THE EFFECTS OF FAMILIAL HYPERCHOLESTEROLEMIA ON ACHILLES TENDON BIOMECHANICS: A CROSS-SECTIONAL STUDY

by

Kip Squier

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

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Examining Committee:

Alexander Scott, Physical Therapy
Co-supervisor

Charlotte Waugh, Centre for Hip Health and Mobility
Co-supervisor

Supervisory Committee Member

Johannes Zwerver, Department of Sports Medicine, University of Groningen
Additional Examiner

Additional Supervisory Committee Members:

Michael Hunt, Physical Therapy
Supervisory Committee Member

David Wilson, Mechanical Engineering
Supervisory Committee Member

Abstract

Familial hypercholesterolemia (FH), a common genetic metabolic disorder characterized by high cholesterol levels, is involved in the development of atherosclerosis and other preventable diseases. FH can also cause tendinous abnormalities, such as thickening and xanthoma (tendon lipid accumulation) in the Achilles and extensor tendons, which may impact and impede tendon biomechanics. There is evidence that high cholesterol may lead to tendon injury or pain, but tendon biomechanics has received little investigation in people with FH. The objective of this study was to investigate how FH can affect Achilles tendon biomechanics, *in vivo*.

16 FH participants were recruited locally from the British Columbia FH Registry database. 16 control participants were recruited from purposeful convenience sampling. All participants completed preferred pace walking trials, shod, on a fully instrumented treadmill to collect gait impact data. Achilles tendon (AT) biomechanical data was obtained according to previously published methodology; simultaneously, lower limb kinematics, and muscle-tendon junction displacement were measured by motion capture and b-mode ultrasound imaging, respectively. AT strain, stiffness and hysteresis were calculated using a custom built Matlab program. AT biomechanical outcomes were assessed for statistical differences using MANCOVA.

16 FH participants (10 males, 6 females, 37±6 years, BMI of 28±4 kg/m^2) and 16 control participants (10 males, 6 females, 36±7 years, BMI of 27±3 kg/m^2) were recruited. FH participants displayed similar AT peak strain (FH: 5.07±0.9%, Controls: 4.95±0.9%; p=0.790), lower stiffness (FH: 87±20 N/mm, Controls: 111±18 N/mm; p=0.001), and higher hysteresis (FH: 56±17%, Controls: of 35±12%; p=0.007), when compared to controls.

To the best of our knowledge, this is the first study that evaluates the impact of FH on AT biomechanics, *in vivo*. The results of the current study provide evidence that cholesterol accumulation can negatively affect AT biomechanics and potentially increase the chance of injury. The findings of this study will be of interest to clinicians, and people with FH and high cholesterol.

Lay Summary

Familial Hypercholesterolemia, also known as FH, is one of the most common inherited disorders causing the buildup of cholesterol in the circulatory system. The high cholesterol buildup of FH causes early heart disease, potentially before the age of 50. Recently, there has also been evidence that suggests that cholesterol accumulation in tendons may compromise their function. Regrettably, there haven't been studies conducted that explore the impact of high cholesterol on tendon health. To fill the gap, we conducted a study that used motion capture cameras, ultrasound and treadmill force platforms to test the mobility and mechanics (stiffness, change in length etc.) of the Achilles tendon in people with FH. We found that people with FH show negatively altered Achilles properties, which suggests that they are at higher risk for tendon injuries. This study lays the framework for future research in the area.

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 (#H16-01358).

I conducted this study under the supervision of Dr. Charlotte Waugh and Dr. Alexander Scott. Development of the cross-sectional study protocol and documents were completed by Dr. Waugh, Dr. Scott and myself, with the assistance of Dr. Michael Hunt and Dr. David Wilson of the University of British Columbia. Recruitment was completed me under the supervision of Dr. Waugh and Dr. Scott. Data collection and biomechanical analysis was completed by me under the supervision of Dr. Waugh. I completed the statistical analysis under the supervision of Dr. Scott. To date, the research presented in the current thesis has not been published.

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List of Abbreviations

2D	Two dimensions
3D	Three dimensions
AT	Achilles tendon
ATMA	Achilles tendon moment arm
ApoB	Apolipoprotein B
ApoE -/-	Apolipoprotein E knockout
BMI	Body mass index
C57BI/6	Mice control group
CG	Control group
CHD	Coronary heart disease
CHHM	Centre for Hip Health and Mobility
CREB	Clinical Research Ethics Board
CSA	Cross-sectional area
DLCNS	Dutch Lipid Clinic Network Score
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
FH	Familial Hypercholesterolemia
GM	Gastrocnemius medialis
GRF	Ground reaction forces
HDL	High-density lipoproteins
IDL	Intermediate-density lipoproteins

OX	Oxidized
LDL	Low-density lipoproteins
LDLR	Low-density lipoproteins receptors
MANCOVA	Multivariate analysis of covariance
MTJ	Myotendinous junction
MTU	Muscle tendon unit
PCSK9	Propoprotein convertase subtilisin/kexin type 9
SBC	Simon Broome Criteria
TC	Total cholesterol
TXT	Tendon xanthoma
VLDL	Very-low-density lipoproteins
VM	Virtual Marker

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I would like to thank both my supervisors, Dr. Scott, for the chance to participate and contribute to his tendon lab group, and Dr. Waugh, for her patient guidance throughout this project. Both of their support and encouragement has been priceless.

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Lastly, I would like to thank Lauren and my family for their support and love throughout this project.

Dedication

Dedicated to Tom,

Although you are now on a separate journey, your impression on my life continues to be immeasurable. Thank you for your love, friendship, and guidance.

Chapter 1: Introduction

Cholesterol is essential for the normal functioning of animal cell membranes and hormone production. However, an abundance of low-density lipoproteins (LDL) cholesterol in plasma (>160 mg/dL) is associated with early coronary heart disease (CHD) (Schaiff, Moe, & Krichbaum, 2008). There is evidence from several studies that high levels of cholesterol in the blood can cause an accumulation of cholesterol in tendons leading to increased rates of tendinopathies (Abboud & Kim, 2010; Mathiak, Wening, Mathiak, Neville, & Jungbluth, 1999; Ozgurtas, Yildiz, Serdar, Atesalp, & Kutluay, 2003). However, the mechanisms of how cholesterol deposits can affect the biomechanics of tendons have received little investigation. Manifestation of cholesterol in tendons introduces lipid deposits (xanthoma) amongst collagen of extensor and Achilles tendons (AT), which enlarges the non-collagenous extracellular matrix (ECM), creating persistent, low-grade inflammation, and potentially impeding function (Abate, Schiavone, Salini, & Andia, 2013; Józsa, Réffy, & Bálint, 1984; Kruth, 1985). This study aims to explore if cholesterol accumulation can lead to reduced tendon function.

1.1 Familial Hypercholesterolemia

Affecting 1 in 250-500 people, familial hypercholesterolemia (FH) is the most common genetic disorder concerning metabolism of lipoproteins (De Ferranti et al., 2016; Goldstein, Schrott, & Bierman, 1973; Levenson & De Ferranti, 2016). Due to mutations in certain genes, uptake of plasma LDL is impaired, leading to elevated LDL levels (Nordestgaard et al., 2013). The National Cholesterol Education Program defines hypercholesterolemia as a serum cholesterol

concentration of 240mg/dL or 6.2 mmol/L and above (Grundy, 1999). High LDL levels can eventually cause deposits of cholesterol in peripheral tissues, skin, tendons, and arterial walls which can set the foundation for premature CHD (Goldstein et al., 1973; Levenson & De Ferranti, 2016). Individuals with FH have a 20-fold increase in risk of (CHD) should the condition be untreated (Hopkins, Toth, Ballantyne, & Rader, 2011). Without treatment, 50% of men with FH and 33% of women with FH will have a cardiovascular incident before they reach 60 years old (Civeira & Plana, 2017). Statins (lipid lowering pharmaceuticals) have become a primary treatment method for FH. Statins increase the survivability of patients with FH, significantly reducing LDL levels by 50-60% and FH related cardiac events by 50% (Civeira & Plana, 2017; Grundy, 1999).

1.1.1 Cholesterol and LDL

Cholesterol is an unsaturated alcohol in the steroid family; it can be ingested from dietary sources and produced by biosynthesis in the liver, intestines, adrenal glands and reproductive organs. Cholesterol is essential for all animal cells in the function and maintenance of cellular membranes and the creation of adrenal and gonadal hormones and acids (Cox & Garcia-palmieri, 1990). Since cholesterol is mostly insoluble in plasma, it is combined with proteins and phospholipids to form the soluble lipoprotein structure, and therefore be transported throughout the circulatory system (Schaiff et al., 2008). Apolipoproteins are proteins that act primarily as the transporters of lipids. Apolipoproteins also function as ligands for lipoproteins to bind to receptors, co-factors for enzymes in the metabolism of lipoproteins and they also moderate lipid transfer (Cox & Garcia-palmieri, 1990). According to density, size, mobility and content of

cholesterol and triglycerides, endogenous pathway lipoproteins are categorized into groups: very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), and high-density lipoproteins (HDL) (Cox & Garcia-palmieri, 1990; Schaiff et al., 2008). VLDL is formed in the liver to carry high amounts of triglycerides to the rest of the body. The triglycerides are removed from the lipoprotein in fat and muscle, transforming the VLDL into a cholesterol rich IDL. A portion of the IDL is removed in the liver and the remaining IDL remains in circulation and is converted into cholesterol-rich LDL. In normal conditions, most of the LDL will bind with LDL receptors on liver and other cells to be removed. HDL binds and esterifies any cholesterol that escapes from cells after LDL removal. The esters are then reallocated into IDL and then LDL to be eventually removed by cells in the liver (Cox & Garcia-palmieri, 1990).

LDL, the major cholesterol-carrying lipoprotein, is approximately 50% cholesterol, 25% protein, 20% phospholipid, and 5% triglyceride (Campos et al., 1992). Apolipoprotein-B (Apo-B) is the exclusive transporter of LDL in humans, and along with LDL, an increase in levels (>160 mg/dL) have been associated with CHD (Cox & Garcia-palmieri, 1990; Li et al., 2017; Schaiff et al., 2008). A number of studies have concluded that lowering LDL, through cholesterol lowering pharmaceuticals, will decrease the incidence of CHD (Grundy, 1999; Schaiff et al., 2008). Atherosclerosis, a narrowing of blood vessels, begins when endothelial cells are activated by risk factors (excess of LDL), which initiates an inflammatory response. The inflammatory response draws monocytes across the endothelium to become macrophages. LDL in the plasma accumulates in the intima, and is bound to the macrophages through scavenger receptors (Libby, 2001). The macrophages consume the LDL and transform into foam cells, known for their large

size and high lipid content. The foam cells become trapped in the lesion, thus constricting the flow of blood through the vessel (Libby, 2001; Robbins et al., 2014).

1.1.2 Genetics

In 1938, Dr. Carl Muller made the first connection between xanthoma and family history. Muller observed a series of families that presented with xanthoma. He noted, "Multiple xanthoma are a hereditary disease" which correlates to increased susceptibility of cardiac diseases (Muller, 1938). While he did not know the cause of the disorder, Muller described the condition to be an inborn anomaly of metabolism resulting in high cholesterol levels, myocardial infarctions and xanthoma in young individuals (Muller, 1938).

In 1964, Khachadurian made the distinction that proved that there are two forms of FH, the less severe heterozygous and more severe homozygous (Khachadurian, 1964). The heterozygous (1 in 250-500) group inherits a gene mutation from one parent at a 50% rate (Nordestgaard et al., 2013). They present with moderately elevated serum cholesterol and xanthoma to a lesser extent (Khachadurian, 1964). For homozygous mutation inheritance (1 in 1,000,000), both parents must have FH and both mutations must be passed down (Nordestgaard et al., 2013). Homozygous FH causes much more severe LDL levels, xanthoma at an early age, and earlier heart disease than those with heterozygous FH (Khachadurian, 1964; Levenson & De Ferranti, 2016).

Originally, FH was thought to only consist of mutations in the low density lipoprotein receptor (LDLR) gene (Goldstein et al., 1973). However, the description of FH broadened to include any

deficiencies in LDL metabolism that lead to a phenotype characteristic of FH. Thus, mutations of the apolipoprotein B-100 (APOB) gene and propoprotein convertase subtilisin/kexin type 9 (PCSK9) gene are also included in the diagnosis of FH (Hopkins et al., 2011; Levenson & De Ferranti, 2016).

In the 1970s, researchers found that the FH phenotype is a result from mutations to the LDLR gene (Goldstein et al., 1973). Mutations in the LDLR gene result in defects that cause the patient to be "receptor negative" (no LDLR activity) or "receptor defective" (reduced receptor activity), which creates a reduced or null ability to bind with and remove LDL from circulation (Goldstein et al., 1973)(Austin, Hutter, Zimmern, & Humphries, 2004). LDLR gene mutations account for 85-90% of all FH cases, and, as of 2011, have over 1600 variations (Austin et al., 2004; Hopkins et al., 2011).

In the late 1980s, research validated that the FH phenotype could also be caused by mutations in the APOB gene (Knott et al., 1985). The APOB mutation is often described as less severe than LDLR gene caused FH (Hopkins et al., 2011). This mutation affects the receptor binding domain of APOB (protein compound of LDL), reducing its ability to bind with LDL receptors (Soutar & Naoumova, 2007). Most commonly, the mutation causes an amino acid (Arg3500) to be replaced by glutamine (Arg3500Gln) on in the LDLR binding site of APOB, which blocks the receptor-binding (Soutar & Naoumova, 2007). APOB gene mutation accounts for 5-10% of FH cases (Hopkins et al., 2011).

The PCSK9 gene mutation is known to cause severe FH conditions (Soutar & Naoumova, 2007). PCSK9 binds with LDLR, post consumption of LDL, causing the receptor to degrade rather than recycle to bind with more LDL (Joseph & Robinson, 2015). Due to the missense, the mutation is gain-of-function, PCSK9 levels rise, and increased LDLR sites are degraded. This mechanism leaves LDL with a reduced opportunity to bind with LDLR and be removed from the system (Hopkins et al., 2011; Joseph & Robinson, 2015). The PCSK9 gene mutation is responsible for less than 5% of all FH cases (Hopkins et al., 2011).

1.1.3 Diagnosis of Familial Hypercholesterolemia

Even with high occurrence and serious implications, FH is heavily under-diagnosed. It is difficult to estimate the actual undiagnosed population. Researchers have deduced that North America has a <1% correct diagnosis rate (Nordestgaard et al., 2013). In contrast, European countries seem to have higher diagnosis rates (the highest being, Netherlands: 71%, Norway: 43%, Iceland: 19%) (Nordestgaard et al., 2013). As diagnosis stands to be a large barrier, criteria have been created in order to facilitate an improved rate of diagnosis. There are three main diagnostic tools at use, the Simon Broome criteria (SBC), the Dutch Lipid Clinic Network criteria (DLCN), and the MedPed criteria (Austin et al., 2004; Sharifi, Futema, Nair, & Humphries, 2017).

