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BIOMEDICAL SCIENCES

Quantitative ultrasound for the evaluation of intratendinous deformation in the pre-insertional Achilles tendon

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Dissertation presented in partial fulfillment of the requirements for the degree of Doctor in Biomedical Sciences

June 2018

KU Leuven
Biomedical Sciences Group
Faculty of Medicine
Department of Development & Regeneration

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June 2018

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Dankwoord

Abstract

Achilles tendinopathy remains a highly prevalent condition among recreational as well as high-level athletes. Mechanical loading has become the gold standard in managing these injuries, but exercises are often generic and prescribed in a “one-size-fits-all” principle. Since static-morphological evaluation does not appear to provide help in guiding rehabilitation, a paradigm shift towards dynamic-functional biomechanical evaluation of tendons might be warranted. By consequence, new evaluation tools are needed to provide the clinician with information on the functional behaviour of the tendon during rehabilitation and active exercises. However, the evaluation of tendon mechanics is less straightforward than structural evaluation. This PhD project aimed to establish and validate a clinically oriented evaluation platform to quantify intratendinous deformation in the Achilles tendon, and to apply this within a patient-centred environment.

In chapter 1, a systematic review of strain mapping in the Achilles tendon was done to get an overview of existing literature in the field of functional evaluation of intratendinous deformation. It was found that the most frequently applied technique is real-time 2D ultrasound with tracking of two reference points during an active maximal voluntary contraction. However, no study evaluated local – intratendinous strain, emphasizing the need for further exploration of techniques enabling this.

In chapter 2, an ultrasound-based speckle-tracking technique was introduced and validated by quantifying the intratendinous deformation patterns of normal Achilles tendons in vivo. The emphasis in this paper lies on the advantages of the use of a high spatial and temporal resolution ultrasound acquisition system, compared to the techniques used in literature so far.

Subsequently, in chapter 3, the aim of the study was to evaluate the impact of different levels of force production and a change in knee angle on the non-uniform behaviour in the Achilles tendon during an isometric contraction. Contrary to the hypotheses, it was found that the non-uniform deformation, i.e. superficial-to-deep variation in displacement with highest displacement in the deep layer, is consistently present, irrespective of the level of force production and knee angle.

Additionally, in chapter 4, the primary aim was to gain more insight in the non-uniform behaviour of the Achilles tendon in a functional setting. Results confirm the presence of a non-uniform deformation pattern in the setting of an eccentric heel drop with bodyweight load, a type of exercise that is often used during rehabilitation of Achilles tendinopathy. The secondary aim was to further elucidate the complex relation between ageing and changes in local mechanical behaviour. Surprisingly, no statistically significant differences were found between the young and the middle-aged group.

In chapter 5, the influence of Achilles tendinopathy on the mechanical properties of the Achilles tendon was evaluated. The aim of this study was to compare local tendon mechanics in healthy versus pathologic Achilles tendons. Tendon tissue displacement was found to be significantly different between asymptomatic and highly symptomatic volunteers, in this controlled setting during an isometric deformation.

The insights gained in this thesis on the non-uniform deformation pattern of the Achilles tendon in different settings helped to enhance our understanding of Achilles tendon mechanics. This knowledge

provides new opportunities to tailor treatments to the specific needs of patients suffering from Achilles tendinopathy. Further research using the developed evaluation platform will definitely improve the interpretation and understanding of the complex relationship between structure and function in Achilles tendons.

Beknopte samenvatting

Achilles tendinopathie is een vaak voorkomend overbelastingsletsel, zowel bij recreationele als elite atleten. Actieve oefentherapie wordt algemeen beschouwd als de gouden standaard qua behandeling, maar deze oefeningen zijn vaak nogal generisch voorgeschreven en niet geïndividualiseerd naar de specifieke noden van de patiënt. Aangezien een statisch-morfologische evaluatie weinig hulp blijkt te bieden in het optimaliseren van revalidatie, lijkt een paradigma shift naar meer dynamisch-functionele vormen van evaluatie van pezen aangewezen. Deze manier van evaluatie is echter niet zo eenvoudig als de structurele evaluatie. Dit doctoraatsproject had dan ook als doel een klinisch bruikbaar evaluatie platform tot stand te brengen en te valideren, waarbinnen de intratendineuze vervorming van een Achillespees gekwantificeerd kan worden. Dit platform werd toegepast binnen een patiënt georiënteerde omgeving.

In hoofdstuk 1 werd een systematisch literatuuroverzicht gemaakt van de bestaande literatuur over functionele evaluatie van intratendineuze vervorming bij Achillespezen. De meest gebruikte techniek bleek echografie te zijn, met simultane registratie van twee anatomische referentiepunten tijdens een actieve beweging. Geen enkele studie evalueerde de lokale - intratendineuze - vervorming, wat de noodzaak naar ontwikkeling van dit soort technieken bevestigde.

In hoofdstuk 2 werd dergelijke nieuwe techniek geïntroduceerd en gevalideerd. Deze techniek kwantificeert de intratendineuze vervorming van Achillespezen door middel van *speckle tracking* op echografische beelden. Het voordeel van het gebruik van een hoge spatiële en temporele resolutie echografie in vergelijking met de huidige technieken werd besproken.

Vervolgens, in hoofdstuk 3, was het doel om de impact van verschillende niveaus van krachtontwikkeling en een verandering van kniehoek op het niet-uniforme gedrag van de Achillespees te evalueren, en dit tijdens een isometrische contractie. De resultaten waren tegen verwachting in. Het niet-uniforme gedrag, meer bepaald de variatie in verplaatsing van de oppervlakkige versus de diepe laag, waarbij de diepe laag het meest verplaatst, is steeds aanwezig. Dit blijkt het geval te zijn ongeacht het niveau van krachtonwikkeling of kniehoek.

Aanvullend, in hoofdstuk 4, was het eerste doel om meer inzicht te verwerven in het niet-uniforme gedrag van de Achillespees tijdens een meer functionele beweging. De resultaten bevestigden de aanwezigheid van niet-uniformiteit tijdens de excentrische fase van tenenstand met lichaamsgewicht, een oefening die gebruikt wordt tijdens de revalidatie bij Achilles tendinopathie. Het tweede doel was om meer inzicht te krijgen in de relatie tussen leeftijd en veranderingen in het mechanische gedrag van de pees. Er bleken echter geen statistisch significante verschillen tussen een jonge groep en een groep van middelbare leeftijd.

In hoofdstuk 5, tot slot, werd de invloed van Achilles tendinopathie op de mechanische eigenschappen van de Achillespees geëvalueerd. De resultaten toonden aan dat er een interactie is tussen de aanwezigheid en graad van pathologie, en de hoeveelheid lokale verplaatsing in de Achillespees, tijdens een isometrische en passieve beweging in een gecontroleerde labo omgeving.

De verworven inzichten over niet-uniforme vervorming van de Achillespees helpen om de mechanica van de pees beter te begrijpen. Deze nieuwe kennis biedt mogelijkheden om de oefenvormen aan te passen aan de specifieke behoeften van patiënten die Achilles tendinopathie hebben. Verder onderzoek met behulp van deze evaluatietechniek zal de interpretatie en het begrip van de complexe relatie tussen structuur en functie bij Achillespezen verbeteren.

Abbreviations

AT – Achilles tendon
GPa – Gigapascal
ICC – Intraclass Correlation Coefficient
LG – Lateral Gastrocnemius
MG – Medial Gastrocnemius
MHz – Megahertz
MRI – Magnetic Resonance Imaging
MTJ – Myotendinous junction
MVC – Maximal Voluntary Contraction
PRISMA – Preferred Reporting Items for Systematic Reviews and Meta- Analyses
ROI – Region of Interest
SAS – Statistical Analysis Software
SD – Standard Deviation
SEM – Standard Error of Measurement
SOL – Soleus
SPSS - Statistical Package for Social Sciences
US – Ultrasound
UTC – Ultrasound Tissue Characterization
VISA-A – Victorian Institute of Sports Assessment Achilles

Table of Contents

General introduction

The introduction of this thesis starts with providing insight into normal Achilles tendon (AT) anatomy, anatomopathological findings and its biomechanics. Subsequently, the anatomopathological findings, pathogenesis and management of Achilles tendinopathy are described, followed by the evaluation of tendon mechanics. Finally, the objectives and the outline of the thesis are described.

1. Normal Achilles tendons

1.1 Anatomy

The AT is the thickest tendon of the human body [1] and is comprised of the subtendons of the three muscles from the triceps surae: the lateral gastrocnemius (LG), the medial gastrocnemius (MG) and the soleus (SOL) [2]. The two-headed gastrocnemius muscle, originating superior to the knee joint at the femoral condyles, forms an aponeurosis below which the soleus, originating from the tibia and fibula, continues as muscle tissue [3]. Where the muscular part of the gastrocnemius ends, the continuing tendinous part is referred to as the gastrocnemius tendon [4]. Further down the lower leg, the soleus gradually joins the gastrocnemius tendon to form the free AT, i.e. free of muscle tissue [1]. In the free AT, the tendinous portions arising from each muscle are still morphologically distinguishable [5]. It has been suggested to use the term 'subtendon' to refer to the distinct portions of multi-muscle tendons [2]. The different layers of the free AT, i.e. superficial, middle and deep, are thereby expected to be part of one of the three subtendons, i.e. LG, MG and SOL.
Interestingly, the free AT undergoes a clockwise or counter clockwise rotation, in the left and right leg respectively, when moving from proximal to distal [5,6]. Therefore, in this area, fascicles originating from the respective muscle bellies are not always found in the expected location. Cummings et al. [7] were the very first to present this finding in 1946. Figure 1 depicts an approximation of the anatomical situation at the free AT [8].

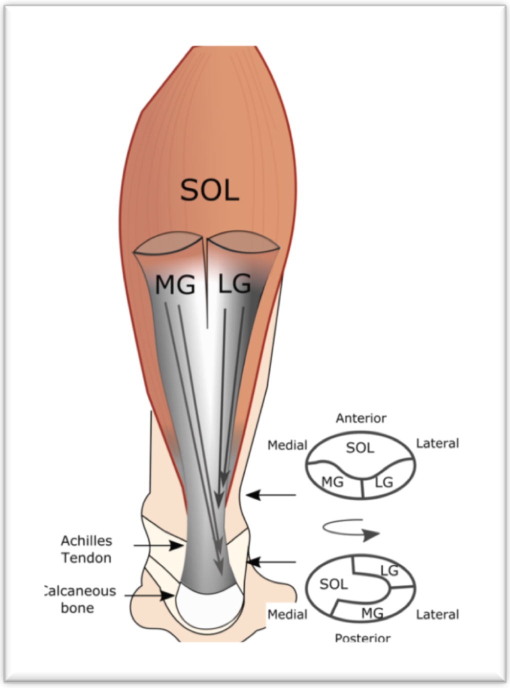


Figure 1 - Posterior view of the right triceps surae. Oval cartoons depict approximated cross sections at the free and insertional level. Adapted with permission from Bojsen-Moller et al. 2015.

Yet, further research showed inconsistency in anatomical findings related to this rotation. First, Szaro et al. [5] assumed the MG to be represented in the superficial and more lateral edge of the superficial layer, while the LG and SOL were expected to be represented in the and the deep-medial layer, respectively.

Second, Pekala et al. [6] described three types of rotation from mild (Type I) to more pronounced (Type III). They highlighted the importance of the proximo-distal location on the tendon, to specify the dominant layer per calf muscle. At the pre-insertional level, the superficial layer would represent mostly the MG, which was classified as a type I rotation; a combination of MG and SOL was classified as type II; and a predominantly representation of the SOL was classified as type III [6]. Consequently, the deep layer would represent a combination of LG and SOL in type I; mostly LG in type II; and a combination of LG and MG in type III [6].

Third, Edama et al. [9] also described three types of rotation when evaluating the insertional footprint on the calcaneal tuberosity. Thereby, the SOL solely attached to the deep layer of the calcaneal tuberosity was classified as least twist (Type I), both the LG and SOL attached to the deep layer was classified as moderate twist (Type II), and the LG solely attached to the deep layer represented the extreme twist (Type III).

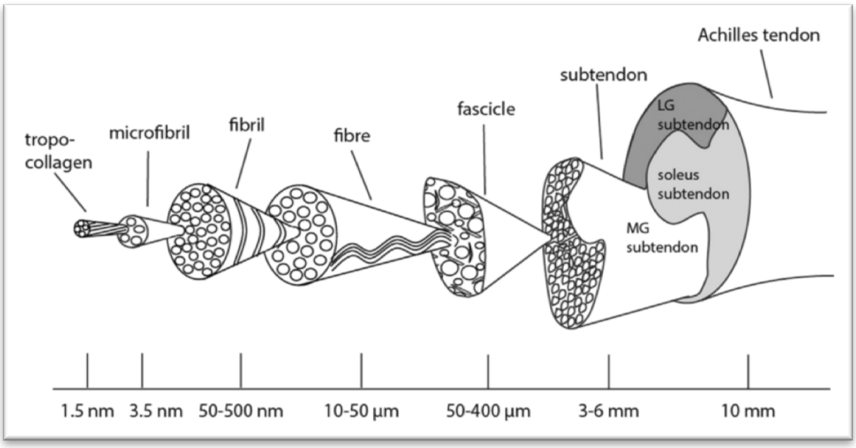
Distally, the tendon inserts on the calcaneal bone in a crescent way to help dissipate the stress that is placed upon the tendon during walking and other activities [3]. This function is supported by subtle changes in the material properties with more fibrocartilagenous tissue in this insertional area [10]. Extratendinous, the AT is surrounded by the fatty tissue of Kager's fat pad and the retrocalcaneal bursa, supporting and protecting the tendon in its function and homeostasis [10].

1.2 Tendon structure

Tendinous tissue consists of three main components: cells, ground substance and collagen [11]. First, tenocytes and the more mature fibroblasts are located between larger bundles of collagen [12]. Cells are spread out in between collagen fibres and make contact to neighbouring cells, which enables cell-cell signalling. This is a crucial aspect of mechanotransduction, i.e. the physiologic process where cells sense and respond to mechanical loads [13]. Second, ground substance consists of proteoglycans, which are capable of retaining large amounts of water [11]. These proteoglycans, e.g. decorin and biglycan, can bind to collagen fibrils and are more abundant in regions of compression, in addition to being important for collagen fibrillogenesis [12]. Third, more than half of the dry matter in adult tendon is type I collagen, which is responsible for the tensile load-bearing capacity of the tissue [14]. The function of type I collagen is further supported by other types of collagen, e.g. type III and type V [10].

Collagen consists of collagen molecules, around 1.5 nm in diameter, that aggregate in groups of five to make microfibrils and from thereon fibrils [15]. These fibrils, around 50-500 nm in diameter, are considered the basic building block of tendons. Fibrils then aggregate to a fibre, and fibres respectively into fascicles, resulting in a diameter of approximately 50-400 mm. This configuration is expected to be the reason for the striated pattern of the tendon visible to the eye [16] (Figure 2).

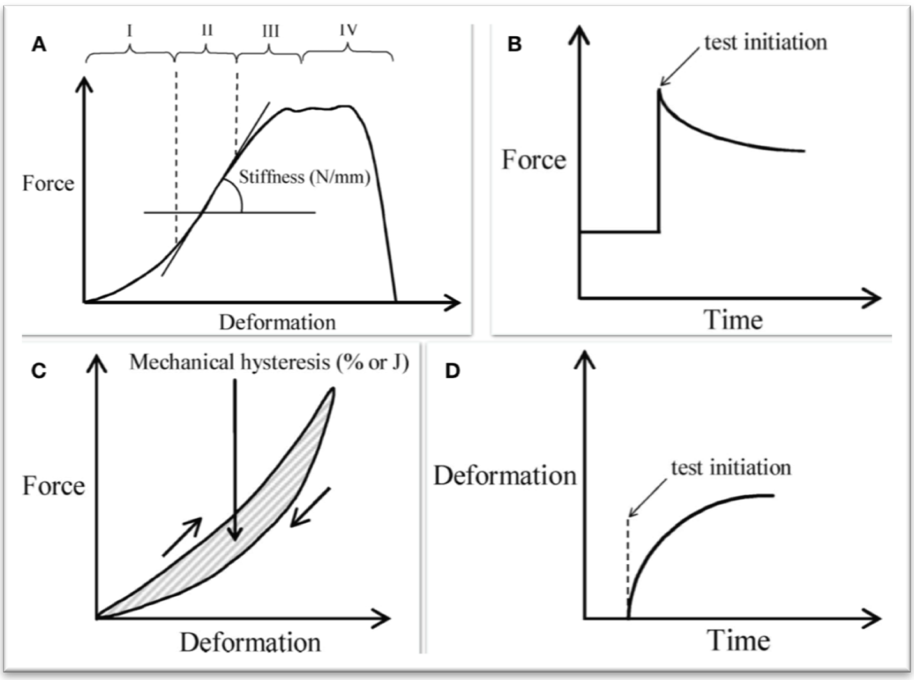
Figure 2 - Achilles tendon hierarchy. Reprinted with permission from Handsfield et al. 2016.



1.3 Basic biomechanics

This unique structural design enables the AT to function in a more complex manner than just transmitting force from muscle to bone. The AT stores and releases energy, protects the muscles of the triceps surae from stretch damage and enhances muscle performance.[17] For example, the AT acts like a spring by means of length change, enabling muscle fascicles to work almost isometric and saving energy expenditure [18]. To evaluate the mechanical properties of the AT, basic ex vivo studies used a material-testing device to put a tendon under tensile load until failure [19]. During tensile testing, the tendon is gradually elongated and, when combined with the force applied by the device, a force-elongation curve can be derived (Figure 3).

Figure 3 - Biomechanics of the Achilles tendon A: Force-deformation curve with 4 regions I-IV. B: Force-relaxation phenomenon. C: Hysteresis phenomenon. D: Creep phenomenon. (reprinted with permission from Maganaris et al. 2008)



The tendon force-elongation curve consists of four different regions (Figure 1A). The 'toe' region (region I) is associated with straightening of collagen fibre crimp. In the 'linear' region II, the aligned fibres are stretched further until small breakages occur at the end point of this region. In region III, additional fibre failure occurs in a rather unpredictable manner. Finally, in region IV, complete failure occurs. The slope of the curve in the 'linear' region then represents the stiffness of the tendon [19].

Importantly, corrections for interindividual differences in tendon dimensions have to be made. Tendon forces are transformed to stress values by normalization to the tendon cross-sectional area. Tendon elongations are transformed to strain values by normalization to the tendon original length. Strain values vary greatly, with results obtained in studies ranging from 5 – 8 % [19]. The shape of the consequent stress – strain curve is similar to the force – elongation curve, but it reflects the intrinsic material properties rather than the structural properties of the tendon. One of the most common material variables taken from a stress – strain curve is Young's modulus, i.e. the slope of stress – strain curve in the 'linear' region of the tendon, ranging between 1 and 2 GPa [19].

If a tendon is stretched, it does not behave perfectly elastically, even if the applied force does not stretch the tendon beyond its 'toe' region. Given the time-dependent properties of the tendon collagen fibres and inter-fibre matrix, the entire tendon exhibits force-relaxation (Figure 3B), creep (Figure 3D), and mechanical hysteresis (Figure 3C). Force-relaxation means that the force required to maintain a given elongation decreases over time in a predictable curvilinear pattern. Creep is the analogous phenomenon when persistent constant force is applied, yielding elongations which increase curvilinearly over time. Mechanical hysteresis is evidenced as a loop formed by the force – elongation (or stress – strain) plots during loading and subsequent unloading of the specimen. The area of the loop represents the amount of elastic strain energy lost as heat in the stretch-recoil cycle, and it is usually expressed as a fraction of the total energy (%) (Figure 3C).

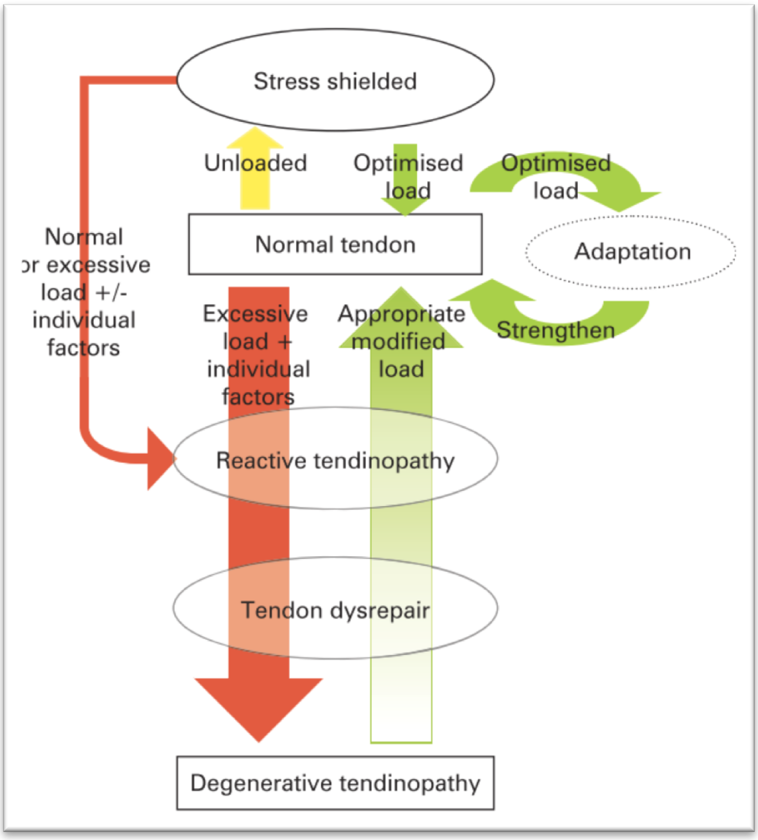
2. Achilles tendinopathy

Despite the appealing AT design, Achilles tendinopathy remains a highly prevalent condition among recreational as well as high-level athletes. The incidence and prevalence of tendon injuries has increased over the last years, likely related to increased sports participation by people worldwide [20]. Tendinopathy as a whole has been found to have an important socio-economic cost, mostly because of long lasting treatment periods [21]. Tendon injuries are estimated to account for 30-50% of all sports-related injuries, with an incidence of AT disorders of 7-9% in top-level runners [22]. In this group of AT disorders, Achilles tendinopathy is the most common clinical finding, with a prevalence up to 12,5% in adults [21]. It is diagnosed as a combination of pain and swelling (diffuse or localized) in and around the Achilles tendon, accompanied by impaired performance [23].

2.1 Anatomopathological findings

Cook and Purdam [24] proposed a generally accepted continuum model that links the anatomopathological findings to the clinical presentation (Figure 4).

Figure 4 - Pathology continuum; this model embraces the transition from normal to degenerative tendinopathy and highlights potential for reversibility early in the continuum. In contrast, reversibility of pathology is unlikely in the degenerative stage. (Reprinted with permission from Cook & Purdam 2009.)



In the first stage of reactive tendinopathy, a non-inflammatory proliferative response in the cell and matrix occurs from acute overload, usually a burst of unaccustomed physical activity. It is a short-term adaptation that thickens the tendon, reduces stress and increases stiffness. The tendon has the potential to revert to normal if the overload is sufficiently reduced or if there is sufficient time between loading sessions. On ultrasound, the tendon is swollen in a fusiform manner with an increased diameter [24].

In the second stage, tendon dysrepair is an attempt at tendon healing, similar to reactive tendinopathy but with greater matrix breakdown. There is an overall increase in number of cells, with a marked increase in protein production. The increase in proteoglycans and further attraction of water results in separation of the collagen and disorganization of the matrix. Ultrasound imaging reveals swollen tendons, with some discontinuity of collagen fascicles and small focal areas of hypoechogenicity (i.e. darker areas on ultrasound image). The increase in vascularity may be evident on colour or power Doppler [24].

The third and final stage, degenerative tendinopathy, is most commonly described in literature. In this stage, there is progression of both matrix and cell changes with areas of cell death. As a result, areas of acellularity have been described, and large areas of the matrix are disordered and filled with vessels, matrix breakdown products and little collagen. Little capacity for reversibility of pathological changes is seen at this stage. There is considerable heterogeneity of the matrix in these tendons, with islands of degenerative pathology interspersed between other stages of pathology and normal tendon. These appear on ultrasound as hypoechoic regions with few reflections from collagen fascicles. Numerous and larger vessels are usually visible on Doppler US. Clinically, this stage is primarily seen in the older person, but is also seen in a younger person or elite athlete with a chronically overloaded tendon [24].

2.2 Pathoaetiology

The exact injury mechanism in tendinopathy is still poorly understood. Tendons are known to be mechanosensitive, reacting to mechanical stimuli with metabolic changes [13]. An acute bout of loading is followed by an increase in synthesis as well as degradation of collagen. A net loss of collagen over the first 24-36 hours is noted, but this is countered by a net synthesis 36-72 hours after exercise [25]. Repeated training bouts with insufficient rest periods can subsequently result in a net degradation of matrix and lead to overuse injury [26]. The exact initiating insult remains unknown, but it is assumed that one or more 'weak links' in the tendon structure result in a pathological response of the tendon cells [26]. In this multifactorial aetiology, several extrinsic and intrinsic factors have been identified. From an extrinsic perspective, general factors (e.g. fluoroquinolone use, corticosteroid injection, etc.) and sports-related factors (e.g. training errors, poor equipment, etc.) have been reported, while from an intrinsic perspective, also general factors (e.g. age, diabetes, etc.) and local-anatomic factors (e.g. malalignment, muscle weakness) should be considered [22].

2.3 Clinical management

Despite this still incomplete understanding of the multifactorial aetiology of tendinopathy, rehabilitation through active exercise has become the golden standard in managing these injuries. The evidence supporting active exercise widely outweighs the potential small benefit of adjuvant therapies, e.g. shockwave [27]. The first to report an active approach to tendinopathy were Stanish et al. in 1986 [28]. They stated that "chronic tendinitis (sic), particularly of the AT, frequently outwits traditional programs of therapy including surgery and/or prolonged immobilization". The study used a comprehensive program of stretching, and eccentric exercises with progression in the speed of execution [28]. It was only in 1998 that the first prospective trial was conducted, evaluating the effect of a 12-week heavy-load eccentric calf training regimen [29]. Further on, studies have evaluated other forms of exercise, e.g. eccentric-concentric progressing to eccentric [30] or heavy-slow resistance [31], incorporating the eccentric component. Indeed, a recent review highlights the lack of evidence for isolating the eccentric component and a paucity of good quality evidence [32]. Furthermore, there is great heterogeneity in studies with respect to parameters such as load magnitude, speed of movement, and recovery period between exercise sessions [33].

In line of these findings, most often exercises are generic and prescribed in a "one size fits all" principle depending on therapist preference. This probably leads to the limited success rates, with non-responders to exercise therapy in some studies estimated to be as high as 45% [34].

To further improve outcome rates, tools are required to evaluate the tendon and guide the treatment to the patient's tendon specific needs. The static evaluation of structural characteristics of tendons has become common clinical practice, e.g. using ultrasound to measure tendon diameter, or to detect hypoechoic areas or neovascularization [35]. However, it is currently generally accepted that there is only a weak correlation between structural findings and therapeutic outcome. A recent review indicated that observable structural change could not be considered as an explanation for the response of therapeutic exercise in tendinopathy, except potentially for heavy-slow resistance training [36]. It could be debated that recent improvements in processing of structural imaging, e.g. ultrasound tissue characterization (UTC) [37], might improve this. UTC quantitatively evaluates tendon structure and categorizes four different echo-types, going from “good” type I to “bad” type IV [37]. It has indeed been shown that UTC appearance of symptomatic, tendinopathic ATs improves after 24 weeks of treatment [38]. However, no association between tendon structure and symptoms was found, demonstrating that restoration of UTC structure type is not required for an improvement of symptoms [38]. Furthermore, there is great heterogeneity in the UTC appearance of ATs, even in a young and healthy study population [39].

3. Evaluation of tendon mechanics

Since static-morphological evaluation does not appear to provide help in guiding our rehabilitation outcome, a paradigm shift towards dynamic-functional biomechanical evaluation of tendons might be warranted. By consequence, new evaluation tools are needed to provide the clinician with information on the functional behaviour of the tendon during rehabilitation and active exercises. However, the evaluation of tendon mechanics is less straightforward than the structural evaluation.

3.1 Ex vivo and global in vivo

To evaluate the mechanical properties, basic ex vivo studies used a material-testing device to put a tendon under tensile load until failure, as previously mentioned in the part on basic biomechanics [19]. The breakthrough for in vivo evaluation of these properties came in the late 1990’s, induced by technical improvements in ultrasound [40]. Ultrasound was thereby used to track two anatomical reference points (e.g. MG myotendinous junction and calcaneal insertion) during an isometric contraction on a dynamometer. In analogy with ex vivo testing, the combination of elongation and ankle torque allows an estimation of global tendon mechanical properties [40]. This technique has then been used in a number of studies, evaluating the response of AT properties to exercise, walking, ageing, etc. [41]. While this ultrasound-based approach improved the insight in the global in vivo mechanical behaviour of tendons, it had some technical and physiological limitations, such as the differences between research groups in choice of anatomical structure to scan and track (e.g. MG myotendinous junction (MTJ) or SOL MTJ), lack of standardization of tendon resting length, lack of adequate calculation of the tendon moment arm, etc. [42].

In parallel, advancements in ex vivo research have continued to provide valuable insight in local – intratendinous – mechanical behaviour. It has been shown that local strains at the fascicle level are much smaller than strains estimated at a more global level (i.e. relative elongation of tendon tissue to its original length), with the non-collagenous matrix as an important regulator [43]. Energy storing tendons, like the AT, undergo high levels of strain and need to be able to recoil efficiently for maximum energy storage and return [44]. The interfascicular matrix appears to play a crucial role in this energy transfer, as it shows great capacity for sliding, which helps the guidance of force transmission through the tendon hierarchy [44]. In general, the different hierarchical levels of tendons are believed to behave in distinct functional ways [45]. Therefore, it should be noted that evaluation of global tendon properties does not necessarily reflect local – intratendinous - behaviour.

