UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

PhD Thesis

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Acute Achilles tendon rupture

An investigation of tendon and muscle structure, function, and clinical outcome

Primary supervisor: S. Peter Magnusson

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen, 30th March 2023

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TABLE OF CONTENTS

PREFACE AND ACKNOWLEDGMENTS

Over the past several years, I have worked as a sports physiotherapist at Bispebjerg-Frederiksberg Hospital. Over the same number of years, my curiosity about research has grown. It was therefore a great pleasure for me that I was given the opportunity to be enrolled as a PhD student. Thank you for the confidence!

I deeply appreciate the assistance of everyone—mentioned or not—who made it possible for me to complete this thesis and I want to express my gratitude to the following people.

Firstly, my primary supervisor Peter Magnusson for your competent guidance, unfailing kindness, patience, and support as I worked on this thesis. Thank you for believing in me, for inspiring me, and for always being so quick to reply to my emails. A special thanks to my co-supervisor Michael Kjær for supporting the beginning and development of my research career in the unique research environment you have established with the Institute of Sports Medicine Copenhagen (ISMC). Also, a special thanks to my co-supervisor Michael Krogsgaard for sharing your extensive experience and for consistently seeing possibilities rather than limitations. Rene Svensson, for your help with technical issues, statistical analyses, and enlightening scientific discussions. Your assistance has been invaluable and is greatly appreciated.

Birgitte Randeris, head of the Orthopedic physiotherapist group, Morten Østergaard, former head of the Department of Physical and Occupational Therapy, and Naja Henriksen, current head of the department, for your encouragement, cooperation, and supportive attitude toward me and my research.

Colleagues and friends at the Department of Physical and Occupational Therapy and at the ISMC, to spread joy and provide encouraging and supportive comments. A sincere thank you to my wonderful office coworkers in building 10, Mathilde Lundgaard-Nielsen, Robert Herzog, Camilla Cullum, Rudi Hansen, and Christina Eddy and in building 8, Anne-Sofie Agergaard, Anne Gates, and Mads Bloch-Ibenfeldt. To all the secretaries and especially Maria Bækgaard Kjær and Charlotte Bilde for their helpfulness and answers to all possible and impossible questions.

The Department of Orthopedic Surgery, Copenhagen University Hospital Bispebjerg-Frederiksberg Hospital, to Rasmus Mikkelsen and Peter Rasmussen, for performing most of the operations, to Lars Konradsen for the professional discussions, and to all the nurses at M-Amb. for their friendly manner and their help in collecting the plaster bandages.

The Department of Radiology, Copenhagen University Hospital Bispebjerg-Frederiksberg Hospital, thank you Mikael Boesen, Philip Hansen, Janus Nybing, Erik Høgh-Schmidt, and Frederik Hvid Linden, it has been a pleasure to collaborate with you.

All the participants with a ruptured Achilles tendon who, despite their critical situation, had the energy to be a part of a research project. You were essential in making this research possible. Also, a thank you to the students, who have taken part in the studies, your assistance was very helpful.

Thank you, Birgitte Holst, for helping me continue my strength training and running routine despite a busy daily schedule.

Naturally, I also want to express my gratitude to my family in particular. I value my parents listening to whatever is on my mind. To my spouse Ulrik, my children, Gustav and Astrid, who have been tremendously patient and encouraging with me throughout this journey.

This PhD thesis was made possible by financial support from the Department of Physical and Occupational Therapy, Copenhagen University Hospital Bispebjerg-Frederiksberg, The Ministry of Culture, Team Denmark, The Research Foundation of Copenhagen University Hospital Bispebjerg-Frederiksberg, and The Novo Nordisk Foundation.

LIST OF PAPERS

Study I

The influence of an orthopaedic walker boot on forefoot force.

Hoeffner R, Agergaard AS, Funaro A, Bjerregaard N, Svensson RB, Alkjaer T, Magnusson SP.

The Foot (Edinb). 2020;46:101739. https://doi.org/10.1016/j.foot.2020.101739

Study II

Tendon elongation and function following delayed or early loading of surgically repaired Achilles tendon ruptures – a randomized controlled trial

Hoeffner R, Agergaard AS, Svensson RB, Cullum C, Mikkelsen RK, Konradsen L, Krogsgaard MR, Boesen M, Kjaer M, Magnusson SP.

Manuscript draft.

Study III

Muscle fascicle and sarcomere adaptation in response to Achilles tendon elongation in an animal model.

Hoeffner R, Svensson RB, Dietrich-Zagonel F, Schefte D, Kjær M, Eliasson P, Magnusson SP.

Manuscript in revision.

Study IV

Eccentric Training to restore persistent Functional Loss following Ruptured Achilles tendon: The role of Fascicle Length and Muscle Excursion

Hoeffner R, Svensson RB, Magnusson SP.

Submitted manuscript.

ABBREVIATION LIST

ANOVA	Analysis of variance
ATR	Achilles tendon rupture
ATRA	Achilles Tendon Resting Angle
ATRS	Achilles tendon Total Rupture Score
ATT	Achilles transected tendon
BMI	Body Mass Index
CSA	Cross-sectional area
CTRL	Control
DOMS	Delayed onset muscle soreness
DVT	Deep venous thrombosis
EMG	Electromyography
MET	Metabolic equivalent
MRI	Magnetic Resonance Imaging
MTJ	Myotendinous junction
MVC	Maximum voluntary contraction
N	Newton
PAS	Physical Activity Scale
PCSA	Physiological cross-sectional area
PROM	Patient reported outcome measure
RCT	Randomized controlled trial
ROI	Region of interest
SD	Standard deviation
SEM	Standard error of the mean
US	Ultrasound
3D	Three-dimensional

ENGLISH SUMMARY

Background: Acute Achilles tendon rupture is a frequent injury with an increasing incidence registered worldwide. Professional athletes may have their careers ended or put on hold by an Achilles tendon rupture, while recreational athletes, who face far lower physical demands, also experience inadequate healing and severe long-term functional deficits. The optimal strategy for treatment and rehabilitation following an acute Achilles tendon rupture is still unknown despite several attempts to address the issue.

