platelets and inflammatory (e.g. neutrophils, macrophages) cells accumulate at the injury site (Wang, 2006; Killian et al., 2012; Ackermann and Hart, 2016). In addition to the phagocytic function conducted by neutrophils and macrophages, they also release chemicals which attract tendon fibroblasts to the site to begin

collagen synthesis and formation (Killian et al., 2012). This continues into the repair phase, where tendon fibroblasts continue to synthesize collagen and extra cellular matrix (ECM) components (e.g. proteoglycans) (Killian et al., 2012). Additionally, the peripheral nervous system plays a role in the inflammatory and repair phases of tendon healing (Ackermann and Hart, 2016). During the healing process, nerve ingrowth from the surrounding connective tissues with corresponding expression of neuropeptides, in a time dependent manner after which (during the remodeling phase) neural tissue retracts from the tendon proper and into the surrounding connective tissue (Ackermann and Hart, 2016). Type III collagen production increases early in the repair process and during remodeling but decreases as type I collagen production increases; resulting in the highly organized structure of the tendon (James et al., 2008).

Although tendons receive their blood supply from the endotenon, epitenon and paratenon they have 7.5 times lower oxygen consumption than skeletal muscles (Sharma and Maffulli, 2005). A low metabolic rate and anaerobic energy generation/function are presumably what allow tendons to function optimally and maintain their homeostasis even while under tension for long periods of time. However, during the healing process, the low metabolic rate could result in prolonged healing times (Sharma and Maffulli, 2005).

1.5 Epidemiology of Tendinopathy

1.5.1 Prevalence of Different Tendinopathies

Tendinopathy can occur at any tendon, particularly at the insertion site where the greatest stresses occur (Xu and Murrell, 2008). Subsequently, any activity or situation (e.g. increased activity, weight gain, age) that increase loads experienced by a tendon can lead to tendinopathy (Xu and Murrell, 2008). In the clinical setting, tendinopathy refers to tendinitis, tendinosis, and paratenonitis, In a study conducted by Kannus et al. (1987), they found that a third of the sports injuries that were treated at on outpatient sports clinic involved tendons with the most common sites of injury listed below in Table 1.1.

Tendon involved	Injury
Achilles	Achilles tendinopathy, Achilles paratendinopathy, tendon rupture, calcaneal apophysitis (Sever's disease)
Patella	Patellar tendinopathy, patellar peritendinopathy, patellar apicitis (jumper's knee), Osgood-Schlatter lesion, Sinding-Larsen-Johansson lesion
Posterior tibial	Medial tibial syndrome
Iliotibial tract	Iliotibial tract syndrome
Biceps femoris, semitendinosus, semimembranosus	Hamstring syndrome
Supraspinatus	Supraspinatus syndrome (impingement syndrome, swimmer's shoulder)
Other rotator cuff tendons (infraspinatus, subscapularis, teres minor)	Rotator cuff tendinopathy or tear
Common wrist extensors	Lateral epicondylitis (tennis elbow)
Common wrist flexors	Medial epicondylitis (thrower's elbow, golfer's elbow, little league elbow)

Table 1.1 Common sites of overuse tendon injuries (Maffulli et al., 2003, reproduced with permission from Elsevier).

1.5.1.1 Incidence and Prevalence of Achilles Tendinopathy

Studies have examined the incidence of Achilles tendinopathy in athletic populations (Kujala et al., 2005; Lian et al., 2005; Zwerver et al., 2011; Lopes et al., 2012). For example, Kujala et al. (2005) found that former elite long-distance male runners have a lifetime incidence of 42% while Lian et al. (2005) determined the prevalence of jumper's knee to be 14.2% (87 out of 613 athletes) from a variety of sports. In a systematic review of running-related musculoskeletal injuries, the incidence rate ranged from 9.1% to 10.9% and the prevalence rate ranged from 6.2% to 9.5% for Achilles tendinopathy (Lopes et al., 2012). Lastly, in a study of jumper's knee in non-elite athlete, the overall prevalence was found to be 8.5% (78 out of 891 athletes) based on a variety of sports (Zwerver et al., 2011).

Studies have also examined the distribution of Achilles tendinopathy in the general population (de Jonge et al., 2011; Albers et al., 2016). De Jonge et al. (2011) conducted a cross-sectional study at a Dutch general practice to determine the incidence of Achilles tendinopathy. Their results showed that the overall incidence rate of Achilles tendinopathy was 1.85 per 1,000 registered patients per year and 2.35 for the entire adult population (de Jonge et al., 2011). The authors also found that the prevalence rate for midportion Achilles tendinopathy was higher than that of other tendinopathies (e.g. lateral epicondylitis) (de Jonge et al., 2011). In a recent study in a Dutch general practice population, the incidence and prevalence of lower extremity tendinopathy was found to be 10.52 and 11.83 per 1000-person years respectively (Albers et al., 2016). The authors also found that the prevalence of lower extremity tendinopathy was higher amongst older patients (Albers et al., 2016). Additionally, they determined that the prevalence

rate was 2.35 (per 1000 person-years) while the incidence rate was 2.16 (per 1000 person-years) for Achilles tendinopathy (Albers et al., 2016). Finally, no differences were found between tendinopathy patients and general practice patients with regard to gender, use of medication or comorbidity (Albers et al., 2016).

1.5.2 Risk Factors for Tendinopathy

Risk factors for tendinopathy can be divided into intrinsic and extrinsic factors; with intrinsic meaning originating from within the body and extrinsic meaning originating from outside the body. Intrinsic risk factors for tendinopathy include: age, gender, obesity, and genetics while extrinsic factors include: excessive activity or intensity, occupation, shoes, and environmental conditions (Mokone et al., 2006; Xu and Murrell, 2008). However, it should be noted that while it is known that the etiology of tendinopathy is multifactorial, a clear understanding about risk factors is still a work in progress (Peters et al., 2016, O'Neill et al., 2016).

Out of all of the intrinsic factors, genetics is the strongest intrinsic risk factor for tendinopathy (Rees et al., 2009). One of the first intrinsic risk factors studied was the ABO blood group. Jozsa et al. (1989) found a significant association between the O blood group and risk of tendon ruptures with 53% of patients who had a tendon rupture belonging to the O blood group. Another study looking at the relationship between blood group and risk of tendinopathy, found similar results; the majority of patients who had Achilles tendon rupture and chronic Achilles peritendinopathy belonged to the O blood group (Kujala et al., 2005). This led to the idea that the ABO gene, located on chromosome 9q34, not only determined glycoprotein antigens on red

blood cells but also ground substance protein structure of tendons (Mokone et al., 2006). Subsequently, other researchers tried to see if they could find similar results in their study population with no success (Maffulli et al., 2003). The researchers concluded that peculiarities in the ABO blood group distribution of genetically segregated populations might explain the early positive findings (Maffulli et al., 2003; Rees et al., 2009). Additionally, studies on the roles genes play in Achilles tendinopathy have found that some collagen and tenascin-C glycoprotein genes, such as COL5A1 and TNC, are located close to the ABO genes at chromosome 9q34, and are stronger candidate genes for Achilles tendinopathy (Mokone et al., 2006; Rahim et al., 2016). Gender is another intrinsic risk factor that has been studied in relation to Achilles tendinopathy. Some studies have observed that men are more likely to experience tendinopathy than women (Maffulli et al., 2003; Rees et al., 2009). However, the type of tendinopathy is important since more men than women experience patellar tendinopathy while elderly women experience more hip tendinopathy (e.g. iliopsoas and gluteal tendons) (Longo et al., 2009; Bancroft and Blakenbaker, 2010). Additionally, studies have found that women have an increased risk of Achilles tendon rupture after menopause has begun (Maffulli et al., 1999). However, other studies did not observe any post-menopausal spike in Achilles tendon rupture (Longo et al., 2009; Scott et al., 2014).

Age is a risk factor that can predispose an individual to tendinopathy by changing the mechanical properties of tendon (Wang et al., 2012; Jonely et al., 2016). In a study that compared the Young's modulus between young (aged 29 – 50 years) and old (aged 64 – 93 years) donor patellar tendons, old tendons were found to have a significantly lower Young's modulus (504 ± 222 MPa compared with 660 ± 266 MPa in the young tendons) (Wang et al., 2012). Through the

glycation mechanism (previously discussed), increases in stiffness of tendon may occur resulting in reduced energy storage capacity and reduced tolerance of loads (Bailey, 2001; Rees et al., 2009). Previous work has also found that aging tendon demonstrates decreased elasticity which puts the elderly at an increased risk of developing tendinopathy (Kubo et al., 2003; Jonely et al., 2016). However, with low-load resistance training, tendon elasticity increased (Kubo et al., 2003). Furthermore, aging has also been linked to aberrant changes in tendon stem cells negatively impacting tendon health (Jonely et al., 2016). Specifically, previous work found a decrease in stem cell numbers and activity as well as atypical differentiation of tendon stem cells into nontenocytes (Jonely et al., 2016).

Body composition, specifically the amount of adipose tissue an individual has, has been associated with tendinopathy (Gaida et al., 2009; Rees et al., 2009; Scott et al., 2013; Abate et al., 2016). In their study of anthropometric risk factors, Malliaras et al. (2007) found that male volleyball players had higher weight, BMI, waist and hip circumferences, and waist-to-hip ratios putting them at greater risk of developing patellar tendinopathy. In their study to assess the relationship between Achilles tendinopathy and BMI, Scott et al. (2013) found that, after accounting for age, the Achilles tendinopathy group had a significantly higher BMI than the control group. In their systematic review, Gaida et al (2009) found that in nearly half the studies reviewed, symptomatic tendinopathy was associated with significantly higher levels of adiposity. In a study on asymptomatic Achilles tendon pathology and fat distribution, men with tendon pathology were found on average to have a more central fat distribution while women with tendon pathology were found to have a peripheral fat distribution (Gaida et al., 2010). The mechanisms which have been speculated to underlie the potential relation between adiposity and

tendon pathology may be considered as either systemic or mechanical (Gaida et al., 2009; Rees et al., 2009; Abate et al., 2016). The mechanical hypothesis suggests that with an increase in adiposity, weight-bearing tendons experience higher loads, leading to tendon pathology (Gaida et al., 2009). The systemic hypothesis, through direct and indirect mechanisms, suggests that either adipose tissue releases bioactive peptides which affects the structure of the tendon (direct mechanism) or metabolic changes throughout the body may affect the structure of tendons (indirect mechanism) (Gaida et al., 2009). Critics of the mechanical hypothesis argue that with other tissues, such as bone and muscle, loading of these tissues results in strengthening (Rees et al., 2009). Additionally, Gaida et al. (2009) found that the literature they reviewed supported the systemic hypothesis. They reasoned that low-levels of inflammation (associated with cardiovascular disease) seen with a central distribution of fat released cytokines which could impact tendon metabolism or healing (Gaida et al., 2009).

Additionally, tendon homeostasis can be influenced by metabolic conditions such as hyperuricemia, thyroid disease, diabetes mellitus and a handful of congenital metabolic conditions (Ackermann and Hart, 2016). Within the scope of this thesis project, the conditions of note involve elevated cholesterol, namely hypercholesterolemia (Ackermann and Hart, 2016). In high cholesterol environments, the accumulation of oxidized LDL occurs (Ackermann and Hart; 2016). When native LDL leaves the circulation and enters the tendon matrix macrophages release cellular oxidants oxidizing the LDL (Ackermann and Hart, 2016). Macrophages then phagocytize the oxidized LDL particles resulting in the accumulation of foam cells (or lipid-laden macrophages) (Ackermann and Hart, 2016). The resulting accumulation of foam cells is referred to as a xanthoma and can be found in tendons such as the Achilles tendon (Ackermann

and Hart, 2016). In studying the possible connection between elevated serum cholesterol and Achilles tendon ruptures, Mathiak et al. (1999) assessed the outcome of 41 patients following surgery to repair Achilles tendon ruptures. With sport activities being the major cause of Achilles tendon rupture (90%) and with an almost equal number of right (22 patients, 54%) and left (19 patients, 46%) included in their study, Mathiak et al. (1999) checked serum cholesterol levels during the follow-up period after surgery (2-12 years post trauma, average of 5.2 years). Ultrasound evaluation revealed that none of the patients had xanthomatosis present in their Achilles tendons but that in 19 patients (43%) mild degeneration was observed on the non-operated tendon (Mathiak et al., 1999). Out of the 31 patients who consented for serum cholesterol testing, 26 (83%) had elevated cholesterol levels (>200-355 mg/dl) (Mathiak et al., 1999). In their prospective study, Ozguratas et al. (2003) assessed the relationship between an Achilles tendon rupture and concentrations of serum lipids. None of the patients (47 individuals) or controls (26 individuals) had systemic acute or chronic conditions requiring drug therapy or had a history of previous tendon rupture (Ozgurtas et al., 2003). Additionally, with an almost equal distribution of left (21 patients, 45%) and right (26 patients, 55%) tendon ruptures, all of the ruptures occurred while participating in a sport activity (soccer: 26 patients, tennis: 6 patients, basketball: 5 patients, and running: 10 patients) (Ozgurtas et al., 2003). For the tendon rupture group, blood samples were collected six to eight hours after the rupture while blood samples were collected while controls subjects were fasting (Ozgurtas et al., 2003). The mean serum lipid concentrations for the control and Achilles tendon rupture group can be seen below in Figure 1.4.

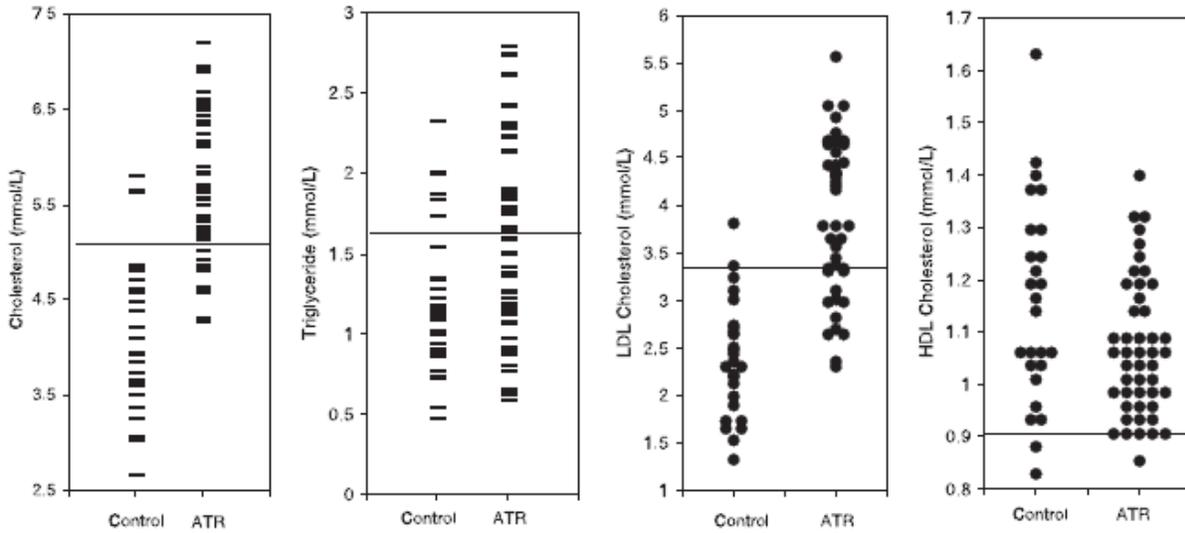


Figure 1.4 Comparison of mean serum lipid concentrations of patients with Achilles tendon rupture and control subjects (Ozgurtas et al., 2003, reproduced with permission from Elsevier).