Furthermore, results of cadaver studies, with invasive estimations of local deformation patterns, have underlined the heterogeneous behaviour of tendons. It has been found that strain differs between the posterior and anterior region of the insertional AT during a movement into dorsiflexion [46]. Also, it has been shown that local distribution of tendon strain is rather influenced by kinematics of the subtalar joint than by muscular imbalances within the triceps surae muscle [47]. It has been suggested that future research should focus on methods monitoring heterogeneous distribution of strain in vivo [47].

3.2 Local in vivo

Advancements in the field of real-time ultrasound scanning in cardiology created the possibility to perform in vivo, non-invasive measurements of local deformation patterns by automated speckle-tracking algorithms [48]. In short, a speckle is part of a pattern, derived from the summation of signals from scatters in the tissue under investigation [49]. Speckles have a granular texture of bright and dark spots [50]. When these speckles are tracked during a deformation, an estimation of local tissue displacement and other parameters can be made [48]. The quantification of tissue deformation on ultrasound images can be done either during acquisition (e.g. colour flow Doppler [35], or shear wave elastography [51]), or after acquisition. When data are evaluated after acquisition, some authors have used the radiofrequency data to estimate local tendon deformation [52]. Others have used the direct B-mode images, either through a block matching approach [53] or an image registration approach [54] for the speckle tracking algorithm. From cardiac strain estimation, it was previously shown that image registration methods resulted in slightly better results than for example block matching approaches [55]. However, translation of this technique from cardiology to the field of musculoskeletal medicine has proven to be technically challenging [56] and leading to instable strain estimation errors [57].

Furthermore, the accuracy and relevance of speckle tracking is related to the spatial resolution of the US acquisition system. If lower spatial resolutions are used, speckle patterns will result from large structures; when using higher spatial resolutions, speckle patterns will result from smaller structures [54]. Most studies evaluating local tendon deformation so far have used ultrasound acquisition central frequencies in range of 10MHz-14MHz [52,53], enabling visualisation up to fascicle scale (dimension 0,05 – 0,4 mm) [2]. Importantly, it has been shown that there are different features and modes of deformation throughout the fibril, fibre, fascicle and tendon scale [45]. Therefore, speckle tracking using higher-frequency ultrasound images could shed further light on the complex multiscale mechanics in the AT.

4. Objective and aims

The overall objective of this PhD project is to establish and validate a clinically oriented evaluation platform to quantify intratendinous deformation in the Achilles tendon, and to apply this within a patient-centred environment. This development and clinical implementation will make further investigation, interpretation and understanding of the complex relationship between structure and function in Achilles tendons possible.

The first novelty of this PhD project lies in the use of a high spatial resolution ultrasound system (central probe frequency of 21 MHz) in the AT, to allow for evaluation at lower hierarchical levels than before (e.g. fibres versus fascicle). Also, the high temporal resolution (frame rate of 100 frames per second) minimizes the influence of the viscoelastic behaviour of the tendon and allows for smoother evaluation of speckle motion on consecutive acquired images. Second, to achieve translation of the engineering-based techniques towards an easy-to-use tool for clinicians to use bedside with patients, an interactive application was created and used. This enables clinicians to select the region inside the tendon and time-interval during the motion of main interest.

To reach these goals, in **chapter 1**, a systematic review of strain mapping in the Achilles tendon was performed to provide an overview of existing literature in the field of functional evaluation of intratendinous deformation, i.e. strain mapping. The primary objective of this review was to outline of the methods already used in the literature and to summarize the current insights based on ultrasound evaluated mechanical properties of the AT, both at the local and global level.

In **chapter 2**, the existing high-frequency ultrasound-based speckle-tracking technique at KU Leuven was adapted to the musculoskeletal field. This could be achieved through a close collaboration between engineers and clinical experts. Given the lack of a solid ground-truth for in vivo experiments, the reliability and validity were defined through comparison with previous, lower resolution ultrasound-based, research.

In **chapter 3 and 4**, potential influencing factors for the mechanical properties of the AT were evaluated in asymptomatic subjects. The influence of a change in knee angle and amount of force development on AT deformation was evaluated in chapter 3. Likewise, the influence of age was evaluated in chapter 4.

Subsequently, in **chapter 5**, the influence of Achilles tendinopathy (i.e. symptoms and/or structural pathology) on the mechanical properties of the AT was evaluated, to determine the sensitivity of the tool to subgroup patients depending on mechanical properties compared to structural abnormalities.

5. Methodological considerations

Throughout the chapters 2 to 5 of this thesis, differences in methodology should be noted. For reasons of clarity, these differences will already be listed here to facilitate interpretation and comparison of the results of the different chapters. The relevance of certain aspects of these differences will be highlighted in the general discussion at the end of this thesis.

Study subjects

The sample under investigation varies between the different chapters. In the first studies, to validate the technique and test for reliability, asymptomatic healthy adult subjects were chosen. In the following studies, for example to investigate the influence of age or tendinopathy, healthy adult subjects of different age categories were chosen, as well as patients with Achilles tendinopathy.

Ultrasound device

The ultrasound device remained the same throughout all tests in this thesis. It is a high-spatial and high-temporal resolution ultrasound system: Vevo 2100 (FujiFilm VisualSonics Inc., Toronto, Canada). The transducer, MS250 (FujiFilm VisualSonics Inc., Toronto, Canada), has a central probe frequency of

21MHz. The acquired images thus have a spatial image resolution of 0.02x0.09mm, with an image width of 23mm and an image depth of 21mm. The location where the probe was positioned differed slightly between the studies.

Setting and subject positioning

In most chapters, the tests were performed under controlled laboratory setting with the subjects positioned on and fixated to an isokinetic device. One study evaluated subjects while standing on a decline board, leaning more towards the clinical setting.

Procedure

The procedure for obtaining movement differed per study, mainly depending on the aims. In the first studies, to validate the technique and test for reliability, a standardized passive elongation and maximal isometric contraction was used. In later studies, an isometric contraction at different percentages of the subject's maximum force was used, as well as the eccentric heel drop on a decline board.

Chapter	**Sample**	**Setting**	**Subject positioning**	**Location of ultrasound probe during measurement**	**Procedure**
2	10 asymptomatic subjects	Lab	Prone with foot fixated to isokinetic device, knee extended	Midportion AT (4 cm proximal from calcaneus bone)	Passive elongation and maximal isometric contraction in neutral ankle position
3	19 asymptomatic subjects	Lab	Prone with foot fixated to isokinetic device, knee extended and flexed	Pre-insertional AT (calcaneus edge in sight on ultrasound images)	Isometric contraction in neutral ankle position at 25, 50 and 75% of maximum force
4	19 young (age range 25-30 years) and 7 middle-aged (age range 46-56 years) asymptomatic subjects	Clinic	Standing on decline board	Pre-insertional AT (calcaneus edge in sight on ultrasound images)	Eccentric ankle plantar flexion

5	10 asymptomatic subjects and 6 symptomatic subjects	Lab	Prone with foot fixated to isokinetic device, knee extended	Midportion AT (4 cm proximal from calcaneus bone)	Passive elongation and maximal isometric contraction in neutral ankle position

Chapter 1: Strain Mapping in the Achilles Tendon – A Systematic Review

Adapted from: Bogaerts S, Desmet H, Slagmolen P, Peers K. Strain mapping in the Achilles tendon – A systematic review. J Biomech 49 (2016): 1411-1419.

You only live once, but if you do it right, once is enough.

Mae West

Abstract

Achilles tendinopathy remains one of the most prevalent overuse injuries in elite as well as recreational athletes. Regardless of the fact that the aetiology of tendinopathy has not been fully understood, therapeutic mechanical loading programs have emerged as being the treatment of choice. In this light, mechanical properties of the tendon and their response to changes in loading or unloading have been the subject of many previous investigations. One of these properties often investigated is strain, a measure of relative deformation. By means of a systematic review, an overview was given of research in this field, with a primary objective to list the methods used and secondary aim to synthesize data on strain mapping in the Achilles tendon. Following the guidelines of the PRISMA statement, 47 articles were found appropriate for qualitative assessment. Achilles tendon strain has been investigated across a variety of contexts, including the response to exercise, walking, unloading, ageing, hormonal changes and weight. Only three studies investigated the effect of the presence of tendinopathy on strain. Ultrasound was the most often used imaging modality to measure or estimate strain. Further methodological parameters, e.g. the location of measurement, differed greatly between all different studies. Nearly all studies considered global strain. Some studies investigated the transverse strain response of the Achilles tendon. Recently, however, the role of local – intratendinous – strain distribution has been found to be of critical importance and further studies should focus on imaging modalities to investigate these local changes.

Introduction

Tendinopathy is known to be the Achilles heel for both athletes and clinicians. Although the Achilles tendon is the thickest and strongest tendon in the human body, its structure is often affected. Achilles tendinopathy - the clinical syndrome characterized by a combination of pain and swelling in and around the Achilles tendon, accompanied by impaired performance [23,58] – is the most common overuse injury of the Achilles tendon, representing 55 – 65 % of the injuries in this tendon [22]. Furthermore, the incidence is still increasing as a result of the rising recreational and competitive sports participation in the population.

Many risk factors, both extrinsic and intrinsic, may predispose to injury, but pathoaetiology remains poorly understood. Tendons play an important role as a force transducer from the muscle to the bone and are designed to withstand considerable loads. Mechanical loading of tendon tissue is however an important cause as well as potential therapeutic goal in case of tendinopathy [13,26]. Strain induced by this mechanical loading seems to be one of the key aspects [19].

Strain is a description of mechanical deformation, referring to the relative differences in particle displacement within a material. In tendon mechanics, the muscle contraction is an external force that creates a local stress field between the muscle and the bone attachment, which then induces strain [59]. The relation between stress and strain is well described in mechanics using a so-called elastic modulus. In the case of linear elastic materials, this elastic modulus is known as Young's modulus, describing a linear relationship between stress and strain. Young's modulus can thus be regarded as a measure for the 'stiffness' of a material. The mechanical properties of tissues can be described using either material characteristics (e.g. Young's modulus, if we assume it to be linear elastic) or using a description of the deformation under stress itself (e.g. strain) [60]. As opposed to Young's modulus, strain can be measured non-invasively and is thus the preferred means to characterise the biomechanical properties of tissue [42].

The measurement or estimation of strain has been the target of many previous studies during the last decades. The methodologies of these different studies have however been heterogeneous in for instance the imaging technique used [61,62], the population studied [63,64] or the effect investigated [65,66]. This systematic review will give an overview of the research in this field, with a primary objective to list the methods used and secondary aim to synthesize data on strain mapping in the Achilles tendon.

Methods

This systematic review was conducted following the guidelines of the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [67].

Search

Relevant studies were identified conducting a computer-based literature search in the MEDLINE database using PubMed. The following main keywords were used: “Achilles tendon”, “Tendinopathy” and “Strain”. These keywords were then expanded to aim for an inclusion of all the relevant literature (see figure 1).

Figure 1 - Search terms

1) Achilles tendon: achilles tendon OR calcaneal tendon OR triceps surae

2) Tendinopathy: tendinopathy OR tendinosis OR tendinitis

3) Strain: strain OR stress OR tension OR elasticity OR Young's modulus OR elongation OR lengthening

To obtain the desired content for this literature search, the above-mentioned expansions were combined as follows:

[(1) AND (3)] OR [(2) AND (3)]. Thus, the complete search consisted of the following query:

[(Achilles tendon OR calcaneal tendon OR triceps surae) AND (strain OR stress OR tension OR elasticity OR Young's modulus OR elongation OR lengthening)] OR [(tendinopathy OR tendinosis OR tendinitis) AND (strain OR stress OR tension OR elasticity OR Young's modulus OR elongation OR lengthening)]

Study selection

Only studies published in English were considered. In addition, 'humans' was marked as a limit for 'species'. Studies involving subjects with normal Achilles tendons as well as subjects with Achilles tendinosis were included. Only in vivo studies were included. Studies involving subjects with an Achilles tendon rupture and/or subjects who underwent Achilles tendon surgery were not covered by this systematic literature search. Studies concerning subjects with other diseases or disorders such as neurological, systemic or neuromuscular problems were beyond the scope of this research work.

Review Process

In the first stage of selection, all titles and abstracts were assessed for inclusion using the selection criteria. The primary reviewers (S.B. and H.D.) assessed the content of all full text articles, making the final inclusion/exclusion decision by consensus of three reviewers (S.B., H.D. and K.P.). There was no blinding to study author, place of publication or results.

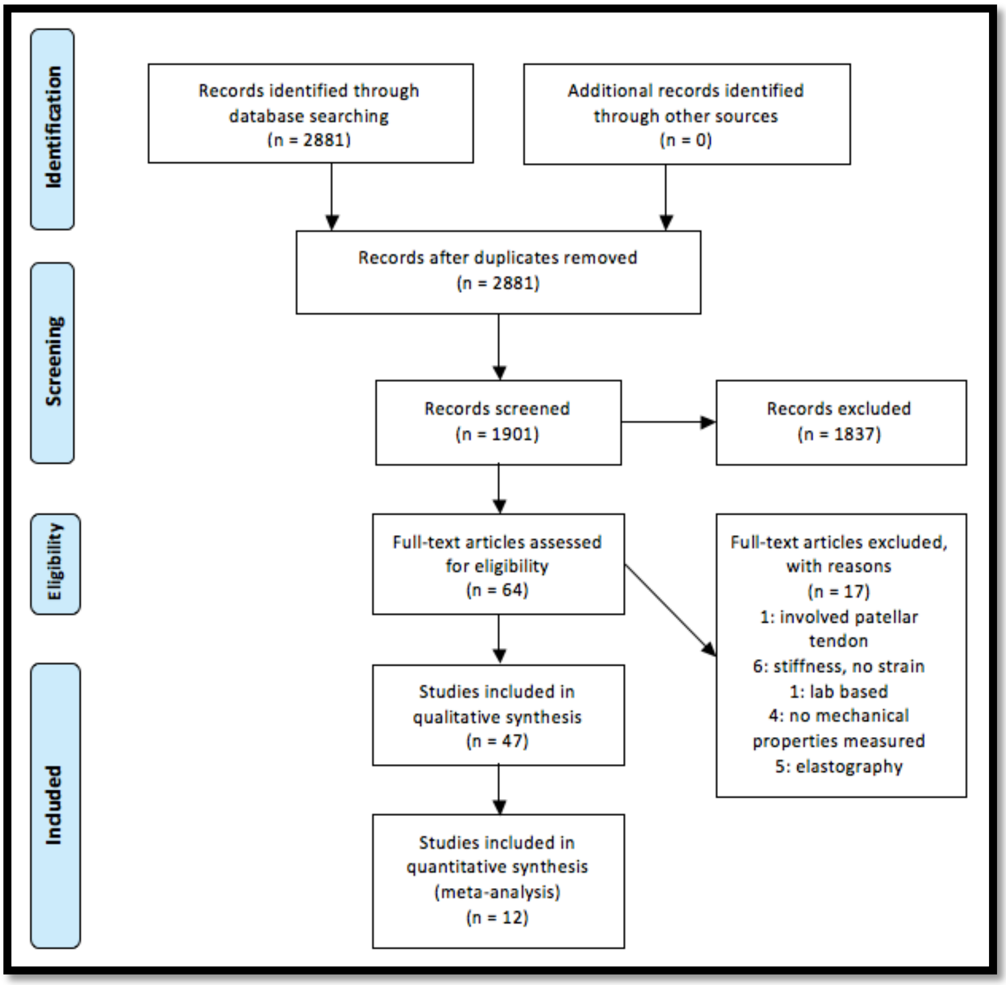
Data items

Key data were extracted from each study. This included measurement technique, location, procedure and strain direction; population; and Achilles tendon strain values (longitudinal or transverse).

Results

The literature search was conducted on the 17th of October 2014 and updated the 21st of August 2015. It yielded 1901 articles to be screened. Sixty-four articles were eligible for full text assessment. Of these 64 articles, 47 articles were found to be appropriate for qualitative assessment. The full PRISMA flow diagram is depicted in figure 2.

Figure 2 - PRISMA flow diagram



Technical aspects

1. MRI versus ultrasound

Real-time 2D-ultrasound imaging is the most often used medical imaging technique to assess Achilles tendon strain. Only four of the included studies used MRI to visualize the reference points of interest in order to quantify tissue lengthening and shortening [62,66,68,69]. Three studies used 3D ultrasound as imaging technique to determine longitudinal and transverse strain [4,70,71]. Details of the above-mentioned studies using MRI and 3D-ultrasound are listed in table 1 and 2.

Table 1 - MRI based studies

Study	Location	Procedure	Population
Iwanuma 2011 [68]	Aponeurosis and free Achilles tendon	Active contraction (30 & 60% MVC)	6 men and 6 women
Shin 2008 [69]	Whole tendon	Active contraction (40% MVC)	5 men

Lee 2006 [66]	Soleus MTJ - calcaneal insertion	Active contraction (20% MVC)	3 men and 5 women
Finni 2003 [62]	Soleus MTJ - calcaneal insertion	Active contraction (20 & 40% MVC)	4 men and 4 women

Table 2 - 3D-ultrasound based studies

Study	Location	Procedure	Population
Farris 2013 [70]	Distal and proximal Achilles tendon	Active contraction (50% MVC)	6 men and 5 women
Lichtwark 2013 [70]	Myotendinous junction and free Achilles tendon	Active contraction (50% MVC)	9 men
Obst 2014 [71]	Whole tendon	Active contraction (70% MVC)	8 men

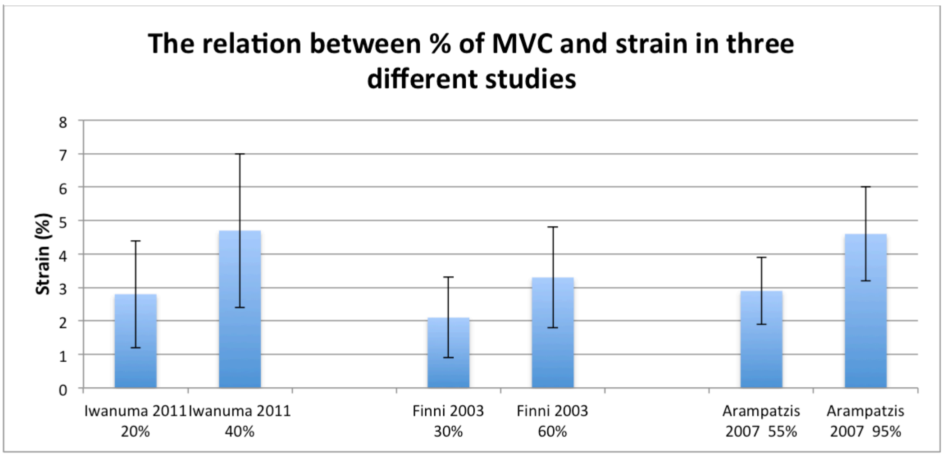
2. Longitudinal versus transverse

Strain direction was measured longitudinally in the vast majority of cases. However, in seven studies, strain was also measured in a transverse direction [63,68,70–74]. It is noteworthy that strain values were different depending on the strain direction; positive strains were recorded in the longitudinal direction and negative strains in the transverse direction. In addition, all included studies dealt with global Achilles tendon strain measurements, mostly using the gastrocnemius-Achilles musculotendinous junction and calcaneal insertion as reference points for strain tracking. None of the studies measured the local strain differences within the tendinous structure of the Achilles tendon.

3. Procedure

The preferred activation procedure to obtain a quantitative measure of Achilles tendon strain is maximum voluntary isometric contraction (MVIC). Further, three studies [75–77] used passive elongation as testing procedure and two studies [72,74] examined the strain values during rest in a neutral ankle position (0° of dorsiflexion). These last authors investigate a “strain response”, being the change in tendon diameter over the course of a day, thus after loading. This is different from the strain investigated by other authors, being the relative change in length of the tendon during a contraction or passive elongation. Also, the amount of active contraction or passive elongation has to be considered when interpreting strain values. Figure 3 shows the relation between the percentage of maximum voluntary contraction and measured strain values in three different studies [62,68,78]. This shows that a higher level of contraction leads to higher level of strain in the Achilles tendon. A further analysis and comparison between the results of these 3 studies is not possible due to the differences in methodology.

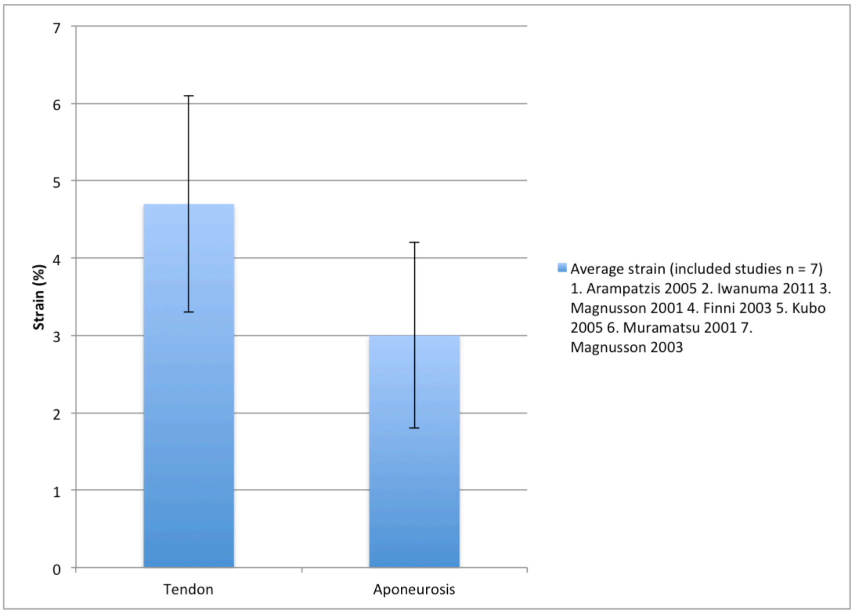
Figure 3 – Influence of force development on strain



4. Location of measurement

Nomenclature is important, but is in this anatomical area sometimes unclear. For example, Achilles tendon, aponeurosis, free tendon, gastrocnemius tendon, myotendinous junction and many other, are sometimes used in conflicting manner between different studies, which may cause misunderstandings and difficulty to compare strain values. There are differences in the strain values along the length of the tendon, in the different anatomical regions, for instance before and after the soleus joins the gastrocnemius tendon. Seven studies have investigated the comparison between strain in the aponeurosis versus strain in the Achilles tendon. This has shown that strain in the Achilles tendon is higher than in the aponeurosis region (see figure 4).

Figure 4 - Strain in tendon versus aponeurosis region



Physiological aspects

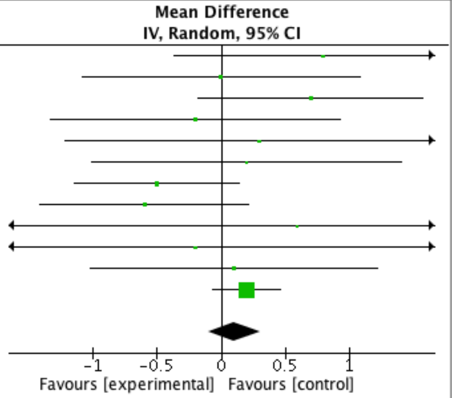
1. Effect of loading

Thirteen studies considered the effects of an applied load on the strain behaviour of the Achilles tendon through exercise [61,62,68,72,73,78–85]. In addition to the effects of exercise on Achilles tendon strain, several studies examined Achilles tendon strain in relation to walking or sports activities [4,18,64,65,75,86–88]. Furthermore, the effects of chronic unloading on tendon strain distribution were examined as well [66,69]. After further selection of 8 studies investigating the effect of loading in a longitudinal design, a meta-analysis was performed. Four of these studies investigated two different subgroups, making the total 12 included groups (see figure 5). No statistically significant effect was found of loading on Achilles tendon strain values pre- versus post-intervention.

Figure 5 - Forest plot – Effect of loading on strain – The experimental group are the strain values after loading/exercise. The control group are the strain values before loading/exercise.

	Experimental			Control				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
Arampatzis 2007	5.4	1.3	11	4.6	1.5	11	3.0%	0.80 [−0.37, 1.97]
Arampatzis 2007	4.8	0.9	11	4.8	1.6	11	3.5%	0.00 [−1.08, 1.08]
Arampatzis 2010	5.8	1.1	11	5.1	1	11	5.4%	0.70 [−0.18, 1.58]
Arampatzis 2010	4.9	1.5	11	5.1	1.2	11	3.2%	−0.20 [−1.34, 0.94]
Farris 2012	3.8	2	12	3.5	1.8	12	1.8%	0.30 [−1.22, 1.82]
Hawkins 2009	5.6	1.9	18	5.4	1.8	18	2.8%	0.20 [−1.01, 1.41]
Mademli 2007	4.5	0.9	12	5	0.7	12	10.0%	−0.50 [−1.15, 0.15]
Mademli 2007	4.3	1.1	14	4.9	1.1	14	6.3%	−0.60 [−1.41, 0.21]
Park 2011	7.7	4.6	10	7.1	5.5	10	0.2%	0.60 [−3.84, 5.04]
Park 2011	8.5	4.7	10	8.7	4.7	10	0.2%	−0.20 [−4.32, 3.92]
Shin 2008	2.3	0.8	5	2.2	1	5	3.3%	0.10 [−1.02, 1.22]
Urlando 2007	5.6	0.3	10	5.4	0.3	10	60.2%	0.20 [−0.06, 0.46]
Total (95% CI)			**135**			**135**	**100.0%**	**0.10 [−0.10, 0.31]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 10.33$, $df = 11$ ($P = 0.50$); $I^2 = 0\%$
Test for overall effect: $Z = 0.99$ ($P = 0.32$)



2. Influencing factors

Achilles tendon strain behaviour has been investigated across a variety of contexts. Some studies [83,85,87,89–92] examined the age-related differences in mechanical properties of the Achilles tendon, the effect of oestrogen on the strain behaviour of the Achilles tendon [93] and the effect of weight [63].

3. Tendinopathy

Only three research groups considered the in vivo Achilles tendon strain in the presence of Achilles tendinopathy [72,94,95]. S. Arya and K. Kulig found a 15 % higher strain ($P \leq 0.05$) in the Achilles tendinopathy group (5.14 +- 0.57 %) compared with the control group (4.36 +- 0.31 %). The outcome of the study of Child et al. was in line with the previous study: the Achilles tendon strain was significantly higher ($P \leq 0.05$) in male athletes with Achilles tendinopathy (5.2 +- 2.6 %) compared with those without it (3.4 +- 1.8 %). In both studies, strain was measured in a longitudinal direction and the Achilles tendon length was defined as the distance between the insertion on the calcaneus and the medial gastrocnemius myotendinous junction. On the other hand, N. L. Grigg et al. determined the anteroposterior (transverse) strain 40mm proximal to the calcaneal insertion in response to eccentric exercise. Eccentric exercises resulted in anteroposterior (transverse) strains of -14.6 %, -13.0 % and -8.3 % for the control, asymptomatic and symptomatic tendons, respectively. Participants in the symptomatic tendinopathy group were found to have a significantly ($P < 0.05$) lower strain when compared with pain-free counterparts and controls.

Discussion

Key findings

This systematic review gives an overview of research in the field of strain mapping in the Achilles tendon, with a primary objective to list the methods used and synthesize data on strain in the Achilles tendon. The technique most often used is real-time 2D ultrasound with tracking of two points (e.g. musculotendinous junction and calcaneal insertion) during an active maximal voluntary contraction. The relative change in tendon length then leads to an estimation of tendon strain (expressed as a percentage). Average strain values range from 1.1 % [68] to 9.2 % [90]. Maximal absolute transverse strain was – 19.5 +/- 7.4 % [73]. Given the heterogeneous aspect of research in this field (with regard to population, procedure of activation, location of measurement, effect investigated) only the effect of loading was investigated by means of a meta-analysis on 12 subgroups (see figure 5). This showed no statistically significant effect on strain values in the Achilles tendon.

Technical aspects

Ultrasound versus MRI

Concerning the applied measurement technique of the included studies, initial observations showed real-time ultrasound scanning (two-dimensional) has been used in the majority of the cases as the preferred non-invasive measurement technique in the assessment of mechanical properties of the Achilles tendon complex. This technique allows in vivo tendon strain measurement mostly by tracking a reference point within the field of view. Multiple arguments support the choice for using US as the preferred measurement technique to assess mechanical tendon properties. Firstly, unlike MRI, an ultrasound device is readily available and overall less costly. Furthermore, the dynamic aspect during measurements is still more advanced when compared to MRI, although recent developments in cardiac imaging with 4D (3D + time) MRI look promising [96]. Lastly, spatial resolution is better in ultrasound images, which is needed when aiming to use speckle tracking to measure strain (see further), which tends to use the secondary or tertiary fibre bundles of the tendon as reference point [53]. In their excellent paper, Seynnes et al. highlight the most important factors to take into consideration when investigating mechanical properties of tendons by means of ultrasound [42].

Other techniques

On-going research in the field of strain mapping in tendons has led to some new and interesting findings. Firstly, fibre optic sensors appear to be a promising new tool [97]. These reflect a narrow bandwidth of light, which responds faithfully to strain and temperature. The downside to this technique remains its minimally invasive nature, making it hard to be used in vivo and clinical practice. This technique can however be useful in research setting as a ground-truth for ex vivo studies trying to validate new non-invasive techniques. Secondly, computational studies have examined global and more recently local strain in Achilles tendon. These studies use biomechanical and mathematical models to calculate local strain [98]. The use of these models has become an important research area in the field of biomechanics, but validation of individual models remains an important issue to take into consideration [99]. Since these models are often compared with or validated against ex vivo material or isolated tendons [100], these studies did not fall within the scope of this review.