Purpose: The overall aim of this thesis was to investigate the healing processes of tendon after an acute Achilles tendon rupture, and to identify a loading pattern of the tendon during the rehabilitation period that would ensure a good recovery of the tendon, muscle structures and overall function.

Methods: Study I investigated Achilles tendon load during gait in a weightbearing orthopedic boot using one or three wedges and crutches. In Study II, the effect of delayed loading after surgically treated Achilles tendon rupture on functional, clinical, and structural outcomes for muscle and tendon during the first year after surgery was investigated in a randomized controlled trial (RCT). In Study III an animal model was used to explore how a muscle adapts to an elongated tendon and in Study IIII we investigated if a muscle, that has undergone shortening secondary to elongation of the tendon, can increase in fascicle length and improve function after 12 weeks of eccentric training.

Results: Study I demonstrated that adding heel wedges to an orthopedic boot and using crutches while walking can lower the force on the forefoot and subsequently the tensile load on the Achilles tendon. In Study II the timing of loading initiation did not influence the heel-rise height, with deficits between the injured and uninjured leg of -2.2 cm for the Early group and -2.1 cm (p= 0.719) for the Delayed group at 12 months. At one week after surgery, the soleus part of the tendon had significantly elongated in both groups without a between-group difference (side-to-side difference, Early 16.3 mm and Delayed 17.5 mm, p= 0.997). Up to 12 months, the soleus tendon length did not further change significantly. The gastrocnemius tendon length remained constant at 1 week, but it significantly elongated over time (side-to-side difference at 12 months, Early 10.5 and Delayed 13.0, p= 0.899) without a between-group difference. The Delayed group had less Doppler activity at three months (p= 0.006), and a better ATRS score (Early 59.6 and Delayed 72.2 points, mean difference of 12.5±5.7, 95%CI [1.1 to 23.2], p= 0.032) at 1 year. In Study

III the tendon lengthened by 15%, while the muscle mass and length decreased 12-18% and 8-10%, respectively after Achilles tendon transection. The reduced overall muscle length was accompanied by a reduced fascicle length and serial sarcomere number such that sarcomere length retained normal length in the distal portion of the fascicle but was unexpectedly greater than normal in the mid and proximal portion of the fascicle. In Study IIII, no difference was found in fascicle length after 12 weeks of eccentric training. Still, plantarflexion toque, heel-rise count, and patient reported outcome improved (mean difference 11.6 points, 95% CI [4 – 19], P<0.0001).
Conclusion: Delayed loading was not superior compared to early loading in the primary endpoint of reducing the heel-rise height difference at one year after acute surgery following an Achilles tendon rupture. Regardless of the rehabilitation protocol, all patients in the RCT had a significant 35% soleus tendon elongation compared to the contralateral side already at one week. In an animal model, it was demonstrated that Achilles tendon transection leads to elongation of the tendon and atrophy and shortening of the muscles. Moreover, a smaller medial gastrocnemius muscle results in shorter fascicles, which then promote adaptation by reducing serial sarcomere counts. Unexpectedly, this adaptation seems to "overshoot" and increase sarcomere length. Finally, eccentric isokinetic training for 12 weeks did not alter the fascicle length of the medial gastrocnemius muscle in persons with a more than one-year-old Achilles tendon rupture who were dissatisfied with their physical function. Nevertheless, plantarflexion torque, heel-rise count and, importantly, patient reported outcome improved.

DANSK RESUME

Baggrund: Akut akillesseneruptur er en hyppig skade med en stigende incidens registreret på verdensplan. For professionelle atleter kan en akillesseneruptur betyde enden på deres karriere eller en lang pause fra sport. For motionister, om udsættes for betydeligt lavere fysiske krav, kan det også have store konsekvenser gå grund af de varige gener, som ofte er til stede. Den optimale strategi for behandling og genoptræning efter en akut akillesseneruptur er stadig ukendt på trods af flere forsøg på at finde løsningen.

Formål: Det overordnede formål med denne afhandling var at undersøge helingsprocesserne af senen efter en akut akillesseneruptur, og at identificere et belastningsmønster af senen under genoptræningsperioden, der ville sikre en god heling af sene- og muskelstrukturer samt den overordnede funktion.