Based on these results, serum concentrations of (total) cholesterol, triglycerides, and LDL-C were significantly higher than the control group while HDL-cholesterol was lower than the control group (Ozgurtas et al., 2003). However, in their study assessing the relationship between Achilles tendon rupture and lipid profiles, Huisman et al. (2016) found that while higher levels of total cholesterol, triglycerides, and total cholesterol:HDL-cholesterol ratio were detected in the rupture patients, a considerable overlap between the lipid profiles of the rupture and control patients was observed (Figure 1.5).

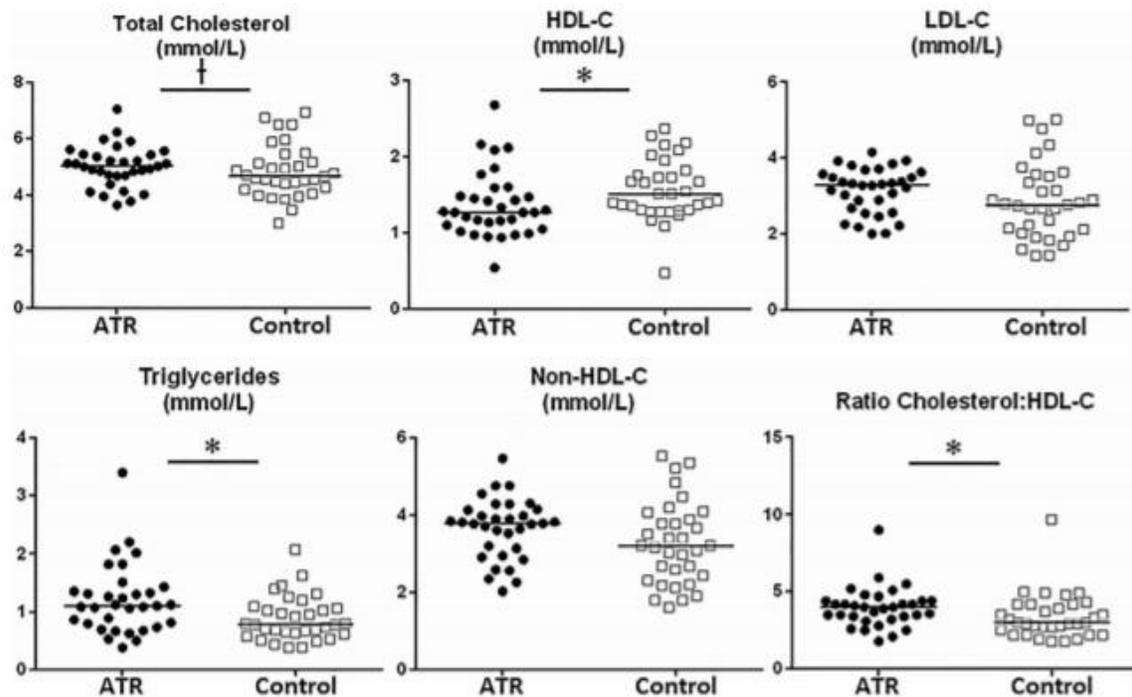


Figure 1.5 Comparison of lipid profiles between patients with Achilles tendon rupture and control subjects (Huisman et al., 2016, reproduced with permission from BMJ). The line indicates the median value. Significance levels of † $p < 0.01$ and * $p < 0.05$ are noted.

With similar patient characteristics to previous literature, Huisman et al., (2016) had 33 patients in the tendon rupture group and 34 controls with unilateral Achilles tendon rupture in their study. No differences in age, BMI and physical activity (assessed with the use of IPAQ) between the tendon rupture and control groups was observed (Huisman et al., 2016). The authors noted that while their results were similar to previous reports, their conclusion was that lipid profiles are weakly associated with Achilles tendon rupture, and that further research is needed to determine the mechanisms underlying the relationship between lipids and tendon health (Huisman et al., 2016).

Extrinsic factors include load, distance and duration of exercise which can affect the rate of wear on tendon tissue (Mokone et al., 2006; O'Neill et al., 2016). As mentioned previously, clear

understanding of risk factors is still emerging (Peters et al., 2016, O'Neill et al., 2016). However, a recent study aimed to improve understanding on what the risk factors are for Achilles tendinopathy since many reported factors in the literature yield conflicting results (Peters et al., 2016; O'Neill et al., 2016). In this Delphi study, tendon experts were invited to complete an online survey that gradually narrowed the list of potential risk factors down, within two categories: an Active/Athletic group and Inactive/Sedentary group (O'Neill et al., 2016). The authors defined the Active/Athletic group as individuals who engage in vigorous physical activity at least 3 times per week while the Inactive/Sedentary group included individuals who did not engage in physical activities or hobbies (O'Neill et al., 2016). Based on the feedback received from the tendon experts, the highest ranked intrinsic risk factor for the Active/Athletic group was previous lower limb tendinopathy while advancing age, obesity, and gender ranked the highest for the Inactive/Sedentary group (O'Neill et al., 2016). For both Active and Inactive groups, the highest ranked extrinsic factor was tendon loading, with changes in loading consistently being ranked first (O'Neill et al., 2016). With a sample size of 14 and the inclusion of published researchers rather than clinicians, the list of risk factors developed in this study is a starting point for future epidemiological studies (O'Neill et al., 2016).

In addition to the extrinsic factors listed above, tendon disorders, mainly at the Achilles tendon, have been linked to the usage of certain drug classes (Kirchgesner et al., 2014; Ackermann and Hart, 2016). Quinolone antibiotics and glucocorticoid therapy have long been associated with tendon pathology (Kirchgesner et al., 2014). However, recently, statins and aromatase inhibitors have also been reported with tendon pathology (Kirchgesner et al., 2014; Ackermann and Hart, 2016). While the specific mechanisms by which these drug classes induce tendinopathy are not

yet fully understood, the lower limb tendons (particularly the Achilles tendon) seem to be the most common sites of tendon issues (Kirchgesner et al., 2014). Usage of medications that fall within these drug classes in combination with the other risk factors discussed above could predispose an individual to developing tendinopathy (Kirchgesner et al., 2014).

1.6 Link between Statins and Tendinopathy?

Several studies have suggested a link between statin use and tendinopathy. Most of the published research linking statins and tendon issues comes from case studies (Chazerain et al., 2001; Movahed and Samsamsharalat 2006, Pullatt et al., 2007, Carmont et al., 2009, Celik et al., 2012; Kearns and Singh, 2016). One of the first observational reports found that 10.5% of patients reported muscular symptoms while taking statin therapy (Bruckert et al., 2005). Bruckert et al. (2005) used the Prediction du Risque Musculaire en Observationnel (Prediction of Muscular Risk in Observational conditions, PRIMO) survey to characterize mild to moderate muscular symptoms in 7,924 patients using statins. Based on the results of the PRIMO survey, tendonitis-associated pain was reported by nearly 25% of statin users for which more than 80% of patients never experienced symptoms before starting their statin. Additionally, Bruckert et al. (2005) found that age, gender, and BMI were not predictive of muscular symptoms while the strongest risk factor was a history of muscle pain while using another lipid-lowering therapy. While Bruckert et al. (2005) determined risk factors for muscular symptoms, they did not differentiate between muscle and tendon complications. In their landmark study, Marie et al. (2008) conducted a retrospective review of reported adverse events to evaluate tendon complications ascribed to statin use. Using a French Pharmacovigilance database, they found 96 out of 4,597

stain adverse event reports of patients who either experienced tendinitis (63 patients) or tendon rupture (33 patients) while on statin therapy (Marie et al., 2008). By specifically looking for reports of tendon complications, Marie et al. (2008) found a larger amount of men than women reported tendon complications (67 men and 29 women with a median age of 56 years). The Achilles tendon was the most frequent tendon injured, along with the quadriceps femoris tendon, tendon of the musculus gluteus medius, tibialis anterior and rotator cuff (Marie et al., 2008). It was noted that none of the 96 patients were taking medications which could increase statin toxicity (e.g. fibric acid derivatives and CYP3A4 inhibitors). The median time for the occurrence of tendon symptoms was 243 days (or 8 months), with one patient experiencing tendinitis within the first day after statin use. Additionally, 59 per cent of patients reported tendinopathy symptoms within the first year of their statin treatment and 26 patients experienced bilateral tendon symptoms (Marie et al., 2008). In terms of symptoms, pain was reported by all 96 patients, with stiffness and difficulty with movement of the involved tendon reported for 62 patients (Marie et al., 2008). Additional symptoms included swelling (n = 59) and warmth and erythema (n = 28) (Marie et al., 2008). All 96 patients discontinued the suspected statin but for 7 patients who reinitiated statin use, they all experienced a recurrence of their tendon symptoms (Marie et al., 2008). However, Marie et al. (2008) also reported that 27 patients had comorbidities that would favour the onset of tendon complications including a history of diabetes (n = 7), tendinopathy (n = 11) with Achilles tendon involvement in 90 per cent of cases, hyperuricemia (n = 5), and sport practice (n = 15) (Marie et al., 2008). Amongst the statins atorvastatin (n = 35), simvastatin (n = 30), pravastatin (n = 21), fluvastatin (n = 5) and rosuvastatin (n = 5) were related to tendon issues (Marie et al., 2008). Moreover, it was not clear from this study whether the rate of tendon injury was higher than expected for such a population

sample as studies on the Dutch general population have reported a prevalence rate of 11.83 per 1000 person-years (de Jonge et al., 2011; Albers et al., 2016). Based on these findings, it is not clear that statins do in fact negatively affect tendons as Marie et al. (2008) conducted a retrospective study and could not control for variables such as previous tendinopathy and diabetes; factors that can predispose individuals to developing tendon complications. Additionally, Marie et al. (2008) do not account for adherence rates, which can account for the fact that more men were included in the study. Non-adherence of medications is an area of research that still has many questions to answer (Goldstein et al., 2016). When specifically considering statins, most of the research has not either differentiated between non-adherence versus non-responders as well as the gender differences observed in the population (Goldstein et al., 2016; Trompet et al., 2016). However, heart disease researchers are starting to assess the gender-based differences as well as the factors that determine non-adherence (Goldstein et al., 2016; Trompet et al., 2016). Based on the literature, women are more likely to be non-adherent, have gender-specific cardiovascular disease risk factors, present with atypical chest pain, and have a greater risk for statin intolerance (Goldstein et al., 2016). Additionally, genetic testing has revealed that variants in the apoE gene significantly affect statin responsiveness (Thompson et al., 2009). Therefore, based on these observations, there are many factors that influence statin use and its subsequent impact on tendons.

In an effort to determine the potential impact of statins on pulmonary health, Schaafsma et al. (2011) found that simvastatin inhibited type I collagen synthesis in human smooth muscle. Additionally, an earlier study by Turner et al. (2005) determined that simvastatin inhibited synthesis and activity of matrix metalloproteinase-9 (MMP-9). Based on these findings, de

Oliveira et al. (2013) sought to determine the biochemical effects of statins on the rat Achilles tendon. In their study, three groups of healthy male Wistar rats fed a normal diet were exposed to two dosages of atorvastatin (A-20, A-80), simvastatin (S-20, S-80), and nothing (the control group) (de Oliveira et al., 2013). Plasma low-density lipoprotein (LDL), non-collagenous proteins, glycosaminoglycans and hydroxyproline were measured. de Oliveira et al. (2013) found that the A-20 group had lower amounts of type I collagen compared to the C group. Additionally, for MMP-2, there was a significant increase in pro-MMP-2 activity in the A-80 group and in active MMP-2 in the S-20 groups. For MMP-9, a significant increase in the latent MMP-9 activity was found in both the A-80 and S-20 groups. de Oliveira et al. (2013) determined that the presence of MMP-2 and MMP-9 meant degeneration and remodeling were taking place in the extracellular matrix of the tendons. They concluded that the statins induced an imbalance of extracellular matrix components which may have resulted in tendon damage (de Oliveira et al., 2013). Additionally, they found that the tendon responded differently to the two dosages (de Oliveira et al., 2013).

Recently, de Oliveira et al. (2015) conducted a further study to determine the structural and biomechanical changes in the rat Achilles tendon after chronic exposure to statins. Using the same methodology as their previous experiment, de Oliveira et al. (2015) exposed rats to two dosages of atorvastatin (A-20, A-80), simvastatin (S-20, S-80), and nothing (the control group). With hematoxylin-eosin (HE) staining, tendon sections of the A-20, S-20 and S-80 groups appeared paler than the pink-red observed in the control and A-80 groups; the authors interpreted this subjective histological finding as indicating a degenerative state. When comparing the epitenon, de Oliveira et al. (2015) found that all of the statin-treated groups showed decreased

thickness compared to the control group. With their previous findings that collagen I levels were lower and MMP-9 activity was increased in the statin-treated groups, de Oliveira et al. (2015) concluded that these factors may have contributed to the reduction of the epitenon observed. Through biomechanical testing it was found that statins had a detrimental effect on maximum load and stress in the A-20, A-80 and S-20 groups (de Oliveira et al., 2015). Surprisingly, the S-80 group fared better in biomechanical tests for which, de Oliveira et al. (2015) did not have an explanation for. Lastly, de Oliveira et al. (2015) conducted birefringence tests to determine the degree of organization of the tendons in each group. This testing revealed that the A-20, A-80, and S-80 groups had lower organization compared to the control group. de Oliveira et al. (2015) concluded that their findings of degeneration and higher disorganization of the tendons indicated that statins made tendons weaker and susceptible to rupture. However, there are certain limitations of these animal studies that should be kept in mind when considering these findings. First, considering the use of animal models in tendon research, it has been noted that while animal models are valuable to study every stage of tendon pathology, the majority of animals used are quadrupeds (Hast et al., 2014). The implications of this are that the loads experienced by quadruped tendons do not truly reflect the loads and subsequent pathology observed in humans (Hast et al., 2014). In modeling the Achilles tendon, rats and rabbits are commonly used (Hast et al., 2014). Next, considering the various animal models used in atherosclerosis research which include the rat and rabbit, there are important limitations that should be noted (Xiangdong et al., 2011). The main limitation of rabbits and rats are that the majority of circulating lipoprotein particles are HDL and rats lack cholesteryl ester transfer protein (CETP) which is found in humans and plays a role in reverse cholesterol transport, allowing HDL-cholesterol particles to travel to the liver (Rader et al., 2009; (Xiangdong et al., 2011)). However, with the

use of transgenic species; particularly transgenic rats expressing human CETP, these limitations can be overcome (Xiangdong et al., 2011).