The SBC criteria is used mainly in the United Kingdom. SBC takes an approach of meeting certain requirements for a “definite” FH diagnosis. The criteria takes into account a) total cholesterol concentration (>7.5 mmol/l), LDL (>4.9 mmol/l), b) physical examination

(xanthoma), c) DNA mutations, and d) family history of premature CHD and total cholesterol levels (>7.5 mmol/l)(Austin et al., 2004; Sharifi et al., 2017). A “definite” FH diagnosis is assigned when the patient meets criteria from a) and b) or c) (Austin et al., 2004).

MedPed was formed in the USA and is the most basic of the three diagnostic tools (Sharifi et al., 2017). MedPed is based on cut points for total cholesterol levels when applied to age and family history. For an FH diagnosis cholesterol cut points are lower for patients with a 1st relative diagnosed with FH compared to a patient with a 2nd or 3rdrelative diagnosed with FH. The theory behind this model is that there is a higher probability of having an FH mutation when a family member has the gene mutation (Austin et al., 2004).

The DLCN is was created in the Netherlands and uses the sum of a points system in order to provide an FH diagnosis (Sharifi et al., 2017). The DLCN has 41 available points over five sections; a patient with a score of ≥8 points would be diagnosed with “definite” FH. 1. Family history takes into account first degree relatives, and if they have been subject to premature CHD or high LDL levels (1 point), xanthoma, or children with 95th percentile LDL levels (2 points). 2. Clinical history takes into account premature CHD (2 points), cerebral or peripheral vascular disease (1 point) that the patient may have shown. 3. Physical examinations look for cholesterol accumulations in the form of tendon xanthoma (TXT) (6 points) or Arcus cornealis (4 points). 4. The cholesterol levels section scores the severity of LDL levels, ranging from 1 point for 4.0-4.9 mmol/l to 8 points for ≥8.5 mmol/l. 5. The DNA analysis will score the patient should it be found that they have a mutation in the LDLR gene (8 points) (Austin et al., 2004). With

increased understanding of the gene mutations causing FH, gene mutations are directly detectable, leading to more consistent treatment and diagnosis (Nordestgaard et al., 2013).

1.2 Tendon Composition, Structure, and Biomechanics

1.2.1 Tendon Composition

Tendons are a fibrous, collagen-rich, tissue that transfer forces from muscle to bone, which changes the angle in joints and results in movement of limbs (James, Kesturu, Balian, & Chhabra, 2008; Thorpe & Screen, 2016; Wang, 2006). Tendons are made of collagens, proteoglycans, glycoproteins, glycosaminoglycans, a variety of cells and water (Killian, et al., 2012).

Collagen is abundant in connective tissue throughout the human body. It is a fibrous protein that offers a degree of compliancy to structures depending on their function (Lapiere, C.M., Nusgens, B., Pierard, 1977; Thorpe & Screen, 2016; Wang, 2006). There are around 28 types of collagen proteins present in the human body, though collagen type I is the most prominent in tendons (Magnusson, Langberg, & Kjaer, 2010). Collagen type I makes up 60-80% of the dry mass of the tendon, and 90-95% of all collagens in the tendon (James et al., 2008; Józsa, Lehto, Kvist, Bálint, & Reffy, 1989; Thorpe & Screen, 2016). Type I collagen supports a high mechanical strength and low flexibility profile (Silver, Freeman, & Seehra, 2003). Type III and V collagens make up the other 5-10%. Type III collagens are often found in the endotenon and epitenon, and can also be found more often in aging tendons (Józsa et al., 1989; Thorpe & Screen, 2016;

Wang, 2006). Type III collagen is more compliant than type I collagen, and make up smaller, less organized, fibrils, which may lead to a reduced mechanical strength (Jozsa et al., 1989; Lapiere, C.M., Nusgens, B., Pierard, 1977; Silver et al., 2003). Type V collagen are larger than type I collagen, and are interwoven into type I fibrils in order to regulate fibril growth (Silver et al., 2003; Wang, 2006). Other collagens types (II, VI, IX, X and XI) are found in minor quantities in tendons, with the purpose to increase the fibrocartilage connection strength at the enthesis (Wang, 2006).

Proteoglycans are the most common non-fibrous proteins in the tendon, making up 1-5% of tendon dry weight (Thorpe & Screen, 2016). The content and type of proteoglycans seem to be dependent on the conditions that the tendon will have to endure, or its function (Thorpe & Screen, 2016; Wang, 2006). For example, if the tendon must endure compression, proteoglycans have more of a presence in the form of aggrecan which holds water in the fibrocartilage to resist compression (Wang, 2006).

Glycoproteins are present in the ECM of the tendon in the form of tenascin-C, fibronectin, and elastin. Tenascin-C is found at low levels, though it seems be more present in sections of tendons that experience high tension. Tenascin-C interacts with collagen to increase the mechanical stability of the ECM (Thorpe & Screen, 2016; Wang, 2006). Fibronectin is established on the exterior of collagen fibres. When the tendon is damaged, fibronectin synthesis is increased to aid in the healing process (Jozsa et al., 1989). Elastin constitutes around 2% of the dry weight of tendons, when combined with other microfibrillar proteins, they comprise up to 10% of the dry tendon weight (Józsa et al., 1989; Thorpe & Screen, 2016; Zatsiorsky & Pirlutsky, 2012). Elastin

is distributed throughout the tendon surrounding tenocytes and fascicles. Elastin travels longitudinally along the tendon and is extremely elastic, resistant to fatigue, can store and return energy, and may contribute to crimp pattern recovery (Grant, Thompson, Urban, & Yu, 2013; Thorpe & Screen, 2016; Zatsiorsky, V.M., Pirlutsky, 2012).

Of the cells that reside in tendons, fibroblasts are the most prominent. Fibroblasts (tenocytes and tenoblasts) synthesize ECM proteins, such as collagens and proteoglycans, while also being responsible for collagen alignment and reorganization post damage (Thorpe & Screen, 2016; Wang, 2006).

1.2.2 Tendon Structure

As previously stated, tendons attach muscles to bone (Wang, 2006). The tendon attaches to the muscle via the myotendinous junction (MTJ), and to the bone via the enthesis (Knudsen et al., 2015; Wang, 2006). The MTJ is a specialized structure that uses a finger like recesses to maximize the surface area contact between the muscle filaments and the collagen fibres, and therefore transfer forces generated from the muscle to tendon (Knudsen et al., 2015; Wang, 2006). Even with the deep recesses to increase mechanical strength, the MTJ is still the weakest point of the musculotendinous unit and is therefore prone to injury (Knudsen et al., 2015). The MTJ is also a site for blood vessels to supply the tendon (Benjamin, Kaiser, & Milz, 2008).

The enthesis is a specialized structure, it is able to endure tensile, compressive and shear forces (McGonagle, Marzo-Ortega, Benjamin, & Emery, 2003). McGonagle et al. (2003) reported that forces at the enthesis may be up to four times the force at the mid-body of the tendon. The

enthesis is either fibrous or fibrocartilaginous. The fibrous enthesis is a direct attachment from the collagen in the tendon to the bone, while the fibrocartilaginous enthesis bears a four zone transition from tendon to bone (Benjamin & McGonagle, 2009; Wang, 2006). The first zone is the tendon of normal composition and alignment. The second zone is constructed of uncalcified fibrocartilage, proteoglycans and various collagens. Zone three is comprised of calcified fibrocartilage, various collagens, and bone. Zone four is solely bone. Each zone has an increased level of stiffness and rigidity (Benjamin & McGonagle, 2009; McGonagle et al., 2003).

Tendons express a multi-unit hierarchical structure that run parallel to the geometrical axis (Silver et al., 2003). The structure is composed of collagen molecules, fibrils, fibre bundles and fascicles, resulting in the tendon unit seen in Figure 1 (Thorpe & Screen, 2016; Wang, 2006; Zatsiorsky & Pirlutsky, 2012).

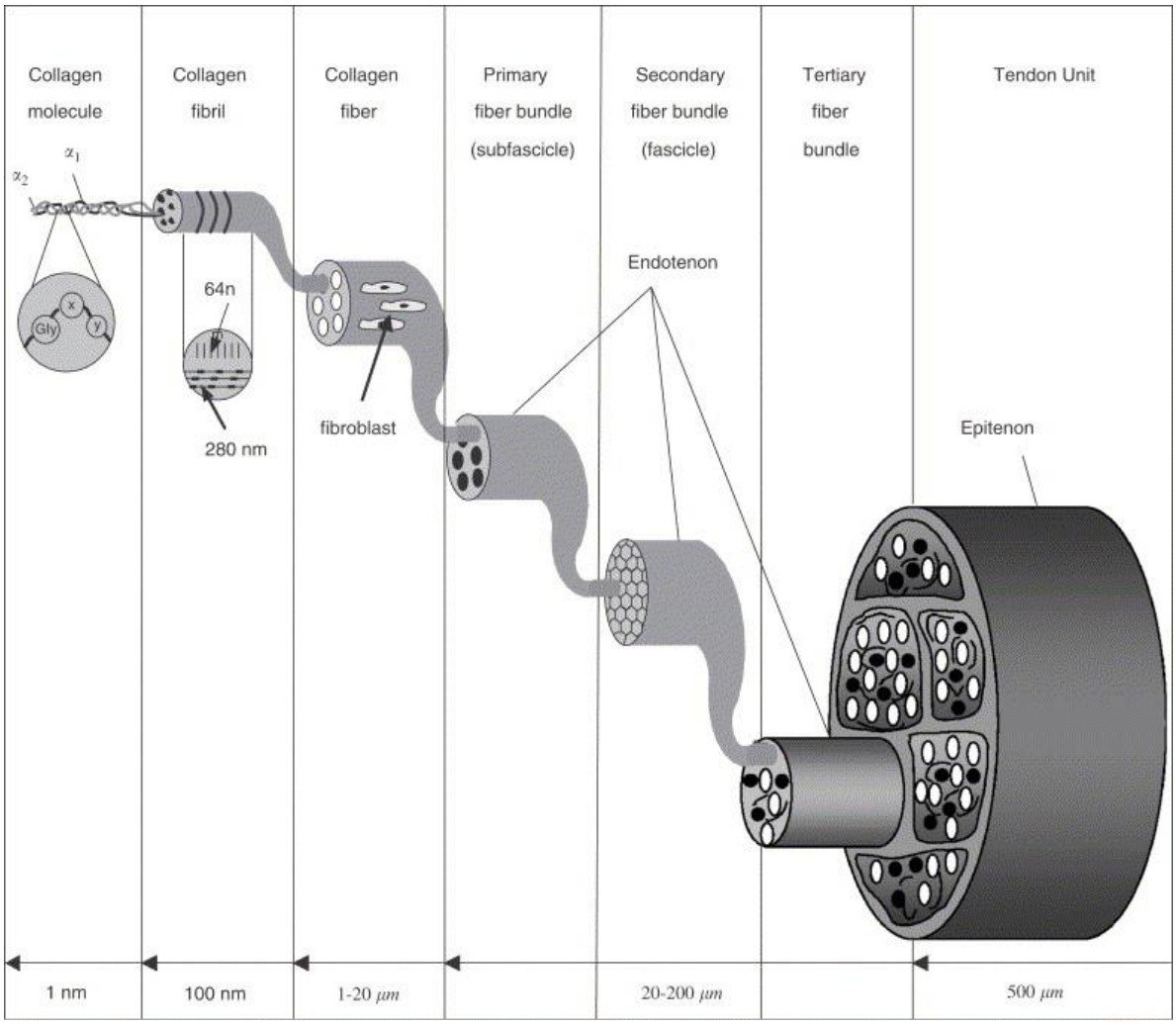

Figure 1. Illustration of the multi-unit hierarchical structure of the tendon (Wang, 2006, reproduced with permission from Elsevier).

Much like a braided rope, the hierarchical structure gives the tendon significant tensile strength to withstand forces generated by the muscles and external events (Zatsiorsky & Pirlutsky, 2012). The collagen unit is the building block in the tendon unit. Arranged longitudinally, collagen molecules form a quarter staggered triple helix pattern. Five collagen molecules are secured together via molecular crosslinks, forming microfibrils. Microfibrils are packed together to build

fibrils, which are stabilized by crosslinking, and range from 10-500nm in diameter. Fibrils are deemed to be a primary structural unit, and are fundamental in a variety of different tissue types (Thorpe & Screen, 2016). Fibres are made up of a collection of fibrils, which are bound together by a thin layer of connective tissue that contains blood vessels, lymph, and nerves, called the endotenon. When in a relaxed state, fibres follow a sinusoidal wave pattern, known as a "crimp" (Zatsiorsky, V.M., Pirlutsky, 2012). Fibres range from 1-20µm in diameter. Fibres are grouped to form fascicles, which range from 20-200µm in diameter. To make up the tendon unit, the fascicles are also bundled together, multiple times, and wrapped with a layer of loose connective tissue called the epitenon. Like the endotenon, the epitenon contains blood vessels, lymph, and nerves. Additionally, the tendon is also surrounded by a third layer of connective tissue, the paratenon. The Paratenon and the epitenon together form the peritendon, whose function is to reduce friction between the tendon and surrounding tissues (Thorpe & Screen, 2016; Wang, 2006). The level of friction or resistance is based on the compression from surrounding tissues, amount of fluid and size of the tendon (An, 2007).

1.2.3 Tendon Biomechanics

1.2.3.1 Elongation profile

Tendons are able to withstand large forces, repetitively. The AT tendon is subject to 12.5 times body weight in common movements like jumping (Komi, 1990). As tendons are submitted to tensile forces, the tendon can pass through three main phases when lengthening, the toe region, linear region and failure region (Figure 2).