Resultater: Studie I viste, at ved at lægge hælkiler i en Walker bandage og bruge krykker under gang, kan kraften på forfoden reduceres og dermed også trækket på akillessenen. I Studie II påvirkede tidspunktet for opstart på belastning i form af vægtbæring ikke hælløft-højden. Der var en difference mellem det skadede og raske ben på henholdsvis -2,2 cm i Early gruppen og -2,1 cm i Delayed gruppen (p= 0,719) efter 12 måneder. En uge efter operationen var soleus-delen af senen signifikant forlænget i begge grupper uden forskel mellem grupperne (sideforskellen var: Early 16,3 mm og Delayed 17,5 mm, p= 0,997). Soleus-delen forlængede sig ikke yderligere frem til 12 måneder. Gastrocnemius-delen af senen var ikke ændret efter 1 uge, men den forlængede sig signifikant over tid (sideforskellen ved 12 måneder: Early 10,5 mm og Delayed 13,0 mm, p= 0,899) og der var ingen gruppeforskel. Delayed gruppen havde mindre Doppler-aktivitet efter tre måneder (p= 0,006), og en bedre ATRS-score (Early 59,6 point og Delayed 72,2 point, gennemsnitlig forskel på 12,5±5,7, 95 % CI [1,1 til 23,2], p= 0,032) ved 1 år. I Studie III blev senen forlænget med 15%, mens muskelmassen og længden faldt med henholdsvis 12-18% og 8-10% efter overskæring af akillessenen. Den reducerede muskellængde var ledsaget af en reduceret fascikellængde og antal af sarkomer i serie således, at sarkomer længden bevarede normal længde i den distale del af fasciklen, men overraskende var større end normalt i den midterste og proksimale del af fasciklen. I Studie IIII blev der ikke fundet nogen forskel i fascikellængden efter 12 ugers excentrisk træning. Alligevel forbedredes plantarflexion toque, antallet af hælløft og de patientrapporterede oplysninger (gennemsnitlig forskel 11,6 point, 95 % CI [4 – 19], p <0,0001).

Konklusion: Udskudt belastning var ikke bedre sammenlignet med tidlig belastning i forhold til at reducere forskellen i hælløft-højde (det primære outcome) et år efter akut operation af en akillesseneruptur. Uanset hvilket genoptræningsregime, Early eller Delayed, så havde alle patienter i RCT-studiet en signifikant forlængelse på 35% af soleus delen af senen i forhold til deres raske side allerede efter en uge. I en dyremodel blev det påvist, at overskæring af akillessenen fører til forlængelse af senen og atrofi og forkortelse af muskulaturen. Desuden resulterer en kortere medial gastrocnemius muskel i kortere fascikler, som derefter fremmer adaptionen ved at reducere antallet af sarkomer i serie. Uventet ser denne tilpasning ud til at overkompensere og øge sarkomer længden. Endelig ændrede excentrisk isokinetisk træning i 12 uger ikke fascikellængden i den mediale gastrocnemius muskel hos personer med en mere end et år gammel akillesseneruptur, som var utilfredse med deres fysiske funktion. Ikke desto mindre blev plantarflexion toque, antallet af hæl-løft og ikke mindst de patientrapporterede oplysninger forbedret.

1. INTRODUCTION

Hippocrates, known as the “Father of medicine”, provided one of the first descriptions of an Achilles tendon rupture. He stated:” This tendon, if bruised or cut, causes the most acute fevers, induces choking, deranges the mind and at length brings dead” (1). Fortunately, the prognosis is not quite as bleak today, while the course of treatment is still debatable.

Acute Achilles tendon rupture, which has an incidence of 31-35 per 100 000/year (2-4), is a frequent injury observed in middle-aged men (and women) who resume high-impact activities like soccer, tennis, and badminton after years of inactivity. Despite the frequency of the injury, and despite numerous studies being published, the optimal treatment and rehabilitation regime are still lacking, and extensive functional deficits are often seen years after an Achilles tendon rupture (5, 6). The increasing number of Achilles tendon ruptures and the severe, long-lasting physical discomfort they frequently cause highlight the importance of additional research to enhance patient prognosis. The effects of Achilles tendon rupture on tendon and muscle structure and physical function will be investigated in this PhD thesis and hopefully contribute new knowledge to the area so that the treatment can be optimized.

2. THE ACHILLES TENDON

2.1 Anatomy

Figure 1. *The anatomy of the Achilles tendon and the triceps surae muscle. (License from Colourbox)*

The Achilles tendon is the largest and strongest tendon of the human body (7, 8). It begins near the middle of the calf and is the conjoint tendon of the gastrocnemius medialis, gastrocnemius lateralis, and soleus muscles and inserts on the calcaneus bone (Figure 1). The medial and lateral gastrocnemius muscles together with the soleus muscle make up the triceps surae (7). The medial head of the gastrocnemius originates from the posterior part of the medial epicondyle and the lateral head of the gastrocnemius from the posterior part of the lateral epicondyle. The broad and flat soleus muscle lies profund to the gastrocnemius. It is a pennate muscle and originates from the soleal line of tibia, the head of fibula, and the posterior border of fibula (7).

The average length of the Achilles tendon is 15 cm, where the gastrocnemius part of the tendon varies between 11-26 cm and the soleus part of the tendon (also called the free tendon) between 3-11 cm (7, 8). The Achilles tendon is clearly divided into separate parts, each connected to one of the three muscular compartments of the triceps surae and is mechanically separated well into the free tendon such that tissue bundles that originate from each of the three muscle portions can be distinguished close to the tendon insertion on the calcaneal bone (9). The fibers of the tendon has a twisted structure which spirals up to 90° counterclockwise in the right leg and clockwise in the left leg (Figure 2), when viewed from proximal to distal (10).

Figure 2. *Posterior view of the right triceps surae muscle tendon unit and the Achilles tendon insertion on the calcaneal bone. (Bojsen-Møller et al. 2015 (9))*

The blood supply of the Achilles tendon is typically divided into three areas: the paratenon, the musculotendinous junction, and the osteotendinous junction (7), where the proximal and distal part of the tendon are supplied by the posterior tibial artery and the middle

part is supplied by the peroneal artery (11). The middle part of the tendon is the least vascularized and therefor perhaps more prone to rupture in this area (7, 11).

