INFLUENCE OF AGE AND PATHOLOGY ON ACHILLES TENDON

PROPERTIES UNDER FUNCTIONAL TASKS

An academic thesis submitted to
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Potsdam in 2016
Affidavits according to doctoral degree regulations (§ 4 (2), sentences No. 4 and 7) of the
Faculty of Human Sciences, University of Potsdam:

Hereby, I declare that this thesis entitled “Influence of age and pathology on Achilles tendon
properties under functional tasks” or parts of the thesis have not yet been submitted for a
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work presented in this thesis is the original work of the author. I did not receive any help or
support from commercial consultants. All parts or single sentences, which have been taken
analogously or literally from other sources, are identified as citations. Additionally,
significant contributions from co-authors to the articles of this cumulative dissertation are
acknowledged in the authors’ contribution section.

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Place, Date  Konstantina Intziegianni
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<tr>
<td>AT (s)</td>
<td>Achilles tendon(s)</td>
</tr>
<tr>
<td>ATL</td>
<td>Achilles tendon length</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-sectional area</td>
</tr>
<tr>
<td>MTJ</td>
<td>myotendinous junction</td>
</tr>
<tr>
<td>MVIC</td>
<td>maximal voluntary isometric contraction</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
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<tr>
<td>TRV</td>
<td>Test-Retest Variability</td>
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<tr>
<td>IRV</td>
<td>Inter-rater Variability</td>
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<tr>
<td>LoA</td>
<td>limits of agreement</td>
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<td>SEM</td>
<td>Standard-Error of Measurement</td>
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Abstract

Prevalence of Achilles tendinopathy increases with age, leading to a weaker tendon with predisposition to rupture. Previous studies, investigating Achilles tendon (AT) properties, are restricted to standardized isometric conditions. Knowledge regarding the influence of age and pathology on AT response under functional tasks remains limited. Therefore, the aim of the thesis was to investigate the influence of age and pathology on AT properties during a single-leg vertical jump.

Healthy children, asymptomatic adults and patients with Achilles tendinopathy participated. Ultrasonography was used to assess AT-length, AT-cross-sectional area and AT-elongation. The reliability of the methodology used was evaluated both Intra- and inter-rater at rest and at maximal isometric plantar-flexion contraction and was further implemented to investigate tendon properties during functional task. During the functional task a single-leg vertical jump on a force plate was performed while simultaneously AT elongation and vertical ground reaction forces were recorded. AT compliance [mm/N] (elongation/force) and AT strain [%] (elongation/length) were calculated. Differences between groups were evaluated with respect to age (children vs. adults) and pathology (asymptomatic adults vs. patients).

Good to excellent reliability with low levels of variability was achieved in the assessment of AT properties. During the jumps AT elongation was found to be statistical significant higher in children. However, no statistical significant difference was found for force among the groups. AT compliance and strain were found to be statistical significant higher only in children. No significant differences were found between asymptomatic adults and patients with tendinopathy.

The methodology used to assess AT properties is reliable, allowing its implementation into further investigations. Higher AT-compliance in children might be considered as a protective factor against load-related injuries. During functional task, when higher forces are acting on the AT, tendinopathy does not result in a weaker tendon.
Zusammenfassung

Die Prävalenz der Achillessehenentendinopathie steigt mit zunehmendem Alter, was zu einer geschwächten Sehnenstruktur mit Prädisposition zur Ruptur führt. Frühere Studien, welche Achillessehnen (AS)-Eigenschaften untersuchten, beschränkten sich auf standardisierte isometrische Bedingungen. Der Einfluss von Alter und Pathologie auf das AS-Verhalten während funktioneller Bewegungen ist unklar. Das die Ziel der vorliegenden Arbeit war es daher, den Einfluss des Alters und Pathologie auf AS-Eigenschaften während einen einbeinigen vertikalen Sprung zu untersuchen.


Die Methodik zur Bestimmung der AS-Eigenschaften ist zuverlässig, was deren Implementierung in weitere funktionelle Untersuchungen ermöglicht. Die höhere funktionelle AS-Compliance bei Kindern kann als Schutz vor Überlastungsschäden diskutiert werden. Während funktioneller Aufgaben, bei denen höhere Kräfte auf die AS wirken, ging das Vorhandensein einer Tendinopathie nicht mit einer schwächeren Sehne einher.
1. Introduction

The Achilles tendon (AT) is the strongest tendon in the human body, subjected to substantially loads reaching up to 12.5 times body weight during running [1]. However, the high magnitude of loads on the AT and the continual stresses placed on it during locomotion also makes it one of the most common tendons to sustain overuse injuries and ruptures [2]. Diagnosis of AT disorders is usually made on clinical grounds by use of history and clinical examination supported by diagnostic US to detect structural lesions and changes in echogenicity or neovascularization [3;4]. Despite the extensively use of US in tendon research and diagnostics, it is often perceived as an imperfect and operator dependent tool [5]. This is compounded by the lack of data regarding its reproducibility as well as absence of measurement standardization which makes comparison between studies difficult and limits its value.

Degenerative disorders of the AT, described as tendinopathies, cause considerable morbidity and functional impairment among athletic and general population with an incidence of 1.85 per 1.000 individuals [6]. The etiology of tendinopathy is considered to be multifactorial [7-9] and its pathogenesis still not fully understood [7;10]. Excessive repetitive overload of the AT is regarded as the main stimulus that leads to degeneration and subsequently to tendinopathy [11]. Previous studies, found that degeneration leads to a weaker tendon resulting in higher strains with lower forces and stiffness [2;12]. However, those results were based on standardized isometric conditions and due to methodological restrictions it remains unclear whether tendinopathy results also in a weaker tendon during dynamic functional situations.

Previous in-vitro studies have reported that strain levels of 4%-8% cause microruptures and eventually at 12% a complete tendon rupture [13;14]. However, previous in vivo studies reported strains of the AT ranging from 5%-10% during isometric contractions at forces below the maximum force during dynamic movement [15;16]. It has previously been thought that such high strain would
cause tendon rupture and failure. However, in real life activities such as one leg hopping can induce much greater strains (11.4%) on the AT without being injured [17]. However, limited information exists in regards to the functional response of the tendons under physiological loads.

Age is noticeably a predisposition factor for the development of Achilles tendinopathy [7] as it’s prevalence increases with age. Typically, this overuse injury is more prevalent in men in the 4th-5th decade of life, and its occurrence is highest among individuals who participate in sports involving running and jumping [6;18-23]. In adult runners, a point prevalence of up to 36% has been reported for Achilles tendinopathy [24] whereas in children at an average of 13 years, only 1.8% is known [25]. It has been reported that children, under isometric contractions, demonstrated more compliant tendinous structures as their elongation and strain was significantly greater at a given force for patellar [26] and vastus medialis [27] tendon. On the other hand, Waugh et al. found no age-related differences in AT strain under isometric conditions. Although the reason for the discrepancies among those studies is unclear; their results gave rise to the hypothesis that compliance found in young tendons might play a protective role against load related injuries [27]. However, this hypothesis has not been empirically tested under functional tasks.

As outcomes of previous studies are based on in-vitro models and standardized isometric conditions [2;12;26-28], knowledge regarding the influence of age and pathology on tendon properties under dynamic load is still limited. Moreover, to ensure accurate diagnosis and evaluation of tendon response, standardized and reliable US measurements independent of investigators experience are needed. This cumulative thesis comprises 3 studies which were recently published in peer-reviewed journals. The studies addressed initially the influence of repeated and between investigators US measurements on AT properties by intra and inter-reliability, and latter evaluates the AT response during single-leg vertical jump among children, asymptomatic adults and patients with Achilles tendinopathy.
2. Literature Review

2.1. Historical Perspectives

Achilles, the ancient Greek hero of the Trojan War, gives his name to the Achilles tendon (AT). Achilles was the son of the nymph Thetis, who tried to make him immortal by dipping him in the river Styx. However, he was left vulnerable at the part of the body she held him, his heel. Achilles was killed by the Trojan prince Paris whose arrow, embedded in his only vulnerable point. This has given rise to the description of a person’s weakest point being called their “Achilles heel” (fig. 1).

Hippocrates, is the earliest description of the AT, stated that “this tendon, if bruised or cut, causes the most acute fevers, induces choking, deranges the mind and by time brings death”[29]. The oldest known written record using the term “Achilles tendon” is found in the work Corporis Humani Anatomia published in 1693 by the Dutch anatomist Philip Verheyen. Previously it was known as “tendo magnus of Hippocrates” [4].

2.2. Functional Anatomy and Structure of the Achilles Tendon

The AT is the thickest and strongest tendon in the human body. Its origin lies close to the middle of the calf, and fuses with the gastrocnemius muscle proximally. The gastrocnemius is a fusiform muscle formed by two heads, medial and lateral, each separately crossing the knee joint. Deep to the gastrocnemius there is the soleus, a large flat, pennate muscle [30]. Together with the gastrocnemius, it forms the three-headed triceps surae, which acts to plantarflex the ankle joint via AT, while the gastrocnemius is also a knee flexor [31]. The gastrocnemius muscle is active in walking, jumping and running and its composed predominantly of type II fibres [32].
The AT is the longest tendon in the human body with an average length of 15 cm, ranging from 11 to 26 cm. The mean width of the AT is 6.8 cm (4.5 - 8.6 cm) at its origin and becomes gradually thinner at its midsection reaching a width of 1.8 cm (1.2 - 2.6 cm) (fig. 2). Approximately 4 cm above the calcaneus where the soleus muscle contributes fibres, AT becomes more rounded reaching a mean width at its insertion of 3.4 cm (2.0 – 4.8 cm) [33].

AT structure is dominated by type I collagen, which explains its considerable strength. The collagen accounts for 65-80% of the dry weight and elastin approximately 1-2%. The collagen is embedded in a proteoglycan-water matrix. Collagen is produced by fibroblasts and fibrocytes that lie between the collagen fibers in a complex structure [34]. Tendon stem cells have recently been found in human tendons [35]. The synthesis of collagen fibrils follows first as an intracellular step assembling and secreting procollagen. The extracellular step converts the procollagen into tropocollagen. Five tropocollagen molecules (or microfibrils) are cross-linked and aggregated into collagen fibrils [36].

Figure 2: Anatomy of the Achilles tendon
The stability and quality of the collagen is largely based on the cross-links [8]. Multiple collagen fibrils are embedded in the extracellular matrix and form collagen fibers. This is the basic unit of a tendon and the smallest visible (light microscopy) tendon unit [34]. Those collagen fibres which are tightly packed in parallel bundle contain nerve, blood and lymphatic vessels. These fiber bundles are surrounded by endotenon and group together to form the macroscopic tendon. The tendon is enveloped by epitenon, which in turn is surrounded by paratenon (fig. 3). The paratenon and epitenon are separated by a thin layer of fluid to reduce friction during tendon motion [30].

![Diagram of tendon structure](image)

**Figure 3:** The hierarchical structure of a tendon (Taken from Kirkendall & Garret, 1997).

The AT has 3 sources of vascular supply which are (1) the perimyseal vessels at the musculotendinous junction, (2) the periosteal vessels at the Osteotendinous junction and (3) the vessels around the tendon within the paratenon, forming a capillary system. The mid-section has less good vascularization than the proximal and distal tendon ends, resulting in a relative avascular area with a higher prevalence of injuries [37]. The neural supply to the AT and the surrounding paratenon is provided by nerves from the attaching muscles and by small fasciculi from cutaneous nerves, in particularly the sural nerve [38;39]. The number of nerve endings is relatively low, and the tendon relatively aneuronal [40;41].
2.3. **ULTRASONOGRAPHY ASSESSMENT OF TENDON STRUCTURAL PROPERTIES**

Tendon structural properties could be determined by several imaging techniques such as MRI and ultrasonography [42;43]. MRI is considered to be the gold-standard imaging modality in the assessment of musculoskeletal system as it provides images with excellent soft tissue characteristics of high sensitivity and specificity independent by the operator [42]. However, as this technique is expensive and time consuming its availability in most clinical and research centers is limited [44]. Ultrasonography is an alternative and more convenient method in the assessment of musculoskeletal system [44]. Its initial use was limited in investigating larger joints; however, technological improvements in the early 1990s improved greatly the image resolution and tissue contrast allowing access to smaller joints [5]. Today ultrasonography has become an integral tool for musculoskeletal investigations in both the clinical and research context. Some of its distinct advantages include low cost, time efficiency and the ability to image tissues dynamically [45].

The US technology involves transmitting US waves from transducer (probe) to the body generated by use of piezoelectric crystals [46]. As the US echoes penetrate body tissues of different acoustic impedance, some are reflected back to the transducer and some continue to penetrate deeper. The returned waves are processed and combined to generate an image [46]. US waves can be described in terms of their frequency, wavelength and amplitude. The wavelength and frequency of US waves are inversely related, e.g., US of high frequency has a short wavelength and vice versa. The higher the frequency used, the greater the axial and the lateral resolution of image, but at the cost of reduced tissue penetration. Therefore, a higher-frequency transducer is best used for superficial structures, such as the small joints of the hand and feet (7.5-20 MHz), and a low frequency transducer is used for deeper joints, such as the hip or shoulder (<7.5 MHz) [46].

Assessment of AT requires a 7.5 MHz (or higher) probe placed perpendicular to the tendon. The AT normally is highly fibrillar and echogenic. The fibrillary anatomic architecture can be appreciated as tightly packed thin echogenic lines on longitudinal scanning and echogenic punctate foci in the axial
plane [47] (fig.4). One the main indication of US is the need to distinguish between normal and pathological anatomical structures [48]. US imaging can aid diagnosis of tendinopathy and confirm presence of specific pathological elements such as fibrillar disruption, tears, thickening and calcifications [49]. Ultrasonography, and in particular Brightness mode (B-mode), has been commonly used to quantify AT length, thickness and cross-sectional area (CSA) [2;48]. However, due to the anatomical dimensions of AT, US assessment can be challenging.

