UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

PhD Thesis

Rikke Beyer

Achilles tendinopathy. The effect of loading on clinical outcome and intratendinous sliding at the fascicle level.

Academic Advisors: Peter Magnusson and Michael Kjær

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PREFACE AND ACKNOWLEDGEMENTS

This PhD thesis is based on work performed at the Institute of Sports Medicine (ISMC) and the Department of Physical & Occupational Therapy at Bispebjerg & Frederiksberg Hospitals and the Faculty of Health Sciences, University of Copenhagen from 2011 to 2018.

This work would have been impossible to complete without the help of some remarkable people, who supported me and made this thesis possible.

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Most importantly and last, I wish to thank my family for their support, understanding and faith in me and to *Sebastian* for helping me to retain focus on other things in life.

LIST OF PAPERS

This thesis is based on the following 2 papers:

R. Beyer, M. Kongsgaard, B.H. Kjær, T. Øhlenschlæger, M. Kjær, S.P. Magnusson
Heavy Slow Resistance Versus Eccentric Training as Treatment for Achilles Tendinopathy : A Randomized Controlled Trail.
The American Journal of Sports Medicine. 43(7):1704-11, 2015

R. Beyer, A.-S. Agergaard, S.P. Magnusson, R.B. Svensson
Speckle tracking in healthy and surgically repaired human Achilles tendons at different knee angles – A validation using implanted tantalum beads.
Translational Sports Medicine. (1):79-88, 2018

Abbreviations

BMI	Body-mass index
ECC	Eccentric
GL	The lateral head of Gastrocnemious
GM	The medial head of Gastrocnemious
HSR	Heavy Slow Resistance
MTJ	Musculotendinous junction
NSAID	Non Steroidal Anti-Inflammatory drug
RM	Repetition maximum
ROI	Region of Interest
ROM	Range of Motion
VAS	Visual analogue scale
VAS^{RUN}	Visual analogue scale running
VAS^{HEEL}	Visual analogue scale heel-rises
VISA-A	Victorian Institute of Sports assessment -Achilles
2D	Two-dimensional space
3D	Three-dimensional space

DANSK RESUME

Senen er bindeleddet mellem muskler og knogler, hvor den fungerer som kraftoverførsels enhed ved at konvertere muskel sammentrækninger om til led bevægelse. Achillessenen er den største og stærkeste sene vi har i kroppen og den kan modstå en vægt på op til 12 gange kropsvægten. Til trods for denne styrke ses der ofte overbelastningsskader i senen, så som tendinopati , som er karakteriseret ved; lokal smerte ved palpation, hævelse og nedsat ydeevne. Prævalensen er høj både hos professionelle og almindelige sportsudøvere og dette kunne pege i retning af, at belastning spiller en central rolle i udviklingen af tilstanden tendinopati. Sene overbelastningsskader er en kæmpe klinisk udfordring, idet denne skadestype udgør 30 til 50% af alle sports skader. Træningsøvelser med belastning på senen, er den mest anerkendte form for behandling til Achillessene tendinopati, hvor patienten udfører hæl-løft med strakt og bøjet knæ. Der eksisterer allerede adskillige typer af disse træningsprogrammer, men op til 40-50% af patienterne respondere ikke på denne type træning og bliver ikke raske. De bagvedliggende skadesmekanismer af tendinopati stadig ikke vel forstået. Derfor vil forskning i hvordan senen responderer på belastning være nøglen til at forstå de forandringer der fører til udviklingen af tendinopati og dette kan på sigt medvirke til en forbedret behandling og skadesforebyggelse.

Det overordnede formål med denne afhandling var at undersøge de strukturelle forhold, de mekaniske egenskaber og den kliniske effekt ved forskellige former for belastning og muskel aktivering, til at belyse skadesmekanismer og helingsprocesser i Achillessenen. Til besvarelse af ovenstående, udførte vi først et 'comparative treatment study' (RCT) til undersøgelse af de kliniske og strukturelle forandringer ved Heavy Slow Resistance træning og Eccentric træning, for at belyse hvilken af de 2 trænings-programmer der gav det bedste resultat i behandling af Achillessene tendinopati. Dernæst undersøgte vi hvordan aktivering af forskellige muskler påvirkede de intratendinøse glidninger på fasicle niveau, for at belyse koblingen mellem senens funktionelle egenskaber og behandlings strategier.

Langt fra alle spørgsmål omhandlende effekten af belastning på Achillessenen er blevet besvaret i denne afhandling, men med disse indledende undersøgelser har vi belyst klinisk vigtige og relevante resultater omhandlende seneskade, seneheling og behandlings strategier til Achillessenen.

ENGLISH SUMMARY

Tendon tissues are the connective link between muscles and bones working as force transducers, thus converting muscle contractions into joint movement. The Achilles tendon is the largest and strongest tendon in the human body and can withstand loads up to 12 times the bodyweight. Yet, repetitive use often results in overuse injuries such as tendinopathies, which are characterised by localised pain upon palpation, swelling and impaired performance. The prevalence is high in both elite and recreational athletes, suggesting that load plays an essential role in the development of tendinopathy. Tendon overload injuries pose an enormous clinical challenge as they accounts for 30 to 50% of all injuries in sports. Loading is the first-line treatment modality for Achilles tendinopathy, where the patient performs heel-rises with bent and straight knee. Several loading regimes exist however, since as many as 40-50% of all patients may not respond to a loading-induced treatment program. To date the injury mechanism is poorly understood. Investigating the influence of how tendon tissue adapts to mechanical loading will be the key to understand the pathogenesis of tendinopathy, which may lead to improved rehabilitation and prevention.

The aim of the present thesis was to investigate the structural, mechanical and clinical adaptations of tendon tissue in response to different kinds of loading and differential muscle activation to elucidate the mechanism of injury and healing in the Achilles tendon. To do so we conducted a comparative treatment study (RCT) to investigate the clinical and structural outcome of Heavy Slow Resistance training compared to Eccentric training in order to see which of the two had the best outcome in the treatment of Achilles tendinopathy. Secondly, we investigated intratendinous sliding at fascicle level in response to differential muscle activation in order to identify links between tendon function and rehabilitation strategies.

Although this present thesis answers far from all questions regarding the effect of loading on tendon tissue, these initial investigations identified clinically important and relevant results in relation to the injury, healing and rehabilitation of the Achilles tendon.

TABEL OF CONTENT

1. INTRODUCTION
1.1 General introduction

Tendons form the connective link between muscles and bones working as force transducers, and thus converting muscle contractions into joint movement. Therefore, proper and optimal tendon function plays a key role in daily movement and sporting activities. Tendon overload injuries pose an enormous clinical challenge, as they accounts for 30% to 50% of all injuries in sports[1, 2] Tendon overload injuries are frequent, difficult-to-treat clinical problems and limit physical performance for months and potentially years.[3, 4] To prevent tendon overload injuries it is necessary to elaborate the understanding of tendon tissue adaptation to mechanical loading of both the healthy and injured tendon.

1.2 Achilles tendinopathy (prevalence, definition and diagnosis)

The Achilles tendon is the largest and strongest tendon in the human body and can withstand loads up to 12 times the bodyweight.[5-7] However, when the tendon is exposed to repetitive high-magnitude loading it can result in tendinopathy, which is a failed healing response.[8] The prevalence varies among different ages and groups of people, but is most commonly found in middle-aged men between the ages of 35 and 45 years.[3, 9] The prevalence of Achilles tendinopathy is high in individuals who participate in physical activities that involve running, jumping and change of speed and direction, suggesting that loading plays an essential role in the development of tendinopathy.[10-12] Achilles tendinopathy is clinically defined as a syndrome characterised by pain during activity, impaired physical performance, localised pain upon palpation, and swelling and stiffness in the tendon.[13]. The most frequently affected region (55-65%) is the mid-portion area, localised 2-6 cm proximal to the calcaneal insertion.[14] The clinical diagnosis is stated on a careful history and clinical testing supplemented with ultrasonographic findings.[15].

1.3 Aetiology of tendinopathy

The aetiology of Achilles tendinopathy remains largely unresolved but most tendinopathies occur during sporting activities, in which greater load is applied onto the tendon.[1, 16] Many sports injuries arise from a multifactorial interaction between intrinsic and extrinsic risk factors[17] making it difficult for the clinician to point out the exact aetiology in each individual case. It is beyond the scope of this thesis to conduct a review of all the suggested risk factors, but the most commonly mentioned intrinsic and extrinsic risk factors for mid-portion Achilles tendinopathy will be listed here. Intrinsic factors are: gender, age, BMI, anatomical abnormalities, Soleus – Gastronemius dysfunction.[9, 14, 15, 18, 19] When looking at the extrinsic sports-related factors it is well established that change in load magnitude, changed ROM, changed speed of movement, changed training volume, changed training intensity, changed length of restitution periods, training error, new equipment or changed training surface or a combination of these are risk factors.[9, 14, 15]

Extensive research on histopathology has tried to clarify the role of inflammation in the development of tendinopathy. Chronic tendinopathy is now widely accepted as a non-inflammatory condition and is considered as a degenerative condition due to repetitive microtrauma from overload and the failed healing of tendon tissue.[20, 21] The inflammatory process might be present in the acute stage of microtrauma[22], but little or no biochemical or histological evidence of inflammation is found in biopsies from tendons with chronic tendinopathy.[23] Taken the viewpoint of tendinopathy being a degenerative process and failed regeneration of the tendon tissue, this will then consequently affect tendon structure, mechanics and function.

On a structural level, the tendinopathic tendon is characterised by a change in the collagen structure, including thinner and disorganised collagen fibres where some might be ruptured.[24, 25] In the healthy tendon the size of the cross-sectional area is an indication of how strong the tendon is. However, in the tendinopathic tendon the cross-sectional area is greater due to increased water content as a reaction to an increased amount of proteoglycans and additionally small capillaries growing into the tendon tissue. The ingrowths of capillaries are often followed by sensory nerve fibres that can trigger pain – collectively referred to as neovascularisation.[26, 27] Tendinopathy is often, but not always[28, 29] followed by neovascularisation when visualised with ultrasonography.[30, 31] Figure 1 illustrates the structural changes in the Achilles tendon.

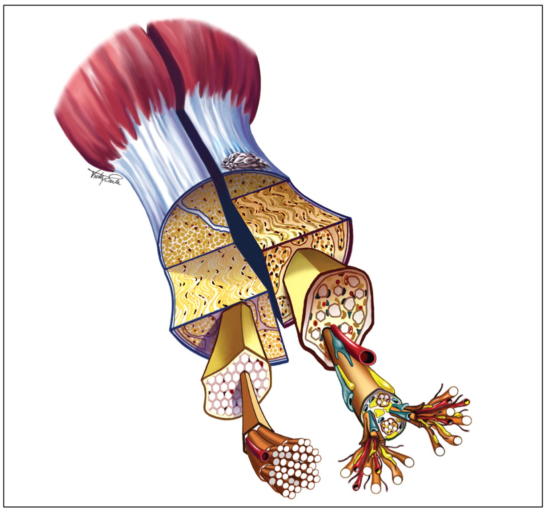

Figure 1: Structural changes in the Achilles tendon with chronic tendinopathy. The left side of the image shows healthy tendon tissue including bundles of tightly collagen fibres. Blood vessels are few and located in the connective tissue that surrounds the collagen bundles. The right side of the image shows the structural changes in the tendinopathic tendon including thinner and dis-organized collagen fibres, increased water content and neovascularization.[27] Image reprinted from Brukner P, Khan K. *Clinical Sports Medicine.* 3rd ed. Sydney, Australia: McGraw-Hill; 2006.

1.4 Management of Achilles tendinopathy

Conservative or loading-induced treatment protocols are generally accepted as the first line approach in the treatment of midportion Achilles tendinopathy.[8, 32-34] However, Achilles tendinopathy is a difficult-to-treat clinical problem and as many as 40-50% of all patients may not respond to a loading-induced treatment programme.[35] Management of this injury is particular challenging as the lack of understanding about the basic pathophysiology of tendinopathy makes it difficult to make decisions about optimal loading, including: load magnitude, frequency (sets and repetitions), load progressions and restitution between treatment sessions.

Back in the mid 80's Stanish[36, 37] was the first to introduce the concept of Eccentric training in the treatment of tendinopathies. Three years later, Alfredson et al. 1998[38] published the well-known paper on Eccentric training for Achilles tendinopathy with very promising results. Later, Scandinavian researchers demonstrated that up to 89% of patients with Achilles tendinopathy responded well to the Eccentric training programme,[39-41] with improvements in pain level during activity dropping from 6.7 to 1.0 during 12 weeks of rehabilitation.[39] However there are also studies that cannot reproduce these findings and their results are less convincing.[35, 42] One study has indicated that physically active patients might respond more positively than non-psychically active patients to the Eccentric loading programme.[42] To date, only a limited number of high-quality RCT studies have investigated the isolated effect of Eccentric exercise compared to other loadings regimes.[43-45] The majority of all studies investigating the effect of eccentric loading have prescribed three sets of 15 repetitions twice a day. However, no research has proven that this is the most optimal load magnitude, frequency (sets and repetitions) and restitution time between treatment sessions.

Loading is generally accepted as first–line treatment for Achilles tendinopathy, but can be supplemented with several treatment interventions. The use and management of these supplemented treatment interventions are not all scientifically based, even though they are widely used clinically: rest, splintage, taping, cryotherapy, electrotherapy, shock wave therapy, hyperthermia, NSAIDs, Corticosteroids, Platelet-rich plasma.[8, 26] Operative interventions are also used, but are usually reserved until conservative treatment fails.[46]

The long-term prognosis for patients with Achilles tendinopathy is generally good, as it has been found that after 5 to 8 years about 80% of the patients were fully recovered and returned to their pre-injury physical activity level.[47, 48] The results from these studies indicate that there might be a group of patients (about 20%) that do not respond to load-induced training.

1.5 Tendon structure

Like other tendons, the Achilles tendon is organised in a hierarchical structure where collagen molecules are grouped together in a highly organised style, forming fibrils, fibres and fascicles into bundles. The fibre bundles and fascicles are bound together by the interfascicular matrix, also referred to as the *endotenon* that contains blood vessels, nerves and lymphatics. Bundles of fascicles group together to form the whole tendon that is surrounded by the *epitenon.*[49] Some tendons including the Achilles tendon are further surrounded by a *paratenon* to protect and ensure free movement of the tendon relative to the surrounded tissue. Each structural level in this hierarchical composition influence the mechanical properties of the whole tendon and how it responds to tensile loading. Figure 2 illustrates the hierarchical structure of the Achilles tendon.

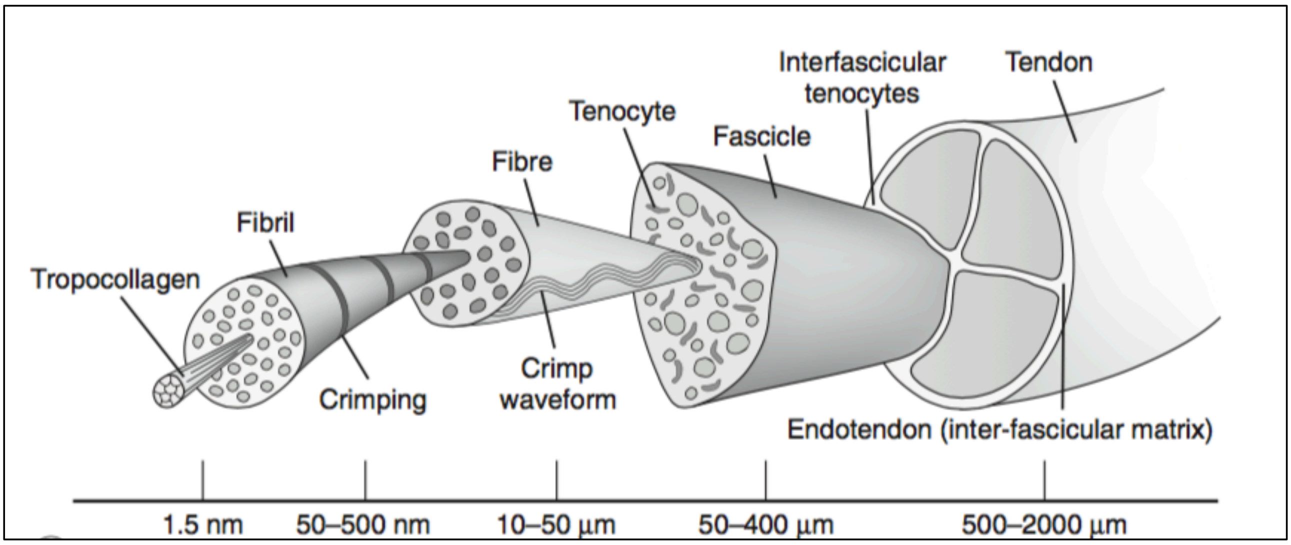

Figure 2: The hierarchical structure of the Achilles tendon. Image reprinted from Gwendolen et al. *Grieve's Modern Musculoskeletal Physiotherapy.* 4th ed. Elsevier; 2015.

The Achilles tendon is very interesting to investigate due to its unique and complex anatomical design. The Achilles tendon originates from the merging of the three muscles; the Solues, the lateral head of the Gastrocnemious (GL) and the medial head of the Gastrocnemius (GM). The tendon consists of multiple fascicles arranged into separated bundles from each of the three muscles. [50-52]

Overall, the fascicle bundles from the lateral and medial head of the gastrocnemius muscles are positioned more superficially and the Soleus is positioned on the deeper part of the tendon. Along the length of the tendon, the individually separated fascicle bundles from each of the three muscle compartments undergo an internal rotation from proximal to distal. [50] The individual difference of this internal helical rotation is varies greatly, but no differences were observed between sexes or between the left and the right side. [51] Figure 3 illustrates the pattern of the twist in the Achilles tendon.