Procedure

Given the biomechanical characteristics of tendinous tissue, a distinction was made between testing procedures using active contraction and those using passive elongation as activation procedure to obtain Achilles tendon strain. Thus far, Achilles tendon strain has been investigated through active contraction in the vast majority of the included studies. However, the load applied on the Achilles tendon varied greatly between the different studies. Since many injuries occur during sports activities, it may be desirable to impose loads mimicking Achilles tendon strain during sports, which tend to be at higher speeds of movement and higher absolute load [65]. Also, whether or not there was preconditioning of the Achilles tendon may influence the mechanical properties measured, given the fact that a tendon behaves as a viscoelastic structure [19,101]. Lastly, another important factor is ankle joint rotation. During experiments with voluntary contraction, when the foot moves into plantar flexion, there is always some degree of ankle joint rotation, which influences elongation of muscle and tendon [102]. Since this change in position of the reference points for tracking is not taken fully into account, this can lead to an overestimation of strain. Farris et al. solved this by making sure the dorsal aspect of the foot always stayed in contact with the foot plate and have taken correction factors into account for passive displacement [70]. Even then, Arampatzis and colleagues have shown that an absolute difference in calculated strain may be as high as 0.3% [103].

Location of measurement and role of local strain

All included studies in this review concerned global strain in which the strain has usually been measured in a longitudinal direction and the musculotendinous and osteotendinous junction were selected as reference points for strain tracking. Global strain has provided us with plenty of information regarding the behaviour of tendon tissue in vivo, but there is however a need for further investigation on local – intratendinous - level. Sub-regional strain differences appear on cadaveric Achilles tendons [46,47,104]. J. Lyman et al. found that strain in the posterior sites of the distal Achilles tendon increased significantly as the movement into dorsiflexion occurred, whereas the anterior sites showed a trend towards a decrease in strain. In addition, C. Lersch et al. found intratendinous strain differences on cadaveric Achilles tendons during different muscle loading conditions and at different calcaneus positions. Finally, Wren et al. investigated strain behaviour of cadaveric Achilles tendons during repetitive cyclic loading to failure. They found different strain distributions within the proximal and distal regions of the Achilles tendons. Based on the findings of these studies on cadaveric Achilles tendons, it may be concluded that knowledge on local – intratendinous strain differences in the Achilles tendon in vivo is valuable.

Further supporting these needs for local strain research are some laboratory-based studies. Arnoczky et al. microscopically investigated rat tail cells with staining of the cell nucleus and witnessed an in situ cell nucleus deformation when under tensile load [105]. Cheng and Screen looked at bleached collagen fibres and fibrils and saw an inhomogeneous strain response in the matrix [43]. They found a large variation between samples, but overall found the local strain values to be much smaller than the applied values of strain in the loading axis, which would be suggestive for compression, fibre sliding and rotation of the collagen. Furthermore, in two different equine based studies [16,106], evidence was found for a local and rotational component in tendon movement, hypothesized by the authors as providing a better recoil and that way a possibly enhanced energy saving system. Lastly, Arndt et al. investigated healthy tendons during passive dorsiflexion and found a difference in strain between deep, middle and superficial parts of the Achilles tendon [53].

Physiological aspects

The meta-analysis performed in this paper (see figure 5) shows no effect of loading programs or exercise on the strain values measured. An important fact to take into consideration however is the heterogeneity in the definition of "loading". Table 3 gives an overview of the differences in duration, volume and intensity of the loading parameters in the included studies. The methodological design of these studies differs, but since the effect is measured using the same methodology this does not influence the comparison between the effect measured in the studies. It should however be noted again, that the methodology of strain measurement has its limitations and that small changes in strain will be difficult to detect.

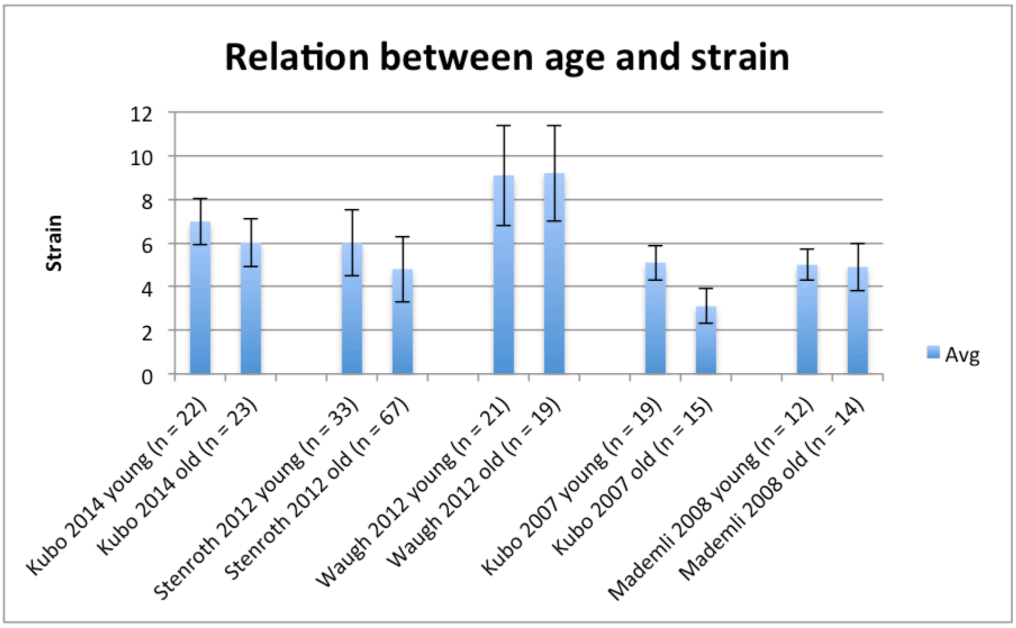
Table 3 - Content of loading programs

Shin 2008	6 weeks rehab after 4 weeks unloading
Farris 2012	30 minutes run
Peltonen 2010	Hopping
Hawkins 2009	7-minute warm-up after 1 hour immobilization
Urlando 2007	8 weeks strength program
Mademli 2008	40% MVC as long as possible on isokinetic device
Arampatzis 2010	14 weeks cyclic load at a certain frequency
Arampatzis 2007	14 weeks cyclic load at a certain magnitude
Park 2011	6 minutes warm-up jog and 5 x 30 seconds static stretch

Mechanical loading

The effect of loading on mechanical parameters of tendinous tissue has been the subject of many previous studies. Two interesting studies by the group of Arampatzis et al. have investigated the response of tendon to cyclic loading at different magnitudes and frequencies of the loading stimuli. Firstly, there appears to be a threshold of strain magnitude of mechanical loading, which has to be exceeded in order to influence the homeostasis of the tendon [78]. This was shown by the fact that after a 14-weeks exercise intervention, only in the leg exercised at high strain magnitude changes were found in the mechanical parameters. Secondly, surprisingly, a threefold increase in the strain frequency (from 0.17 to 0.5 Hz) caused lower adaptational effects on the Achilles tendon mechanical and morphological properties [82]. Given the fact that the mechanical load can only cause changes in these properties by triggering cells and matrix or affecting collagen, the authors hypothesize that a lower strain frequency may lead to more effective fibre recruitment and therefore a greater transfer of the external tendon strain magnitude to the cellular level. In another longitudinal study, Urlando et al. investigated the effect of an 8-week strength program [80]. This showed no difference in strain of the Achilles tendon, despite a significant change in muscle strength. The authors hypothesize that tendons have a preferred strain limit that is maintained despite changes in triceps surae strength. It remains to be seen for which reasons and through what exact mechanisms a tendon reacts to loading after an acute bout of exercise as well as in more chronic (over)loading conditions. Changes could be a physiological reaction to reduce stress as well as an attempt to repair damaged tissue [26] and might be a structural as well as mechanical adaptation. As mentioned earlier, also ageing seems to have an influence on tendinous material with overall lower strain levels measured in the elderly (figure 6). Although no statistically significant finding, this conflicts with the overall perception that stiffness decreases during ageing due to worsening material properties, which would contrastingly lead to higher strain values. However, as mentioned earlier, it is important to take into consideration that great inter-individual differences exist, leading to difficulties to interpret results of cross-sectional studies. Stenroth et al. suggest that regardless of age, Achilles tendon mechanical properties adapt to match the level of muscle performance [92]. Unfortunately, no studies have investigated the change in strain values in response to ageing in a longitudinal design longer than 6 months. Given the fact that mechanical properties of tendon material appear mostly related to muscle strength with little influence of ageing or loading, it might be interesting to look at the tendon during growth. As was shown by Heinemeier et al., most of the tendon core is formed during height growth, reaching a steady state around the age of 17 [107]. This leads to the hypothesis that the influence of mechanical loading of tendinous tissue during growth could have a much more profound and possibly long-lasting effect on mechanical and structural properties, then any loading program or exercise during later stages in life.

Figure 6 - Relation between age and strain



Implications for further research

It is noteworthy that only three research groups considered the in vivo Achilles tendon strain in response to Achilles tendinopathy [72,94,95]. Since the ultimate objective is to help patients and athletes with Achilles tendinopathy through optimizing their therapeutic exercise program, future research regarding strain measurement on subjects with symptomatic and asymptomatic Achilles tendinosis is warranted.

Recently, the strain measurement technique using real-time ultrasound has been further automated using speckle-tracking algorithms. These allow tracking frame-to-frame displacements of speckle patterns within the US image. Strain rate imaging using two- and three-dimensional ultrasound speckle tracking has been used for several years in cardiology [48] and seems to be a promising technique in a number of musculoskeletal applications. Y.S. Kim et al. described an in vivo strain analysis of the intact supraspinatus tendon by ultrasound speckle tracking imaging [108], but there are limitations to be taken into consideration [56]. J.W. Korstanje et al. evaluated the accuracy of tracking the flexor digitorum superficialis of a human cadaver hand [109] and the study of Y. Yoshii demonstrated that an ultrasound method based on speckle tracking had the potential to evaluate finger flexor tendon motion in vivo [110]. Although these studies have several limitations, they prove ultrasound speckle-tracking is a promising technique for in vivo strain analysis of tendons.

Chapter 2: Evaluation of tissue displacement and regional strain in the Achilles tendon using quantitative high-frequency ultrasound

Adapted from: Bogaerts S *, De Brito Carvalho C *, Scheys L, Desloovere K, D'hooge J, Maes F, Suetens P, Peers K. Evaluation of tissue displacement and regional strain in the Achilles tendon using quantitative high-frequency ultrasound. Plos One (2017) 12(7): e0181364.

I never worry about the future, it comes soon enough.

Albert Einstein

Abstract

The Achilles tendon has a unique structure-function relationship thanks to its innate hierarchical architecture in combination with the rotational anatomy of the subtendons from the triceps surae muscles. Previous research has provided valuable insight in global Achilles tendon mechanics, but limitations with the technique used remain. Furthermore, given the global approach evaluating muscle-tendon junction to insertion, regional differences in tendon mechanical properties might be overlooked. However, recent advancements in the field of ultrasound imaging in combination with speckle tracking have made an intratendinous evaluation possible. This study uses high-frequency ultrasound to allow for quantification of regional tendon deformation. Also, an interactive application was developed to improve clinical applicability. A dynamic ultrasound of both Achilles tendons of ten asymptomatic subjects was taken. The displacement and regional strain in the superficial, middle and deep layer were evaluated during passive elongation and isometric contraction. Building on previous research, results showed that the Achilles tendon displaces non-uniformly with a higher displacement found in the deep layer of the tendon. Adding to this, a non-uniform regional strain behavior was found in the Achilles tendon during passive elongation, with the highest strain in the superficial layer. Further exploration of tendon mechanics will improve the knowledge on etiology of tendinopathy and provide options to optimize existing therapeutic loading programs.

* Joint co-authorship
CC covered ultrasound and speckle tracking related items in the introduction and methods section. SB conceptualised the study, ensured interpretation of results from a clinical perspective, was responsible for writing and acted as corresponding author.

Introduction

Structure and function of tendons

Tendons in the human body have a hierarchical structure consisting of collagen triple helices, fibrils, fibers and fascicles [26]. The Achilles tendon (AT) has an extra hierarchical level as it is comprised of the subtendons of three muscles of the triceps surae (lateral gastrocnemius, medial gastrocnemius and soleus) [2]. The macrostructure of the AT is even more complex due to its twisted anatomy where fascicles undergo some degree of a counterclockwise (right AT) or clockwise (left AT) rotation, moving from proximal to distal [5]. This complex structure-function relationship leads to a fine balance between resisting tension and allowing compliance [10] as the function of tendons is more complex than just transmitting force from muscle to bone. They store and release energy, protect muscle from stretch damage, allow favorable muscle output and enhance muscle performance [15].

Quantification of the mechanical behavior of the muscle-tendon unit in vivo during deformation is needed to further elucidate the interplay between structure and function. The most common approach to do this has so far been the tracking of a reference point during an isometric contraction. The most commonly used reference points are the myotendinous junction (of the medial gastrocnemius or soleus) and the calcaneal insertion [41]. This then typically leads to a "global" force-elongation curve, of which the slope is a measure for the stiffness of tendon, relating to the global mechanical properties of the tendon. Force divided by the cross-sectional area of the tendon then leads to stress, elongation divided by the resting length of the tendon on the other hand leads to strain. From this, a stress-strain curve can be derived, of which the slope can be interpreted as Young's modulus, relating to the intrinsic material properties of the tendon, irrespective of its dimensions [19].

However, quantification of the "global" deformation of myotendinous junction to insertion of the Achilles tendon has its technical limitations [42]. Furthermore, recent studies have shown an important non-uniform deformation pattern in the muscle-tendon unit of the triceps surae, along its length as well as antero-posterior, as could be expected from the rotational anatomy described above [6]. It is known that in the majority of tendons, force transmission occurs mainly along the individual fascicles that can act as independent structures [111]. This intratendinous non-uniform behavior would go undetected when using a global evaluation from myotendinous junction to insertion. In a proximo-distal direction, it has been shown that strain and stiffness levels in the aponeurosis region of the tendon differ from the tendon proper [41]. The bulging of underlying muscles at the aponeuroses level has been suggested as a possible explanation [70]. Even more interesting, at intratendinous level, a cadaver study by Lyman et al. [46], using an invasive technique, has shown a non-uniform strain distribution with the superficial layer of the AT demonstrating a higher strain than the deep layer. Lersch et al. [47] have also shown the influence of calcaneal position on the intratendinous strain distribution, where an eversion led to higher strain in the medial and central distal AT.

Recent evolution in dynamic ultrasound imaging has also led to non-invasive evaluation of these deformation patterns. Arndt et al. [53] were the first to show a larger displacement in the deep layer of the AT during a passive elongation. These findings were confirmed by the group of Slane et al. [52] who later also showed that aging led to a more uniform deformation pattern [112]. Handsfield et al. [15] have recently developed a computational model and found that intratendon sliding and differential muscle force output appear to be the main contributors to this non-uniform deformation pattern. Further knowledge on the biomechanical behavior at intratendinous level is needed to elucidate the role of this non-uniform behavior in pathogenesis of tendinopathy and its place in treatment.

Ultrasound and speckle tracking

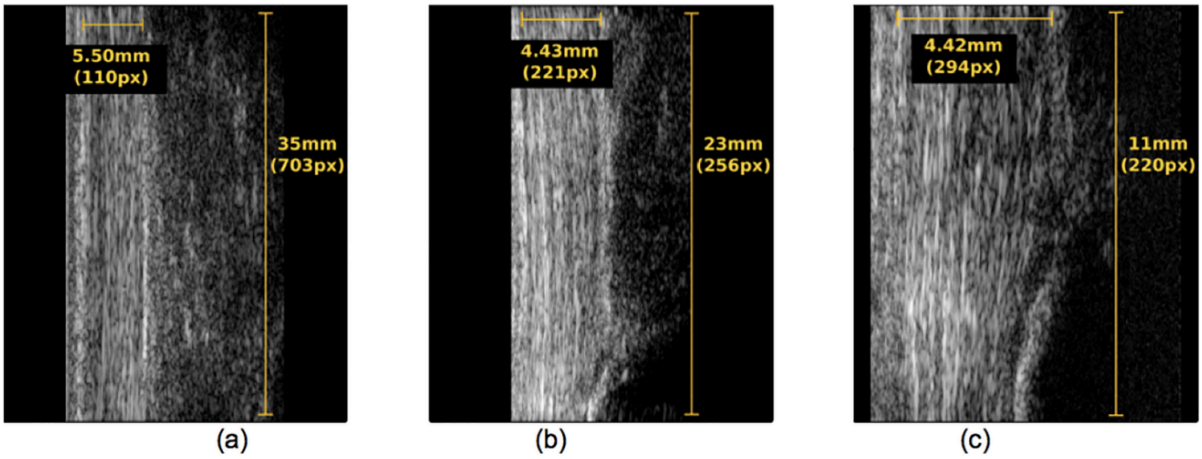
Most of the in vivo studies mentioned above used ultrasound (US) imaging with speckle tracking approaches to investigate mechanical properties of tendons [52,53]. In summary, an US wave is emitted into the body and the US transducer converts the reflected and scattered US waves into radiofrequency (RF) data. These RF data are afterwards converted to B-mode data and contain information on the internal structure of the scanned tissue and is obtained by coherent summation of signals from scatterers (reflectors), which are typically smaller than the spatial resolution of the imaging system [49]. When this summation occurs, an interference pattern, named speckle, is obtained [50]. Speckles have a granular texture of bright and dark spots. Although classified as noise, speckles are deterministic artifacts, meaning that if the same tissue is evaluated at two different time points, without any changes to the structure of the tissue, the speckle pattern will remain constant. Speckle patterns are correlated with the spatial resolution of the US acquisition system. In other words, if lower spatial resolutions are used, speckle patterns will result from large structures. When using higher spatial resolutions, speckle patterns will result from smaller structures.

Different transducers or acquisition systems can be used to acquire US images of tendons. Depending on the type and properties of the transducer, different spatial resolutions can be obtained. Spatial resolution describes the ability to distinguish between objects located at different positions in space and it is commonly defined in two directions: along the beam propagation direction (axial resolution) and perpendicular to this (lateral resolution). By definition, the axial resolution of the US system corresponds to the capability of the system to distinguish between echoes originated from two objects lying one behind the other. In the case of tendon imaging, it would correspond to discriminate between two fascicles with one lying superficial and one lying deeper. Lateral resolution is then the ability to discriminate between two objects situated side by side. In the case of tendons, it would correspond to discriminate an object lying proximal from one lying more distal.

One of the novelties of this work is that the use of a higher frequency (21 MHz) US acquisition system would enable tracking speckle patterns of smaller structures and hence allow for a description of the inter-fiber and inter-fascicular deformation.

In order to demonstrate the advantages of such a system, a qualitative comparison was performed (Fig 1) between a conventional 10 MHz US acquisition system (L14-5/38 Linear transducer, Ultrasonix Medical Corporation, Canada) and two transducers of the Vevo2100 (MS250 – central frequency 21 MHz and MS550 – central frequency 40 MHz).

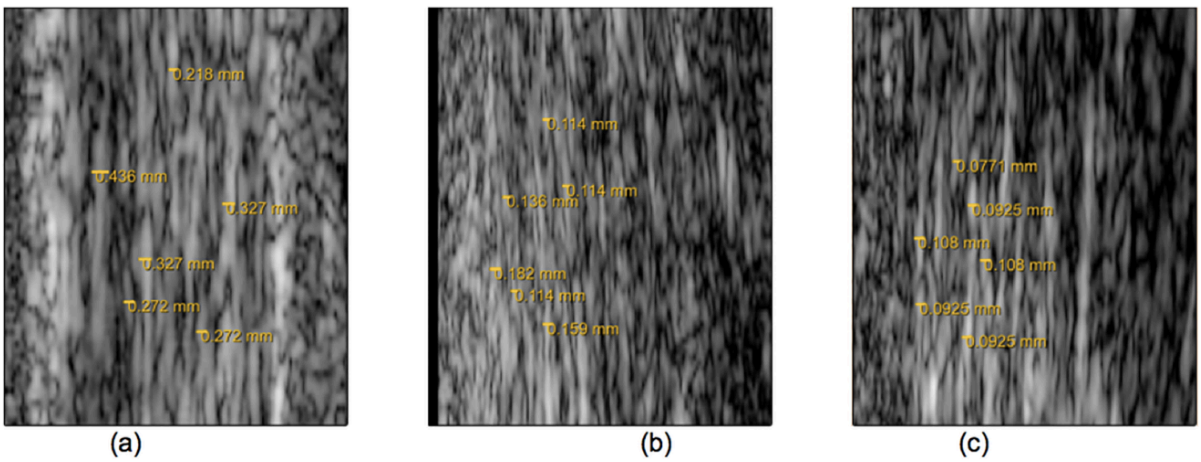
Figure 1 - US images of an Achilles tendon acquired with different central frequencies. (a) 10MHz transducer, (b) 21MHz transducer (c) 40MHz transducer. Tendon width, length and corresponding image resolution is annotated in yellow.



As figure 1 shows, the AT is represented with more detail in figure 1(b) and figure 1(c) than in figure 1(a). The ultrasound axial resolution of these images is 0.308 mm, 0.0367 mm and 0.0192 mm respectively.

A better perspective on the actual resolution of the different US systems is presented in figure 2, which are close-ups of figure 1.

Figure 2 - Close-up of Figure 1. Speckle pattern width is measured for the 10MHz image (a), 21MHz image(b) and 40MHz image(c).



As these images demonstrate, with the 10 MHz system it is possible to measure a striated speckle pattern with an average width of +/- 0.30 mm, which would correspond to structures smaller than the system's axial resolution (0.308 mm), being large fascicles [2]. On the other hand, with the 21 MHz and 40 MHz system, the striated speckle pattern that can be measured has an average width of +/- 0.14 mm and +/- 0.0951 mm, respectively. The speckle pattern obtained from the 21 MHz and 40 MHz transducers are then assumed to represent tendon structures with sizes smaller than the axial resolution of these transducers (0.0367 mm and 0.0182 mm, respectively). This corresponds to tendon fibers [2]. However, this higher resolution along the beam propagation direction is compromised by a reduction of the field of view along the lateral direction (35 mm for 10 MHz, 23 mm for 21 MHz and 11 mm for 40 MHz). The ultrasound lateral resolution of these three images is then 0.039 mm, 0.10 mm and 0.033 mm, respectively.

Considering both lateral and axial resolution, together with the field of view, the US acquisition system used in this work was 21 MHz, which was assumed to allow improved tracking of inter-fiber and inter-fascicle deformation, due to its higher axial resolution (0.0367 mm), while maintaining a reasonable field of view (23 mm). Lateral resolution was not strongly considered because fibers and fascicles have been reported to be elongated structures with longer dimensions [14] and lateral force transmission to be small [111].

From speckle tracking perspective, Heyde et al. [55] compared the performance of two speckle tracking approaches, being block-matching and image registration methods, for cardiac strain estimation. They found that image registration methods yielded slightly better results. Considering also the non-uniform deformation of tendons and the better performance of image registration methods, a non-rigid image registration method was considered the preferred speckle tracking approach.

Purpose

The objective of this study was to quantify the intratendinous deformation patterns of normal Achilles tendons in vivo by means of high-frequency ultrasound-based speckle tracking. The displacement and regional strain in the superficial, middle and deep layer were evaluated during passive elongation and isometric contraction. Since non-invasive techniques to establish ground-truth are lacking, validation was based on results of previous research in this field. It was hypothesized that highest displacement would be found in the deep layer, as was previously shown by others [52,53]. Quantification of regional strain has so far been even less successful and more challenging [56,57], but based on a cadaver study by Lyman et al. [46], it was hypothesized that the highest regional strain would be found in the superficial layer.

Methods

Set-up

The KU UZ Leuven ethics committee approved this specific study and gave it number s57302. After providing written consent, participants filled in a document with demographic questions and completed the VISA-A questionnaire, a validated measure to evaluate tendon health and function [113]. Subjects with VISA-A scores lower then 100, previous history of rupture, surgery, and systemic or neuromuscular diseases were excluded. A convenience sample of subjects was recruited from a group of co-workers at the lab. Participants were asked to refrain from physical activity the day before and the day of the test, other than normal ambulation required in daily life. The participant lay prone on a table, knees extended, with the foot fixated in an isokinetic testing device according to the manufacturer's guidelines (Biodex system 4 PRO, Biodex Medical Systems, Inc., Shirley, New York). After a standardized warm-up of 5 repetitions of concentric plantar- and dorsiflexion through 20° range of motion, starting from a neutral position, the ultrasound probe was attached to a custom-made holder at the mid-portion of the

Achilles tendon. The position of the holder was marked on the skin with a marker to allow reproducible positioning on day 2 for test-retest reliability trials. Two motions were used in randomized order by coin toss between subjects and between days: 2 repetitions of maximal voluntary isometric contraction in a neutral ankle position during 5 seconds, and 2 repetitions of passive elongation from 10° plantarflexion to 10° dorsiflexion and back at 15°/sec. Data from the Biodex machine were collected after finishing all testing procedures. The same procedure was repeated the next day at the same time of the day using the same setup.

Ultrasound

2-dimensional B-mode US images were acquired during each motion using a high-frequency US system from FujiFilm VisualSonics Inc. (Vevo 2100 - Amsterdam, The Netherlands). As mentioned in the introduction, a transducer (MS 250) with a central frequency of 21MHz was used and the acquired images had a spatial image resolution of 0.02x0.09mm. A dynamic sequence of 2D images was acquired during 5 seconds with a temporal resolution of 100 frames per second.

Tissue displacement and strain estimation

As mentioned in the introduction, a non-rigid image registration method was the preferred speckle tracking method due to its ability to recover non-uniform deformation. Tissue displacement was then estimated by finding the transformation T(x) that maximized the spatial correspondences when deforming a moving image (IM) to match a fixed image (IF). These correspondences are maximized by optimizing a cost function (C), defined according to a similarity measure (S) and a penalty term (P), with respect to T(x) as presented in equation 1.

Equation 1

$$T(x) = \underset{T(x)}{argmin} C(T(x); I_F, I_M)$$
$$C(T(x); I_F, I_M) = -S(T(x); I_F, I_M) + \gamma P(T(x))$$

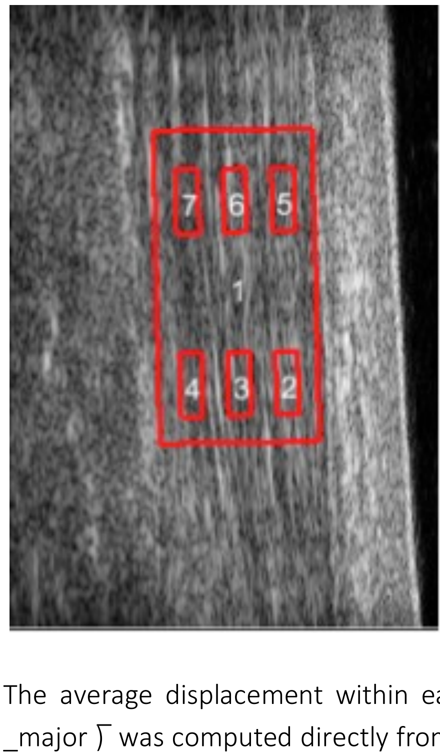
A cubic Bspline transformation T was used in this work. In this type of transformation, the control points, placed in a uniform grid, are displaced to deform the moving image to match the fixed image. The spacing between the control points defines the locality of the transformation: the smaller the spacing, the more local deformations are obtained. The displacement of the pixels at non-control positions is obtained by cubic interpolation. Sum of squared differences between intensity values of corresponding locations in both images was used as similarity measure and a bending energy penalty P with a weight γ of 0.5 was used [114]. This penalty term penalizes sharp discontinuities in the transformation. A three-level multi-resolution strategy using a pyramidal scheme was used with a grid spacing of 4, 2 and 1 pixels from coarser to finer resolution. As optimizer, a quasi-Newton limited memory BFGS (Broyden–Fletcher–Goldfarb–Shanno) [115] was employed to minimize the cost function C because of its speed and memory efficiency [55].

After all trials were visually reviewed to ensure that there were no artifacts (e.g. probe release, air bubble) and a stable speckle pattern was visualized, a region of interest (ROI) containing only tendon material was selected, to limit the calculation of deformation to the tissue of interest. In order to reduce computational effort, an interval of 100 frames, out of 500 frames of the cine-loop, was selected.

A pair-wise registration of consecutive frames in the sequence was favored due to the high speckle correlation between images acquired with a low temporal difference. Once every consecutive pair of images was registered, the transformation between the last frame of the cine-loop (100th) and the first one was obtained by composition of the intermediate pair-wise image transformations. At the end, point-wise displacement maps were obtained along the major deformation direction (〖Δd〗 _major) representing the tissue displacement in the longitudinal direction (i.e. the principal strain direction), relative to the starting position in the first frame. This entire framework was implemented using elastix [116,117].

The ROI selected at the pre-processing step was re-used to automatically define 6 sub-regions (3 proximal and 3 distal), consisting of a deep, middle and superficially located sub-region as represented in figure 3. The deep, middle and superficial sub-regions were automatically placed at 25, 50 and 75% of the selected global ROI width, and the proximal and distal at 25 and 75% of the global ROI length. The deep-middle-superficial sub-division was based on clinical reasoning where it was expected that the three muscles of the triceps surae account for three layers of the subtendons (lateral gastrocnemius, medial gastrocnemius and soleus) in the Achilles tendon. The proximal versus distal sub-division was done to include the potential to evaluate possible longitudinal differences.

Figure 3 - 2D US image of volunteer with selected regions of interest (1) and subregions (2-7) delimited in red.



The average displacement within each sub-region along the major deformation direction (〖Δd〗 _major ͞ was computed directly from the point-wise displacement maps. Regional strain (equation 2) was computed along the major deformation direction between two different regions (Ri and Rj). Superficial strain was estimated between R5 and R2 (Ri=R5 and Rj=R2, equation 2), medial strain was computed between R3 and R6 (Ri=R3 and Rj=R6, equation 2) and deep strain was computed using R7 and R4 (Ri=R7 and Rj=R4, equation 2). L_initial represents the distance between the central position of Ri and Rj.