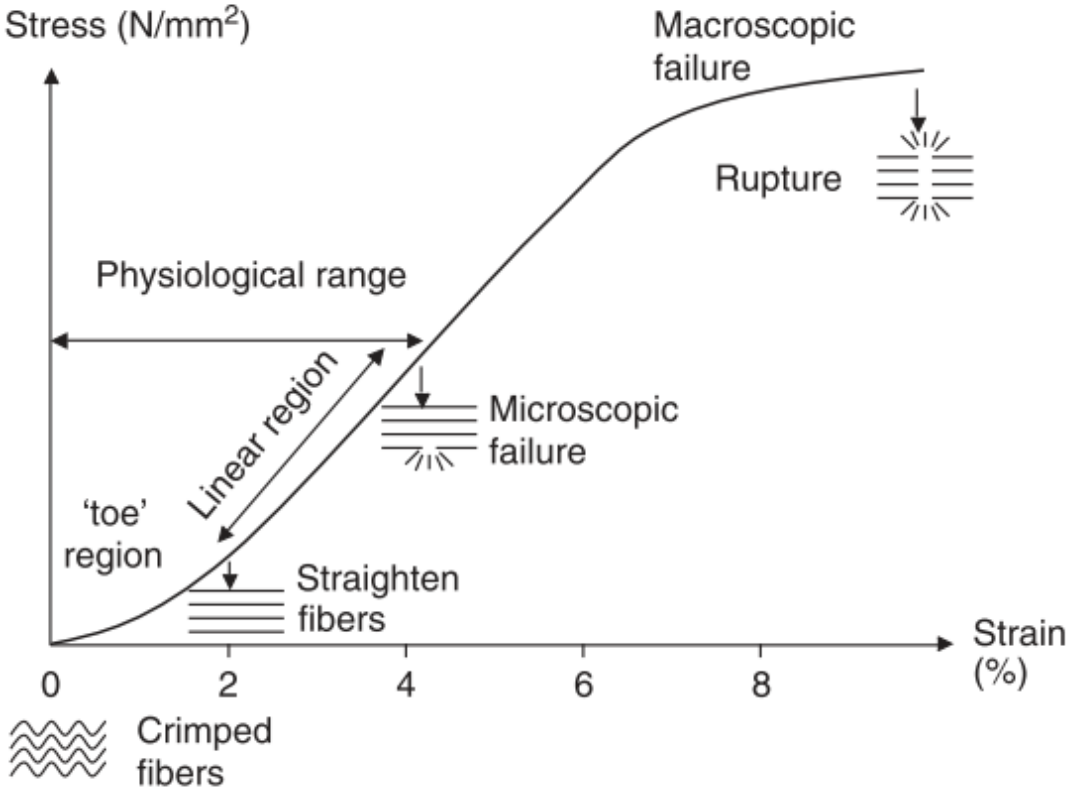

Figure 1. Stress-strain curve of a tendon (Wang, 2006, reproduced with permission from Elsevier).

While the mechanical properties of tendons are relatively similar across vertebrates, variations in stresses, strain, hysteresis or modulus can be attributed to the cross-sectional, biological composition of the tendon and testing conditions (In vitro and in vivo) (Maganaris & Paul, 1999; Pollock & Shadwick, 1994). In vitro conditions require the tendon sample to be tested outside of their normal biological environment. Caution should be employed when inferring results of in vitro conditions apply to in vivo (normal biological environment) tendon functioning (Maganaris, Narici, & Maffulli, 2008). The difference between the conditions could be due to a difference in tendon loading due to clamping in testing rigs, the impact of tendon sample

preservation, and lack of support from other structures (Maganaris et al., 2008). Historically, accuracy has been an issue in measuring tendon length while using in vivo methods. Newer techniques using non-invasive imaging (ultrasound) and motion capture systems have increased reliability of capturing in vivo tendon length changes (Lichtwark & Wilson, 2005; Maganaris & Paul, 1999).

In the toe region, tendons are generally strained to approximately 2% (range of 1.5%-4%) (Butler, Grood, Noyes, & Zernicke, 1978; Zatsiorsky, V.M., Pirlutsky, 2012). During activities of daily living, tendons usually perform within the upper-limit of the toe region (Zatsiorsky & Pirlutsky, 2012). The tendon resistance during this phase is due to the straightening out of the collagen crimped pattern (Maganaris et al., 2008). Depending on the function of the tendon, the crimp patterns can vary in angle and length. Fibres with larger crimp pattern angles will have a higher failure length, as well as provide a greater potential for storing energy (Thorpe & Screen, 2016; Wang, 2006). Collagen fibres have different levels of crimp; as elongation rises, more fibres are engaged to resist the deformation forces. The modulus of elasticity continues to rise with the change in length, contributing to the toe region's non-linear profile. As the tendon is elongated, the modulus of elasticity will eventually reach a constant value which signifies the beginning of the linear region (Maganaris & Paul, 1999; Zatsiorsky & Pirlutsky, 2012). Elongation of the tendon in the linear region takes place from around 2-5.5% strain for the majority of tendons tested in vivo (Arampatzis, A. S. Stafilids, G. DeMonte, K. Karamanidis, G. Morey-Klapsing, 2005; Butler et al., 1978). This is due to the stretching of collagen fibres and is dependent on their material stiffness (Maganaris & Paul, 1999). The linear region begins at a stress of 5-30 MPa, and the failure region at 70-100 MPa, in vitro (Zatsiorsky, V.M., Pirlutsky,

2012). In a study conducted by Magnusson et al. (2001), the AT tendon endured a stress of 41.6±3.9 MPa and a strain of 4.4-5.6% at maximal voluntary isometric plantarflexion. These findings are in accordance with Arampatzis et al. (2005), who found a similar strain of 5.1±1.1%. However, in some tendons, strains (in vivo) can safely rise to around 8-10% during certain exercises, without obvious damage to the tendon (Lichtwark & Wilson, 2005; Zatsiorsky, V.M., Pirlutsky, 2012).

The failure region takes place beyond the linear region; in vitro, the tendon sample can experience microscopic tearing of the collagen fibres at 4-8% strain, and macroscopic failure beyond 8-10% strain (Butler et al., 1978; Wang, 2006). Failure rates of tendons can vary significantly when comparing results from in vivo and in vitro conditions (Maganaris et al., 2008).

1.2.3.2 Tendon Viscoelasticity

Tendons represent both viscous and elastic properties while undergoing deformation. The viscoelastic properties are most likely due to exchanges between water, collagen, and non-collagenous proteins (Wang, 2006). Viscoelastic bodies are time and history dependent through four main features that are fundamental to tendon operation (Hawkins, Lum, Gaydos, & Dunning, 2009; Zatsiorsky & Pirlutsky, 2012). First, tendons are sensitive to the rate at which they are strained. The rate at which the tendon is deformed will influence stress versus strain characteristics (Zatsiorsky & Pirlutsky, 2012). Second, creep, defined as an increase in length while under constant load or a gradual increase of strain or deformation with each successive

loading cycle, until the tendon reaches a steady state (Hawkins et al., 2009). Third, stress-relaxation is characterized by the tendon's decrease in stress while under a constant load and length. As time passes, the amount of force necessary to hold the tendon at the set length is reduced (Schatzmann, 1998). Lastly, hysteresis represents the amount of energy lost when loading and unloading a tendon. Energy is lost, mainly in the form of heat, due to friction during deformation. Hysteresis is graphically explained as the difference between the loading and unloading of the force-displacement cycle (Maganaris & Paul, 2000, Zatsiorsky & Pirlutsky, 2012). Hysteresis values tend to be different depending on the location and function of the tendon, and can range from 5-25% (Lichtwark & Wilson, 2005; Maganaris & Paul, 2000). The viscoelastic properties allow the tendon to be more compliant and to absorb more energy at low strain rates, but in turn make them less efficient at transferring forces. At high strain rates, tendons are stiffer, making them more effective at transferring loads from muscles to bone (Maganaris & Paul, 2000; Wang, 2006, Zatsiorsky & Pirlutsky, 2012), but reducing their capacity to absorb and return energy.

1.2.3.3 Achilles Tendon Biomechanics

The AT is the thickest tendon in the human body, connecting the triceps surae to the calcaneus in order to transmit forces created by the muscles into foot plantar flexion. The biomechanical properties of in-vivo AT are measured using ultrasound and plantar flexion force data. However, the results of these methods can vary considerably. In a summary of biomechanical, in vivo, testing conducted by Maganaris et al. (2008), they found a wide range of reported outcomes (Stiffness: 17-760 N/mm, strains: 5-8 %, AT force: 200-3800 N). The differences in results are

likely due to the methodological differences in the way that AT forces are calculated, location of the landmark being tracked by ultrasound (MTJ, tendon or muscle), and positions of markers for kinematics.

The AT has energy saving abilities; the tendon stores and returns plantar flexion energy to minimize the metabolic cost of ambulation (Maganaris & Paul, 1999). Recent research indicates that the AT and other energy storage tendons benefit from high strain capacities. This is in part due to greater interfascicular sliding allowing the tendon a greater length (Thorpe, Godinho, Riley et al., 2015).

In walking, the AT acts in a catapult like action; the tendon is strained throughout the stance phase, storing energy. Just before toe off, the majority of the stored energy is returned to assist with push off. In repetitive hopping and running, the majority of elastic energy comes from the initial negative work. The AT stores energy in a spring-like fashion, straining to store energy in the first half of ground contact, to recoil and return energy in the second half (Ishikawa, 2005). Because of the stored energy being returned in the late stance phase, the AT is subject to high stresses, which can rise up to 110 MPa during running. The AT stress surpasses the average ultimate tensile tendon stress of 100 MPa, which may explain in part why the AT is so commonly injured (Maganaris, 2002).

Even though the AT has the ability to store and return energy, the tendon can also lose stored energy in the form of heat (hysteresis) due to friction. The AT displays low hysteresis values that

range from ~10-35%, maximizing energy returned and minimizing heat damage (Finni et al., 2012, Lichtwark & Wilson, 2005).

1.2.4 Tendon Adaptation

1.2.4.1 Exercise

Tendons change their structure and size depending on the demands of loading. Exercise tends to have a short and long term impact on the properties of tendons (Couppe et al., 2008; Kjær et al., 2006; Miller et al., 2005). In order for a tendon to remain healthy, older, damaged fibres are degraded, while younger, more elastic, fibres are synthesized. Exercise has an immediate effect on collagen synthesis rates and blood flow. Collagen synthesis rates increase by 100% after 60 minutes of exercise, and blood flow increases by 3-7 fold (Magnusson et al., 2010; Miller et al., 2005). The increased collagen expression is due to the fibroblast responding to the strain applied to the tendon (Magnusson et al., 2010). Post-exercise, collagen synthesis peaked around 24 hours and returned to baseline synthesis at ~72 hours. It is important to note that degradation of collagen peaks just before 24 hours post exercise, at which point degradation is more pronounced than synthesis. Collagen degradation has a faster return to baseline which allows for a net production in collagen (Miller et al., 2005). After repetitive loading, the net increase in collagen synthesis can result in tendon hypertrophy and increased cross-section (Couppe et al., 2008; Miller et al., 2005).

1.2.4.2 Aging

Tendons, much like most other structures, are affected by aging. Ageing tendon related changes are characterized by a decline in structural consistency, leading to altered mechanical properties (Kubo, Kanehisa, Miyatani, Tachi, & Fukunaga, 2003; Tuite, Renstrom, & O'Brien, 1997; Zatsiorsky & Pirlutsky, 2012). Major cellular changes include decreased density and activity of tenoblasts, decreased ability for protein synthesis, and decreased number of capillaries (Birch, Peffers, & Clegg, 2016; Tuite et al., 1997). These changes directly affect the ability of the tendon to regenerate and turn over old or unaligned collagen fibres(Diamant, Keller, Baer, Litt, & Arridge, 1972; Tuite et al., 1997). Stenroth et al. (2012) found that young adults (18-30 years), in comparison to elderly adults (70-80 years), had higher AT stiffness (170±37 N/mm vs. 141±37 N/mm) and smaller CSA (53.49±9.75 mm2 vs. 61.98±12.64). The authors hypothesized that the stiffness and CSA changes to the elderly's AT could be an optimization of low loading conditions and lack of need for a rapid energy return (Stenroth, Peltonen, Cronin, Sipila, & Finni, 2012).

1.2.4.3 Gender

Tendons portray mechanical and physical differences between genders. Men have a tendency to have larger muscles that provide a greater strength than women (Kanehisa, Ikegawa, & Fukunaga, 1994). To accommodate the larger muscle forces, men have greater tendon CSA and stiffness (Kubo, Kanehisa, & Fukunaga, 2003; Muraoka, Muramatsu, Fukunaga, & Kanehisa, 2005; Taş et al., 2017). In a study conducted by Kubo et al. (2003), researchers measured GM

aponeurosis mechanical values. During a maximal voluntary contraction of the foot plantar-flexors, the researchers noted differences in stiffness (women had significantly lower stiffness than men), strain (women had higher strain for forces over 8 MPa), hysteresis (women: 11.1%, men 18.7%) and CSA (women: 46 mm2, men: 60 mm2) (Kubo, Kanehisa, & Fukunaga, 2003). The difference in values is due in part to circulating estrogen, which inhibits collagen synthesis leading to less hypertrophy after exercise related loading (Hansen & Kjaer, 2014)

1.3 Cholesterol and Tendon Health

1.3.1 Tendon Xanthoma

In a high cholesterol environment, the body can respond by developing TXT (Soslowsky & Fryhofer, 2016). The dry weight make up of a TXT is around 33% lipids and 24% collagen fibres (Soslowsky & Fryhofer, 2016). TXT may form when blood serum levels of LDL are extremely high for a prolonged period (Kruth, 1985). TXT growth occurs when LDL leaves circulation and becomes trapped in the tendon's matrix. For this process to take place, LDL that enters the ECM becomes oxidized by cellular oxidants produced by macrophages and local inflammatory cells. The oxidation (OX) stimulates the entry of monocytes from circulation into the tendon matrix. The monocytes mature into macrophages and in an attempt to rid the matrix of the OX-LDL, the macrophages consume the OX-LDL. However, macrophages have had their ability to exit the tendon matrix impaired by the oxidized-LDL. The macrophages filled with OX-LDL turn into foam cells which then accumulate amongst the collagen fibres in the ECM, forming TXT (Soslowsky & Fryhofer, 2016). The TXT directly affects the alignment of the

collagen fibres, making a less uniform region, more variation in size and potentially reducing the collagen's ability to function properly (Józsa, Réffy, Kannus, Demel, & Elek, 1990; Józsa et al., 1984).

TXT formation is dependent on various factors such as age, gender and cholesterol levels. They are most commonly observed in superficial tendons such as the AT and extensor tendons in areas of high stress (Kruth, 1985; Tsouli, Kiortsis, Argyropoulou, Mikhailidis, & Elisaf, 2005). Accumulation of cholesterol in tendon is comparable to that in atherosclerotic vessels (Adams, Bayliss, Baker, Abdulla, & Huntercraig, 1974). TXT are most common in people with high cholesterol disorders such as familial hypercholesterolemia, though TXT are not exclusive to the condition. Patients with homozygous FH can start to develop TXT in childhood (Civeira et al., 2005). TXT are less common in those with heterozygous FH and tend not to produce TXT until later adulthood (>40 years of age) (Soslowsky & Fryhofer, 2016). Approximately 30-50% of people with heterozygous FH have TXT (Civeira et al., 2005).

While not a lot is known about the biomechanical impact of TXT on tendons, it is thought that the increase in tendon size may interfere with overall tendon function (Kruth, 1985). Research, up until this point, has looked at the effect of high cholesterol environments on tendon biomechanics in animal models and studies have reported mixed results. Soslowsky & Fryhofer (2016) summarized the findings of multiple animal model studies. They found that the supraspinatus tendons tended to have a higher stiffness and increased modulus (ratio of stress and strain) in genetically modified apolipoprotein E knockout (ApoE -/-) high cholesterol mice, monkeys (high cholesterol diet) and rats (high cholesterol diet). Contrary to these findings,

researchers using high cholesterol porcine bicep tendons, found that there was a significant drop in stiffness and modulus (D. Beason et al., 2004). The changes in stiffness and modulus could be due to changes in protein synthesis and ECM composition and changeover (Ronnemaa et al., 1975).