In an effort to determine the effects of statins on tendon cells, human primary tenocytes were cultured with differing concentrations of lovastatin for up to a week (Kuzma-Kuzniarska et al., 2015). With therapeutic doses, there were no changes in cell viability and morphology while cell migration decreased with short-term exposure to lovastatin concentrations outside the therapeutic range (Kuzma-Kuzniarska et al., 2015). With exposure to simvastatin and atorvastatin, cell migration also decreased (Kuzma-Kuzniarska et al., 2015). Additionally, with high concentrations of lovastatin, the cytoskeleton was affected resulting in cell rounding. mRNA levels of matrix proteins (for collagen types I and III) were decreased, and bone morphogenetic protein-2 expression was increased (Kuzma-Kuzniarska et al., 2015). Subsequently, these results provide additional insights into potential mechanisms underlying the link between statins and tendons (Deren et al., 2016).

Another potential mechanism linking statins and tendon injury could be related to the statin's intended effect (Deren et al., 2016). In preventing cholesterol synthesis, cell membranes may be weakened, specifically those of tenocytes (Deren et al., 2016). Due to their role in synthesis and maintenance of the extracellular matrix, compromised tenocyte cell membranes may result in alterations in the structural components of tendons (Deren et al., 2016). However, there is no evidence to directly support this hypothesis.

In an effort to examine the purported relationship between statins and tendon rupture, a large propensity score (PS) matched cohort study was recently conducted by Spoenclin et al. (2016). Patients (≥ 45 years) who had at least one new statin prescription between the years 1995 and 2014 were PS-score matched to control patients with no statin prescriptions (Spoenclin et al., 2016). Spoenclin et al. (2016) followed patients until an Achilles or biceps tendon rupture (ATR or BTR) occurred, completed 5-years of follow-up, or no longer met the inclusion criteria for the study (Spoenclin et al., 2016). With a cohort of 526,351 PS-matched pairs, an equal distribution of covariates was found in the matched groups along with 50% of women in each group (Spoenclin et al., 2016). In the overall cohort, similar levels of ATR or BTR events were recorded for statins users (0.11%) and non-statin users (0.10%) for a total of 8893 events (Spoenclin et al., 2016). In the PS-match cohort, 1205 diagnoses of ATR or BTR were recorded during the follow-up period (Spoenclin et al., 2016). Statistical analysis resulted in a crude hazard ratio (HR) of 1.3 (before PS matching), multivariable adjustment HR of 1.02 (before PS matching) and a PS-matched cohort HR of 0.95 (Spoenclin et al., 2016). A crude HR of 1.6 was observed in women, but statin use was not associated with ATR or BTR in men or women after multivariable adjustment and PS matching. Based on the results of this large, observational cohort study, the authors concluded that statins do not increase the risk of Achilles and biceps tendon rupture, regardless of covariates such as gender, age, statin dose and duration of statin use (Spoenclin et al., 2016).

1.6.1 Nature of Reported Tendon Complications

When examining the possible relationship between statin and tendon health, it is important to consider either where or which part of the tendon may be affected by statins. While the larger observational studies do not report the precise location of where rupture or pathology within the observed tendons (and only state which tendons are affected), some case reports do provide details on the precise nature of the tendon complication experienced by patients. One of the first case reports conducted by Chazerain et al. (2001), described the experiences of four patients who experienced tendinopathy while using statins. With three men and one women included in their case report, Chazerain et al (2001) noted that risk factors (e.g. familial hypercholesterolemia, hyperuricemia, sports-related injuries, and medications known to instigate tendinopathy) for tendon pathology were not present for the four patients. However, for two of the male patients, one had left Achilles tendinopathy in 1994 and the other had long-term glucocorticoid therapy usage (Chazerain et al., 2001). The tendons involved for the four patients is as follows: Case 1 (male aged 56 years) – finger extensor tendons on both hands, Case 2 (female aged 53 years) – right tibialis anterior tendon, Case 3 (male aged 49 years) – right Achilles tendon, and Case 4 (male aged 65 years) – both Achilles tendons and the right supraspinatus tendon (Chazerain et al., 2001). Two patients were using atorvastatin (Case 2 used 20mg/day dosage and Case 4 used 40 then 80mg/day dosages) while the other two used simvastatin (Case 1 used 10mg/day dosage and Case 3 used 20 mg/day dosage) (Chazerain et al., 2001). One patient (Case 1) had a clinical picture of tenosynovitis and the remaining three had acute tendinitis (Chazerain et al., 2001). While no imaging modality (e.g. ultrasound or MRI) was used for Case 1, either ultrasound, MRI, or both were used for the remaining three patients (Chazerain et al., 2001). With Case 2,

ultrasound examination revealed widening and thickening of the tendon when compared to the left side with relative hypoechogenicity but no fibrillar structure alterations (Chazerain et al., 2001). With Case 3, ultrasound examination revealed that for his right Achilles tendon, a thickening of the upper third and junction between the upper and middle thirds of the tendon was present, with no nodules or fissures observed within the tendon; the middle and lower thirds of the Achilles tendon were observed to be normal (Chazerain et al., 2001). Lastly, Case 4 had both an ultrasound scan and MRI done for both of his Achilles tendons (Chazerain et al., 2001). MRI revealed changes that were consistent with inflammatory tendinopathy for the right supraspinatus and both Achilles tendons; with microfissures observed on the left Achilles tendon (Chazerain et al., 2001). With discontinuation of their statin, recovery from tendon symptoms was observed for all four patients (Chazerain et al., 2001).

In their case report, Movahed and Samsamsharaiat (2006) described two cases of where patients had tendinitis-like symptoms while using a statin. Case 1 was a male aged 43 years who had a history of hyperlipidemia, hypertriglyceridemia, and low HDL who used the 40 mg/day dosage of simvastatin (Movahed and Samsamsharalat 2006). Eight weeks after statin initiation, he experienced pain near the medial aspect of the left knee as well as near the quadriceps and Achilles tendons (Movahed and Samsamsharalat 2006). Upon physical examination, tenderness was found in the peritendon area around the quadriceps and Achilles tendons (Movahed and Samsamsharalat 2006). When the simvastatin dose was decreased to 20 mg/day, the quadriceps tendinitis-like pain resolved after 4 weeks but the Achilles tendon pain increased (Movahed and Samsamsharalat 2006). With continued use of simvastatin 20 mg/day, Achilles tendon pain continued to be an issue for the patient with a new onset of pain in the deltoid tendon (Movahed

and Samsamsharalat 2006). After discontinuation of simvastatin, the tendinitis-like symptoms took 6 weeks to resolve (Movahed and Samsamsharalat 2006). However, when the patient started using 2.5 mg/day of rosuvastatin, the tendinitis-like pain in both the Achilles and deltoid areas started up again for which it took 8 weeks for his symptoms to resolve when rosuvastatin was discontinued (Movahed and Samsamsharalat 2006). Case 2 was a male aged 70 years who had a history of hypercholesterolemia and was started on lovastatin 20 mg/day and gemfibrozil 300 mg/day (Movahed and Samsamsharalat 2006). Tenderness near both Achilles tendons began after a few months (Movahed and Samsamsharalat 2006). A physical exam determined that the tenderness was localized to the peritendon of both Achilles tendons (Movahed and Samsamsharalat 2006). Lovastatin was discontinued after the patient reported that his walking was effected by the severity of pain for which it took 2 weeks for the symptoms to resolve (Movahed and Samsamsharalat 2006). Like with Case 1, Case 2 was started on a lower dosage of lovastatin (10 mg/day) for which a recurrence of the tendinitis-like symptoms occurred (Movahed and Samsamsharalat 2006). 2 weeks after lovastatin was discontinued, all tendinitis-like symptoms resolved (Movahed and Samsamsharalat 2006). For both cases, symptoms were revealed to involve the peritendon and the authors noted that the pain was not associated with creatine kinase elevation or abnormal liver enzymes (Movahed and Samsamsharalat 2006). Movahed and Samsamsharalat (2006) concluded that with the high mechanical stress on the muscle around the tendons, implicated in their case report, the peritendon could be predisposed to developing symptoms, such as the ones observed in the two men (Movahed and Samsamsharalat 2006).

While the previous case reports describe symptoms of tendon pain, tenderness, and tendinitis, Carmont et al. (2009) describe the case of a 47 year old male who had been using simvastatin 40 mg/day for twelve weeks before both Achilles tendons ruptured. With a longstanding history of elevated serum lipoprotein level, which predisposed him to developing ruptures, he regularly rock climbed once a week (for 27 years) (Carmont et al., 2009). Preceding the bilateral rupture, there were no symptoms of tendon pain in either Achilles tendon (Carmont et al., 2009). An ultrasound exam revealed that for the right Achilles tendon, there was a complete rupture 4cm from the insertion while the left Achilles tendon had a proximal rupture of the Achilles tendon 8cm from its insertion (Carmont et al. 2009). The ultrasound scans also detected no lipid deposition in either Achilles tendon (Carmont et al., 2009). Carmont et al. (2009) determined that the benefits of eccentric loads (since eccentric exercises can normalize structure in tendinopathic tendons) were not enough to overcome the risk factors of hyperlipidemia and statin use for this patient.

While Carmont et al. (2009) described a case of bilateral Achilles tendon rupture Celik et al. (2012) describe a case of spontaneous bilateral quadriceps tendon rupture. The 56 year-old male had a BMI of 25.7 kg/m², weight of 92 kg, did not participate in regular sport activities or heavy labor (Celik et al., 2012). While he had normal cholesterol levels, he had been using a variety of statins for 14 years as a preventative measure for atherosclerosis; including rosuvastatin (Celik et al., 2012). Additionally, he did not report any experience of myalgias or the use of medications that can predispose individuals to developing tendon complications (Celik et al., 2012). MRI examination revealed bilateral complete ruptures of the quadriceps tendon located at the patellar insertions (Celik et al., 2012).

Lastly, Kearns and Singh (2016) describe a case of bilateral patellar tendon rupture. The 56 year-old male had no reported risk factors for tendon rupture and long-term statin treatment presented with bilateral knee pain after falling on ice (Kearns and Singh, 2016). It was noted that the patient was a long-time smoker and had a history of excessive alcohol use (Kearns and Singh, 2016). Surgical examination revealed bilateral complete rupture of the patellar tendon at the inferior pole attachment was observed (Kearns and Singh, 2016).

Based on these case reports, it is understandable why the relationship between statins and tendon health does seem plausible and why further research on the mechanisms underlying such a relationship as well as assessing the relationship in larger prospective studies has been undertaken. The location of tendon complications as well as the temporality of symptoms experienced by patients mentioned in these case reports lends credence to the possibility of statins negatively impacting tendon health. However, with the findings of the large Spöndlin et al. (2016) study as well as a previous systematic review, the strongest available evidence indicates no relationship between statin use and tendon complications (Teichtahl et al., 2016).

1.7 Ultrasound Tissue Characterization

Standard medical ultrasound technology is considered to be a very safe diagnostic technology, and it is currently being used for many patient groups (e.g. from fetus to adults) and for a variety of organ systems (e.g. brain, heart, ovaries, etc.). Additionally, medical ultrasonography results in real-time images and does not expose the patient to ionizing radiation. However, this imaging

technique has poor reproducibility since the resulting 2-D images are heavily dependent on the user (van Schie et al., 2010). Furthermore, since ultrasound images are the product of reflections and echoes of sound waves, only soft tissues (e.g. tendons, muscles, blood vessels, etc.) can be observed.

For these reasons, a novel ultrasound technology was recently developed: computerized Ultrasound Tissue Characterization (UTC). UTC (Smartprobe 10L5; Terason 2000, Teratech, USA) captures multiple transverse 2-D images every 0.2mm over a total length of 12cm. The 2-D images are then collected and stored on a computer where they are combined to produce a 3-D image. The 3-D image can then be used for tomographic visualization where the transverse, sagittal, and coronal planes can be viewed. With the use of UTC algorithms, the stability of the echo pattern, or tendon tissue structure, can be quantified (Cook and Purdam 2013; de Jonge et al., 2015). This results in the discrimination of four colours in the tendon, referred to as echo-types (Cook and Purdam 2013; de Jonge et al., 2015). Echo-type I is coloured green and based on comparison with histological evaluation of horse tendon tissue has been suggested to indicate intact and aligned tendon bundles (cite original study); echo-type II is coloured blue and is suggested to indicate waving or discontinuous tendon bundles, echo-type III is coloured red and is suggested to indicate interfering echoes from fibrillar components, and echo-type IV is coloured black and is suggested to indicate cellular components and fluid (de Jonge et al., 2015). This ultrasonography method, developed and validated on isolated horse SDFT, has been used in human studies (van Schie et al., 2001; van Schie et al., 2003; van Schie et al., 2009; van Schie et al., 2010) despite the fact that it has not yet been validated in humans. Additionally, it should be noted that the only parameter that can be modified by a user is the Window Size which relates

to how many scans are used to determine the proportion of echo-type patterns. With three options available to a user for Window Size (9, 17, and 25) the usage of these three sizes can yield different amounts of echo-type patterns for the same UTC scan (Barry and O'Neill, 2016). Furthermore, validation of which window size corresponds to tendon histology has not yet been done.

In its use with the horse, UTC has enabled researchers to monitor the effects of maximal exercise and healing of the superficial digital flexor tendon (van Schie et al., 2009; Bosh et al., 2011; David et al., 2012; Docking et al., 2012). With humans, UTC has been used in a variety of studies involving healthy athletes, individuals with Achilles tendinopathy and those with type 2 diabetes to name just a few (Rosengarten et al., 2015; de Jonge et al., 2015; Docking et al., 2015). Based on these studies, researchers found that in response exercise, a significant reduction in echo-type I patterns with a corresponding increase in echo-type II patterns were observed 2 days after exercise in a group of Australian football players with no history of tendinopathy (Rosengarten et al., 2015). These observable changes in echo-type patterns returned to baseline levels in 4 days after exercise (Rosengarten et al., 2015). Additionally, individuals with type 2 diabetes and Achilles tendinopathy were found to have decreased Achilles tendon organization (de Jonge et al., 2015; Docking et al., 2015). Recently, Wezenbeek et al. (2016) used UTC to study the structure of 70 normal Achilles tendons in physiotherapy students with no history of tendon injury. They found higher amounts of echo-type II at the insertion compared to midportion (Wezenbeek et al., 2016). Additionally, they found higher insertional and midportion echo-type II patterns in female tendons (Wezenbeek et al., 2016).