The Achilles tendon is primarily innervated by the sural nerve with a minor supply from the tibial nerve through the connecting triceps surae muscle. The majority of the nerves are sensory and mainly located close to the musculotendinous junction and the osteotendinous junction (7).

2.2 Structure

Collagen and elastin comprise 60-70% and 2 % respectively of the dry weight of the tendon (7, 8, 12). In a healthy tendon collagen type I is dominant and constitutes 95% of the total collagen and the last 5% consists of collagen type III and V. In a ruptured tendon there is a higher proportion of type III collagen, which is less resistant to tensile forces (7). The tendon has a hierarchical structure (Figure 3) composed of collagen molecules that assembles into fibrils that, in turn, form fiber arranged in parallel bundles containing nerve, blood, and lymphatic vessels, creating fascicles. The fascicle bundles are bound by endotenon that forms the next layer of the tendon structure and finally the tendon unit. The tendon is surrounded by an outer layer of connective tissue, epitenon, which in turn is surrounded by another layer of connective tissue, the paratenon. The two outermost layers are separated by a thin layer of fluid to ensure movement with reduced friction (7, 8, 12, 13).

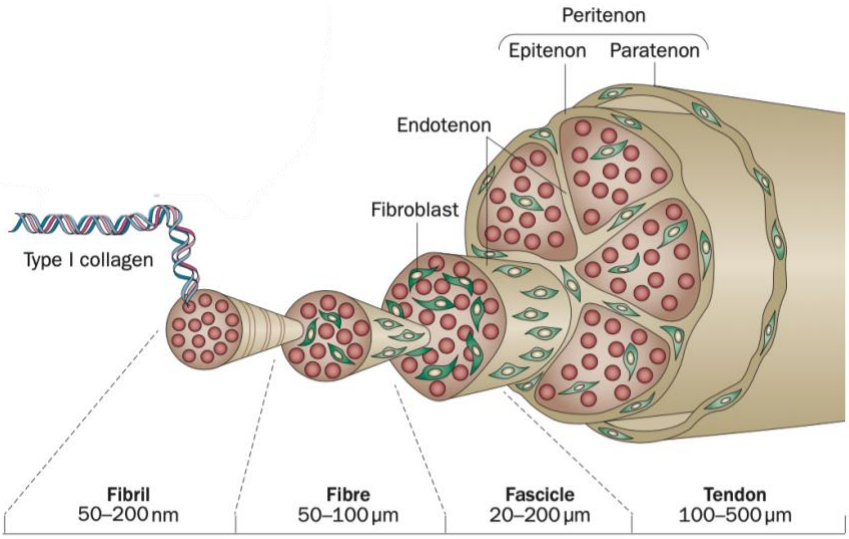

***Figure 3**. The structure of the tendon from the smallest collagen molecule to the entire tendon. (Modified from Nourissat et al. 2015 (13))*

2.3 Biomechanical properties and function

Tendon connects muscle and bone, and movement is produced when the force generated by a muscular contraction is transferred to the bone via the tendon. Consequently, the function of the tendon is important for the entire function of the muscle-tendon unit (12, 14). Due to the distinct tendon parts deriving from the three muscular compartments of the triceps surae, the Achilles tendon is loaded asymmetrically during human movement, and as a result, the tendon experiences heterogeneous deformation (9). Measurements of selected physical activities have displayed the peak strain on the tendon to be highest when running, where the tendon is exposed to loads up to 9000 N, equivalent to about 12.5 times the body weight (15, 16). At rest the collagen fibers of the tendon have a wavy appearance (Figure 4).

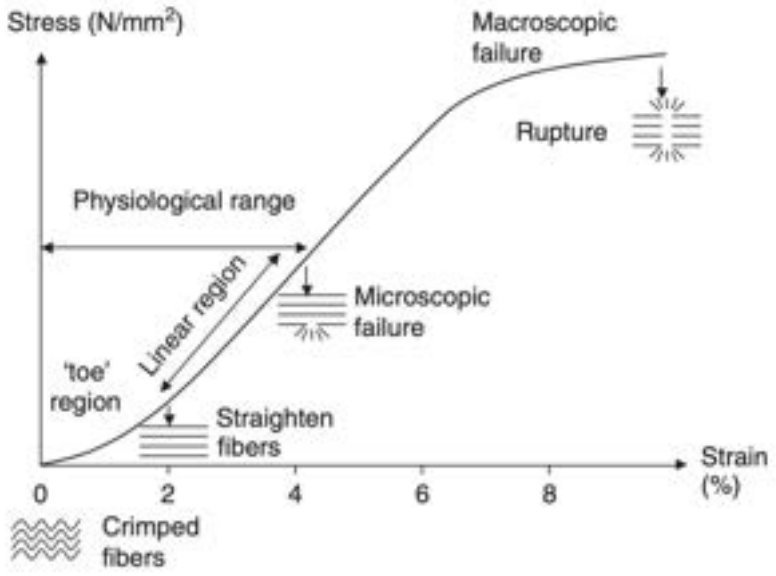

Figure 4. *Stress-strain curve of a tendon. (Wang 2006 (12))*

This wavy configuration disappears when the tendon is stretched more than 2% and as all the fibers become stretched the stress-strain curve increases linear until the tendon is stretched more than 4% and microscopic micro tearing of the tendon fibers occurs. At strain levels beyond 8% macroscopic tearing and rupture of the tendon occurs (12).

3. ACUTE ACHILLES TENDON RUPTURE

3.1 Epidemiology

Achilles tendon rupture is a relatively common injury, and the incidence has been rising over the last decades and presently stands at 31–35 per 100 000/year (2-4). Mainly because of an aging population and an increased interest in sporting activities. Thus, a middle-aged man (30-50 years old), who occasionally plays sports is most likely to sustain the injury (2).