Equation 2

$$\boldsymbol{RegionalStrain} = \frac{\overline{\Delta \boldsymbol{d}_{\boldsymbol{major}} \boldsymbol{R}\mathrm{i}} - \overline{\Delta \boldsymbol{d}_{\boldsymbol{major}} \boldsymbol{R}\mathrm{j}}}{\boldsymbol{L}_{\boldsymbol{initial}}} \times \mathbf{100}$$

Statistics

Statistical analysis was performed using SPSS (IBM, New York, NY, USA). Test-retest reliability (intraday: between 2 repetitions on the same day - interday: between average of 2 repetitions on 2 consecutive days) was evaluated for results of displacement in the superficial layer during passive trials in the right leg of all 10 subjects. Results were assessed using intraclass correlation coefficients (ICC). Standard error of measurement (SEM) was then derived from ICC's.

After confirming normal distribution of results with Shapiro-Wilk's test, non-uniform deformation of the different layers was evaluated using a two-sided paired t-test with alpha-level set at 0.05. A comparison between the superficial, middle and deep layer during passive as well as isometric trials was made. Also, the comparison between the absolute difference in mean displacement between layers (superficial to deep) during passive versus isometric trials was made.

Results

10 asymptomatic subjects (6 male – 4 female; 26 (SD 3) years; 72 (SD 14) kg; VISA-A 100 (SD 0) %) participated in this experiment with both Achilles tendons tested, leading to a total of 20 tendons investigated. The mean torque, relative to body weight, generated on the Biodex was 79 N/kg (SD 32 N/kg) during the isometric trials and 7.5 N/kg (SD 3.4 N/kg) during the passive trials.

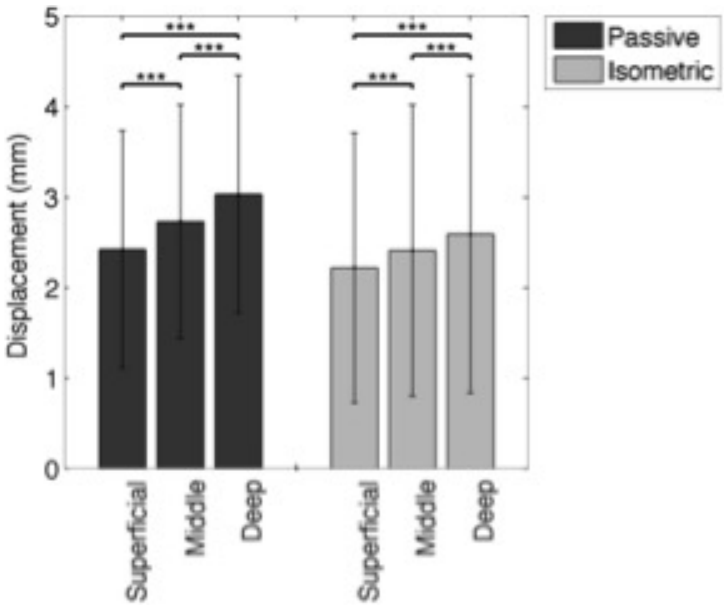
Test-retest reliability

ICC was 0.86 for intraday and 0.72 for interday measurements, leading to a SEM of 0.35 mm and 0.44 mm respectively.

Tissue displacement

There was a significantly different tissue displacement when comparing the three layers during passive as well as isometric trials ($p < 0.001$). The deep layer of the tendon moved most with an average displacement of 3.03 mm during passive elongation and 2.59 mm during isometric contraction (Fig 4).

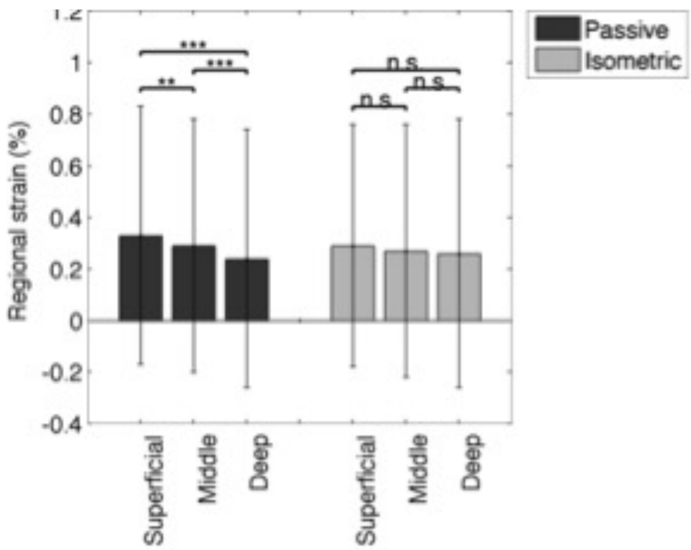
*Figure 4 - Non-uniform displacement along the major deformation direction (*** = p < 0.001)*



Regional strain

There was a significantly different regional strain when comparing the three layers, but only during passive elongation. The non-uniform regional strain was not significantly different during the isometric trials. Highest strain was found in the superficial layer with an average of 0.33% during passive elongation and 0.29% during isometric contraction (Fig 5).

*Figure 5 - Non-uniform regional strain along the major deformation direction (** = p = 0.002 / *** = p < 0.001 / n.s. = non significant).*

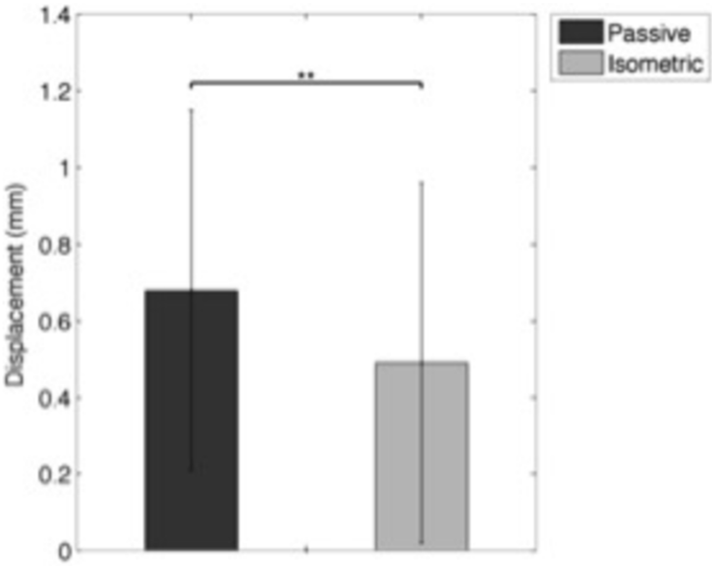


Relative displacement

There is an absolute difference in displacement of 0.34 (SD 0.24) mm between the superficial and middle layer and of 0.34 (SD 0.23) mm between the middle and deep layer during passive elongation. For

isometric contraction, the absolute differences in displacement are 0.25 (SD 0.24) mm for superficial-middle and 0.24 (SD 0.23) mm for middle-deep.
Looking at absolute difference in mean displacement between the superficial and deep layer there is a significantly larger difference during the passive elongation, compared to the isometric deformation (Fig 6).

*Figure 6 - Absolute difference in mean displacement of superficial versus deep layer (** = p = 0.002).*



Discussion

The objective of this study was to quantify the intratendinous deformation patterns of normal Achilles tendons in vivo by means of high-frequency ultrasound based speckle tracking. Results showed that the Achilles tendon displaces non-uniformly with a higher displacement found in the deep layer of the tendon. This is in line with the findings of Slane et al. and Arndt et al. [52,53]. Adding to this, this study showed, using a non-invasive method, a non-uniform regional strain behavior in the Achilles tendon during passive elongation, with the highest strain found in the superficial layer. There was a similar trend observed during isometric contraction, however not significant. This is in line with the cadaver work of Lyman et al. [46] and in vivo work by Chimenti et al. on the insertional Achilles tendon . Previous cadaver studies using invasive techniques on patellar tendons [118] and supraspinatus tendons [119] also showed that joint-sided strain was lowest, which would relate to the superficial strain of the Achilles tendon being highest.

A few possible reasons for the non-uniform behavior in Achilles tendons have been recently described [8]. The rotational anatomy of the Achilles tendon [5] provides a basis for non-uniform deformation between layers. At the insertional level of the Achilles tendon, the superficial fibers relate mostly to the gastrocnemius medialis subtendon and the deep fibers mostly to the gastrocnemius lateralis subtendon [6]. In their anatomical study, Pekala et al. [6] hypothesize that the deep layer of the AT in general twists more than the superficial. This could lead to a higher pre-tension in the deep part of the tendon, leaving little to no margin for extra straining during deformation and therefore more displacement in this layer of the tendon. For the same level of force going through the whole tendon, the superficial part on the other hand could then still undergo more straining during deformation. Besides differences in morphology and neuromuscular activation patterns in the triceps surae, there might also be local material differences at the level of the tendon [8]. This was already described in the patellar tendon, where tendon fascicles from the anterior portion of the human patellar tendon in young men displayed considerably greater peak and yield stress and tangent modulus compared with the posterior portion of the tendon, indicating region-specific material properties [120,121].

The difference in absolute difference in mean displacement between passive and isometric trials (Fig 6) falls in line with previous research from Finni et al. [122]. They have described the influence of active versus passive muscle contribution to the behavior of muscle shear, which is believed to have an influence on tendon behavior. During passive conditions, there is a slack and compliant connection between muscle bellies of the triceps surae, leaving margin for an independent non-uniform behavior. During active conditions, the tensing of muscle connections might lead to more uniform behavior.

As described in the introduction, since we are evaluating at fascicle and fiber level, the non-uniform behavior described in this paper supposedly links to sliding at intratendinous level. This sliding might even be more important than pure tensile – longitudinal strain, as Thorpe et al. [44] nicely summarize ex vivo and in vitro research, stating that "… at low force, there is sliding between fibers/fibril, but no real fiber extension. At higher force there is interfascicular matrix sliding". It has also been shown that changes at the interfascicular matrix is an important factor to consider in the pathogenesis of tendinopathy [123]. With respect to pathogenesis and the effects of ageing and loading, other research has shown that ageing leads to changes in the tendon morphology and mechanics [124] with increase in non-enzymatic crosslinks and as a consequence an expected increase in stiffness [125]. However, stiffness seems to decrease as we age [12]. This might be due to the loss of interfascicular sliding because of non-enzymatic crosslinks, turning fascicles more vulnerable to local tensile strain, which can consequently lead to damage. This local damage and loss of local tensile stiffness from the fascicles could then be the tipping point where the decline of stiffness starts as seen in elderly, but also leading to stress-shielding [126] and development into pathologic tendons [127]. The possibility to evaluate in vivo tendon sliding could therefore be a valuable tool to detect changes in tendon function before onset of structural pathology or symptoms.

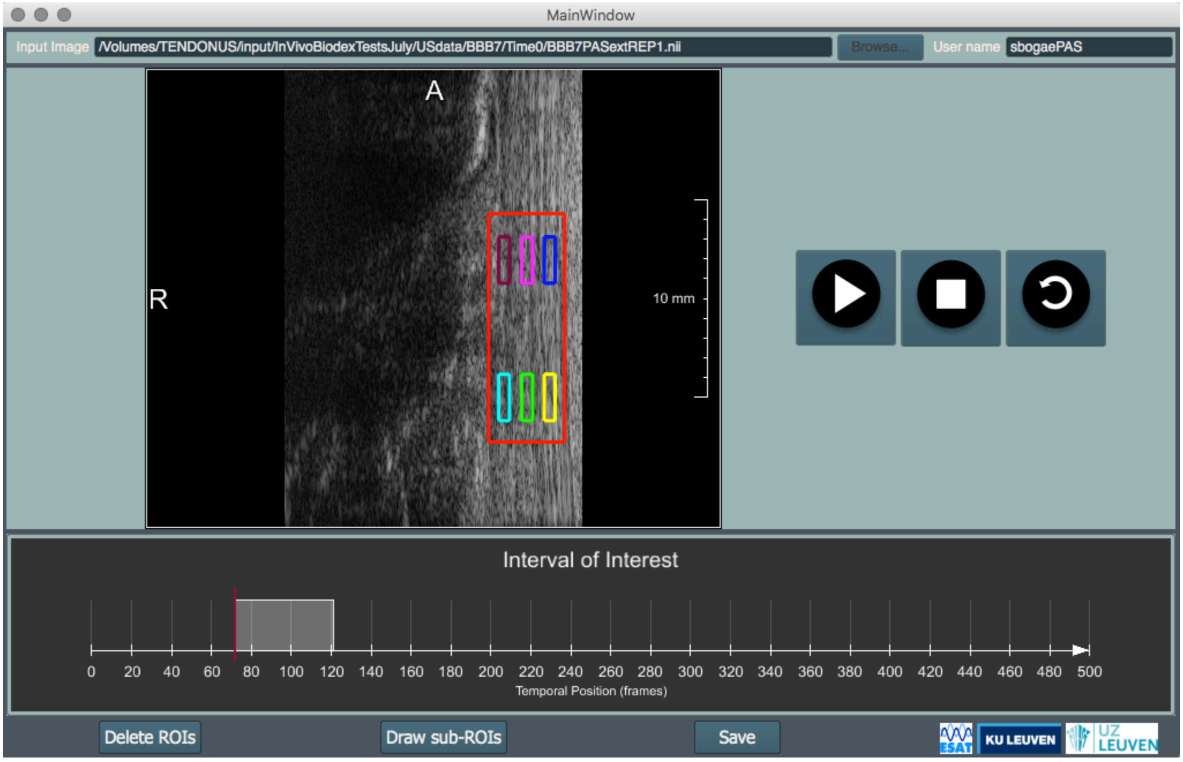
The absolute values of tendon displacement in the data presented here are smaller when compared with previous research applying similar techniques. Arndt et al. [53] reported displacements ranging from 4,6 to 13,5 mm. The results from Slane et al. [52] ranged between 4 and 8 mm. Results in the current study showed displacements only up to 2,95 mm. This could be explained by the differences between studies in range and speed of motion, and position of measurement along the free tendon. The study of Arndt et al. used a similar set-up to this study, but with a larger range of motion, going from 20° plantarflexion up to 15° dorsiflexion at an angular velocity of 15°/sec. The study of Slane et al was slightly different with a position from 0° to 30° plantarflexion at a rate of 0,5 Hz (correlating with a peak angular speed of 90°/sec at mid range of motion), much higher then 15°/sec as used in this study. The Achilles tendon can be considered a visco-elastic tissue, where at low loading rates and slower loading the viscous behavior more important with high energy absorption, leaving more time for creep phenomena and higher local shear strain [33,128] and so conversely lower displacement values. This means that in the set-up used, with a low loading rate (15°/sec) during the passive trials, the tendon will have enough time to deform and creep.

The absolute values of strain in our study are small and much less than values that have been previously reported in the literature documenting global in vivo strain measurement values from 2 up to 11% [41]. However, these small values are in line with results from in vitro research and cadaver studies at intratendinous level. Screen et al. [129] showed, using microscopic imaging-based analysis that local strains at fascicle level are smaller than globally applied strains and never exceeded 1.2%, even at 8% gross applied strain. A study by Arnockzy et al. [105] on rat tails reported similar findings. The regional strain values in this current study are nicely in line with the intratendinous strain measured on cadavers by Lyman et al. [46] where values ranged from 0,07% to 1,11% in different areas of the insertional Achilles tendon. The only other study evaluating tensile strain in vivo, by Chimenti et al. [118], used radiofrequency data from an ultrasound device of lower resolution (10 MHz). Importantly, the set-up was different, as subjects in those experiments were standing or positioned in partial squat during the ankle dorsiflexion motion. Only results from the standing position trials can be compared to our results, given the fact that subjects in our study had their leg extended during all trials (passive and isometric). Strain results in the standing trials by Chimenti et al. [118] varied around 3.33% for the deep and 3.57% for the superficial region. It could be expected that strains are higher during a weight-bearing exercise when compared with a passive elongation or isometric contraction. An important limitation in their results is the high degree of variability, as was also the case in our results.

A limitation of this study is the small sample size with only 10 subjects (20 tendons) evaluated. As was previously mentioned in other research [130], the range of mechanical parameters in healthy controls varies based on many factors (external, e.g. activity, and internal, e.g. material properties). The ICC values were moderate for interday to good for intraday analysis. A second limitation is the high variability in strain values. It was stated previously by other authors that a high variability in strain estimation remains when using commercially available speckle tracking techniques [57]. It is beyond the scope of this article to go in depth on these technical details. However, it is reasonable to believe that a certain degree of variability in local strain results, when measured using ultrasound-based speckle tracking, is physiological and not only due to technical limitations or noise. The high amount of interindividual variation in degree of rotation as seen by anatomical studies [5,6] could already partly explain the high interindividual variation in strain and deformation patterns. An important technical consideration is the fact that tracking of tendon motion with 2D-ultrasound is confronted with out-of-plane motion, which is always to be expected due to the complex 3D-deformation of tendons. However, the impact of this is limited thanks to the high frame-rate of 100 frames per second that is used in these experiments. It has been shown that small frame-to-frame displacements increased the potential to keep scatterers in view, which decreases the effect of out-of-plane motion and therefore enhances the ability to accurately track motion [131]. Also, as mentioned above, all trials were visually reviewed to ensure there were no artifacts (e.g. probe release, air bubble) and a stable speckle pattern was visualized. In the future, 3D-ultrasound will most likely become a tool to overcome the problem of out-of-plane motion artifacts.

To improve clinical applicability, this study also developed an interactive application (Fig 7) to provide researcher SB with an adequate environment to segment and select the ROI's and time-intervals. This could potentially provide clinicians with a tool to track intra-individual change over time. Still, attention has to be made to use a reproducible set-up with adequate image acquisition and good control of image quality. The definition of population-based cut-off values will be much harder, given the previously stated methodological and physiological between-subject variability.

Figure 7 - Interactive application.



Future research should include pathological tendons in different stages of symptomatology, pathology and age, since only a few studies have previously investigated the mechanical deformation in degenerated tendons with computational models [132] or other techniques [94,95,127]. Also, compressive or axial strain [133] is an interesting way of further investigating the local mechanical behavior of healthy and pathologic tendons. Another possible addition could be the combined evaluation of global and regional strain with parallel tracking of the musculotendinous junction and insertion on the calcaneus and torque evaluation. This would provide further insight in the complex structure-function relationship of the triceps surae muscle-tendon unit.

Conclusion

The objective of this study was to quantify the intratendinous deformation patterns of normal Achilles tendons in vivo by means of ultrasound-based speckle tracking. The first novelty of this work was the use of a high-frequency (21 MHz) ultrasound acquisition system. This allowed the tracking of speckle patterns of smaller structures and henceforth a better description of the inter-fiber and inter-fascicular deformation. Secondly, an interactive application was used to improve clinical applicability. The displacement and regional strain in the superficial, middle and deep layer were evaluated during passive elongation and isometric contraction. Building on previous research, results showed that the Achilles tendon displaces non-uniformly with a higher displacement found in the deep layer of the tendon. Adding to this, a non-uniform regional strain behavior was found in the Achilles tendon during passive elongation, with the highest strain in the superficial layer.

Chapter 3: Non-uniformity in pre-insertional Achilles tendon is not influenced by changing knee angle during isometric contractions

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Scand J Med Sci Sports (2018). In press.

In the end, it is not the years in your life that counts. It is the life in your years.

Abraham Lincoln

Abstract

Achilles tendinopathy remains a prevalent condition among recreational and high-level athletes. Mechanical loading has become the gold standard in managing these injuries, but exercises are often generic and prescribed in a “one-size-fits-all” principle. The aim of this study was to evaluate the impact of knee angle changes and different levels of force production on the non-uniform behaviour in the Achilles tendon during isometric contractions. It was hypothesized that a flexed knee position would lead to a more distinct non-uniform behaviour, due to greater differential loading of soleus versus gastrocnemius, and that this effect would be attenuated by higher levels of force production. Contrary to the hypotheses, it was found that the non-uniform deformation, i.e. superficial-to-deep variation in displacement with highest displacement in the deep layer, is consistently present, irrespective of the level of force production and knee angle ($n = 19$; mean normalized displacement ratio 6,32, 4,88 and 4,09 % with extended knee versus 5,47, 2,56 and 6,01 % with flexed knee, at 25, 50 and 75% MVC respectively; $p > 0,05$). From tendon perspective, aside from the influence on muscle behaviour, this might question the mechanical rationale for a change in knee angle during eccentric heel drops. Additionally, despite reaching high levels of plantar flexion force, the relative contribution of the AT sometimes appears to be decreased, potentially due to compensatory actions by agonist muscle groups. These results are relevant for optimizing AT rehabilitation as the goal is to reach specific local tendon loading.

Introduction

The Achilles tendon (AT) is the thickest tendon of the human body and is, like every tendon, structured in a hierarchical manner from collagen molecule up to fascicle bundle [22]. The AT is the tendinous continuation of the three muscles from the triceps surae: the lateral gastrocnemius, the medial gastrocnemius and the soleus [2]. It rotates medially from proximal to distal and inserts on the calcaneus bone at the ankle [5]. Despite this design, Achilles tendinopathy remains a highly prevalent condition among recreational as well as high-level athletes [134]. Although understanding of the multifactorial aetiology of tendinopathy is still incomplete, mechanical loading appears to be of crucial importance in managing this injury, and rehabilitation through active exercise has become the gold standard [13,26]. However, these exercises are often generic and prescribed in a "one size fits all" principle. The classic exercise used in patients with midportion Achilles tendinopathy is the eccentric heel drop [29]. Interestingly, minor modifications to an exercise can make an important clinical outcome difference for patients with insertional tendinopathy [135].

In the currently most often used Alfredson eccentric heel drop program, the calf muscle is eccentrically loaded both with the knee straight and also with the knee bent [29]. The latter has been included to maximize the activation of the soleus muscle [29]. Considering the multi-muscle and rotatory anatomy of the tendon, it can be assumed that changing the knee angle at which the heel drop is performed can influence the local AT deformation pattern, due to differential impact on soleus versus gastrocnemius. Recently, it was indeed confirmed that a change in knee angle has an impact on the non-uniform behaviour of the different layers in the Achilles tendon [52]. This non-uniform behaviour of the AT [8], i.e. a superficial-to-deep variation in displacement and strain of the layers of the AT, has been shown to be present during different contraction modes, e.g. passive [53], eccentric [52] and isometric [136]. An eccentric and passive motion in a flexed knee position have been shown to lead to relatively more displacement in the deep and middle layer of the AT than in the superficial layer, when compared to the extended knee position [52]. This is hypothesized to be linked to proportionally more loading of the soleus muscle in the flexed knee position, with relatively less gastrocnemius contribution, following its origin proximal to the knee joint [52]. Given the fact that the middle and deep layers of the AT are considered to be originating from the soleus muscle [6], relatively higher activation of the soleus muscle is hypothesized to result in relatively higher displacement in the middle and deep layers of the AT [52].

However, it is unclear to what extent these intratendinous changes occur due to the positional changes or due to the subsequent biomechanical advantages or disadvantages that arise. It has been shown that knee flexion causes a drop in force production of ankle plantar flexion because of the more isolated contribution of the soleus muscle, as the gastrocnemius can contribute less because of its origin proximal to the knee joint [137]. Additionally, Finni et al. [138] described the influence of active versus passive muscle contribution to the behaviour of muscle shear, which is believed to have an influence on local tendon behaviour. During passive conditions, there is a slack and compliant connection between muscle bellies of the triceps surae, leaving margin for a heterogeneous non-uniform behaviour, which might also be the case at low levels of force production. During more active conditions, the tensing of muscle connections would lead to more uniform behaviour, which will potentially cancel out any heterogeneous loading at the tendon level, associated with a specific knee position.

Therefore, the goal of this study was to evaluate whether there is a mechanical rationale behind the currently used rehabilitation protocol to perform the eccentric heel drop both with knee in extension as well as in flexion. Also, another goal was to evaluate whether higher levels of force production would attenuate a possible impact of knee angle on local AT deformation. To reach this goal, the local AT deformation pattern was evaluated in an extended as well as flexed knee position, both at three levels of force production (25%, 50% and 75%). To facilitate interpretation and maximize standardization, activation was achieved by isometric contractions, performed on an isokinetic device. The local AT deformation was evaluated by means of high-frequency ultrasound-based speckle tracking [136]. Firstly, it was hypothesized that a flexed knee position would lead to a more distinct non-uniform deformation, and, secondly, that this non-uniform deformation would be attenuated by higher levels of force production.

Material and methods

The study was approved by the UZ / KU Leuven ethics committee (s-number 59330).

Subjects

A convenience sample of healthy subjects was recruited from a group of co-workers at the department of Physical and Rehabilitation Medicine of the University Hospitals Leuven. Candidates were asked to participate and given the opportunity to read the study protocol and sign the informed consent. Subjects with previous history of Achilles tendinopathy, rupture, surgery, and/or systemic or neuromuscular diseases were excluded. Nineteen (19) subjects agreed to participate.

Procedure

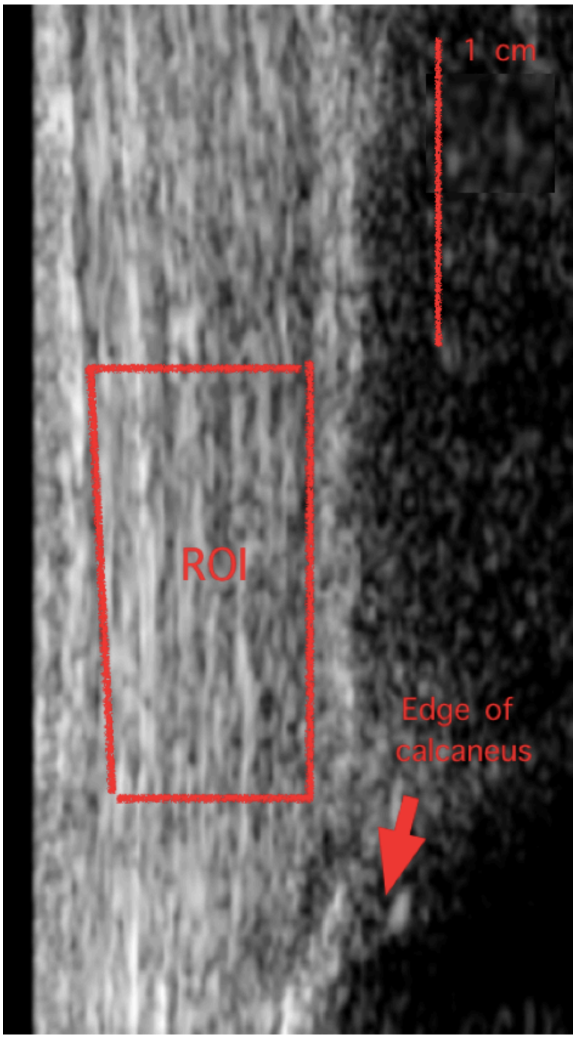
Participants were asked to refrain from physical activity, other than normal ambulation required in daily life, the day before and the day of the test. During the testing, the participant lay prone on a table, knees extended, with the foot fixated to an isokinetic testing device (Biodex system 4 PRO, Biodex Medical Systems, Inc., Shirley, New York).

The knee was extended in a comfortable range for the subject, with goniometer - aligned with the midline of the femur at the iliotibial band and the midline of the fibula along the axis between the fibula head and lateral malleolus – confirming not more than 5° flexion was reached. The axis of rotation of the dynamometer was aligned with the lateral malleolus. The ankle was set in a neutral position. A standardized warm-up of 5 repetitions of concentric plantar- and dorsiflexion through 20° range of motion was performed. A maximal isometric voluntary contraction (MVC) was performed, firstly to get acquainted to the device and secondly to estimate the 25%, 50% and 75% MVC values. Subjects received visual feedback on force development during all tests. They were asked to reach the required level of force production during approximately 2 seconds. Two trials were recorded per level of force production, with exclusion of a trial if there was insufficient image quality (e.g. probe slipping). After reaching 2 trials at 25%, 50% and 75% MVC in the extended knee position, the same was repeated in a flexed knee position. Subjects were kneeling upright and again a maximal MVC was performed to get acquainted with this new position and estimate the 25%, 50% and 75% MVC levels. Knee angle was again measured with the goniometer, confirming 90 +/- 5° was reached. Afterwards, procedure was similar to the extended position. Torque data for each motion trial were extracted from the Biodex machine after finishing all testing procedures.

Ultrasound

A high-spatial and high-temporal resolution US system (Vevo 2100, FujiFilm VisualSonics Inc., Toronto, Canada) was used to acquire 2D + time US images during each motion. The transducer (MS250, FujiFilm VisualSonics Inc., Toronto, Canada) had a central probe frequency of 21MHz and images thus had a spatial image resolution of 0.02x0.09mm. The images were acquired in the pre-insertional tendon area, with the edge of the calcaneus bone always in sight, in the sagittal plane (Figure 1).

Figure 1 - Position of the region of interest (ROI) at the pre-insertional level of the AT.



Tendon deformation evaluation

The entire speckle tracking approach used in this work was described elsewhere [136,139]. In short, after acquisition, ultrasound images were visually reviewed to ensure that there were no artifacts (e.g. probe release, air bubble) and a stable speckle pattern was visualized. A region of interest (ROI) containing only tendon material was selected, to limit the calculation of deformation to the tissue of interest. To reduce computational effort, an interval out of the 500 available frames was selected. The total length of the interval was on average 200 frames, as subjects were asked to reach the level of force in approximately 2 seconds and considering a frame rate of 100 frames per second. This interval was then long enough to capture the complete displacement, always starting registration just before the cue was given to the subject to start the contraction. Point-wise displacement maps were obtained along the major deformation direction representing the tissue displacement in the longitudinal direction (in mm), relative to the starting position in the first frame. The ROI selected at the pre-processing step was then re-used to automatically define 6 sub-regions (3 proximal and 3 distal), consisting of a deep, middle and superficially located sub-region.