1.8 Objective

The objective of this cross-sectional study is to compare Achilles tendon morphology as revealed by UTC examination in individuals who have been taking statins for at least a year, compared to a control group; who have never taken statins.

1.9 Aims

This thesis aimed to answer the following questions:

1. With the use of UTC, can we see an increase in the cross-sectional area of the Achilles tendon in individuals who have been taking statins for at least a year, compared to those who have never taken statins?
2. With the use of UTC, can we see any signs of reduced collagen organization in the Achilles tendon in individuals who have been taking statins for at least a year, compared to those who have never taken statins?

1.10 Hypothesis

Based on the literature and our aims for this pilot, cross-section study, the following hypothesis was formed:

H: Individuals who have been taking statins for at least a year will have a greater cross-sectional area and reduced collagen organization (corresponding to a decrease in the proportion of echo-type I) in their Achilles tendons compared to controls who have never taken statins.

1.11 Significance of this Research

The relationship between statins and the risk tendinopathy or tendon rupture has been a topic of interest. Based on the literature, there is evidence supporting a potential relationship between statins and risk of tendinopathy from cell, animal and case studies (Movahed and Samsamsharalat 2006; Bruckert et al., 2005; Pullatt et al., 2007; de Oliveira et al., 2013, 2015; Kuzma-Kuzniarska et al., 2015). Additionally, with a handful of potential mechanisms, a relationship between statins and tendon injury does seem plausible (Deren et al., 2016). However, with the recent study conducted by Spöndlin et al. (2016), there is conclusive evidence that there is no association between statins and the risk of Achilles or biceps tendon rupture. The findings from this study will provide new information regarding the relative health of Achilles tendon tissue in statin users.

Chapter 2: Methods

2.1 UTC Reliability

A UTC reliability experiment was conducted to establish the UTC methodology and determine the user (Ad) reliability.

2.1.1 Subjects

10 healthy control subjects were recruited from April 15 to May 8 2015 from CHHM at Vancouver General Hospital. The rationale behind using young, healthy individuals for the reliability portion of this study was based on the need to determine the best UTC configuration as well as the user (Ad) reliability using an easily accessible group of participants. Inclusion criteria for this study were an age of 19 years and older and being able to give informed consent. Exclusion criteria were a BMI greater than 35kg/m^2 , the use of statins, oral corticosteroids or fluoroquinolones within the past year of enrolment in the study and the presence of a systematic inflammatory, thyroid or kidney disease as well as the presence of any condition which affects mobility. All of the patients had no history of Achilles tendinopathy or rupture. Written informed consent was obtained from all subjects before participation in the study for which ethics approval was obtained from the University of British Columbia (Clinical Research Ethics Board Certificate number is H14-01808). Analysis was conducted on all 10 subjects for which the demographics can be found in Table 3.1.

2.1.2 Study Visits

Each subject attended two study visits (at the same time of day, a day apart). Weight and height were measured at the first (of two) study visit to calculate BMI. Additionally, subject's self-reported dominant leg, age and physical activity before the study visit were recorded. All subjects were told to avoid moderate to vigorous physical activity before both study visits. One examiner (Ad) collected four UTC scans at each study visit, resulting in a total of eight scans from two visits, for each subject.

2.1.3 UTC Configuration

Through various trials we assessed the UTC images that resulted from different positions of not only the user, but the subject. In doing so, the user (Ad) observed that the position of the subject and UTC tracker were important variables to control for. Additionally, it was observed that a system that a system where the UTC tracker was not help by the user (Ad) when taking a scan was needed in order to ensure that movement of the tracker was eliminated. All this lead to the development of the following UTC configuration.

Subjects were lying prone on an examination bed with their feet placed on a foot and ankle stabilizer (FAS) (Figure 2.1) (5° increments from 0° - 20°) needed to achieve a 90° alignment of

the Achilles tendon with the UTC transducer and allowed us to standardize the position of each subject's foot. The FAS degree for each subject was recorded.

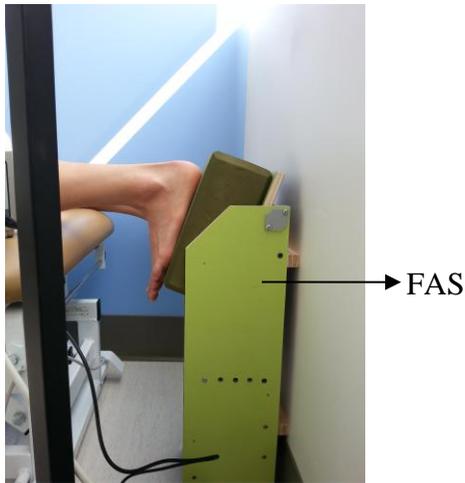


Figure 2.1 Placement of subject legs. The foot of each subject was placed on the FAS (bright green) before placing the UTC machine on the leg to acquire a scan.

The UTC was held by an arm (with 360° of freedom) which was secured to a pole to prevent any movement of the UTC while scanning (Figures 2.2A-C). A 10MHz fixed liner-array transducer (Terason 2000, Teratech, USA) moved along the long axis of the Achilles tendon.

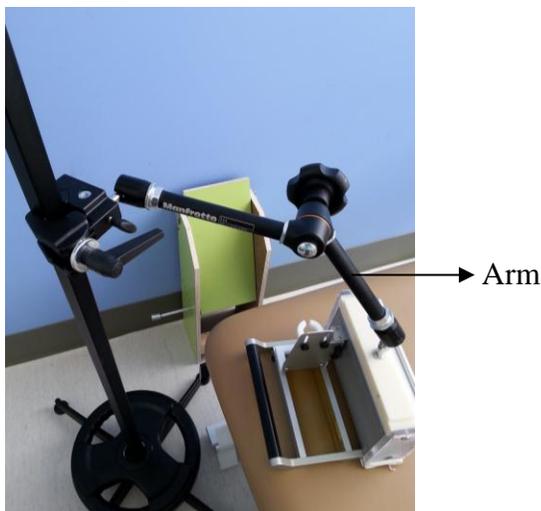
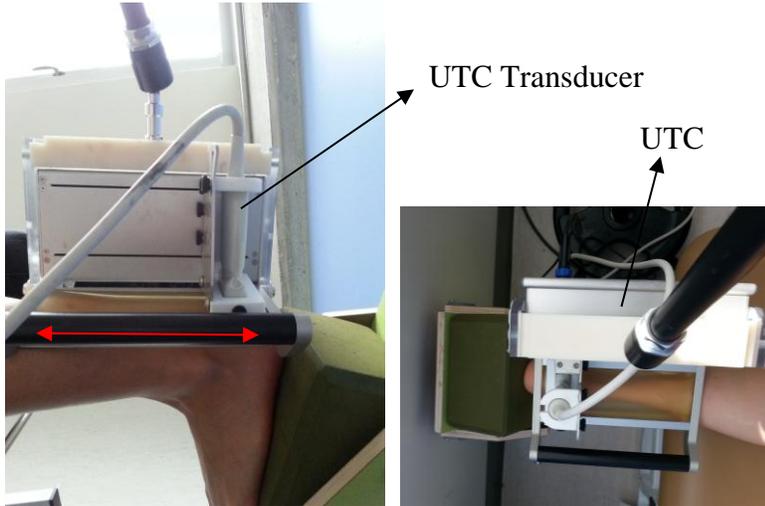


Figure 2.2A UTC Setup. The arm held the UTC, fixing it in place with the use of a weighted pole. The arm prevented the user (Ad) from holding the UTC, thus preventing movement, while the scan was taken.



Figures 2.2B and 2.2C UTC Placement. The arm, allowed user (Ad) to adjust the position of the UTC for each subject. Additionally, the UTC transducer was always places at the subject's heel to capture the insertion as well as get better quality images. Right: Distance UTC Transducer moves. The red arrow shows how far the transducer moves. For this UTC machine, the total length is 12cm.

2.1.4 UTC Analysis

Transverse 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 data block. With this 3-D data block, tissue characterization (differentiating between different tissues), quantification of architecture, and integrity of collagenous matrix was determined. The 3-D stability of the echo pattern, intensity and distribution over contiguous transverse images was analyzed and quantified by means of custom-designed algorithms (UTC 2012, UTC imaging). This resulted in the discrimination of four echo-types. The developers of UTC technology have suggested that echo-type I represents intact and aligned tendon bundles (coloured green in processed images), echo-type II represents discontinuous or waving tendon bundles (coloured

blue in processed images), echo-type III represents fibrillar components and echo-type IV represents cellular and fluid components in amorphous tissue (coloured black in processed images) (UTC Imaging). However, a recent study found that when window size decreased, a reduction in echo-type I percentage was observed, while the percentages of echo-types II-IV increased (Barry and O'Neill, 2016). The authors noted that the echo-types for each window size were highly correlated resulting in high internal validity of UTC (Barry and O'Neill, 2016). The following UTC setting was used: Window Size = 25. All images were de-identified to ensure the examiner (Ad) was blinded during analysis. The examiner determined the landmark (2cm from the calcaneal insertion) in the sagittal plane. The tendon border was outlined (referred to as a contour) at 2mm proximal and distal to the landmark. The two contours were interpolated to create a 4mm region of interest used for tissue characterization. Proportions of the four echo-types were calculated within the region of interest.

2.1.5 Validity of UTC

UTC scans of the Near Field Ultrasound Phantom (Model 050, Computerized Imaging Reference Systems, INC, Virginia, USA) were taken to determine the calibration for cross-sectional area measurements (Figure 2.3).

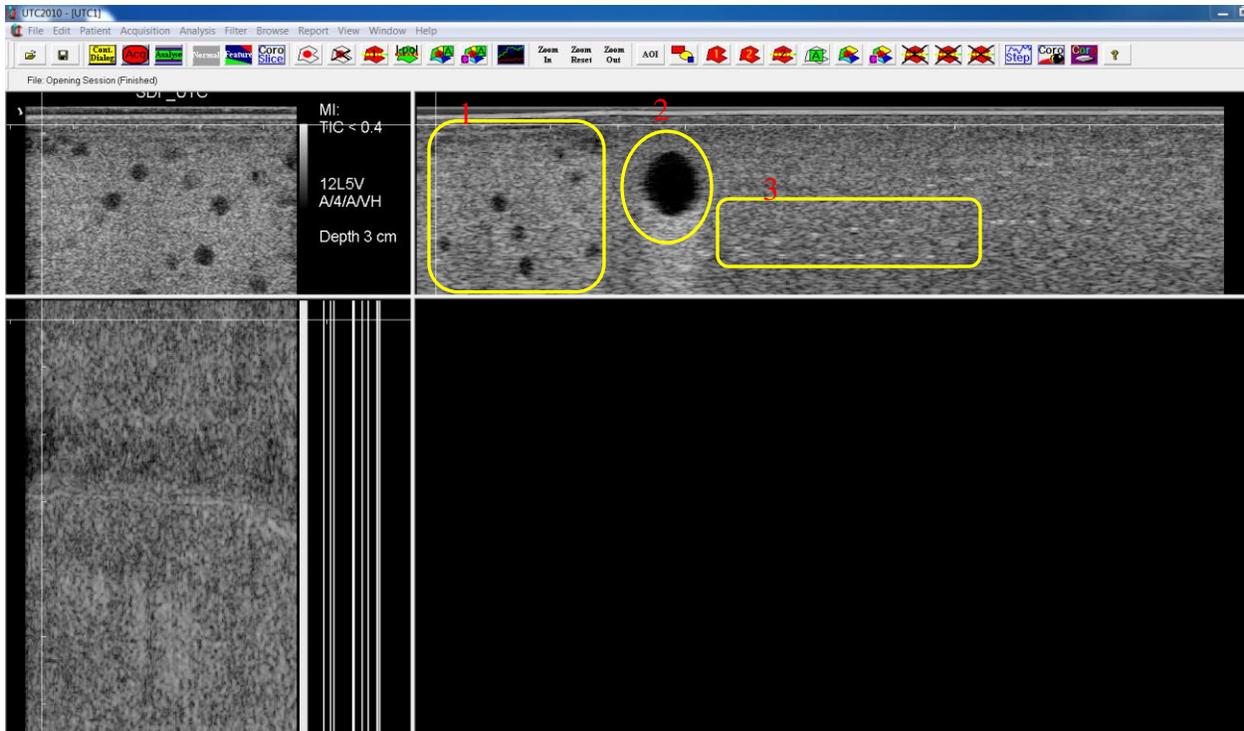


Figure 2.3 UTC scan of the Ultrasound Phantom. In this image, 3 sections of the Phantom can be observed: 1. 5mm hyperechoic masses, 2. 10mm anechoic cylinder, and 3. axial resolution target.

2.1.6 Calibration for Tendon Cross-sectional Area Measurements

In order to measure the CSA of the Achilles tendons, the calibration settings had to be determined. With UTC scans of the Ultrasound Phantom, the pixel aspect ratio (PAR) and calibration scale were determined.

Once UTC images were saved, Image J was used to analyze the images. Using the combination cyst-like/hyperechoic masses (section 1 in Figure 2.4), the pixel aspect ratio (PAR) was determined. Using the circularity function in Image J, the results showed that the PAR was 1.0 (Figure 2.4).