3.2 Etiology and risk factors

Achilles tendon rupture is a multifactorial injury, and several risk factors has been presented (17, 18). Corticosteroids and Fluoroquinolones are two medications that have been associated with impaired healing and risk of necrosis and thus weakening the tendon tissue (17). Other risk factors that have been suggested are obesity, age, smoking, a previous Achilles tendon rupture, Achilles tendinopathy, type 2 diabetes, spring season, and genetic factors (18).

Three various injury mechanism has been described: 1) Push-off with the weightbearing forefoot and the knee stretched as seen in racket sports, or when jumping in sports such as basketball, or in sprint starts. 2) Sudden and unexpected dorsiflexion in the ankle as seen when falling from stairs or suddenly falling forwards when skiing. 3) Powerful dorsiflexion of a plantar flexed ankle as seen when falling or jumping from a height (19). As all three of the mechanisms indicate to high force as a triggering factor, this implies that during high load activities, the tendon works closely to the rupture point.

3.3 Diagnosis

The diagnose of an acute Achilles tendon rupture is often verified partly by the anamnesis and by the clinical examination. The typical patient presents a history with a sudden onset of pain, often accompanied by a snapping sound, and the feeling of being kicked over the heel although no one is near, when looking for the person who kicked (20). To establish the diagnosis of acute Achilles tendon rupture, following physical examinations are recommended to perform: Thompsons test (i.e., Simmonds squeeze test), increased ankle dorsiflexion (i.e., Matles test) decreased plantar flexion strength, and the presence of a palpable gap (21).

The Thompsons test is performed with the patient lying prone on the bench with the lower legs extended and the feet hanging freely. The calf muscles of the injured leg are squeezed, and the test is positive if no or minimal plantar flexion occurs, indicating a ruptured tendon. The test has a high sensitivity (0.96) and specificity (0.93) (22).

The Matles test is performed with the patient lying prone on the bench with the knees flexed 90 degrees and the ankles relaxed. The test is positive if the foot on the injured side falls into a neutral or dorsiflexed position. This test likewise has a high sensitivity (0.88) and specificity (0.85) (22).

Ultrasound and magnetic resonance imaging are not recommended for routine use but can be used to determine whether a rupture is partial or complete (21).

3.4 Initial treatment (operative and non-operative)

Both operative and non-operative treatment can be used initially after an acute Achilles tendon rupture and despite a large number of conducted studies, reviews and meta-analyses, evaluating operative versus non-operative treatment (23-30), there is still no consensus on which treatment to prefer. However, there is a general agreement that surgery reduces the risk of rerupture but is associated with a higher risk of other complications, such as infections and nerve lesions (23-30). In a resent published RCT (23) with more that 500 included patients, the numbers of tendon rerupture was 6.2% in the non-operated group versus 0.6% in the open surgery group. Complications in terms of nerve lesions was reported to be 0.6% in the non-operated group compared to 2.8% in the open surgery group.

3.5 Healing of the tendon

Healing of the tendon can be divided into three overlapping phases (12, 31, 32). The first acute inflammatory phase starts with bleeding from the injury, activation of inflammatory cells and platelets, and hematoma formation in the injured area (31). After a few days the second proliferative or repair phase starts. During this phase the inflammatory cells and tenocytes proliferate and form the granulation tissue. The immature tissue starts to produce collagen type III, which initially is not parallel aligned, but still helps to strengthen the biomechanics of the tissue (33). In the third remodeling phase, which begins after about 6 weeks, the collagen I synthesis

becomes most dominant, and the extracellular matrix becomes more aligned. The repaired tissue will most likely never fully reach the same biomechanical properties as before the injury and will act more like scar tissue. This final phase can last more than a year (31, 33).

3.5.1 Impact of mechanical loading

Studies in rats have shown that mechanical loading stimulates tendon healing and therefor is very important in the healing process of an injured Achilles tendon, however, the biochemical response is not fully understood (13). There is a balance between preventing tendon damage (as rerupture or elongation) and supplying the tensile forces required to facilitate healing that is still up for debate regarding the ideal loading and when to begin loading the tendon (34). In an animal study (35), it was found that mild loading has a strong stimulating effect on tendon healing without altering inflammation or causing damage to the tissue. The same study found that high mechanical loading enhances tissue mass and drive tendon regeneration even more but is also related with altered inflammatory responses and causes microdamage to the tissue. Most animal studies on Achilles tendon rupture (36, 37) have concentrated on the consequences of different loading regimes; however, they have not paid as much attention to the length of the tendon or the implications of a potential elongation.

3.5.2 Elongation during healing

Regardless of initial treatment with or without surgery, substantial elongation of the Achilles tendon during healing has been described in various studies (38-46) and according to the studies, this elongation can reach levels of 10–20 mm. The first study to report tendon elongation was published 40 years ago (43). Here, an initial separation of the tendon ends of 3 mm (range 2-4 mm) was found already a few days after surgery, and after 40 days, a total separation of 11 mm (range 10-12 mm) was seen. In this study, metal markers were placed at the tendon ends, and repeated radiography examinations were used to measure elongation. More than 20 years later, a similar study, also using radiographic markers to measure tendon elongation, was conducted (45). Here, an increase in tendon elongation was found in the first 6 weeks after tendon repair, followed by a slow decrease until the last timepoint at 60 weeks. They also found a significant correlation between tendon elongation and clinical outcome. Patients with less elongation achieved a better clinical outcome. Furthermore, tendon elongation has also been found to

correlate with functional outcome measures in terms of the heel-rise test at both 6 and 12 months after tendon repair (39), where patients with less elongation achieved better function. In the latter study, a different measurement technique was applied. Here ultrasonography was obtained of the tendon length, defined as the distance between the musculotendinous junction of the gastrocnemius and the calcanea osteotendinous junction. Moreover, tendon length difference between injured and uninjured leg was reported to be 3.1 cm at 3 months, 2.9 cm at 6 months, and 2.6 cm at 12 months. It has been demonstrated that elongation of the tendon is not only present in the initial months but continues up to six months after the injury regardless of early or late loading of the tendon (38). These results suggest that, to achieve optimal function, tendon length restoration is probably crucial.