**Figure 4:** Longitudinal (a) and transversal (b) ultrasound views of a normal Achilles tendon (unpattern arrows) the tightly packed thin echogenic lines on longitudinal scanning and echogenic punctate foci in the axial plane indicate the fibrillary anatomic architecture. The thin paratenon is seen surrounding the tendon as a slightly more echogenic border (dashed arrows)

AT is the longest tendon in the human body, which makes its assessment by ultrasonography difficult, as the field of view in most US probes is insufficient to visualize its entire length. Previous studies, have used surface markers corresponding to the proximal and distal tendon ends, identified using separate US images, and with the distance in-between measured by a ruler [2;50]. However, this method lacks of standardization, as it is unclear how the surface markers were defined on the skin. Compared to the assessment of AT length, measurements of AT CSA present a different challenge as its dimensions are not the same along its length [51] and thus, it’s unclear which
distance point along the tendon, CSA should be measured. Previous studies assessing AT CSA used as a reference the level of medial malleolus [50;52;53]; on the other hand, in an attempt to have a more representable value of CSA, Arya and Kulig, averaged 3 different distance points along its length at 2, 4 and 6 cm proximal the attachment on the calcaneus [2]. Thus, it remains unclear whether single-site measurement of CSA or average of different distance points along the AT, is more beneficial for the assessment of AT CSA.

Ultrasonography is a technically demanding tool which requires considerable examiner experience, as the measurement quality of the US images is solely depending on the investigator skills and thus; it is often perceived as an imperfect and operator-dependent tool [5;44;45]. In most US studies investigating tendons structures, the experience of the investigators is often neglected and not reported. Investigators experience and knowledge is crucial in order to perform a consistent and high quality scanning and further analysis on the images taken. In regards to tendon examinations the examiner should hold the transducer stable and be vigilant about the amount of pressure that is being placed on the tissue. The stability is essential when the instrument is placed on prominences such as the AT and medial malleolus, small and controlled movements are needed to differentiate pathologic findings from anisotropy [54].

Anisotropy is an artefact produced by the linear configuration of tendons whereby hypoechoic change is seen if the transducer is slightly angulated (fig. 5) [55;56]. This artefact can mimic hypoechoic tendinopathy (fig.8), but minor changes to transducer angulation make anisotropy disappear whereas true pathologic findings do not [54]. Moreover, excessive pressure can alter the shape of the structure leading to an incorrect measure. The correct position of transducer on the area of interest is also of clinical importance for an accurate diagnosis, as tendon or muscle image captured slightly oblique to the structure’s longitudinal axis may appear larger in diameter than a true axial-plane image [57].
Tendons form the structural link between muscle and bone and due to their anatomical location their primary role is to transmit contractile force to the bone, facilitating joint movement [58]. Tendons are not however completely rigid structures, they do elongate when subjected to a tensile load imposed upon them by muscle contraction [13;14;58;59]. A reference point on the tendon e.g. its myotendinous junction is visualized by US and its displacement during contraction is measured representing the tendon elongation [58]. The forces acting on the tendon can be calculated from dynamometer based measurements of joint torque. The degree to which tendons elongate to a given level of muscle force depends partially upon their dimensions. In the case of a healthy tendon a shorter one with larger CSA is expected to have lower elongation indicating a stiff tendon, whereas a longer tendon with smaller CSA is expected to have a higher elongation indicating a compliant tendon (Fig. 6)

Figure 5: A: Longitudinal ultrasound image shows a normal fibrillar pattern (arrows) at the Achilles tendon calcaneal insertion with minor anisotropy (arrowheads).
B: Longitudinal ultrasound image shows transducer angulation producing anisotropy (arrowheads) more marked than A, arrows indicating normal fibrillar pattern
C: Transverse ultrasound image of Achilles tendon cross-sectional area shows normal echogenic tendon (arrows)
D: Transverse ultrasound image shows transducer angulation producing tendon anisotropy (arrows). Taken from Robinson P. Sonography of common tendon injuries. AJR Am J Roentgenol 2009; 193(3):607-618.[54]

2.4. MEASUREMENT OF TENDON MECHANICAL PROPERTIES AND BEHAVIOUR
Characterization of tendon mechanical and material properties such as stiffness and Young’s modulus are essential for understanding mechanisms that enable to optimize the functional behavior of the muscle-tendon complex [1;2]. Tendon stiffness is calculated by dividing the estimated tendon force by the tendon elongation and it’s thereby influenced by tendon structural properties such as tendon length and CSA. On the contrary, Young’s modulus is defined as stiffness normalized to tendon CSA and length, described from the linear relation between stress (force/CSA) and strain (elongation/length), providing a measure of tendon material properties, irrespective of its structural dimensions [2;60]. Currently models used to calculate stiffness and young’s modulus are somewhat diverse between research groups, which underlies the large variability in the reported values [61]. Potential reasons for the matter are ranging from inherent variation within/between populations to methodological reasons e.g. moment arm estimations, contraction time and portion of the tendon measured (distal/proximal) [61]. However, even when all things are equal and
standardised between studies, there is also a variety of calculation methods to determine those properties [61]. These calculations are based on mathematical and in-vitro models and although they have provided insights into musculoskeletal function, their outcome is model-based estimation and subject to deviation from true values [62].

Initially the mechanical properties of tendons have been studied using isolated animal or cadaver human tendons undergoing elongation to failure in which the tissue is gripped on both ends and stretched to a predetermined length [14]. Based on these studies the mechanical behaviour of the tendon is characterized by its stress-strain curve. A typical stress-strain curve has three phases. In the resting phase, tendons have a wavy or crimped configuration because of the crimped shape of collagen fibers, which disappears when the strain exceeds 2%. After this initial toe region of as much as 4% strain, the tendon can return to its original length. Between 4% and 8% strain there are microscopic collagen fiber ruptures. Beyond this level of strain, there are macroscopic tears, which eventually lead to complete tendon rupture at approximately 12% strain (Fig. 7). Despite the widely acceptance of this model in literature, several limitations raise doubts as to whether results from excised tendons can be extrapolated to directly interpret the in-vivo physiological function of the tendon [16]. For example, when stretching a gripped fibrous structure it results in larger elongations in the tendon regions near the clamps leading to a premature failure [63]. Moreover, many of the experiments have been performed using preserved or deep frozen tendons which may have altered properties [63-65]. Recent in-vivo studies reported strains of the AT ranging from 5% - 10% during isometric contractions at forces below the maximum force [15;16], underestimating those classic values of tendon strains. In addition, a study by Lichtwark et al. investigated the AT strain during one-legged hopping reaching strain values of as much as 11.4% [17]. This fact indicates that the tendon can elongate far more than once considered without rupturing which yields further investigation into more dynamic and functional movements in order to understand the physiological response of tendons.
2.5. Functional Implications of Tendon Mechanical Behaviour

Tendons feature both viscous and elastic properties due to the content of collagen, elastin and water and as well between the interactions of collagenous and non-collagenous proteins (e.g. proteoglycans) [13]. One example of viscoelastic behaviour is the sensitivity of tendons to different strain rates [14]. It was found that during lower strain rates the elongation of the tendon is higher indicating a more compliant tendon. Thus, the tendon absorbs more strain energy but is less effective in transferring loads. On the other hand, with higher strain rates, the tendon elongates less indicating a more stiffer tendon and thus, the load transfer becomes more efficient [63;66]. This viscoelastic behaviour of the tendon has a direct effect and important functional implications on the in-series muscle. Having a muscle attached to a compliant tendon makes it more difficult to control the position of the joint spanned by the tendon and results also to a reduction of muscle force as the length of the muscle shortens to take up the excess tendon elongation [67-69]. However, those observations were done under standardized isometric contraction.
Most dynamic human movements involved a stretch-shortening cycle (SSC) where an agonist muscle contracts after being stretched in an eccentric movement phase [70]. The SSC of muscle function comes from the observation that body segments are periodically subjected to impact or stretch forces. Running, walking and jumping are typical examples in human locomotion of how external forces lengthen the muscle [71]. During a SSC the viscoelastic characteristics of the tendon play an important role in enhancing both the effectiveness and the efficiency of human performance [72]. It was reported that under human locomotion such as ankle bending [73], jumping [74] and walking [75] the muscle fibers contract at a nearly constant length, whereas the tendon performs a stretch-shortening cycle and thus, higher forces are produced without significant mechanical work being performed by the muscle [76].

As previously discussed in vivo measurements of the AT during isometric conditions have demonstrated different results compared to those measured in vitro. However, it remains largely unknown how AT properties behave during functional tasks as only one study has been able to directly measured it during real life movements [17]. Lichtwark et al. investigated the AT strain during one-legged hopping in ten participants by use of US and motion analysis [17]. The results demonstrated that one-legged hopping elicited high tendon strain of as much as 11.4% with an average of 8.3%. According to Lichtwark et al. one-legged hopping was chosen specifically because it is an activity that should elicit very high stress and strain on the tendon [17]. This estimation is strengthen by a previous study indicating that during one leg jump high forces are acting on the tendon [77]. Fukashiro et al. investigated the elastic behaviour of the human muscle-tendon during various types of jumps. This was done by determination of the in vivo AT force by use of an invasive buckle-type transducer around the AT of only one participant [77]. The AT force was recorded during a squat jump (SJ), countermovement (CMJ) and one leg hopping (HP) by use of ground reaction forces. The peak AT force and mechanically work done by the calf muscles were 2233N and 34J in the SJ, 1895N and 27J in the CMJ, and 3786N and 51J when HP [77]. The changes in tendon length in
gastrocnemius muscle-tendon complex were estimated and not measured, assuming a constant stiffness calculated from the tendon architecture ranging from 26 mm during SJ, 32 mm in CMJ and 46 mm during HP [77]. This estimation however is subjected to deviation from true values as muscle-tendon architectural parameters (e.g. pennation angle) and subsequently stiffness are not constant during movements and vary as a function of the kinematic configuration of the joints that a muscle spans [62]. Thus, a comprehensive characterization of dynamic muscle-tendon function requires in vivo measurements of these parameters away from model-based calculations.

As described above in the study of Fukashiro et al., [77] direct measurements of forces during movements is usually limited to minimal invasive measurements [1;78] with a force transducer placed on the tendon. Non-invasive methods rely on the basic principle that muscle produce skeletal movements and ground reaction forces. A non-invasive technique known as inverse dynamic analysis has been developed based on computational modelling of the dynamics of linked body segments. The analysis produces estimates of joint torques, each of which represents the resultant action of all muscles crossing the joint [79]. Actual estimates of tendon force can only be obtained with computational models based on inverse dynamics which makes the applicability in clinical context difficult and often not possible. In order to evaluate the tendon behaviour during human movements, it is important to observe the forces as well the length changes. The need to establish a simple and clinical applicable method in both the research and clinical field is high, as the understanding of tendon functional behaviour is still limited.

2.6. Tendon Loading and Adaptation

Tendons are spring-like structures and their tensile stiffness is adaptable to the mechanical environment they operate in, increasing in response to chronic loading and decreasing with chronic unloading. The AT is the strongest tendon in the human body and is exposed to high forces during daily activities and athletics. Previous studies have reported that AT experiences forces ranging from 3kN during maximal isometric contractions [67], 5 kN during unilateral hopping [80] and 9 kN during
running exceeding 12 times the body weight [1]. The magnitude of AT loading results in positive adaptation but excessive and repetitive forces are a precursor to degeneration and tendinopathy. Adaptation to repetitive force occurs through alterations in tissue composition and biomechanical behaviour of the tendon [67].

In response to chronic long-term loading, indication of adaptation was found in cross-sectional studies investigating the thickness of tendons. Male runners were found to have ~ 30% larger AT CSA than non-runners [81], and similarly male athletes who perform frequent weight-bearing exercise (running, jumping) had relatively thicker tendons compared to athletes in non-weight bearing sports (kayakers) (~20% larger CSA) [82]. AT adaptation was also found during a high-strain-magnitude intervention of 14 weeks of isometric plantar flexion contractions resulting in a decrease in tendon-aponeurosis of 4.6% at a given tendon force, with no adaptation found in the same period with low-strain magnitude intervention [83]. Thus, those findings indicates that strain magnitudes should exceed a given threshold to trigger adaptation effects on the mechanical and structural properties of the AT [83].

Changes in tendon properties were also reported in times of unloading [84-86]. A study by Reeves et al. investigated the effect of AT unloading after 90 days of bed rest. It was found that tendon stiffness was reduced by 58% however the dimensions of the tendon e.g. CSA remained unaltered which is reflected by the fact that the corresponding young’s modulus decreased after loading by 57% (266 – 144 MPa). Indicating that 90 days of unloading reduces AT stiffness due to a change in tendon young’s modulus, with no measurable tendon atrophy [84]. The reduced tendon stiffness following unloading means that for any given level of force production the tendon elongation would be greater post-intervention, suggesting that muscle fibres would shorten more [58].

Another study by Kinugasa et al. investigated the influence of unloading by chronic (4 weeks) unilateral lower limb suspension on dimensional (volume, CSA, segmented length) and elastic (Young’s modulus) properties of the AT, resulting in an increase in tendon volume but a decrease in
Young’s modulus [85]. Moreover, the magnitude of tendon hypertrophy was not correlated to the degree of reduced tendon stiffness, indicating that the change in tendon elastic properties could not be attributed to tendon volume and thus implies a role for a change in material properties (Young’s modulus) [85]. On the other hand, Kubo et al. investigated the effects of 20 days bed rest on the viscoelastic properties of the tendon structures in knee extensor and plantar flexor muscles and found decreased tendon stiffness in knee extensors but not in the plantar flexors [86].