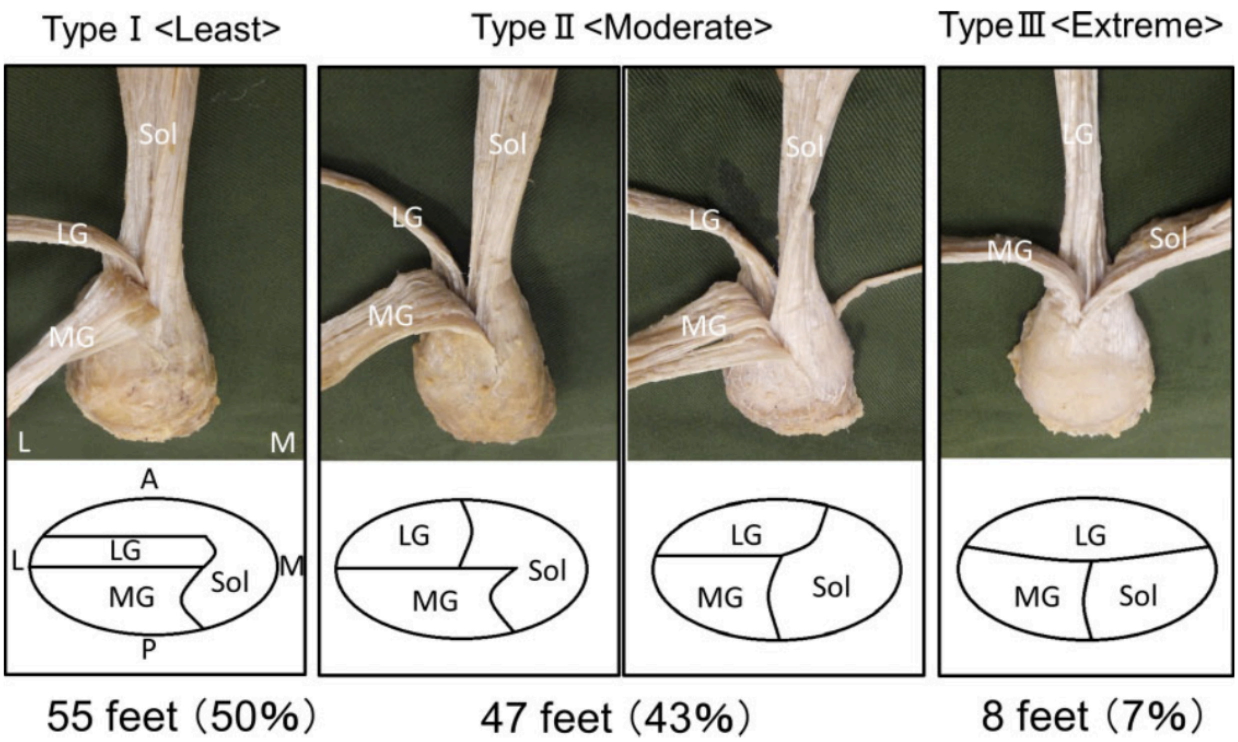

Figure 3: The patterns of “twist” of the left Achilles tendon, posterior-superior view. The lower schema: transverse cross-section through the left Achilles tendon, 1 cm above the calcaneal bone. A=anterior, L=lateral, LG=fibres from the lateral head of Gastrocnemius, M=medial, MG=fibres from the medial head of Gastrocnemius, P=posterior, SOL=fibres from the Soleus muscle.[51]

1.6 Tendon mechanics

The Achilles tendon has earlier been considered as one functional entity, but recent studies have demonstrated that tendon tissue undergoes heterogeneous deformation during stretch and loading, which suggest a certain degree of free interfasciclular movement. [53, 54] It is well known that that the contribution of the 2-joint Gastrocnemius muscle to force production declines with increasing knee flexion angle and this creates an intratendinous sliding and shear inside the tendon during locomotion.[55, 56] It has been shown that tendon fascicles work functionally as independent structures and only a minor force transmission between individual fascicles takes place.[54] This biomechanical quality may be beneficial in enabling the muscles to work at individual fibre lengths while maintaining optimal force transmission during motion and loading.[57] Another group have used selective activation of the 3 triceps surae muscles and concluded that intratendinous sliding and shear could be associated with the 3 muscles not always being activated to the same extent.[58, 59] This could be related to the great variance of cross-sectional size (GL=7%, GM=20% and Soleus=73%), as it is proportional to the muscles ability to generate force.[50]

It has been stated that the Achilles tendon has a lower safety margin compared to other tendons as it works closer to its yielding or rupture point.[60] The explanation for this low safety margin is related to its structural and mechanical properties where the relation between stiffness and strengths together with the ability to store and release energy are combined.[50]

1.7 Tendon function

Tendons can be divided into two groups according to their function: positional and energy-storing tendons. Energy-storing tendons are submitted to a much higher strain rate compared to positional tendons, where strains up to 11% have been reported in the Achilles tendon.[61] Thorpe et al. 2015[62] showed that energy-storing tendons have greater interfascicle sliding during loading, combined with specialised mechanical properties of the interfascicle matrix allowing the tendon to transmit and take up more energy when subjected to load.

Free inter-fascicle sliding acts as a protective mechanism against excessive strain and shear loads during muscular contraction.[57] One might assume, that a reduction in interfascicle sliding would increase the shear and frictional forces and increase the stress concentration on individual fascicles. LI Y et al. 2013[63] showed that increased stress could be located to a specific area and tendon region in the Achilles tendon, where the interfascicle sliding was inhibited, resulting in local fibre damage. Approximately 90% of all Achilles tendon ruptures occur 3-6 cm proximal to the calcaneal insertion indicating the presence of a local variation in strain and stress concentration along the tendon. Supporting this, an elevated concentration of the proteoglycan *Lubricin* has been located at the surface of individual fascicles in exactly this area (2.1 to 6.4 cm proximal of the insertion to the calcaneal bone) of the Achilles tendon, indicating that this local region undergoes significant inter-fascicle shear and sliding and is more vulnerably to injury.[64] Supporting this, studies suggest that micro strains often occur in the area 2-6 cm proximal of the calcaneus due to a smaller amount of cross-links.[65] It still unknown what the optimal distance for interfascicle sliding in the healthy Achilles tendon is? Therefore, we cannot measure whether a specific interfascicle sliding is increased or decreased. However, it is believed that tendon fascicles run all along the length of the tendon and are continuous throughout the mid-section of the tendon.[66] Thus, if optimal tendon function largely depends on free fascicle sliding, then an irregular arrangement of the fascicles in a local area of the tendon might have great consequences for the entire tendon.

1.8 Speckle tracking

Ultrasonography B-mode (Grey-scale) is frequently used to examine Achilles tendon properties, function and pathological changes.[67, 68] Real-time evaluation of tendon function and mechanical properties during stretch and loading is a considerable advantage of ultrasound imaging. Within recent years the B-mode technique has been supplemented by speckle tracking, which has become an accepted method in research environments to study the complex sliding and displacement pattern in the Achilles tendon during stretch and loading.[53, 69, 70] Speckle tracking imaging is a non-invasive and inexpensive method and recent evidence shows that it is useful to quantify tendon tissue movement without using any anatomical landmark as a reference point.[71] The method is, therefore, widely used on the Achilles tendon due to its long nature, where one scan cannot reach the most used anatomical landmarks: the calcaneal bone and the muscolotendon junction. The speckle tracking technique was originally developed by cardiologists to study contraction of the heart, conducted with 1 or 2 ultrasound probes and post-process resolved into a 2D or 3D tissue deformation and motion sequence.[72] The speckle image is developed by reflected sound waves coming in from the underlying tissue and is built up as a granular texture of small white, grey and black dots (Figure 4). Each dot does not correspond to an exact anatomical structure in the tendon, but represents the interference of sound waves coming in from the local tissue.[73] Each speckle image has it own unique speckle pattern that allow the image to be recognised and tracked from one frame to the next. The distance the individual speckles travels during the physiological movement can then be analysed and quantified.

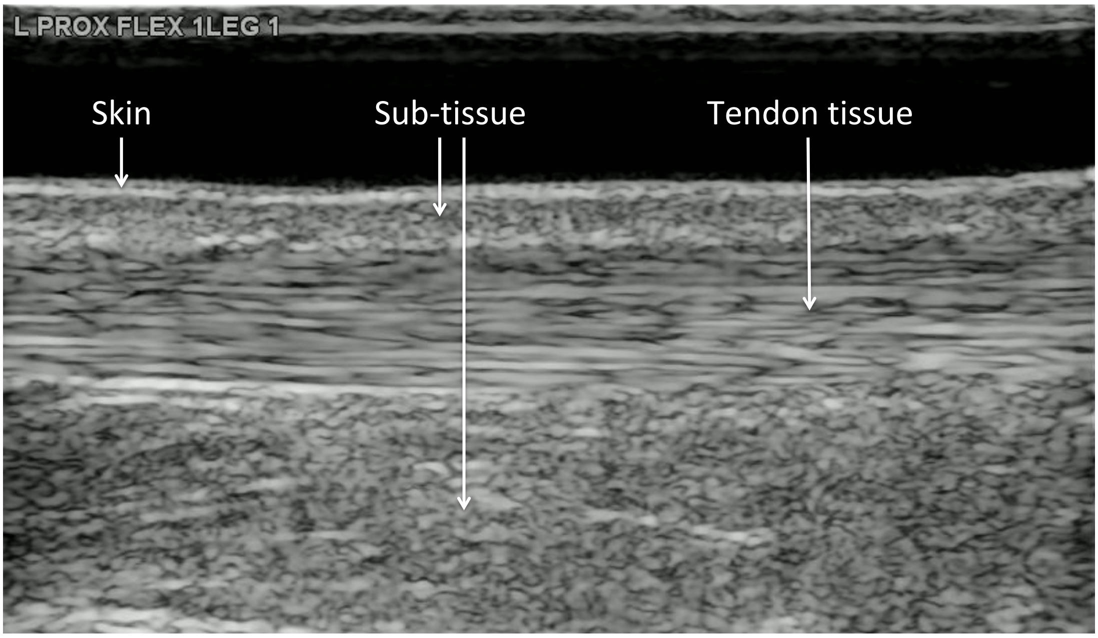

Figure 4, Speckle tracking image of the Achilles tendon. At the top of the image the small white speckles represent the skin, the mixed black and white speckles underneath represent the sub-tissue and the longitudinal speckles represent tendon tissue.

But how can we be sure that the speckle motion actually correlates to the true movement of the tendon tissue? Korstanje et al. 2010[71] validated the speckle method in vitro using aluminium platelets inserted into porcine tendons compared to the movement of the speckles and found a mean error of 1.3%. Based on these novel findings Arndt et al. 2012[53] conducted a study on the human Achilles tendon and was the first to show the complex non-uniform displacement between the tendon layers during passive movement. These findings were confirmed by Slane et al. 2015[70] who later showed that aging is associated with reduced intratendinous sliding and shear.[74] Within recent years several studies have followed and elaborated these novel findings and demonstrated a non-uniform displacement in the healthy Achilles tendon under different settings and loading conditions.[69, 75-77] However, despite the fact that the method is widely used in several research groups, it has never been validated in an in vivo human model.

2. AIMS

2.1 Aim of the thesis

The aim of the present thesis was to investigate the structural, mechanical and clinical adaptations of tendon tissue in response to different kinds of loading and differential muscle activation to elucidate the mechanism of injury and healing in the Achilles tendon

Loading is the first-line treatment modality for Achilles tendinopathy, where the patient performs heel-rises with bent and straight knee. Several loading regimes exist (Eccentric, concentric, isometric or a mix of these) and, though they all work very well it is unknown which one is most effective. The purpose of the present work was to compare the Heavy Slow Resistance (HSR) training regime to the already well-established Eccentric training regime to see which of the two had the best outcome in the treatment of Achilles tendinopathy.

The speckle tracking method has recently shown to be a promising tool to quantify the intratendinous movement in the Achilles tendon. Elaborating the understanding of intratendinous sliding and shear could be an important aspect of tendon aetiology and infer basic information of the mechanics of tendon injury. The purpose of the present work was to validate the speckle tracking method and investigate whether it has the capability to address clinically relevant questions in tendon function, in order to elucidate links between tendon function and rehabilitation strategies.

2.2 Specific study aims (Paper I)

The aim of this study was to investigate the clinical and structural outcome of the heavy slow resistance training regime and eccentric training regime in patients with Achilles tendinopathy

2.3 Specific study aims (Paper II)

The aim of this study was 1) to validate the speckle tracking method in vivo using patients that had tantalum beads inserted in their Achilles tendon following rupture. 2) to investigate intratendinous sliding at fascicle level in response to differential muscle activation in the healthy tendon and surgically repaired one.

3. METHODOLOGICAL CONSIDERATIONS

The following section will present methodological considerations including a discussion of particular concerns regarding the use of that method. More detailed information about material and method can be found in each paper located at the end of this thesis.

3.1 Comparative treatment study (Paper I)

3.1.1 Design and participants

The study was designed as a prospective randomised controlled trail with a 12-week intervention period and a 52-week follow-up period. An active comparative treatment design was applied to compare the outcome in two groups. We decided not to include a placebo group (a wait-and-see group) due to ethical reasons. A study by Rompe et al. 2007[35] investigating the effect of Eccentric training on Achilles tendinopathy included a wait-and-see group and found minimal clinical changes after 12 weeks (the VISA score decreased by 7 out of 100 points in total). Therefore, we do not believe that a placebo group would achieve clinical detectable changes to the outcomes measures of the present study. A pain duration of at least 3 months was considered essential to secure the presence of a chronic condition in the Achilles tendon.[27] The clinical diagnosis of Achilles tendinopathy was supplemented with ultrasonography findings as pain alone appears only to be moderately correlated with pathological changes in the Achilles tendon.[78]

The study was originally designed also to investigate: collagen content, cross-link composition and fibril composition including tendon biopsies to elaborate the structural and mechanical properties and not only the clinical effects of loading. However, due to some pain problems related to the invasive procedure of a biopsy in the Achilles tendon conducted in an already on-going study in the department, we decided to leave this part out. The VISA-A was chosen as the primary outcome measure as is a disease-specific and validated questionnaire evaluating both pain and function.[79, 80]

Designing this study pragmatically in a primary-care setting produced some methodological limitations in the recruitment phase. All participants were recruited via the Institute of Sports Medicine (Copenhagen) by seven sports physicians employed at the department. They were contacted at the beginning of the study and they were all willing to help out recruiting patients with mid-portion Achilles tendon problems on an on-going basis through the practice. If a patient seemed to meet the initial inclusion criteria, the patient was invited to participate in the study and assured that their treatment would not be affected, regardless of participation. Patients were informed that two active exercise strategies were to be compared and that both groups were expected to improve. If a patient gave consent, the patient was handed over to the responsible sports physician, who conducted all baseline assessments and determined whether the patient should be included in the study or not. Due to practicalities, the total number of patients assessed for eligibility by the seven sports physicians is unknown. That also applies to the total number of patients handed over to the responsible sports physician, because a new computer system was introduced 8 months into the inclusion period and registered data was lost. The present study is, therefore limited by not knowing how many patients were excluded in the study and what the reason for this was. The remaining saved data shows that patients were mainly excluded for two reasons: they had received a corticoid-steroid in the past 12 months; or there was absence of neovascularization in the tendon, even when all other clinical signs were present.

In order to decrease sampling error and secure two homogenous groups we implemented a stratified-randomisation procedure using the software programme *Minimize.*[81] The stratified-randomisation was conducted by weighting equally the three characteristics: *activity level* (hours of exercise/week before injury), *symptom duration* and *age*. A wide age span (31 – 60 years) and a long duration of symptoms (3 – 120 months) were chosen as relevant population characteristics, as we wanted to reflect the patient group we see in the clinic.

Retrospectively, choosing *level of pain* as randomisation characteristic could in the present study have been relevant in order to ensure that both groups were present with the same level of pain at baseline. Theoretically, if one group had a higher pain level compared to the other group, this could influence the total outcome, as the delta value of change in pain over time could be higher in one group and mistakenly be analysed as the loading regime had a better outcome. In the present study the *level of pain* was entitled as a secondary outcome measure and investigated as pain during running and pain conducting heel-rises. These results are illustrated in Figure 5 and additionally shown in Table 2 in the study (Paper I).

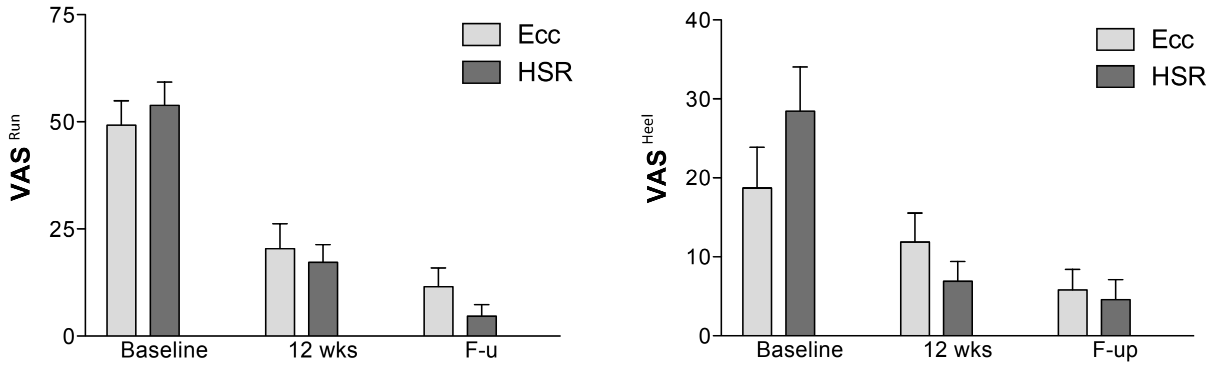

Figure 5: Participants level of pain during running and heel-rises measured at baseline, 12 weeks and 52 weeks follow-up. Values are presented as means ± SEM. The level of pain is measured in mm on the VAS scale.