3.6 Muscle-tendon unit

The triceps surae and the Achilles tendon work as a functional unit, and the muscle produces force by contracting the fibers, pulling the origin and insertion of the muscle-tendon unit towards each other (47). The muscle fibers contain sarcomeres, the basic contractile units, which are arranged both in parallel and series. The number of sarcomeres in parallel corresponds to the physiological cross-sectional area (PCSA) of a muscle, described as the muscle cross-sectional area perpendicular to the muscle fiber axis, which is based on the quantity of muscle fibers. PCSA, or number of sarcomeres in parallel, determines the maximum force-generating capacity of the muscle. The number of sarcomeres in series corresponds to fiber length, which is proportional to the excursion of a muscle, which affects the range of motion of a joint (48, 49).

In association with a tendon rupture and the early rehabilitation period, there will inherently be some period of immobilization, albeit with variation (50), which will result in some muscle atrophy (less sarcomeres in parallel) and reduced strength (51). In the long-term, impairment such as reduced plantarflexion strength (52) and reduced heel-rise height (39, 53) on the injured side are often still present. There seems to be a relationship between the deficit in heel-rise height and tendon elongation (39, 53, 54), and it has likewise been proposed that muscle fascicle length may play a significant role in determining heel-rise height (53-55). As just described, the triceps surae and the Achilles tendon work as a functional unit. If the tendon lengthens during healing as previously suggested, and if this lengthening causes the muscles to shorten (have fewer

sarcomeres in series), this will reduce the muscle excursion and may be the cause of a persistent decrease in heel-rise height.

3.7 Treatment (rehabilitation)

The optimal rehabilitation after an Achilles tendon rupture/repair is still debated. However, the trend across the literature in the last decades has moved towards more early or accelerated rehabilitation to avoid the loss of muscle mass and function (50, 56, 57) but this approach has not demonstrated efficacy in reversing these muscle and function impairments (57). Early weightbearing and ankle joint range-of-motion activities are the different types of early functional rehabilitation that are frequently described in studies and are typically permitted between week 0 and 2 (50, 56). Despite considered to be effective, early rehabilitation has been linked to a higher rate of postsurgical tendon elongation (58).

3.8 Tendon imaging

Various techniques have been used to measure tendon elongation, including radiography with metal markers, ultrasonography, and magnetic resonance imaging. The various procedures have different advantages and weaknesses and makes it challenging to compare study results because of the diverse approaches.

3.8.1 Radiography

Using metal markers or tantalum beads, which are placed during operative repair, coupled with repeated radiography examinations makes it possible to measure separation of the tendon ends during healing, and this method has been used for decades (38, 43, 45, 59, 60). Hence, this measurement method more accurately reflects tissue elongation than the full length of the tendon and precludes differential tendon assessment between the soleus and gastrocnemius parts of the tendon.

3.8.2 Ultrasonography (US)

B-mode US is a simple and low-cost measurement and doubtless the most used to image tendon length (39, 42, 46, 61-66). Extended field of view ultrasound has been found to be a valid and reliable method to measure tendon length in healthy individuals (67). However, measurements

performed on injured people are more complicated, especially in the initial phase after surgery, where the myotendinous junction of the soleus muscle can be quite challenging to define. Thus, it is common practice to measure the Achilles tendon length, when using ultrasound, between its insertion on the calcaneus and the myotendinous junction of the medial gastrocnemius (61, 67). It is extremely challenging, if not impossible, to assess the tendons differentially with US after an Achilles tendon rupture.

3.8.3 Magnetic Resonance Imaging (MRI)

The use of MRI is more expensive and less available than US or radiography, which is probably the reason why only a few studies have used MRI for tendon imaging (40, 68). The precision of repeated sagittal images on conventional MRI can be difficult and inaccurate because the length can change drastically from one slice to the next. Assessment of the different parts of the tendons initially after surgery can be challenging, and often only the gastrocnemius part of the tendon is measured (40, 68). A more precise method is to use 3D MRI, which makes it possible to look at the sagittal, horizontal, and coronal plane simultaneous when performing the measurements. The use of 3D MRI has never been employed before, but was developed in our lab and showed no systematic bias on repeated measures and the correlation coefficient and typical error percent were 0.98, and 5.1% for the injured soleus muscle, 0.99 and 4.6% for the uninjured soleus muscle, 0.94 and 3.1% for the injured gastrocnemius muscle, and 0.99 and 0.6% for the uninjured gastrocnemius muscle (53).

4. AIMS AND HYPOTHESIS

4.1 Aim of the thesis

The **overall aim** of the thesis was to investigate the healing processes of tendon after an acute Achilles tendon rupture, and to identify a loading pattern of the tendon during the rehabilitation period that would ensure a good recovery of the tendon, muscle structures and overall function.