2.7. AGE AND TENDONS

In addition to the loading-induced changes in the properties of the tendon the biological process of maturation [87-91] and ageing [92-94] can also alter tendon properties due to muscular, neuronal, hormonal and biomechanical factors [95]. During childhood, the stiffness of weight-bearing tendons has been shown to increase with age from 9 to adulthood [26;27]. Age-related increases in body mass and force production capabilities have been postulated to contribute to these observed increases in tendon stiffness [26]. Increases in stiffness could also be explained by increases in tendon size and/or Young’s modulus and the latter due to tendon microstructural changes, including an increased fibril diameter [96-98], fibril packing [99], collagen cross-linking [100] and a reduced collagen crimping [101]. Previous studies reported that children demonstrate more compliant tendinous structure compared to adults as their tendon elongation and strain was significantly greater at a given force during isometric conditions [26;27].

Kubo et al. investigated the growth changes in the elastic properties of vastus lateralis tendon among nine younger boys (10.8 ± 0.9 yrs) nine older boys (14.8 ± 0.3 yrs) and fourteen young adult men (24.7 ± 1.6 yrs) during isometric extension of their knee joint [27]. It was found that the vastus lateralis tendon is more compliant in younger boys than in adults as their tendon strain at a given force was significantly greater [27]. In the same line O’Brien et al. [26] investigated whether there are differences in the mechanical and material properties of the patellar tendon between adults
Literature Review

(N=10 males; N=10 females; 23-31yrs) and pre-pubertal children (N=10 boys; N=10 girls 8-10 yrs.) during isometric knee extension. They found that adults have significant stiffer tendon compared to children with no differences in between gender in both the adult and children group.

A study by Waugh et al. investigated the contribution of age, body mass and muscular strength on prepubertal development increases in AT stiffness, and documented the development of its mechanical properties during isometric plantar-flexion contraction. It was found that body mass and peak force are the primary factors underpinning both the dimensional and maturational aspects of tendon stiffness rather than age per se [28]. In contrast to the previous studies mentioned above [26;27], Waugh et al. found no differences in the maximal strain of AT between children (N=52; 5-12yrs) and adults (N=19; 22-29yrs) as elongation increased in proportion to its resting length, resulting in a consistent peak strain across all age groups. Moreover, in the above studies, the tendon structural dimensions, e.g. CSA, thickness and length, were found to be greater in adults compared to children [26-28].

In regards to older ages, changes in tendon mechanical properties caused by aging seem to be in the same direction with those caused by immobilization. Onambele et al. examined the mechanical properties of the AT in younger (24 ± 1 yr.), middle-aged (46 ± 1 yr.) and older ages (68 ± 1 yr.) subjects. They found that both the stiffness and Young’s modulus of tendon decreased gradually with age, with the differences between the younger and the older group reaching 36% and 48%, respectively. The CSA of the tendon decreased with age by 19% and tendon length by 16% which could be attributed to anthropometric differences rather than tendon atrophy [93].

The age related changes found in tendons have been postulated to contribute towards the development of certain tendon ailments. As it will be discussed in the next chapter, the prevalence of tendon pathologies e.g. tendinopathies increases with age (adults 36 % vs. children 1.8%) [24;25]. The reason behind the lower prevalence of these overuses injuries in children is still unclear. Kubo et al. have made the interesting suggestion that the greater the tendon compliance found in children
might be an important factor in reducing the risk of tendon injuries [27]. Conversely, O’Brien et al. assumed that due to the greater tendon compliance, children have lower safety margin; but have less exposure to exercise-related microdamage accumulation over time compared to adults [26]. On the other hand, Waugh et al. argued that the high prevalence found in adult population could be explained by the fact that increases in tendon stress with age to adulthood are a result of strength gains (~310%) exceeding those of tendon hypertrophy (~93%), assuming an age-related imbalance between muscle and tendon [28]. As outcomes of the previous reported studies [26-28] are based on standardized isometric conditions, knowledge regarding the influence of age on tendon response under dynamic situations is limited and thus, its association with tendon injuries is still unclear.

2.8. Achilles mid-portion tendinopathy

The most common clinical diagnosis of AT overuse injuries is mid-portion tendinopathy with an incidence of 55% to 65%, followed by insertional problems such as retrocalcaneal bursitis and insertional tendinopathy of 20% to 25% [6;102-106]. Achilles tendinopathy is most prevalent in men in the forth to fifth decade of life and its occurrence is highest among individuals who participate in sports involving running and jumping [6;18-23]. In adult runners, a point prevalence of up to 36% has been reported for Achilles tendinopathy [24]. Whereas, recent data in 760 adolescent athletes at an average of 13 years of age a point prevalence of 1.8% was reported for Achilles tendinopathy [25]. In the general population, the incidence rate of mid-portion Achilles tendinopathy was reported to be 1.85 per 1.000 individuals, with sport activity being recorded in only 35% of these cases [107].

The etiology of tendinopathy is considered to be multifactorial [7-9] and its pathogenesis unclear [7;10]. In the epidemiological studies, various alignment and biomechanical faults are claimed to play a causative role in two-thirds of the athletes with AT disorders [20]. Increased foot pronation [108;109], varus deformity of the forefoot [6;108-110], increased hind foot inversion and decreased ankle dorsiflexion with the knee in extension has shown to be associated with Achilles tendinopathy
Literature Review

[111]. However, excessive loading of tendons during vigorous physical training is regarded as the main pathological stimulus for tendinopathy and degeneration [112].

In general, tendinopathy is regarded as an overuse condition implying that excessive and repetitive loading elicits symptoms [8;113]. A well-known mechanical theory of tendon overuse, states that when a tendon has been strained repeatedly to 4-8% strain it is unable to endure further tension, whereupon overuse injury occurs. The tendon becomes fatigued as its basal reparative ability e.g. the ability of the tendon cells to repair the fiber damage is overwhelmed by repetitive microtraumatic process leading to a failed healing process. The structure of the tendon is disrupted micro- or macroscopically by this repetitive strain and collagen fibers begin to slide past one another causing breakage of their cross-linked structure and by time leading to tendon degeneration including pain and swelling [114;115].

The cardinal symptom of Achilles tendinopathy is pain which occurs at the begin and end of a training session, with a period of diminished discomfort in between [112]. As the pathology progress pain may occur during exercise interfering with activities of daily living. In the acute phase the tendon is diffusely swollen and on palpation tenderness is usually greatest 2-6 cm proximal to the tendon insertion [112]. The diagnosis of Achilles tendinopathy is made on clinical grounds by use of history and clinical examination supported by diagnostic US to detect structural lesions, changes in echogenicity, neovascularization or perfusion [3;4]. In US imaging tendinopathy initially appears as tendon thickening and at progress the fibrillar pattern is lost and replaced by hypoechoic changes with further swelling e.g. spindle shape (fig. 8) [4;116].

The degenerative process associated with tendinopathy may also affect the tendons inherent mechanical and material properties. Previous studies which have investigated the tendon properties in the presence of tendinopathy found that degeneration may lead to a weaker tendon. These alterations in mechanical characteristics may put the AT in a higher risk to sustain further injury and prolong the time to recovery [2]. Arya and Kulig investigated the material and mechanical properties
of the AT in the presence of tendinopathy during isometric plantar-flexion contraction between asymptomatic adults (N=12; 45±7yrs) and patients with Achilles midportion tendinopathy (N=12; 47±8yrs) [2]. It was found that tendinopathic tendons present greater CSA, lower stiffness and lower Young’s modulus leading to a weaker tendon [2]. In the same line Child et al. compared AT strain between male athletes with (N=14; 40±8yrs) and without Achilles tendinopathy (N=15; 35±9yrs) during maximal isometric plantar flexion on a calf-raise apparatus [12]. It was found that AT-aponeurosis strain is higher on patients with tendinopathy (5.2%) than those without (3.4%) [12]. However, results from those studies are shadowed by methodological restrictions as they were perform under artificial conditions e.g. standardized isometric conditions resulting in lower forces acting on the tendon compared to real life activities which yields further consideration when interpreting the results. Despite immense knowledge provided by the above studies the influence of tendinopathy on tendon response under functional task still remains unclear and yields further investigation as this will enable a better understanding of the pathology leading also to better treatment strategies and prevention.

Figure 8: Longitudinal and transverse grey-scale ultrasound images of normal (A and B) and tendinopathic (C and D) AT from 1 control and 1 individual with Achilles tendinopathy. C and D: significant focal thickening of the tendon. Black dotted lines outline the tendons. White curved line on the right denotes the calcaneus. *Taken from Arya and Kulig, Tendinopathy alters mechanical and material properties of the Achilles tendon J Appl Physiol 2010; 108(3):670-675. [2]*
3. Research Objectives

In summary, the previous paragraphs have demonstrated that several studies have examined how tendons respond in terms of loading, age and pathology. Despite important information provided, there are still gaps in literature and insufficiencies that needs to be elucidated for an enhanced understanding of the influence of age and pathology on tendon behaviour. Furthermore, in terms of methodology used, US assessment remains a dubious tool due its operator-dependency, which yields further investigation.

Ultrasonography is technical demanding device, which requires a considerable experience by the investigators in order to perform a consistent and high quality scanning and further analysis [42]. In most US studies, the experience of the investigators is often neglected and not reported. Experience and knowledge in ultrasonography assessment is crucial in order to be able to distinguish between artefacts and clinical outcomes. However, not only the experience is important but also the assessment protocol. In literature no standardised US protocol exists for the assessment of AT properties and thus, limited information exist regarding its reproducibility. Therefore, to ensure accurate diagnosis of a condition and to correctly evaluate tendon properties, use of clinical reliable tools, independent of investigators experience, is needed.

Regarding the effects of tendinopathy, studies have shown that tendinopathy results in a weaker tendon with high predisposition to further injury, resulting in higher tendon strains with lower forces and stiffness [2;12]. However, due to methodological restrictions the influence of pathology remains unclear. As prevalence of Achilles tendinopathy increases with age, age-related changes found in tendon properties have been considered as a predisposition factor. However, little is known about the growth related changes in the human tendon [26-28]. Among them, the tendinous structures of knee extensors [27] and patellar tendon [26] were found to be more compliant in children than in adults. It was thereby assumed that higher compliance found in young tendon might play a
protective role against load-related injuries [27]. However, this hypothesis has not been empirically tested under functional tasks. On the other hand, Waugh et al. did found no age-related changes in maximal strain in AT between children and adults [28].

In addition, there is still gap in literature regarding the tendon behaviour under physiological loads. As a result, the pathophysiological mechanism of tendinopathy remains largely unknown. Although computational models have provided an insight into musculoskeletal function, the outcome is a model-based estimation and subject to deviation from true values as they are based in in-vitro models and standardized isometric conditions. Therefore, it is crucial to understand the response of tendon and the influence of age and pathology in functional task in order to enable further investigation into injury mechanism, prevention and treatment strategies of tendinopathies.

Based on these queries, the research objectives of this thesis can be summarized as follow:

1. The first objective was to investigate the consistency of repeated US assessment on tendon properties by a contribution of a single rater. It is hypothesis that by a standardized and yet simple protocol excellent reliability could be achieved.

2. The second objective was to investigate the influence of two raters with different levels of experience on a single US assessment on tendon properties. It is hypothesis that by a standardized protocol excellent reliability could be achieved irrespective of investigators experience.

3. The third objective was to investigate the influence of age and pathology on tendon properties under dynamic conditions. It is hypothesis that tendon will respond differently in regards to age and pathology.
4. Studies

**Table 1**: Characteristics of the studies included in the present thesis

<table>
<thead>
<tr>
<th>Study</th>
<th>Journal</th>
<th>Design</th>
<th>Participants</th>
<th>Measures</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isokinetics and Exercise Science (peer-reviewed)</td>
<td>Test-Retest 1 week interval</td>
<td>Asymptomatic adults Males: (N=7); Females: (N=9), Mean age 29 ± 5 yrs</td>
<td>Intra-rater reliability, Tendon properties</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>Sport Orthopaedics and Traumatology (peer-reviewed)</td>
<td>Cross-sectional</td>
<td>Asymptomatic adults Men (N=3), Female (N=7), Mean age: 30 ± 7</td>
<td>Inter-rater reliability, Tendon properties</td>
<td>4.2</td>
</tr>
</tbody>
</table>

**Asymptomatic adults**

Men (N=7), Female (N=3), Mean age: 37 ± 8

**Tendinopathic patients**

Men (N=7), Female (N=3), Mean age: 40 ± 7
4.1. Study 1

Ultrasonography for the assessment of the structural properties of the Achilles tendon in asymptomatic individuals: An intra-rater reproducibility study.

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Reference

4.1.1. ABSTRACT

**BACKGROUND**: Reproducible measurements of tendon structural properties are a prerequisite for accurate diagnosis of tendon disorders and for determination of their mechanical properties. Despite the widely used application of Ultrasonography (US) in musculoskeletal assessment, its operator dependency and lack of standardization influences the consistency of the measurement.

**OBJECTIVE**: To evaluate the intra-rater reproducibility of a standardized US method assessing the structural properties of the Achilles tendon (AT).

**METHODS**: Sixteen asymptomatic participants were positioned prone on an isokinetic dynamometer with the knee extended and ankle at 90° flexion. US was used to assess AT-length, cross-sectional area (CSA), and AT-elongation during isometric plantarflexion contraction. The intra-rater reproducibility was assessed by ICC (2.1), Test-Retest Variability (TRV,%), Bland-Altman analyses (Bias±LoA[1.96*SD]), and Standard-Error of Measurement (SEM).