At baseline no statistical differences (VAS^{Run} $p< 0.57$ and VAS^{Heel} $p< 0.21$) were found between the two groups, meaning that the two groups were not different in terms of *pain level* at baseline.

3.1.3 Questionnaires and clinical assessment

The VISA-A score is a patient-administrated questionnaire to assess the severity of Achilles tendinopathy. The questionnaire consists of eight questions assessing pain level, function in daily living and ability to perform sporting activities. The total maximum score is 100 points, which indicates no pain, full function and full participation in sports. The theoretical minimum is 0. The VISA-A questionnaire has been proven to be a reliable and validated tool for monitoring the clinical severity of Achilles tendinopathy and progress in rehabilitation.[80] A Danish version of the VISA-A questionnaire was adapted from the Swedish VISA-A questionnaire, which in 2005 was translated and validated from the original Australian VISA-A score.[82]

All clinical evaluations including the VISA-A questionnaire, the two VAS scores and treatment-satisfaction were completed by the participants themselves and with as little assistance as possible in order to minimise potential bias. The risk of participants misunderstanding the questions is of cause a potential source of error. To accommodate this, a physiotherapist assisted each participant in fulfilling the self-reported clinical evaluations at baseline, 12 week and 52 weeks assessment. To reduce patient bias, the clinical evaluation were completed before the ultrasonography examination, so that the patient administrated rating would not be affected by the sonographic findings or the presence of the investigator.

3.1.2 Ultrasound Grey-scale and colour Doppler assessments

Ultrasound Grey-scale imaging is a reliable method for studying tendon structure[83] and when supplemented by colour Doppler, additional information about blood flow can be gained.[67, 84]

Grey-scale ultrasonographic imaging of Achilles tendinopathy is characterised by fibrillar/fascicle disorientation and disorganisation, increased water content and areas with signs of neovascularization within the tendon.[85-87] As previously described the degenerative changes in tendinopathy are related to the increased amount of proteoglycans and water, leading to swelling corresponding to the painful area in the tendon. Measurement of tendon thickness is, therefore, a well-established and accepted parameter for quantifying the level of degeneration in the injured tendon.[88] Side-to-side comparison, where the healthy tendon acts as the control is of great advantage in ultrasonographic imaging. It has to be acknowledge that ultrasonographic-detected degenerative changes can be present in asymptomatic tendons and are therefore not unambiguously linked to tendinopathy.[78]

Colour Doppler imaging is often recorded as a video, in order to visualise and quantify the amount of blood flow (neovascularization) inside the tendon. In the normal resting tendon no blood flow is detectable with Colour Doppler due to the low circulation, thus Doppler activity is detectable in the pathological tendon due to the higher blood flow.[86, 89] Several studies have shown that acute physical activities can generate a physiological response in tendon tissue, which can increase Colour Doppler activity[67] and tendon water accumulation /thickness.[90] Therefore, participants were asked to refrain from any sporting activities for 24 hours prior to testing, as ultrasonographic findings are an essential part of both Study I and Study II.

3.1.4 Intervention

Standing on a step on a staircase performing eccentric heel-rises, has become the dominant conservative choice of treatment for Achilles tendinopathy.[40, 44, 91, 92] The Eccentric exercise programme applied and included in the present study was identical to that used in previous studies.[38] The HSR regime is novel in tendinopathy management, but showed good clinical, structural and mechanical improvements on patella tendinopathy superior to the eccentric regime.[93] The reason for including the HSR intervention in the study was to investigate if the HSR regime had the similar positive outcome on patients with Achilles tendinopathy. The HSR regime differs from most other loading-based exercise regimes for the management of Achilles tendinopathy in several aspects: slowly performed repetitions, high load magnitude and longer restitution periods between training sessions. Figure 6 illustrates similarities and differences between the two loading regimes.

Eccentric training	
Load	15 – 20 RM
Exercise program	Two types of exercises 3 sets of 15 repetitions
How often?	Twice a day 7 days a week for 12 weeks

Heavy slow resistance training	
Load	< 15 RM
Exercise program	Three types of exercises 3-4 sets x 15–6 repetitions
How often?	3 times a week for 12 weeks

Figure 6: The exercise protocol for Eccentric training and Heavy Slow Resistance training, including load magnitude, frequency (sets and repetitions) and restitution periods between training sessions.

For both intervention groups, a certain pain level was allowed throughout the exercise programme according to the pain monitoring model (Thomee 1997)[94] and modified by Silbernagel et al. 2001,[95] following these three conditions: 1) Pain was allowed to reach up to 5 cm on the VAS scale during exercise, if pain subsided immediately after the end of exercise; 2) Pain was allowed to reach up to 5 cm on the VAS scale after finishing the entire exercise program, if pain had subsided the following training session; and 3) Pain and stiffness in the Achilles tendon were not allowed to increase from day to day. All participants were carefully informed that pain during exercise was a 'normal' tendon reaction and they were encouraged to perform the exercises with a pain level reaching 4-5 on the Pain Monitoring Model (Figure 7).

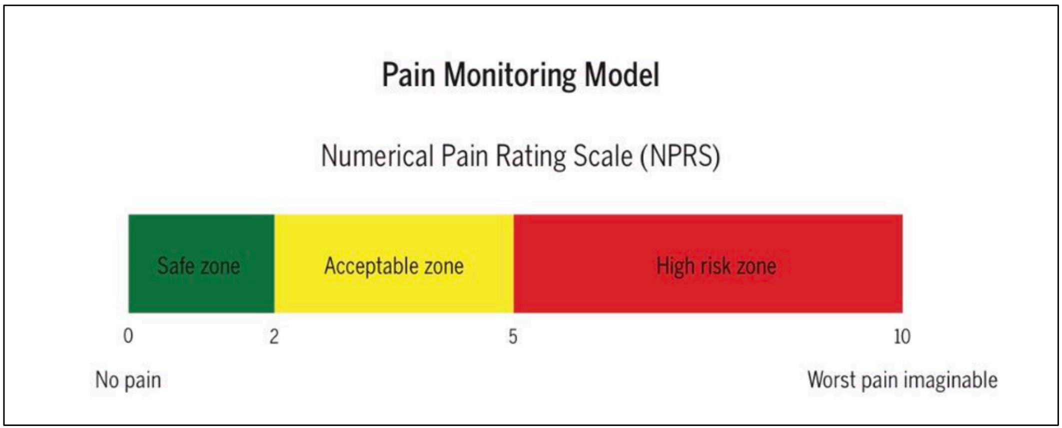

Figure 7. Pain monitoring Model[94, 95]

3.1.5 Training diary

Methods such as information manuals, training diaries, practical demonstrations and supervision were implemented in the present study to improve compliance. The consistent use of a written training diary made it possible to make an individual training progression and adjustments to help avoid adverse events caused by too high load progression.

3.2 Validation and investigation study (Paper II)

3.2.1 Design and participants

The study was designed as a validation study combined with some additional clinical investigations of intratendinous sliding and shear in the Achilles tendon. Participants were recruited from a larger on-going study in the department,[96] investigating the rehabilitation of Achilles tendon ruptures at the Institute of Sports Medicine, Copenhagen. Recruitment of participants was based on sampling of convenience, where all patients who met the inclusion and exclusion criteria were chosen as they became available >52 weeks post-surgery.

Figure 8: During surgery four tantalum metal beads with a diameter of 1.0 mm were implanted into the tendon tissue on both sides of the rupture (two beads in each end). From Eliasson et al. (2018).[96]

Sample size estimation was not conducted in the current study as the speckle tracking method was tested for the first time in the department and no reference values were available. Sometimes one can adopt and use reference values from other research groups.[97] However, we concluded that our ultrasonography settings and tracking algorithm deviated extensively, and reference values were incompatible. Several speckle tracking algorithms have been used over time and the majority of these have been custom-developed for application to tendon tissue, as no commercially algorithm has yet been developed for this purpose.[71, 98, 99] We estimated that a sample size of 10 participants, each with one healthy and one surgically repaired Achilles tendon (20 tendon in total), was suitable for conducting a within-subject comparison detecting a change between the bead and reference boxes and between Test 1 and Test 2. Related to the number of participants, we decided to select a group with a certain level of homogeneity. We eliminated the variable, gender (only males) and narrowed the range of age (23-53 years) as we expected these variables might interfere with the results.[74, 75]

3.2.2 Familiarisation procedures and warm up

When assessing tendon mechanical and functional properties in vitro, it is very important to conduct a so-called pre-conditioning in order to secure reproducible data.[100, 101] But when assessing tendon mechanical and functional properties in vivo this problem is probably less significant as during locomotion the tendon, especially the Achilles tendon, is moved constantly. However, in order to secure an identical starting-point at baseline, all participants performed 5-minutes walking on a treadmill prior to testing and had to refrain from all heavy loading 24 hours before testing.

Because repeated measures were made in Study II, a series of familiarisation trials were conducted prior to each exercise. It has to be acknowledged, that the test protocol of exercises were performed in the same order and it can not be ruled out that exercises performed at the end of the test protocol may have been influenced by a learning effect (Table 1).

	Recording 1	Recording 2
Heel-rise with 2 legs and extended knee		
Heel-rise with 1 leg and extended knee		
Heel-rise with 2 legs and flexed knee		
Heel-rise with 1 leg and flexed knee		
Heel-rise with 1 leg and sitting on a chair		

Table 1: Test protocol, showing the order of exercises performed by the participants. Heel-rises were performed at 3 different knee angles: standing with fully extended knee (0°), standing with flexed knee (40°) and sitting on a chair (100° flexed knee angle), to differentiate between the contribution from the Gastrocnemius and Soleus muscle. Standing exercises were performed with either full body weight (1 leg heel rise), or half body weight (2 legs heel rise), and the seated exercise was performed with a 15 kg weight placed on the knee. The 3 exercises are illustrated by images in Paper II.

3.2.3 Ultrasonography speckle imaging

The use of ultrasonography (B-mode) with one probe to determine intratendinous sliding and shear in the Achilles tendon has some inherent advantages, limitations and error sources.
In general ultrasonography has the advantages of being safe, non-invasive and inexpensive compared to other methods investigating tendon mechanical properties and function. However, out-of-plane movement is a limiting factor of any 2D technique trying to capture the motion of a complex 3D-deformation of the Achilles tendon and must be considered as an established source of error.[102] As speckle tracking is a method to quantify intratendinous sliding without using any anatomical landmark as reference point, we had several considerations in terms of how to place the probe at the exact same location within and between participants. The region of interest (ROI) was defined and located with the following procedure. First, the anatomical landmark of the apex of the Musculotendinous junction (MTJ) was identified. Next, the probe was placed such that the proximal tantalum bead was at the distal end in the image and the distance from the bead to the MTJ was estimated. This distance was reproduced on the healthy side and the ROI defined. The method worked out well for all tendons. However, we overlooked the fact that surgically repaired tendons are often elongated by as much as to 5-8 mm.[96] By using this method we only measured the relative distance and not the absolute distance and consequently placed the probe and ROI more distally on the healthy tendon. This has to be acknowledged, as a potential source of error, as regional differences of intratendinous sliding show along the length of the Achilles tendon.[77] That said, all measurement in the present study was conducted in the proximal part of the tendon. Consequently, we do not suspect that this significantly influenced our findings or interpretation.

To ensure validity and to exclude biased findings, all ultrasonographic settings were kept constant for all participants at all time points throughout the study. All measurements were conducted with the patient standing straight to ensure identical tendon tension for all participants and to avoid tendon fascicle crimp.

3.2.4 Post process speckle image analysis

The study was originally designed to validate the bead against four tracking nodes placed in the tendon tissue to the left, right, bellow and above the bead. The bottom node had to be excluded, as it would have tracked too well. It would end up tracking the shadow from the bead and thereby be too strongly correlated to the bead (i.e. it would have falsely improved the correlation between the bead and the box movement). See Figure 9. We had many considerations in terms of how far distant from the bead the tracking nodes should be placed. One could argue that if the tracking nodes were too close to the bead, the risk of being interfered with became high. On the other hand if the tracking nodes were too far away from the bead, the movement of the individual fascicles might in its nature be different from the ones the bead was attached to. Balancing this task was not easy. During the post process analysis no sign of interference between the bead and the three tracking nodes was present.

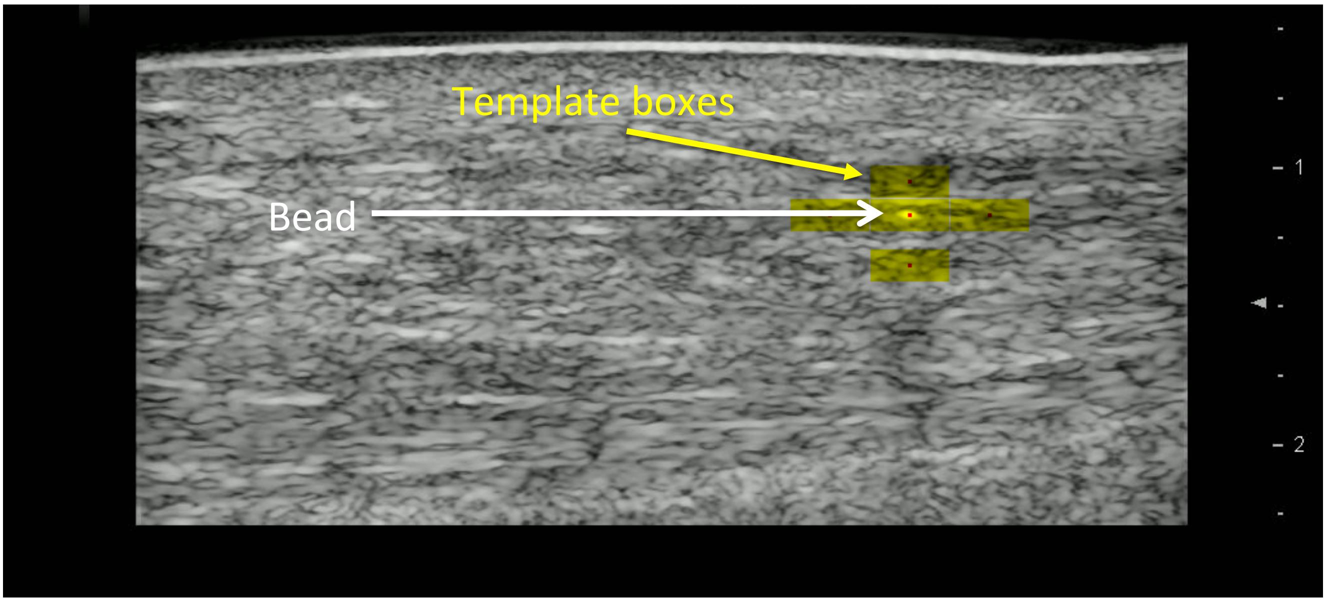

Figure 9: Validation analysis of the speckle method. The 4 template boxes each include a tracking node are located on each side of the bead.

4. STATISTICS

Statistical analysis in both studies was performed using the statistical software GraphPad Prism® version 7. Prior to analyses, all data was investigated for Gaussian distribution or non-Gaussian distribution.

4.1 Study I (paper I)

All data is reported as group mean ± standard error of mean (SEM) and 95% CIs. *P*-values <0.05 were considered statistically significant. The unpaired Students *t*-test was used to analyse baseline patient characteristics. Outcome measures were analysed using 2-way ANOVA (treatment x time) with repeated measures. When appropriate the Bonferroni post-hoc test was carried out. Patient satisfaction at 12 and 52 weeks and patient activity level at 0, 12 and 52 weeks were analysed using Fishers exact test. Patient compliance was analysed with an unpaired Students *t*-test. To examine whether changes in Colour Doppler activity over time were related to changes in the VISA-A score over time, a Pearson correlation was carried out. In accordance with the CONSORT statement for reporting randomised controlled trials, all statistical analyses were conducted on an intention-to-treat basis to avoid overestimation of the clinical effectiveness. All participants were included for statistical calculation, regardless of deviation from the protocol or subsequent withdrawal i.e. loss to follow-up. Missing responses were included as the last observation carried forward.

4.2 Study II (paper II)

4.2.1 Validation and reproducibility

All data is reported as means ± standard deviation (SD). *P*-values <0.05 were considered statistically significant. A paired *t*-test was performed to assess differences in absolute values between the bead and the 3 template boxes. The correlation coefficient (R^2) and typical error (%) were calculated to analyse the validity of the speckle tracking method.[97] Reproducibility was assessed on the basis of the same parameters.

4.2.2 Clinical tests

All data is reported as means ± standard error of mean (SEM). *P*-values <0.05 were considered statistically significant. The mean displacement of each of the four layers was determined for each measurement and differences between the 4 layers were analysed using 1-way ANOVA and paired statistics to determine the influence of knee-joint angle. If the ANOVA was significant, a post-hoc test was made using Dunnett's multiple comparisons test, with layer 1 (superficial) as comparator. To compare the 3 different test positions, Turkey's correction for multiple comparisons was used.

5. RESULTS
5.1 Paper I (the RCT study)

The main results are an equally effect of Eccentric training compared to Heavy Slow Resistance training measured on the VISA-A index score, tendon tissue thickness and neovascularization in the treatment of Achilles tendinopathy. The compliance was significantly greater after 12 weeks intervention in the HSR group compared to the ECC group, as well patient satisfaction also tended to be greater after 12 weeks but not after 52 weeks.

5.1.1 Participants

In the inclusion period (2009 – 2012) patients with Achilles tendinopathy were referred to the trial from the Institute of Sports Medicine, Copenhagen. A total of 58 patients with chronic unilateral mid-portion Achilles tendinopathy were included in the study and randomised into one of the two intervention groups. The randomisation procedure was based on activity level, symptom duration and age. A total of 11 patients withdrew (n=5 ECC and n=6 HSR) due to ankle pain, back pain, lack of time or moving to another location, leaving 45 patients to complete the intervention period. Another 3 patients withdrew (n=1 ECC and n=2 HSR) for the 52-week assessment, leaving 44 patients to complete the trial. The flows of the participants are shown in Figure 10.