1.3.2 Cholesterol Based Tendinopathies

Recent evidence has shown that high levels of total cholesterol (TC) and low-density lipoproteins (LDL) can have negative impacts on tendons, leading to tendinopathies (Abboud & Kim, 2010; Klemp, Halland, Majoos, & Steyn, 1993; Mathiak et al., 1999; Ozgurtas et al., 2003). In a study, directed by Mathiak et al. (1999), which looks at surgical treatment of AT ruptures, researchers noted that 83% of the rupture patients had elevated serum cholesterol levels (>200-335 mg/dL). These findings imply that high lipid levels may predispose individuals to tendon ruptures (Mathiak et al., 1999). Ozgurtas et al. (2003) found a significant difference in cholesterol levels between an AT rupture group compared to a control group (rupture: TC:5.69 mmol/l, LDL:3.91 mmol/L, TG: 1.53mmol/l; control: TC:4.09 mmol/L, LDL:2.35 mmol/L, TG: 1.22 mmol/L); whilst Abboud & Kim (2010) found that a rotator cuff tendon tear group had higher TC (237mg/dL) than a shoulder pain group (194 mg/dL); 64% of patients in the rotator cuff tendon tears group had TC levels of >240mg/dL, compared to 28% of patients in the shoulder pain group.

1.4 Purpose

Due to a lack of research in the area, the purpose of this cross-sectional study is to gain an understanding of the effects of FH and TXT on the mechanical properties of the AT, in vivo.

1.5 Hypothesis

Based on prior research, the following hypothesis has been formulated:

Individuals with FH will have altered AT mechanical properties when compared to controls. The AT of participants with FH will display lower stiffness (increased compliance), higher maximum strain, and increased energy lost to hysteresis, resulting in reduced energy storage and return during cyclic loading (walking).

Chapter 2: Methods

2.1 Study Design

By the use of a cross-sectional study design and established methods, AT data of FH participants was collected during standardized gait trials. When compared to control participants, this data could be important in substantiating whether FH and TXT lead to impairment of tendon function and predisposition to tendinopathy. The study was approved by the Clinical Research Ethics Board at the University of British Columbia (#H16-01358).

2.2 Recruitment

All participants provided written consent prior to participation in the current study. The participant's eligibility for the study was determined using the following inclusion and exclusion criteria:

2.2.1 Inclusion criteria

- FH participants must have a definite diagnosis of FH, deemed to be ≥8 on the DLCNS (Austin et al., 2004). The BC FH registry uses the DLCNS for diagnosis of FH. The use of participants with FH minimizes the variability and duration of high cholesterol levels. The FH participants are born with the condition which leads to a more consistent timeline of elevated cholesterol (Soslowsky & Fryhofer, 2016).

- FH Participants must have a positive TXT diagnosis on the BC FH Registry. Physicians with the BC FH Registry include a “Physical Examination” section, in which they identify if the patient has TXT.
- Participants must be aged between 19-50 years old. Aging has a direct impact on tendon mechanical properties. An aging tendon has increased hysteresis, less stiffness and a reduced maximum strain (Zatsiorsky & Prilutsky, 2012).
- Participants must be able to participate in moderate physical activity for 20+ minutes. Due to the data collection trial of exercising on the instrumented treadmill, participants must be able to walk for a reasonable amount of time. This requires that the participant is of reasonable activity levels and not in the category of tendon disuse. Tendon disuse or tendon unloading reduces tendon stiffness. Only complete unloading of the tendon due to spinal injuries seem to cause atrophy (Kannus, Józsa, Natri, & Järvinen, 1997; Zatsiorsky, V.M., Pirlutsky, 2012). Activity levels will be determined using the recommendations outlined in the Global Recommendations on Physical Activity for Health (Who, 2010). Participants will be allocated into four categories defined by accumulated minutes of moderate activity per week (<75 mins/week, 75-150 mins/week, 150-225 mins/week, >225 mins/week). Vigorous-intensity activity will count as double the minutes to moderate activity.
- Participants must be of a BMI of under 35. People that are morbidly obese have decreased tendon stiffness, increased tendon thickness, and are more likely to have undiagnosed disorders that could confound the findings of the study (Kyrou, Randeva, & Weickert, 2000; Taş et al., 2017).

2.2.2 Exclusion criteria

- The participants must not have any other conditions that could negatively influence musculoskeletal properties or impede the gait cycle, such as Parkinson's disease or diabetes mellitus (Cronin et al., 2010).
- The participants must be free of AT ruptures or tears. Ruptured AT, post injury, become more compliant and are associated with reduced strength and calf atrophy (Cetti, Christensen, Ejsted, Jensen, & Jorgensen, 1993; Geremia et al., 2015). Geremia et al. (2015) found that in patients with unilateral AT ruptures, the tendon showed a reduced stiffness and Young's modulus when compared to the uninjured side. These changes are due to the sub-optimal collagen alignment and increased type III collagen fibres (lower tensile strength than type I collagen) in post-injury tendons (Kannus et al., 1997).
- The participants must be non-smokers, or have stopped smoking ≥ 1 year. Smoking has not yet been seen to have an effect on tendon mechanical properties. However, cigarettes are known to have nicotine as a main additive. Nicotine has been shown to impair the overall ability of tendons to heal (Duygulu, Karaoğlu, Zeybek, Kaymaz, & Güneş, 2006). This impairment is due to degeneration of fibroblasts, leading to irregular collagen fiber alignment (Abate et al., 2013).
- The participants must not have any lower limb musculoskeletal injury (grade 2 tear) within the last 6 months. Must not have any lower shank grade 3 tears where reconstruction was required. Must be free of lower limb fractures in the past year.
- The participants must not have participated in a structured resistance training specifically targeting the triceps surae, in the year preceding data collection. (Kannus et al., 1997)

2.2.3 FH Participants

FH participants were recruited from the FH registry at St. Paul's Healthy heart program (Vancouver, British Columbia). FH Registry access was approved by Providence Health and St. Paul's Hospital. FH participants were identified from the BC FH Registry website (June, 2017). The participants were recruited between June 15th and September 15th, 2017. FH participant flow is available in Figure 3.

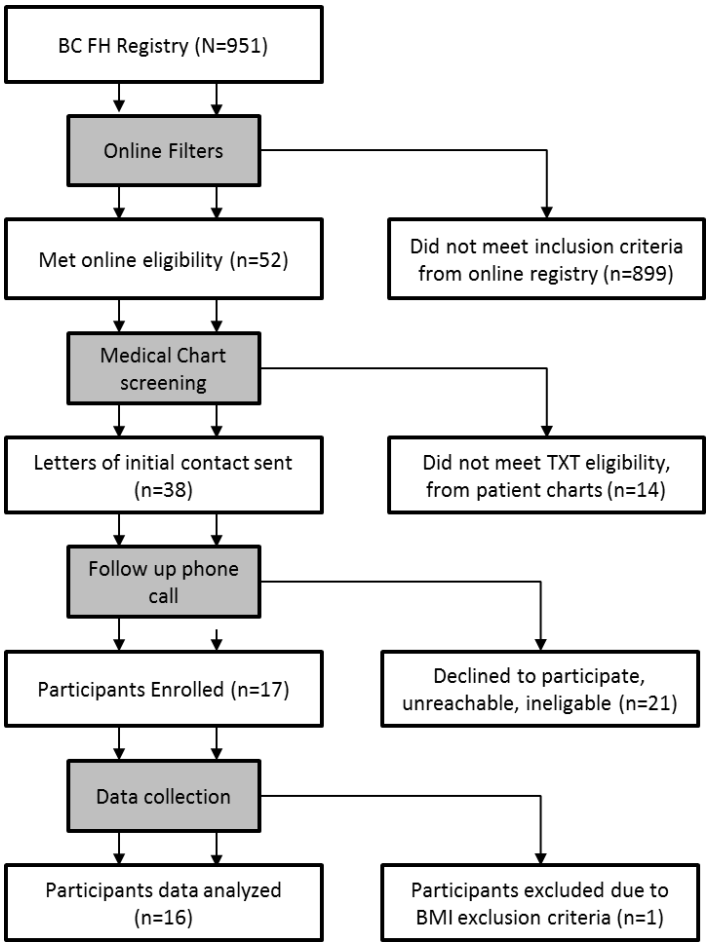

Figure 3. FH participant flow chart.

After online registry filters for diagnosis level, TXT presence, age, location and registry status, 52 participants met our criteria. We followed up by checking the 52 potential participant's medical charts for the presence of TXT. This left 38 participants with TXT diagnosis, all of whom had letters of initial contact send to their mailing addresses. After a two week grace period, we followed up with telephone interviews to assess if the participant met all our criteria. Of the 38 participants, 11 participants were not interested or didn't have time, 7 could not be reached, and 3 were not eligible due to BMI, recent injury or pregnancy. 17 participants were deemed willing and eligible, they were given an appointment time for data collection. One participant was excluded after data collection, due to their BMI being over the cut off, which left us with data from 16 participants.

2.2.4 Control Participants

16 control group (CG) participants were recruited using purposeful convenience sampling, May 1st to Aug 9th, 2018. The CG participants were recruited to mirror the FH participant's age, BMI, gender, and activity level. CG participant flow is shown in Figure 4. Methods for recruitment were word of mouth, posters, social media and online forums. Word of mouth recruitment took place in the Vancouver General Hospital and Centre for Hip Health and Mobility (CHHM) area, calling upon staff and students. Word of mouth presented eighteen potential participants, ten were enrolled; eight were excluded due to age, BMI or activity levels. Eight, Clinical Research Ethics Board (CREB) approved, posters were placed on bulletin boards at CHHM and University of British Columbia, two at CHHM, two at local coffee shops and four at bus stops. Posters yielded four potential participants; two were enrolled and two were

excluded due to activity levels. A social media (Facebook) post was made using the CREB approved poster. The social media post yielded eight potential participants, four were enrolled and four were excluded due to age and activity levels. An online forum post on reddit.com/r/Vancouver (CREB approved poster) was used to no effect, two potential participants were in contact, and both were excluded due to activity levels.

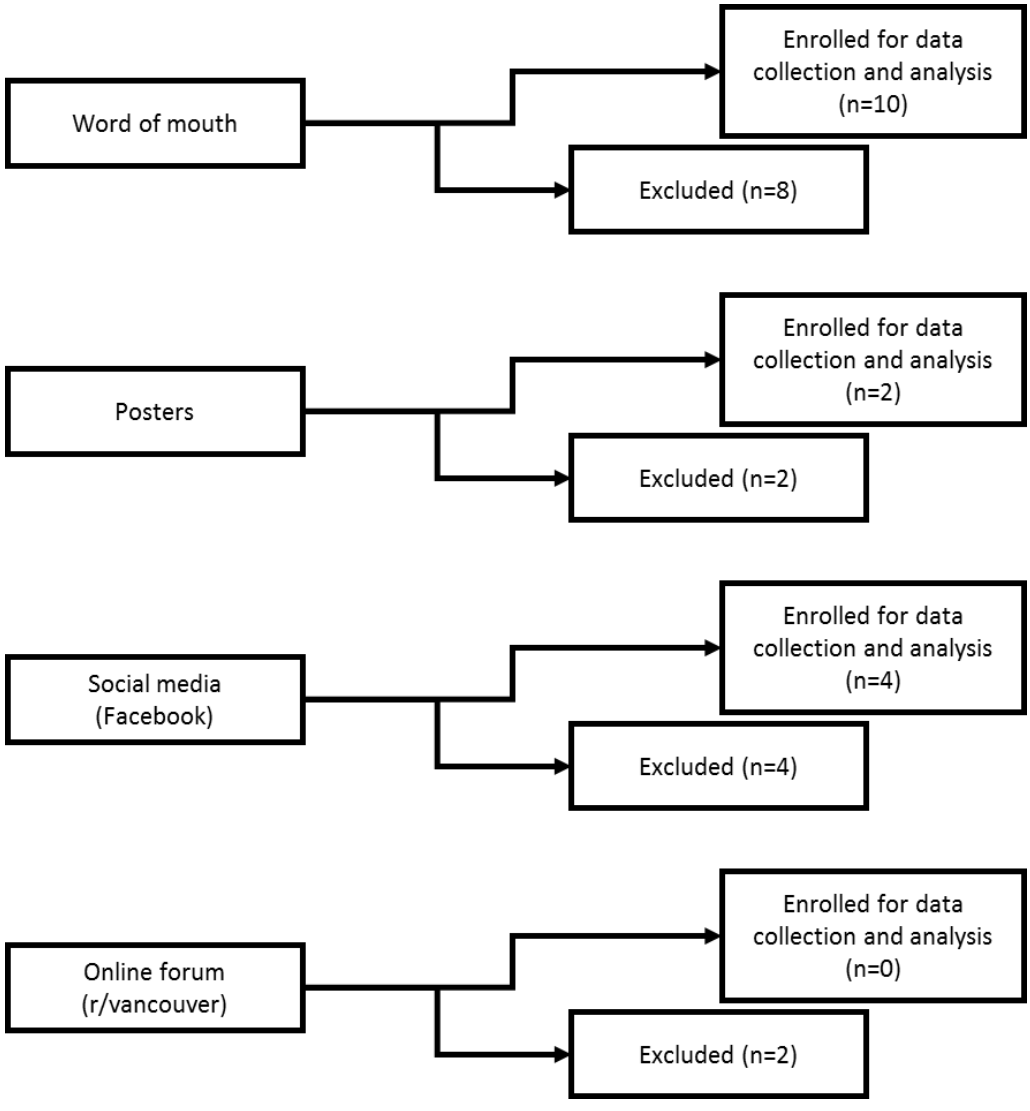

Figure 4. CG participant flow chart.

2.3 Data Collection

2.3.1 Study Visits

Each participant was required to attend a single study visit at CHHM (Vancouver, BC), which lasted no longer than 90 minutes. The participants were asked to avoid moderate to vigorous physical activity 48 hours prior to the appointment. Upon arrival, the participants were introduced to the researchers and familiarized with the data collection process. The participants were screened again for previous AT or lower limb injuries and any other conditions that could affect the participant's gait and musculoskeletal properties. Written and informed consent was collected from each participant prior to any data collection. The participants' anthropometrics (height and weight) were collected, along with age, self-reported dominant leg and activity levels. Previous AT pain that couldn't be attributed to exercise habits was also noted. The participants were then monitored through the walking trials using modified methods developed by Lichtwark & Wilson (2005) (Figure 5).