**RESULTS**: Measurements of AT-length demonstrated an ICC of 0.93, TRV of 4.5±3.9%, Bias±LoA of -2.8±25.0mm and SEM of 6.6mm. AT-CSA showed an ICC of 0.79, TRV of 8.7±9.6%, Bias±LoA of 1.7±19.4mm² and SEM of 5.3mm². AT-elongation revealed an ICC of 0.92, TRV of 12.9±8.9%, Bias±LoA of 0.3±5.7mm and SEM of 1.5mm.

**CONCLUSIONS**: The presented methodology allows a reproducible assessment of Achilles tendon structural properties when performed by a single rater.

**Key words**: Ultrasonography, Achilles tendon, Reproducibility, Isokinetic
4.1.2. INTRODUCTION

The Achilles tendon (AT) is the strongest tendon in the human body [1] and one of the most common sites of injuries [117-121]. Degenerative AT disorders, commonly described by the term tendinopathy, cause considerable morbidity and functional impairment among athletic and general population [6;122] with an incidence of 1.85 per 1.000 individuals [107]. Degenerative tendons usually present a larger cross-sectional area (CSA) and a higher strain compared to a healthy tendon[2]. Sonographic measurements of tendon structural properties have thereby been established as important markers for the diagnosis of AT disorders [2;82;120], as well for the determination of tendon mechanical and material properties [2] underlining their importance in both the clinical and research context.

Characterization of tendon mechanical and material properties, such as stiffness and Young’s modulus, are essential for understanding mechanisms that enable to optimize the functional behaviour of the muscle tendon complex [1;2]. Tendon stiffness is calculated by dividing the estimated tendon force by the tendon’s elongation and it’s thereby influenced by tendon structural properties such as tendon length and CSA. In the case of a healthy tendon, a shorter one with larger CSA is expected to have greater stiffness, whereas a longer tendon with lower CSA is expected to be more compliant. On the contrary, Young’s modulus is defined as stiffness normalized to tendon CSA and length. It is described based on the linear relation between stress (force/CSA) and strain (elongation/length), providing a measure of tendon material properties, irrespective of its structural dimensions [2;60].

Methods to determine in-vivo human tendon mechanical and material properties using non-invasive techniques have been described [2;84;123-125] through the combined use of dynamometry, electromyography, and B-mode linear ultrasound (US) imaging. Measurements of the proximal
displacement of the distal myotendinous junction (MTJ) of the medial gastrocnemius during ramped isometric muscle contractions [84;126-128] are typically used to give an estimation of AT mechanics.

By the use of the above mentioned methodology, previous studies have determined AT stiffness and Young’s modulus in several populations, developing a better understanding of the tissue in response to age [28], loading [81;82], gender [127;129] and pathology [2]. Despite its widely used application, few studies have evaluated the reproducibility of the AT structural properties from which they are defined such as tendon length, CSA, and tendon elongation during maximal voluntary isometric contraction (MVIC).

These studies were based on small pre-pilot measurements which consisted of a few participants (N=5-7) [2;50], reporting excellent reproducibility on the measure of tendon elongation and muscle torque. However, due to the lack of measurement standardization, it remains unclear whether CSA and length can be measured in a reproducible manner. It has to be noted that summation of small errors in each of the structural properties measured (e.g. CSA, length), can lead to a larger variability in the calculation of stiffness and Young’s modulus, highlighting the need to determine a standardized and reproducible setup.

Several US methods, such as the extended field of view or panoramic ultrasound technique, have been reported to be reproducible and valid in the assessment of AT. Although these techniques are reproducible, they are not available in most medical and research centres, highlighting the need for simple, time-efficient, and reproducible US methods.

As US measurements are operator dependent external factors, such as the experience of different rater involved, can influence the consistency of the measurement [130]. Intra-rater reproducibility can thereby improve the consistency of measurement by eliminating external factors with the contribution of a single rater. It is hypothesized that by using a standardized and yet simple US
protocol with the contribution of a single rater, excellent reproducibility with low levels of variability could be achieved. Therefore, the purpose of this study was to assess the intra-rater reproducibility of a simple ultrasonographic method assessing AT structural properties at rest and MVIC.

4.1.3. MATERIAL AND METHODS

Study Design
A test-retest design was used in the present study with a time interval of one week. The measurements were supported by a single rater with one year of experience in musculoskeletal US assessment in both image acquisition as well as image analysis.

Participants
Sixteen recreational active participants (7 males and 9 females, 29 ± 5 yrs., 1.77 ± 0.10 m., 73 ± 12 kg) were included in the present study. Participants were excluded if they reported any acute or chronic musculoskeletal injury of the lower limb and/or signs of tendinopathy on US imaging [116]. The study was approved by the local ethics committee. All participants signed an informed consent.

Tendon properties
*Tendon length*: To obtain AT-length, participants lay in a prone position with their ankles hanging over the examination table being manually flexed at 90° by the investigator knee. A diagnostic ultrasound device (Vivid q; GE Healthcare, Tirat Carmel, Israel) with a 7.5 MHz continuous linear ultrasound array (4 -13 MHz) was used. Presets were standardized at a frequency of 13 MHz and a depth of 3 cm. The most distal part of AT, attaching to the calcaneus and medial gastrocnemius myotendinous junction (MTJ), were detected sonographically. Metal fine-wires were then placed between the skin and the transducer (Fig. 9), providing an acoustic shadow visual to the US, overlaying the corresponding tendon structures (Fig. 10). The location of the corresponding structures was accurately marked on the skin. The distance between the two markers was measured using a measuring tape and represented ATL.
Methodology used to assess Achilles tendon length by the use of metal fine-wires (placed between skin and transducer) at the distal Achilles tendon insertion on the calcaneus (a) and at the Medial Gastrocnemius myotendinous junction (b). The distance between these markers was assessed by a measuring tape.

Ultrasound images demonstrating the acoustic shadow produced by the use of metal fine-wires placed between skin and transducer at the most distal part of Achilles tendon attaching on the calcaneus (a) and at the Medial Gastrocnemius myotendinous Junction (b). Structures are identified by the arrows (c).
Tendon Cross-sectional area: Previous studies have shown that AT-CSA dimensions are not the same throughout its length [51], with the narrowest part being the most vulnerable to injuries. Furthermore, it’s unclear at which distance point CSA should be assessed. Thereby, AT-CSA was measured at three distance points at 2, 4 and 6 cm from the tendon distal insertion on the calcaneus (Fig. 11) defined by the use of a measuring tape. The US probe was placed in a transversal scan over the corresponding points and the CSA at these three distance points was measured [2].

Figure 11: Methodology used to assess tendon cross-sectional area at different distance points at 2, 4 and 6 cm proximal the most distal insertion on the calcaneus defined by the use of a measuring tape (a) and measured with the US probe placed in a transversal scan over the corresponding points (b).

MVIC protocol

As tendon stiffness is calculated by dividing the estimated tendon force by the tendon’s elongation, maximal isometric contractions are typically used in the assessment of tendon properties in order to shorten the muscle and elongate the tendon [131]. Thus, participants were positioned prone on the isokinetic dynamometer (Contrex MJ, Physiomed, Germany) with the hip and knee extended and ankle at 90° of flexion. The axis of rotation was carefully aligned to the lateral malleolus. The left foot was strapped securely to the footplate by the use of Velcro straps. The probe was placed perpendicularly to the skin surface above the MTJ, and was fixed in position using a custom-made
holder (Fig. 12). A thin strip of echo absorptive tape was placed on the skin visible in the sonographic picture which provided a reference to detect any probe movement.

For warming up and getting accustomed to the measurement situation, participants performed three sub-maximal and two maximal isometric plantar flexion contractions of 5 s, with 1-min rest in between. After these practice trials, participants performed 3 maximal isometric plantar flexion contractions within a period of 5 s with 1 min rest while simultaneously the displacement [mm] of the medial gastrocnemius MTJ, defined as tendon elongation was sonographically recorded (Fig. 12).

**Figure 12:** Probe placement perpendicularly to the skin surface above the Medial gastrocnemius Myotendinous Junction (MTJ), fixed in position using a custom made holder (a). MTJ displacement defined as tendon elongation from rest (b) to maximal voluntary isometric plantarflexion contraction(c). Errors point the MTJ, dashed error indicates the direction of the displacement

**Data analysis**

*Tendon Cross-sectional area:* The ultrasound images of AT-CSA where stored digitally as JPEG file and processed on a PC using public domain software (Image J, [http://rsbweb.nih.gov/ij/](http://rsbweb.nih.gov/ij/)). The freehand selection tool was used to outline the tendon and measure the CSA at each of the three distance points of the tendon. In addition to the single site measures, the average of all 3 distance points was calculated.
Tendon elongation: The ultrasound video-clips were stored digitally as .avi files, converted into stacks of images (http://www.virtualdub.org/), and processed by use of Image J. Tendon elongation was then measured by manually tracking the displacement of the medial gastrocnemius MTJ from rest to MVIC.

In order to decrease the variability within participants, each image and video clip was digitized three times and the average was taken [2]. To minimize a possible bias, all images and video-clips were stored under a three digit random number, assigned prior to testing and stored in an identification file. As consequence, the investigator was blind to the participants, measurement days and examination order. After finalizing the analysis of the data, the results were assigned to the corresponding participants.

Data were initially analysed descriptively (mean ± SD). An ICC (2,1) value of ≤ 0.50 was considered as low, 0.50 - 0.75 was considered as moderate, ≥ 0.75 was considered as good and ≥ 0.90 was considered as excellent reproducibility [132]. The agreements between the measurements were verified qualitatively using Bland-Altman analysis (Bias ± 1.96*SD [LoA]) [133]. Test-retest variability (TRV) was calculated as the absolute differences between the two measurements, divided by their average, and expressed as percentage [%]. Additionally, to provide an estimate of the precision of measurement, the standard error of measurement (SEM=SD*√(1−ICC)) was calculated [134]. All statistical calculations were performed using SPSS (SPSS Statistics 20, IBM, USA) and Microsoft Excel version 14.0 (Microsoft Corporation, 2010).

4.1.4. RESULTS

The average values for both measurement days and the evaluated parameters of AT-length, CSA, elongation, and associated muscle torque are present in Table 2. All average measures revealed good to excellent reproducibility with muscle torque being the least reproducible (Table 3). AT-elongation was assessed with excellent reproducibility and very low systematic error. In comparison AT-length revealed higher systematic and random error. For the AT-CSA at 2, 4 and 6 cm it was
revealed that the position 2 cm proximal of the tendon insertion had the least reproducibility. In comparison to the position at 6 cm which was most reproducible (Table 3).

**Table 2:** Achilles Tendon structural properties and associated muscle torque

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test</th>
<th>Retest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tendon length [mm]</td>
<td>220.6 ± 28.5</td>
<td>223.3 ± 22.1</td>
</tr>
<tr>
<td>CSA at 2cm [mm²]</td>
<td>88.8 ± 20.3</td>
<td>88.1 ± 10.9</td>
</tr>
<tr>
<td>CSA at 4cm [mm²]</td>
<td>69.6 ± 13.6</td>
<td>66.4 ± 9.5</td>
</tr>
<tr>
<td>CSA at 6cm [mm²]</td>
<td>62.5 ± 12.7</td>
<td>61.3 ± 11.7</td>
</tr>
<tr>
<td>CSA average [mm²]</td>
<td>73.6 ± 14.3</td>
<td>71.9 ± 8.6</td>
</tr>
<tr>
<td>Tendon elongation [mm]</td>
<td>18.2 ± 5.6</td>
<td>17.9 ± 5.2</td>
</tr>
<tr>
<td>Muscle Torque [Nm]</td>
<td>97.5 ± 22.9</td>
<td>100.8 ± 29.3</td>
</tr>
</tbody>
</table>

*Values are mean ± SD averaged for each individual between test and retest; CSA=Cross-Sectional Area of Achilles tendon.*

**Table 3:** Reproducibility values for Achilles Tendon structural properties and the associated muscle torque

<table>
<thead>
<tr>
<th>Variable</th>
<th>ICC</th>
<th>TRV [%]</th>
<th>Bias ± LoA</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tendon length [mm]</td>
<td>0.93</td>
<td>4.5 ± 3.9</td>
<td>-2.8 ± 25.0</td>
<td>6.6</td>
</tr>
<tr>
<td>CSA at 2cm [mm²]</td>
<td>0.26</td>
<td>14.9 ± 15.7</td>
<td>0.7 ± 41.6</td>
<td>13.8</td>
</tr>
<tr>
<td>CSA at 4cm [mm²]</td>
<td>0.86</td>
<td>9.4 ± 6.8</td>
<td>3.2 ± 15.5</td>
<td>4.4</td>
</tr>
<tr>
<td>CSA at 6cm [mm²]</td>
<td>0.94</td>
<td>7.7 ± 6.2</td>
<td>1.2 ± 11.9</td>
<td>2.9</td>
</tr>
<tr>
<td>CSA average [mm²]</td>
<td>0.79</td>
<td>8.7 ± 9.6</td>
<td>1.7 ± 19.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Tendon elongation [mm]</td>
<td>0.92</td>
<td>12.9 ± 8.9</td>
<td>0.3 ± 5.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Muscle Torque [Nm]</td>
<td>0.88</td>
<td>13.8 ± 9.9</td>
<td>-3.4 ± 34.5</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*Measures of reproducibility: ICC= Intraclass correlation coefficient, TRV= Test-Retest variability, Bias ± LoA= 95% Limits of agreement, SEM= standard error of measurement. CSA= Cross-Sectional Area of Achilles tendon.*
4.1.5. DISCUSSION

The present study was designed to evaluate the intra-rater reproducibility of a sonographic method assessing AT structural properties at rest and at MVIC. The results indicate that by the use of a standardized and simple US protocol and by the contribution of a single rater, the methodology used and variables measured are reproducible. This finding suggests that a potential calculation of stiffness and Young’s modulus could be determined with a relatively low variability.