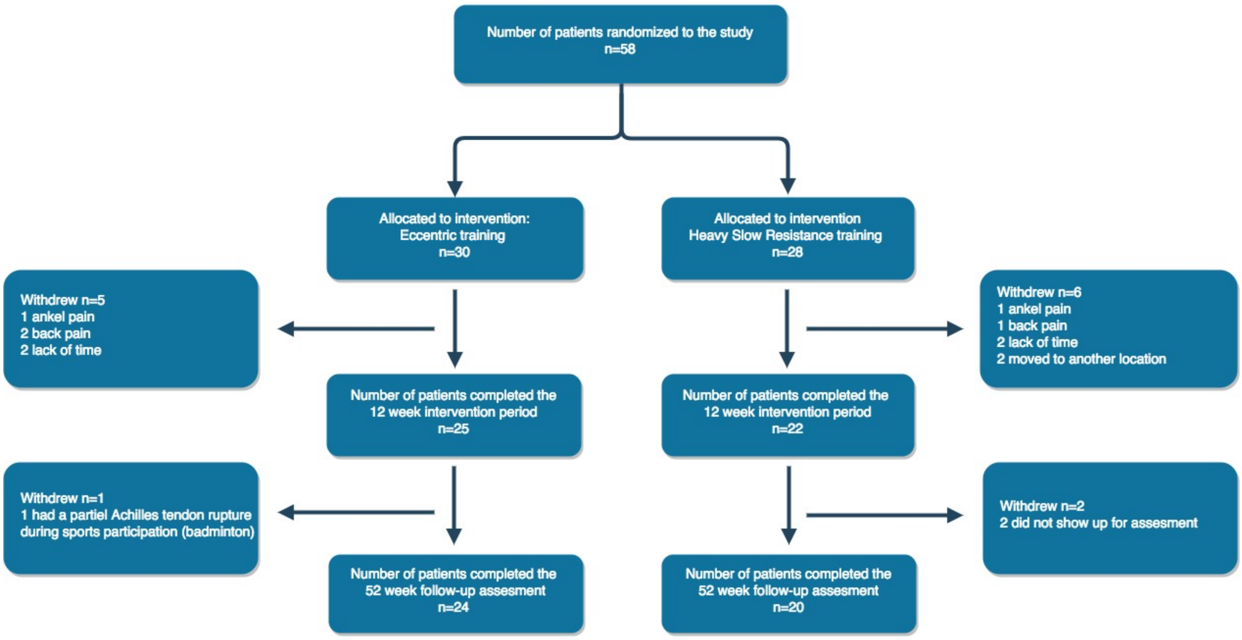

Figure 10: Flow diagram depicting the flows of participants through the trial.

5.2 Paper II (The speckle tracking method)

The speckle tracking method appears to be a valid method for measuring intratendinous sliding and displacement in the Achilles tendon. The displacement in tendon tissue surrounding the bead correlated strongly with the displacement of the bead. However, it systematically underestimates the displacement with a typical error of 1.1% - 2.7%. This is in line with earlier studies that have reported similar conservative results of the speckle algorithm[103] and tracking error.[71] There was a significant difference in displacement between the superficial and deep tendon layer for all three heel-rise exercises in the healthy, but not the surgically repaired tendon. The sliding was significantly greater when performing heel-rises with the knee flexed 100° compared to when the knee was flexed 40°.

The results of the present study show that intratendinous displacement and sliding are characteristic for the behaviour of healthy tendon tissue and decreased displacement and sliding are characteristic for patients following tendon rupture, even 52 weeks post surgery.

5.2.1 Participants

In the inclusion period (October 2015 – February 2016) a total of 11 participants were referred to the trial. One participant was excluded, as he had a total Achilles tendons rupture 7 years earlier on the non-injured side and, therefore, his tendon tissue could not be considered as healthy. Participant characteristics are shown in Table 2.

Participant number	Age (years)	Height (cm)	Weight (kg)	Activity level (hours/week)
1	28	180	76	1
2	37	183	79	2
3	52	192	97	2
4	31	180	66	2
5	28	189	75	6
6	27	189	77	5
7	52	185	105	3
8	31	185	83	2
9	23	179	82	3
10	36	184	86	4
Mean	**35**	**185**	**83**	**3**

Table 2: Participant characteristics (n=10) including: age, height, weight and activity level.

5.2.2 Load and intratendinous movement (Additional data to Paper II)

The following data, statistical analyses and results presented are not included in the published paper and should be considered as additional data to the original dataset. This additional data contains measurements of two different load volumes applied onto the tendon: heel-rises performed with 1 leg (equals 100% bodyweight) and heel-rises performed with 2 legs (equals 50% bodyweight). A post-hoc test was conducted and the results on healthy tendon tissue are now shown for the first time in the present thesis (Figure 11).

We found no difference in the intratendinous displacement between loading the tendon with heel-rises performed with 1 leg or 2 legs, measured on healthy tendon tissue. This indicates that there is no difference in the amount of sliding and shear between the four layers, whether the Achilles tendon is strengthened with one or two-legged heel-rises. As expected, a similar result was found in the surgically repaired tendon with no difference between loading the tendon with 1 leg or 2 legs in the two test positions. These results are not presented, but are similar to the data presented in Figure 11.

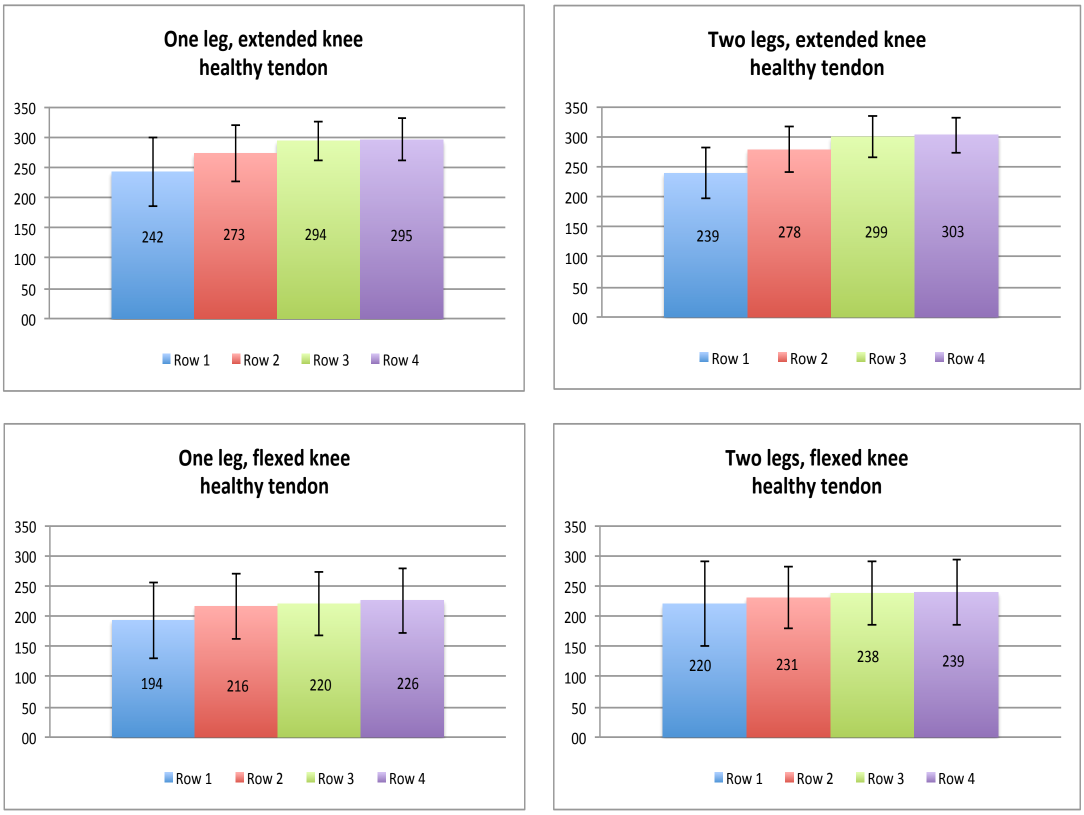

Figure 11 A-D: Speckle movement (pixels) of the 4 layers in the healthy tendon for 2 different exercises performed on one or two legs. Group mean ± SD. The mean bodyweight was 83 kg (range 66 – 105). There was no significant difference (*P* < .05) between layers when loading the tendon with 1 leg or 2 legs.

6. DISCUSSION

In the following, some of the most important results from the two studies will be presented and discussed. Please see the two manuscripts at the end of this thesis for a more detailed presentation and discussion of the results.

6.2 The concept of loading in ECC and HSR training regimes (Paper I)

Load is the key component in both training regimes, thus the parameters such as load magnitude, frequency (sets and repetitions) and restitution periods between sessions are implemented with different amounts and/or time in the two regimes.

In the ECC training regime one-legged heel-rises are performed on the step of a staircase, with rising on two legs and lowering on one leg. The load applied onto the tendon is the bodyweight of the patient, where the lowering part of the heel-rise equals 100% and the lifting part equals 50%. The exercise is conducted with both bend and straight knee. In the HSR training regime two-legged heel-rises are performed in the leg-press machine, calf-raise machine and with barbell lifting. Individual load for each of the 3 exercises is estimated on the concept of 1RM (repetition of maximum).

The training concept of the ECC regime is very ‘locked’ as all patients regardless of muscle strength are prescribed 3 sets of 15 repetitions. Some patients might only be able to implement 8, 9 or 10 repetitions, especially at the beginning of the rehabilitation programme where pain persists and muscle strength is at its lowest point. If a patient is able to perform more than the prescribed repetitions, load is added in a backpack. We are not aware of any studies that have described how to manage patients that cannot perform 15 prescribed repetitions of heel-rises in the ECC training programme. In the present study a few patients had trouble performing the rising part of the heel-rise and we decided to handle this issue by encouraging them to increase the load on the healthy leg. This worked out well. As the intervention period progressed the ECC training group became more homogeneous (total load volume), as muscle strength increased by exercise and additional load was added for all patients. In the present study all patients completed 3 sets of 15 on the first day of intervention and a follow-up training session was carried out one week after, to adjust load, and pain monitoring, and to give instruction on how to progress.

The HSR regime is based on the concept of individual calculated loading of the tendon in each of the three exercises. It is a well-known clinical challenge to test maximal muscle strength in patients who have a pain-related injury as the onset of pain occurs before reaching the true level of maximum strength. If pain is very pronounced it will be the limiting factor and the risk of testing too low maximum muscle strength will be present. We had several considerations to front this challenge in the study, as testing muscle strength was required in order to calculate one repetition of max (1RM) in the HSR regime. A submaximal strength test including 3-5 repetitions where pain was allowed to reach 40-50 mm on the VAS scale was conducted for each of the three exercises. Accepting pain during the test was related to fact that the same level of pain was allowed during the intervention. Based on the test result the 1RM was calculated and thereafter converted to 15 RM and that was the starting point. All patients completed 3 sets of 15 repetitions on the first day of intervention in order to test and verify that the calculated load (%RM) was correct. Furthermore, a follow-up training session was carried out one week after the start of intervention, to adjust load and pain monitoring and to give instruction on how to progress.

To sum up, the total load volume applied onto the tendon is the same for each patient in the HSR group at the first day of intervention, but not for each patient in the ECC group. Load is individually controlled during the entire rehabilitation programme in the HSR regime, but not to the same extent in the ECC regime. One might speculate, whether this could be the reason why athletic patients respond more positively to the ECC programme, simply because the non-athletic patients are overloaded at the beginning of the programme?

6.3 Training session compliance (Paper I)

All patients were informed that they would be randomly allocated into one of the two treatment groups. The patients were informed that if allocated into the HSR group, it required a self-pay 3-month partnership in the local fitness centre. Some patients had never before in their life been in a fitness centre before but were positive in their response to the idea: especially when reassured that prior to the training they would receive a careful individual instruction in the fitness centre located at the hospital. Patients were also informed that they could be allocated into the ECC group, even though they had received this treatment previously. A few patients had unsuccessfully tried the ECC training regime before attending this study, but were all positive about giving the training regime a second try, as none of them had completed the programme. However, it has to be acknowledged that the compliance of these patients might have influenced the results in the ECC group if they lost motivation again during the training.

The compliance rate for the ECC group was lower than the HSR group. One key aspect could be the considerable difference in time allotment between the two groups (ECC= 308 min/week, HSR=36 min/week). If the compliance rate had been higher and more patients had completed the ECC training programme, would the results for the ECC group then have been better? There are studies that show repeated training with rest periods that are too short can result in a negative balance between collagen degradation and synthesis.[104] The missed training sessions may actually have had a positive effect on the clinical results for the ECC group, but this is only speculation. Age could also be a confounding factor, as the rate of collagen turnover slows down when aging.[105] In this case the longer restitution periods between training sessions in the HSR group or missed training sessions in the ECC group might be in advantageous for older persons.

6.4 What can the speckle tracking method be used for? (Paper II)

The speckle tracking method can provide baseline data on how tendon tissue moves during loading and stretching. The method can quantify the distance each layer moves, but it can not tell, whether this distance of movement (sliding) is increased or decreased compared to its normal distance, nor if the shear between the different layers is balanced. In the present study it was clear that, compared to healthy tendon tissue, tendon tissue, which had undergone surgery, had a different displacement pattern with impaired sliding and shear between the different tendon layers. The speckle tracking method is an investigation tool that can provide baseline data on tendon tissue behaviour, when examining the influence of factors such as: different loading, different knee and ankle angles, pace, aging, injury and healing. This data may be important for future investigations in order to elaborate the understanding of the complex intratendinous sliding and shear and perhaps the aetiology of Achilles tendon injuries.

The speckle tracking method appears to be a valid method when used as a research tool investigating the effect of an intervention in two groups, using within subject comparison. This conducted on a participant group with a certain inter-individual mechanical properties in the tendon (e.g. activity level linked to material properties) and the previously stated high amount of inter-individual anatomical variation in degree of fascicle rotation.[52, 106] The speckle method had a high reproducibility when tracking twice on the same recording (% typical error 1.2 to 5.4) and had a fair reproducibility when tracking on two separate recordings (% typical error 3.9 to 8.8), presuming that it is the same investigator performing the tests and that the speckle tracking equipment is not moved between tests. It is reasonable to believe that the reproducibility will further decrease when measuring changes over time (day to day variation) added up with the test equipment is being moved. One inherent limitation of the speckle tracking method used in the present study is that the validations between the bead and reference boxes are only conducted on surgically repaired tendon tissue. The intratendinous sliding and shear are impaired due to the non-linear fibrillar composition after surgery and the whole tendon moves like a block. How accurate the speckle tracking method is on healthy tendon tissue is unknown, but we would expect an increase in the typical error value due to the increased movement between tendon layers.

The speckle tracking method is not a user-friendly investigation tool, as it requires high technical skills, it is time consuming (large amount of work in relation to out-put) and it is difficult to use by a novice investigator. The speckle tracking method is in its state-of-art-technology most suitable for research purposes, but not yet for clinical use.

6.5 Load and intratendinous movement (Paper II)

One of many interesting results found in this study is the observation of how load does not influence intratendinous sliding and shear in the Achilles tendon. The present study found no significant change in intratendinous sliding and shear between loading with 100% or 50% bodyweight during a single heel-rise. The tensile load applied onto the tendon (the force from the activated Triceps Surae muscles) influences tendon mechanical properties on the force/length curve. One interpretation may be that both measurements (100 % and 50% bodyweight) were conducted in the linear region of the force/length curve, where tendon stiffness is high.

Due to the high stiffness a detectable change in tendon elongation would require considerable load applied onto the tendon. The load difference between performing heel-rises on one or two legs in the present study was too small and that could be the reason why no change in intratendinous displacement was shown even by doubling the load. Theoretically, if load were applied onto the tendon at its toe-region of the force/length curve the intratendinous displacement would probably be different. Due to the low stiffness in this region, a detectable change in tendon elongation would only require a small load volume applied onto the tendon. Several studies have investigated displacement patterns at the toe-region of the tendon (passive loading) and found that all layers had a higher displacement compared to active loading at the linear region.[70, 75] These results are in line with the assumption that, in the toe-region the tendon slack would imply a change in intratendinous displacement of the individual layers prior to the onset of force transmission. The results in the present study point imply that, when force transmission is set and all muscle units activated the intratendinous displacement and sliding seem to reach a load threshold. Beyond this threshold, only small detectable changes in tendon tissue movement and strain are present, when additional load is applied.

7. CONCLUSIONS

The main focus of the present thesis was to investigate the effect of loading on clinical outcome and intratendinous sliding at fascicle level, to elucidate mechanism of injury and healing in the Achilles tendon. Although there are still several aspects of tendon tissue adaptation to loading that are unclear, we believe that the present work have elaborated some important aspects.

The ECC and HSR loading programmes both yield positive, equally good and lasting clinical results in patients with Achilles tendinopathy, leaving the choice of treatment intervention up to the patient, therapist and physician. This study confirms that loading-induced exercise leads to good clinical and structural improvements in the treatment of Achilles tendinopathy. In agreement with earlier conducted research, this study confirms that not all patients recover 100% after 12 weeks or after 52 weeks, which may indicate a group of patients that cannot reach 100 points on the VISA-A scale. The reason for the lack of complete recovery is unknown.