Outcome parameters

Based on the speckle tracking output, 2 outcome parameters were calculated. First, local tendon tissue displacement (mm) of the different layers was defined separately, computed directly from the point-wise displacement maps as an average displacement within each sub-region along the major deformation direction. Intraclass Correlation Coefficient (ICC) for this parameter has been shown to be 0.86 for intraday and 0.72 for interday measurements, leading to a Standard Error of Measurement (SEM) of 0.35 mm and 0.44 mm respectively [136]. Second, the normalized displacement ratio (%) was calculated. The normalized displacement ratio is computed as the difference in local tendon tissue displacement between the deep and the superficial layer, and then divided by the average displacement in all three layers combined [112]. This is supposed to take probe slipping and displacement during acquisition, and therefore relative displacement errors, into account. Participants were asked to fill in a document asking their body mass, body height, hours of activity per week, and the validated VISA-A questionnaire 18, questioning tendon health. Subjects with VISA-A score lower than 90 were excluded from this study.

Statistics

All statistics were performed using Statistical Package for Social Sciences (SPSS Inc. version 24., Chicago, Illinois, USA) and the alpha level for all tests was set at 0.05.

First, to evaluate the presence of non-uniform behaviour, local tendon tissue displacement of the different layers of the AT was compared with a two-way ANOVA with repeated measures, separately at each level of force production (25%, 50% and 75% MVC) and knee angle (extended and flexed). Second, to evaluate the magnitude of non-uniform behaviour, the normalized displacement ratio in extended and flexed knee position at three levels of force production was compared, using a repeated measures ANOVA with post hoc Bonferroni adjustment for multiple comparisons.

A paired t-test was used to compare the mean force levels reached at the pre-set levels.

The influence of length, weight and hours activity per week was evaluated using a linear regression.

Results

Subject characteristics (n = 19) are given in table 1.

An overview of outcome parameters is given in table 2.

For the different layers separately, mean (+/- SD) local tendon tissue displacement is given in Table 2 and Figures 2 (knee in extended position) and 3 (knee in flexed position). The results show a significantly different displacement between all three layers for both knee positions at all three levels of force production, except for middle-to-deep at 50% MVC in a flexed knee position (Figure 3). These differences are generally greater than the previously calculated SEM of 0.35 mm for intraday measurements [136].

Table 1 - Subject characteristics (mean +/- standard deviation).

Subjects	11 male – 8 female
Height (cm)	176 +/- 9
Weight (kg)	71 +/- 14
Age (years)	28 +/- 3
Activity per week (hours)	6 +/- 4
VISA-A (/100)	99 +/- 1

Table 2 - Overview of outcome parameters (mean +/- standard deviation).

Knee angle	Force production % of MVC	Displacement superficial layer (mm)	Displacement middle layer (mm)	Displacement deep layer (mm)	Normalized displacement ratio (%)
Extended	25%	3.81 (+/- 1.75)	3.92 (+/- 1.75)	4.02 (+/- 1.74)	6.32 (+/- 7.63)
	50%	5.04 (+/- 2.33)	5.17 (+/- 2.32)	5.27 (+/- 2.31)	4.88 (+/- 5.86)
	75%	6.09 (+/- 2.59)	6.22 (+/- 2.62)	6.32 (+/-2.64)	4.09 (+/- 3.86)
Flexed	25%	3.06 (+/- 1.85)	3.15 (+/- 1.86)	3.22 (+/- 1.85)	5.47 (+/- 5.72)
	50%	5.03 (+/- 2.39)	5.10 (+/- 2.41)	5.15 (+/- 2.42)	2.56 (+/- 3.25)
	75%	4.50 (+/-2.93)	4.58 (+/- 2.90)	4.64 (+/- 2.87)	6.01 (+/- 9.44)

Figure 2 - Mean local tendon tissue displacement during trials with knee in extended position +/- standard deviation. (= p < 0.05; ** = p < 0.01; *** = p < 0.005; **** = p < 0.001)*

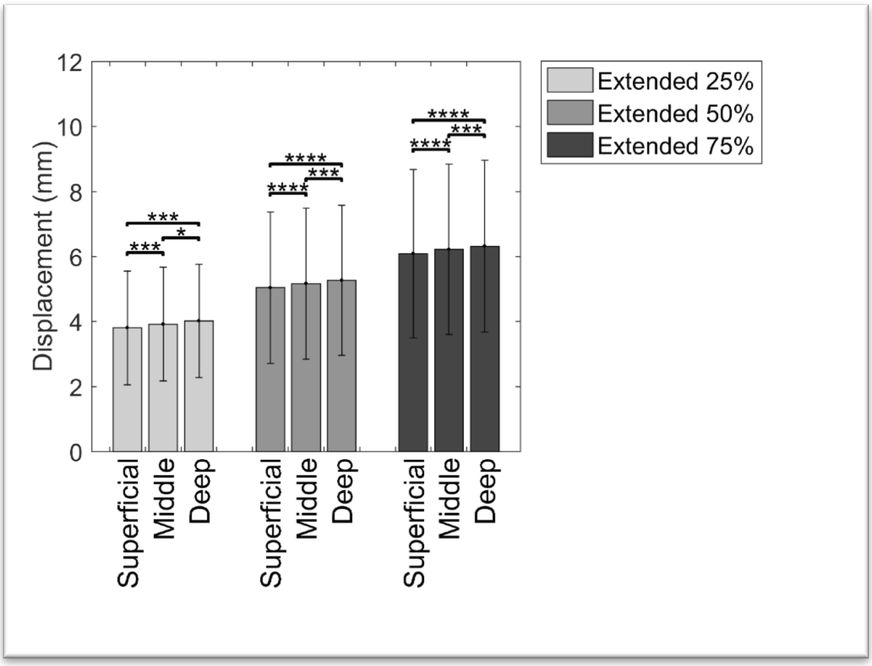
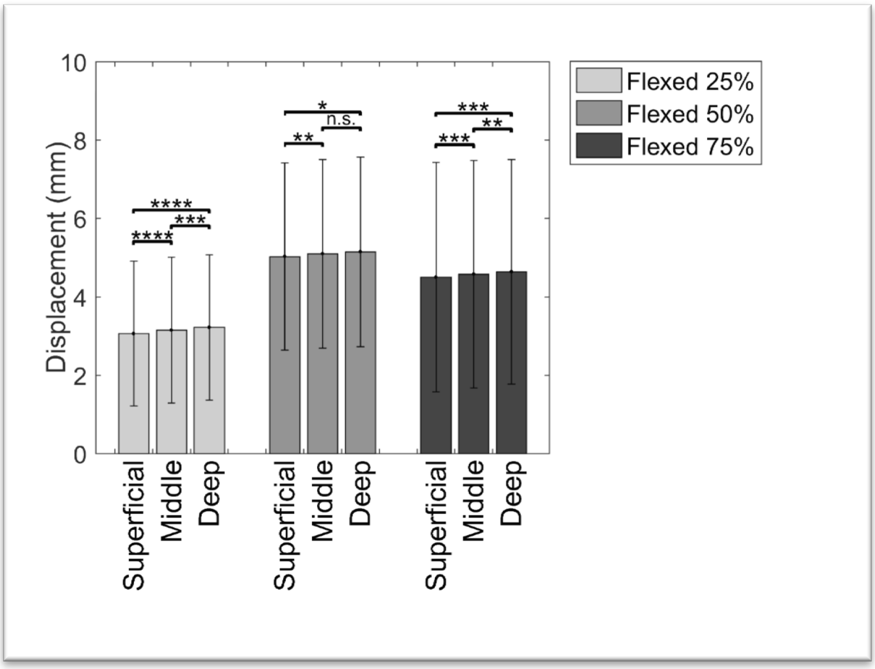
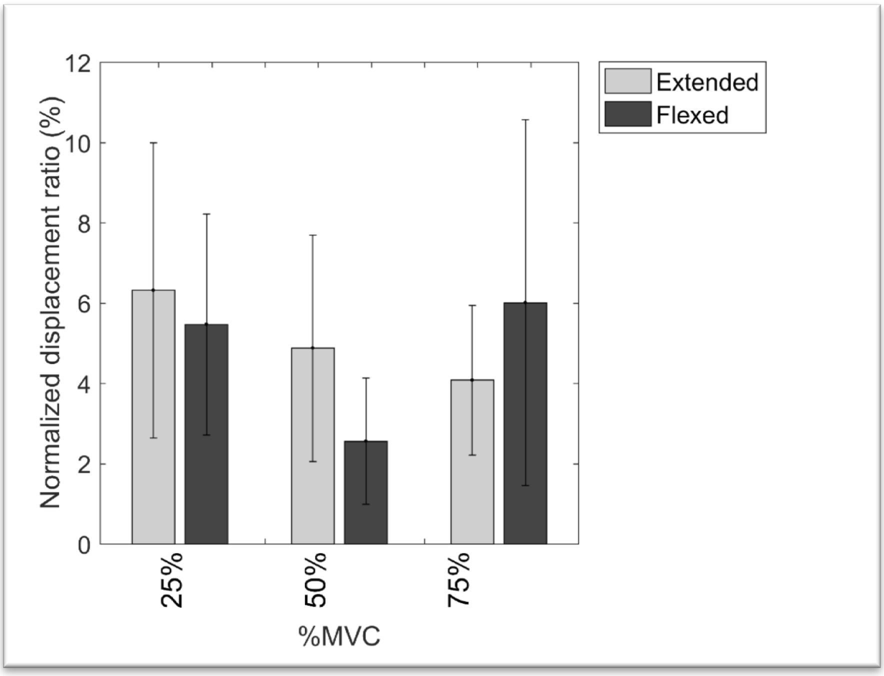


Figure 3 - Mean local tendon tissue displacement during trials with knee in flexed position +/- standard deviation. (= p < 0.05; ** = p < 0.01; *** = p < 0.005; **** = p < 0.001; n.s. = non significant)*



The amount of non-uniform behaviour, as expressed by the normalized displacement ratio, did not significantly differ between different settings, i.e. flexed or extended knee position at three levels of force production (Figure 4). A trend can be seen towards a more uniform behaviour (i.e. decrease in ratio) at higher levels of force production, except for 75% MVC in a flexed knee position. Also, the flexed knee position appears to lead to a more uniform tendon deformation, except at the 75% MVC where the non-uniform behaviour rises again.

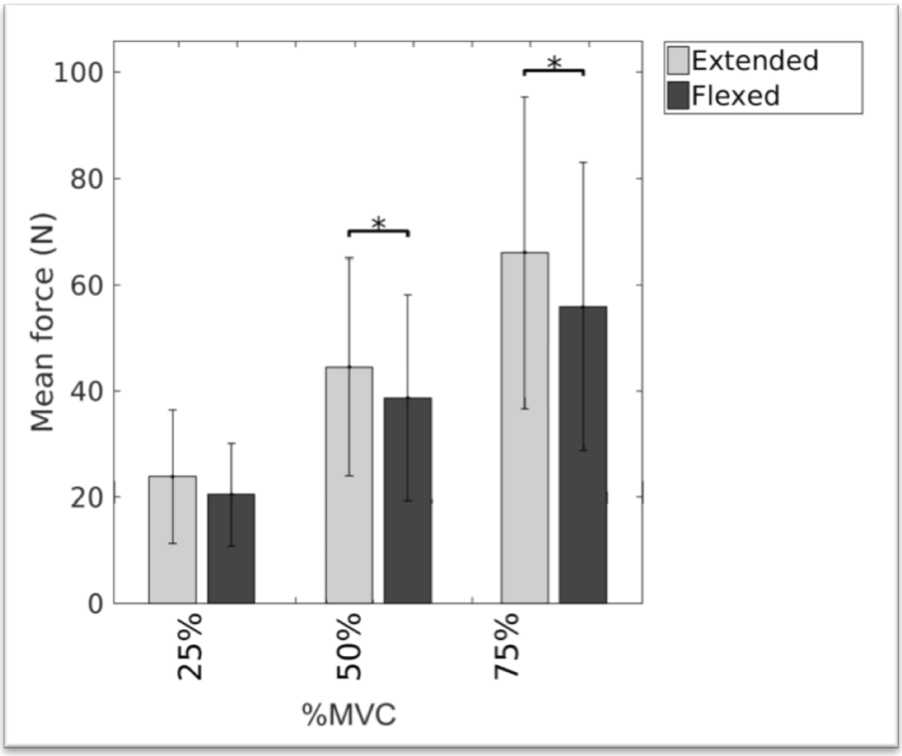
Figure 4 - Mean normalized displacement ratio +/- confidence interval.



Linear regression analysis revealed that none of the independent variables had a significant impact on normalized displacement ratio (body mass: p = 0,28; body height: p = 0,27; hours activity per week: p = 0,77).

Mean levels of force subjects reached at the pre-set levels of 25%, 50% and 75% MVC, can be found in figure 5. The mean level of force reached is always higher in the extended knee position when compared with the flexed knee position, but only significantly different at 50% and 75% MVC (p < 0,02), not at 25% MVC (p = 0,08).

Figure 5 - Mean levels of force at different %MVC at both knee angles. (= p < 0.05)*



Discussion

This study evaluated the impact of different levels of force production and a change in knee angle on the non-uniform behaviour in the Achilles tendon of healthy subjects during an isometric contraction. It was hypothesized that a flexed knee position would lead to higher normalized displacement ratio, and that this effect would be attenuated by higher levels of force production. Contrary to these hypotheses, it was found that the non-uniform deformation in the AT, i.e. superficial-to-deep variation in displacement with highest displacement in the deep layer, is consistently present, irrespective of the level of force production and knee angle.

The results of this study confirm the presence of non-uniform behaviour at all levels of force production (25%, 50% and 75% MVC) and both tested knee angles (extended and flexed). More specific, there is always significantly more local tissue displacement in the middle layer compared with the superficial layer, the deep compared with the middle layer, and the deep compared with the superficial layer. Only deep-to-middle local tissue displacement during trials at 50% MVC in the flexed knee position are not significantly different (Figure 3). This is generally in line with previous research where the same behaviour was found during passive [53], eccentric [52] and isometric loading [136]. Multiple potential reasons for this non-uniform behaviour of the AT have been described. Besides a possible influence from the rotatory anatomy of the tendinous area [2], the multi-muscle origin, with differences in neural activation and cross sectional area of the separate muscles, is hypothesized to contribute [8]. Because of this potentially differential impact of soleus versus gastrocnemius, it could be expected that a change in knee angle, which affects the bi-articular gastrocnemius more than the uni-articular soleus, would impact the intratendinous non-uniform deformation pattern. Indeed, it has previously been shown that with the knee joint in extension, the displacement in proximal gastrocnemius musculotendinous region was higher than in soleus; with the knee joint in flexion this was reversed [140]. In general, total displacement of the proximal musculotendinous area was also higher in extension versus flexion [140]. Previous research by Slane et al. [52] has also shown that when the knee was flexed, eccentric loading significantly altered motion in the mid and deep portions of the tendon, but not in the superficial tendon.

However, results in the current study show no significant impact of changes in knee angle on the amount of non-uniformity in the AT. The amount of non-uniform behaviour, as expressed by the normalized displacement ratio, did not significantly differ between different settings, i.e. flexed or extended knee position at three levels of force production. This is contrary to the hypothesis where it was expected that a flexed knee position would lead to a more distinct non-uniformity due to greater differential loading of soleus versus gastrocnemius. This could be an indication that the assumption of soleus and gastrocnemius having separate sub-tendons is incorrect. Indeed, there is still uncertainty whether the tendinous portions arising from each muscle are still morphologically distinguishable in the free AT. Some papers indicate discernable sub-tendons in the free AT [6], while others do not [3].

Importantly, there are a few differences in methods used to evaluate the non-uniform behaviour between the current study and previous research. Firstly, the region of the Achilles tendon under investigation is different. Others [52] evaluated the midportion of the tendon, whereas this study evaluated the pre-insertional region. It has been shown that there are differences in mechanical behaviour between these regions [46,133], so a different non-uniform behaviour could be expected. Furthermore, the rotational component has a high degree of inter-individual variation. Three types of torsion are described [6], but they commonly demonstrate the most pronounced rotation at the midportion level [5]. This could cause a more distinct non-uniform behaviour at this level of the tendon. Towards the insertion, tendon rotation appears to be less present [6], corroborating a different morphology and mechanical behaviour of this area [46,133]. Secondly, the study of Slane et al. [52] evaluated local tendon behaviour during an eccentric (dynamic) deformation, whereas this study used an isometric (static) contraction in a neutral ankle position. A dynamic motion will cause changes in calcaneus position, an effect that is limited in the fixed position during isometric contractions. The calcaneus position has an important impact on local tendon deformation, as Lersch et al. [47] showed that eversion will differentially impact the deformation of the proximal and distal tendon. Therefore, future studies should evaluate the differences in local tendon behaviour of the Achilles tendon in-vivo during different angles of the ankle joint during isometric contractions. Finally, ultrasound images in this study are obtained at fiber level [136], whereas others [52,53] have evaluated this deformation at fascicle level, using lower resolution ultrasound. Thorpe et al. [44] state, summarizing ex vivo and in vitro research, that ". . . at low force, there is sliding between fibers/fibril, but no real fiber extension. At higher force, there is interfascicular matrix sliding". Consequently, the higher level of ultrasound resolution used in this study might capture a different level of ultrastructural behaviour.

In this study, the local AT deformation pattern was not altered by a change in knee angle and no attenuation of the non-uniform behaviour at higher levels of force production could be shown. However, a trend can be seen towards a more uniform behaviour (i.e. decrease in normalized displacement ratio) at higher levels of force production, except at 75% MVC in a flexed knee position, where this trend is broken. This rise in normalized displacement ratio (Fig 4), and also drop in local tissue displacement (Figs 2 and 3), at 75% MVC in the flexed position, is unexpected. A possible explanation for this finding might be compensatory actions by subjects trying to reach the requested force level. The kneeling upright position used during the knee flexed trials is an adverse position to develop maximal plantar flexion force. Therefore, subjects might be tempted to use agonist muscle groups (e.g. tibialis posterior, flexor hallucis longus, gluteus maximus, etc.). Similar compensatory actions have been found after AT rupture where a high plantar flexion torque appears to be achieved by compensatory action of the flexor hallucis longus muscle [141,142]. Therefore, the AT might be relatively under-used during these trials and the local AT displacement consequently unexpectedly low. During trials with the knee in extension, the position is adequate enough to reach the requested levels of force with sufficient contribution of the AT. It is generally accepted that knee angle has an important influence on force generation capacity in the triceps surae [137,143], resulting in a higher force output in extended position. This was also found in this study as subjects reached statistically significant higher levels of force production during trials with knee in extension when compared to the flexed position (Fig 5). This is again supposed to be linked to the biomechanically more beneficial position for the gastrocnemius muscle-tendon unit in the extended position, whereas the muscle partly loses its contribution in the flexed knee position due to a drop in fascicle length [144].

It is generally known that tendons react to loading with changes in their metabolism [13], although it remains unclear if changes are to be expected in material or mechanical properties [12]. Nonetheless, to achieve a positive outcome with therapeutic loading programs, the data strongly suggest that loading magnitude in particular plays a key role for tendon adaptation, with the need for an adequate local stimulus to the tendon [145]. However, as discussed above, having a patient perform a high load exercise does not necessarily reflect adequate local AT deformation. Too heavy exercises may induce compensation mechanisms using relatively less local AT contribution. Then again, current evidence shows that heavy slow resistance training [31] is equally effective as the isolated eccentric loading program [29]. As discussed in a clinical commentary by Couppé et al. [33], parameters such as load magnitude, speed of movement, and recovery periods between exercise sessions should be more carefully controlled. Therefore, the tool used in this study might be ideally positioned to monitor local AT loading during rehabilitation exercises. However, the clinical relevance of the non-uniform deformation pattern of the AT remains to be further investigated. It has been hypothesized that less non-uniform behaviour at older age [112], due to crosslinks at interfascicular level [16], would limit the possibility for stress dissipation through the tendon by interfascicular sliding. Tendon fascicles themselves then become more vulnerable to tensile damage. A more distinct non-uniformity might therefore be a sign of tendon health, but more research is needed to investigate this hypothesis.

A few limitations should be considered. Firstly, an inherent limitation of studies using speckle tracking to evaluate local tendon mechanics, is out-of-plane motion. However, the high frame-rate that is used in these experiments limits this potential influence [136] and all acquired images were visually reviewed to ensure there was a stable speckle pattern without artefacts. Secondly, the isometric contraction in a neutral ankle position is not exactly a functional position and careful interpretation is warranted. Future research should investigate the local AT deformation pattern during an isometric contraction in different ankle joint angles and during more functional movements like walking or during eccentric heel drops. Thirdly, the mechanical behaviour of normal ATs differs from that of pathological ATs [41]. Therefore, translation of results from this study to the clinical rehabilitation setting should be done with care. A strength of the present study is the use of high-frequency ultrasound-based speckle tracking, leading to evaluation of local tendon mechanics at a smaller hierarchical level than performed so far in literature [136].

Perspectives

Results of this study show that non-uniform behaviour is consistently present within the Achilles tendon, but that its magnitude is not significantly influenced by changes in level of force production or knee angle. From clinical perspective, this might question the mechanical rationale for a change in knee angle during the Alfredson eccentric heel drop program [29]. However, further research is needed to evaluate the local AT deformation patterns during more functional movements. Additionally, it was found that despite reaching high levels of force, the contribution of the AT might sometimes be relatively smaller, potentially due to compensatory actions. These results are relevant for optimizing AT rehabilitation as the goal is to reach specific local tendon loading. Therefore, the tool used in this study might be ideally positioned to monitor local AT loading during rehabilitation and individually tailor tendon exercises.

Chapter 4: Non-uniform behavior of the pre-insertional Achilles tendon during a functional eccentric heel drop in young and middle-aged asymptomatic subjects

Bogaerts S, De Brito Carvalho C, De Groef A, Suetens P, Peers K.

We don't stop playing because we grow old; we grow old because we stop playing.

George Bernard Shaw

Abstract

The Achilles tendon behaves in a non-uniform manner with superficial-to-deep variation in displacement. However, this pattern has been mostly evaluated during passive, isometric or controlled eccentric motions. Parallel to this, the deformation pattern is thought to be influenced by ageing. The primary aim of the present study was to gain more insight in the non-uniform behavior of the AT in the functional setting of an eccentric heel drop with bodyweight load, a type of exercise that is often used during rehabilitation of Achilles tendinopathy. The secondary aim was to evaluate the influence of age on this deformation pattern. This will help further elucidate the complex relation between ageing and changes in local mechanical behavior. First, 25 healthy subjects participated in this study and results confirmed the presence of a non-uniform deformation pattern. Interestingly, a greater range of motion during the eccentric heel drop was found to be related to a greater normalized displacement. Therefore, range of motion should be taken into account during exercises in rehabilitation for Achilles tendinopathy as it appears to influence local deformation. Second, out of those 25 participants, two groups of different ages were matched for sex, height and weight: 7 young (age range 25-30 years) and 7 middle-aged (age range 46-56 years). Surprisingly, no statistically significant differences were found in normalized displacement (i.e. amount of non-uniform behavior) between the young and the middle-aged group. However, there appears to be a trend towards more uniform behavior at older age.

Introduction

The Achilles tendon (AT) has a unique structure as the continuation and intertwining of the subtendons of the three muscles of the triceps surae [2], rotating approximately 90 degrees inwards towards its insertion on the calcaneus bone [5]. Partly because of this anatomical feature, the tendon itself deforms in a non-uniform manner, with superficial-to-deep variation in displacement [53]. This pattern was first shown ex vivo by cadaver studies [47]. In vivo studies focused mainly on the major proximo-distal differences in behavior between the aponeurosis region and the free tendon, inherently failing to take intratendinous patterns into account [146]. However, recent advances in ultrasound [55] have made it possible to also evaluate intratendinous displacement patterns by means of speckle tracking [53]. As such, this technique enabled the confirmation of a non-uniform behavior of the AT in vivo [52,53,112,136]. Methodological set-up has mainly focused on laboratory setting during passive [52,53], isometric [136] or controlled eccentric [112] movements so far. However, question remains whether this non-uniform behavior has a specific clinical significance [147]. Furthermore, recent evidence indicates that functionally relevant higher tendon loads might cause a more uniform displacement pattern, overruling the subtle anatomical superficial-to-deep variation (Bogaerts et al. 2017. Under review). Therefore, the primary aim of the present study was to gain more insight in the non-uniform behavior of the AT in a functional high-load setting.

Aside from methodological issues, ageing might also be an important factor influencing this non-uniform behavior of the AT [112]. Slane et al. [112] have previously shown that ageing influences the non-uniform deformation, with loss of non-uniformity at older age, which is hypothesized to be related to loss of intrafascicular sliding [16]. However, the relation with gross tendon mechanics remains partly unclear, as older age is also related with lower strain [41]. This loss of strain is possibly more related to loss of strength than to intrinsic morphological (i.e. cross sectional area) or material (i.e. Young's modulus) changes in the tendon [92]. Hence, it needs to be confirmed if changes at micro level at older age are related to mechanical and/or material changes at macro level [12]. Research by Slane et al. [112] was during controlled eccentric movements. Therefore, the secondary aim was to evaluate the influence of age on the non-uniform deformation pattern during the high-load eccentric heel drop used in this study.

Therefore, there are two hypotheses. Firstly, it was hypothesized that the non-uniform deformation pattern of the AT would also be present during the functional eccentric heel drop with bodyweight load, an exercise typically used in tendon rehabilitation [29]. Secondly, it was hypothesized that ageing would be associated with a more uniform deformation pattern.

Methods

This study was approved by the Ethical Committee of UZ / KU Leuven (reference number: s59330).

Subjects

A convenience sample of healthy adults was recruited from the department of Physical and Rehabilitation Medicine of the University Hospitals Leuven and through peers of the researchers. Inclusion criteria were: age between 20 and 60 years, and absence of Achilles tendon pain. Subjects with a systemic and/or neuromuscular disease, or previous surgery at the foot and/or ankle, were excluded.

Procedure

After signing the informed consent, subjects filled in a VISA-A questionnaire [113] to rule out ongoing AT problems, were weighed, measured and questioned about their weekly hours of activity. Subjects then took place on a box with 20° inclination, putting the ankle in dorsiflexion. From this position, they were asked to perform a maximal heel rise in 2 seconds and go back down through a controlled eccentric motion in 3 seconds. This timing was guided by the sound of a metronome. The ankle angle was measured with a manual goniometer at the bottom and top position of the motion; the difference between these two angles was then calculated as the ankle range of motion.

During this motion, 2-dimensional ultrasound images were acquired with a high-frequency ultrasound device (Vevo 2100 – FujiFilm VisualSonics Inc., Amsterdam, Nederland) with a probe central frequency of 21 MHz. Images thus had a spatial image resolution of 0.02x0.09mm. The probe was manually positioned longitudinally along the tendon length at the pre-insertional area of the AT of the dominant foot of the participant, with the edge of the calcaneus bone always in sight as reference point. Acquisitions were performed by researcher SB. The participant was allowed to perform a few heel rises and eccentric drops without acquisition, to get accustomed to the motion and the metronome pace. After this, three repetitions were performed during which images were acquired.

Tendon deformation evaluation

The speckle tracking approach used in this work was described elsewhere [54,136]. In summary, all ultrasound images were visually reviewed after acquisition, where trials with artefacts (e.g. probe release, air bubble, shaking image) were excluded. A region of interest (ROI) inside the tendon boundaries was selected. Point-wise displacement maps were obtained along the major deformation direction representing the tissue displacement in the longitudinal direction (in mm), relative to the starting position in the first frame. Six sub-regions (3 proximal and 3 distal) were automatically defined; a deep, middle and superficial. The average displacement within each sub-region along the major deformation direction was computed directly from the point-wise displacement maps. The outcome parameter of interest for non-uniformity was normalized displacement, computed as the difference in tissue displacement between the deep and the superficial layer, and then divided by the average displacement in all three layers combined. The mean of outcome measures during three repetitions was used for analysis.

Statistics

All statistics were performed using Statistical Package for Social Sciences (SPSS Inc. version 24, Chicago, Illinois, USA) and the alpha level for all tests was set at 0.05. All variables were tested for normality with the Shapiro-Wilk test and found to be normally distributed.

A repeated measures ANOVA was used to evaluate for non-uniform displacement between the three tendon layers, in the total population. Post hoc Bonferroni for multiple testing was used for the pairwise comparisons.

An unpaired T-test with unequal variances was used to test for the difference in normalized displacement between both age groups.

The influence of range of motion, body mass, height and hours activity per week, was evaluated by means of separate simple linear regression analyses.

Results

In total, 25 healthy adults participated in the present study (Table 1). To test the second hypothesis on age-related influences, of these 25 subjects two groups of different ages were matched for sex, height and body mass: 7 young (age range 25-30 years) and 7 middle-aged (age range 46-56 years) (Table 2).