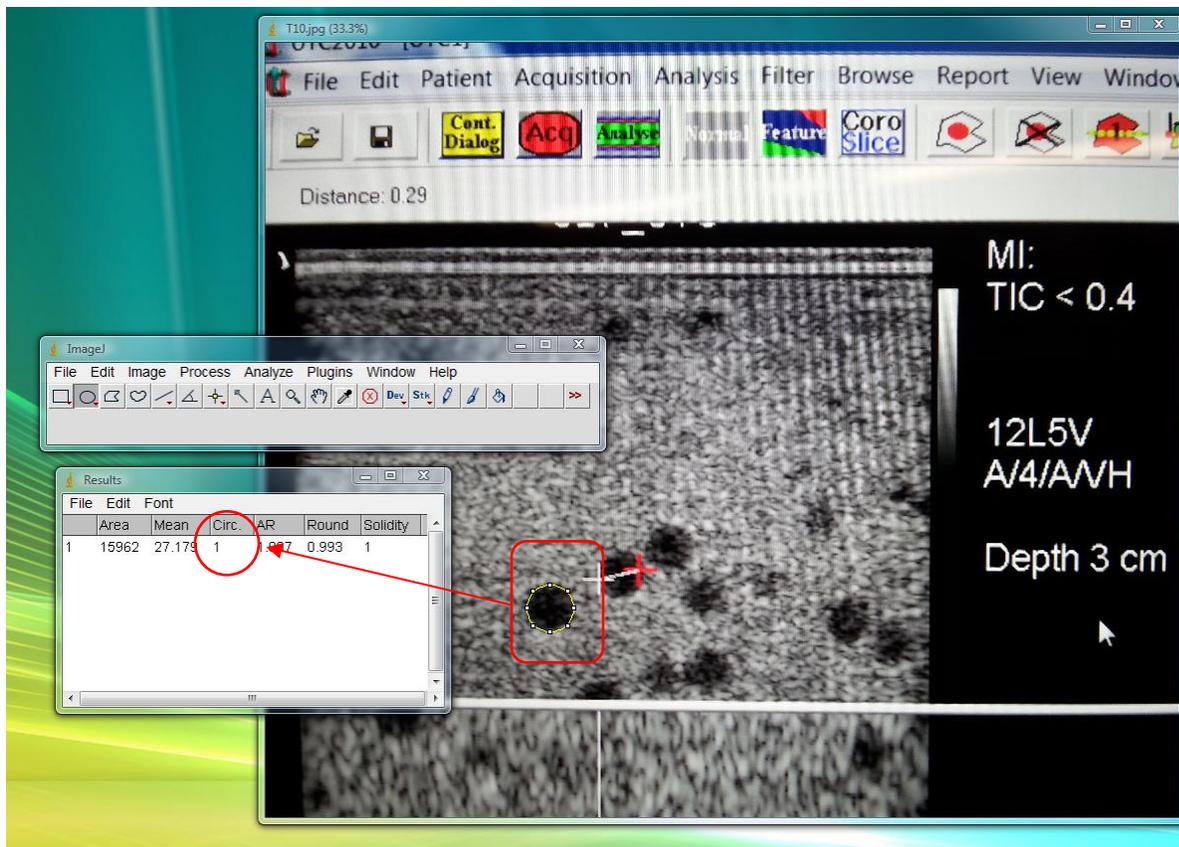


Figure 2.4 PAR Determination. A scan of the 5mm masses in the Ultrasound Phantom is shown above. The UTC image of the Phantom scan was analysed in Image J. In Image J, circularity values range from 0 (infinitely elongated polygon) to 1 (perfect circle) (Ferreira & Rasband, 2012).

Following this, the examiner (Ad) used the Axial Resolution Target, composed of a series of six pairs of nylon wires 0.1mm in diameter, to determine the calibration scale (Figure 2.5). The Straight line tool in ImageJ was used to measure the distance in between the wires which was then compared to the known distances provided by CIRS, Inc. of the Ultrasound Phantom. Analysis resulted in measurements that were within 0.2mm of the known values. Additionally, the calibration scale was determined to be 9.167 pixels/mm.

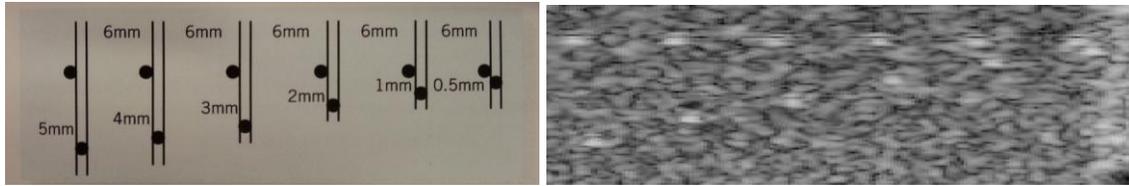


Figure 2.5 Axial Resolution Target. Left: Diagram of the axial resolution target provided by CIRS, Inc. of the Ultrasound Phantom with distance measurements. Right: UTC image of the axial resolution target. The resolution of the UTC device is at least 0.5mm (the highest resolution typically examined during ultrasound image calibration).

2.1.7 Tendon CSA

Utilizing the calibration scale developed according to the method described above, two CSA measurements were taken of each de-identified image using the Freehand tool in Image J.

2.2 Study of Statin Users and Controls

2.2.1 Study Design

This was a cross-sectional study, to use UTC to determine effects of statins on the Achilles tendon in individuals who have been taking statins for at least a year, compared to those who have never taken statins. Inclusion criteria for this study were an age of 19 years and older and being able to give informed consent. In order to get a clear understanding on the how statins might affect the Achilles tendon, we did not include any subjects who had taken any medications (such as corticosteroids, fluoroquinolones, and antibiotics) or had any condition which would impact Achilles tendon health (including diabetes, gout, rheumatoid arthritis, and a previously torn Achilles tendon). In addition to these exclusion criteria, we also did not include any subjects

whose BMI was over 35 kg/m² or had peripheral edema in their lower extremities since we were utilizing UTC which has a depth of 3cm. Similarly, because we were taking ultrasound scans of both Achilles tendons, we did not include subjects who had either pain conditions (such as fibromyalgia) or mobility issues (the use of a cane or walker) which would influence tendon health. Written informed consent was obtained from all subjects before participation in the study for which ethics approval was obtained from the University of British Columbia (Clinical Research Ethics Board Certificate number is H14-01808). Enrolled subjects were assigned a study ID and were matched to either a statin or control subject. Once a matched pair was formed, the examiner (Ad) did not modify or re-match any subjects (unless the pair had to be changed for gender balancing).

2.2.2 Recruitment

Upon receiving ethics approval from the University of British Columbia (Clinical Research Ethics Board Certificate number is H14-01808), recruitment for this study ran from May 10 2015 till February 17 2017.

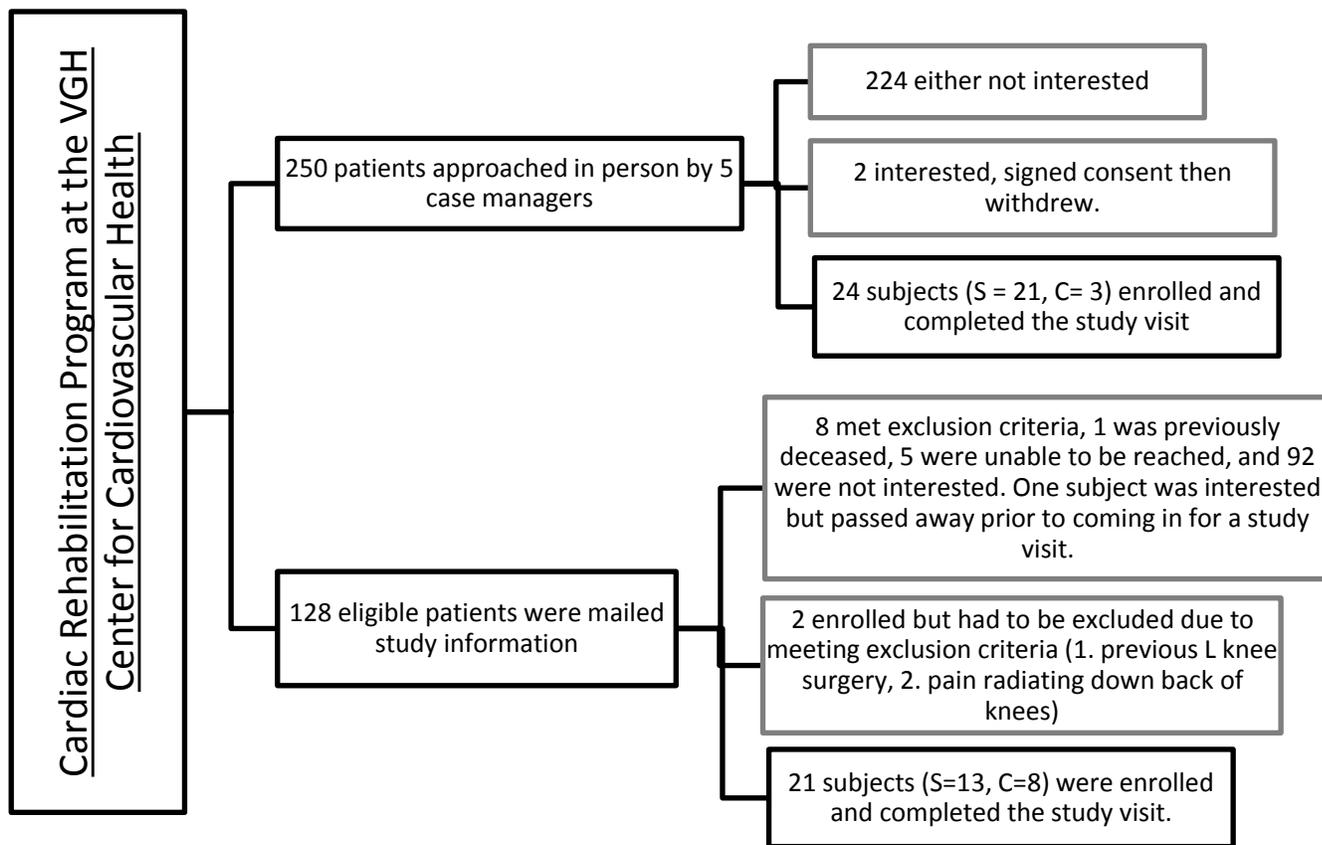


Figure 2.6A Initial recruitment method through cardiac rehabilitation program.

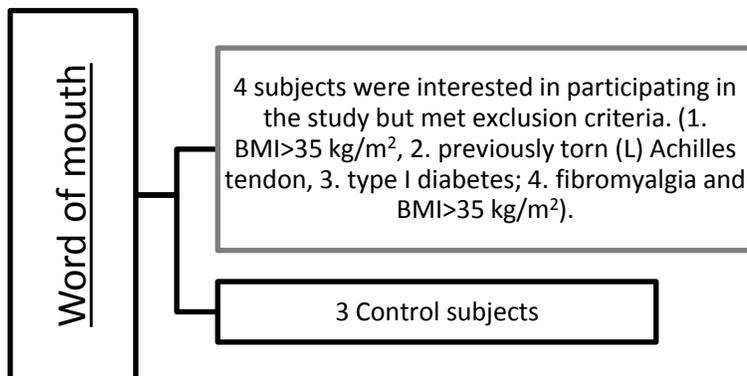


Figure 2.6B Initial recruitment method for controls through word of mouth.

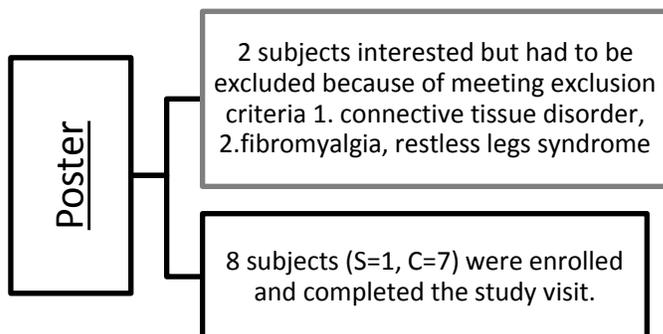


Figure 2.6C Initial recruitment method for controls through poster.

Initially, Statin and Control subjects were recruited from the Cardiac Rehabilitation Program at the VGH Center for Cardiovascular Health with the help of the case managers at the Centre. Due to a low recruitment rate of Control subjects, the recruitment process was modified to include

word of mouth and a recruitment poster placed at various businesses around Vancouver. This recruitment method resulted in Statin and Control groups having unequal numbers of males and females. Thus, in order to eliminate an imbalance of males and females in the statin and control groups, a second recruitment phase was undertaken to increase the recruitment of healthy male controls (19 more control men were needed in order to create gender-balanced groups). During this second phase, the primary method of recruitment was posting the recruitment poster on Craigslist (under the Community - Volunteers and Jobs - Nonprofit sector and Education sections) so that potential eligible subjects could be recruited in a timely method. Additional avenues of recruitment were also implemented and included the distribution of the recruitment poster to local organizations whose members included healthy men over the age of 50 (the target age group, based on the demographic of the Statin group). Local organizations that helped distribute the recruitment poster to their members included: the Vancouver Metro Soccer League, the South Vancouver Neighborhood House, the Seniors Hub Program, the South Granville Seniors Centre, and the Vancouver Prime Timers. In addition, recruitment also took place via word of mouth to staff and students at CHHM. This included emailing past participants from the Men on the Move study and speaking to a FitBrain class. Men on the Move and FitBrain are studies based out of CHHM. With help from CHHM Staff (Paul Drexler) the Principal Investigator (Dawn Mackey) for the Men on the Move study was contacted to ask if participants (who noted on their Men on the Move consent forms if they would like to be contacted about future research) could be contacted since they matched the target demographic for which she granted permission. In addition, the research manager (Lisanne Ten Brinke, MSc) and the Principal Investigator (Dr. Teresa Liu-Ambrose) for the FitBrain study were contacted to ask if I could speak to their participants since they were the target demographic for which they

granted permission for me to speak to their participants. These recruitment methods to find additional control subjects are described in Figures 2.7A-C below.

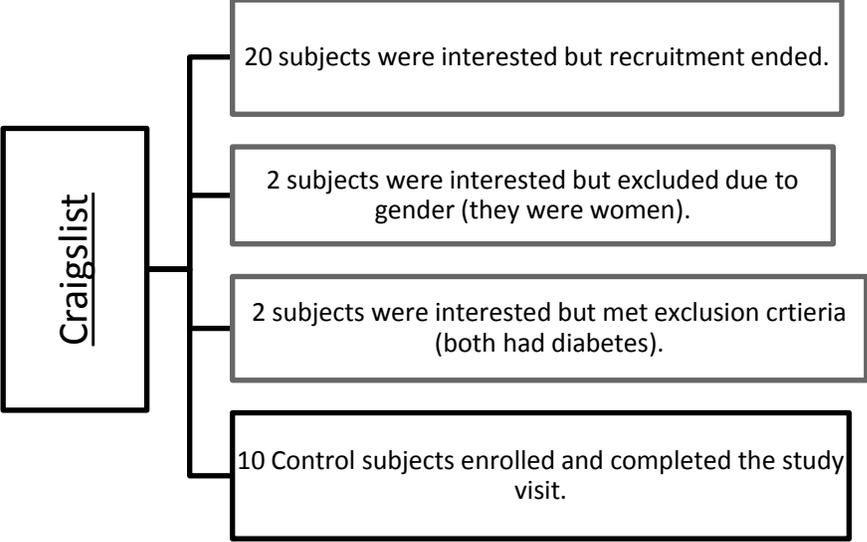


Figure 2.7A Recruitment method for additional control subjects - Craigslist.