We found the speckle tracking method to be a valid approach to investigating intratendinous displacement and sliding in the Achilles tendon. The study confirms that the superficial layer moves significantly less than the deeper layer, indicating that sliding and shear of tendon fascicles take place during heel-rise. We found that sliding and shear occur mostly when keeping the ankle joint at 90° (standing and sitting position) and is enhanced when the ankle joint is >90° (the 40° flexed knee position). This behaviour could be relevant knowledge to the clinician who wants to re-establish interfascicle sliding in the injured tendon. Secondly, we found that a tensile loading of 50% or 100% bodyweight did not influence intratendinous sliding and shear, which points in the direction of a load threshold. These results provide evidence that investigating the whole tendon as one functional entity fail to capture the complex intratendinous sliding and shear during loading, which could be an important aspect of tendon aetiology and infer basic information about the mechanics of tendon injury.

7.1 Perspectives

Both studies provide valuable information about tendon tissue responses to different modes of exercise in the healthy and injured Achilles tendon, but also give rise to further questions, which can hopefully be answered in future research.

The present thesis extends the knowledge of optimal conservative treatment of mid-portion Achilles tendinopathy and presents an alternative loading strategy to the traditional ECC training regime. However, scientifically proven treatment strategies are still limited and there are still many unanswered questions related to the management of tendinopathy. Most research groups[35, 41, 43, 45, 107, 108] including the RCT study in this present thesis, have used an intervention period of 3 months, but the effect of a prolonged intervention period of 6, 9 or 12 months remains unknown. Could the lack of complete recovery for a certain group of patients be related to a too short intervention period? In the present RCT study, all patients were considered as having chronic tendinopathy (<3months). However, this terminology is wide, as symptom duration ranged from 3 to 120 months. Is there a relationship between how long patients have had the symptoms and the ability to return fully rehabilitated? These aspects are important to consider when planning future studies in order to better understand tendon function and healing.

The conclusions made in previous section imply that loading is the key component in the treatment of Achilles tendinopathy, and thereby that 'optimal exercise loading' should be the primary focus for future research. The effect of loading parameters such as: type of loading, type of exercises, load magnitude, frequency, length of intervention and length of restitution periods between sessions, should therefore in future research be elaborated. Thus, the best way to proceed in order to investigate the influence of each individual parameter on tendon tissue adaptation is to test a single parameter at a time and match all other parameters in the intervention groups.

The speckle tracking method seems to be of future value for investigating tendon function during stretch and loading and might be a step toward understanding the aetiology of tendon injury, which may in turn lead to improved rehabilitation strategies. On the basis of the findings of this thesis, it would be interesting to investigate the effect of training on interfascicle sliding and shear in the Achilles tendon.

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9. PAPERS
9.1 Paper I

Study I

Heavy Slow Resistance Versus Eccentric Training as Treatment for Achilles Tendinopathy

A Randomized Controlled Trial

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Background: Previous studies have shown that eccentric training has a positive effect on Achilles tendinopathy, but few randomized controlled trials have compared it with other loading-based treatment regimens.

Purpose: To evaluate the effectiveness of eccentric training (ECC) and heavy slow resistance training (HSR) among patients with midportion Achilles tendinopathy.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A total of 58 patients with chronic (>3 months) midportion Achilles tendinopathy were randomized to ECC or HSR for 12 weeks. Function and symptoms (Victorian Institute of Sports Assessment–Achilles), tendon pain during activity (visual analog scale), tendon swelling, tendon neovascularization, and treatment satisfaction were assessed at 0 and 12 weeks and at the 52-week follow-up. Analyses were performed on an intention-to-treat basis.

Results: Both groups showed significant ($P < .0001$) improvements in Victorian Institute of Sports Assessment–Achilles and visual analog scale from 0 to 12 weeks, and these improvements were maintained at the 52-week follow-up. Concomitant with the clinical improvement, there was a significant reduction in tendon thickness and neovascularization. None of these robust clinical and structural improvements differed between the ECC and HSR groups. However, patient satisfaction tended to be greater after 12 weeks with HSR (100%) than with ECC (80%; $P = .052$) but not after 52 weeks (HSR, 96%; ECC, 76%; $P = .10$), and the mean training session compliance rate was 78% in the ECC group and 92% in the HSR group, with a significant difference between groups ($P < .005$).

Conclusion: The results of this study show that both traditional ECC and HSR yield positive, equally good, lasting clinical results in patients with Achilles tendinopathy and that the latter tends to be associated with greater patient satisfaction after 12 weeks but not after 52 weeks.

Keywords: Achilles tendon; tendinopathy; eccentric training; heavy slow resistance training

Tendon tissue is uniquely designed to withstand considerable forces to produce joint movement, and it may at times see loads exceeding 9 kN.[8,14] However, when the tendon is exposed to repetitive high-magnitude loading, it can result in tendinopathy, which is a painful and disabling tendon injury that can persist for months to years.[13,18] The Achilles tendon is one of the largest and strongest tendons in the body,[11] yet it is frequently afflicted by tendinopathy. The incidence of tendon injuries has been estimated to be as high as 30% to 50% of all sports injuries, 50% among elite endurance runners, and 6% among sedentary people.[17,19] In fact, it has been reported that Achilles tendinopathy is the clinical diagnosis in 55% to 65% of all Achilles tendon disorders.[11]

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The exact cause of Achilles tendinopathy and other similar injuries remains elusive, which may be one reason for the lack of an apparent evidence-based best treatment practice. Histopathologic data and recent gene expression data indicate that chronic tendinopathy is not an inflammatory condition but rather a result of a failed healing process that causes degenerative changes of the hierarchical tendon structure, neovascularization, and nerve ingrowth.[22,30] The associated pain is due to the neovascularization and nerve ingrowth in the tendon[26,27] and is therefore a useful parameter in the diagnosis and subsequent clinical monitoring.

Research on the treatment of Achilles tendinopathy is somewhat scarce despite its prevalence and that it represents a major clinical problem. In the past decade, loading-based treatment in the form of eccentric training (ECC) has become the principal nonsurgical choice of treatment for Achilles tendinopathy,[3] although there is no convincing evidence that it is the most effective exercise regimen. In fact, a recent systematic review concluded that there is little clinical or mechanistic evidence that supports using the eccentric component alone and that well-conducted studies comparing different loading programs are largely lacking.[21] Nevertheless, it seems that loading itself yields positive clinical, structural, and biochemical effects with respect to tendinopathy.[1,15,16,20,39] However, Achilles tendinopathy remains challenging to manage successfully, and as many as 45% may not respond to eccentric exercises,[36] which may be related to lack of knowledge about the effect of loading parameters: load progressions, load magnitude, frequency (sets and repetitions), and restitution between treatment sessions. New loading-based exercise regimens—such as isolated concentric training,[20] heavy slow resistance training (HSR),[15] and eccentric-concentric progressing to ECC[37,39]—have been suggested but lack firm scientific evidence for their efficacy in Achilles tendinopathy. It was recently shown for patellar tendinopathy that HSR performed 3 times weekly yielded superior long-term results compared with the traditional eccentric loading regimen.[15] However, the effect of HSR in Achilles tendinopathy has never been investigated. On the basis of the aforementioned findings, we hypothesized that HSR would yield a more favorable clinical outcome compared with ECC. Therefore, we sought to investigate the effect of a 12-week HSR regimen compared with the traditional eccentric loading regimen in patients with midportion Achilles tendinopathy in a randomized controlled trial with a 52-week follow-up.

METHODS

Study Design

The study was designed as a prospective randomized single-blind controlled trial with a 12-week intervention period and a subsequent 52-week follow-up period from July 2009 through October 2012. A total of 58 recreational athletes (32 men, 15 women; age range, 18-60 years) with a diagnosed chronic unilateral midportion Achilles tendinopathy were included in the study. Patients were recruited from the Institute of Sports Medicine, Bispebjerg Hospital, Copenhagen, Denmark. An experienced sports medicine physician determined the diagnosis on the basis of defined clinical findings (Victorian Institute of Sports Assessment–Achilles [VISA-A] and visual analog scale [VAS]), physical examination, and pain duration of at least 3 months. In addition, the following ultrasonography findings needed to be present: local anterior-posterior (A-P) thickening of the midtendon level with a hypoechoic area and a color Doppler signal within the hypoechoic area.[15] Exclusion criteria were as follows: <4-week washout period from any other treatments, corticosteroid injections in the previous 12 months, bilateral Achilles tendinopathy, insertional Achilles tendinopathy, systemic disease (eg, rheumatoid arthritis, diabetes), any surgery, or any confounding lower limb and ankle injury.

TABLE 1
Patient Characteristics at Baseline[a]

Variable	ECC (n = 25)	HSR (n = 22)
Sex, n (male:female)	18:7	14:8
Age, y	48 ± 2 (31-60)	48 ± 2 (31-60)
Height, cm	179 ± 2 (164-196)	178 ± 2 (164-195)
Weight, kg	81 ± 2 (62-96)	81 ± 3 (65-112)
Body mass index, kg/m^2	25 ± 1 (21-35)	26 ± 1 (18-40)
Symptom duration, mo	19 ± 5 (3-120)	17 ± 3 (3-80)
Activity level before injury, h/wk	5 ± 1 (0-16)	5 ± 1 (2-11)

[a]Values are reported as mean ± SEM (range) unless otherwise indicated. There were no differences between groups for any parameter at baseline. ECC, eccentric training; HSR, heavy slow resistance training.

A sample size calculation was performed a priori on the basis of the primary outcome of VISA-A. A total of 18 patients was needed in each group to establish a clinically significant mean difference of 10 points (maximum score, 100 points) in the VISA-A score, with 80% power and an alpha level of .05. To account for dropouts, we sought to include a total of 25 patients in each group. The total of 58 patients was randomly allocated into 1 of the 2 intervention groups—ECC (n = 30) and HSR (n = 28)—using a computer-generated minimization randomization procedure.[12] The minimization procedure was based on activity level, symptom duration, and age. The subject characteristics are shown in Table 1, and there were no significant differences between the 2 groups (unpaired Student *t* tests). The study complied with the Declaration of Helsinki, was approved by the local human ethics committee for medical research (KF256131), and was registered at ClinicalTrials.gov (NCT00952042). All patients gave their informed consent before experiment.

Treatment Intervention

The eccentric loading program (ECC) was performed according to the protocol previously described,[3] and it included 3 sets of 15 slow repetitions of eccentric unilateral loading while standing on the step of a staircase. One

Figure 1. Depiction of applied heavy slow resistance exercises: (A) heel rises with bended knee in the seated calf raise machine, (B) heel rises with straight knee standing on a disc weight with the forefoot with the barbell on shoulders, (C) heel rises with straight knee in the leg press machine. All exercises are performed bilateral with equal weight on both legs.

exercise is performed with straight knees and one with bent knees, twice a day (morning and evening), 7 days a week, for 12 consecutive weeks. Patients were instructed to spend approximately 3 seconds completing each repetition and to have a 2-minute rest period between sets and a 5-minute rest period between the 2 exercises. All patients were instructed by a trained physical therapist on how to perform the 2 exercises, and they were given instructions and a written manual on how to progress. Load was increased gradually using a loaded backpack as pain diminished.

The HSR program previously described[15] was performed 3 times per week using resistance equipment in a fitness center. Each session consisted of three 2-legged exercises: heel rises with bended knee in the seated calf raise machine, heel rises with straight knee in the leg press machine, and heel rises with straight knee standing on a disc weight with the forefoot with the barbell on shoulders (Figure 1). The patients completed 3 or 4 sets in each exercise with a 2- to 3-minute rest between sets and a 5-minute rest period between the 3 exercises. The number of repetitions decreased, and load gradually increased, every week as the tendon got stronger. The repetitions and loads were as follows: 3 times, 15-repetition maximum (15RM), in week 1; 3 times, 12RM, in weeks 2 to 3; 4 times, 10RM, in weeks 4 to 5; 4 times, 8RM, in weeks 6 to 8; and 4 times, 6RM, in weeks 9 to 12. All exercises were performed in the full range of motion of the ankle joint, and patients were instructed to spend 3 seconds completing each eccentric and concentric phase (ie, 6 seconds per repetition). All patients were instructed by a trained physical therapist on how to perform the 3 exercises, and they were given instructions and a written manual on how to progress.

The main difference between the 2 exercise regimens is the total loading time “seen” by the tendon and the calculated session time, which includes rest periods. The time of tendon loading was estimated to be approximately 63 min/wk for ECC and 41 min/wk for HSR. The session time was 308 min/wk for ECC and 107 min/wk for HSR. Note that the estimated time for the HSR regimen represents only the first week of the protocol since repetitions and sets decrease over the intervention period.

To improve compliance, information manuals were provided to all patients in both groups. Furthermore, a follow-up supervision session was carried out by a trained physical therapist for all patients 1 week after intervention start. All patients were required to keep a standardized training diary from weeks 0 to 12, in which they documented every training session. The training record included number of repetitions, sessions, and load, and it was used to assess compliance and training progression.

Patients in both groups were not allowed to engage in sporting activities in the initial 3 weeks of the intervention period, in an attempt to minimize their risk of symptom exacerbation while adjusting to the exercise regimen. Subsequently, the patients were allowed to engage in sporting activities throughout the intervention period, provided that these could be performed with a discomfort not exceeding 30 mm on the VAS. While performing the intervention exercises, the patients were allowed to reach 40 to 50 mm on the VAS, but pain should have subsided by the following training session. If pain in the tendon had not subsided, the patient was advised to adjust the load of the program and/or one’s daily living or sporting activities. A similar approach to pain management was included successfully by other randomized controlled trials in the management of Achilles tendinopathy.[15,39] All patients were instructed to refrain from taking anti-inflammatory drugs during the intervention. When the intervention period ended, the patients did not receive any further treatment or guidelines but were encouraged to maintain the obtained activity level.

Clinical Evaluation

All patients completed a written VISA-A questionnaire to assess the symptoms, function, and pain during sporting activities. The questionnaire consists of 8 questions in which the patients rate the magnitude of pain during rest, function, and activity. The maximum score is 100 points, and a lower score indicates more symptoms and a greater limitation of function and activity. The VISA-A questionnaire has been shown to be a valid and reliable outcome measure for patients with Achilles tendinopathy.[33] Improvement on the VISA-A >10 points is considered clinically significant.[35] In addition, the pain level in the tendon was assessed during 5 heel rises on stairs (VAS_H) and during running (VAS_R) and indicated on a 100-mm VAS. Patients completed the VISA-A questionnaire and VAS with a trained physical therapist at weeks 0 and 12 and at the 52-week follow-up. The VISA-A score was the primary clinical outcome measure of this study. The activity level of sporting activities (h/wk) was documented: before injury, at weeks 0 and 12, and at the 52-week follow-up. At weeks 12 and 52, the patients indicated

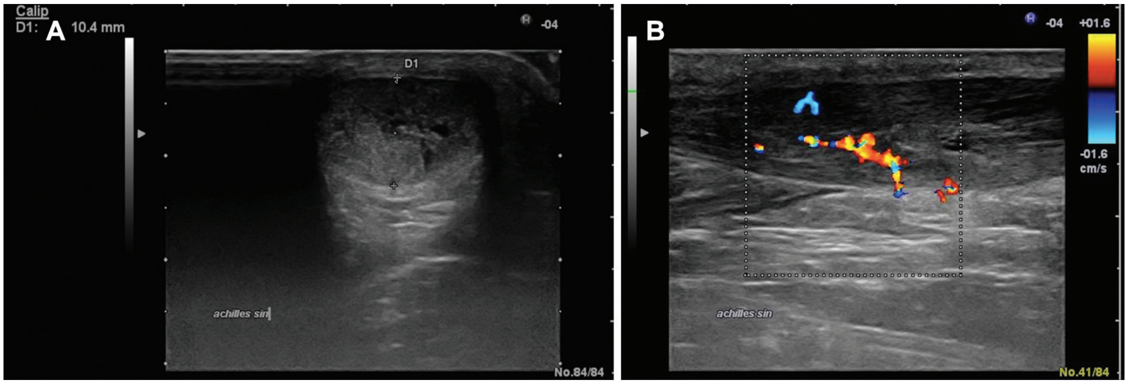

Figure 2. Illustration of ultrasonography assessments. (A) Assessment of anterior-posterior tendon thickness. (B) Color Doppler activity.

whether they were satisfied with the result of the treatment as part of the questionnaire.

Ultrasonography Measurements and Doppler Evaluation

Ultrasonography was performed on the injured Achilles tendon using a Hitachi Ascendus with a EUP-L75 18-MHz linear-array transducer (GE Medical Systems). Patients were examined in a prone position with the feet hanging free in a neutral position over the end of the table. All patients were instructed to avoid physical activity 24 hours before the examination. Grayscale and color Doppler settings (Figure 2) were identical for all examinations.

Grayscale examination was performed with a depth of 2.0 cm: auto optimizing [AO] = 100%, depth resolution = 84, and gain = 53). The tendon was scanned longitudinally and transversally to get a 3-dimensional impression of the tendon structure and its rotation. When the thickest point of the tendon was identified, the A-P distance was measured in a transversal scan, and the epitendon and paratendon were not included (Figure 2A). The measurement was standardized according to the method previously described.[9] The mean of 3 A-P thickness measurements of each image was used for analysis.

The color Doppler scans were obtained with a visual thin layer of gel between the transducer and the skin. The investigator applied minimum transducer pressure during scanning to demonstrate as much flow as possible. Color Doppler settings were optimized for low flow: frequency = 7.5 MHz (gain just below random noise level), AO = 100%, pulse repetition frequency = 0.4 kHz, and wall filters = 48 Hz. A standardized color box of 2.5 × 2.5 cm was positioned at the midtendon area that had the highest color Doppler activity (Figure 2B), according to the method previously described.[15] The scans were recorded as a 4-second sine loop in the sagittal plane with the highest color Doppler activity. Three 4-second sine loops were recorded, each containing 86 single images. From each sine loop, the image with the highest amount of Doppler activity was selected and saved for subsequent analysis. All analyses were conducted by the same researcher to avoid interindividual variations in the results; each sine loop was analyzed 3 times; and the image with the highest amount of Doppler activity was chosen for further statistical calculations. Activity was quantified as Doppler color fraction (ie, as the total number of colored pixels within the region of interest using a custom-made macro) in the software program ImageJ (v 1.48; National Institutes of Health). The Doppler color fraction measurement (%) was used for analysis. One investigator conducted all analyses in blinded fashion.