AT-length was assessed by the use of a consistent protocol and well-defined anatomical landmarks, which were identified by the use of US and metal fine-wires, achieving excellent reproducibility with low levels of variability and SEM. Transferring the results to the clinical setting, the systematic bias of 2.8 mm found in measuring ATL, seems negligible; however, the relative high random error of 25 mm should be discussed critically. A possible explanation to this result could be the manual positioning of the ankle by the investigator; as different range of motion of the ankle results in different tendon length. These findings suggest that implementation of correction tools e.g. goniometer could improve the reproducibility of the method. Previous studies have used surface markers on the skin corresponding to the proximal and distal tendon ends, measured manually by the use of a ruler [2;50]. However, the absence of standardized US protocols and the limited field of view of most US transducers restricted the wide implementation of US for this use. A recent method overcoming these issues is the panoramic US technique, where a study by Ryan et al. reported excellent test-retest reproducibility (ICC: 0.95, SEM: 4.34 mm) [135]. Despite that this method is easy to perform, and clinically applicable, its availability in most research and clinical centers is still limited.

Compared to the assessment of AT-length, measurements of AT-CSA present a different type of challenge, as AT-CSA dimensions are not the same along its length [51;136]. Furthermore, it’s unclear at which distance point CSA should be assessed. AT-CSA was thereby measured at three
Study 1

different distance points at 2, 4 and 6 cm from the most distal insertion on the calcaneus [2;51]. In addition to the single values, for a more representable value of CSA, an average of the three distance points was calculated [2]. Based on the present results, averaging the CSA seemed non-beneficial compared to the single measures, since the reproducibility at each point measured was different, with the lowest reproducibility and highest variability found at 2 cm. This finding is attributed to the fact that 2 cm is located at the calcaneus bone which leads to a less clear image quality. Additionally, in that region, the tendon is wider which makes borders of the CSA difficult to identify with the used methodology. This discrepancy is also represented by the high random error given by the limits of agreement and SEM at 2 cm compared with the location at 4 and 6 cm. These findings indicate that for a reproducible assessment of CSA, measures on a single site of the tendon at the region of 4 - 6 cm proximal the distal insertion on the calcaneus have to be obtained. Moreover, the area of 4 cm defined always in the present study based on the distance from the tendon distal insertion, falls well within the level of medial malleolus. This is in line with the methodological approach of previous studies assessing the CSA with a single measure at the level of malleolus [50;52;53], reporting good to excellent reproducibility [50;52].

In the present study by the use of standardized probe positioning the displacement of the MTJ from rest to MVIC defined as tendon elongation demonstrated excellent reproducibility and low systematic error. This finding agrees with previous studies reporting excellent test-retest (ICC: 0.95, 0.99) [2;50] and intra/ inter-session (ICC: 0.99/0.93, SEM: 0.41/1.59 mm) reproducibility [137]. Furthermore, torque measures revealed good reproducibility between the tests with a relative low systematic error, a finding which also agrees with previous studies reporting high test-retest (ICC: 0.80, 0.93) [2;50] and intra/inter-session (ICC: 0.99/0.93, SEM: 3.52/7.77 N m) [137] reproducibility. However, compared to the other variables measured, muscle torque presents one of the highest SEM and variability as indicated also by the high random error given by the limits of agreement. This could be attributed to the participants’ prone position on the isokinetic dynamometer, which led to
a propelling of the body during MVIC, leading to variability of the torque exerted. Subsequently, as tendon elongation is depended on muscle torque, this could explain the variability found also in tendon elongation. However, in order to be able to properly attach the US on the shank, the seated position was not chosen.

To improve the consistency of the measurements, reproducibility in the present study was assessed by a single rater, with an experience of one year in the assessment of musculoskeletal US achieving good to excellent reproducibility. This finding suggests that the implementation of one rater in the musculoskeletal US assessment for reproducible measurements is beneficial. However, for a comprehensive statement about reproducibility inter-rater must also be evaluated. For example, during the course of a clinical follow-up or intervention; patients may be examined by more than one physician [138].

Although this study presents a reproducible method, the variability of the data has to be discussed critically. Methods and tools have to be sensitive enough to be able to distinguish between even the smallest changes e.g. in the case of early stages of pathology or during a clinical follow up. Variability of data can thereby overshadow the outcome leading to a false diagnosis. Moreover, it has to be kept in mind that data of the present study were derived from only healthy young participants. This fact limits the implementation of the findings in other age groups and questions its application into the field of pathology. Future research should thereby be conducted involving symptomatic patients with signs of tendon degeneration since degenerative tendons have different clinical presentation altering the AT properties [2].

4.1.6. CONCLUSION

The study presents a reproducible and simple ultrasonographic method for the assessment of Achilles tendon structural properties, achieving good to excellent intra-rater reproducibility with low levels of variability. Moreover, for a reproducible assessment of AT-CSA, measurements on a single
site of the tendon, at the region of 4-6 cm proximal the distal insertion on the calcaneus should be obtained. Reproducible methods can enable further investigation in the field of pathology and into the effects of current treatment strategies. However, for a comprehensive statement about reproducibility, inter-rater should also be addressed in future studies.

4.1.7. Gab leading to study 2 of the thesis

Despite that the methodology presented in study 1 of the thesis allowed for a reproducible assessment of AT properties; the investigation was limited to a single rater. As US is a technically demanding tool which requires a considerable experience by the investigator; the influence of different raters should also be evaluated. Inter-rater reliability in combination with intra-as presented in study 1 will allow for a comprehensive statement about reliability. For that purpose a second reliability study was conducted in order to investigate the influence of between investigators with different level of experience in the assessment of a method measuring AT properties.
4.2. Study

Original Article

Measuring Achilles Tendon Length: A Simple and Reliable Method

Die Bestimmung der Achillessen-Länge: eine einfache und reliable Methode

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Reference

4.2.1. Abstract

**Background:** The purpose of the study was to evaluate the inter-rater reliability of the Achilles tendon length (ATL) by use a simple US marker-based method. **Methods and Materials:** By the use of metal fine-wires placed between skin and transducer, distal Achilles tendon insertions and medial gastrocnemius myotendinous-junctions of 10 healthy subjects were detected, marked and finally, ATL was measured on the skin. Reliability was analysed between an experienced and an inexperienced examiner using Intraclass Correlation Coefficient (ICC, 2.1), Inter-Rater-Variability (IRV, %), Bland-Altman-analyses (Bias ± Limits of Agreement, [LoA]) and Standard-Error of Measurement (SEM, mm). **Results:** Results revealed an ICC of 0.91, IRV of 3.1 ± 2.5%, Bias ± LoA of -2.4 ± 16.6 mm and SEM of 5.7 mm. **Conclusion:** The present novel method provides a reliable assessment of ATL in healthy subjects in a simple and time-efficient manner, independent of observer’s experience.

**Key words:** Achilles tendon length, Ultrasound, Inter-rater reliability
4.2.1. Zusammenfassung

**Hintergrund:** Ziel der Studie war die Bestimmung der „inter-rater“ Reliabilität in der sonographischen Messung der Achillessehnen-Länge (ASL) unter Nutzung einer einfachen, markerbasierten Methode. **Material und Methoden:** Bei 10 gesunden Probanden wurde mithilfe eines feinen Drahtes, der zwischen Schallkopf und Haut platziert wurde, die Achillessehnen-Insertion und der Muskel-Sehnen-Übergang des medialen M. gastrocnemius detektiert, markiert und anschließend die ASL auf der Haut gemessen. Die Reliabilität zwischen einem erfahrenen und einem unerfahrenen Untersucher wurde bestimmt durch den Intraklassen Korrelations-Koeffizienten (ICC, 2.1), die Inter-Rater-Variabilität (IRV, %), die Bland-Altman-Analyse (Bias ± Limits of Agreement, [LoA]) und den Standardfehler (SEM, mm). **Ergebnisse:** Die Ergebnisse zeigen einen ICC von 0.91, einen IRV von 3.1 ± 2.5 %, einen Bias ± LoA von -2.4 ± 16.6 mm und einen SEM von 5.7 mm. **Schlussfolgerung:** Die vorliegende Studie stellt eine neue und einfache Methode zur Bestimmung der Achillessehnen-Länge bei gesunden Probanden dar, die unabhängig der Erfahrung des Untersuchers reliabel angewendet werden kann. **Schlüsselwörter:** Achillessehnen-Länge, Ultraschall, Inter-rater Reliabilität
4.2.2. Introduction

The Achilles tendon (AT) is the strongest tendon in the human body [1] and one of the most common sites of overuse injuries [120]. The high incidence of AT disorders and their unresponsiveness to current treatments is a compelling factor underscoring the need to investigate the tendon properties [2]. Diagnosis of AT disorders (such as bursitis subachillae, Haglund exostosis, tendinopathy as well as sever’s injuries) is usually made on clinical grounds by use of history and clinical examination supported by diagnostic ultrasound to detect structural lesions, changes in echogenicity, neovascularization or perfusion [2;4]. The structural measures of the AT such as the cross-sectional area [2] and length [139] have also been established as important markers for AT disorders [2;82], and thus are important in both the clinical and research context.

Achilles tendon length (ATL) has been found to be influenced by various conditions that include neurologic disease [140], stroke [141] and aging [142]. In the research context, ATL is a critical parameter for an accurate calculation of tendon strain which describes the elongation/deformation of the tendon relative to the resting length, adapting in terms of loading by decreasing strain and unloading by increasing strain [60]. Previous studies have shown that tendinopathic tendons present with higher strain and reduced stiffness as a result of tendon degeneration in comparison to healthy tendons [2]. It is assumed that higher strains result in microscopic disruptions of collagen fibers making the tendon vulnerable to further injury and potentially predisposing the tendon to the danger of rupture [2]. Ruptures of AT are a common long-term problem in sports medicine and their incidence is increasing [143]. In view of the decision about surgery or conservative treatment [144] it is important to assess the rupture zone and the adaptability of tendon stumps in different range of motion for tendon length [145], highlighting the need for accurate and reliable methods of ATL.

Measurements of ATL are challenging due to the limited field of view of most ultrasound (US) transducers which makes it impossible to visualize the entire length of the tendon. Several US
methods have being reported in the literature overcoming this difficulty, such as the extended field of view (EFOV) imaging [53] or panoramic ultrasound [135] technique. These techniques enable the generation of images of tendon length by sweeping the probe manually across it. Studies evaluating the above US guided techniques have reported excellent reliability [135] and validity [146;147]. However, despite that these techniques are reliable, they are not available in most medical centre’s, highlighting the need of simple and time-efficient ultrasonographic methods. Another US method assessing tendon length used surface markers on the skin corresponding to the proximal and distal tendon ends. The distance between those markers represents the tendon length and is measured manually by the use of a ruler [2;50], however is unclear how these studies defined the surface markers on the skin and whether the experience of a rater played a role. Though, the absence of standardized US protocols and the operator dependence on scanning techniques have limited the wide implementation of US for this use.

For the interpretation of different pathologies investigators knowledge and experience is of highly importance in clinical practice. During clinical follow-up examinations, patients are imaged by several investigators with different levels of experience. Thereby, its clinical application requires standardized methods that are independent of investigators’ experience. The degree to which different investigators agree on the measurement is referred to as inter-rater reliability. This type of reliability assessment is relevant in interpretation of measurements administered by different clinicians over time [130]. However, no previous studies have evaluated inter-rater reliability of an ultrasonographic method assessing ATL and more importantly between investigators of different level of experience. Therefore, the purpose of the present study was to examine the inter-rater reliability of a simple and time efficient US method assessing Achilles tendon length in healthy subjects between investigators of different level of experience.

4.2.3. Materials and Methods
Subjects

Ten healthy subjects (7 females and 3 males, 30 ± 7 yrs., 1.7 ± 0.1 m, 69 ± 13 kg) participated in the present study. Subjects were excluded if they reported any acute or chronic musculoskeletal injury of the lower limb and/or signs of tendinopathy on US imaging [116]. Before testing, all participants signed an informed consent document. The study was approved by the local ethics committee.

Investigators

The study was conducted in a cross-sectional design by an experience (EI) and inexperience investigator (II). The EI was a sport orthopaedic physician with five years of intensive clinical practice and study participation using muscle and tendon ultrasound in both image acquisition as well as image analysis [148;149]. The II had no sonography experience before in image acquisition as well as image analysis and only focus training of two weeks prior to the study on healthy tendons. Thus, to ensure equal understanding of ATL measurement, both investigators were introduced and practise to this protocol before commencing the study.

Test Procedure

Subjects were positioned prone with their knees straight in a neutral position and ankles hanging over the examination table being passively flexed at 90° manually by the investigators knee, in order to avoid hind-foot flexion/extension as well as inversion/eversion movements. A diagnostic ultrasound device (Vivid q; GE Healthcare, Tirat Carmel, Israel) with a 7.5 MHz continuous linear ultrasound array (4 -13 MHz) was used. Presets were standardized at a frequency of 13 MHz with a focus of 1 cm and a depth of 3 cm. The most distal part of AT insertion to the calcaneus and the medial gastrocnemius myotendinous junction (MTJ) were detected sonographically. Metal fine-wires were then placed between the skin and the transducer (Fig. 13), providing an acoustic shadow visual to the US, overlaying the corresponding tendon structures (Fig. 14). The locations of the
corresponding structures were accurately marked on the skin. The distance between the two markers was measured using a measuring tape and represented ATL. Both ankles were assessed by the two investigators (EI, II) in a random order. The measurements were performed in the same session by the use of the same equipment with the investigators being blinded to each other’s measurements. Due to the subjects’ personal time schedule, ATL from two subjects was assessed only at one side of the ankle.