Table 1 - Total population characteristics; mean +/- standard deviation

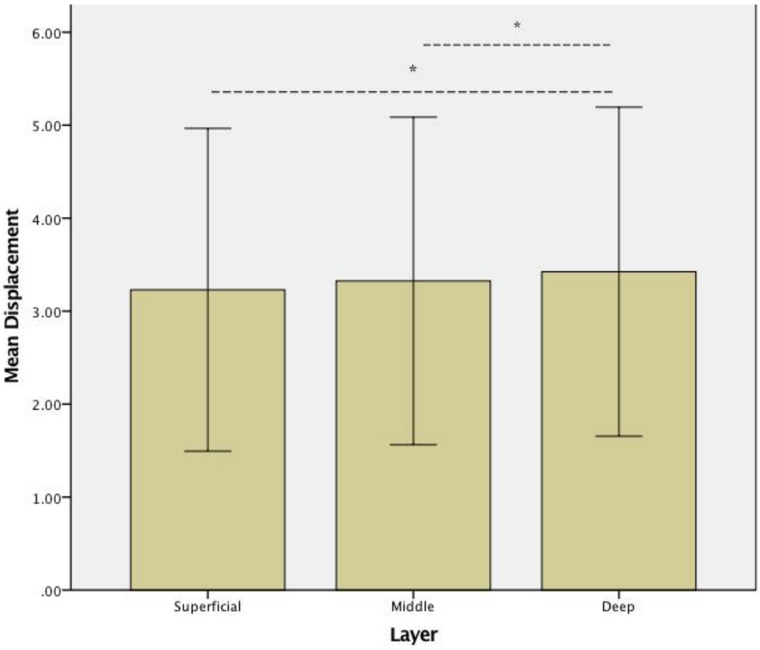
N	13 M / 12 F
Age (year)	34.3 +/- 10.9
Height (cm)	176.1 +/- 9.2
Bodymass (kg)	71.8 +/- 12.7
Activity per week (hours)	5.4 +/- 3.2
Range of motion (°)	40.4 +/- 8.9

Table 2 – Matched age groups characteristics; mean +/- standard deviation (* = p < 0,05)

	Young	Middle-aged
N	3 M / 4 F	3 M / 4 F
Age (year) *	27 +/- 1,5	50,7 +/- 4,2
Height (cm)	176,3 +/- 8,5	175,9 +/- 8,2
Bodymass (kg)	71,7 +/- 9,2	74,1 +/- 8,3
Activity per week (hours)	5,4 +/- 1,9	4 +/- 1,4
Range of motion (°) *	45,9 +/- 8,2	36,3 +/- 7,3

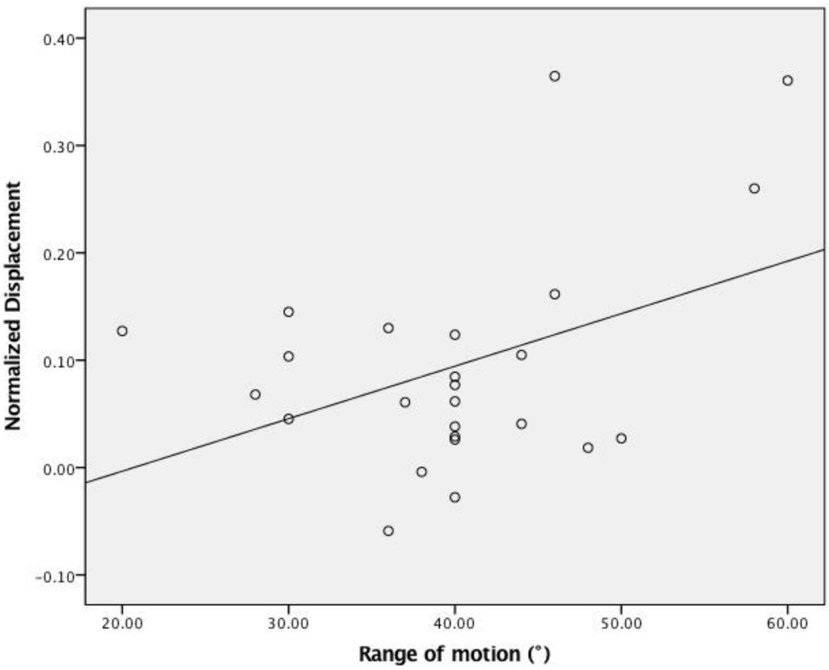
Mean displacement was 3,23 mm (SD 1,74) for the superficial layer, 3,33 mm (SD 1,76) for the middle layer, and 3,43 mm (SD 1,77) for the deep layer (Figure 1). There was a significantly different tissue displacement between the superficial and deep (p=0.004), and between the middle and deep tendon layer (p=0.045) in all 25 subjects. Tissue displacement between the superficial and middle layer was not significantly different (p=0.287).

Figure 1 - Mean (SD) displacement of the superficial, middle and deep layer of the Achilles tendon in healthy subjects (n=25). * p < 0,05.



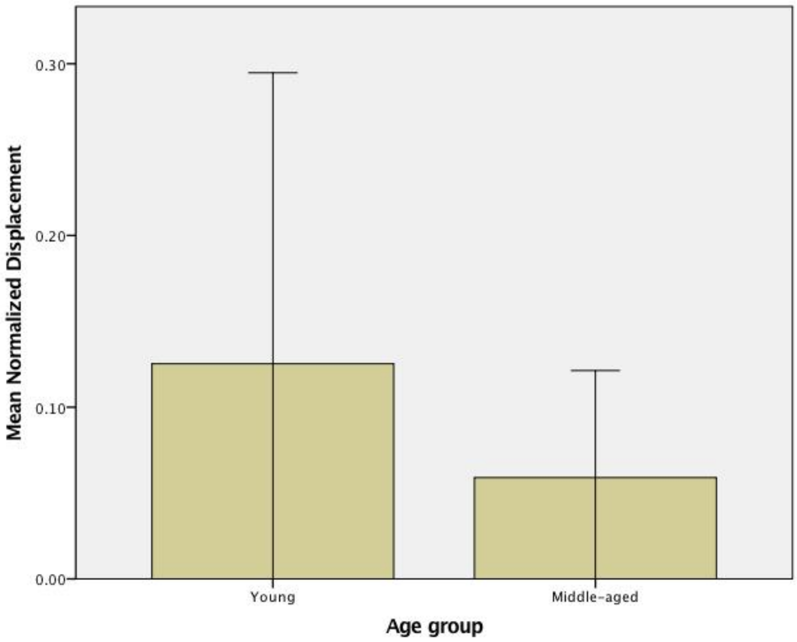
There were no significant interactions between body mass, height or hours activity per week, and the local tissue displacement or normalized displacement. Only for total ankle range of motion, a significant, but very weak [148], correlation with normalized displacement (r^2=0.172; p = 0,04) was found. A more non-uniform deformation pattern was found when there was a higher range of motion during the trial (Figure 3).

Figure 2 - Scatter plot of normalized displacement and range of motion (n = 25).



Mean normalized displacement was 0.13 (SD 0.16) for the young subgroup, and 0.06 (SD 0.06) for the middle-aged subgroup (Figure 2). There was no significantly different normalized displacement between both age groups (p=0.36), despite a trend towards more non-uniform behavior in the younger group.

Figure 3 - Mean (SD) normalized displacement for a young group (n=7) and sex-, height- and body mass matched middle-aged group (n=7).



Discussion

The purpose of this study was to evaluate the presence of the non-uniform displacement pattern of the healthy Achilles tendon during a functional eccentric movement by means of high-frequency ultrasound based speckle tracking [136]. In addition, the possible influence of age on this displacement pattern was evaluated.

Previous research already showed a non-uniform displacement pattern, with the deep layer of the tendon showing highest displacement during passive [52,53], isometric [136] and controlled eccentric [112] movements. The present study confirms that this pattern is present during a more functional movement as well (Figure 1). This is the first time this pattern has been demonstrated examining an eccentric heel drop with bodyweight load, a type of exercise that is often used during rehabilitation of Achilles tendinopathy [29]. The potential reasons behind this non-uniform deformation and heterogeneous loading are numerous; e.g. differences in cross sectional area and neural activation of the muscles of the triceps surae [8], and region-dependent differences in material tendinous properties [121]. However, the anatomy with three subtendons from three separate muscles [2] intertwining into one tendon in a rotatory manner [5] appears to be of potentially critical importance [15].

Nonetheless, only superficial-versus-deep and middle-versus-deep comparisons were statistically significantly different (Figure 1). The superficial layer did not move significantly less than the middle

layer in the present study, as would have been expected from previous research [112,136]. As observed power was found to be 0.69, by means of the software G*Power (version 3.1.9.2, Germany), the sample size appears to be adequate. However, there are a few possible alternative reasons for this finding.
First, the functional eccentric movement is expected to produce high torque levels. In previous research investigating a controlled eccentric contraction, torque levels reached around 30 Nm [112]. An eccentric contraction with bodyweight load as in the present study leads to much higher torque levels, roughly estimated to be around 100 Nm for an average weight of participants in this study around 70 kg. This would probably provoke a more uniform tendon deformation, as it was previously shown that more active conditions cause tensing of muscle connections, which would lead to more uniform behavior [138], as observed in our study specifically between the middle and the superficial layer. Unfortunately, there was no continuous monitoring of ankle angle and force development during the trials in this study, so no quantification of torque development was possible.
Second, an anatomical reason for this lack of significant difference can be postulated as well. The superficial layer of the AT in the midportion region is generally assumed to be the continuation of the medial gastrocnemius muscle, whereas the middle and deep layer are arising for a small part from the lateral gastrocnemius muscle and mainly from the soleus muscle [5,6]. Therefore, it could by hypothesized that the non-uniform pattern is mainly caused by differential contribution to the heel drop by the soleus muscle versus the gastrocnemii muscles [8]. Meanwhile, there might only be little differential displacement between the fascicles arising from the medial versus the lateral gastrocnemius muscle, leading to a more homogeneous deformation in the superficial and middle layer. However, it should be stated that there is a huge inter-individual variation in the twisting anatomy [9] and so interpretation from group-level data should be done with care.
Third, the present study evaluated the deformation pattern in the pre-insertional AT, where others have evaluated the more proximal midportion region [52,53]. This was done to improve repeatability between trials by keeping the calcaneus edge in sight [136]. It is generally assumed that the anatomy of the insertional area is relatively different from the midportion level [10] as well as its mechanical behavior [133]. Therefore, this could potentially have influenced the non-uniform deformation pattern observed in this area.

Interestingly, a greater range of motion during the eccentric heel drop was found to be related to a greater normalized displacement (Figure 3). Given an expected decrease in interfascicular sliding due to crosslinks at older age [16], it was suggested that crosslinking might be a risk factor for the development of tendinopathy; as less non-uniform behavior would limit the possibility for stress dissipation through the tendon by interfascicular sliding, tendon fascicles themselves would be more vulnerable to tensile damage [149]. Therefore, using a higher range of motion during exercises in rehabilitation would emphasize a more non-uniform behavior in the tendon. This could potentially limit the progress of crosslinking and promote interfascicular sliding. Adding to this, duration of the eccentric movement might influence the findings as well. Given the time-dependent changes in mechanical properties of the visco-elastic structure that a tendon is [19], it could be expected that a longer movement during a greater range of motion would cause different estimations of these properties. However, this was prevented by guiding subjects through the eccentric movement with a metronome, making the duration 3 seconds for all subjects.

In view of the second hypothesis, no statistically significant differences were found in normalized displacement (i.e. amount of non-uniform behavior) between the young and the middle-aged group

(Figure 2). However, there appears to be a trend towards more uniform behavior at older age, in line with previous research [112]. A hypothesis supporting increased uniformity with ageing, suggests that increased non-enzymatic collagen cross-linking at older age [150] might reduce interfascicular sliding, leading to a more uniform tendon deformation [16]. However, it would be expected that these changes also lead to a higher stiffness at macro-tendon level, but the contrary has been found with ageing supposedly leading to lower stiffness [41]. A recent review by Svensson et al. [12] supports this finding, but highlights that changes with ageing are multifactorial, as morphological (i.e. tendon cross sectional area), material (i.e. Young's modulus) and neuromuscular (i.e. strength) changes all relate to tendon stiffness. Further research in this area is needed to elucidate this interplay.
Post-hoc power analysis of normalized displacement results revealed a power of only 0.26, which indicates caution should be taken when interpreting the results. Several reasons can contribute to the lack of power and resulting non-significant findings, e.g. small sample size and high standard deviation. It is indeed well known that ultrasound-based measurements of mechanical properties are confronted with methodological challenges [42] as well as inherent physiological and technical variability [57].

Interestingly, as can be seen by the standard deviation crossing the zero-line, some individuals presented with a negative normalized displacement (Figure 2). This means that the superficial layer of the tendon was displacing more than the middle and deep layer, which is in contradiction with previous research [52,53,112,136].
A first possible reason for this finding, would be the rotatory anatomy of the Achilles tendon. A recent rat-based study suggests that the different subtendons of the triceps surae in the AT might have different mechanical properties, with the soleus subtendon demonstrating a lower stiffness [151]. When extrapolating this to humans, it would possibly mean that the observed non-uniform behavior is for a large part due to mechanical differences in the subtendons of the AT. Additionally, as described above, a great variation between individuals in the amount of rotation of the tendon has been described [9]. Possibly, in some individuals the rotation could be that extreme, leading to rotation of the soleus-subtendon to the superficial area. This in turn may lead to the highest displacement in the superficial layer and a non-uniform deformation pattern opposite to what is normally found.
Second, recently a reversed deformation pattern has been shown in highly pathological Achilles tendons, suggesting the hypothesis that this might be a risk factor for injury (Bogaerts et al. 2017. Under review). Therefore, considering the findings in this study, it could be that a certain sub-group of Achilles tendinopathy is related to an extreme rotation of the tendon, leading to a mechanical disadvantage and vulnerability. It has been stated before that Achilles tendinopathy likely consists of smaller sub-groups of specific pathology, e.g. plantaris involvement [152]. Unfortunately, anatomical reconstruction of the rotation of the AT was beyond the scope of this study. A combination of morphological 3-dimensional tendon appearance together with the intratendinous deformation pattern as measured in this study, would help guide further interpretation of local tendon mechanics.

A limitation to acknowledge is the fact that studies using ultrasound-based speckle tracking are inherently confronted with the issue of out-of-plane motion, especially in this research area where the 3D-rotational anatomy of the AT appears to play an important role [9]. To limit this effect, a high frame-rate was used in these trials to limit the potential influence [136] and all images were reviewed to exclude trials where there was faulty positioning or slipping of the probe during acquisition.

Conclusion

This is the first study to show that the non-uniform deformation pattern of the AT is also present during an eccentric heel drop with bodyweight load. The total range of motion of the heel drop might be of relevance and should be taken into account during exercises in rehabilitation for Achilles tendinopathy. Surprisingly, although knowingly underpowered, an age-related difference of deformation pattern could not be significantly proven in this study.

Chapter 5: Healthy and pathological Achilles tendon mechanics assessment using quantitative ultrasound - exploratory study

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Poster presentation at Tendon UK Conference, Oxford, UK – 11 & 12 April 2018.

Abstract published in Translational Sports Medicine, volume 1, issue S1, April 2018.

The problem with quotes on the internet is that it is hard to verify their authenticity.

Abraham Lincoln

Abstract

Knowledge on the mechanical behaviour of the healthy Achilles tendon is growing, but research on pathological tendon mechanics is lacking. The aim of this study was to evaluate the influence of tendinopathy on local tendon mechanics.
Tendon tissue displacement and regional strain of 10 healthy and 10 tendons of subjects with Achilles tendinopathy were evaluated by means of speckle tracking based on high resolution ultrasound imaging data.
Tendon tissue displacement was found to be significantly different between asymptomatic and highly symptomatic volunteers during isometric trials. Also, the non-uniform deformation pattern of the superficial-to-deep tendon layer was reversed in 2 highly symptomatic tendons.
The data thus show an influence of degree of pathology and symptoms on local tendon mechanics. Further exploration of this technique can shed light on the pathogenesis of tendinopathy and may point to mechanical changes occurring prior to the onset of symptoms or morphological changes.

Introduction

Tendinopathy is a major burden for society [21] and an important cause of injury and performance loss in athletes [134]. It is well known that tendinopathy occurs when there is an imbalance between load and capacity [24] and that balanced mechanical loading in a rehabilitation program has a crucial role in managing these injuries [153]. It is generally accepted that tendons respond to mechanical load by consequent mechanotransduction and molecular anabolic signalling [154]. However, the response of the tendon at macrolevel remains unclear. Morphological changes, e.g. increasing cross sectional area, in response to load, appear to occur mainly during youth and adolescence [107]. Changes in mechanical properties on the other hand, e.g. stiffness or strain, might also occur during later stages of life [12,145].
To shed further light on adaptations of the tendon to load, in vivo techniques to quantify morphology and function are needed. Evaluation of tendon morphology is common practice and achieved by means of e.g. ultrasound (US) or magnetic resonance imaging [155]. The evaluation of mechanical properties in vivo is less straightforward. Global tendon stiffness and strain have previously been investigated mainly by using US and tracking of the musculotendinous junction and insertion [41,61]. More recently, improvements in US technology created the possibility of evaluating tendon deformation at intratendinous level, using radiofrequency data [156] or direct B-mode images [53]. Further developments have provided a new direct imaging technique with speckle tracking of US images with higher temporal and spatial resolution [136]. Use of higher resolution allows evaluation of smaller structures and henceforth quantification of mechanics at a different hierarchical level.

In contrast to numerous papers investigating the mechanical behaviour of normal Achilles tendons, only little is known on the relation between pathology, symptoms and function. Arya et al. [94] and Child et al. [95] both found higher strain in Achilles tendinopathy subjects compared with controls. However, in both studies, only global strain was measured, evaluating the relative Achilles tendon lengthening between the medial gastrocnemius myotendinous junction and the insertion on the calcaneus. This global estimation fails to take local non-uniform behaviour into account, which might have a role in the aetiology of tendinopathy [8]. Furthermore, recently no difference in mechanical parameters during a single-leg jump between tendinopathy patients and healthy controls were found, rejecting the hypothesis that the presence of tendinopathy weakens the tendon [157].

The aim of this study is to explore the link between the presence of pathology and tendon mechanics by comparing local tendon mechanics in healthy subjects versus subjects diagnosed with Achilles tendinopathy, the clinical syndrome of pain and dysfunction of the tendon [22]. A recently developed US-based speckle tracking technique was used to evaluate local tendon tissue displacement and regional strain [136].

Materials and methods

Protocol

After approval by the UZ KU Leuven ethics committee (s-number 57302), a convenience sample of healthy subjects was recruited from a group of co-workers. For the pathologic sample, patients with Achilles tendon pathology were recruited from the consultation of physical and rehabilitation medicine at the University Hospitals Leuven. Clinical examination and diagnostic US was performed to confirm the diagnosis of midportion Achilles tendinopathy, and/or exclude differential diagnoses (for example peritendinous changes, rupture, insertional changes). Patients were then asked to participate and were given the opportunity to read the study protocol and sign the informed consent. Subjects with previous history of rupture, surgery, and systemic or neuromuscular diseases were excluded.

Symptomatic subjects were classified based on two factors: (a) symptom severity, objectified using the validated VISA-A questionnaire [113]; and (b) morphological appearance on grey-scale US, objectified using the Archambault score [35], which classifies a tendon as: grade I, normal; grade II, enlarged tendon > 6 mm; and grade III, tendon containing a hypoechoic area, regardless of size.

Participants were asked to refrain from physical activity, other than normal ambulation required in daily life, the day before and the day of the test. The participant lay prone on a table, knees extended, with the foot fixated to an isokinetic testing device (Biodex system 4 PRO, Biodex Medical Systems, Inc., Shirley, New York). The axis of rotation of the dynamometer was aligned with the lateral malleolus. After a standardized warm-up of 5 repetitions of concentric plantar- and dorsiflexion through 20° range of motion, the US probe was attached to a custom-made holder at the mid-portion of the Achilles tendon. Two motions were used, in randomized order by coin toss: 3 repetitions of maximal voluntary isometric contraction in a neutral ankle position of 0° dorsiflexion during 5 seconds, and 3 repetitions of passive elongation from 10° plantarflexion to 10° dorsiflexion and back, at an angular velocity of 15°/sec. Torque data for each motion trial were extracted from the Biodex machine after finishing all testing procedures.

Ultrasound

2D + time US images were acquired during each motion using a high-spatial and high-temporal resolution US system (Vevo 2100, FujiFilm VisualSonics Inc., Toronto, Canada) with a transducer (MS250, FujiFilm VisualSonics Inc., Toronto, Canada) with central probe frequency of 21MHz. The acquired images thus had a spatial image resolution of 0.02x0.09mm, with an image width of 23mm and an image depth of 21mm. The maximum width allowed by the transducer was always used, while the depth was adapted per tendon to visualize the entire tendon. Gain remained the same through the acquisitions since this only affects the image globally and is only useful for visualization purposes. Time gain compensation (TGR) and dynamic range (DR) also remained the same throughout the experiment to avoid subjectivity and variation of the acquired images. The images were acquired from the midportion region of the Achilles tendon, approximately 2 cm proximal to where the calcaneus bone was no longer visible on ultrasound.

Tendon deformation evaluation

Speckle tracking is commonly used for quantification of US images. The entire approach used in this work was described elsewhere [54,136]. In short, after acquisition, ultrasound images were visually reviewed to ensure that there were no artefacts (e.g. probe release, air bubble) and a stable speckle pattern was visualized. A region of interest (ROI) containing only tendon material was selected, to limit the calculation of deformation to the tissue of interest. To reduce computational effort, an interval of 100 frames, out of 500 frames of the cine-loop, was selected. The transformation between the last frame of the cine-loop (100th) and the first one was obtained by composition of intermediate pair-wise image transformations (1 vs 2, 2 vs 3, …, 98 vs 99, 99 vs 100). At the end, point-wise displacement maps were obtained along the major deformation direction representing the tissue displacement in the longitudinal direction (in mm), relative to the starting position in the first frame. The ROI selected at the pre-processing step was then re-used to automatically define 6 sub-regions (3 proximal and 3 distal), consisting of a deep, middle and superficially located sub-region. The average displacement within each sub-region along the major deformation direction was computed directly from the point-wise displacement maps. Regional strain (in %) was computed along the major deformation direction between the proximal and distal regions for the superficial, middle and deep layer respectively.

Statistics

Discrimination between classes was estimated by means of a mixed model approach using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina). This model had the repetition of the measurement per leg, torque and class as fixed variables. Tendon tissue displacement and regional strain in the superficial, middle and deep layers were the response variables with the subject being defined as a random intercept variable. As described before in studies on the patellar tendon [158], relative displacement was afterwards evaluated as a measure of non-uniformity, computed as the average displacement in each layer (superficial, middle and deep) minus the average displacement in all these three layers combined. The significance level was set at $p \leq 0.05$. Given the normal distribution of the data (tested through Shapiro-Wilk test), mean tendon tissue displacement, regional strain and relative displacement were compared between the different classes in all three layers using an unpaired 2-sided t-test.

Results

The total sample (Table 1) consisted of 10 healthy subjects with the dominant leg being tested, and 8 patients with the symptomatic leg being tested. In 2 patients, there was a bilateral Achilles tendinopathy, leading to a total number of 10 pathologic tendons tested. Asymptomatic tendons were grouped as C0 (n=10 tendons), while symptomatic tendons were grouped according to Archambault score as C1 (grade I, n=3), C2 (grade II, n=4) or C3 (grade III, n=3).

Table 1 - Patient data (* = significant difference, M = male, F = female, SD = standard deviation, gr = grade)

	Pathologic (n = 10)	Healthy (n = 10)	Between group difference (p-value)
Male - Female	7 M - 3 F	6 M – 4 F	N/A
Age (years)	45 (SD 18)	26 (SD 3)	0.003*
Weight (kg)	76.6 (SD 21.3)	72,0 (SD 14.0)	0.55
Archambault score	3 gr I - 4 gr II - 3 gr III	10 gr 0	N/A
VISA-A (%)	75.1 (SD 24.4)	100 (SD 0)	0.005*

Tissue displacement

Mean superficial, middle and deep displacement during isometric trials (Table 2 - Figure 1) were found to be significantly different between subgroups C0 and C2 (p = 0.01) and between C0 and C3 (p = 0.02), as well as between C1 and C3 (p = 0.01).

No significant differences in displacement were found during passive trials (Table 2 - Figure 2) between any subgroups, although there was a trend towards higher displacement in the more pathologic tendons.

*Figure 1 - Displacement during isometric trials in the superficial, middle and deep layer. * = < 0,05; ** = < 0,01; *** = < 0,005.*

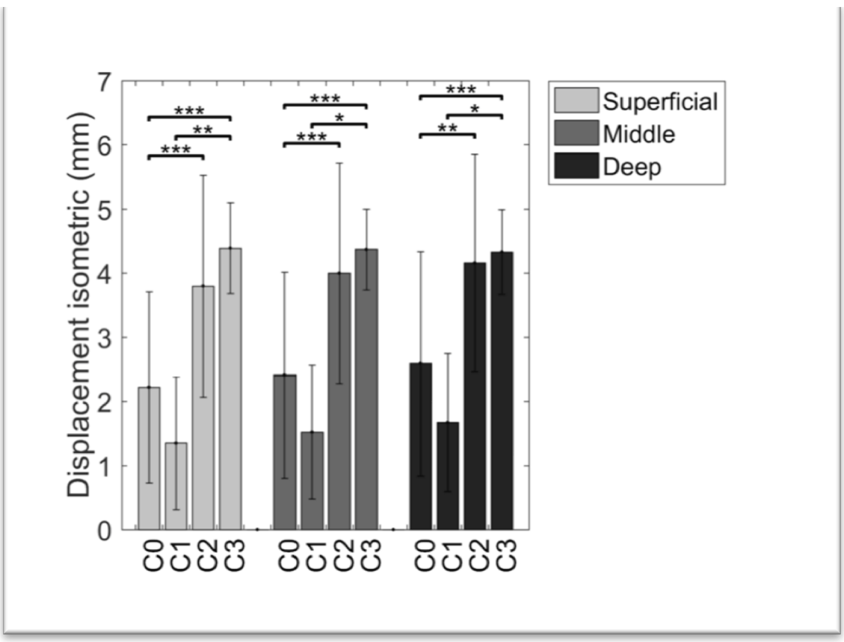


Table 2 - Main outcome parameters for all sub-groups (tissue displacement - mm and regional strain - %). Values represented as mean (+/- standard deviation) for a) isometric trials and b) passive trials.

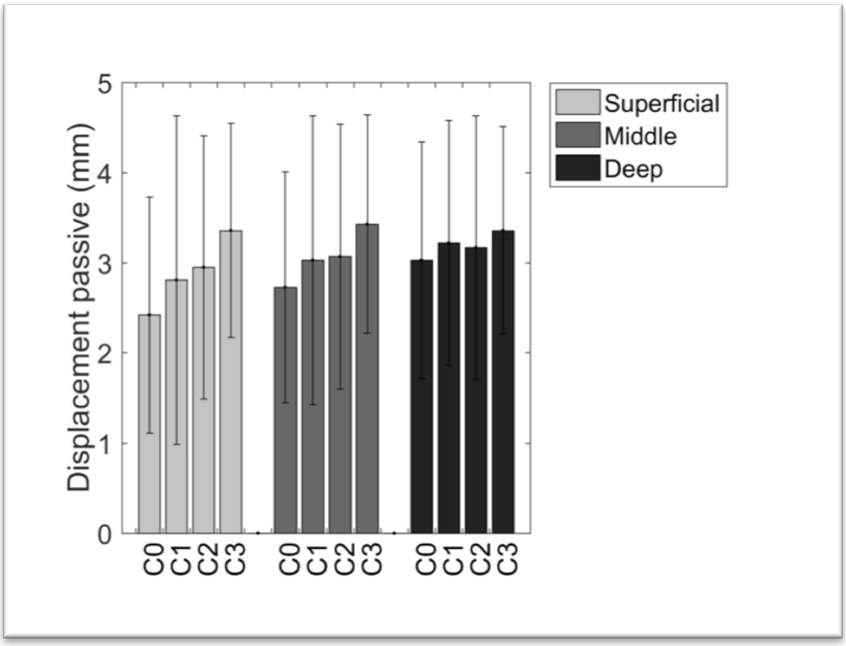
2a) Isometric trials

		C0	C1	C2	C3
Tissue displacement	Superficial	2.22 (+/- 1.49)	1.35 (+/- 1.04)	3.80 (+/- 1.73)	4.39 (+/- 0.71)
	Middle	2.41 (+/- 1.61)	1.52 (+/- 1.04)	4.00 (+/- 1.72)	4.37 (+/- 0.63)
	Deep	2.59 (+/- 1.75)	1.67 (+/- 1.07)	4.16 (+/- 1.70)	4.33 (+/- 0.66)
Regional strain	Superficial	0.29 (+/- 0.47)	0.16 (+/- 0.11)	0.23 (+/- 0.14)	0.11 (+/- 0.06)
	Middle	0.27 (+/- 0.49)	0.10 (+/- 0.10)	0.19 (+/- 0.11)	0.19 (+/- 0.18)
	Deep	0.26 (+/- 0.52)	0.13 (+/- 0.10)	0.14 (+/- 0.10)	0.21 (+/- 0.23)

2b) Passive trials

		C0	C1	C2	C3
Tissue displacement	Superficial	2.42 (+/- 1.31)	2.81 (+/- 1.82)	2.95 (+/- 1.46)	3.36 (+/- 1.19)
	Middle	2.73 (+/- 1.28)	3.03 (+/- 1.60)	3.07 (+/- 1.47)	3.43 (+/- 1.21)
	Deep	3.03 (+/- 1.31)	3.22 (+/- 1.36)	3.17 (+/- 1.46)	3.36 (+/- 1.15)
Regional strain	Superficial	0.33 (+/- 0.50)	0.15 (+/- 0.12)	0.23 (+/- 0.21)	0.18 (+/- 1.14)
	Middle	0.29 (+/-0.49)	0.16 (+/- 0.10)	0.22 (+/- 0.18)	0.14 (+/- 0.07)
	Deep	0.24 (+/- 0.50)	0.16 (+/- 0.8)	0.19 (+/- 0.16)	0.11 (+/- 0.09)

Figure 2 - Displacement during passive trial in superficial, middle and deep layer.