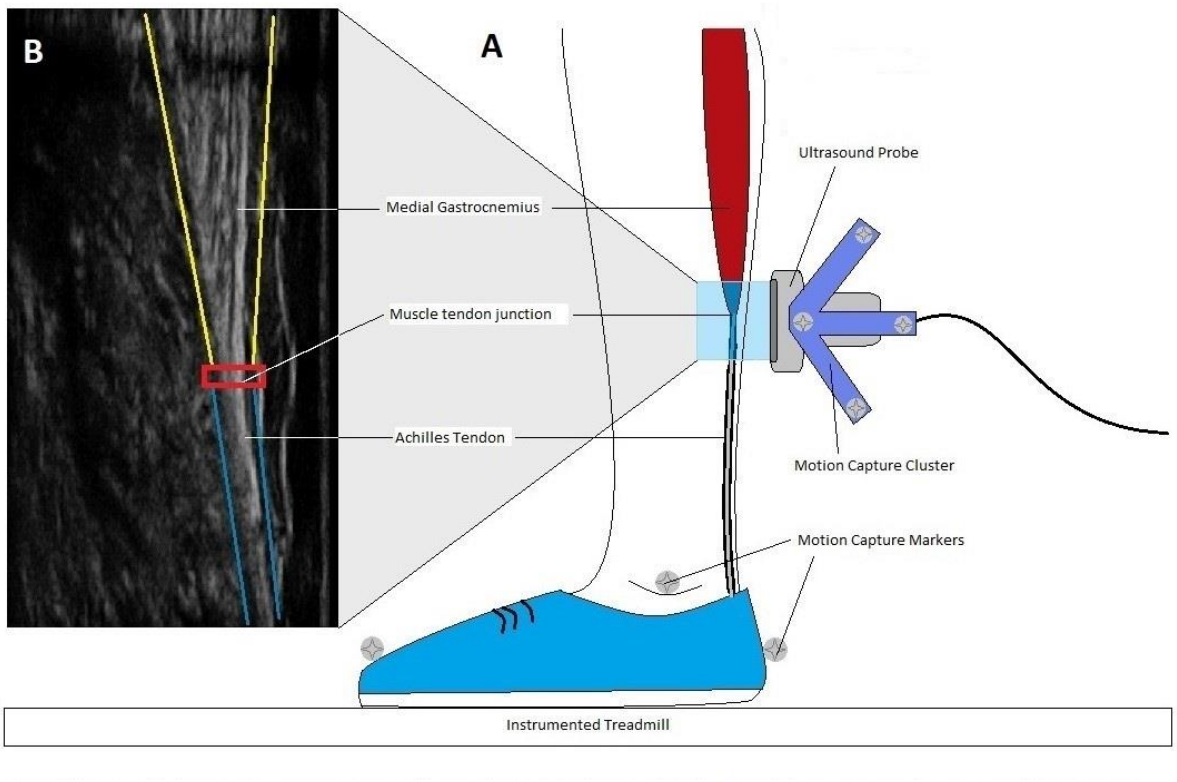

Figure 5. (A) Methodological set-up of ultrasound and motion capture markers. (B) Ultrasound visualization of the GM, MTJ and AT to quantify AT displacement during data collection.

2.3.2 Motion Capture

To provide lower limb kinematic data, passive motion capture markers were tracked in 3D space by a calibrated infrared motion capture system (Cortex 5, Motion Analysis, Santa Rosa, CA, USA). Motion capture markers and a custom-made ultrasound probe housing were fixed to the participant's dominant leg using Transpore™ tape (3M, St. Paul, MN, USA). Motion capture markers were fixed to the hallux, calcaneus (marker was placed on exterior of shoe, the distance between skin and marker was measured with a caliper and calculated into the AT moment arm

length), medial and lateral malleoli, head of the fibula, and lateral epicondyle of the femur. A four-marker cluster was also permanently fixed to the ultrasound probe housing.

2.3.3 Ultrasound

B-mode ultrasonography with a 60 mm linear array probe (RP Sonix, Ultrasonix, Burnaby, BC, Canada) was used to measure two-dimensional displacement of the GM muscle-tendon junction (MTJ) during the stance phase. The ultrasound was set to 10 MHz scanning frequency, 60 Hz sampling frequency at a maximum depth of 3.0 cm. The ultrasound image was synchronized to analog and lower limb kinematic data with a pulse sent from a synchronizing trigger. The ultrasound probe housing was fixed to the participant's skin over the GM MTJ, medial to the septum of the gastrocnemius lateralis, on their dominant leg. In order to find the correct location for the ultrasound housing, the MTJ had to be visible in frame during triceps surae contraction upon fixing of the ultrasound housing. A thin strip of Nexcare™ (3M, St. Paul, MN, USA) tape was used as echo-absorptive marker. The thin strip of echo tape was placed perpendicular to the probe interface, reflecting ultrasound waves, which makes a reference point in the event of probe displacement in relation to the participant's skin. The echo tape was fitted to the participant's skin prior to the fixing of the ultrasound housing. The ultrasound output an image of the displacement of the GM MTJ that was tracked manually with Tracker (v4.9.8, physlets.org/tracker).

2.3.4 Instrumented Treadmill and Protocol

A split-belt instrumented treadmill (Bertec, OH, USA) collected ground reaction forces (GRF) at 1500 Hz during walking trials. The motion capture system is calibrated and synchronized with the treadmill so that the GRF and center of pressure align with gait kinematic events. To ensure that treadmill gait is as natural as possible, the participants were asked to wear their own exercise footwear throughout data collection. The participants were made familiar with the treadmill and the trial protocol in advance of locomotion. Due to the nature of the split belt treadmill and tendon biomechanics, participants engaged in an acclimatization period (8-10 minutes) at a preferred walking pace before commencing data collection (Hawkins et al., 2009). Preferred walking pace was determined by incrementally increasing the speed of the treadmill, while the participant was asked for feedback on comfort. When the participant described the pace as too fast, the treadmill belt speed was lowered until the participant designated the pace as comfortable and of a normal speed that they walk during their everyday life (described as a pace that the participants walk to the grocery store-- purposeful, but not a rush). At a level, preferred walking pace, the tendon provides the majority of the displacement within the muscle tendon unit (MTU), enhancing force production, which allows the muscle to do less work by working quasi-isometrically (Takeshita, 2006).

To keep the participant ambulating in the correct position, their vision was directed to a vertical line in front of them (4 meters), which was set in accordance with the split of the treadmill belts. By watching the line, the participant minimized the likelihood of contaminating ground reaction

forces with their non-dominant foot and diverted their attention away from the walking task to encourage natural walking.

2.3.5 Control Group Protocol

The CG participants engaged in the same protocol to the FH participants in order to follow a direct comparison using natural walking pace.

2.3.6 Potential Data Collection Errors

During pilot testing, some potential areas for error were identified. Due to the complexity of the systems involved with data collection, the participant and the devices were monitored to ensure that data would be as complete as possible.

- MC markers not being visible by the IR cameras. Markers can be hidden by wires, clothing and limbs. To ensure this is minimized, wires and loose clothing were comfortably taped in place.
- Reflectors on participant's clothing that may interfere with MC marker tracking. Prior to marker placement, the participants clothing and shoes were checked for reflective areas. If clothing caused reflection, the area would be masked with tape.
- Ultrasound slippage in relation to the participant's skin or unclear ultrasound image. Echo absorptive tape was placed within the image of the ultrasound to display if there was any slippage during ambulation. To check for an unclear image, prior to data collection, the

participant was asked to jog and hop on the spot. During which, the ultrasound image was monitored to ensure that the MTJ is visible at all times.

- Improper foot placement on the split belt treadmill. During data collection, the GRF was monitored to indicate if the participant is splitting stance phase GRF between the split belts.
- Researcher error is a possibility in the placement of MC marker, which would influence the coordinates of virtual markers. Bony protrusions were used as landmarks for marker placement.

2.4 Analysis and Outcomes

2.4.1 Virtual Markers

In order to calculate AT force and MTJ 3D position, two virtual markers (VM) were produced within the motion capture software (Cortex 5, Motion Analysis, Santa Rosa, CA, USA). VMs were based on the positions of the motion capture markers and the ultrasound cluster. The first VM represented the ankle joint center, which was calculated from the positions of calcaneus, lateral malleolus and hallux markers. The VM for the ankle joint center is required to calculate the ankle joint plantar flexor moment, which in turn is used forthe calculation of AT force. The second VM was based on the marker cluster attached to the ultrasound probe housing. The probe VM represents the superior edge of the ultrasound scanning array. The probe VM acts as a reference point to transform the 2D coordinates of the MTJ from the ultrasound image into 3D coordinates.

2.4.2 Gait events

Gait cycle and stance phase events were obtained from the GRF from the instrumented treadmill. A gait cycle is defined as starting with initial contact of heel strike and finishing with the subsequent heel strike of the same leg. The stance phase is defined as the initial contact of heel strike to the toe off of the same leg. The GRF of stance phase is separated into four segments, brake I, brake II, push I and push II, shown in the example in Figure 6A (Ishikawa, 2005). Initial contact is defined as the vertical portion of the GRF exceeding two standard deviations of its baseline. Eight gait cycles were analyzed for each trial in a custom-written analysis program (Matlab v14, Mathworks, Cambridge, United Kingdom).

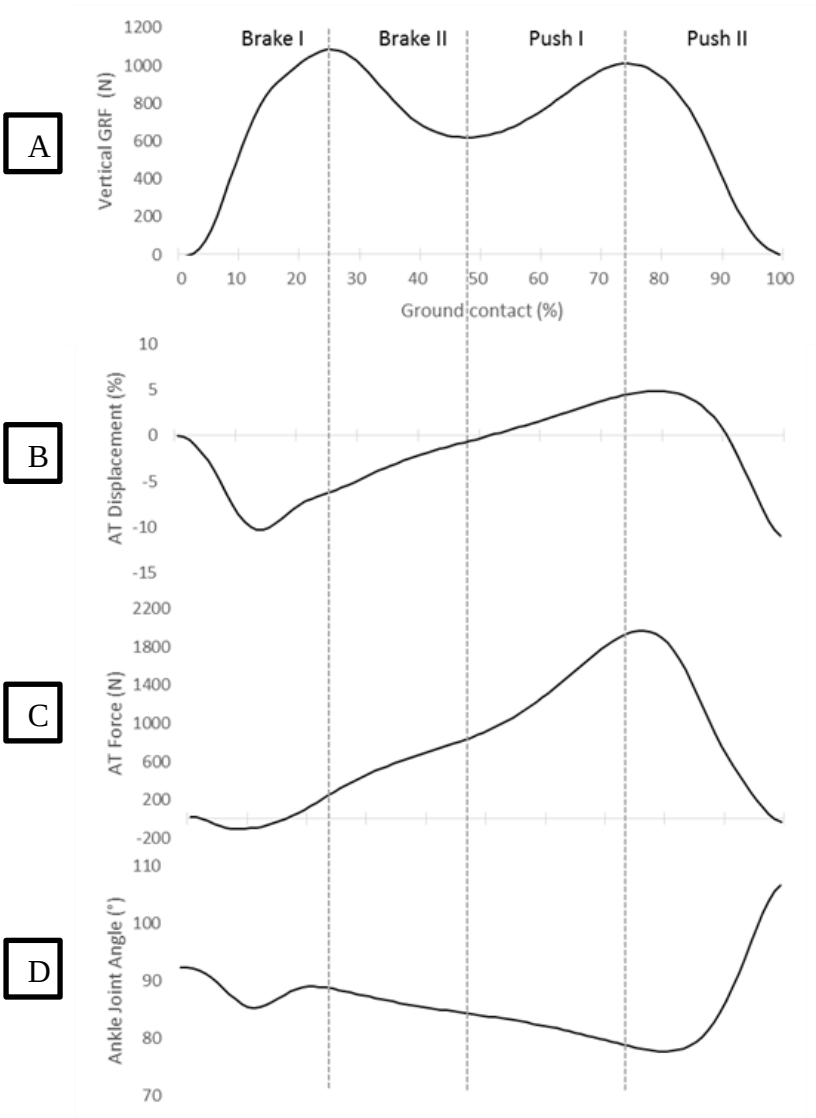

Figure 6. Example of vertical ground reaction force (A), AT displacement (B), AT force (C), and ankle joint angle (D) in accordance with ground contact events, brake I, brake II, push I, and push II. GRF and AT displacement are displayed as a percentage of maximum. AT force is displayed in N. Ankle joint angle is displayed in degrees. Heel strike transires at 0% and toe-off at 100% ground contact.

2.4.3 AT Length Calculation

Instantaneous tendon length change was calculated using a custom-written analysis program (Matlab v14, Mathworks, Cambridge, United Kingdom). The AT length was calculated as the distance from the calcaneus to the instantaneous 3D coordinates of the GM MTJ. GM muscle length was calculated as the distance from the 3D MTJ coordinates to the lateral epicondyle. Figure 4B shows an example of AT displacement in relation to ground contact events during the stance phase.

2.4.4 AT Force Calculation

In order to obtain the AT force, four variables are required; the ankle joint center position, the GRF, center of pressure, and the AT moment arm length. The ankle joint center is the position of the center of rotation and was determined by a VM, as previously described. The plantar flexor moment was calculated in the sagittal plane using instantaneous GRF components, center of pressure and ankle VM coordinates. The AT moment arm was calculated as the perpendicular distance from the ankle joint VM to where it intersects the AT line of force action (calcaneus to MTJ coordinates). Under the assumption that the AT was the sole contributor to the plantar flexor moment, AT force was equal to the instantaneous plantar flexor moment divided by the AT moment arm length. An example of AT force during the stance phase is displayed in Figure 4C. AT force was calculated in the custom-written analysis program (Matlab v14, Mathworks, Cambridge, United Kingdom).

2.4.5 Outcome Measures

Stiffness is the primary outcome of the study. Stiffness is the extent to which the tendon resists deformation during an applied force. As seen in previous literature altered stiffness can be a precursor for increased tendinopathies (Arya & Kulig, 2010). Strain is the tendon's change in length as a response to loading. Hysteresis represents the tendon's stored energy lost to heat due to friction.

AT, stiffness, strain and hysteresis were also calculated using the custom-written analysis program (Matlab v14, Mathworks, Cambridge, United Kingdom). Stiffness was defined as the slope of the AT force-deformation curve between 50-100% of peak AT force. Strain was defined as peak AT displacement with the initial AT length at heel strike. Elastic energy stored was calculated as the area under the loading portion (ascending) of the force-deformation curve. Elastic energy returned, during AT recoil, was calculated as the area under the unloading portion (descending) of the force-deformation curve. The AT elastic energy lost to hysteresis was the difference between the elastic energy stored and the elastic energy returned.

2.4.6 Filters

Noise in data was filtered out with a low pass Butterworth filter with cutoff frequencies of 8Hz (motion capture data), 20Hz (analog data) and 20Hz (force data). The cutoff frequencies were determined by residual analysis.