4.2 Aim of the studies

4.2.1 Aim of study I:

The aim of study I was 1) to assess the Achilles tendon load during ambulation in a weightbearing orthopedic boot with the use of different numbers of wedges and 2) to describe changes in muscle activity of the medial gastrocnemius, soleus, and tibialis anterior when using one or three wedges during ambulation in a weightbearing orthopedic boot.

The hypothesis was that more wedges would entail less muscle activity.

4.2.2 Aim of study II:

The aim of study II was to investigate if delayed loading following surgically treated Achilles tendon rupture, influences the functional, clinical, and structural outcomes for muscle and tendon during one year after surgery.

The hypothesis was that delaying the gradual introduction of full loading with 6 weeks in the initial 26 weeks would reduce the heel-rise height difference (primary outcome) between the injured and uninjured leg one year after surgery (primary endpoint).

4.2.3 Aim of study III:

The aim of study III was to explore the muscle adaptation associated with an elongated tendon in an animal model. The model included two groups with different Achilles tendon surgical treatments of Achilles tendon rupture, where one group had further removal of tendon to encourage elongation, and the other group had suture repair to achieve minimal elongation.

The hypothesis was that the muscle fascicle length would shorten, and that the serial sarcomere number would be reduced such that the sarcomere length would remain the same.

4.2.4 Aim of study IIII:

The aim of study IIII was to examine if a muscle that has undergone shortening secondary to elongation of the tendon, and therefore has a reduced overall excursion, can increase in fascicle length, and improve function following 12 weeks of eccentric training.

The hypothesis was that eccentric training would increase fascicle length in persons with a prior Achilles tendon rupture who had been left with an elongated tendon that was coupled with a shortened muscle. We further hypothesized that the training would result in an improved heel-rise function.

5. MATERIAL AND METHODS

In this chapter the study design, participants, materials, and ethical considerations regarding study I-IIII are summarized. A more complete and detailed description can be found in the associated paper for each study. Furthermore, a discussion of the methodological considerations is provided in the following chapter 6.

5.1 Study I

5.1.1 Study design

This study was conducted to investigate Achilles tendon load during gait in a weightbearing orthopedic boot as a part of the preparations for Study II, to gather knowledge for use in the creation of the rehabilitation regime in our intervention group. Electromyography (EMG) recordings of muscle activity in the medial gastrocnemius, soleus, and tibialis anterior muscle was used as an indirect measure of Achilles tendon load. Force sensor insoles to measure forefoot force were likewise used as an indirect measure of Achilles tendon load.

5.1.2 Participants

A convenience sample of 10 (4 women, 6 men) participants were included. All presented healthy Achilles tendons and no history of injuries that could affect their gait.

5.1.3 Materials

Surface electrodes (Ag/AgCl Blue-Sensor N-00-S, AMBU, Ballerup, Denmark) were placed on the skin over the right medial gastrocnemius, soleus, and tibialis anterior muscle and before the ambulation assessment, participants completed a maximum voluntary contraction (MVC) for the plantar- and dorsi flexors for EMG normalization. Then an orthopedic walker boot (Nextep Contour II Walker; DonJoy Nordic) was placed on the participants right foot and a standard sneaker on the left. Pedar force sensor insoles (Pedar®, Novel, Munich, Germany) were placed in both the orthopedic walker boot and the sneaker to register force during ambulation.

Three types of ambulation were performed: 1) gait with no crutches and no instructions, 2) gait with crutches and no instructions, 3) gait with crutches and instructions to put weight on the heel only. The different ambulation types were performed using one and three wedges adding 3 cm and 9 cm respectively underneath the heel. Three trials were performed of each ambulation type and the order of wedge conditions was randomized across participants.

5.1.4 Ethical considerations

The study was approved by the Regional Ethics Committee. Healthy individuals were studied to be able to investigate ambulation conditions without putting a healing tendon at risk.

5.2 Study II

5.2.1 Study design

This trial was a single-blinded prospective randomized controlled superiority trial with 2 parallel arms.

5.2.2 Participants

Patients with an acute Achilles tendon rupture referred to the emergency clinic and the orthopedic surgery department at Copenhagen University Hospital Bispebjerg-Frederiksberg Hospital, Copenhagen, Denmark, from May 2020 to September 2021 were assessed for eligibility (due to Covid-19 lockdowns the recruitment period was longer than expected). A total of 48 participants diagnosed with a traumatic, complete Achilles tendon mid-substance rupture were included. The inclusion criteria were: No contraindications for magnetic resonance imaging (MRI), operated within 14 days from the injury, should understand Danish, and manage transport to and

from the hospital on their own. Exclusion criteria were: Prior Achilles tendon rupture, re-rupture, other injuries affecting their lower limb function, smoking, systemic diseases influencing healing, anticoagulation treatment, inability to follow rehabilitation or complete follow-ups, and immunosuppressive treatment including systemic corticosteroid treatment.