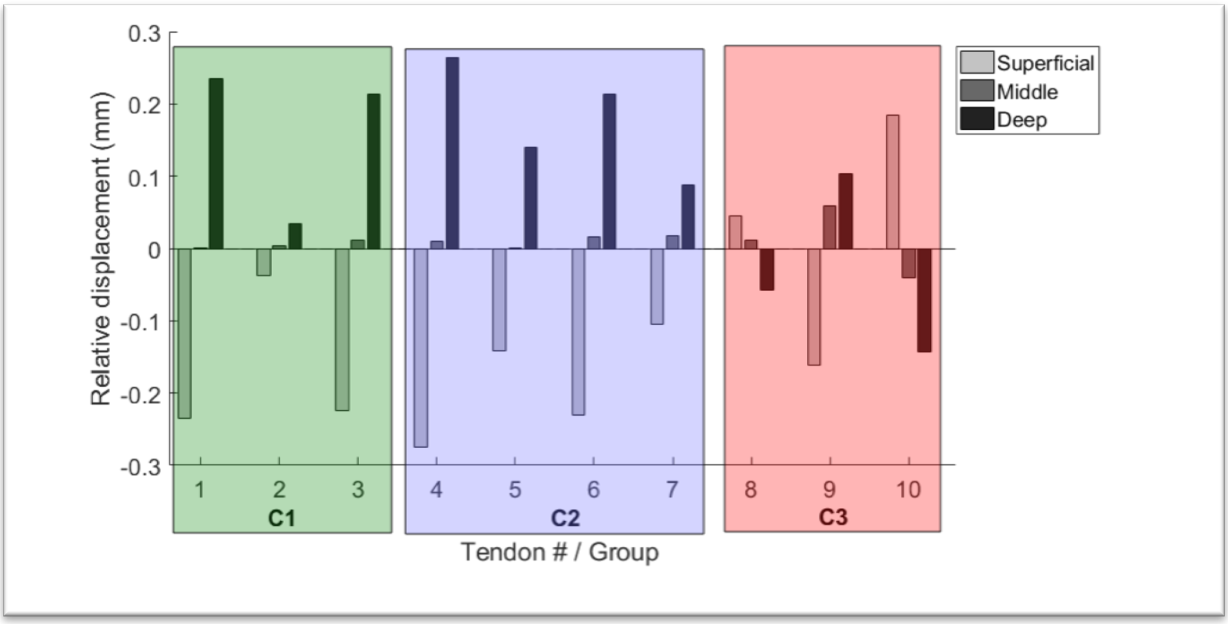
Regional strain

No significant differences in regional strain were found between subgroups during neither isometric nor passive trials (Table 2).

Non-uniformity – Relative displacement

No significant differences in relative displacement were observed between subgroups, nor between symptomatic and asymptomatic tendons. Most symptomatic tendons presented with the well-known higher displacement in the deep layer [52,53], as can be observed by the relative displacement being positive for the deep layer (Figure 3). However, in two cases (tendons 8 and 10), there was an inverse deformation pattern with the relative displacement of the deep layer being negative, meaning that the superficial layer had the highest displacement.

Figure 3 - Relative displacement pattern for each tendon and sub-group.



Discussion

The aim of this study was to compare local tendon mechanics in healthy versus pathologic Achilles tendons using high-frequency US-based speckle tracking. Displacement in all three layers during isometric trials was significantly different between asymptomatic tendons and symptomatic Archambault grade II and grade III tendons, as well as between symptomatic Archambault grade I and grade III tendons.

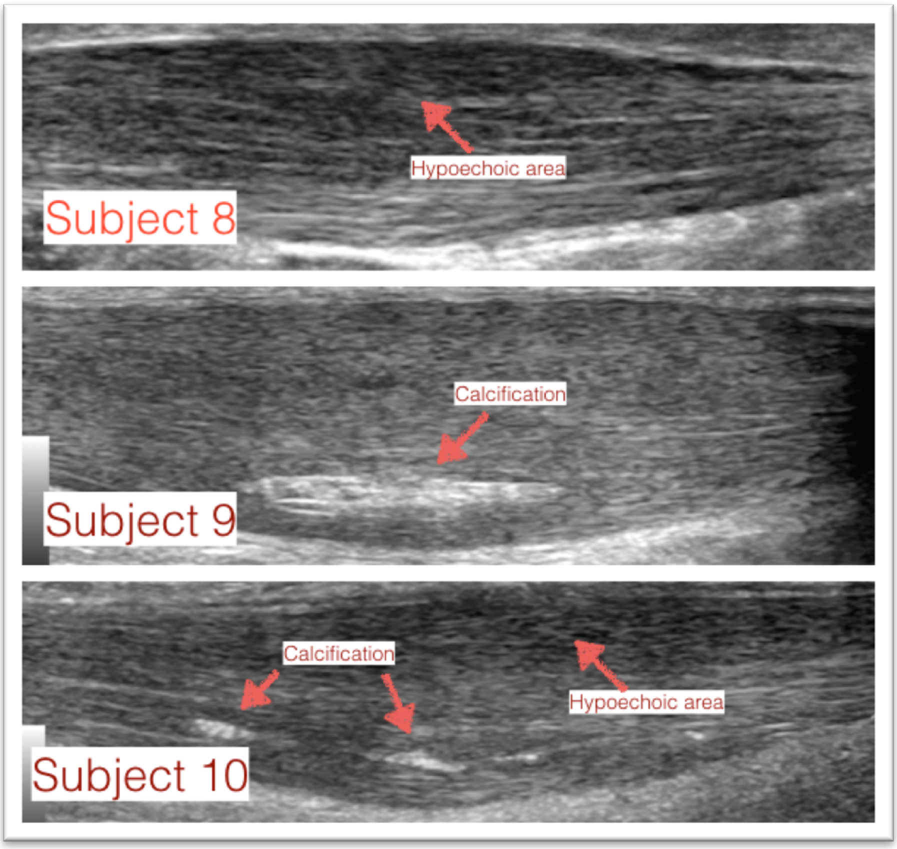
Only a few studies have evaluated the mechanical behaviour of pathologic Achilles tendons. Arya et al. (2010) and Child et al. (2010) both found higher global strain in Achilles tendinopathy subjects compared with controls, leading to the hypothesis that the presence of tendinopathy leads to a weakened tendon. Arya et al. (2010) showed, similar to our results, a higher elongation (≈ displacement) in the presence of tendinopathy, but their mean peak elongation (12.72 mm) was much higher than mean displacement (3.24 mm) in this study. However, it should be noted that they evaluated global tendon mechanics by tracking of the medial gastrocnemius myotendinous junction, as opposed to this study where local tissue displacement was tracked. The regional strain values measured in this study were also much smaller (e.g. regional strain in superficial layer = 0.09%) than the global strain values measured in the study by Arya et al. (2010) (mean = 5.14%). This corroborates the findings from ex vivo studies that showed a major difference between tendon deformation at global versus local level [105,129], with smaller values observed at local level. Furthermore, while the positioning on the isokinetic device was similar between studies (subject lying prone with extended knee and hip), part of the absolute difference could also be attributed to differences in fixation. Unlike the study of Arya et al. (2010), the subjects in this study were not attached to the chair with extra straps over the body and could therefore develop less force. The importance of testing protocol has previously been shown [159]. The study of Child et al. (2010) used a different set-up with the knee in 90° flexion. As it was previously shown that this could have an important impact on deformation patterns in the Achilles tendon [112], no further comparisons with these results were made.

In the present study, we found no differences in regional strain behaviour of pathologic versus normal tendons. It is known that there is a high variability in strain estimation using speckle-tracking algorithms [57]. Taking this technical limitation into account and in view of the lack of differences in this exploratory study, more research is needed to evaluate the role of region-specific strain in normal versus pathological tendon mechanics.

In this study, also the influence of the degree of pathology on mechanical parameters was evaluated. A significantly different isometric displacement in all three tendon layers between asymptomatic (C0) and highly pathologic (C2 and C3) tendons was observed. These differences exceeded the standard error of measurement for this technique, which was previously estimated in repeatability trials on normal tendons as being 0.54 mm during isometric displacement trials, in the superficial layer [136]. To our knowledge, this is the first study to discriminate stages of pathology based on mechanical behaviour of the Achilles tendon, using a direct US-based approach. Previous research in this field applied indirect techniques quantifying structural characteristics, without dynamic functional evaluation [160]. Further studies should investigate the time-dependency of these changes in a prospective manner.

It is generally accepted that a tendon deforms in a non-uniform manner, with highest displacement in the deep layer [52,53]. When evaluating this non-uniform behaviour between the subgroups of the pathologic tendons in this study (Figure 3), no differences were found. This could be attributed mainly to low statistical power, as the subgroups contained only 3 or 4 tendons. However, when evaluating the individual non-uniform displacement patterns, 2 of the 3 most pathological tendons in sub-group C3 (tendon 8 and 10) showed a reversed non-uniform displacement, with the highest displacement measured in the superficial layer. Observation of their grey-scale US images revealed that these 2 tendons had a distinct hypoechoic area located in the superficial layer (Figure 4). The third pathologic tendon in this subgroup C3 (tendon 9) only had a limited hypoechoic area in the medial part, together with a calcification in the deep layer. The calcification did not seem to influence the normal tendon behaviour in tendon 9, as the displacement in the deep layer was in the same line as the other tendons in subgroups C0, C1 and C2 (Figure 3). It could therefore be assumed that the presence of pathology, a hypoechoic area i.e. loss of collagen fibril integrity, is closely related to the intratendinous deformation pattern in the Achilles tendon. The presence of a calcification does not appear to have the same influence. The time course of this relation is unknown, as it might be that mechanical changes (i.e. higher displacement, changes in non-uniform behaviour) precede structural changes (i.e. hypoechoic area). It is known from interventional studies on healthy tendons that material (e.g. Young's modulus) and mechanical (e.g. stiffness) properties change quicker in response to load, as opposed to morphological properties (e.g. cross sectional area), which takes several months [145]. It could thus be that the pathological response of a tendon to overloading is first noticeable in changes in material and mechanical properties and that we should focus more on functional evaluation of tendon deformation, rather than structural characterization.

Figure 4 - Ultrasound images of pathologic AT of subject 8, 9 and 10.



An important limitation of this study is the small sample size; it should therefore be considered exploratory. Yet, sample size was in the same line as previous studies, where Arya et al. [94] evaluated 12 symptomatic and 12 controls, and Child et al. [95] studied 14 symptomatic and 15 controls. Nonetheless, future studies should increase the sample size.

A second potential limitation is the difference in age between the healthy and symptomatic subjects (Table 1). However, with a median age of 31, 38 and 50 in sub-groups C1, C2 and C3 respectively, this effect is partly lost. Furthermore, a recent review has highlighted the fact that most research reporting age-related tendon changes reported changes to occur during maturation into adulthood but not later, and that no clear structural change can be linked with changes in tendon mechanics [12]. Also, during isometric trials, the strength of the subject, which is assumed to decline with age [92], could have an important impact on the estimation of mechanical properties [12]. However, no significant differences in torque levels between sub-groups were found in the current study. Therefore, in this exploratory setting it can be assumed that the differences in mechanical behaviour between sub-groups were linked mainly with the presence of pathology, rather than the age of the subject.

Finally, out-of-plane motion is an inherent limitation when using ultrasound to evaluate the complex 3D-deformation of tendons. However, the impact of this is limited thanks to the high frame-rate of 100 frames per second that is used in these experiments [136]. It has been shown that small frame-to-frame displacements increased the potential to keep scatterers in view, which decreases the effect of out-of-plane motion and therefore enhances the ability to accurately track motion. Also, data were collected with a handheld transducer, which enabled the researcher to better retain transducer alignment during motion. Furthermore, all trials were visually reviewed to ensure there were no artefacts (e.g. probe release, air bubble) and a stable speckle pattern was visualized. In the future, 3D-ultrasound will most likely become a tool to overcome the problem of out-of-plane motion artefacts.

Summary

Local tendon mechanics, i.e. local tendon tissue displacement, were found to be significantly different between asymptomatic and highly symptomatic subjects with Achilles tendinopathy during isometric trials. Also, the non-uniform deformation pattern of the superficial-to-deep tendon layer was reversed in 2 highly symptomatic tendons.

The data thus show an influence of the presence and degree of pathology and symptoms on local tendon mechanics. Further exploration of this technique can shed light on the pathogenesis of tendinopathy and may point to mechanical changes occurring prior to the onset of symptoms or morphological changes.

General discussion

The general discussion of this thesis starts with an overview of the main findings of the PhD project, followed by a discussion of some aspects of the speckle tracking method used in this project. In a next section, a reflection on the non-uniform behaviour of the AT is made, together with a link towards other tendons and the aetiology of tendinopathy in general. From thereon, the clinical implications of results of this thesis towards treatment tailoring is discussed. Finally, suggestions for future research are described.

1. Overview

This PhD project aimed to establish and validate a clinically oriented evaluation platform to quantify intratendinous deformation in the Achilles tendon, and to apply this within a patient-centred environment. This development and clinical implementation established the pathway for further investigation, interpretation and understanding of the complex relationship between structure and function in Achilles tendons possible.

In **chapter 1**, a systematic review of strain mapping in the AT was performed to provide an overview of existing literature in the field of functional evaluation of intratendinous deformation [41]. The primary objective of this review was to outline the applied methods already used in the literature. This was combined with summarizing the current insights from ultrasound-based evaluation of mechanical properties of the AT, both at the local and global level. The real-time 2D ultrasound with tracking of two reference points during an active maximal voluntary contraction was found to be the most frequently applied technique [40]. The relative change in tendon length thereby leads to an estimation of tendon strain (expressed as a percentage) [19]. AT strain has been investigated across a variety of contexts, including the response to exercise, walking, unloading, ageing, hormonal changes and weight. Only three studies investigated the effect of the presence of tendinopathy on strain behaviour [72,94,95]. The most important result was that nearly all studies evaluated global strain, i.e. the relative change in length between musculotendinous junction and calcaneal insertion. One studie investigated the transverse strain response of the AT, i.e. the change in tendon diameter before and after exercise [63]. However, no study evaluated local – intratendinous strain, emphasizing the need for further exploration of techniques enabling this.

In **chapter 2**, a high-frequency ultrasound-based speckle-tracking technique was introduced and validated by quantifying the intratendinous deformation patterns of normal ATs in vivo [136]. An important key point in this paper lies on the advantages of the use of a high-frequency (21 MHz) ultrasound acquisition system versus the lower resolution techniques used in literature so far [52,53]. This advanced technique allowed the tracking of speckle patterns of smaller structures and henceforth a better estimation of the inter-fibre deformation. Besides this, the development of an interactive application to improve clinical applicability was explained. In this application, a clinician can choose time intervals and regions of interest within the tendon, to enable an individual tendon-tailored functional evaluation.
Intra-class correlation was 0.86 for intraday and 0.72 for interday measurements. Results confirmed that the AT displaces non-uniformly, with a higher displacement observed in the deep layer of the tendon. This is in line with the findings of Slane et al. [52] and Arndt et al. [53], both using lower-resolution

ultrasound-based techniques. In addition, when evaluating strain, results showed a non-uniform regional strain behaviour in the AT during passive elongation, with the highest strain observed in the superficial layer. This is in line with results of a cadaver study by Lyman et al. [46], using an invasive technique on the insertional AT.

Subsequently, in **chapter 3**, the aim of the study was to evaluate the impact of different levels of force production and a change in knee angle on the non-uniform behaviour in the Achilles tendon during an isometric contraction. It was hypothesized that a flexed knee position leads to a more non-uniform behaviour, due to greater differential loading of soleus versus gastrocnemius in this position, but that this effect can be attenuated by higher levels of force production. Contrary to the hypotheses, it was found that the non-uniform deformation, i.e. superficial-to-deep variation in displacement with highest displacement in the deep layer, was consistently present, irrespective of the level of force production and knee angle. From clinical perspective, this might indicate the absence of a mechanical rationale for a change in knee angle during eccentric heel drops. Additionally, it was found that at the highest requested plantar flexion force level, the relative contribution of the AT was decreased in the flexed knee position, potentially due to compensatory actions by agonist muscle groups. This is relevant for AT rehabilitation that aims to reach specific local tendon loading.

Additionally, in **chapter 4**, the primary aim was to gain more insight in the non-uniform behaviour of the AT in a functional setting. Results confirm the presence of a non-uniform deformation pattern in the setting of an eccentric heel drop with bodyweight load, which is a type of exercise that is often used during rehabilitation of Achilles tendinopathy. The secondary aim was to further elucidate the complex relation between ageing and changes in local mechanical behaviour. Surprisingly, the results revealed no statistically significant differences in normalized displacement (a measure of non-uniformity, computed as the difference in tissue displacement between the deep and the superficial layer, divided by the average displacement in all three AT layers combined) between the young and the middle-aged group. Interestingly, a greater range of motion during the eccentric heel drop was found to be related to a greater normalized displacement. Therefore, using a higher range of motion during exercises in rehabilitation might be of relevance and should be considered during exercises in rehabilitation for Achilles tendinopathy.

In **chapter 5**, the influence of Achilles tendinopathy (i.e. symptoms and/or structural pathology) on the mechanical properties of the AT was evaluated. The aim of this study was to compare local tendon mechanics in healthy versus pathologic ATs as this would shed further light on the complex relation between pain (i.e. symptoms), structure (i.e. intratendinous pathologic areas) and function (i.e. mechanical properties).
Results showed that displacement during isometric trials was significantly different between asymptomatic tendons and symptomatic Archambault [35] grade II and grade III tendons, as well as between symptomatic Archambault grade I and grade III tendons. No differences in regional strain behaviour of pathologic versus normal tendons were found.
These results highlighted some form of interaction between the presence and degree of pathology, and local tendon displacement, but no strain, in this controlled setting during an isometric and passive deformation.

2. Speckle tracking

The applied technique used in this PhD project to quantify intratendinous deformation was ultrasound-based speckle tracking. As explained in chapter 2, speckles are representations of the tissue under investigation, i.e. tendon material in this thesis. When these speckles are tracked during a deformation, an estimation of local tissue displacement can be made. This technique has been used in cardiology for many years [48], but translation to musculoskeletal applications has proven to be challenging [56]. Several issues related to speckle tracking methodology can cause difficulties and should be considered when using and interpreting results of studies using this technique. Specific technical issues causing this difficulty are partly discussed in chapter 2, but are mainly outside the scope of this project [57]. However, two aspects particularly relevant for the AT should be mentioned, namely out-of-plane motion and the importance of the ultrasound central probe frequency.

2.1 Out-of-plane motion

One of the possible physiological reasons causing difficulties to use speckle tracking in the AT is its twisted anatomy, which was highlighted in in the general introduction. Because of this twisting effect, out-of-plane motion is more likely to occur. Out-of-plane motion occurs when the tracked speckles move into and away from the field of view. Since the anatomical twisting is happening in a 3-dimensional manner, the 2-dimensional ultrasound system is not able to fully capture this phenomenon and prevent out-of-plane motion during dynamic acquisitions. However, the high temporal resolution (frame-rate of 100 frames per second) used in the acquisition system in this project will partly limit this effect; the small frame-to-frame displacements increases the potential to keep speckles in view, thereby decreasing the effect of out-of-plane motion and therefore enhancing the ability to accurately track motion [131]. Furthermore, there was subjective quality control in all studies in this thesis, as all trials were visually reviewed and controlled for accurate probe positioning and clear image quality.

2.2 Ultrasound central probe frequency

The tendon deformation obtained from a speckle tracking technique is dependent on the images acquired by the ultrasound device. If lower spatial resolutions are used, speckle patterns will result from large structures; when using higher spatial resolutions, speckle patterns will result from smaller structures [54]. Therefore, the central probe frequency of the device used is crucial, as probe frequency is directly linked to resolution. Most studies evaluating local tendon deformation so far have used central probe frequencies in range of 10-14 MHz [52,53]. As described in chapter 2, tests were performed to compare the image resolution of a 10 MHz with a 21 MHz frequency probe. It was found that these acquisitions would result in an axial resolution (i.e. the capability to distinguish two objects lying behind each other) of approximately 0.308 mm and 0.0367 mm, respectively. In the context of tendon ultrasound, the axial resolution is an important aspect to distinguish the parallel aligned collagen bundles. Relating these axial resolutions to the hierarchical dimensions of the tendon would mean that 10 MHz enables visualisation at fascicle scale (dimensions 0.05 – 0.4 mm), whereas 21 MHz would enable visualisation at fibre scale (dimensions 0.01 – 0.05 mm) [2]. It can thus be stated that the higher resolution ultrasound allows for tracking of deformation at a different hierarchical tendon scale.

This is important, as previous studies have evaluated tendon deformation at lower end scales (fibrils, fibres), using laboratory- and ex vivo-based techniques, and have found relevant differences with higher end scales. Indeed, it has been shown that there are different features and modes of deformation throughout the fibril, fibre, fascicle and whole tendon scale [45]. Tendons are believed to deform through a combination of different deformations; fascicle sliding, i.e. differential displacement of fascicle 'x' parallel to fascicle 'y'; fascicle rotation, i.e. longitudinal rotation of adjacent fascicles like a tightening rope; fibre sliding, i.e. differential displacement of fibre 'x' parallel to fibre 'y' [106]. Therefore, the high-frequency approach used in this project likely enables the additional evaluation of inter-fibre sliding, aside from currently existing techniques at higher scales, visualising rotation and/or sliding of fascicles.

Furthermore, Cheng and Screen focused on bleached collagen fibres and fibrils and noted an inhomogeneous strain response in the matrix [43]. They found the local strain values to be much smaller than the applied values of strain in the loading axis, likely caused by different protective mechanisms in the non-collagenous matrix. This would potentially aid in energy transfer through the different tendon scales. Direct tensile cell overload would be prevented, and instead deflected into a more shearing load on the cells [123], as tendon cells are located between fibres and dispersed in the interfascicular matrix [161]. Therefore, the tool used in this project could be a valuable asset in the evaluation of multiscale mechanics. However, it should be stated that the clinical relevance of information from this microscale is currently unclear. Undoubtedly, further research with this technique, when combined with advancements in in vitro research, will provide valuable insights in the linkage between multiscale tendon mechanics.

3. Non-uniform behaviour of the AT

In all clinical studies of this thesis, the non-uniform behaviour of the AT, i.e. superficial-to-deep variation in displacement and strain of the different layers of the tendon, has been found. From these findings, combined with results from previous research, several reasons for and influencing factors of this non-uniform behaviour seem to exist, which will be discussed here. Moreover, the presence of a non-uniform behaviour in other tendons will be reviewed, as well as its possible role in the aetiology of tendinopathy in the AT.

3.1 Reasons of non-uniform behaviour

Multiple reasons for this non-uniform behaviour have already been described. First, through differences in morphology and neuromuscular activation patterns, the multi-muscle composition of the triceps surae inherently leads to the possibility of differential muscle activation and therefore tendon deformation [8]. Indeed, mechanical modelling simulations found intratendinous deformation mechanisms (e.g. fibre-fascicle sliding) with differential muscle forces being the largest contributing factors to non-uniform behaviour [15].

Second, the rotational anatomy of the AT provides an extra reason for some unexpected biomechanical observations in the non-uniform deformation pattern of the AT. It is counterintuitive that the deep part of the AT would displace more, given the moment arm is greater for the superficial part. However, it has been hypothesized that the deep layer of the AT, representing mainly the GL and SOL subtendon, in general twists more than the superficial [6]. This would lead to a higher pre-tension in the deep part of the tendon, leaving little to no margin for extra straining during deformation and therefore more displacement in this layer of the tendon. For the same level of force going through the whole tendon, the superficial part on the other hand could then still undergo more straining during deformation. This falls in line with the findings of chapter 2 where highest strain was observed in the superficial layer, as was also shown in cadaver-based work in earlier research [46]. However, it should be stated that there is still uncertainty whether the tendinous portions arising from each muscle are still morphologically distinguishable in the free AT [3].

3.2 Influencing factors of non-uniform behaviour

Interestingly, in chapter 2 the significant difference in regional strain between the three AT layers was only found during passive trials. Adding to this, in chapter 5, the absolute difference in mean displacement between the superficial and deep layer of the AT was significantly larger during the passive elongation, compared to the isometric trials. This might be related to previous findings of an activity-induced decrease in relative displacement of SOL and LG [122], which in turn could be expected to have an influence on tendon behaviour. During passive conditions, there is a slack and compliant connection between muscle bellies of the triceps surae, leaving margin for an independent, i.e. non-uniform, behaviour. During active conditions, the tensing of muscle connections would then lead to a more uniform behaviour.

These findings led to the investigation of the impact of amount of force development on local AT mechanics, as described in chapter 3. It was hypothesized that the non-uniform deformation pattern would be less pronounced at higher levels of force production. Contrary to this, it was found that the non-uniform deformation in the AT, i.e. superficial-to-deep variation in displacement with highest displacement in the deep layer, was consistently present, irrespective of the level of force production. Yet, a possible limitation might be the methodological set-up, i.e. activation mode was an isometric contraction on an isokinetic device at 25, 50 and 75% MVC. The lack of motion at the ankle joint likely inhibits the direct translation of these results to functional movements like walking or running. As mentioned, the calcaneus has an important impact on local tendon deformation, as it has been shown that eversion will differentially impact the deformation of the proximal and distal tendon [47]. Therefore, future studies should evaluate the impact of different levels of force development in a functional setting, e.g. weighted heel drops with progressively increasing external loads, on local non-uniform behaviour of the AT.

Besides the amount of force development, the rate of loading is another important factor to consider. In the isometric trials of chapter 3, subjects were asked to reach the requested MVC (25, 50 or 75%) in 2 seconds. Therefore, the loading rate in these trials was low, as e.g. a mean load reached by subjects during the 75% MVC trials of 60 N would equal a loading rate of only 30 N/sec. This is in sharp contrast to loading rates during functional movements, which have been shown to be as high as 600 N/sec during treadmill running [162]. This is important, as loading rate will influence the preferential loading of muscle versus tendon. As explained in chapter 1, the mechanical behaviour of muscle and tendon is closely related. An increase in muscle force generating capacity will be accompanied by a change in the mechanical properties of the associated tendon, supposedly to avoid potential harm to the tendinous tissue [163]. However, muscle and tendon respond differently to different rates of loading. It has been reported that mechanical (i.e. stiffness) and morphological (i.e. size) properties of human Achilles tendon were more responsive to a low number of loads with low loading rate (6-second cycle) than to a high number of loads with high loading rate (2-second cycle) [82]. Conversely, plyometric movements, i.e. exercises with fast stretch-shortening cycles, appear to promote muscle strength development, while it does not appear to change tendon stiffness [163].

The AT can be considered a visco-elastic tissue, from which it is known that they are stronger and stiffer when loaded at high loading rates, while at low loading rates the viscous behaviour is more important. These lower loading rates involve more energy absorption at the tendon level, leaving more time for creep phenomena and higher local shear strain [33,128]. It can therefore be hypothesized that at lower loading rates, the non-uniform deformation pattern will be more enhanced, whereas at higher loading rates this pattern will be attenuated by a preferential and thus higher muscle activation. As discussed in chapter 1, mechanical load can only cause changes in mechanical properties of the tendon by triggering tenocytes. Therefore, a lower strain frequency may lead to more effective fibre recruitment and henceforth a greater transfer of the external tendon strain magnitude to the cellular level. Because of this, it is important to realize that besides level of force development, as shown in chapter 3, also the loading rate is an important determining factor in estimation of mechanical properties.

3.3 Non-uniform behaviour in other tendons

As mentioned, the AT has several unique properties that contribute to the observed non-uniform deformation pattern. However, to further improve our understanding of this phenomenon, it is interesting to look at other tendons and explore how a non-uniform behaviour might be relevant in normal or pathological tendon mechanics. The non-uniform deformation of the rotator cuff and patellar tendon will now be discussed, respectively. The rotator cuff was chosen, given its crucial part in upper limb biomechanics and its tendency to get injured in athletes performing overhead sports [164]. The patellar tendon was chosen as this tendon has already been thoroughly investigated towards its non-uniform behaviour.

Rotator cuff

The anatomical footprint of the AT and the rotator cuff display a high level of similarity [165]. In comparison to the complex loading deformation pattern in the AT, the mechanical behaviour of rotator cuff tendons is even more difficult to interpret. Despite the absence of rotatory intertwining of different subtendons as in the AT, an inhomogeneous deformation pattern has also been shown inside the intact supraspinatus tendon [166]. Differences in histological features throughout the joint- and bursal-side layers of the rotator cuff have been indicated as a possible reason [167]. On top of this intratendinous heterogeneity, there is a complex interaction between the separate muscles and tendons of the rotator cuff (supraspinatus, infraspinatus, subscapularis, teres minor), further complicating the deformation pattern. For example, it has been shown that an interaction exists between the infraspinatus tendon and the strained supraspinatus tendon, leading to increases in infraspinatus tendon strain when the supraspinatus tendon is injured [168]. In the same line, it can be hypothesized that injury at a certain area in the AT will have an impact on the mechanical behaviour at other areas. This will likely contribute to the pathogenic cascade and increase risk of future injury, if not adequately and fully rehabilitated.

Research investigating these intratendinous deformation patterns in the rotator cuff has so far mainly been cadaver-based. In these studies, a significant difference in strain between the joint- and bursal-side tendon layer of the supraspinatus has been found during glenohumeral abduction [119,169]. It has been hypothesized that differences in bursal versus joint-side strain may account, at least in part, for the initiation and propagation of intratendinous tears of supraspinatus [169]. Interestingly, as in the AT, the lowest strains in the supraspinatus tendon were found on the layer closest to the joint [119]. However, the strain distribution appears to be angle dependent, with a relatively uniform strain distribution seen throughout the tendon at 45 and 60 degrees of abduction [119]. Interestingly though, at higher degrees of glenohumeral abduction, absolute strain values in the supraspinatus tendon were also significantly higher, pointing towards higher force development [119]. Therefore, a more uniform behaviour at higher degrees of abduction corroborates with the findings from chapter 3 in this thesis, where a trend was noticed towards more uniform behaviour at higher levels of force production during trials with the knee in extension. However, the relative contribution of joint angle versus amount of force development to the attenuation of non-uniformity is unclear.

When attempting to quantify local deformation patterns of the rotator cuff in vivo, no studies have been successful thus far. There has been one ultrasound-based speckle tracking approach using 2D speckle tracking echocardiography [108], but with important limitations to be taken into account [56]. The main shortcoming lies in the fact that cardiac speckle tracking techniques cannot readily be applied to musculoskeletal tracking, as mentioned in chapter 2 and in the discussion of the speckle tracking technique. The technique used in this thesis might be technically well suited, but the local anatomy of the shoulder region poses difficulties to overcome, e.g. bony acromial coverage of the cuff and the bended trajectory of the tendons.