2.5 Statistics

It was determined, from a sample size calculation, 18 FH participants and 18 control participants will required. Due to a lack of research on the impact of cholesterol on the biomechanics of the human AT, unpublished data from a previous study using stiffness values from high cholesterol and control mice (10.47±4.98 and 13.07±4.02 N/mm, a 20% difference, respectively) was used to calculate the sample size (Grewal et al., 2014). The control mice's stiffness values were normalized (116±24 N/mm) to pilot data from the current study's FH participant's stiffness (93±37 N/mm). A level of significance of 5% ($p=0.05$) and a power of 80% was used to determine that 36 participants will be required.

Multivariate analysis of covariance (MANCOVA) tests were used to test for statistically significant differences between the dependent variables of the FH and CG groups while accounting for the covariance of age, BMI, gender and walking velocity. To ensure that the data fit with the MANCOVA model, the data was tested for missing values, outliers, linear relationships between covariate and dependent variable, distribution of covariate across groups, homogeneity of regression for the covariate. Significance was established at $p \leq 0.05$.

2.6 Reliability Testing

Following the same protocol for all participants, reliability testing was performed on a single participant on July 26th, 2018. The reliability participant consented to three data collection bouts. The participant had their data collected following the walking trial protocol, and then would have

the full “set-up” removed from their body. They were asked to wait for 35-45 minutes and all visible signs of the equipment on their leg was removed. The equipment was reattached to the participant’s leg for the next data collection. The walking speed was consistent in each trial. Analysis, digitizing and tracking of the MTJ was done on different days in order to minimize recall. This was repeated for three data collections, in order to determine if the researcher’s equipment placement was consistent leading to minimal variations in the target dependent variable (stiffness). The stiffness values for the three trials were 100, 97, and 93 N/mm. These variations were consistent with those seen with Lichtwark and Wilson (2005), who reported 188, 187, 170 N/mm for one leg-hopping trials. Variation could be due to marker and ultrasound placement, as well as small variability in the participant’s gait cycle from step to step.

Chapter 3: Results

32 participants (16 FH participants, 16 CG participants) partook in the study (Table 1). 30 participants had data collection performed on their right leg, and two participants on their left leg.

Participants (n=32)	Gender	Age	BMI	Physical Activity level
FH participants (n=16)	10 males, 6 females	37.4±6.1 years	28.2±4 kg/m²	Moderate (~150 mins/week)
CG participants (n=16)	10 males, 6 females	36.1±6.6 years	26.7±2.5 kg/m²	Moderate (~150 mins/week)

Table 1. FH and CG participant demographics.

The main effects of FH and TXT on tendon stiffness (p=0.001) and hysteresis (p=0.007) were statistically significant, while the effect on tendon strain was not significant (p=0.790). The FH participants displayed an average strain: 5.07±.85%, stiffness: 87±20 N/mm and hysteresis: 56±17%, while the CG participants displayed an average strain: 4.95±0.9%, stiffness: 111±18 N/mm and hysteresis: 35±12% (Table 2). MANCOVA test results are shown in Supplementary Table 3.

Participants	Strain	Stiffness*	Hysteresis*	Walking velocity
FH participants	5.07±0.9 %	87±20 N/mm	56±17 %	1.17±0.1 m/s
CG participants	4.95±0.9 %	111±18 N/mm	35±12 %	1.22±0.1 m/s

Table 2. FH and CG participant average strain, stiffness, hysteresis and walking velocity. * Indicates a significant difference between FH and CG participants (p=0.005).

The CG participants displayed a mean AT stiffness 23.7% higher and a mean hysteresis 45.9% lower than the FH participants. Boxplots of participant strain, stiffness and hysteresis are shown in Figure 7.

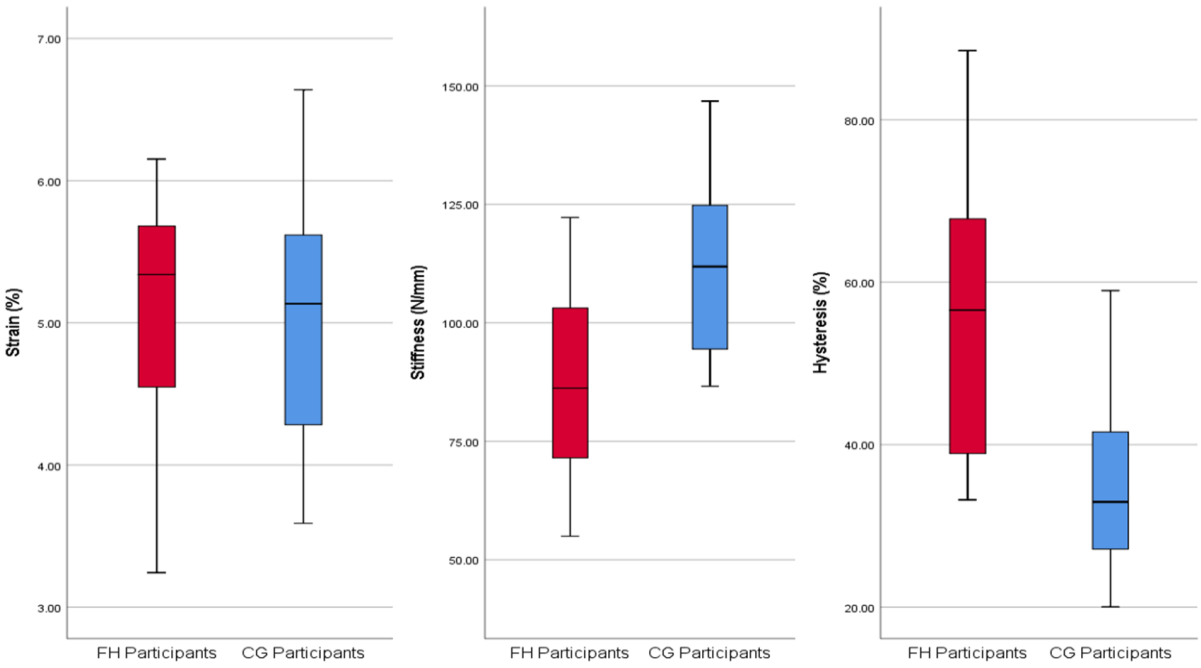

Figure 7. Box plot displaying participant distributions for outcome variables (AT strain [left], AT stiffness [middle] and AT hysteresis [right]).

BMI, walking velocity and age were not significant predictors for stiffness (p=0.183, 0.606, 0.108), hysteresis (p=0.266, 0.231, 0.367), or strain (p=0.781, 0.924, 0.427). Gender was also not a significant predictor for strain (p=0.958) and hysteresis (p=0.593), however gender was a significant predictor for stiffness (p=0.038, $\eta 2$=0.156).

Figure 8C presents AT loading during the stance phase, which revealed that the FH participants on average display reduced unloading of the AT during the brake I phase, just after heel strike, followed by a greater rate of loading into the brake II phase. Figure 8D displays loading rates for both groups. FH participants displayed a higher mean maximum loading rate in the brake I and push I phases (FH: 6.64 and 8.82 kN/sec, CG: 5.01 and 7.23 kN/sec, respectively), as well as a lower minimum loading rate in the brake II (FH: 0.1 kN/sec, CG: 2.68 kN/sec).

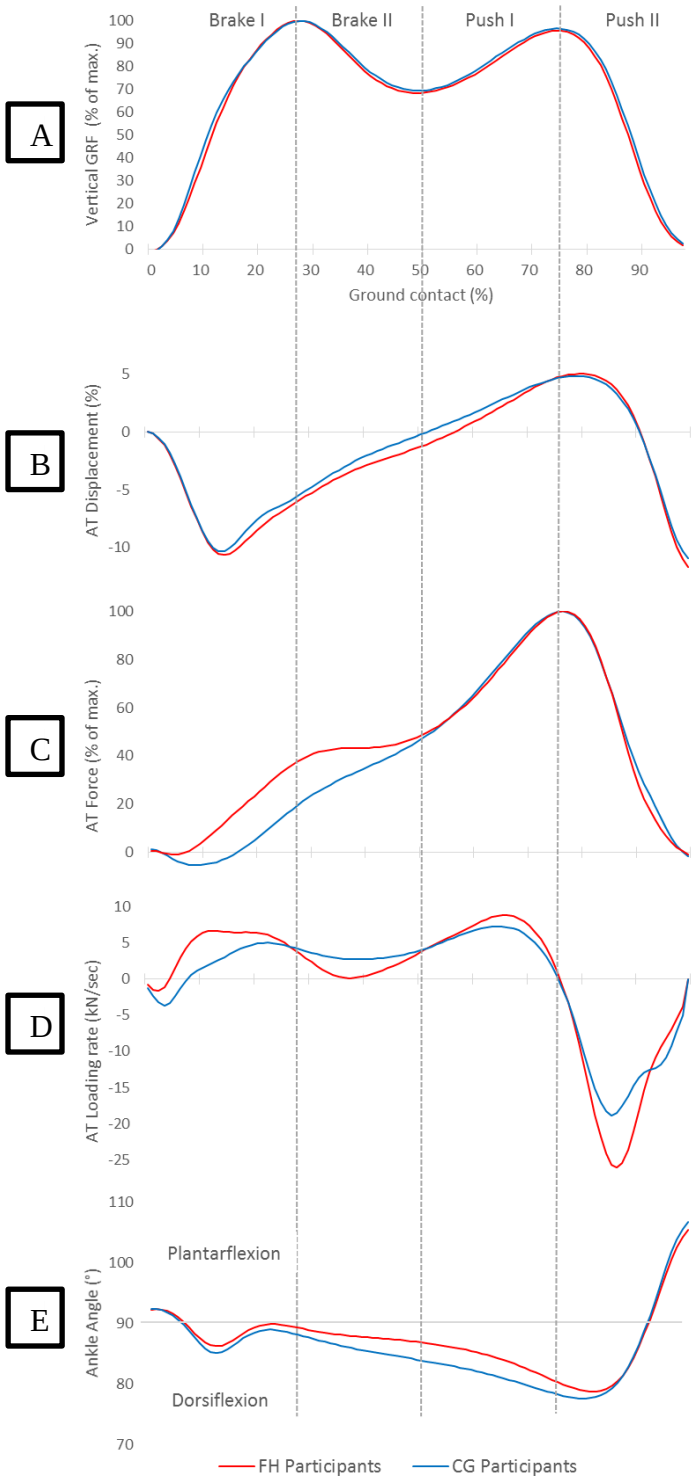

Figure 8 A-E. Participant vertical GRF (A), AT displacement (B), AT force (C), AT loading rate (D), and ankle joint angle (E) in accordance with ground contact events, brake I, brake II, push I, and push II.

The FH and CG had similar mean MTU lengths, 488±27 and 498±35 mm, respectively. The AT made up 44% of the FH group's MTU length and 42% of the CG participant's. FH participants displayed muscle displacement of 29±13% of the MTU length, compared to the CG participant's 23±9%. he majority of the MTU displacement was due to AT displacement.

Figure 9 exhibits the differences in force-deformation curves for the two groups. The FH participants display high initial loading and increased hysteresis which is represented in the curves as the area between the ascending and descending curves. The CG participants display a greater stiffness which is represented by the steeper slope of linear portion of the ascending curve. Energy stored is represented as the area under the ascending portion of the curves, and energy returned is represented as the area under the descending portion of the curve.

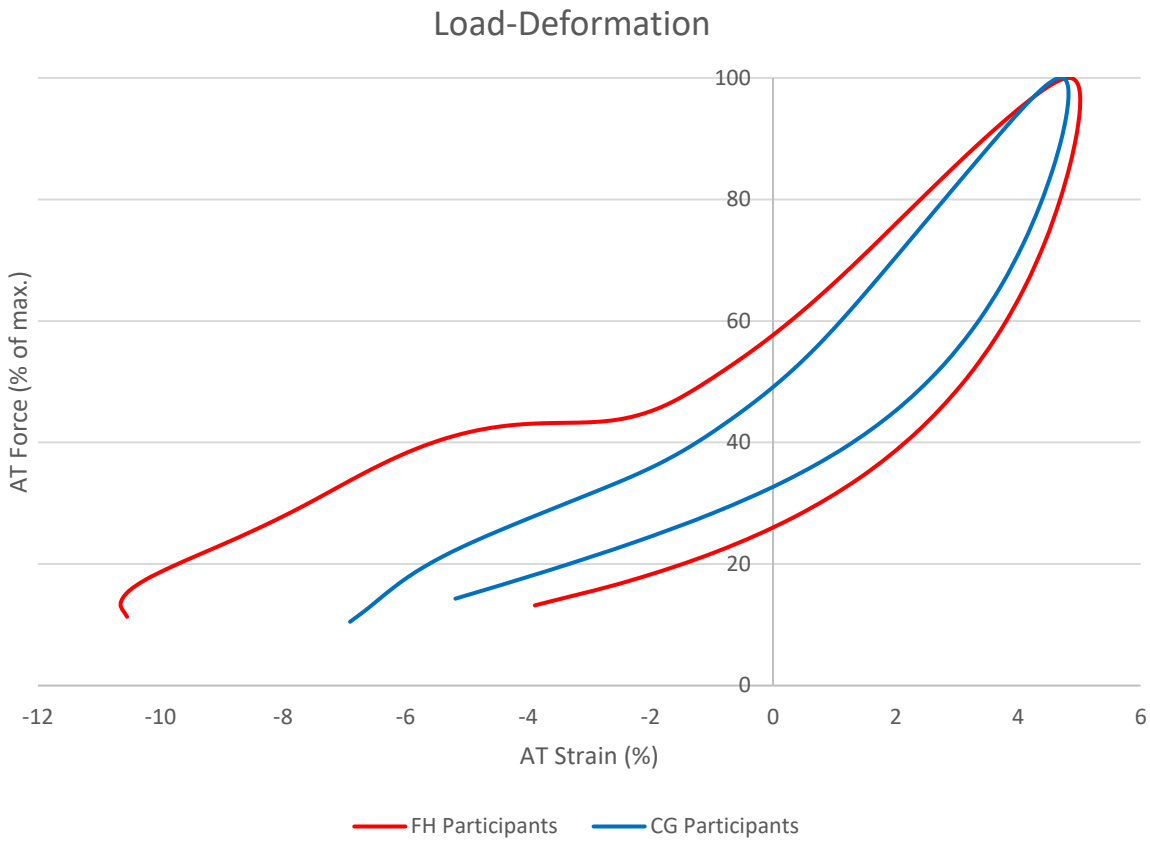

Figure 9. Force-deformation curve for FH participants and CG participants. Tendon deformation displayed as strain (%). The AT force is displayed as percent of maximum force in order to standardize the two curves to allow comparison. AT force percentage values below 200 N were excluded.

FH participants showed a higher average maximum tendon load than the CG participants (2600±641 N and 2061±461 N, respectively), displayed in Figure 10.