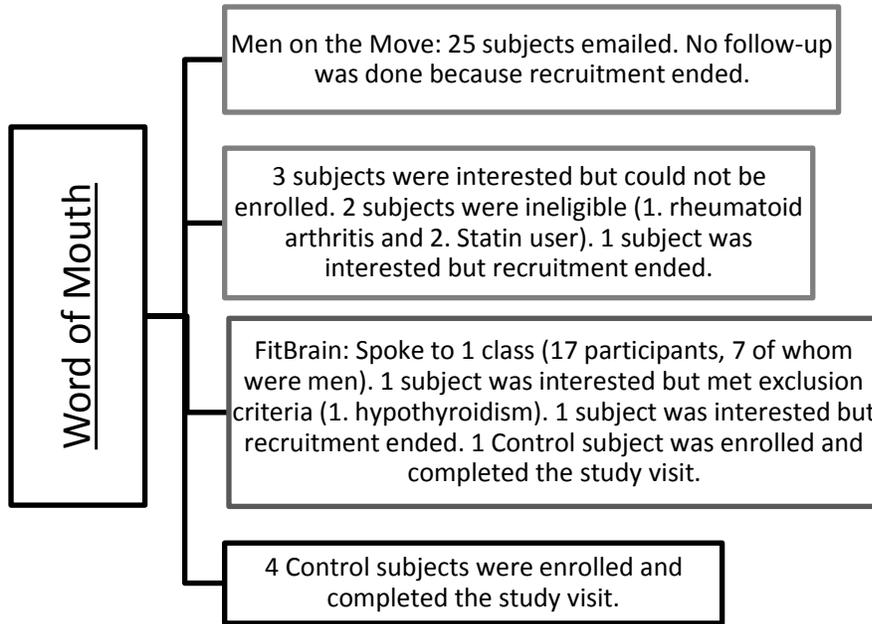


Figure 2.7B Recruitment method for additional control subjects - word of mouth.

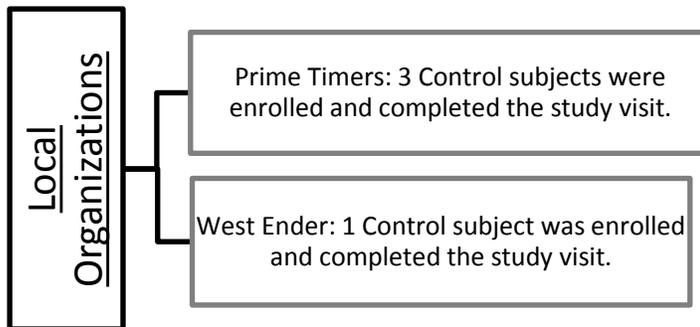


Figure 2.7C Recruitment method for additional control subjects - local organizations.

Written informed consent was obtained from all subjects before participation in the study for which ethics approval was obtained from the University of British Columbia (Clinical Research Ethics Board Certificate number is H14-01808).

2.2.3 Study Visits

Similar to the methodology used for the reliability experiment, each subject attended one study visit. Weight and height were measured at study visit to calculate BMI. Additionally, subject's self-reported dominant leg, age and physical activity (IPAQ questionnaire) were recorded at the study visit. One examiner (Ad) collected four UTC scans at the study visit. Furthermore, cholesterol values from within the past year (and closest to the study visit date) were collected for subjects in the Statin group along with length of statin use, and a list of current medications. Subjects were also asked if they had experienced any muscle pain they could not attribute to exercise or injury.

2.2.4 UTC Analysis

Similar to the reliability experiment, the following UTC setting was used: Window Size = 25. All images were de-identified to ensure the examiner (Ad) was blinded during analysis. The examiner determined the landmark (2cm from the calcaneal insertion) in the sagittal plane. The tendon border was outlined (referred to as a contour) at 2mm proximal and distal to the landmark. The two contours were interpolated to create a 4mm long region of interest used for tissue characterization. Proportions of the four echo-types were calculated within the region of interest.

2.2.5 Tendon CSA

Utilizing the methodology established from the reliability experiment, de-identified images were analyzed using Image J to determine the tendon CSA. Utilizing the same calibration scale from the reliability experiment, two measurements of the CSA were taken for each de-identified image using the Freehand tool in Image J.

2.3 Statistical Analysis

To compare the percentage of Type I echo values and CSA between statin users (n=33) and controls (n=33), student's independent t-test was performed following examination of the data for skewness and outliers. After determining that there was no significant impact of statin use on either variable, we conducted Pearson correlation analyses to determine whether age or BMI were significantly correlated with the percentage of echo-type I values or CSA, and Spearman correlation analysis to see if IPAQ category score correlated with the percentage of echo-type I values or CSA. This was done by me, Agnetha de Sá, under the supervision of Dr. Alexander Scott.

Chapter 3: Results

3.1 Reliability Experiment

Demographics for the 10 subjects can be found below in Table 3.1

Subject Demographics Variable	All Subjects (n=10)
Age (yr), mean \pm SD	28 \pm 4
Male	1
Female	9

Table 3.1. Reliability Experiment Subject Demographics.

While most UTC papers publish the minimum detectable change (MDC), the intraclass correlation coefficient (ICC) was deemed to be a measurement better suited to this study. MDC is sensitive to measurements taken of an individual over time, whereas ICC is typically used when assessing the ability of a measure to detect differences between groups. Table 3.2, below, shows the tendon CSA and echo-type I values for each subject for both visits. The interclass correlation coefficient (ICC) from the echo-type I measurements was 0.90. Combining the echo-types 1-3 measurements, the ICC was the same (0.90). Additionally, the ICC result of tendon CSA was >0.80 (right leg = 0.87).

Visit 1								Visit 2							
Subject	Side	CSA		Echo-types				Side	CSA			Echo-types			
		Replicate	Value	Type I	Type II	Type III	Type IV		Replicate	Value	Type I	Type II	Type III	Type IV	
1	L	1	71.167	0.57	0.41	0.016	0.0031	L	1	60.278	0.63	0.32	0.033	0.02	
		2	71.631						2	60.432					
	R	1	49.615	0.7	0.28	0.0054	0.0066	R	1	60.873	0.78	0.2	0.0092	0.0085	
		2	49.698						2	60.528					
2	L	1	41.724	0.46	0.49	0.043	0.0093	L	1	47.139	0.53	0.46	0.0089	0.0046	
		2	41.105						2	47.651					
	R	1	42.545	0.46	0.47	0.061	0.015	R	1	54.054	0.28	0.57	0.13	0.02	
		2	42.724						2	54.018					
3	L	1	51.328	0.34	0.63	0.025	0.0044	L	1	61.67	0.3	0.52	0.16	0.026	
		2	51.114						2	61.373					
	R	1	43.569	0.5	0.5	0.0057	0.00075	R	1	50.781	0.51	0.44	0.038	0.012	
		2	43.26						2	50.483					
4	L	1	41.248	0.77	0.22	0.0052	0.003	L	1	45.318	0.76	0.22	0.0088	0.0075	
		2	41.486						2	45.663					
	R	1	51.435	0.77	0.22	0.0043	0.0025	R	1	49.341	0.75	0.24	0.0038	0.0014	
		2	51.911						2	49.377					
5	L	1	71.726	0.74	0.25	0.0058	0.006	L	1	72.071	0.74	0.26	0.0026	0.00081	
		2	71.643						2	72.262					
	R	1	66.204	0.75	0.25	0.002	0.00018	R	1	93.564	0.76	0.23	0.002	0.0039	
		2	66.085						2	93.41					
6	L	1	70.703	0.73	0.25	0.014	0.0083	L	1	72.357	0.82	0.17	0.0025	0.0016	
		2	70.953						2	71.143					
	R	1	87.328	0.81	0.18	0.0032	0.0061	R	1	66.109	0.74	0.26	0.0038	0.0031	
		2	87.412						2	66.228					
7	L	1	59.29	0.66	0.32	0.011	0.0019	L	1	64.038	0.69	0.3	0.006	0.0017	

		2	59.219						2	64.478				
	R	1	78.962	0.7	0.3	0.0021	0	R	1	62.372	0.73	0.27	0.002	0.00054
		2	78.653						2	62.384				
8	L	1	47.937	0.69	0.28	0.022	0.014	L	1	42.26	0.63	0.35	0.015	0.0071
		2	47.651						2	42.153				
	R	1	50.912	0.73	0.27	0.0016	0.00026	R	1	47.27	0.72	0.27	0.0045	0.0015
		2	50.579						2	47.282				
9	L	1	50.257	0.63	0.37	0.0036	0.0018	L	1	47.853	0.54	0.46	0.0043	0.00058
		2	50.281						2	48.496				
	R	1	46.104	0.56	0.44	0.0022	0.0022	R	1	57.047	0.58	0.39	0.026	0.0033
		2	46.08						2	57.088				
10	L	1	48.817	0.74	0.26	0.0042	0.0015	L	1	46.865	0.78	0.22	0.0032	0.0042
		2	48.222						2	46.556				
	R	1	44.414	0.82	0.16	0.0077	0.0072	R	1	45.33	0.76	0.21	0.016	0.015
		2	44.212						2	45.152				

Table 3.2 Tendon CSA and echo-type results for visits 1 and 2.

3.2 Study of Statin Users and Controls

For this study, there were two response variables: tendon CSA and echo-type I values. Analysis was done on 66 subjects (n=33 statin users, n=33 controls) for which the demographics can be found below in Table 3.3.

Variable	All Subjects (n=66)	Statin Subjects (n=33)	Control Subjects (n=33)
Age (yr), mean \pm SD	66 \pm 9	69 \pm 10	63 \pm 8
Male	58	29	29
Female	8	4	4

Table 3.3 Study subject demographics.

3.2.1 Echo-type I

Every subject in the study group had 4 UTC scans taken (2 scans/leg), resulting in 4 UTC echo-type I values for each subject. On the advice of the statistician, the average of these four values was used in subsequent analyses with the covariates, such that each individual was represented by one value in the analysis. The mean echo-type I value of the statin users was 0.67 (0.12), and the mean echo-type I value of the control group was 0.71 (0.10) (Figure 3.1).

Since the statin and control groups contained equivalent numbers of men and women and since they displayed similar levels of physical activity, and because statin use had no measurable effect on either response variable, simple correlation analyses were done on the data. Specifically, we were interested in how the echo-type I results correlated with BMI, age, and activity level (IPAQ category score). These can be seen in scatterplots (Figures 3.2-3.4) below.

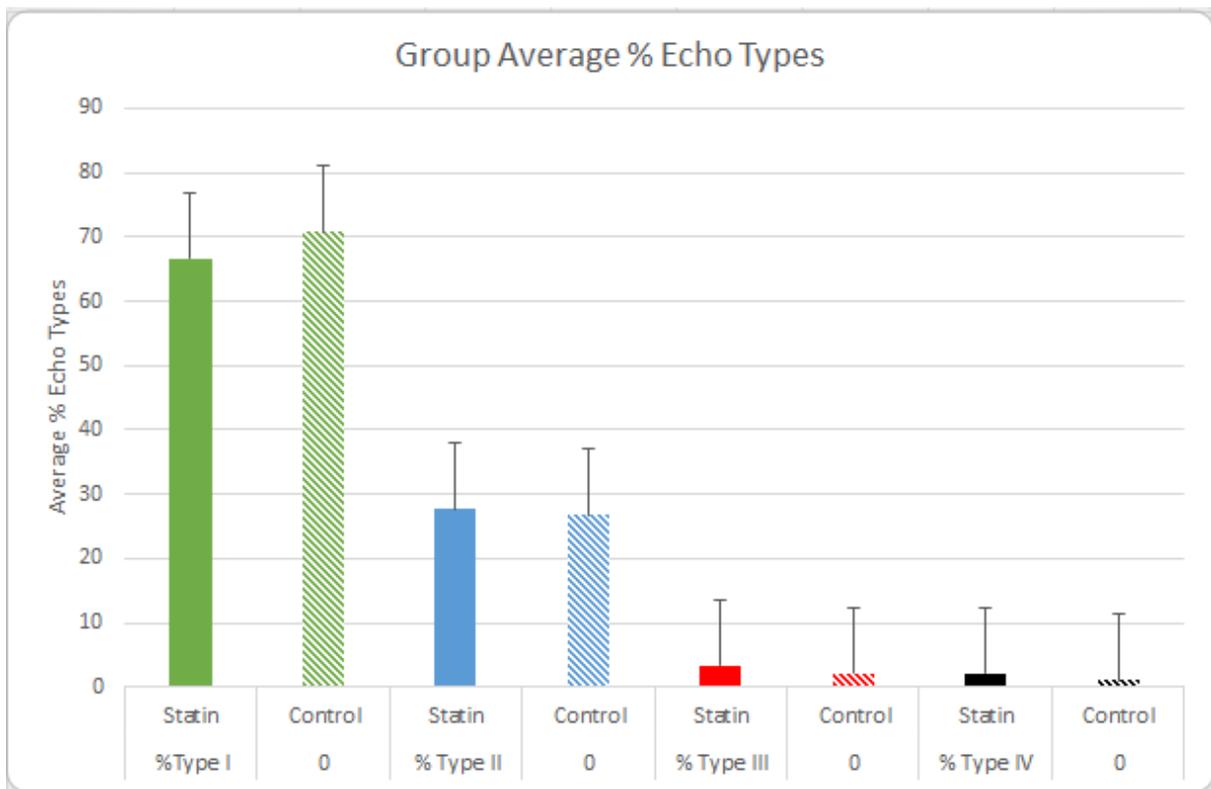


Figure 3.1 Bar graph of the per cent of each echo-type pattern for Statin and Control groups.

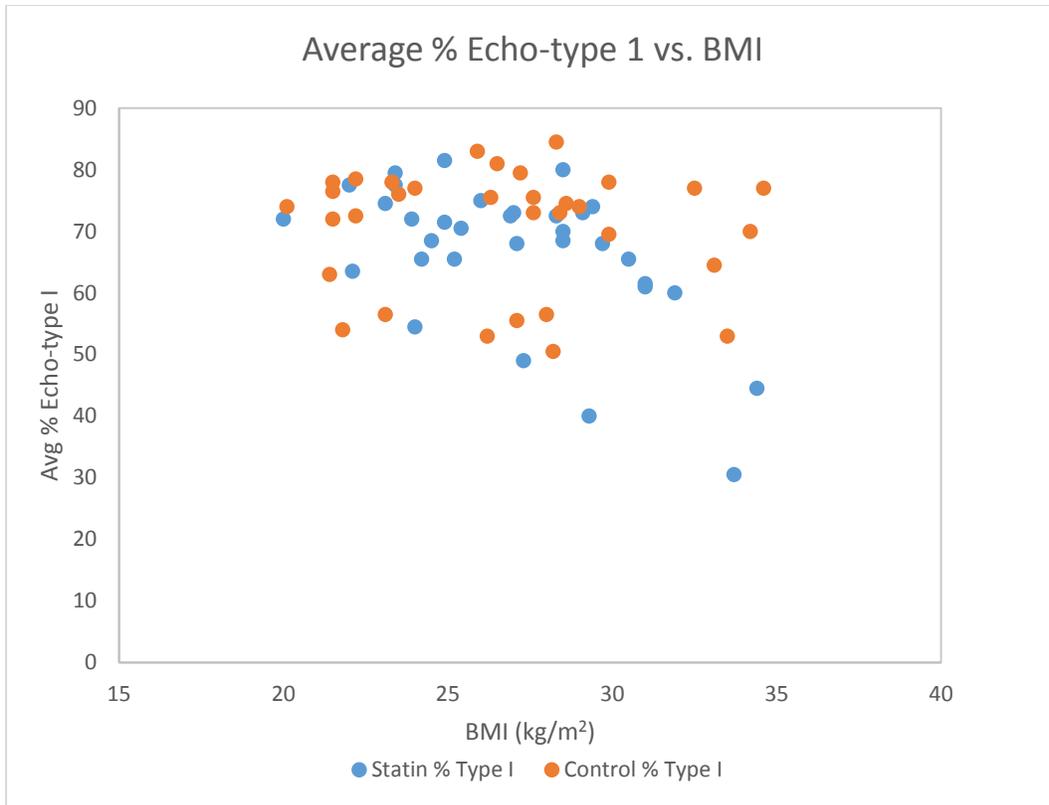


Figure 3.2 Scatterplot of the average echo-type I percentages vs. BMI. The color of a point indicates whether the subject used statins.