Patellar tendon

In contrast to the lack of research, especially in vivo, on the rotator cuff, the patellar tendon has been the subject of numerous cadaver- as well as in vivo ultrasound-based studies. However, it should be stated that these studies were also performed with lower resolution (spatial and temporal) ultrasound. In one of the these cadaver-based studies, strain measurements of the most proximal part of the patellar tendon revealed low strains in the posterior, joint-sided part of the tendon [170]. This is thus in line with findings in the rotator cuff [119] as well as results in chapter 2 in this thesis, where regional strain was lowest in the deep, i.e. joint-sided, layer of the AT. However, these results have been contradicted by a later study combining cadaver-testing with computational modelling, where it was found that localized tendon strain at the classic location of the jumper's knee lesion, proximal-posterior, was increased in association with an increase in the magnitude of applied patellar tendon strain and deeper knee flexion [132]. Again, in vivo research of deformation patterns in the patellar tendon has supported the finding of lower strain in the deep-proximal region of the patellar tendon. Using a speckle tracking approach, lower proximal strains in the posterior tendon were found compared to the anterior region [171]. Furthermore, this pattern has recently been evaluated across a range of knee angles. Anterior strain was most often found to be significantly greater than posterior strain, and this for all knee angles. In the knee, similarly as in the rotator cuff, this might as well be related to region-specific material properties. It has been shown that tendon fascicles from the anterior portion of the human patellar tendon in young men displayed higher material stiffness when compared with the posterior portion of the tendon [121]. Unfortunately, so far, no research has investigated the possible contribution of region-specific material properties in the AT. However, this will be less straightforward, given the interindividual variation in rotatory anatomy, making it difficult to directly link certain areas to specific subtendon-regions.

Finally, previous research also indicated that the knee joint angle affects the regional strain differentially, resulting in greater shear between the tendon layers with force application when the knee is in greater degrees of flexion [172]. This again highlights the importance of methodological set-up when comparing estimations of mechanical properties, since as a change in joint angle will have an influence on the measured parameters.

3.4 Link with aetiology of tendinopathy

The observed deformation pattern in the AT is a representation of the force transfer through the different tendon scales. In the aetiology of tendinopathy, one hypothesis entails the phenomenon of "stress shielding", i.e. the phenomenon where areas demonstrate smaller local deformation. In the stress shielding hypothesis it is thought that the aetiopathogenic stimulus for the degenerative cascade of tendinopathy is the catabolic response of tendon cells to mechanobiologic under-stimulation [126]. In vitro research has shown that a healthy cell has a solid cell-matrix interaction. When a region is overloaded, this interaction is damaged and the cell undergoes changes in shape and markers of inflammation and matrix degeneration rise [123]. From thereon, the cell is relatively "stress shielded" from its surrounding environment and the degenerative cascade can proceed. For example, in the patellar tendon, lowest strain and thus stress shielding is observed at the deep-proximal location, which is also the location where patellar tendinopathy typically occurs [170]. However, the translation of this hypothesis from the patellar to the AT is challenging, as tendon pathology in the AT does not appear to represent in one preferential location. Sonograms of symptomatic tendons revealed that 81% had abnormalities in the proximal two-third of the AT, 8% in the distal third, and 11% at both sites. In the group with proximal two-third involvement, 91% had medial tendon involvement, 19% lateral [173]. The rotatory anatomy is the likely cause of this, as pathology appears to be linked to certain subtendons with particular fascicular involvement [174]. However, as previously mentioned, high variability in anatomy exists between individuals [5,6,9].

The role of the observed non-uniform deformation pattern in this cascade remains up for debate. It has been hypothesized that a loss of non-uniformity with ageing could be considered a risk factor for the development of tendinopathy, given the rise in incidence of tendon disorders at older age [112]. However, in chapter 4 of this thesis, no statistically significant differences were found in normalized displacement (i.e. amount of non-uniform behaviour) between a young and middle-aged group. This interaction is hypothesized to be related to increased non-enzymatic collagen cross-linking at older age [150], impeding normal intratendinous deformation modes (e.g. fibre-fascicle sliding), and henceforth causing a more uniform tendon deformation pattern [16]. However, it could be expected that increased cross-linking also leads to a higher stiffness at macro-tendon level. However, as highlighted in chapter 1, the contrary has been found with ageing, supposedly leading to lower stiffness. A recent review by Svensson et al. [12] indeed reported stiffness to be decreased at older age, but highlights that changes with ageing are multifactorial, since morphological (i.e. tendon cross sectional area), material (i.e. Young's modulus) and neuromuscular (i.e. strength) changes all relate to tendon stiffness. Further research is needed to elucidate the interplay between the non-uniform deformation pattern, ageing and aetiology of tendinopathy.

4. Treatment tailoring

The overall objective of this PhD project was to establish a clinically oriented tool to quantify intratendinous deformation in the AT. Hereby, the intention was to gain further insight in its structure-function relationship with an ultimate goal to improve and fine-tune existing therapeutic algorithms for patients with Achilles tendinopathy. In this part of the general discussion, the clinically most relevant findings of the different studies in this thesis towards treatment tailoring are discussed. A short background on the main intentions of Achilles tendinopathy rehabilitation and the link with AT mechanics is laid out. This is followed by an outline of factors influencing AT deformation, as found in this thesis (knee angle, ankle angle, and range of motion). Finally, a summary highlights the clinically most relevant findings.

4.1 Background

It is generally accepted that tendons react to loading with changes in their metabolism through the mechanism of mechanotransduction [13]. Nonetheless, it remains unclear whether the mechanical properties of tendinous tissue are adapted only in longer term to match changes in the associated muscle [147], or are changed more quickly in response to specific training stimuli (e.g. eccentric training, heavy slow resistance training) [145].

It has indeed been suggested that regardless of age, AT mechanical properties adapt to match the level of muscle performance [92]. Unfortunately, no studies have investigated changes in both tendon mechanical properties as well as strength in response to loading or ageing in a longitudinal design spanning further than 6 months. However, during the maturation phase in adolescence, it has been shown that most of the tendon core is formed during height growth, reaching a steady state around the age of 17 [107]. This suggests that the influence of maturation-associated changes is much more profound and its effect on mechanical and structural properties possibly longer lasting then any loading program or exercise during later stages in life.

Nonetheless, as discussed in chapter 1, there indeed appear to be ranges of magnitude and loading rate in mechanical loading, in which changes in mechanical properties of tendons are more likely [78,82]. For example, during a 14-weeks exercise intervention, changes in the mechanical properties were only found in legs of subjects who exercised at high strain magnitude [78]. It has also been shown that an 8-week pure strength program caused no difference in strain of the Achilles tendon, despite a significant change in muscle strength [80]. It could therefore be hypothesized that tendons have a preferred strain limit that is maintained despite changes in triceps surae strength.

Overall, these findings highlight that healthy human tendons become stiffer when mechanically loaded. The exact mechanisms through which the tendon achieves this change in mechanical properties is unclear. A tendon can become stiffer through a change in morphology (i.e. increase in tendon cross sectional area through hypertrophy) as well as a change in material properties (e.g. improved collagen enzymatic crosslinking and therefore increase in Young’s modulus). As discussed in chapter 1, plenty of research supporting both of these theories exists. An important limiting factor in interpretation of current research is the fact that tendon mechanical properties can change regionally and not homogeneously throughout the entire length and width of the tendon. It has for example been shown that habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon differently in the proximal versus distal tendon region [175]. Consequently, changes are sometimes missed when using currently existing global evaluation techniques.

Therefore, the technique used in this thesis appears to be a promising tool, ideal to quantify local deformation patterns in combination with the possibility to take regional heterogeneity into account. The local AT deformation patterns, measured trough local tissue displacement, regional strain and non-uniform behaviour, provides useful parameters for the interpretation of the impact of mechanical loading on tendinous tissue.

In clinical practice, Achilles tendinopathy is currently most often rehabilitated through the use of eccentric exercises [29]. However, recent research has shown that other forms of strength training, e.g. heavy slow resistance training [31], might be equally effective as the eccentric heel drop program [29]. It is currently hypothesized that isolating the eccentric component of exercises might not be essential, but that the force, speed and duration of the applied load to the tendon is crucial [33]. The data strongly suggest that loading magnitude in particular plays a key role to achieve tendon adaptation, in contrast to muscle contraction type [145]. Local AT deformation and the parameters used in this thesis, could therefore be used as a direct measure for AT load during rehabilitation exercises. From this perspective, results from this thesis highlight three clinically relevant factors that should be discussed, as these influence local AT deformation: knee angle, ankle angle, and range of motion.

4.2 Influencing factors

4.2.1 Knee angle

As mentioned, Achilles tendinopathy is currently often rehabilitated through the use of the Alfredson eccentric heel drop program [29]. In this program, the calf muscle is eccentrically loaded both with the knee straight and, to maximize the activation of the soleus muscle, also with the knee bent [29]. To evaluate the importance of this change in knee angle, the impact of such a change on the non-uniform behaviour in the Achilles tendon was evaluated in chapter 3 of this thesis.

Recently, it has been shown that during certain non-functional laboratory circumstances, a change in knee angle has an impact on the non-uniform behaviour of different layers in the Achilles tendon [52]. An eccentric and passive deformation in a flexed knee position led to preferential displacement in the deep and middle layer of the AT. This is more pronounced than in the superficial layer, thereby leading to a higher non-uniform behaviour overall (i.e. superficial-to-deep displacement) [52]. This is supposedly linked to preferential loading of the soleus muscle in the flexed knee position [52], as the middle and deep layers of the AT are considered to be originating from the soleus muscle [6]. However, several factors could interfere with these findings: active versus passive motion, positional-related anatomical changes, biomechanical or force-related advantages or disadvantages that arise from the positional change, etc. For example, previous research has shown that higher levels of force production will cause load-sharing and a more uniform activation of the muscles of the triceps surae [138], which will potentially cancel out any heterogeneous loading at the tendon level, associated with a specific knee position.

Therefore, in chapter 3, it was hypothesized that a flexed knee position would lead to more non-uniform behaviour, due to greater differential loading of soleus versus gastrocnemius in this position, but that this effect would be attenuated by higher levels of force production. Contrary to these hypotheses, it was found that the non-uniform deformation in the AT, i.e. superficial-to-deep variation in displacement with highest displacement in the deep layer, is consistently present, irrespective of the knee angle or level of force production.

Thus, it appears that a change in knee angle only has a limited effect on local AT deformation. Therefore, it could be hypothesized that rehabilitation of Achilles tendinopathy with eccentric heel drops [29] might not require a change in knee angle, considering that the impact on local AT deformation might be clinically not relevant. Although, as mentioned, some methodological differences between the study in this thesis and previous research [52] have to be taken into account, as discussed in chapter 3. Furthermore, during all trials in chapter 3, force production was consistently higher at the different requested levels of force development during extension compared to flexion. It has indeed been shown that knee flexion causes a drop in force production of ankle plantar flexion because of the more isolated contribution of the soleus muscle, as the gastrocnemius can contribute less because of its origin proximal to the knee joint [137]. This might mean that a change from knee extension to flexion has no direct impact on local AT deformation, but an indirect effect through loss of force production in the flexed position. In chapter 3, it was found that at the 75% MVC level in the flexed knee position, subjects still reached sufficiently higher force levels than at the 25% and 50% MVC level, but with relatively less local AT tissue displacement. This is in contrast with the goal of striving for sufficient local AT loading during rehabilitation. Therefore, importantly, when patients are requested to reach a certain goal during an exercise in rehabilitation in a possibly adverse position, compensatory actions by synergistic muscle groups might relatively underload the tissue of interest.

These results, taken together with the growing knowledge that load is the most determining factor during tendon rehabilitation [32], indicate that a change in knee angle might still be useful to incorporate during rehab. The effect would only be indirect through enabling a patient to reach higher levels of force development in their individually most preferred knee angle.

4.2.2 Ankle joint

Contrary to the presumed indirect influence of a change in knee angle on local AT deformation, a change in ankle angle might be expected to influence this local deformation pattern more directly.

Comparison of the methodological set-up in the different studies of this thesis, reveals an important difference in the motion at the ankle joint. More specifically, in chapter 3, the ankle is fixed on the isokinetic device in a neutral position during an isometric contraction at different levels of force production. In chapter 4, on the other hand, the ankle goes through a range of motion during an eccentric heel drop with bodyweight load. This difference appears to influence the magnitude of local AT deformation that is found in these different trials.

In chapter 3, the mean local tissue displacement was 6.09 mm for the superficial layer, 6.22 mm for the middle, and 6.32 mm for the deep layer at 75% maximal voluntary isometric contraction. In chapter 4, during the eccentric trials, the mean displacement was overall much lower; 3.23 mm for the superficial layer, 3.33 mm for the middle layer, and 3.43 mm for the deep layer.

An important related factor in this comparison is the amount of force development. Force development in chapter 3 is roughly around 20 Nm during the 25% MVC trials, around 40 Nm during the 50% MVC trials, and 60 Nm during the 75% MVC trials. During the eccentric trials in chapter 4 on the other hand, it can be estimated that a subject weighing 70 kg would have an external ankle plantar flexor moment of approximately 105 Nm (considering a moment arm of 0.15 m between the ankle joint and the metatarsophalangeal joints where the subjects would find support during heel rise on a decline board).

It is therefore interesting to notice that local AT deformation during isometric trials is much higher at the highest levels of force production, despite the external load at these levels still being smaller then during eccentric trials. It could be hypothesized that the dynamic motion and changing joint angle during the eccentric trials provides the MTU with a more efficient way to develop force, requiring less absolute AT deformation. This means that during dynamic motions, the tendon can be loaded much higher with equivalently less tendon deformation. From rehabilitation perspective this is interesting, given the fact that we are aiming to reach heavy loads on the tendon. Isometric contractions can therefore be useful at the starting phase of rehabilitation but will probably reach a plateau as to how much local AT deformation and force they can develop.

This is in line with recent clinical research, where isometric exercises have been placed in the first stage of classic rehabilitation progression with an emphasis on pain management, and less on strength gain [176]. For example, in patellar tendinopathy, it has been shown that five repetitions of 45-second isometric exercises at 70% of maximal voluntary contraction can reduce patellar tendon pain for up to 45 minutes after exercise [176]. This response is associated with a reduction in motor cortex inhibition of the quadriceps, with the clinical implication that isometric exercises are indicated to reduce and manage tendon pain and initiate loading of the muscle-tendon unit when pain limits the ability to perform isotonic – dynamic exercises [177].

From the mechanical insights gained in this thesis, it could be hypothesized that these isometric exercises at sufficient load magnitude represent the perfect balance between high local tissue displacement (i.e. positive stimulus for tendinous tissue from mechanobiologic perspective), positive neurophysiological adaptations and limits the risk of overloading the global tendon area in these early stages of rehabilitation. Isotonic deformations, e.g. eccentric trials as used in this thesis, would not provoke sufficient local tissue displacement when the same load would be applied or would require too much load to reach the same level of local tissue displacement, potentially irritating a painful tendon.

When progressing through rehabilitation, a switch to more dynamic motions is strived for to achieve a sufficiently high load on the tendon, but only when they can be performed with minimal pain. This kind of load is important to restore muscle bulk and strength through functional ranges of movement [176]. In the latter stages of rehabilitation, reintroduction of energy-storage loads on the myotendinous unit is critical to increase load tolerance of the tendon and improve power as a progression to return to sport [178]. The major change through these activities is rate of loading of the tendon, which should be progressed gradually through relevant energy-storage activities for the individual athlete, depending on the demands of the individual's sport [176]. The importance of loading rate and its relation to mechanical insights gained in this thesis has been discussed in an earlier paragraph on influencing factors of non-uniform behaviour. In short, a lower loading rate may lead to more effective fibre recruitment and henceforth a greater transfer of the external tendon strain magnitude to the cellular level.

A limitation to consider when interpreting results of chapter 3 and 4 is the fact that there was no registration of kinematics at the ankle joint. This would be useful as it was previously shown that not so much the tibiotalar motion (e.g. plantar – dorsiflexion), but more the subtalar motion (in – eversion) plays an important role for AT deformation. Lersch et al. [47] found that changes in calcaneus position resulted in intratendinous AT strain differences. In particular rear foot eversion appeared to be of crucial importance, as this motion led to increased strain heterogeneity in the AT. The authors hypothesized this to be related to a reduction of AT fascicle twist, i.e. fascicles that originate in the region of the medial gastrocnemius cross the dorsal aspect of the free AT and insert in the lateral portion of the AT insertion. Hence, during eversion these fascicles should experience a reduction in strain as their origin and insertion are approximated. This highlights the fact that the progression from isometric contractions to dynamic motions is not only progressive in the amount of force development, but also in the complexity of kinematic changes that occur.

4.2.3 Range of motion

In chapter 4, a greater range of motion during the eccentric heel drop was found to be related to a greater normalized displacement, i.e. non-uniform behaviour. It has previously been suggested that crosslinking might be a risk factor for the development of tendinopathy. Since less non-uniform behaviour would limit the possibility for stress dissipation through the tendon by interfascicular sliding, tendon fascicles themselves would be more vulnerable to tensile damage [149]. Therefore, using a higher range of motion during exercises in rehabilitation would emphasize a more non-uniform behaviour in the tendon. This could potentially limit the progress of crosslinking and promote interfascicular sliding.

This is in line with the current usage of isometric exercises in the first stage of rehabilitation as asset for pain management, and less for strength gain. Hereby most often a comfortable midrange position is sought. For example, patellar tendon isometrics are routinely performed in midrange knee flexion around 30-60 degrees [176]. Differences between isometric and isokinetic loading, partly explaining this contrast, have been touched upon. Nonetheless, it would be interesting to evaluate whether there might be a mechanistic rationale to perform AT isometric exercises at different ankle angles, outside the comfortable midrange position.

4.3 Clinical implications

The clinically oriented results from this thesis support the generally accepted rehabilitation protocols [176] and provide it with more rationale from mechanical perspective. Starting rehabilitation with isometric exercises seems beneficial, as these provide sufficient local tissue deformation, besides their known positive impact on pain mechanisms. The knee angle does not appear as important from pure local AT mechanical perspective but is preferably changed to the position which enables highest ankle plantar flexion force production. Dynamic – isotonic exercises appear to be a more efficient combination of motion and force production, leading to less local tissue deformation in early loading stages. Loading rate is another important factor to consider and can be progressively increased, in the end going onto plyometric exercises. It is advisable to aim for the maximum range of motion through which the dynamic movements are performed.

5. Structure – function – pain

As discussed above, it is generally accepted that healthy human tendons become stiffer when mechanically loaded, but the exact mechanisms through which the tendon achieves this change in mechanical properties, is unclear. Moreover, in pathological ATs, research is even more scarce and possibly even less clear.

A recent review found no major observable structural change as an explanation for the response of therapeutic exercise in tendinopathy, except maybe for heavy-slow resistance training [36]. And despite recent advancements in structural imaging, e.g. ultrasound tissue characterization (UTC) [37], no association between tendon structure and symptoms has been found, demonstrating that restoration of tendon structure is not required for improvement of symptoms [38].

In contrast to these papers evaluating morphological changes in tendinopathy and during rehabilitation, only a few studies have evaluated the mechanical behaviour in pathologic Achilles tendons. Arya et al. [94] and Child et al. [95] both found higher strain in Achilles tendinopathy subjects compared with controls, leading to the hypothesis that the presence of tendinopathy leads to a weakened tendon. However, they evaluated global strain (i.e. relative change in length from musculotendinous junction to insertion). Therefore, the aim of the study in chapter 5 was to compare local tendon mechanics in healthy versus pathologic Achilles tendons using the newly developed tool from this thesis. It was found that displacement in all three layers during isometric trials was significantly different between tendons with a different degree of symptomatology and pathology.

Interestingly, when evaluating the individual displacement patterns, 2 of the 3 most pathological tendons showed a “reversed” non-uniform displacement, with the highest displacement measured in the superficial layer. This is in clear contrast with the “classic” non-uniform displacement, with the highest displacement in the deep layer. Observation of the grey-scale US images revealed that these 2 tendons had a distinct hypoechoic area located in the superficial layer. In contrast, the third pathologic tendon only had a limited hypoechoic area in the medial part, together with a calcification in the deep layer. It could therefore be assumed that the presence of a distinct hypoechoic area, i.e. loss of collagen fibril integrity, is closely related to an increase in local tissue displacement in the relevant tendon layer or area.

The time course of this structure-function relation is unknown. It might be that mechanical changes (e.g. change in local tissue displacement) precede structural changes (e.g. distinct hypoechoic area) or vice versa. It is known from interventional studies on healthy tendons that material (e.g. Young’s modulus) and mechanical (e.g. stiffness) properties change quicker in response to load, as opposed to morphological properties (e.g. cross sectional area), which takes several months [145]. It could thus be that the pathological response of a tendon to overloading is first noticeable in changes in material and mechanical properties and that one should focus more on the functional evaluation of tendon deformation patterns, rather than on the structural characterization. This is in line with the hypothesis investigated in chapter 4, where it was expected that ageing would be related to changes in mechanical behaviour of the AT. Given an expected decrease in interfascicular sliding due to crosslinks at older age [16], it has been suggested that crosslinking might be a risk factor for the development of tendinopathy. Since less non-uniform behaviour would limit the possibility for stress dissipation through the tendon by interfascicular sliding, tendon fascicles themselves would be more vulnerable to tensile damage [149]. In chapter 4 of this thesis, a trend in line with previous research [112] was found, which warrants further investigation.

Nonetheless, it should be stated that there is still uncertainty on the exact factors at play in tendon pain, which remains the most classic symptom in tendinopathy [179]. Also, use of the biopsychosocial model instead of the classic biomedical structure-pain model has drawn attention to associated factors as sleep, fatigue, anxiety, etc. [180]. Achilles tendinopathy has been shown to be associated with a significant psychosocial impact. A better understanding of the experiences and personal impacts of AT may thus also enhance its management [181]. It should always be kept in mind that the painful tendon is part of a patient.

Overall, there appears to be a clear need for prospective studies, evaluating the time-dependent changes of mechanical properties and possible relation with the onset of symptoms and anatomopathological changes, as seen in tendinopathy. As an example from structural perspective, a recent systematic review investigated whether the presence of structural abnormalities in asymptomatic tendons predicts the development of future tendon symptoms [180]. It was found that tendons with an abnormal ultrasound had a nearly fivefold increased risk of becoming symptomatic compared to tendons with a normal ultrasound. However, when considering the fact that tendon abnormalities are estimated to be present in 59% of asymptomatic populations [182], the clinical implications of a relative risk ratio around five are only limited. With a baseline risk of developing symptoms with a normal tendon being only 3%, the actual risk of asymptomatic athletes with a structural abnormality in their tendon developing tendinopathy is only 15%. Hence, in a theoretical sample of 100 athletes, as many as 59 athletes will have asymptomatic structure abnormality on US [182], and only as few as 9 of these 59 athletes may go on to develop future symptoms [180].

6. Continuing research

Further exploration of the role of (changes in) mechanical properties in the development of symptoms and pathology is needed. In chapter 5 of this thesis, the aim of the study was to evaluate the influence of tendinopathy on local tendon mechanics. As mentioned, tendon tissue displacement was found to be significantly different between asymptomatic and highly symptomatic volunteers during isometric trials. Also, the non-uniform deformation pattern of the superficial-to-deep tendon layer was reversed in 2 highly symptomatic tendons. However, possible limitations of this study are the small sample size, as well as the difference in age between the healthy subjects and subjects in the three symptomatic subgroups.

Furthermore, as discussed, the interpretation of the interplay between structure (e.g. abnormalities as seen on diagnostic ultrasound) and function (e.g. local tissue displacement as quantified with the tool used in this thesis) is likely clouded by pain (e.g. quantified by the VISA-A questionnaire). To further elucidate this interplay, the choice was made to restart the study as described in chapter 5. Building on the insights gained so far, a more robust methodology was defined.

The aim of this additional study was to evaluate whether structural or functional abnormalities are the most predictive factors to explain the level of pain and dysfunction in subjects with Achilles tendinopathy. It was hypothesized that differences in the deformation pattern of the AT would be more related to symptom severity than pure structural characteristics.

6.1 Participants

To improve homogeneity in the sample under investigation, inclusion criteria are made stricter when compared to chapter 5. Most importantly, only subjects between 18 and 45 years are considered eligible for participation, to prevent age differences from possibly influencing interpretation of results, as was the case in chapter 5. All patients in this age range visiting the sports medicine consultation at the University Hospitals Leuven with a clinical diagnosis of midportion Achilles tendinopathy are asked to participate. An ultrasound is performed by an experienced radiologist (PB), mainly to exclude differential diagnoses (Achilles tendon rupture, accessory soleus muscle, retrocalcaneal bursitis).

Patients are informed about the possibility to participate in the study. Patients who had already received treatment (i.e. physical therapy, shockwave therapy, infiltrations and/or surgical interventions) are excluded. If none of the exclusion criteria are present, a VISA-A questionnaire is taken. Patients with a VISA-A score above 90 are excluded. After signing the informed consent, the following data are registered: age, gender, duration of the pain (months), hours of sports per week and which sports, history and/or type of diabetes and smoking habits.

6.2 Outcome parameters

From structural perspective, on ultrasound images, the diameter at the widest point is noted, together with the distance from the calcaneal tip to this point, as well as the presence of a hypoechoic area and/or neovascularisation.

The VISA-A questionnaire is used as an outcome measure for pain and dysfunction.

As in previous studies in this thesis, two outcome parameters from a mechanical perspective are evaluated. First, local tendon tissue displacement (mm) of the different layers (superficial, middle, and deep layer) is defined separately. Second, the normalized displacement ratio (%) is calculated, computed as the difference in local tendon tissue displacement between the deep and the superficial layer, and then divided by the average displacement in all three layers combined. Both the symptomatic and asymptomatic side are evaluated.

6.3 Statistics

A one-way ANOVA will be performed to evaluate differences in mechanical parameters between the painful and non-painful limb of participants. Where non-parametric variables are identified, independent samples Kruskal-Wallis tests will be performed.

To assess which structural or mechanical parameters are most important to explain variations in VISA-A questionnaire, a backward step-wise regression will be performed. Variables included in the analysis will be: diameter of the tendon at the widest point, presence of neovascularisation, presence of hypoechoic area through Archambault score, mean local tissue displacement across three layers, and normalized displacement ratio.

Given that there are 3 predictors in the model (structure, pain/dysfunction, and mechanics), with a rule of thumb of 10 cases of data per predictor, a sample of 30 subjects was estimated to be the minimum to achieve adequate power.

6.4 Preliminary data

Currently, six subjects (6 male; age 32 +/- 7,1 years) have been included and tested. Recruitment, image processing and data analysis are currently ongoing. Hereby, the aim is to evaluate whether structural or functional abnormalities are the most predictive factors to explain the level of pain and dysfunction in subjects with Achilles tendinopathy.

7. Future directions

As always, progressing insights cause new research questions to arise and highlights certain limitations of the current work to be solved. For example, as mentioned in chapter 2, speckle tracking techniques using 2D-ultrasound images are inherently confronted with out of plane motion. This is due to the rotatory anatomy and the complex deformation patterns that occur in 3D. In the future, 3D-ultrasound will overcome this problem and will provide the possibility to analyse individual rotatory anatomy and possibly integrate this with an analysis of the tendon-specific deformation pattern.

Nonetheless, the progress that has and is being made in the field of ultrasound-based speckle tracking has provided exciting insights into local tendon mechanics. As discussed, the high-frequency ultrasound used in this thesis provides a tool to evaluate local tissue displacement at other hierarchical tendon scales then before. Combining this technique with existing macroscale evaluation techniques (e.g. strain derived from musculotendinous junction to insertion) or integrating it with insights from micro- and nanoscale research (e.g. laboratory-based microscope techniques) will further enhance our understanding of the interaction between different scales.

From biomechanical perspective, the activation modes used during this thesis were passive and isometric on an isokinetic device, and an eccentric heel drop on a decline board. The complex interaction of kinematics and kinetics, as discussed in the example of calcaneus motion, is crucial in the evaluation and interpretation of local tendon deformation. Therefore, future studies should further explore the impact of biomechanical changes on local AT deformation; e.g. isometric contractions in different ankle angles, at different loading rates; eccentric heel drops with added external load, at different loading rates; etc.

This would also aid advancements in aetiological research, as the exact role of the local deformation patterns in the onset of Achilles tendinopathy is unknown. Prospective studies are needed to evaluate if certain local AT deformation patterns might be considered a risk factor for the development of Achilles tendinopathy. Ideally, other prognostic factors could be incorporated, including tendon-specific morphological and patient-specific psychosocial factors. This kind of study design should help shed light on the time-dependent changes that might occur at morphological versus mechanical level.

Further research in the area of rehabilitation is inevitable, as the exact mechanism through which mechanical loading therapy works in patients with tendinopathy is not yet known. Longitudinal interventional studies, evaluating if local AT deformation patterns change during a progressive tendon rehabilitation program, are needed.

8. Final conclusion

This PhD project established a clinically oriented evaluation platform to quantify intratendinous deformation in the AT and applied this within a patient-centred environment. The insights gained in the non-uniform deformation pattern of the AT helped to enhance our understanding of Achilles tendinopathy and provided opportunities to tailor treatments to the specific needs of patients suffering from this overuse injury. Further research using this tool will definitely improve the interpretation and understanding of the complex relationship between structure and function in Achilles tendons.

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Acknowledgments

Scientific acknowledgement

Koen Peers supervised conceptualisation and design of the research project, helped analyse data and corrected papers and the thesis manuscript. Kaat Desloovere gave methodological advice and corrected papers and the thesis manuscript. Lennart Scheys provided engineering background and corrected papers and the thesis manuscript. Catarina De Brito Carvalho conducted experiments and provided technical support on all ultrasound-related matters, with supervision of Paul Suetens, Frederik Maes, Jan D'hooge and Pieter Slagmolen. An De Groef helped analyze data, gave statistical advice and helped correct papers and the thesis manuscript. Pieter-Jan Loos helped in preparing part of the data for analysis and Elien Apers helped during some of the data acquisition.

Personal contribution

SB conceptualized and designed the research project, conducted experiments, ensured interpretation of results from a clinical perspective, was responsible for writing and acted as corresponding author for all papers.

Conflict of interest statement

There are no conflicts of interest to declare that would be directly relevant to the content of this thesis.

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