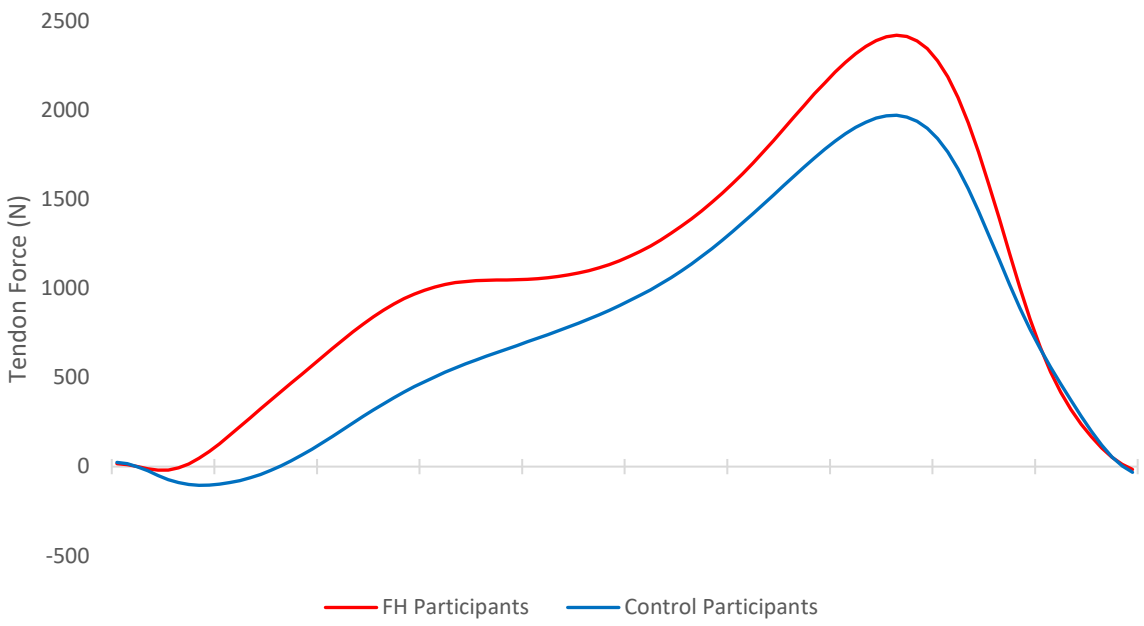

Figure 10. AT force (N) during the stance phase.

Chapter 4: Discussion

The current study measured the AT biomechanical properties in FH participants compared to the CG, measured during cyclic loading (walking). Due to cholesterol accumulation in the tendon, we hypothesized that the FH participant's AT would have decreased stiffness, increased hysteresis and increased strain. As predicted, we found that the FH participants had reduced material stiffness and increased hysteresis, confirming part of our hypothesis. However, the FH participants did not show a significant difference for strain values. Biomechanical testing of the FH participants revealed the significant effect of cholesterol accumulation in the AT. Previous data has shown that cholesterol accumulation results in less homogenous collagen fibre size, disruption of collagen alignment, TXT formation in areas of high stress, increased vascularity and increased LDL oxidation leading to inflammation (Józsa et al., 1984; Ronnemaa et al., 1975; Soslowsky & Fryhofer, 2016). These disruptions alter the mechanical properties of the tendon, potentially resulting in less efficient gait and a higher rate of injury.

4.1 Gait Efficiency

During locomotion, the AT consistently acts as storage and provider of plantar flexion energy. In ideal circumstances, the triceps surae contract near isometrically, while the majority of the work placed on the AT from ground contact is returned during the plantarflexion of the ankle at push off (Fukunaga et al., 2001; Ishikawa, 2005). This underlines the importance of the AT, demonstrating that the AT operates to minimize the expenditures of the triceps surae. The results of the current study show that FH participants had a significantly higher AT hysteresis than the

CG participants. The reduced return of stored energy could lead to an increased metabolic cost of locomotion in people with FH by demanding more work from muscles of the calf, knee and hip (Sawicki, Lewis, & Ferris, 2013). The FH participant's AT energy efficiency or energy returned was ~44%, while the CG participant's AT energy returned was ~65%. To add to the argument, the FH participants' muscle contributed 29±13% of the overall MTU displacement, compared to the CG participants' 23±9%. This could suggest that the compromised energy saving abilities of the AT are compensated in FH participants by increased muscle work. However, because there was not a significant difference (exploratory t-test, $p=0.13$) between the two groups, this hypothesis will need to be tested in a future study to fully explore the phenomenon.

FH participant hysteresis increases could be due to the cholesterol accumulation increasing tendon CSA, which results in more friction against the tissues surrounding the tendon (Kruth, 1985). Another possible contributor to the hysteresis increase, as well as stiffness decease, is the disorganization and variation in size of collagen fibres; due to changes in protein synthesis, extracellular matrix composition and turnover (Soslowsky & Fryhofer, 2016). These microstructural changes could lead to more friction between non-parallel, unaligned, collagen in the tendon.

4.2 Past Research Comparison

As previously mentioned, prior literature concerning the effects of high cholesterol on tendon mechanics is limited to animal models. However, it is interesting to compare results from the FH participants to other disorders that affect tendon mechanics. Patients with AT tendinopathy and

diabetes also show reduced AT energy saving properties. Patients with diabetes exhibit thickening of the AT, as well as, increased density, misalignment of collagen fibres and calcification (Batista et al., 2008). Cronin et al. (2010) reported that diabetic changes in the AT may cause higher stiffness and reduced energy storage due to attenuated AT length changes. Glycation of collagen fibres and non-enzymatic cross-linking increases stiffness by restricting the movement among fibers(Reddy, Stehno-Bittel, & Enwemeka, 2002). The stiffness increase may also restrict and decrease the ankle joint rotation which results in altered gait patterns. The diabetes patients display a different form of impairment, however the result may be similar to FH participants, with a less efficient push II phase where the AT is supposed to rapidly recoil to return the majority of stored energy.

Similar to the current study's main findings, tendinopathies of the AT also display changes to the mechanics of the tendon. Tendinopathic ATs display decreased stiffness, increased strain and CSA, signifying decreased energy storage and return (Arya & Kulig, 2010). Arya and Kulig also suggest that the altered morphology puts the AT at risk for additional injury and extended recovery time. Tendinopathic tendons have increased matrix separation, reduced expression of type I collagen and increased type III collagen, which is fundamentally weaker (Paavola et al., 2002). Due to the reduction in stiffness, the tendon is unable to resist higher forces which would result in the tendon being subjected to higher strains and micro trauma (Arya & Kulig, 2010). Despite the differences in morphological structures, the FH participants of the current study portray similar biomechanical effects from the cholesterol accumulation compared to tendinopathy. In conjunction with hypercholesterolemia research from Mathiak et al. (1999),

Ozgurtas et al. (2003), and Abboud & Kim (2010), our research suggests that patients with FH show mechanics that could put them at higher risk for tendinopathies.

4.3 Loading Differences between FH and CG participants

The FH participants showed some interesting results in terms of their tendon loading. FH participants exhibited less AT unloading in the brake I phase, followed by higher maximum AT force (Figure 6C and Figure 10, respectively). This is apparent from the AT loading rate in Figure 8D; the FH participants had a 32.5% higher maximum loading rate in the brake I phase. The maximum loading rate occurred earlier in the brake I phase as well. The CG participant's AT force displayed the expected pattern, unloading just after heel strike in the brake I phase. The AT is unloaded as the tibialis anterior eccentrically contracts in order to control plantar flexion (Procter & Paul, 1994). The AT is then gradually loaded and lengthened through the brake II and push I phases until rapid recoil in push II (Figure 8C). This motion is described as a catapult action, differing from the spring-like bouncing action in running and hopping, where the energy comes from the initial negative work in the first half of ground contact and is returned in the second half (Ishikawa, 2005). Future research could look into the EMG activity of the anterior tibialis and GM to determine if the FH participants displayed different patterns of muscle activation that would affect the regular unloading of the AT just after heel strike. Unfortunately, this study was unable to explain this phenomenon with regard to EMG.

In order to confirm that the abnormal FH AT loading phenomenon was not due to calculation error in differences between the FH and CG participant's AT moment arm length (ATMA), the

ankle moment was calculated using the FH AT force with the FH and CP ATMA, and CG AT force with the FH and CP ATMA. The values were added into curves for visual inspection of any differences. The curves did not differ from each other, ensuring that the FH AT loading phenomenon was not an error in calculation (Supplementary Figure 12A-B).

4.4 Elongation Profile

There was not a significant difference in strain between the two groups during walking trials, visible in Figure 8B. However, should stiffness be reduced, in theory the tendon will deform more for a given force (Arya & Kulig, 2010). Perhaps the forces during the walking trails were too low to reveal increased deformation. Future research could be conducted using one leg hopping or maximal voluntary contraction trials that induce high loading rate or high load, and perhaps in these conditions a significant difference between the deformation of high cholesterol tendons and controls could be witnessed.

4.5 Pain and Inflammation

AT pain and inflammation is fairly common in patients with FH. They are 6.75 times more likely to have AT pain than the general population (Beeharry et al., 2006). In the current study, it was not surprising to learn that 44% of the FH participants exclaimed that they had experienced past AT pain for consecutive days without an obvious mechanism of injury. One study, concerning the detection and treatment of xanthoma (73 patients with heterozygous type II hyperlipidemia), found that 18% of the patients had AT pain (Tsouli et al., 2005). Another study

reported 46.6% of patients with FH had one or more incidents of AT pain that lasted 3 or more days (Beeharry et al., 2006). Pain and inflammation could be explained by tenosynovitis (inflammation of the synovium surrounding the tendon) (Beeharry et al., 2006; Soslowsky & Fryhofer, 2016). FH patients with TXT have higher plasma levels of cytokines (TNF-alpha, IL-8 and IL-6) due to altered foam cell gene expression and oxidized LDL, leading to increased inflammation (Martin-Fuentes et al., 2009).

4.6 Covariates

Similar to results from past studies, we found that gender was a significant predictor of tendon stiffness. The CG and FH males had higher stiffness values than the females of the same group (CG males: 116±17 N/mm, CG females: 102±17 N/mm; FH males: 92±23 N/mm, FH females: 80±15 N/mm). Male's tendons tend to have a greater CSA and be stiffer to accommodate larger muscle forces (Muraoka et al., 2005). Females also have higher levels of estrogen, which can inhibit hypertrophy of the tendon after exercise (Hansen & Kjaer, 2014).

BMI and age have also been known to affect tendon physiology and mechanics (Kubo, Kanehisa, & Fukunaga, 2003; Taş et al., 2017). However, the current study did not find BMI and age to be to be significant predictors for strain, stiffness and hysteresis. The current study's sample size was not powered to test this hypothesis. Future research could be undertaken in this area in the form of a larger, longitudinal, study. This could enable us to obtain data just after patient diagnosis, and ask questions relating to the impact of cholesterol on tendons at diagnosis, as well as the impacts of statin treatment over time.

The participants were instructed to walk at their preferred pace for the walking protocol. The FH group did walk slightly slower than the CG (1.17±0.1 m/s and 1.22±0.1 m/s, respectively), however the difference in walking pace was deemed not significant. Walking pace was not a significant predictor for the stiffness, hysteresis, and strain (Appendix Table 1). Efforts to match walking speed would require participants to walk at unnatural velocities, which is known to affect balance and muscle activation leading to altered muscle-tendon length and loads (Cronin et al., 2009). Small alterations in walking velocity do not render a comparison between groups inappropriate, given that dramatic differences in loading rates are required in order to see changes in tendon stiffness (Cronin et al., 2010; Lichtwark & Wilson, 2008).

4.7 Testing Conditions

It is important to show caution when comparing results from different models and dissimilar testing conditions. Beason et al. has conducted multiple studies concerning the impact of high cholesterol on tendons of porcine, mice, rats and monkeys (Beason et al., 2004; D. P. Beason et al., 2013). In keeping with the current study, Beason et al. (2004) found that there was a significant decrease in in-vitro bicep tendon stiffness of porcine. Even with their small sample size, they concluded that the hypercholesterolemic porcine tendons were severely compromised. The second study found that in-vitro, supraspinatus tendons of high cholesterol mice, rats and monkeys had elevated stiffness when compared to controls, differing from the current study (Beason et al., 2013). Grewal et al. (2014) found that tendons of ApoE -/- mice (simulating hypercholesterolemia) on a high cholesterol diet had increased tendon failure at lower loads

when comparing to C57BI/6 (controls) mice. Grewal et al. did not find a significant difference in stiffness values between the ApoE -/- and C57BI/6 groups. However, the ApoE -/- mice with the high fat diet has significantly lower stiffness when compared to the C57BI/6 mice with the regular diet. The differences in results between the animal studies could be due to the variations in the species, the functions of the tendons, and the testing conditions (Soslowsky & Fryhofer, 2016).

This is the first study that has used in-vivo methods of testing the effects of cholesterol on the AT, therefore the results cannot be directly compared to in-vitro conditions. In-vitro tendons are clamped in testing rigs, and may be stored or preserved in different ways, which could cause the tendon samples to display altered properties (Maganaris et al., 2008). In-vivo testing allows the tendon to be observed in its natural position, which receives support from surrounding tissues and therefore the tendon will display different mechanics than in-vitro testing. There are multiple ways of testing the AT using an in-vivo ultrasound image of the GM MTJ, e.g. dynamometer, force platforms, etc. Each methodological condition will output results that may or may not be comparable to the other (Maganaris et al., 2008). For example, the methods of the current study, were to use a force platform and motion capture, to allow for testing during a normal activity in which the participant walked as naturally as possible. The results showed that the CG, the normal participants, displayed average stiffness and hysteresis values of 111±18 N/mm and 35±12%, respectively. When comparing the current results to other studies that have different testing conditions, there are a wide range in AT values for stiffness (17-760 N/mm) and hysteresis (4-45%) (Finni, Peltonen, Stenroth, & Cronin, 2012; Lichtwark & Wilson, 2005; Maganaris, 2002).

Because of the inter-study methodological differences, it is important that the methods within the study are systematic in order to identify differences between the focus group and the controls.

4.8 Clinical Significance

The findings from the current study are relevant to FH, high cholesterol disorders, as well as clinicians. Physical activity can be an important aspect to the management of FH and high cholesterol disorders, so it is imperative for the patients to be able to exercise without interruption due to injury. However, results from the current study, as well as past studies, have shown that the patients could be at risk for tendinopathies (Mathiak et al., 1999; Ozgurtas et al., 2003). The study's findings could assist clinicians in the creation of physical activity guidelines for those with lipid disorders. Guidelines could suggest certain safe activities as well as activities to avoid that have high rates of tendon loading. Furthermore, future research should be undertaken to determine if targeted exercises could reverse some of the negative impacts of cholesterol in tendons. Results from the proposed studies could be incorporated into hypercholesterolemia physical activity guidelines.

4.9 Limitations

Due to this unique form of testing the AT, systematic errors could have been introduced through the methods and analysis. We assume that the methodological systematic errors in calculation and measurement are minor and did not have serious effects on the interpretation of the results.

Our calculations were based on the calculation of the plantar flexion moment in solely the sagittal plane, as a result we were not able to take into account forces that may be acting from other planes.