**Figure 13:** Methodology used to assess Achilles tendon length by the use of metal fine-wires (placed between skin and transducer) at the distal Achilles tendon insertion on the calcaneus (a) and at the Medial Gastrocnemius myotendinous junction (b). The distance between these markers was assessed by a measuring tape (c).
Figure 14: Ultrasound images demonstrating the acoustic shadow produced by the use of metal fine-wires placed between skin and transducer at the most distal part of Achilles tendon attaching on the calcaneus (a) and at the Medial Gastrocnemius myotendinous Junction (b).
Statistics

Data were initially analysed descriptively (mean ± SD). The inter-rater reliability was assessed by Intraclass Correlation Coefficient (ICC, 2.1) with 95% confidence interval (CI: 95%). An ICC value ≤ 0.50 was considered as low, 0.50 to 0.75 was considered as moderate, ≥ 0.75 was considered as good and ≥ 0.90 was considered as excellent [132]. The agreement between the measurements was verified qualitatively using Bland-Altman analysis (Bias ± Limits of Agreements, [LoA]) and was calculated by the following equation (1):

\[
(1) \quad \text{Bias} \pm 1.96 \times \text{SD}
\]

Inter-rater variability (IRV) was calculated as the absolute differences between the two investigators, divided by their average and expressed as percentage [%]. Additionally, to provide an estimate of the precision of measurement, the Standard Error of Measurement (SEM) [134] was calculated by the following equation (2):

\[
(2) \quad \text{SEM} = \text{SD} \times \sqrt{1 - \text{ICC}}
\]

All statistical calculations were performed using SPSS (SPSS Statistics 21, IBM, USA) and Microsoft excel version 14.0 (Microsoft corporation, 2010).

4.2.4. Results

A total of 18 ATs were assessed. Descriptive analysis demonstrated an ATL of 220.9 ± 19.2 mm measured by the experienced investigator and 223.4 ± 20.2 mm measured by the inexperienced investigator. The inter-rater reliability measures revealed an ICC of 0.91, IRV of 3.1 ± 2.5 %, Bias ± LoA of -2.4 ± 16.6 mm (Fig. 15) and SEM of 5.7 mm.
4.2.5. Discussion

The present study describes a reliable and time-efficient novel US method for the assessment of ATL by the use of metal fine-wires, providing a practical tool for both the clinical and research context. ATL in the present study was defined as the distance between the most distal part of insertion on the calcaneus and the MTJ of medial gastrocnemius muscle, as defined in several studies in both healthy [135] and pathological tendons [2;144;150]. The results indicate that by the use of a consistent protocol and well defined anatomical landmarks, excellent inter-rater reliability can be achieved, independent of investigators experience when measuring the ATL in healthy subjects. Thus, this is the first study to report inter-rater reliability of an US method assessing ATL.

Previous studies assessing ATL have shown excellent day-to-day and intra-rater reliability of methods used. A study by Fouré et al. investigated the intra-rater day-to-day reliability of a method assessing ATL defined as the distance between the most distal identifiable portion of the medial
gastrocnemius muscle and AT insertion on the calcaneus determined by US and measured by a ruler, reporting an ICC of 0.95, coefficient of variation 1.9% and a standard error of 4 mm [50]. Silbernagel et al. reported similar values (ICC: 0.97, SEM: 4.0 mm) of a method assessing ATL by a combination of US and motion analysis in a test-retest design study evaluated on 2 different days within one week by the same investigator [150]. A recent study by Ryan et al. investigated the test-retest reliability and the minimal detectable change for ATL by panoramic US assessment and reported excellent reliability (ICC: 0.95, SEM: 4.34 mm) suggesting that panoramic ultrasound imagining is a reliable technique for the detection of clinical relevant changes of ATL [135].

The findings of the above cited literature indicate that independent of the method used excellent intra-rater reliability can be achieved. However, as the above studies were only performed by one investigator, factors such as the investigator experience and preference may have influence the outcome of reliability measures.

In the present study investigators of different levels of experience were involved. The measurements were performed by an experienced investigator with more than 5 years of experience in US assessment both in image acquisition and image analysis and by an inexperienced investigator with only focused training of several weeks. The findings indicate that the method used preserves not only reliable results between investigators but also its independence from investigators experience. The use of clinical reliable tools, independent of observer experience, is thereby necessary to ensure accurate diagnosis of a condition and to evaluate clinical outcomes in order to formulate effective treatments. However, as in the present study only healthy subjects were included the applicability of the method in the clinical context is unclear and needs to be examined.

Although this study presents a reliable method, its limitations should not be ignored. During the measurement procedure both the ankle as well as the knee was positioned manually by the investigators, a correction method (e.g. knee orthosis or goniometer) to ensure their exact position has not been used. This could be a factor that had might influence the reliability of measuring ATL,
as different range of motion results in different tendon lengths. Moreover, only the inter-rater reliability was investigated. However for a comprehensive state of reliability, intra-rater has to be evaluated as well, especially in the inexperienced examiner.

4.2.6. Conclusion

The study presents an alternative method by the use of metal fine-wires in the assessment of ATL in healthy subjects, achieving even between investigators with different level of experience, excellent reliability. The present method can provide a reliable assessment of ATL in a simple and time-efficient manner; however its applicability in clinical context is unclear and should be discussed critically.

4.2.7. Transferability to study 3

In the previous studies of the thesis it was shown that the methods used to assess the tendon structural properties are reliable. Findings of these studies enable the implementation of the methodology used into the further investigations of tendon properties. Thereby, the described methodology used in the previous studies (1&2) was transferred in study 3 in order to reliably investigate the structural properties of AT among children, asymptomatic adults and patients with tendinopathy.
4.3. Study

Influence of Age and Pathology on Achilles Tendon Properties during a single-leg jump

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Original Article

Running title: Tendon Properties during one leg jump

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Reference

4.3.1. Abstract

Prevalence of Achilles tendinopathy increases with age leading to a weaker tendon with predisposition to rupture. Conclusive evidence of the influence of age and pathology on Achilles tendon (AT) properties remains limited, as previous studies are based on standardized isometric conditions. The study investigates the influence of age and pathology on AT properties during single-leg vertical jump (SLVJ). Ten children (C), ten asymptomatic adults (A), and ten tendinopathic patients (T) were included. AT elongation [mm] from rest to maximal displacement during a SLVJ on a force-plate was sonographically assessed. AT compliance [mm/N]) and strain [%] was calculated by dividing elongation by peak ground reaction force [N] and length respectively. One-way ANOVA followed by Bonferroni post-hoc correction (α=0.05) were used to compare C with A and A with T. AT elongation (p=0.004), compliance (p=0.001), and strain were found to be statistically significant higher in C (27±3mm, 0.026±0.006[mm/N], 13±2%) compared to A (21±4mm, 0.017±0.005[mm/N], 10±2%). No statistically significant differences (p≥0.05) was found between A and T (25±5mm, 0.019±0.004[mm/N], 12±3%). During SLVJ, tendon responded differently in regards to age and pathology with children having the most compliant AT. Higher compliance found in healthy tendons might be considered as a protective factor against load-related injuries.

Key words: children, tendinopathy, compliance, dynamic, ultrasonography
4.3.2. INTRODUCTION

Degenerative Achilles tendon (AT) disorders commonly described by the term tendinopathy, cause considerable morbidity and functional impairment among athletic and general population [6;122]. The etiology is considered to be multifactorial [7;9;151] and its pathogenesis not fully understood [7;10]. Typically this overuse injury is more prevalent in men in the 4th-5th decade of life, and its occurrence is highest among individuals who participate in sports involving running and jumping [6;18-23]. In adult runners, a point prevalence of up to 36% has been reported for Achilles tendinopathy [24] whereas in children at an average of 13 years, only 1.8% [25].

Excessive loading of tendons during vigorous physical training is regarded as the main pathological stimulus for tendon degeneration [112]. In vivo experiments have measured peak forces for AT ranging from 3kN-9kN exceeding 12 times the body weight [1]. Ultrasonography (US) and magnetic resonance imaging (MRI) studies have recently reported strains of the AT ranging from 5% - 10% during isometric contractions at levels below the maximum force during dynamic movements [15;16]. It was thought that such high strain would cause tendon rupture and failure [13;152]. However, activities such as one-leg hopping can induce much larger strains on the AT without inducing injuries [153].

Structural changes accompanying tendinopathy includes a larger cross-sectional area (CSA) with histological evidence of increased ground substance, hypercellularity, and collagen fiber disruption [2;154]. This degenerative process might also affect the tendon’s mechanical and material properties. Previous studies, which have investigating the AT properties in presence of tendinopathy, found that degeneration leads to a weaker tendon, resulting in higher tendon strains with lower forces and stiffness [2;12]. However, those results were based on standardized isometric conditions and due to methodological complications; it remains unclear whether tendinopathy results in a weaker tendon.
Age-related changes in AT properties are thought to contribute towards the development of such tendon ailments. Previous studies investigating the age-related changes of tendon properties under isometric conditions, found that during childhood, the stiffness of weight-bearing tendons increases with age from 9 to adulthood [26;27]. Age-related increases in body mass and force production capabilities have been postulated to contribute to these observed increases in tendon stiffness [26]. It was reported that children demonstrate more compliant tendinous structure compared to adults, as their tendon elongation and strain was significantly greater at a given force at vastus lateralis [27] and patellar tendon [26]. It was thereby assumed that higher compliance found children’s tendon might play a protective role against load-related injuries. However, this hypothesis has not been empirically tested under functional tasks [27]. Conversely, a study by Waugh et al., found no age-related differences in AT strain under isometric contractions between children and adults [28].

Generally, knowledge regarding the influence of growth as well as pathology in AT properties is still limited, as outcomes of previous studies are based on standardized isometric conditions [2;12;26-28]. Thus, it is still unclear how or if those factors can influence the dynamic response of the tendon properties during functional tasks. Therefore, the aim of the present study was to investigate the influences of age and pathology on AT properties during a single-leg vertical jump among children, asymptomatic adults, and tendinopathic patients. An enhanced understanding of the tendon response during functional tasks will enable further investigations into injury mechanism, prevention, and treatment strategies of tendinopathies.

4.3.3 MATERIALS & METHODS

Participants

Ten children (5 boys & 5 girls), ten asymptomatic adults (7 males & 3 females), and ten patients with Achilles tendinopathy (7 males & 3 females) volunteered to participate in the present study (Table 1). Sample size was determined via power analysis [155] done on preliminary data. An effect size $f$ of
1.37 was calculated for tendon elongation based on an α-level of 0.05 and power values of 0.80 requiring a minimum number of nine participants. Participants were recreationally active in different sports with an average time per week of 3 ± 2 hours in children and 4 ± 2 hours in both asymptomatic adults and patients with tendinopathy. Children and asymptomatic adults were excluded if they reported chronic musculoskeletal injury or pain of the lower limb and/or signs of tendinopathy on US imaging [116]. For the tendinopathic group, the following criteria was used to determine their inclusion into the present study: history of recurrent episodes of AT pain lasting more than 6 consecutive weeks, pain on palpation at the AT midportion, palplable focal thickening at the mid-portion, and sonographic evidence of tendinopathy e.g. focal thickening and hypoechocity (Fig. 16) [116]. Patients were excluded if they reported: history of previous surgery involving AT, systemic and/or rheumatic disease and current active stage of tendinopathy restricting them from functional activities.

**Study design**

A cross-sectional design was used in the present study. A standardized clinical examination including US was performed by a sports orthopaedic physician. Tendinopathic patients had to complete the Victorian Institute of Sports Assessment-Achilles questionnaire (VISA-A) [156]. Asymptomatic adults and tendinopathic patients were matched accordingly to gender, age, weight, and site of injury. In the children group, an equal number of both genders [26] of the same age were included and their dominant limb was measured according to a previous study [53]. The study was approved by the local ethics committee of the University. The subjects and/or their parents signed the written informed consent prior to the examination.

**Tendon Structural Properties during rest**

A diagnostic US device (Vivid q; GE Healthcare, Tirat Carmel, Israel) with a 7.5 MHz continuous linear ultrasound array (4 -13 MHz) was used. Presets were standardized at a frequency of 13 MHz and a depth of 3 cm. AT length was measured as the distance between the most distal part of AT insertion
to the calcaneus and the medial gastrocnemius myotendinous junction (MTJ) by use of a measuring tape. Structures were identified by use of US and metal fine wires and marked accurately on the skin as described in previous studies [157;158]. AT CSA was measured at 4 cm from most distal attachment on the calcaneus defined by use of a measuring tape [157]. The area of 4 cm falls well within the level of malleolus and has been previously reported to be one of the most reliable site to measure [157]. Tendon thickness was measured at its maximal (antero to posterior) thickness in the sagittal plane identified by visual inspection [159].

![Figure 16: Longitudinal and transverse grey-scale ultrasound images of tendinopathic (A and B) and asymptomatic (C and D) Achilles tendon. Black dotted lines outline the tendons. White curved line denotes the calcaneus bone.](image)

### Tendon Structural Properties during a single-leg vertical jump

A standardized warm up was chosen to ensure similar loads prior to the test based on a previous study assessing AT properties under isometric conditions [2]. Participants were positioned prone on the isokinetic dynamometer (Contrex MJ, Physiomed, Germany) with the hip and knee extended and ankle at 90° of flexion. Three sub-maximal and two maximal isometric plantar flexion contractions of 5 s with 1-min rest in between were performed.

After the warm up, a high resolution US device (Xario SSA-660A; Toshiba, Tokyo, Japan) with 8 MHz (PLT-805AT, 56mm at 12 MHz) continuous linear US array (6.2 – 12.0 MHz) was used to measure the...
tendon elongation during the jumps as the displacement of the medial gastrocnemius myotendinous junction (MTJ). A thin strip of echo absorptive tape was placed on the skin visible in the US image providing a reference to detect any probe movement. The US transducer was placed in a custom-made holder made by orthotic specialists (CCtec, Germany) perpendicularly to the skin surface above the MTJ fixed by use of Velcro straps (Fig. 17).