Data Reduction and Statistical Analysis

Forty-seven patients completed the intervention period (ECC, n = 25; HSR, n = 22). Five patients withdrew from the ECC group (1 with ankle pain, 2 with back pain, and 2 because of lack of time). Six patients withdrew from the HSR group (1 with ankle pain, 1 with back pain, 2 because of lack of time, and 2 who moved away). Forty-four patients attended the 1-year follow-up control (ECC, n = 24; HSR, n = 20). One patient in the ECC group sustained a partial Achilles tendon rupture during sports participation (badminton), and 2 patients did not show up for the 52-week follow-up. Four patients (2 in each group) did not return the training diary. The data were analyzed with the intention-to-treat approach, with the last observation carried forward.

Baseline characteristics were analyzed with unpaired Student t tests. Outcome measures were analyzed using 2-way analysis of variance (treatment × time) with repeated measures with Bonferroni-adjusted post hoc tests when appropriate. Results are reported as mean ± SEM and 95% CIs.

Patient satisfaction at 12 and 52 weeks and patient activity level at 0, 12, and 52 weeks were analyzed using Fisher exact tests. Patient compliance was analyzed with unpaired Student t tests. A Pearson correlation coefficient was employed to examine if changes in color Doppler activity over time were related to changes in VISA-A score over time.

RESULTS

All data are shown in Table 2. For VISA-A, there was a significant effect of time ($P < .0001$), but there was no

TABLE 2
Clinical and Sonographic Results[a]

	ECC					HSR				
	0 wk	12 wk	52 wk	Δ (%), 0-12 wk	Δ (%), 0-52 wk	0 wk	12 wk	52 wk	Δ (%), 0-12 wk	Δ (%), 0-52 wk
VAS–running[b]	49 ± 5.5 (38.3, 60.1)	20 ± 5.7 (9.3, 31.5)	12 ± 4.2 (3.2, 19.8)	29 ± 5.1 (18.9, 38.8)	38 ± 6.2 (25.6, 49.9)	54 ± 5.4 (43.3, 64.3)	17 ± 4.1 (9.3, 25.2)	5 ± 2.6 (–0.5, 9.8)	37 ± 6.7 (23.4, 49.8)	49 ± 7.0 (35.5, 62.8)
VAS–heel rises[b]	19 ± 5.0 (8.8, 28.6)	12 ± 3.6 (4.8, 18.9)	6 ± 2.6 (0.9, 11.0)	7 ± 3.9 (–0.8, 14.5)	13 ± 5.9 (1.3, 24.3)	29 ± 5.5 (17.7, 39.2)	7 ± 2.4 (2.1, 11.7)	5 ± 2.5 (–0.2, 9.4)	22 ± 5.5 (10.8, 32.3)	24 ± 5.7 (12.7, 35.0)
VISA-A[b]	58 ± 3.9 (50.6, 65.8)	72 ± 3.7 (64.7, 79.3)	84 ± 3.5 (78.0, 91.9)	–14 ± 2.5 (–18.8, –8.8)	–27 ± 4.5 (–35.6, –18.0)	54 ± 3.2 (48.6, 61.6)	76 ± 3.7 (70.5, 83.1)	89 ± 2.8 (83.6, 94.8)	–22 ± 2.7 (–26.9, –16.4)	–34 ± 3.9 (–41.8, –26.5)
A-P, mm[b]	8.3 ± 0.3 (7.7, 9.0)	8.1 ± 0.4 (7.4, 8.8)	7.3 ±0.3 (6.8, 7.9)	0 ± 0.1 (0, 0.5)	1.0 ± 0.3 (0.5, 1.5)	8.6 ± 0.5 (7.7, 9.5)	7.9 ± 0.4 (7.3, 8.8)	6.9 ± 0.3 (6.4, 7.4)	0.6 ± 0.2 (0.2, 0.9)	1.7 ± 0.3 (1.1, 2.4)
Doppler, %[c,d]	2.8 ± 0.6 (1.7, 3.7)	2.8 ± 0.5 (1.8, 3.8)	1.6 ± 0.5 (0.6, 2.6)	0 ± 0.4 (–0.9, 0.8)	4.0 ± 0.9 (2.7, 6.1)	4.0 ± 0.8 (2.5, 5.8)	2.0 ± 0.5 (1.0, 2.9)	1.0 ± 0.4 (0.1, 1.8)	2.3 ± 0.8 (0.8, 3.7)	1.1 ± 0.4 (0.2, 1.9)

[a]Values are presented as means ± SEM (95% CI). Significance based on 2-way analysis of variance; significant difference between groups set at $P < .01$. A-P, anterior-posterior thickness of the midtendon; Doppler, color Doppler fraction (ie, total number of colored pixels within the region of interest); ECC, eccentric training; HSR, heavy slow resistance training; VAS, visual analog scale; VISA-A, Victorian Institute of Sports Assessment–Achilles; 0 wk, baseline; 12 wk, postintervention; 52 wk, follow-up; Δ (%), relative change in time interval.
[b]Significant effect of time, $P < .0001$.
[c]Significant effect of time, $P < .005$.
[d]Significant treatment interaction, $P < .01$.

TABLE 3
Patient Activity Level at Weeks 0, 12, and 52[a]

Activity Level, h/wk	ECC (n = 25)	HSR (n = 22)
Wk 0	2 ± 1 (0-16)	2 ± 1 (0-8)
Wk 12	3 ± 1 (0-14)	4 ± 1 (1-10)
Wk 52	4 ± 1 (0-12)	5 ± 1 (2-20)

[a]Values are reported as mean ± SEM (range). There was a significant effect of time ($P < .05$) but no differences between groups or interaction. ECC, eccentric training; HSR, heavy slow resistance training.

significant interaction ($P = .26$) or difference between groups ($P = .62$), indicating that both treatments yielded similar improvements from 0 to 52 weeks. For VAS_H and VAS_R, there was a significant effect of time (VAS_H, $P < .0001$; VAS_R, $P < .0001$), but there was no significant interaction (VAS_H, $P = .08$; VAS_R, $P = .38$) or difference between groups (VAS_H, $P = .77$; VAS_R, $P = .71$), indicating that both treatments yielded similar improvements in pain from 0 to 52 weeks.

Tendon A-P thickness decreased significantly with time ($P < .0001$), but there was no difference between groups or treatment interaction. Tendon color Doppler area also decreased significantly with time ($P < .005$), and the interaction was significant ($P < .01$), but there was no difference found between groups (Table 2). The Doppler color fraction did not correlate significantly with the VISA-A score over time (0-12 weeks) in the HSR group ($r = 0.008$, $P = .95$) or the ECC group ($r = 0.14$, $P = .49$).

For activity level, there was a significant effect of time ($P < .05$), but there was no significant interaction ($P = .43$) or difference between groups ($P = .16$) (Table 3). The mean activity level during the 12-week intervention period was not different from the baseline level for either group.

The mean training session compliance rate was 78% in the ECC group and 92% in the HSR group, with a significant difference between groups ($P < .005$). The patient satisfaction with the clinical outcome at 12-week follow-up was 20 of 25 for ECC (80%) and 22 of 22 for HSR (100%; $P = .052$ between groups). At 52-week follow-up, 19 of 25 ECC patients (76%) and 21 of 22 HSR patients (96%) were satisfied ($P = .10$).

DISCUSSION

The main finding of the present study was that both the traditional ECC and the HSR yielded a positive clinical result in patients with Achilles tendinopathy in both the short- and long-term ranges. There was a pronounced improvement in physical activity level and pain during sporting activities (VAS_R, VAS_H, and VISA-A) in both groups. Concomitant with the clinical improvement, there was a reduction in A-P thickness and neovascularization as measured with color Doppler. None of these robust clinical and structural improvements differed between the ECC and HSR groups. However, patient satisfaction tended to be greater after 12 weeks with HSR (100%) than ECC (80%) but not after 52 weeks (HSR, 96%; ECC, 76%).

The effectiveness of a loading regimen for the treatment of Achilles tendinopathy is supported by the fact that there is often considerable improvement in pain and function.[3,20,23,35,39] In the current study, the patients had baseline VISA-A and VAS scores comparable with those previously reported in patients with Achilles tendinopathy.[6,20,39,40] Over the course of the 12-week intervention period, the VISA-A score improved by >10 points, and the VAS_R score was reduced by $\geq$30 points (mm) in both groups on average. These are clinically meaningful improvements and, moreover, corroborate previous reports of the effect of loading regimens on tendinopathy.[15,34,39-41]

After the 12-week intervention, the mean VISA-A score was 74 for all patients (ECC, 72; HSR, 77) after having undergone a loading-based exercise regimen alone, and this increased to 87 (ECC, 85; HSR, 89) after 52 weeks. So although clinical progress took place there, symptoms and decreased function remain 1 year after the end of intervention. A recent study by Silbernagel et al[38] demonstrated an average VISA-A score of 90 at a 5-year follow-up, with 20% of patients still having symptoms, which indicates that patients may not necessarily fully recover. The reason for the lack of complete recovery for patients is unknown. In the present study, it was not possible to obtain a tendon biopsy specimen as in other studies,[15] which may have shed light on the effect that the treatment may have had on the biochemical and structural composition of the tendon.

It was recently reported that both HSR and ECC yield good clinical effects in patients with patellar tendinopathy and that long-term patient satisfaction with HSR exceeded that of ECC.[15] We therefore sought to compare these loading regimens in patients with Achilles tendinopathy, which have not been examined before to the best of our knowledge. The results of the current study suggest that HSR and ECC improve symptoms and physical activity level equally well in patients with chronic midportion Achilles tendinopathy.

Eccentric loading regimens for tendinopathy have been widely accepted as the treatment of choice. However, the direction of the movement of the entire muscle-tendon unit at a given load and range of motion should have little or no differential effect on the tendon. In fact, peak forces and tendon length changes are similar during concentric and eccentric contractions.[31] Moreover, eccentric and concentric contractions yield a similar expression of collagen,[10] indicating that the fibroblast is similarly affected. Finally, habitual training with concentric and eccentric contractions appears to produce similar tendon growth.[7] Thus, it is not entirely clear why avoiding the concentric component should produce a more favorable clinical outcome. Studies have been designed to address the question,[20,23] but none have controlled carefully for magnitude of load (ie, tendon elongation). Although the present study was not designed to answer the effect of contraction mode per se, it appears that HSR, which includes a concentric as well as an eccentric component, produced similar results as the traditional ECC regimen.

Treatment satisfaction is an important clinical outcome and likely represents several components. In the present study, 80% of ECC patients and 100% of HSR patients were satisfied after the 12-week intervention, and the corresponding values were 76% and 96% at the 52-week follow-up, respectively. The clinical outcome was the primary study goal, and we did not chiefly aim to assess patient satisfaction. Nevertheless, we find it interesting that HSR tended to result in a higher satisfaction among patients than ECC did. The reason for this remains unknown, but it appears to corroborate the findings of an earlier investigation of patients with patellar tendinopathy.[15] Moreover, patient satisfaction of the ECC regimen in the current study is akin to that previously reported.[6,20] A possible reason for the difference in patient satisfaction between ECC and HSR could be the time necessary to complete the 2 regimens: ECC requires two 22-minute training sessions per day, 7 days a week, for a total of 308 min/wk. The corresponding session time for HSR patients is three 36-minute training sessions per week, for a total of 107 min/wk, and this is a considerable difference in time allotment. The actual reason for the reduced compliance rate in the ECC group (78% vs 92% in HSR) is unknown, but is one aspect that may be considered when loading regimes are offered to patients.

In contrast to our data, prior studies[3,6,20] showed that ECC patients resumed their previous activity levels at the end of the 12-week intervention period. It is difficult to reconcile these differences, since the same eccentric loading regimen was used in these studies. However, it was recently shown that mild pain can persist up to 5 years despite the use of the ECC regimen[41]; therefore, it is not surprising that patients in the present study did not completely regain their activity levels even at the 52-week follow-up.

Ultrasonography is commonly used to image tendinopathy, and color and power Doppler mode can be used to assess neovascularization.[27] It has been suggested that neovascularization is present in tendinopathy and that the associated nerve endings are the cause of the pain.[2] In the literature, there is conflicting evidence whether a tendinopathic Achilles tendon normalizes A-P thickness and color Doppler area after the ECC training regimen.[5,24-26,29,34,41] In the present study, tendon A-P thickness was reduced in both groups with time, which agrees well with some prior reports.[24,26,41] Tendon color Doppler area also decreased with time (Table 2), alongside the clinical symptoms, but it did not seem to be appreciably affected by the loading regimen. This decline in color Doppler area in response to a loading regimen corresponds with previous reports.[15,26,41] The absolute values for Doppler activity were quite low (1.0%-4.0%) and similar to that reported by Boesen.[4] There was a significant time $\times$ treatment interaction between the 2 exercise regimens, but this is likely a function of the coincidental dissimilarity at baseline (ECC, 2.8%; HSR, 4.0%). It has been suggested that ultrasound Doppler does not always reveal neovascularization in Achilles tendinopathy patients, and not all tendons with neovascularization are symptomatic.[28,42] In the present study, the change in Doppler color fraction did not relate to the change in the VISA-A score over time in the HSR group (r = 0.008, P = .95) or ECC group (r = 0.14, P = .49). The lack of relationship between color Doppler activity and clinical symptoms is supported by some[28] authors but not all.[32]

There are some inherent limitations associated with the current study. As previously mentioned, it would have been desirable to obtain a tendon biopsy specimen to examine if the interventions influenced the collagen content, cross-link composition, and fibril composition, but this was not possible within the confines of the study. It could also be argued that strength testing would provide some information about the effect of the intervention. However, it is challenging to strength test a person with a painful condition, since the pain or injury itself would likely influence the result. An alternative method to examine the effect of the intervention on the muscular tissue would be to obtain magnetic resonance imaging to determine muscle hypertrophy, but this was not possible in the present study. Another drawback was the lack of registration regarding to what extent the patients continued the training program after the 12-week intervention period.

In conclusion, the results of this study do not support our hypothesis that HSR would yield a more favorable clinical outcome compared with the traditional ECC. The data show that ECC and HSR are effective in the treatment of chronic midportion Achilles tendinopathy and that the improvement achieved after 12 weeks of training lasts for 1 year, irrespective of exercise strength mode.

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9.2 Paper II

Study II

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ORIGINAL ARTICLE

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Speckle tracking in healthy and surgically repaired human Achilles tendons at different knee angles—A validation using implanted tantalum beads

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The exact injury mechanism of Achilles tendinopathy remains unknown, but sliding of fascicles relative to each other during loading may be an important factor. This study validated the motion of ultrasound speckles against actual tendon movement using tantalum beads as reference markers. In addition, the effect of different knee joint angles (ie, muscle activation) on tendon tissue shear and displacement during a single heel rise was investigated. The 10 male participants had tantalum beads inserted in the tendon during surgery for a unilateral Achilles tendon rupture at least 1 year prior to the study. Ultrasound speckle displacement in the tissue surrounding the bead correlated strongly with displacement of the bead ($R^2 \geq .9987$). Speckle tracking systematically underestimated the displacement of the tendon tissue with a typical error of 1.1%-2.7%. There was a significant difference in displacement between the superficial and deep tendon layer for the 3 exercises in the healthy, but not in the surgically repaired Achilles tendon. The displacement difference was significantly greater when performing heel rises with the knee flexed 100° compared to knee flexed 40°. In conclusion, speckle tracking appears to be a valid approach to investigate intratendinous displacement.

KEYWORDS
Achilles tendon rupture, deformation pattern, fascicle sliding, speckle tracking, tendinopathy, tendon shear

1 | INTRODUCTION

The free Achilles tendon has a complex architecture that consists of distinct fascicle bundles that arise from 3 separate muscles: the soleus and the medial and lateral gastrocnemii. The tendon displays a helical rotation in the medial direction toward its insertion on the calcaneal bone with the superficial fascicles arising from the medial gastrocnemius and deeper fascicles arise from the soleus and lateral gastrocnemius muscles.[1,2] It has been shown that non-uniform displacement of these distinct fascicles bundles can be observed when the Achilles tendon is exposed to load and stretch,[3,4] and that human tendon fascicles may function as independent structures with only minor force transmission between individual fascicles.[5] It has been suggested that interfascicle sliding serves as a protective mechanism against excessive strain.[6]

Loading of the Achilles tendon with the knee extended as well as flexed is commonly employed in rehabilitation regimes for Achilles tendinopathy.[7] It is well known that the contribution of the 2-joint gastrocnemii muscles to force production declines with increasing knee flexion angle and that this creates differential displacement at the level of the aponeurosis.[8,9] However, whether this shear at the level of the aponeurosis is transferred onto the free Achilles tendon remains unknown.

Interfascicle sliding in Achilles tendon has until recently not been quantifiable in vivo; however, ultrasonographic speckle tracking has made it possible to quantify the intratendinuous movement.[10] The speckle tracking method, which measures tissue displacement without using any anatomical landmarks as reference point, has been validated in vitro.[11] However, the method has never been validated in an in vivo model. In this study, we validate the speckle method in vivo using participants that had tantalum beads inserted in their Achilles tendon following rupture (in conjunction with a previous study). The beads were used as a reference point representing the true movement of the tendon. Secondly, we examine whether the knee joint angle and thereby differential muscle activation influence tissue displacement during a single heel rise. We hypothesized that altering the knee flexion angle would result in non-uniform tendon tissue displacement between the superficial and deeper layer of the tendon. Finally, we compared the intratendinuous movement in the healthy tendon to that of the surgically repaired one.