When calculating the AT length, we measured from the calcaneus marker (minus shoe thickness) to the instantaneous coordinates of the MTJ. Unfortunately, the calculation does not account for the inherent anterior curve of the AT.

In order to acquire a clear image of the MTJ, the US probe had to be slightly rotated to follow the pennation of the GM fascicles. According to Lichtwark and Wilson (2005), this could lead to an overestimation of the AT strain by ~1.08% at initial load and final unloading. Because of the timing of the overestimation, stiffness would be unaffected (Lichtwark & Wilson, 2005).

We encouraged the participant to walk as naturally as possible, however their gait could have been unavoidably altered due to the differences in walking on a treadmill with data collection equipment attached to their dominant leg. We attempted to minimize the impact of the devices by giving the participants an acclimatization period and a visual cue to divert attention away from their feet.

While we did control for hypercholesterolemia length of exposure by recruiting patients with a genetic hypercholesterolemia disorder, we were not able to control the severity of the disorder, length of time on statins or type of statins and other lipid-lowering therapy. The participant's cholesterol levels and size of AT TXT could not be gathered at the time of data collection. As previously mentioned, further longitudinal studies could be undertaken in this regard.

Chapter 5: Conclusion

In conclusion, we found that the AT of the FH participants display altered biomechanical properties, which are likely due to the cholesterol accumulation in TXT. The AT of the FH participants display decreased stiffness and increased hysteresis when compared to controls. Due to the significant hysteresis, the FH participant's AT return less stored energy, suggesting that their gait is less efficient and could require a greater metabolic cost. These findings represent a new contribution to our understanding of how cholesterol accumulation affects the function of the AT in people with FH.

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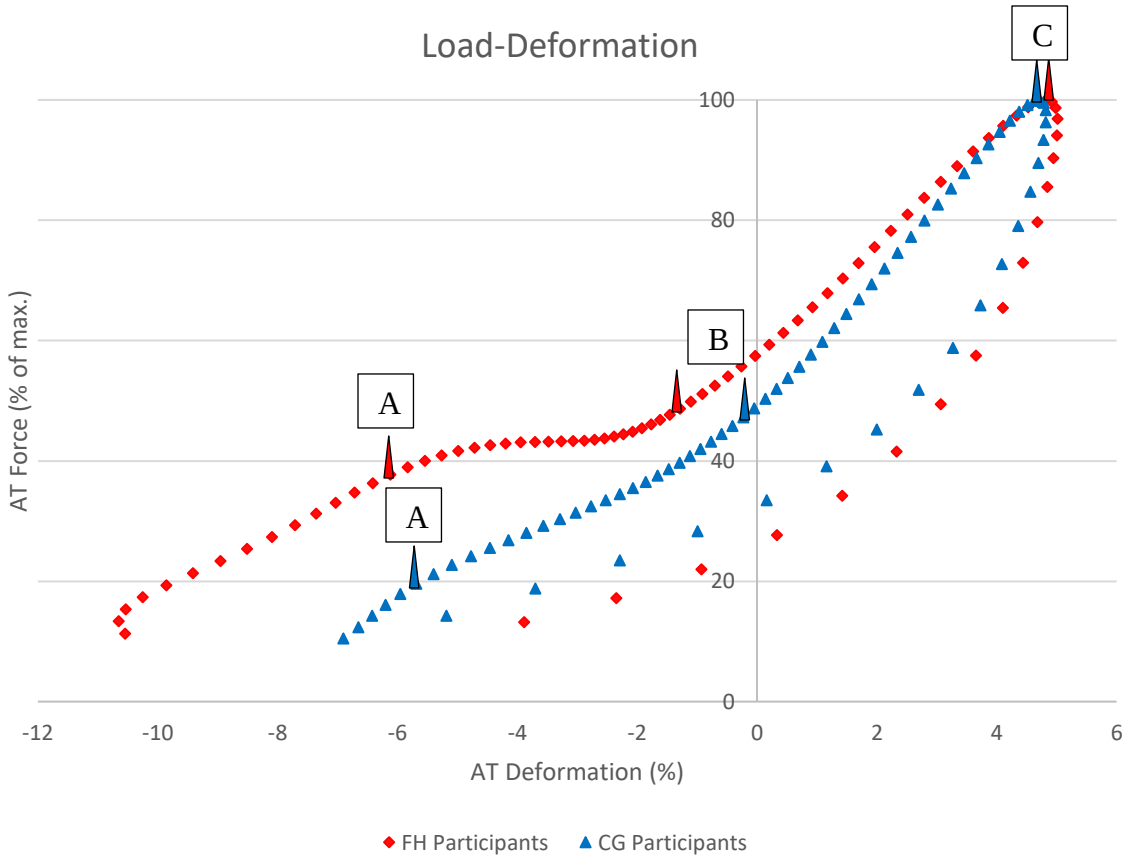
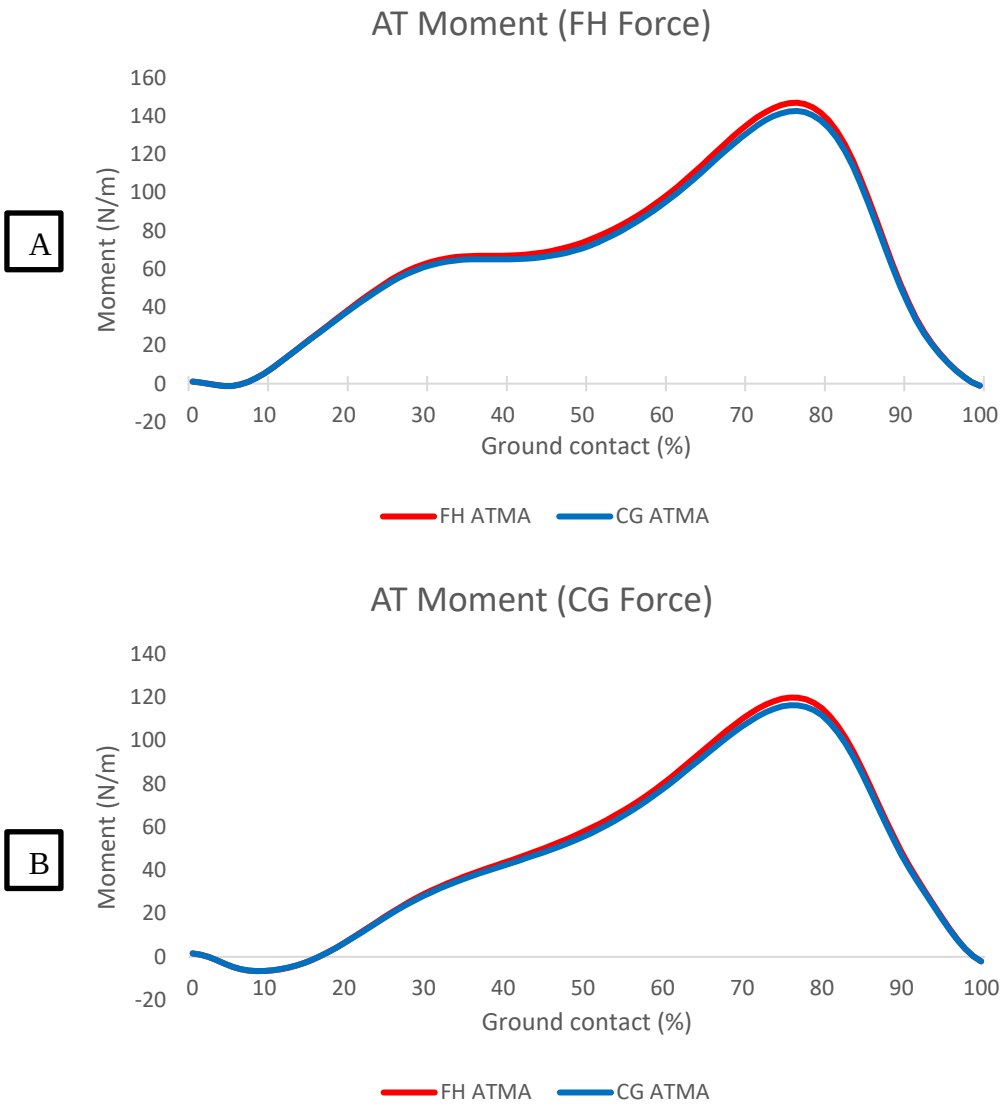
Appendices

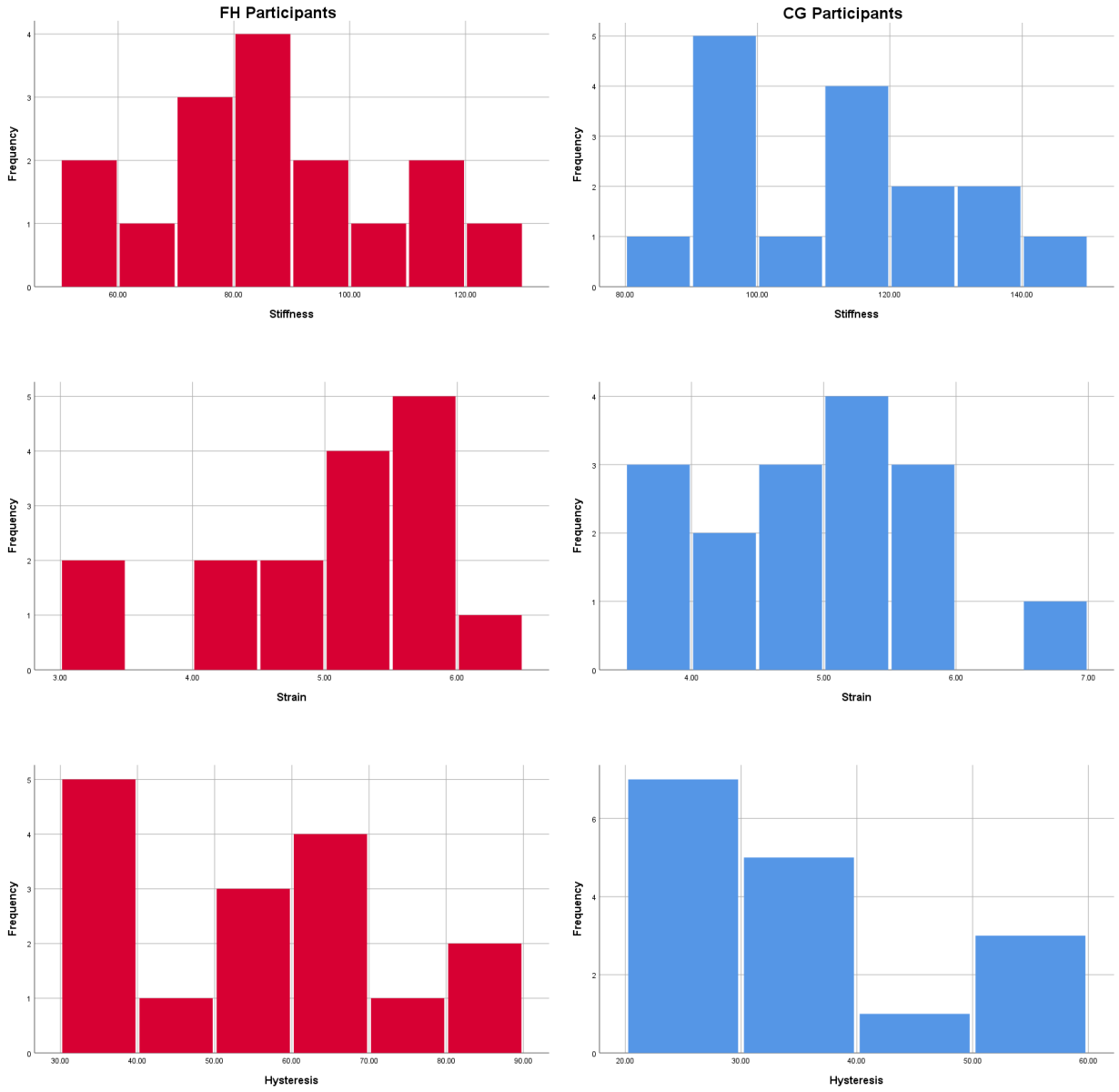
Supplementary Figures



Supplementary Figure 11. Force-deformation curve with stance phases. Tendon deformation displayed as strain (%). The AT force is displayed as percent of maximum force in order to standardize the two curves to allow comparison. AT force percentage values below 200 N were excluded. Stance phases are represented by A (end of Brake I phase and start of Brake II phase), B (start of Push I phase), C (start of Push II phase).



Supplementary Figure 12A-B. (A) AT moment using FH AT force, FH ATMA and CG ATMA. Shows minimal difference in moment with both FH and CG ATMA. (B) AT moment using CG force, FH ATMA and CG ATMA. Both show minimal difference in moment with both FH and CG ATMA.



Supplementary Figure 13. Histograms for FH (red) and CG (blue) distribution for stiffness (N/mm), strain (%) and hysteresis (%).

Supplementary Tables

Source	Variable	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Stiffness	7282.679[a]	5	1456.536	4.777	.003	.479
	Strain	.790[b]	5	.158	.183	.966	.034
	Hysteresis	4303.819[c]	5	860.764	4.045	.008	.438
Intercept	Stiffness	1110.516	1	1110.516	3.642	.067	.123
	Strain	1.509	1	1.509	1.750	.197	.063
	Hysteresis	276.666	1	276.666	1.300	.265	.048
BMI	Stiffness	570.396	1	570.396	1.871	.183	.067
	Strain	.068	1	.068	.079	.781	.003
	Hysteresis	275.017	1	275.017	1.292	.266	.047
Gender	Stiffness	1461.894	1	1461.894	4.795	.038	.156
	Strain	.002	1	.002	.003	.958	.000
	Hysteresis	62.306	1	62.306	.293	.593	.011
Walking Pace	Stiffness	83.082	1	83.082	.272	.606	.010
	Strain	.008	1	.008	.009	.924	.000
	Hysteresis	320.099	1	320.099	1.504	.231	.055
Age	Stiffness	845.433	1	845.433	2.773	.108	.096
	Strain	.562	1	.562	.652	.427	.024
	Hysteresis	179.093	1	179.093	.842	.367	.031
Group	Stiffness	4264.023	1	4264.023	13.985	.001	.350
	Strain	.062	1	.062	.072	.790	.003
	Hysteresis	1789.309	1	1789.309	8.409	.007	.244
Error	Stiffness	7927.636	26	304.909			
	Strain	22.425	26	.862			
	Hysteresis	5532.452	26	212.787			
Total	Stiffness	329636.971	32				
	Strain	828.107	32				
	Hysteresis	76849.676	32				

Supplementary Table 3. MANCOVA results for participants. Dependent variables (stiffness, strain and hysteresis), covariates (BMI, gender, walking pace and age) for FH and CG participants. a. R Squared = .479 (Adjusted R Squared = .379) b. R Squared = .034 (Adjusted R Squared = -.152) c. R Squared = .438 (Adjusted R Squared = .329)