With $r = -0.31$, the relationship between BMI and average per cent of echo-type I is moderate but the result is significant ($p = 0.012$) at $p < 0.05$.

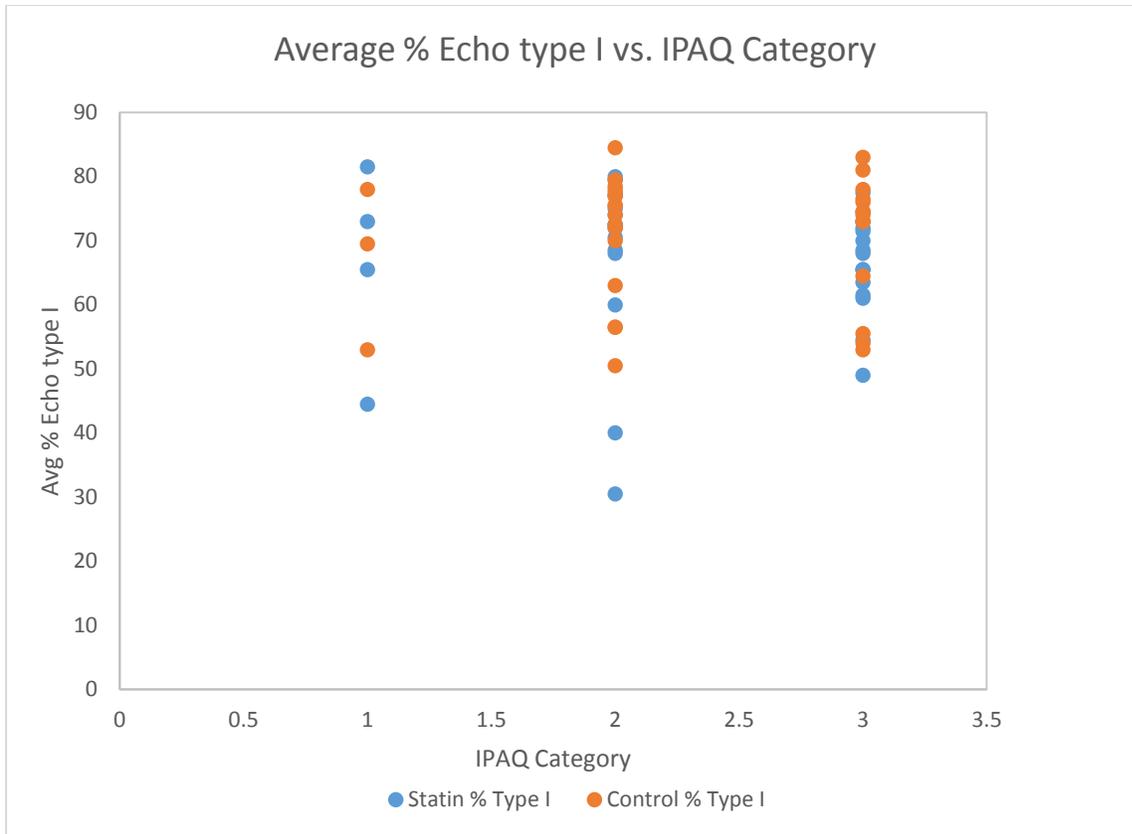


Figure 3.3 Scatterplot of the average echo-type I percentages vs. IPAQ category. The color of a point indicates whether the subject used statins.

With $r = -0.085$ the relationship between IPAQ category and average per cent of echo-type I is not statistically significant ($p = 0.50$) at $p < 0.05$.

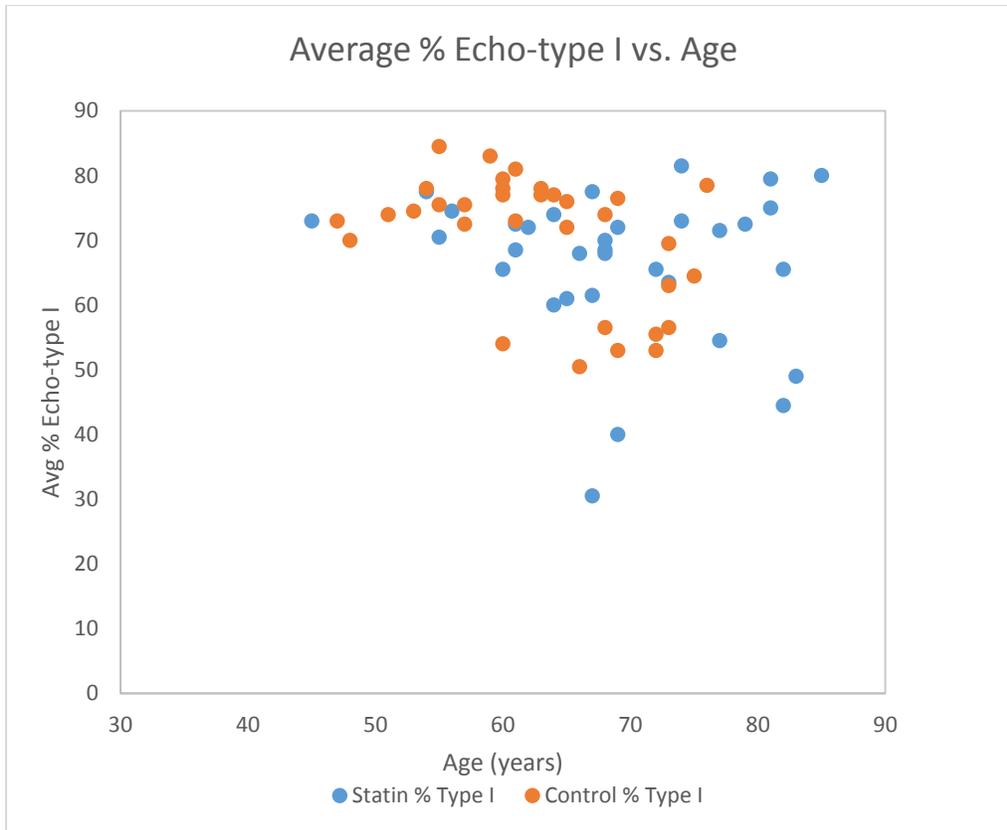


Figure 3.4 Scatterplot of the average echo-type I percentages vs. age. The color of a point indicates whether the subject used statins.

With $r = -0.31$, the relationship between age and average per cent of echo-type I is moderate but the result is significant ($p = 0.012$) at $p < 0.05$.

3.2.2 Tendon CSA

Again, using the same methodology as the reliability study, for each UTC scan taken for a subject, there were 2 Achilles tendon CSA measurements done. This means that with 4 UTC scans/subject there were 8 Achilles tendon CSA measurements for each subject. Again, based on the statistician's advice an average Achilles tendon CSA was calculated for each subject. It was this average that was used in subsequent analyses with the covariates.

	Statin	Control
Minimum value (mm ²)	39.23	38.16
Maximum value (mm ²)	84.85	84.52
Mean	59.74	59.92
Median	60.02	60.34

Table 3.4 Achilles tendon CSA results.

Since the statin and control groups were gender balanced, simple correlation analyses were done on the data. Specifically, we were interested in how the tendon CSA results correlated with BMI, age and IPAQ category. These can be seen in the scatterplots (Figures 3.5-3.7) below.

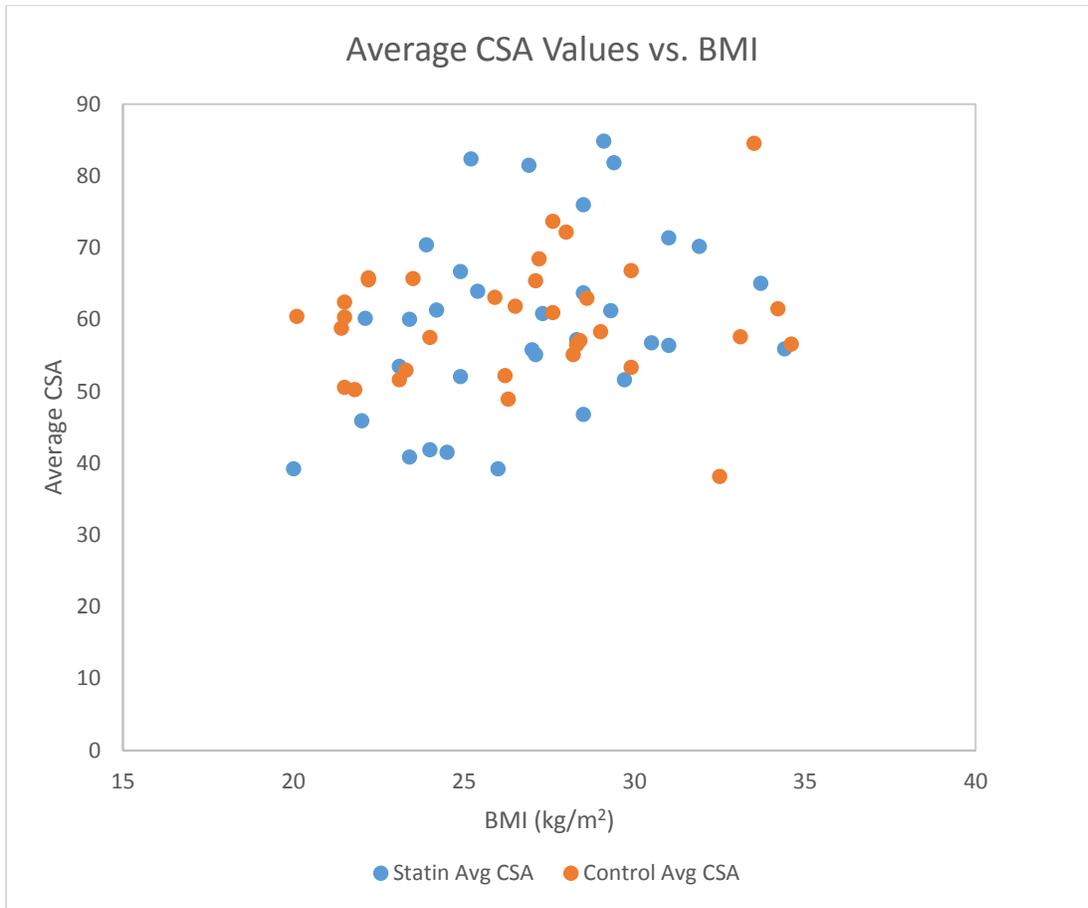


Figure 3.5 Scatterplot of the average Achilles tendon CSA vs. BMI. The color of a point indicates whether the subject used statins.

With $r = 0.23$, the relationship between BMI and average tendon CSA is not statistically significant ($p = 0.058$) at $p < 0.05$.

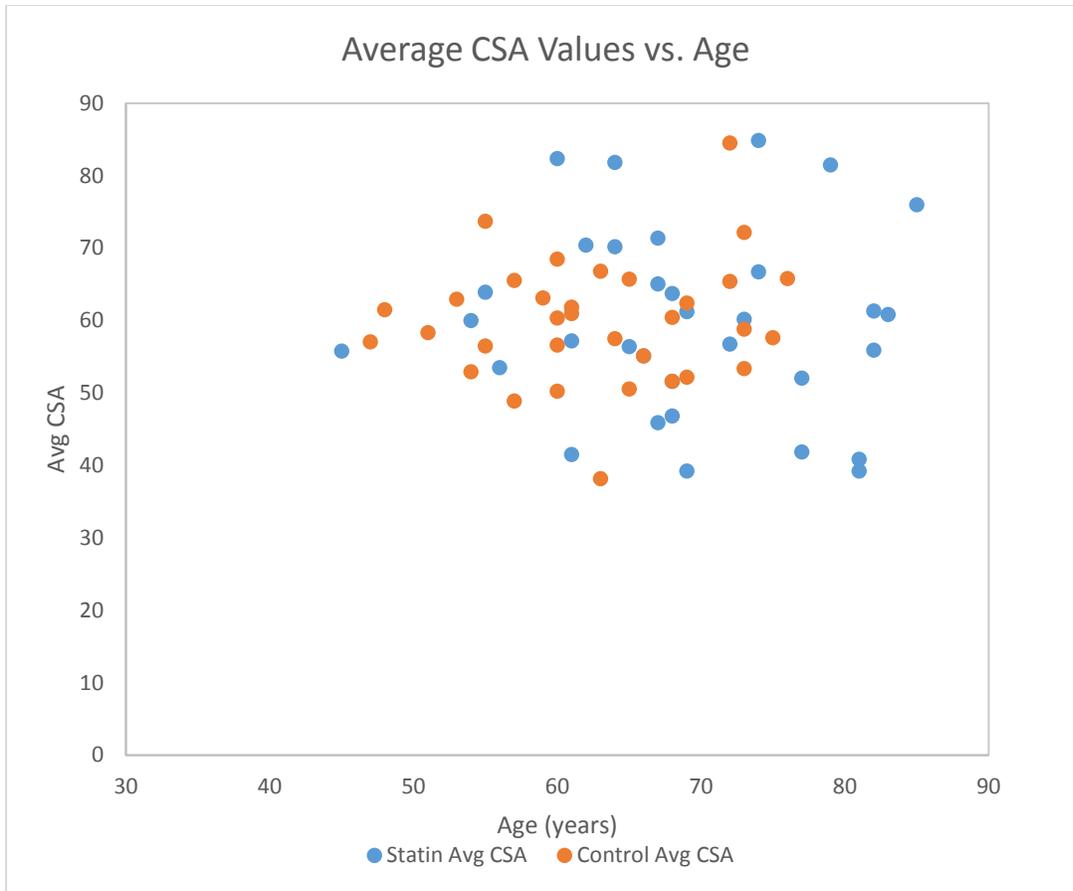


Figure 3.6 Scatterplot of the average Achilles tendon CSA vs. age. The color of a point indicates whether the subject used statins.

With $r = 0.013$ the relationship between age and average tendon CSA is not statistically significant ($p = 0.92$) at $p < 0.05$.