2 | MATERIALS AND METHODS

2.1 | Participants and study design

Ten males (age 23-53 years, mean: 35 years) with previous unilateral surgically treated Achilles tendon rupture were investigated using ultrasound speckle tracking. The first part of the study was a within-subject comparison where tendon tissue displacement in the surgically repaired and healthy tendons was compared and the second part a within leg comparison of 3 different heel-rise exercises. Participants were recruited >52 weeks post-surgery and had all undergone a standardized suture repair (a.m. Kessler) and rehabilitation. In addition to the tendon suture, tantalum beads (0.8 mm diameter) were implanted into the tendon tissue in the areas proximal and distal to the rupture (1-2 beads at each site). At 52 weeks post-surgery, all participants were well rehabilitated with a moderate activity level (1-7 hour/wk sporting activities) and were able to perform single heel rises without any pain or dysfunction. Exclusion criteria were as follows: the presence of mid-tendon or insertional tendinopathy during the last 12 months, corticosteroid injections during the last 12 months, confounding lower limb and ankle injuries, rheumatic disease or diabetes or any systemic diseases affecting tendon tissue properties or composition. In addition, the participants were required to have: no heavy loading on the tendon 24 hour before testing, no pain or discomfort performing a single-leg heel rise, full ROM in the ankle joint, no history of partial or total Achilles tendon rupture on the non-injured leg. Participants were recruited from October 2015 through February 2016 following a larger ongoing study investigating rehabilitation of the Achilles tendon ruptures at the Institute of Sports Medicine, Bispebjerg Hospital, Copenhagen. The study complied with the Declaration of Helsinki and was approved by the local human ethics committee for medical research (H-3-2012-060).

2.2 | Experimental setup

2.2.1 | Ultrasonography

Ultrasound recording was performed on an HI Vision Ascendus machine (Hitachi Medical Systems, Japan), with a 10 MHz linear B-mode array transducer; 38 mm probe (14-6). A custom-made rigid holder was designed to fixate the ultrasound transducer in a set position. A rectangular reusable acoustic standoff pad (10 × 100 × 150 mm; Civco, Kalona, IA, USA) was placed between the transducer and tendon to ensure optimal contact. Additional ultrasound gel was added on both sides of the standoff pad, and possible wrinkles were flattened to avoid artifacts.

2.2.2 | Joint angle measurement

Ankle and knee joint movement were measured with electrical goniometers (inline mechanical goniometer; Noraxon Inc., USA), sampled through a wireless transmitter (8-channel, TeleMyo 2400T G2, Telemetry System; Noraxon Inc.) and a PC-interface receiver (TeleMyo PC-Interface receiver; Noraxon Inc.). Joint angle measurements were visualized onscreen with corresponding software (MyoReseachXP Master edition 1.07; Noraxon Inc.) to ensure adequate range of motion throughout the experiment and to standardize the subsequent comparative analysis with tendon movement. The goniometer sensors were accurately placed at the axis of rotation for the ankle joint (lateral malleolus) and knee joint (lateral femur condyle) and fastened with tape. To ensure corresponding joint measurements, the electrical goniometer data were compared to manual measurements at different joint angles prior to all trials. Joint angle data and ultrasound images were synchronized with a trigger device, allowing the previous 10 seconds of recording to be stored upon activation.

2.3 | Experimental procedure

Heel-rise exercises were performed at 3 different knee angles: standing with fully extended knee (0°), standing with flexed knee (40°), and seated on a chair (100° flexed knee angle), to differentiate between the contribution from the gastrocnemius and soleus muscles (Figure 1). Standing exercises were performed with full body weight (single-leg heel rise), and the seated exercise was performed with 15 kg weight placed on the knee, to provide adequate resistance

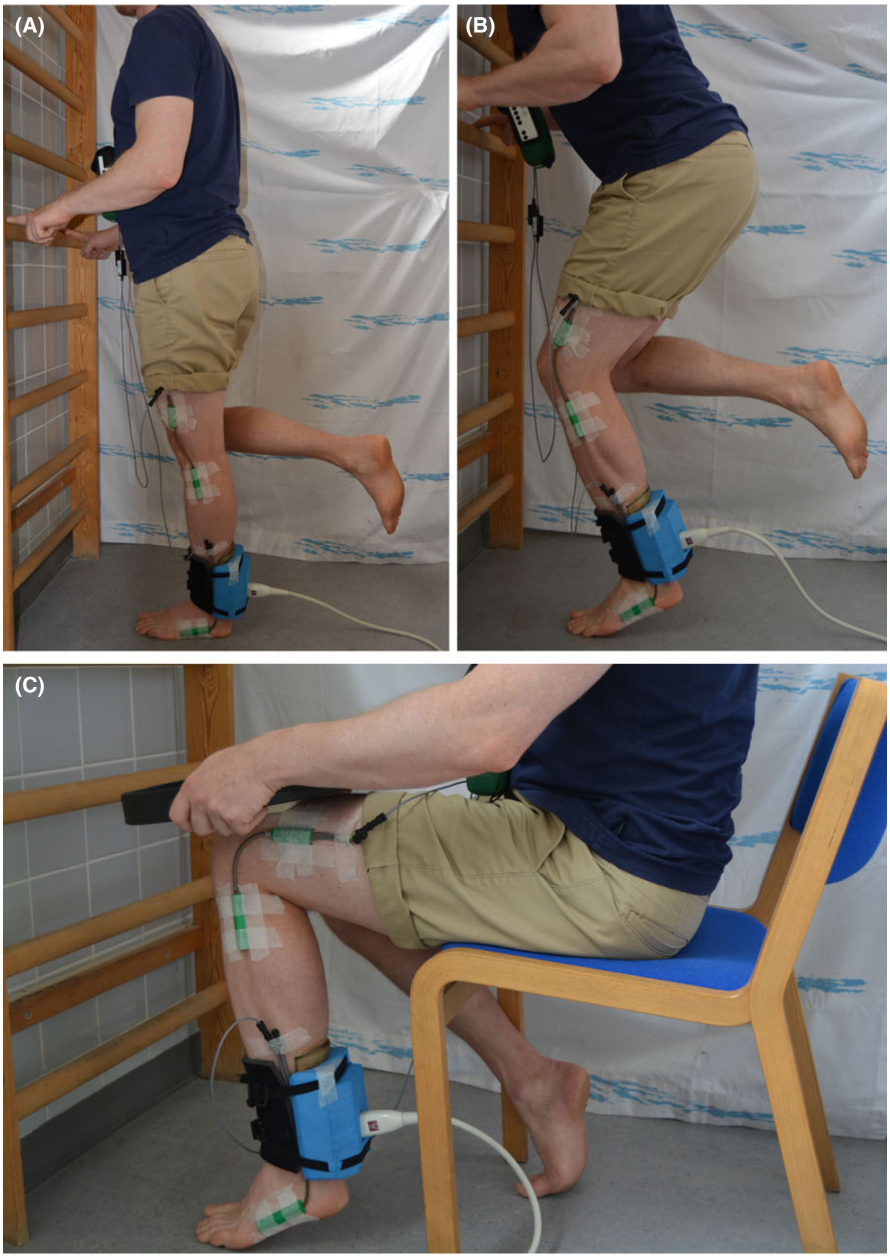

FIGURE 1 The 3 exercises are illustrated: (A) standing with straight knee, (B) standing with 40° flexed knee, and (C) seated on chair with 100° flexed knee

during the single-leg seated heel rise. Although this weight is substantially less than bodyweight, it was determined prior to the experiment that 15 kg was on average the greatest load that the participants could lift consistently throughout the test at this disadvantageous knee angle. Heel rises were targeted at 20° of plantar flexion from the starting position. A greater range of motion would cause the tendon to move out of view in the ultrasound recording.

Prior to testing, participants performed 5 minutes walking on a treadmill (4.5 km/h) for tendon preconditioning. Ultrasound speckle imaging of the Achilles tendon was conducted with the patient standing bare feet on the floor. To avoid fatigue, there were breaks (~5 minutes) between trials and a larger break (~15 minutes) between testing the 2 legs. None of the participants reported that pain impaired their performance or hindered them in completing the present protocol. Two investigators performed the ultrasound measurements, and they were randomly assigned to the trials.

Heel rises were performed cyclically with a 3-second concentric muscular contraction immediately transitioning into 3-second eccentric extension and repeated upon conclusion. Participants were instructed to perform as continuous and smooth heel-rise motion as possible. The investigator provided real-time feedback to the patient to maintain the desired pace, height and knee angle. To aid balance without affecting tendon loading, participants were allowed to touch a support with their fingertips. Participants performed a couple of trials for familiarization prior to data collection. Two measurements were made at each test position.

2.4 | Ultrasound speckle imaging

Ultrasound speckle imaging was collected at 42 frames/s with a resolution of 21.5 pixel/mm. B-mode examination was performed with a depth of 2.25 cm, gain = 30 and focus set individually to optimize quality. The transducer was manually positioned in the selected area and aligned with tendon fascicles and fixed with a custom-made cuff (Figure 2). The surgically repaired tendon was examined first, and the most proximal tantalum bead was located. The distance from the proximal bead to the musculotendinous junction was used as landmark to approximately localize the same area on the contralateral non-injured tendon. The probe was placed with the bead at the distal side of the image, and the recording was visually reviewed after each test to ensure minimal artifacts and that the bead remained in view throughout the recording. At least 1 recording for each of the 2 × 3 trails for each participant met the required criteria and was included in the post-process analysis.

2.5 | Post process speckle image analysis

A custom speckle tracking algorithm was written in MatLab (MatLab R_ 2014b; Mathworks, USA) and used to analyze speckle motion. The algorithm is based on cross-correlation similar to previous reports.[10,11] Subpixel precision was achieved by a parabolic fit to the cross-correlation matrix in a 1-pixel vicinity of the peak point. Tracking parameters were standardized for all recordings.

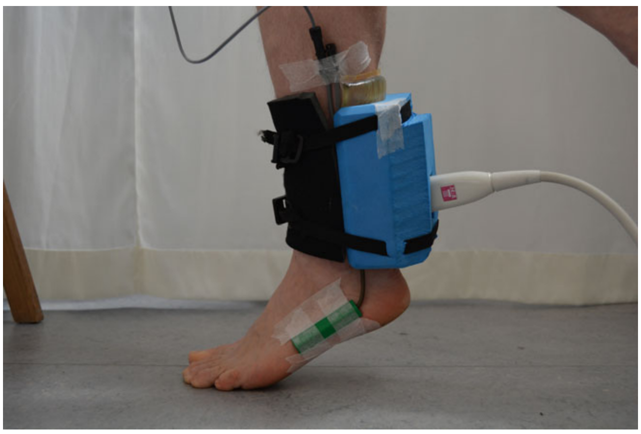

FIGURE 2 The experimental setup. The transducer fixed with a custom-made cuff onto the tendon

2.5.1 | Validation analysis of the speckle method in vivo

A tracking node was placed on the bead, and 3 tracking nodes were placed in the tendon tissue to the left, right, and above the bead (Figure 3A). A bottom node was excluded due to shadows cast by the bead. Displacement of the tantalum bead was compared to the displacement of the 3 boxes tracking on tendon speckles. Around each node, a template box 25 pixels high and 61 pixels long was used for frame-to-frame cross-correlation. Between frames, a matching template box was sought in 5 × 17 pixels around the node, and the dimensions were chosen based on trial tracking. Often, feature tracking algorithms update the template region in every frame; however, this can lead to propagating errors if a poor match is made as subsequent frames will attempt to match the new "incorrect" template box. To ameliorate this issue, the template box was only updated with a fraction of each new frame such that 90% of the intensities in the previous template region were retained and only 10% of the new intensities were added. This approach was inspired by the "evolution rate" in the freeware-tracking program: *Tracker* (http://physlets.org/tracker/).

2.5.2 | Speckle tracking on the healthy tendon and surgically repaired tendon

A standardized region of interest (ROI) was defined and manually positioned over the distal part of the tendon (Figure 3B,C). The ROI was standardized in length (14 mm) but adjustable in height to adapt to the increased thickness on the surgically repaired tendon. A grid of 32 nodes (4 along the width and 8 along the length of the tendon) was generated inside the ROI. The same investigator performed all the speckle image analysis using a standard protocol: (a) The ROI box must not move outside of the image window or connect to the peritendinous tissue throughout the analysis. (b) The position of each row of tracking points should return to the starting position over the movement cycle. If the difference between the start and end point exceeded 1 mm (Figure 4A,B), the recording was tracked in reverse to verify whether the difference was due to accumulating error in the tracking, in which case the deviation would be reversed in the reverse tracking. If the deviation reversed, the recording was discarded but if it remained the recording was kept and the deviation considered representing either a movement of the probe or an incomplete movement cycle by the participant. Displacements of the 4 tendon layers were quantified for each recording, and all speckle data were synchronized and merged with the goniometer data from the ankle joint. Two measurements were made at each test position, and the average was

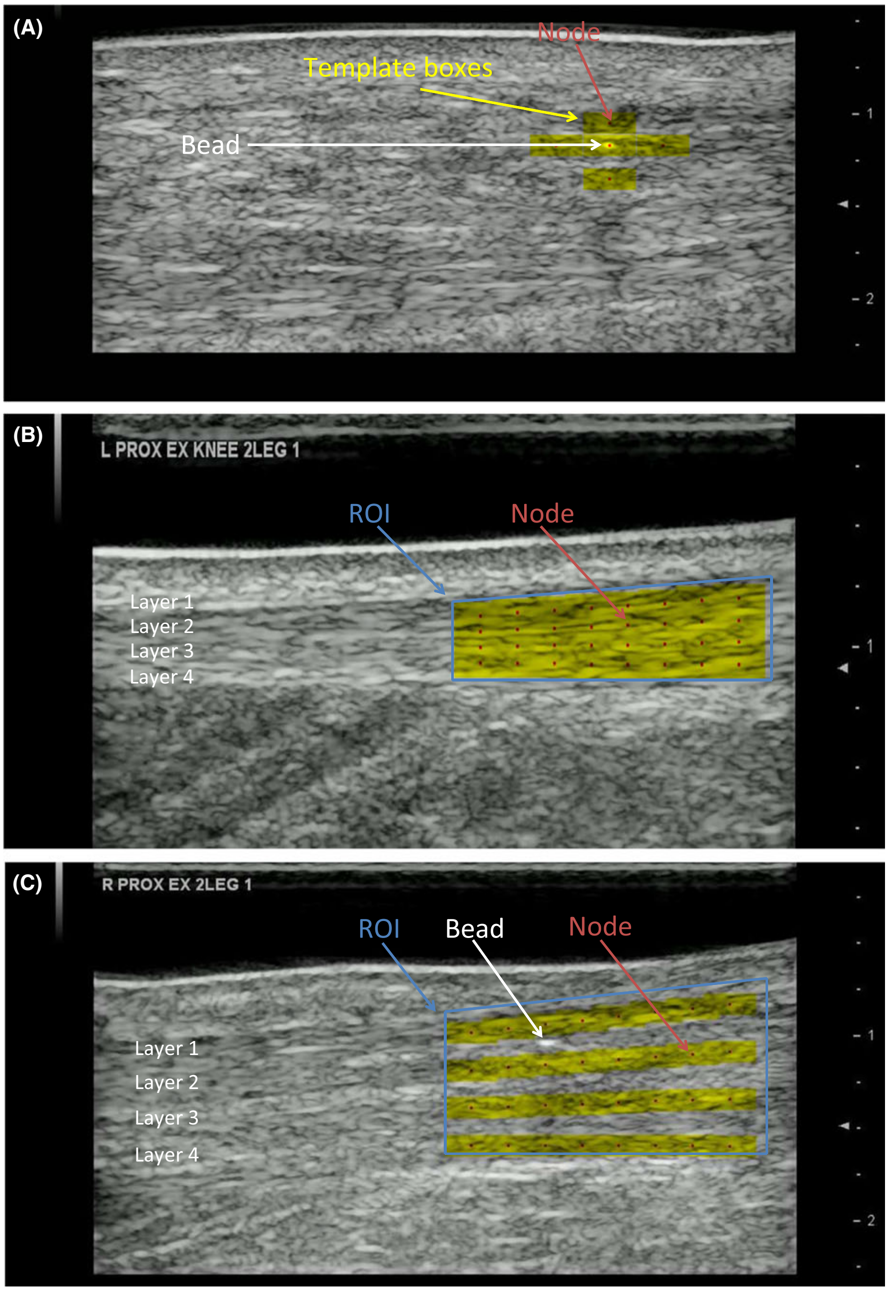

FIGURE 3 A, Validation analysis of the speckle method. B, C, Speckle tracking on healthy- and surgically repaired tendon tissue. The region of interest (ROI) box includes 4 layers with the superficial layer (layer 1) and the deepest layer (layer 4). Each layer includes 8 tracking nodes that follow tendon tissue movement during the single-leg heel rise

calculated. Not all trials achieved the desired 20° ankle motion, and consequently, values were analyzed at a common cutoff point of 17.5°.

2.6 | Statistical analysis

We quantitatively compared the displacement between the Tantalum bead and each of the 3 speckle boxes for each participant. A paired *t* test was performed to assess differences in absolute values between the bead and the 3 template boxes. The correlation coefficient (R^2) and typical error (%) were calculated to analyze the validity of the speckle tracking method. Reproducibility of speckle tracking was assessed by the same parameters in repeated tracking of the same recording, as well as tracking of 2 separate recordings of the same exercise. Data are presented with mean and standard deviation (SD) unless otherwise specified. The most difficult exercise (1 leg, 40° flexed knee) with the highest variation between the participants was chosen for the analysis of the above-mentioned validation parameters.