Participants were initiated to perform a single-leg vertical jump on an AMTI force plate (1000 Hz). The instructions were to jump as high as possible with hands on their waist and perform each repetition from the initial standing position in a non-consecutive manner. After a familiarization trial, participants performed three repetitions while simultaneously the vertical ground reaction force and the maximal tendon elongation were recorded.

Figure 17: Probe placement perpendicular to the skin surface above the Medial gastrocnemius myotendinous junction, fixed in a position using a custom made holder (Cctec, Germany).
Data analysis

AT CSA and thickness: The US images of AT CSA and thickness were stored digitally as DICOM files and processed on a PC using Image J. The freehand selection tool was used to outline the tendon and measure the CSA. The straight selection tool was used to measure the maximal antero-posterior diameter of the tendon. AT elongation: Likewise, the ultrasound video-clips were stored digitally as DICOM files, and processed by use of Image J. The Jumps were divided into five phases: the initial position, the propulsion, the jump, the landing, and back to initial position (Fig. 3). The phases were defined by the movement and subsequently by the direction of the AT elongation. Due to the anatomical position and biarticular function of the AT, its elongation is also influenced by the position of the knee joint. In order to minimize the effect of the knee joint, the phases of propulsion and landing during the jumps were excluded from the analysis, as this would have led to an overestimation of the elongation measured. AT elongation was thereby measured by manually tracking the maximum MTJ displacement from initial position until the maximum displacement at the peak of the jumping phase before landing (Fig. 18).

Peak Vertical ground reaction force (vGRF): The peak vGRF during the jumps was calculated by Matlab software. The peak vGRF was defined by the vertical component (Fz) as the maximum peak value 100ms before the flight phase. Flight phase was defined when values reached below 30N.

AT strain: To describe the changes in elongation in regards to the length of the tendon, tendon strain was calculated by the following equation:

\[
AT \text{ strain} = \frac{AT \text{ elongation [mm]}}{AT \text{ length [mm]}} \times 100
\]

AT compliance: In order to describe the change in length in relation to the force applied to the tendon an equation was plotted by dividing the measured tendon elongation by the force measured:

\[
AT \text{ compliance} = \frac{AT \text{ elongation [mm]}}{force[N]}
\]
All statistical calculations were performed using SPSS (SPSS Statistics- 22, IBM, USA). Data were initially analysed descriptively (mean ± SD). Regarding age, results were compared between children and asymptomatic adults, and concerning pathology between asymptomatic adults and patients with tendinopathy. Variables were compared between groups using one-way ANOVA, followed by Bonferroni post-hoc correction test ($\alpha=0.05$).
4.3.4. RESULTS

The average values (mean ± SD) for anthropometric characteristics, variables measured, and statistical test used (p<0.05), are given in tables 4 and 5. Regarding the influence of age on AT properties no statistically significant differences were found for ATL, CSA, tendon thickness, as well as for force (p>0.05). AT elongation (p=0.004) compliance (p=0.001) and strain (p=0.016) was found to be statistically significant higher in children compared to adults. Patients with tendinopathy demonstrated statistically significant higher CSA (p=0.021) and tendon thickness (p=0.000) compared to asymptomatic adults. No statistically significant differences were found for ATL, force and strain (p>0.05). AT elongation (p=0.055) and compliance (p=0.161) were found to be higher in patients with tendinopathy compared to asymptomatic adults, but without statistically significance.

Patients with tendinopathy reached a VISA-A score of 87 ± 7% with a range between 77-97%.

Table 4: Anthropometric and tendon characteristics of the groups with analysis of variance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children (C)</th>
<th>Asymptomatic Adults (A)</th>
<th>Tendinopathic (T)</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [yrs]</td>
<td>13 ± 1</td>
<td>37 ± 8</td>
<td>40 ± 7</td>
<td>.000* A .763</td>
</tr>
<tr>
<td>Height [cm]</td>
<td>166 ± 9</td>
<td>178 ± 7</td>
<td>175 ± 9</td>
<td>.005* A .963</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>58 ± 9</td>
<td>79 ± 10</td>
<td>77 ± 9</td>
<td>.000* A .000*</td>
</tr>
<tr>
<td>Thickness [mm]</td>
<td>4.6 ± 0.9</td>
<td>4.9 ± 0.5</td>
<td>6.9 ± 1.4</td>
<td>1.00 A .021*</td>
</tr>
<tr>
<td>CSA [mm²]</td>
<td>59 ± 18</td>
<td>58 ± 13</td>
<td>83 ± 24</td>
<td>1.00 A .000*</td>
</tr>
<tr>
<td>Length [mm]</td>
<td>219 ± 17</td>
<td>217 ± 24</td>
<td>220 ± 24</td>
<td>1.00 A 1.00</td>
</tr>
</tbody>
</table>

Values are means ± SD and depict group average of data. *Significant group differences (P≤0.05).

Table 5: AT properties and force during one leg jump of the groups with analysis of variance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children (C)</th>
<th>Asymptomatic Adults (A)</th>
<th>Tendinopathic (T)</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation [mm]</td>
<td>27 ± 3</td>
<td>21 ± 4</td>
<td>25 ± 5</td>
<td>.004* A .055</td>
</tr>
<tr>
<td>Jump Peak Force [N]</td>
<td>1068 ± 208</td>
<td>1287 ± 225</td>
<td>1389 ± 288</td>
<td>.161 A 1.00</td>
</tr>
<tr>
<td>Strain [%]</td>
<td>13 ± 2</td>
<td>10 ± 2</td>
<td>12 ± 3</td>
<td>.016* A 1.00</td>
</tr>
<tr>
<td>Compliance [mm/N]</td>
<td>0.026 ± 0.006</td>
<td>0.017 ± 0.005</td>
<td>0.019 ± 0.004</td>
<td>.001* A .106</td>
</tr>
</tbody>
</table>

Values are means ± SD and depict group average of data. *Significant group differences (P≤0.05).
4.3.5. DISCUSSION

The present study aimed to investigate the influence of age and pathology on AT properties under a single-leg vertical jump between children, asymptomatic adults, and patients with Achilles tendinopathy. The results indicate that age influences the AT response under dynamic task, with children having the most compliant tendinous structure. On the other hand, patients with tendinopathy demonstrated a tendency of altered tendon response; however, no statistically significant differences were found compared to asymptomatic adults.

In the present study children demonstrated a significant higher tendon elongation with a relative lower force indicating a more compliant AT compared to asymptomatic adults. Conversely, a previous study by Waugh et al., found that children demonstrated lower AT elongation with lower levels of force compared to adults during isometric contractions, indicating no age-related differences in compliance [28]. Conversely, previous studies investigating the patellar [26] and vastus lateralis tendon [27] during isometric conditions reported higher compliance in children’s tendons as their elongation and strain were higher under a given force. Thus, findings of the present study support the notion [27] that compliance found in children tendons might indeed play a protective role against load related injuries. Moreover, it is worthwhile to note that despite anthropometrical differences between children and asymptomatic adults, tendon dimensions measured during rest, did not differ. Typically, a thicker tendon is considered to be stiffer due to its ability to withstand higher forces with less elongation [2;60]. Thus, findings of the present study suggest that compliance between those two groups is independent by its tendon dimensions.

Despite tendon alterations found in tendinopathic tendons e.g. larger CSA and thickness, the tendon response was not significantly altered compared to asymptomatic adults. Tendinopathic patients demonstrated a high tendon elongation but also achieved the highest force. This could be partly attributed that during jumps no patient reported any increase of pain; moreover the severity reported based on VISA-A questionnaire differed between patients with some reporting less severe
symptoms than others. In calculation of AT compliance no significant differences were visible between the groups. Thus, this finding implies that during functional task, when higher forces are acting on the AT, tendinopathy does not result in a weaker tendon. Previous studies investigating AT properties during isometric conditions have found that tendinopathy weakness the tendon as higher elongation and strain with lower forces were observed [2;12]. In understanding of tendon response to load, one must also take into account its viscoelastic behaviour and more specifically the sensitivity to different strain rates [14]. During low strain rates the elongation of the tendon is higher indicating a more compliant tendon, whereas, during higher strain rates, the tendon elongates less indicating a stiffer tendon. Compared to the 5 s. ramped isometric contractions used in these studies [2;12], jumping is considered a very high strain activity which could partly explain the no differences found.

The strain levels measured in the present study reached a significant level of 13% in children, 10% in asymptomatic adults, and 12% in the tendinopathic patients. Classic values based on cadaver animal and human tendons, which strained the tendon until it ruptures, reported that high strain levels cause’s tendon injury (4%-8%) and rupture (12%) [14;152]. However, during dynamic movements with the use of the present method, the tendon can strained at levels more than 12%, indicating that during physiological loads the border to pathological loading is still unclear. This fact along with the high prevalence of AT injuries highlights the need for further investigation into the dynamic response of tendons.

The present methodology uses the ground reaction force upon propulsion as the force acting on the structures of the lower leg influencing the response of AT. Actual estimates of tendon force can only be obtained with computational models based on inverse dynamics which makes the applicability in clinical context difficult and often not possible [79]. Previous studies have also used invasive methods where a force transducer is placed on the tendon after a surgery [1;78]. Despite the simplification of the present methodology, it provides important information of the tendon response
in regards to forces under physiological conditions. The need to establish a simple and clinical applicable method in both the research and clinical field is high, as the understanding of tendon functional behaviour is still limited.

The findings of this study provide important information of tendon response during a single-leg vertical jump, taking into account the influence of age and pathology. In the present study, children demonstrated the most compliant AT, supporting the notion that the higher compliance found in young tendons might indeed play a protective role against load-related injuries. Furthermore, the influence of pathology on tendon properties was not significant, suggesting that during functional task, tendinopathy does not result in a weaker tendon. Results of this study, provide an important first step towards gaining a better understanding of the AT functional behaviour enabling further investigation into injury mechanism, prevention, and treatment strategies of tendinopathies.
5. General Discussion

Previous studies, investigating tendon properties are based in in-vitro models and standardized isometric conditions, limiting the understanding of tendon response during functional task. Moreover, due to the lack of measurement standardization and operator dependency in ultrasound assessment it is unclear whether AT properties can be measured reliably. Therefore, one test–retest and 2 cross-sectional studies were conducted to initially evaluate the influence of repeated and between investigators ultrasound measurements, and last to investigate the influence of pathology and age on AT response during a single-leg vertical jump between children, asymptomatic adults and patients with Achilles tendinopathy.

5.1. Reliability of ultrasonographic measurements of Achilles tendon properties

In view of the drawbacks ultrasonography has, the present thesis addressed the reliability of US assessment in tendon properties. The reliability was evaluated with the contribution of a single investigator during repeated measurements; as well as between investigators of different level of experience during single measurements. In general, the findings of this thesis revealed that by a simple and yet standardized US protocol good to excellent reliability can be achieved.

Previous studies investigating the reliability of US measurements on tendon properties are limited on small pre-pilot measurements which consisted of a few subjects (N=5-7). Moreover, due to the lack of measurement standardization, it remains unclear in the literature whether CSA and length can be measured reliably, partly due to the challenging assessment of these properties. AT being the longest tendon in the human body makes its assessment by US challenging as the entire length cannot be visualized by the probe. Methods found in literature trying to visualized the two ends of AT and then measured the distance in between to defined its length.
A study by Foure et al. investigated the intra-rater day-to-day reliability of a method assessing AT length defined as the distance between the most distal identifiable portion of the medial gastrocnemius muscle and AT insertion on the calcaneus determined by US and measured by a ruler, reporting an ICC of 0.95, coefficient of variation 1.9% and a standard error of 4 mm [50]. Silbernagel et al. reported similar values (ICC: 0.97, SEM: 4.0 mm) of a method assessing ATL by a combination of US and motion analysis in a test-retest design study evaluated on 2 different days within one week by the same investigator [21]. However, the absence of standardized US protocols and the limited field of view of most US transducers restricted the wide implementation of US for this use in clinical applications. A recent study by Ryan et al. investigated the test-retest reliability and the minimal detectable change for AT length by panoramic US assessment and reported excellent reliability (ICC: 0.95, SEM: 4.34 mm) suggesting that panoramic US imagining is a reliable technique for the detection of clinical relevant changes of AT length [20]. Despite the fact of recent more sophisticated, reliable and clinically applicable methods such as panoramic US its availability in most research and clinical centers is still limited. As indicated in study 1 and 2, the assessment of AT length by the use of metal fine-wires in a simple and time-efficient manner provided excellent reliability both intra- as well as inter-rater with low levels of variability and SEM highlighting its applicability in both the clinical and research context.

Compared to the assessment of AT length, assessment of AT CSA presents a different challenge, as its dimensions are not the same along its length [51] and thus, it remains unclear which distance point should be assessed. Previous studies assessing CSA used the level of malleolus as a reference site to measure, reporting good to excellent reliability [50;52;53]. However, a recent study by Arya et al. measured the AT CSA at three distance points at 2 cm, 4 cm and 6 cm proximal the distal attachment and calculated the average [2]. In study 1, AT CSA was measured at 2 cm, 4 cm and 6 cm proximal the distal attachment. The average of the three points as well the single values at each point was calculated. The results of the present thesis presented mixed results as different distance...
sites along the length of the tendon presented different results with the region between 4 and 6 cm being the most reliable whereas at 2 cm the least reliable. This finding is attributed to the fact that 2 cm is located at the calcaneus bone which leads to a less clear image quality. Additionally, in that region the tendon is wider which makes borders of the CSA difficult to identify with the used methodology. This discrepancy is also represented by the high random error given by the limits of agreement and SEM at 2 cm compared to the location at 4 and 6 cm. Thus, these findings indicate that averaging different points along the length of the tendon is not beneficial compared to single sites.