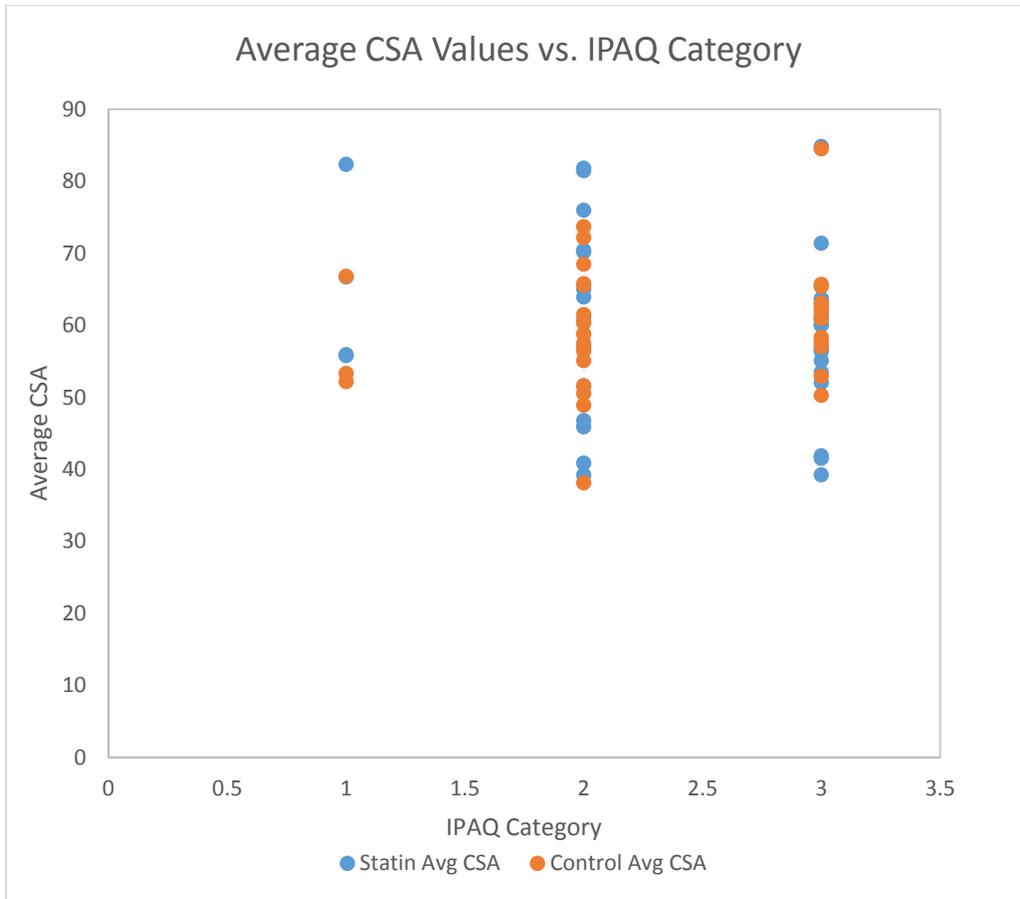


Figure 3.7 Scatterplot of the average Achilles tendon CSA vs. IPAQ category. The color of a point indicates whether the subject used statins.

With $r = -0.035$ the relationship between IPAQ category and average tendon CSA is not statistically significant ($p = 0.79$) at $p < 0.05$.

Chapter 4: Discussion

This was a cross-sectional pilot study with 66 subjects. All of the statin users (n=33) stated that they were consistently taking their statin (adherent to treatment) and had been on their statin for at least a year. All of the control subjects (n=33) had never taken a statin medication.

4.1 Comparison between Statin Users and Controls

In July 2016, Wezenbeek et al. (2016) published the results of their study determining what normal Achilles tendon structure is with using UTC. With a sample of 70 active students (mean age of 17.9 ± 0.5 years) with no history of Achilles tendon injuries, higher amounts of echo-type II were found in females, at both the insertion and midportion, compared to males. Although the ages of the female subjects in our study at the time were much higher (mean age 64 ± 7 years in the age-matched group), the results of Wezenbeek et al. (2016) introduced a limitation (a requirement for gender balance in the control group) that we did not initially foresee when recruitment for this study started. In order to address this limitation, a second round of recruitment was undertaken in order to account for any gender effects with using UTC.

We found that the statin users and the gender balanced controls had similar echo-type (I – IV) patterns which can be seen in Figure 3.1. There was no effect of IPAQ category ($r = -0.085$, $p = 0.50$) on echo-type I patterns. However, we found a moderate but significant effect of BMI on average echo-type I patterns ($r = -0.31$, $p = 0.012$) at $p < 0.05$ meaning that higher BMI values corresponded with lower echo-type I. In addition to the significant effect of BMI, we also found

a moderate but significant effect of age on echo-type I patterns ($r = -0.31$, $p = 0.012$). Similarly with BMI, Figure 3.4 shows that with increasing age, echo-type I levels decrease. Thus, the echo-type I results in this study suggest an asymptomatic deterioration in Achilles tendon structure associated with increased age and BMI. These associations between increasing BMI and age (separately) and poorer tendon organization support findings of previous work; indicating that elevated BMI and increasing age are risk factors for developing tendon pathology (Gaida et al., 2009; Rees et al., 2009; Jonely et al., 2016). The results we found for the effect of age on the Achilles tendon structure may support previous observations that aging tendon demonstrates decreases in energy storage and elasticity (Jonely et al., 2016). Additionally, the decline in echo-type I values we found in aging tendon may support previous findings of age-related structural changes such as an increase in advanced glycation end products and a decrease in collagen content seen in tendon which could predispose older individuals to developing injuries (Coupe et al., 2009; Jonely et al., 2016). The results we found for the effect of BMI on Achilles tendon structure is supported by work done by Abate et al. (2010) and Biancalana et al. (2012). In their study, Abate et al. (2012) observed a significant prevalence of ultrasound abnormalities in asymptomatic overweight male runners. Abate et al. (2012) also observed a significant increase in Achilles tendon thickness in overweight non-runners. Based on their findings, the authors concluded that overweight runners could be predisposed to developing tendon injuries due to factors such as increased stress and an unfavourable environment for tendon repair (Abate et al., 2012). In their study on the deep digital flexor tendon structure in obese Zucker rats, Biancalana et al. (2012) found that the obese rats had decreased collagen integrity compared to lean rats which the authors concluded could lead to tendon pathology. However, it should be noted that Zucker rats are a model of genetic obesity and experience both

hyperinsulinemia and hyperlipidemia and therefore may not represent the effects of obesity unrelated to a genetic mutation (Ackermann and Hart, 2016). On the other hand, the relationship between higher BMI and tendon pathology has been observed in humans in additional studies (Galli et al., 2014; Abate et al., 2016). In comparing tendon and ligament pathology between overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$) and nonoverweight ($\text{BMI} < 25 \text{ kg/m}^2$) individuals, Galli et al. (2014) found that 98% of overweight group experienced tendon or ligament pathology compared with only 62% in the nonoverweight group. While our results found a moderate effect of BMI on Achilles tendon structure, the cross-sectional nature of this study prevents us from giving support to the systemic or mechanical mechanism underlying the effects of BMI on tendon structure. However, recalling the work done by Gaida et al. (2008, 2009, and 2010) BMI might not be the only aspect of obesity to consider (Ackermann and Hart, 2016). Based on their findings, Gaida et al. (2008, 2010) have found that body composition, specifically the location of adiposity may be a better indicator of tendinopathy. In their study of fat distribution and asymptomatic Achilles tendinopathy, Giada et al. (2010) found that in individuals with Achilles tendon pathology, the fat distribution was central in men and peripheral in women. They also observed an interaction in men aged 40 years and older with a waist circumference greater than 83cm and tendon pathology (Gaida et al., 2010). Additionally, changes in the musculoskeletal system occur in obese individuals such as increased plantar pressure (Ackermann and Hart, 2016). Moreover, alterations in the distributions of forces along the knee, for example, can lead to varus malalignment which can lead to the occurrence of knee osteoarthritis (Ackermann and Hart, 2016). Therefore, future studies should not only consider elevated BMI on tendon structure but also fat distribution. Furthermore, assessing these variables over a longer time period would help to determine the mechanism underlying the relationship between obesity and tendon health.

4.2 Link between Statins and Tendinopathy?

While some studies have demonstrated a link between statins and tendon health, our study did not (Pullatt et al., 2007; Marie et al., 2008; Carmont et al., 2009; Celik et al., 2012; Kearns and Singh, 2016). Instead, our findings from this study support recent work done by Spoendlin et al. (2016). In their study, Spoendlin et al. (2016) focused on the occurrence of Achilles and biceps tendon rupture in new statin users. Utilizing a large primary-care database, based in the UK, Spoendlin et al. (2016) were able to study individuals who began their statin treatment. This is important as previous studies found that the time in which individuals can develop tendon symptoms is likely within the first year of statin treatment (Marie et al., 2008). Additionally, new statin users were required to have 3 or more years without statin therapy recorded prior to the initiation of statin treatment (Spoendlin et al., 2016). While patients were excluded if they had a previous Achilles or biceps tendon rupture, patients who had previously recorded events of Achilles or biceps tendinopathy/tendinitis were not excluded since tendon ruptures often develop from tendinopathy/tendinitis (Spoendlin et al., 2016). With a total of 526,351 PS-matched pairs, similar levels of ATR or BTR events were recorded for statins users (0.11%) and non-statin users (0.10%) in the overall cohort (Spoendlin et al., 2016). Before matching, the statin users were more likely to have comorbidities such as obesity, diabetes mellitus, and cardiovascular and chronic kidney disease (Spoendlin et al., 2016). In addition, statin users were older on average than non-statin users before matching (Spoendlin et al., 2016). Taking into account the comorbidities, sex, age, duration of treatment, and statin dose, statins were found to have no association with Achilles or biceps tendon ruptures (Spoendlin et al., 2016).

With the cross-sectional study design for our study, we were unable to prospectively assess any potential effects statins may have on Achilles tendon health. However, we were able to get observations of Achilles tendon structure in individuals who had been consistently using a statin for at least a year. With the hypothesis that statins may impact tendon health, we utilized UTC to observe if any changes were present in the Achilles tendons of individuals consistently using statins. Specifically, we focused on cross-sectional area and collagen alignment, something which Spöndlin et al. (2016) noted should be done in future studies.

If you recall, the hypothesis for this study was that individuals who have been taking statins would have a greater cross-sectional area and reduced collagen organization in their Achilles tendons compared to individuals who have never taken statins. Based on the results of this study, statins do not appear to negatively impact Achilles tendon health. Subsequently, we have to reject our hypothesis and conclude that there are no observable differences in Achilles tendon organization and cross sectional area between statin users and non-users. Moreover, based on these findings, the results we observed in our study add to the work already done in the field. By focusing on Achilles tendon cross-sectional area and collagen organization, we have been able to fill in one of the missing gaps currently found in the literature (Teichtahl et al., 2016; Spöndlin et al., 2016). With the use of UTC, details about Achilles tendon structure, and subsequently pathology can be assessed (Docking and Cook, 2015). Keeping in mind the effect of decreasing window size can have on resulting echo-types, UTC still has validity and is useful in observing tendon structure in pathologic, symptomatic, and asymptomatic individuals (Docking and Cook, 2015; Barry and O'Neill, 2016; O'Neill et al., 2016). Subsequently, given the demographics of our study population and the comorbidities we excluded, our finding that there is no association

between statins and Achilles tendon health support those findings of Teichtahl et al. (2016) and Spoendlin et al. (2016).

It should be noted that this study was unique compared to past research in this field. Past literature has used retrospective chart reviews, animal studies and case reports to draw a link between statins and tendons (Marie et al., 2008; Pullatt et al., 2007; Carmont et al., 2009; Celik et al., 2012; de Oliveira et al., 2013; de Oliveira et al., 2015). Additionally, while Marie et al. (2008) found that only 96 (out of 4,597) patients reported statin-specific tendon injuries in a 10-year period, 27 of these patients had comorbidities which would increase the chances of tendon injury (such as diabetes, hyperuricemia, and sports practice) (Marie et al., 2008). Moreover, there were another 11 patients who had a history of Achilles tendinopathy (Marie et al., 2008).

However, while our study adds to the field, there are limitations. First, due to the cross-sectional study design, we are not able to conclusively confirm that statins do not impact Achilles tendon health over a period of time. Long-term prospective studies are required that specifically focus on tendon structure in individuals taking statins. Second, as Spoendlin et al. (2016) noted, most patients in the UK are prescribed lipophilic statins (such as simvastatin and atorvastatin) which help statins move better into and out of cells (Sathasivam, 2012). This is reflected in the Canadian population where atorvastatin has been the most prescribed statin subsequently, making lipophilic statins candidates for the findings linking statins to tendon complications (Neutel et al., 2007). Third, our analysis only looked at 66 individuals which limits the power of our findings. The resulting implications of this could be that we may be making a type II error and failing to see an effect when there is one. Fourth, while there were many methods which

were used to recruit subjects for both groups, the exclusion criteria for this study were a limiting factor, preventing many individuals from participating. Additionally, while there are individuals who do not have arthritis, diabetes, hyper-/hypo-thyroidism, or a previous Achilles tendon rupture there are more individuals who not only have these diagnoses, but a combination of them. Therefore, future studies should assess the relationship between statins and tendon health in individuals with some of the comorbidities that we excluded in our study. By looking at the impact of statin use and diabetes and/or thyroid disease for example, the resulting findings can be more clinically relevant. Moreover, while the UTC is a good ultrasound tool, allowing for the collection of 3-D images, there are many factors that can impact the quality of a UTC scan. Although we have tried our best to control for many variables, there are still factors that may have affected the quality of the UTC scans collected and analyzed. Such factors include movement of the UTC tracker or the subject while a scan is in progress. Additionally, when conducting echo-type analysis, the UTC program is not yet able to smoothly combine the contours drawn by a user which can affect the resulting proportions of echo-type patterns determined for an ultrasound image. Lastly, it should be noted that there are limitations with the short IPAQ questionnaire. While it is a very useful tool in assessing physical activity, it does not differentiate on the types of vigorous and moderate physical activity subjects participate in as well as duration (looks at the past week but not past months, or years). In tendon research, particularly considering the load-bearing tendons, it is important to consider not only the loads but also the duration of physical activities such as running where the Achilles tendon, in particular, is heavily solicited (Ackermann and Hart, 2016). This has many implications, the main one being that there may be a difference between participating in current (over the past week) and long-term (over a span of years) physical activity.

Chapter 5: Conclusion

In conclusion, we did not find an association between statin and Achilles tendon structure, as visualized with ultrasound tissue characterization. However, we did find some expected correlations between different covariates. We found that advancing age and increasing BMI (separately) had significant negative effects on average echo-type I values. These findings support the work done by Spöndlin et al. (2016) and add to the field by providing information where a gap in knowledge exists. Specifically, by focusing on Achilles tendon structure, our findings suggest that statins do not impact tendon cross-sectional area or collagen organization.

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