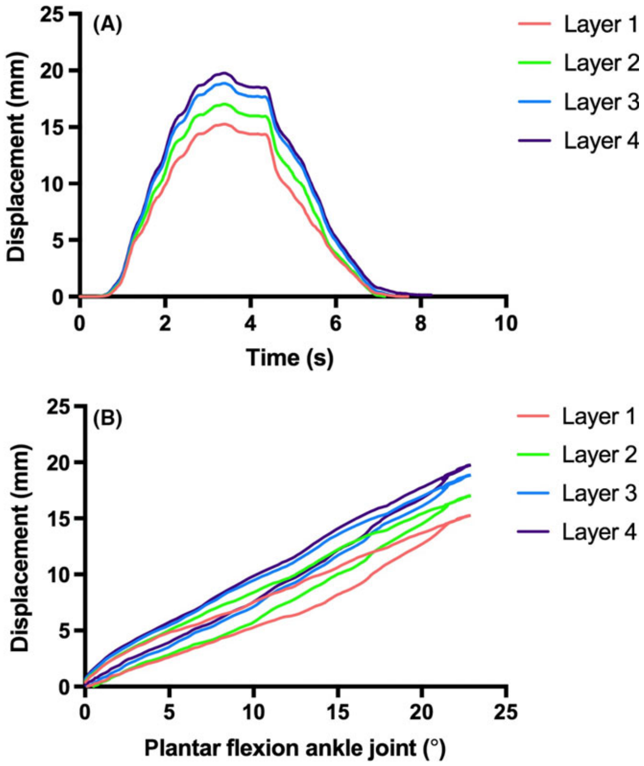

FIGURE 4 A, B, illustrates the shear and displacement within the healthy tendon during a single heel rise. Layer 1 is the most superficial and layer 4 the deepest area of the Achilles tendon. The limit of deviation was set at 1 mm between start- and end point interpreted on the horizontal axis on the Figure 4A graph

For each measurement, the mean displacement of each of the 4 layers was determined and differences between the 4 layers were analyzed to determine the influence of knee joint angle. All statistical analyses were performed in Prism 7 (GraphPad Software, La Jolla CA USA; www.graphpad.com) using 1-way ANOVA and paired statistics; if the ANOVA was significant, a post-hoc test was made with Dunnett's multiple comparisons test, with Layer 1 (superficial) as comparator. To compare the 3 different test positions (knee angles), Tukey's correction for multiple comparisons was used.

3 | RESULTS

The results are based on 189/200 complete UL recordings, where the 11 incomplete UL recordings all were due to insufficient ROM (<17.5°) in the ankle joint. In this case, the second recording was used as the only data for further statistical analysis. Post-process analysis found 16 speckle tracking recordings that exceeded the limit of 1 mm difference between the start- and end point. The reverse speckle analyses found that 14/16 tracking recordings were true displacement of the tendon tissue and they were included in the results. Two tracking recordings were due to tracking error and these were excluded from the data set, and only, the second recording was used for further statistical analysis.

3.1 | Validation of the speckle tracking method

There was no significant difference between the reference displacement of the bead and the speckle tracking displacement of the left box (P = .12), the right box (P = .22), and the top box (P = .283). There was a strong correlation between the bead and the 3 tracking boxes (Table 1); however, the speckle tracking systematically underestimated the displacement of all 3 boxes.

3.2 | Reproducibility of tracking in the healthy tendon

Tracking twice on the same recording had a high reproducibility (Table 2A) with the main variable being placement of the ROI box. The variability was somewhat higher in layer 1 (superficial) than the other layers. Tracking on 2 separate recordings of the same exercise had a fair reproducibility (Table 2B) with the superficial layer again being more variable.

3.3 | Displacement in the healthy tendon

There was a significant difference between layers 1 and 4 in the healthy leg in all 3 exercises: extended knee P = .027, flexed knee (40°) P = .015, and seated on chair (knee flexed 100°) P = .0012. The results for each participant and the calculated mean ± SEM for the 4 layers are shown in Figure 5A-C.

3.4 | Displacement in the surgically repaired tendon

There was no significant difference between layers 1 and 4 in the surgically repaired leg in all 3 exercises: extended knee P = .92, flexed knee (40°) P = .70, and seated on chair (knee flexed 100°) P = .56. The results for each participant and the calculated mean ± SEM for the 4 layers are shown in Figure 5D-F.

3.5 | Difference in displacement between layers 1 and 4 for each of the 3 knee positions

There was a significantly greater shear (P = .045) (displacement difference between layers 1 and 4) in the seated position with the knee flexed 100° compared to the standing position with the knee flexed 40°. There was no significant

TABLE 1 Speckle tracking displacement error

n = 10	Peak displacement	Difference to bead	*P*-value	Typical error %	R^2
Bead	271 ± 44	—	—	—	—
Left box	269 ± 43	−1.9 ± 4.2	.196	1.1	.9996 ± .0007 [.9980-1.0000]
Top box	269 ± 46	−1.4 ± 3.8	.283	1.0	.9996 ± .0004 [.9990-1.0000]
Right box	267 ± 46	−4.2 ± 10.1	.218	2.7	.9987 ± .0034 [.9890-1.0000]

Displacement of the bead and the surrounding tissue regions at the peak of heel rise. Data are presented as pixel displacement ± SD. All 3 boxes underestimated tissue displacement. Correlation coefficients are averages of within measurements correlation to the bead displacement for each of the 3 boxes [range].

TABLE 2 Reproducibility of tracking within recordings (A) and between recordings (B)

A						
n = 10	**Test 1 mean ± SD**	**Test 2 mean ± SD**	**Mean difference ± SD**	***P*-value**	**Typical error %**	R^2
17.5°						
Layer 1	189 ± 70	193 ± 72	3.3 ± 14.5	.49	5.4	.96
Layer 2	219 ± 58	220 ± 60	1.3 ± 4.8	.42	1.6	.99
Layer 3	218 ± 53	217 ± 54	−1.0 ± 3.6	.42	1.2	1.00
Layer 4	225 ± 57	226 ± 57	1.2 ± 5.1	.48	1.6	.99
B						
n = 10	**Recording 1 mean ± SD**	**Recording 2 mean ± SD**	**Mean difference ± SD**	***P*-value**	**Typical error %**	R^2
17.5°						
Layer 1	189 ± 70	198 ± 58	8.8 ± 24.1	.28	8.8	.90
Layer 2	219 ± 58	214 ± 52	−4.4 ± 17.6	.45	5.8	.91
Layer 3	218 ± 53	223 ± 53	5.1 ± 15.3	.32	4.9	.92
Layer 4	225 ± 57	228 ± 52	3.4 ± 12.5	.41	3.9	.95

A: Data are presented as the mean ± SD (pixel) displacement for the 4 rows tracked at 2 separate recordings of the same exercise. Correlation coefficients are averages of correlation between recordings 1 and 2 within each participant.
B: Data are presented as the mean ± SD (pixel) displacement for the 4 rows tracked in 2 separate tracking of the same recording. Correlation coefficients are averages of correlation between tests 1 and 2 within each recording.

difference in displacement between the standing position with the knee extended compared to: the seated position with the knee flexed 100° ($P = .26$) nor the standing position with the knee flexed 40° ($P < .34$). The results for each exercise are shown in Figure 6.

4 | DISCUSSION

In this study, we validated speckle tracking against actual tendon movement using surgically inserted tantalum beads as reference markers of tendon motion. This is the first study, to the author's knowledge, that uses in vivo reference points to quantify the accuracy of the speckle tracking method. There was a strong correlation between the bead and the 3 reference boxes ($R^2 \geq .9987$). The tantalum beads were implanted at different positions (distance from the calcaneus bone) and at different tendon layers (superficial to deep tendon tissue), but in all cases correlated well with speckle tracking. Speckle tracking appears to systematically underestimate the displacement for all 3 reference boxes. The systematic underestimation of displacement seems relatively constant for our speckle tracking method with a typical error ranging from 1.0% to 2.7%. The technical settings used for the speckle tracking will affect this result and using other settings could reduce or increase the systematic error. The settings we chose were based on trial and error to find somewhat optimal settings for our heel-rise recordings. Previous studies have reported similar results showing the speckle algorithm being a bit conservative.[12] Others[11] have performed a similar validation of the speckle tracking method in an in vitro animal model and reported a mean tracking error of 1.6%, which is close to the data of the present human in vivo study.

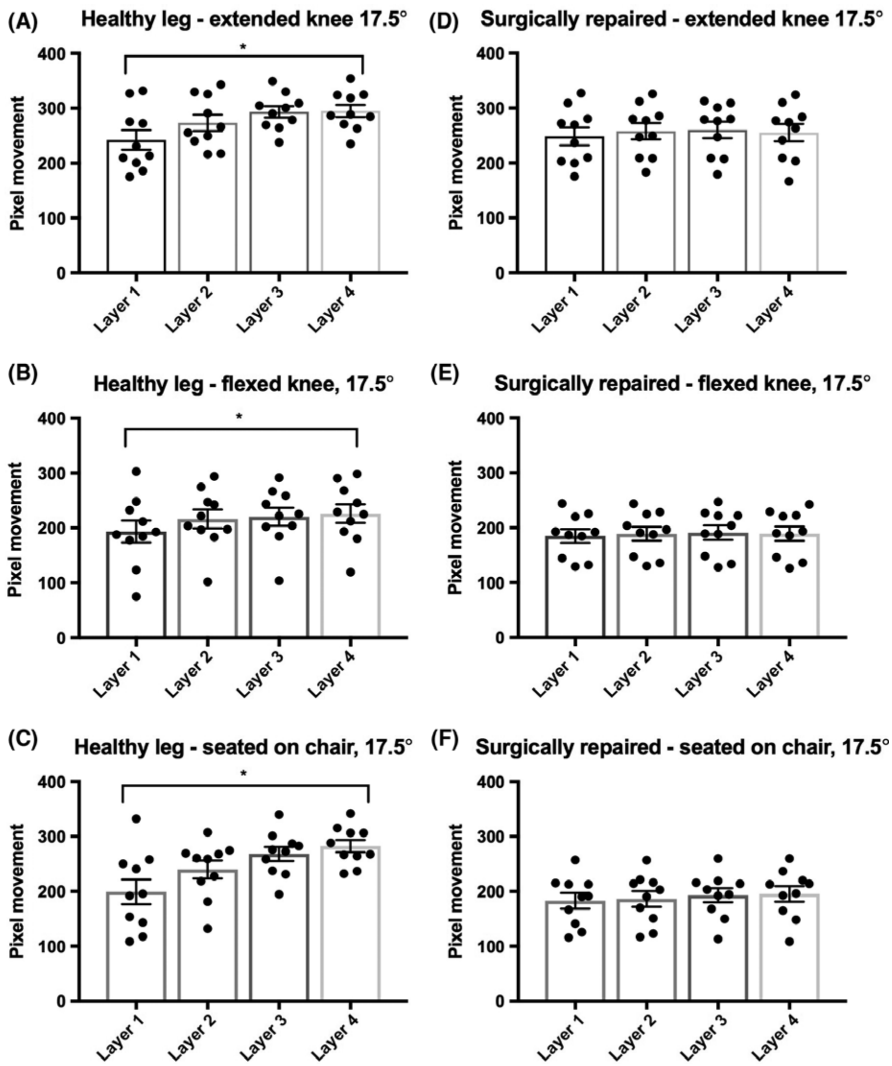

FIGURE 5 A-F, Speckle movement (pixels) of the 4 layers in the healthy and surgically repaired Achilles tendon for 3 different knee angles. Group mean ± SEM and individual results plotted for each participant. There was a significant difference (*$P < .05$.) between layers 1 and 4 in the healthy leg in all 3 knee angles, but not on the surgically repaired AT, indicating decreased shear

In the present study, we were interested in reliability and therefore investigated the reproducibility of 10 participants values obtained within and between 2 speckle recordings by the same operator. The typical error was 1.2%-5.4% within recordings and this increased between recordings (3.9%-8.8%), which represents the variation in reproducing the exercise. The test equipment was not removed and repositioned between these tests, which would likely result in a larger typical error. For both reproducibility tests, the superficial layer (layer 1) was the one with the highest typical error value and this probably relates to the fact that locating the ROI box in this zone between tendon tissue and peritendinous tissue was difficult. During the tracking, it appeared that the superficial layer (layer 1) did not glide freely but that it was somewhat attached to the peritendinous tissue.

Investigating tendon tissue sliding and displacement at different degrees of knee flexion (muscle activation) could improve our understanding of interfascicle sliding in relation to the etiology of tendinopathy and other tendon injuries. The overall results of this study were in line with previous studies[3,13] in which the superficial layer (layer 1) displaces less compared to the deepest layer (layer 4). We expected that flexion of the knee would decrease the activation of the gastrocnemius muscle and thereby reduce the displacement of the superficial layer, while the deeper layer keeps their displacement more or less constant. There was a reduction in the sliding between the superficial and deeper layer as expected moving from 0° to 40° knee flexion (Figure 5A,B), but the displacement of all 4 layers was reduced. The explanation for this unexpected result is likely related to the reduced ankle joint angle when flexing the

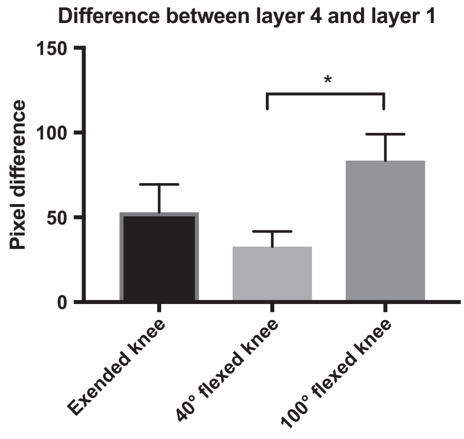

FIGURE 6 Difference in displacement (pixels) between layer 4 and layer 1 for each of the 3 knee positions in the healthy tendon. Group mean ± SEM. There is a significant greater difference in the seated position (100° flexed knee) compared to the 40° flexed knee position. $^{*}P < .05$

knee while standing. We did have this in mind when planning the study and that is why we included the seated position to resemble the conditions of the standing tests with 90° in the ankle joint while greatly reducing the activity of the gastrocnemius muscle (100° flexed knee joint).[14] The result seen in Figure 5A,C is that the deeper layers had approximately the same displacement as they did at 0° knee flexion, but counter to our hypothesis, the superficial layer moved again significantly less compared to the deeper layer. Heel-rise exercises with 40° knee flexion and ankle joint flexion >90° are a standard part of the rehabilitation programs for both Achilles tendinopathy and Achilles tendon rupture. The results in our study suggest that sliding and displacement in the Achilles tendon is greater with the ankle joint at 90°, and to the extent that fascicle sliding is beneficial, this behavior could be relevant knowledge to the clinician who wants to re-establish interfascicle sliding in the injured Achilles tendon.

The study was performed on participants with a surgically repaired Achilles tendon and scar tissue in the ruptured area is a concern. To attend the issue, we included subjects >52 weeks post-surgery to ensure that healing process was largely completed,[15] and we used the bead farthest from the ruptured area to minimize the risk of tracking on scar tissue. However, it appeared that the entire tendon, including the presumed "healthy" region, was altered as a result of the rupture (Figure 5D-F). All 4 layers were affected with decreased sliding, which may relate to disorganized and laterally connected collagen. There was reduced sliding between tendon layers ~3-4 cm proximal to the ruptured area, which indicates that tendon injury also affected tendon tissue far from the injury site. The impaired interfascicle sliding will likely increase shear forces in the tendon and may cause stress concentration on individual fascicles.

Our intention was that the speckle tracking validation would be applicable to healthy tendon tissue using the healthy part of the ruptured tendon for investigation. However, due to the changes in the healthy tissue of the repaired tendon, it was only possible to validate speckle tracking on tissue with impaired movement, which is a limitation of the study. It would also have been preferable to investigate tendon strain in the Achilles tendon, as strain is closely related to tendon injuries like tendinopathy and rupture. However, assessing strain instead of displacement is more challenging as it measures the difference in displacement between the ends of a tracking row (Figure 3B,C). This was not possible for 2 reasons: (a) the load during a single heel rise is relatively small and consequently also the tensile strain; (b) the length of the tracking row was 14 mm (Figure 4A) and with at most 3% strain[16] that would only be 0.42 mm distance. Tracking with that precision on moving tendon tissue was not possible with the current method.

There are some additional limitations in this study to consider. Only 1 location in the Achilles tendon was investigated, and the presented results of tendon tissue sliding and displacement are all related to the proximal area of the Achilles tendon. It remains to be seen how distal and mid-tendon tissue moves during hell rises at different knee angles. Out of plane motion is a common issue in speckle tracking and especially in the Achilles tendon due to its helical twisting nature. Tracking on the proximal bead, most distant to the ruptured area turned out to be beneficial regarding keeping the transducer in line of tendon fascicles as the twisting nature often were located more distal to this. The frame-rate was set at 42 FPS and thereby lower compared to similar studies.[12,13] However, the pace of contraction was 3 seconds for each phase and the displacements were around 300 pixels, so approximately 100 pixels displacement per second. With a frame-rate of 42 FPS that is ~2 pixels per frame which is well within the search range, we used for the speckle tracking. In addition, the majority of speckle recordings were tracked successfully supporting that the technical settings were well balanced to the task.

5 | CONCLUSION

The speckle tracking method appears to be a valid method to investigate tissue sliding and displacement in the Achilles tendon. This study confirms that the superficial layer moves significantly less compared to the deeper layer, indicating that shear and sliding of tendon fascicles takes place during heel rise. The sliding tended to be enhanced in the Achilles tendon when the gastrocnemius muscle activity is reduced by increased knee flexion, but not simultaneous increased ankle dorsal flexion. In conclusion, our results point in the direction that sliding and displacement occurs mostly when keeping the ankle joint at 90°, which may help to re-establish tendon tissue flexibility between tendon fascicles in the injured Achilles tendon.

6 | PERSPECTIVES

The speckle tracking method seems a promising tool to investigate tendon function during stretch and loading and might be a step toward understanding the importance of interfascicle sliding in relation to the etiology of tendinopathy and other tendon injuries—with the aim to adjust future treatment strategies.

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