The findings of study 1 and the above cited literature indicates that excellent intra-rater reliability can be achieved in the assessment of AT properties. However, as the above studies were only performed by one investigator, factors such as the investigator experience and preference may have influenced the outcome of reliability measures. Thus, for a comprehensive statement about reliability, inter-rater must also be evaluated. To address this issue and to evaluate the influence of different level of experience between investigators in the US assessment of AT, a second reliability study was conducted to evaluate the methodology used for measuring AT length. The results of study 2 indicate that the methodology used for the assessment of AT length is reliable between investigators independent of experience. Within the context of inter-rater reliability the methodology of assessing also AT-CSA at 6cm (ICC: 0.83, IRV: 8 ± 4 % Bias ± LoA: 3 ± 7 mm²) and AT elongation (ICC: 0.99, IRV: 3.18) were evaluated in other pilot studies reporting excellent reliability between investigators [160;161]. The use of clinical reliable tools, independent of observer experience is necessary to ensure accurate diagnosis of a condition and to evaluate clinical outcomes in order to formulate effective treatments.

5.2. Influence of age on Achilles tendon properties

Generally, knowledge regarding the influence of age on tendon properties under functional task is still limited. Growth changes found in tendon properties are thought to contribute towards the
development of tendinopathies as their prevalence increases with age; however this association is still unclear. In study 3 of this thesis the influence of age on AT properties was evaluated during functional activities such as single-leg vertical jump. The results of study 3 indicate that age influences the AT response under single-leg vertical jump, with children having the most compliant tendinous structure. The higher compliance found in children compared to adults was attributed to a relative lower force and a significant higher tendon elongation. Conversely asymptomatic adults demonstrated higher force and a significant lower tendon elongation resulting in a less compliant tendon.

Previous studies investigating the influence of age on tendon properties during isometric conditions have demonstrate inconsistent results as different tendon sites were assessed [26-28]. Waugh et al. investigated the age-related properties of AT under isometric conditions and found that children had lower tendon elongation with lower levels of force, indicating no differences in tendon compliance compared to adults [28]. On the other hand, O’Brien et al. [26] investigated whether there are differences in the mechanical and material properties of the patellar tendon in vivo between adults and pre-pubertal children in both sexes during isometric knee extension. It was found that adults have significantly stiffer tendons compared to children with no difference regarding gender in both the adult and children group. In the same line Kubo et al. [27] investigated the growth changes in the Vastus lateralis tendon in younger boys, compared to older boys, and young adult men during isometric extension of the knee joint. They found that tendons are more compliant in younger boys than in adults as their strain at a given force was significantly greater [27].

As prevalence of Achilles tendinopathy is low in children compared to adults, higher compliance found in healthy young tendons, as indicated in study 3, might play a protective role against load related injuries. This finding also agrees with the study by Kubo et al. suggesting that higher tendon compliance found in children might be an important factor in reducing the risk of sport injuries [27]. On the other hand Waugh et al. argued that the high prevalence of tendon related injuries in adults
might be as a result of imbalance between strength gains and those of tendon hypertrophy (≈310% vs. ≈93%) [28]. Whereas O’Brien et al. discussed, that the lower prevalence found in children might be a result of less exposure to exercise-related microdamage accumulation over time [26].

A worthwhile to note finding from study 3, is that despite height and weight differences between children and asymptomatic adults, tendon dimensions e.g. length, tendon thickness and CSA measured during rest, did not differ. This finding contradicts with previous studies reporting that children have smaller structures than adults [26-28]. This, however, could be partly attributed to methodological issues, site measured [27], as well as the age of participants, and training status. Typically, it is considered that a thicker tendon is also a less compliant due to its ability to withstand higher forces across the tendon with less elongation. Thus, this finding indicates that age related compliance found in study 3 is independent by the tendon dimensions. These results however, yields further investigation into the microstructure of the tendon, as other factors e.g. increased fibril diameter [97] and packing [99], increased collagen cross-linking [100] and a reduced collagen crimping [101], might had play a role into the decreased compliance found in asymptomatic adults compared to children.

5.3. Influence of pathology on Achilles tendon properties

Despite immense knowledge provided in literature regarding tendinopathy, its influence on AT properties under functional task is still limited. In study 3 structural comparisons between tendinopathic and asymptomatic tendons demonstrated a significant larger CSA and thickness as a consequence of the pathology. However, despite the structural changes found in tendinopathic patients, tendon response during the single-leg vertical jump was not significantly altered. Tendinopathic patients demonstrated a high tendon elongation but also achieved the highest force. As a consequence, no significant difference was found in tendon compliance between tendinopathic and asymptomatic tendons.
In contrast to the findings of study 3, previous studies under isometric conditions found an influence of pathology on tendon response. A study by Arya and Kulig investigated the in vivo mechanical and material properties of AT in the presence of tendinopathy [2]. It was found that Achilles tendinopathy weakens the properties of the AT due to the degeneration process, leading to a higher elongation and strain with lower forces [2]. In the same line Child et al. compared the AT strain and found that tendinopathic tendons had higher strain compared to healthy tendons with no differences in maximal isometric plantar flexion force [12].

In understanding AT response, one must take into account its viscoelastic behaviour and the specific strain rate sensitivity to loading. Lower strain rates leads to higher tendon elongation which could partly explain the findings of the previous studies as ramped isometric conditions of 5 sec. is considered a low strain activity compared to the single-leg jump used in study 3. Thus, it can be argued based on the results of study 3, that tendinopathy under functional task, does not produce a weaker tendon. However, the higher elongation found in tendinopathic tendons should be discussed critical. In literature there is no defined threshold of tendon elongation which could define a weak tendon and thus further investigation for the definition of a weaker tendon is needed.

Generally, it is still unclear whether degeneration and structural changes found in tendinopathic tendons leads to a weaker tendon. The tendon changes found in tendinopathic tendons have been defined as irregular fibre structure and arrangement with vascular ingrowth. These changes however, are not necessarily degenerative in character as it has never been shown that these changes gradually progress to an end state demonstrating a structurally disorganised and weak tendon. In contrast, several studies have shown remarkable results in treatment of Achilles and patellar tendinopathy with eccentric and heavy resistance training achieving a significant reduction in tendon thickness, neovascularization and a more normal structure after treatment [162-165], indicating a reversible and not a degenerative state. Thus, based on these findings the term degeneration in tendinopathy might need to be reconsidered.
5.4. Achilles Tendon assessment under single-leg vertical jump

It was previously thought that high strain levels (4%-8%) caused microruptures on the tendon, which by time leads to degeneration and subsequently to tendon rupture when strain level reach 12%. This mechanical theory was based on isolated animal and human cadaver tendons and as proven from the results of the present thesis, those values do not reflect how a tendon actually behaves under physiological loads. Thus, in order to prevent AT injuries it is necessary to understand its pathogenesis and more importantly its influencing factors, such as age which is considered one of the main influences. Although load is a major risk factor, it is almost certainly modulated by the age-related changes found in tendons.

In study 3 of the thesis the strain level measured was beyond of what was ones considered a strain level for tendon overuse injuries and rupture. Strain levels reached a range from 10-16 % in children, 6-13% in asymptomatic adults and 8-15 % for patients with tendinopathy without rupturing the tendon. This age-related differences found in tendon strain might explain the high prevalence of injuries in adults. As the stiffness of tendon increases with age, the inability of tendons to sufficiently stretch under force might be responsible for causing micro-ruptures which by time might lead to injury. On the other hand, the higher strain found in tendinopathic tendons compared to asymptomatic adults might play a protective mechanism towards further injury. However, this hypothesis needs to be further investigated.

Regarding the methodology presented in study 3, single-leg jump was chosen specifically as it is a functional activity were higher forces are acting on the AT. In literature limited information exist in the functional assessment of tendon properties, mainly due to the complexity of these measures [79]. Actual estimation of tendon force requires inverse dynamics analysis based on computational models with further calculations of moment arms [79]. Other studies have used invasive methods
where a force transducer is placed on the tendon after a surgery [1;78]. The applicability of these methodologies in the clinical context is difficult and often not possible [79]. In an attempt to develop a simple methodology which could be applicable also in the clinical context, the method applied in study 3 uses the vertical ground reaction force as the force acting on the structures of the lower leg influencing the response of AT. Despite the simplification of the present methodology, it provides important information of the tendon response in regards to forces under physiological conditions. While computational models have provided insight into musculoskeletal function, the outcome is model-based estimation and subject to deviation from true values. Moreover, in literature there is a variety of calculations methods for tendon stiffness resulting in a large range of values for specific populations exist [61]. Hence, these methodological and computation differences make comparisons between studies difficult and often inaccurate [61]. In order to avoid those methodological issues, in study 3 no model based computation or calculation methods were used. AT behaviour was evaluated by its absolute changes in force and length were upon compliance was determined.
6. Practical Relevance

The studies conducted in the present thesis were designed to initially evaluate the reliability of US measurements on tendon properties and second to investigate the influence of age and pathology on AT response during a single-leg vertical jump. The results revealed that by a standardized and simple US protocol good to excellent reliability could be achieved both in longitudinal measurements assessed by a single investigator as well as in cross-sectional measurements between investigators irrespective of experience. Given that ultrasonography is often perceived as an imperfect and operator-dependent tool, the findings of this thesis provide reliable methods for the assessment of tendon properties independent of operator experience. These findings are of clinical and research importance as during follow-up examinations, patients are often imaged by several investigators with different levels of experience. Consistency of an US protocol is thereby necessary to ensure accurate diagnosis of a condition and to evaluate clinical outcomes in order to formulate effective treatments.

The results also revealed that during a single-leg vertical jump tendon responded differently in regards to age and pathology. Children had the most compliant tendinous structure compared to the asymptomatic adults. On the other hand, pathology during the jumps did not result in significant alterations of the tendon response compared to the asymptomatic tendons. The results of this thesis provide important insights regarding the dynamic function of the tendon as well as the influence that pathology and age can have on tendon properties. Moreover, the present thesis describes a simple methodology for the assessment of tendon properties under dynamic conditions which can be applied for the assessment of AT both in the clinical and research context.

As prevalence of tendinopathy increases with age [25], the current findings imply that higher compliance found in children’s tendon might be considered as a protective factor against load-
related injuries. Coaches and trainers are thereby encouraged to maintain the compliance in young athlete’s tendons from early ages as this could hold the key of reducing load-related injuries in adulthood. It was believed that tendinopathy results in weaker tendon with high predisposition to further injury. However, the results of this thesis revealed that under dynamic conditions where higher forces are acting on the tendon no significant alteration in tendon response was found compared to the asymptomatic tendons. These findings have important implications for both the practitioners and physiotherapists in order to formulate more effective and efficient treatments for tendinopathy based on the functional response of the tendon.

Lastly, regarding the practical implication and recommendations, the present thesis further designates that the classical values of strain frequently reported in literature which causes tendon to rupture have been underestimated. Those classical values were based on cadaver and isometric conditions leading to unprecise mechanism of AT overuse injuries stating that the level of strain between 8% -12% to causes rupture of the tendon [13;152]. The present thesis revealed that AT can be strained of as much as 16% during dynamic task without the danger of rupture. Hence, these findings provide new insights into the functional dynamics of the AT and yields further investigation into the mechanism of tendon overuse injuries.
In the present thesis, 1 test-retest study and 2 cross-sectional studies were conducted to evaluate the reliability of ultrasonographic assessment of tendon properties and to investigate the influence of age and pathology on tendon response during a single-leg vertical jump. Regarding the reliability studies, only asymptomatic young participants were included. Thus, it seems reasonable to question how reliability of ultrasonographic assessment on tendon properties in the presence of tendinopathy can change. Thereby, in order to substantiate the results found in study 1 and 2 more research in the reliability of tendon properties in the presence of pathology is needed.

Furthermore, in study 3, the severity of tendinopathy between patients was different on VISA-A questionnaire; despite the presence of pain and tendon alteration on US imaging. This point along with the fact that patients reported no increase of pain during the jumps could be a one possible reason that no differences were found when compared to asymptomatic tendons. This finding yields further consideration into the inclusion criteria of patients with tendinopathy e.g. by including only patients with acute/active symptoms of Achilles tendinopathy or a defined cut-off threshold based on the severity of VISA-A questionnaire.

Regarding the influence of age on tendon properties, it is still unclear why children demonstrated higher compliance compared to asymptomatic adults as their tendon dimensions did not differ. A possible explanation could be differences in the microstructure of the tendon e.g. increased fibril diameter [96-98] and packing [99], increased collagen cross-linking [100] and a reduced collagen crimping [101]. Hence, more research is needed in order to understand the influence of tendon microstructure to its tendon response. To substantiate the results of study 3, future longitudinal studies should focus to investigate the relationship between lower compliance found in healthy tendons and prevalence of tendinopathy.
One last aspect of this thesis which needs to be considered is the number of participants included in these studies. Regarding the reliability studies, a controversy surrounds the relationship between power and reliability [166] e.g. good statistical power can exist with poor reliability and a change in variance unrelated to reliability can change power. Taking this into account it was decided to focus on reliability and therefore did not perform a statistical power analysis for study 1 and 2. On the other hand, in study 3, a power analysis was performed based on preliminary data of tendon elongation. Tendon elongation was chosen as it’s the main variable to describe tendon behaviour and further for the calculation of tendon strain and compliance.
8. Reference List


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Authors’ contribution

The present thesis is designed as a cumulative dissertation. In this regard, three scientific articles have been prepared, submitted to peer-reviewed journals, and accepted for publication. According to the local doctoral degree regulations (§ 7 (4), sentence No. 2), significant contributions to the articles from the respective co-authors were acknowledged and finally confirmed by each co-author:

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### Authors' Contribution

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*Note: First author is highlighted in bold and underlined*