5.2.3 Materials

All patients underwent open surgery, and the Kessler technique was used to repair the tendon. Subsequently a non-weightbearing below-knee plaster cast with the ankle in 30^{o} of plantar flexion was applied to the lower limb. Following surgery, patients were randomized to one of the two rehabilitation groups, Early or Delayed. After two weeks, the skin sutures were removed, and the plaster cast was replaced by an orthosis (AirSelect^{TM} Standard Aircast Walker). Rehabilitation: *The Early group* (The present regime at Bispebjerg-Frederiksberg Hospital and hence considered the control group): The orthosis was used for 4 weeks. Partial weight bearing was permitted from week 3 after surgery and full weight bearing from week 7. The patients were told to remove the orthosis five times a day and in a seated position perform 20 repetitions of active ankle joint range of motion exercises (dorsiflexion not permitted past 0 degrees) with the knee flexed and extended. Swimming and ergometer biking were allowed from week 9, standing heel-rise from week 15, jogging 6 months after surgery and return to contact sports 8-9 months after surgery. *The Delayed group:* The orthosis was used for 10 weeks. No weight-bearing was permitted the first 6 weeks after surgery and partial weight bearing with solely heel pressure was allowed from week 7 and full weight bearing from week 13. Similar instructions were given to the patients in this group to remove the orthosis five times a day and in a seated position perform 20 repetitions of active ankle joint range of motion exercises (dorsiflexion not permitted past 0 degrees) with the knee flexed and extended. Ergometer biking was permitted starting in week 13, swimming starting in week 15, standing heel-rise starting in week 21, jogging 8 months after surgery and return to contact sports 12 months after surgery. Patients in both groups attended four consultations with a physiotherapist (week 2, 6, 12, 18).

5.2.4 Ethical considerations

On the individual level, patients who participated in this study benefitted from a carefully designed rehabilitation program with regular supervision, which was beyond standard treatment.

Furthermore, patients who participated in this study received a careful clinical examination both at the start and at the end of the study and got feedback of the results. It was expected that the benefits would outweigh the risks and potential side effects associated with this study and the study was considered ethically justifiable. Ethical approval was obtained from the Regional Ethics Committee and all participants were given oral and written information about the study and gave written informed consent.

5.3 Study III

5.3.1 Study design

This study was a controlled laboratory study using an animal model.

5.3.2 Participants

In the study 28 female Specific Pathogen Free Sprague Dawley rats, aged 9-10 weeks, were used. Nine animals had to be excluded for various reasons (see paper III for more details) and therefor only 19 animals were included in the analysis.

5.3.3 Materials

The rats were randomized into either group A) where Achilles tendon transection (ATT) on the right leg was performed followed by a 3 mm removal of the Achilles tendon to achieve a significant elongation of the tendon. Or group B) where ATT on the right leg was performed followed by a suture repair to achieve minimal tendon elongation. All rats used a metal boot on their operated leg for two weeks to reduce tendon loading followed by full loading for two weeks after removal of the boot. Then, after four weeks, all rats were euthanized, and their hind limbs were collected for microscopic analysis of the fascicles and sarcomeres.

5.3.4 Ethical considerations

All surgical and experimental procedures were completed in accordance with the institutional guidelines and approved by the regional ethics committee for animal experiments in Linköping, Sweden.

5.4 Study IIII

5.4.1 Study design

This study was a longitudinal within-subject control exploratory investigation of persons with a prior unilateral Achilles tendon rupture more than 1 year after the injury, treated with or without surgery, who still experienced a functional deficit.

5.4.2 Participants

Fourteen participants (2 women and 12 men), who have had an Achilles tendon rupture more than one year ago, were recruited via posts on social media. Inclusion criteria: Their Achilles tendon injury should still compromise their overall daily activities, including recreational activities and they should be unable to perform a heel-rise of equal height when standing on one leg at the time. Exclusion criteria: Less than 3 cm difference in heel-rise height between the injured and uninjured leg, bilateral Achilles tendon rupture, other pathological complications or injuries in the lower extremities, or any severe health related problems.

5.4.3 Materials

The participants performed 12 weeks of supervised isokinetic eccentric training in a dynamometer (Biodex Medical Systems, Shirley, New York). The training was performed at Bispebjerg-Frederiksberg Hospital twice a week. All sessions consisted of isokinetic eccentric plantar flexion contractions first performed with the knee flexed and afterwards with the knee extended. The number of sets performed increased over the 12 weeks starting with 3 set of 10 repetitions and ending with 5 set of 10 repetitions.

5.4.4 Ethical considerations

Ethical approval was obtained from the Regional Ethics Committee and all participants were given oral and written information about the study and gave written informed consent.

6. METHODOLOGICAL CONSIDERATIONS

6.1 Outcomes

An overview of the outcomes used in study I, II, and IIII and when they were obtained are presented in Table 1.

Outcomes	-1 week	Baseline	1 week	2 weeks	12 weeks	26 weeks	52 weeks
Patient reported outcome measures							
ATRS	●	●			●		●
PAS	●	●					●
Satisfaction		●			●		●
Functional evaluation							
Heel-rise test		●			●	●	●
Muscle strength		●			●		●
ATRA		●		●	● ●	●	●
Electromyography		●					
Force loading		●					
Imaging							
MRI (3D)		●	●		● ●	●	●
MRI (Dixon)		●	●		● ●	●	●
US		●		●	● ●	●	●

Table 1: *Outcomes in Study I, II, and IIII and when obtained.*
● Study I, ● Study II, ● Study IIII
ATRS: Achilles Tendon total Rupture Score, PAS: Physical Activity Scale, ATRA: Achilles Tendon Resting Angle, MRI Magnetic resonance imaging, US: Ultrasound. -1 week: Recall one week before injury.

6.1.1 Patient reported outcome measure

Investigating the patient's accounts of function, limitations, and challenges is one technique to gauge the impact of an injury. These reports are known as patient reported outcome measures (PROMs), and they can be used when attempting to comprehend and scale the outcome observed from a patient point of view. Over the last years PROMs have been almost mandatory to use in clinical trials and are frequently used as the primary outcome (69, 70). A variety of PROMs exists and can be divided into generic and condition specific. Generic PROMs are typically used to evaluate general health and can be applied to most patient groups. When evaluating patients with a more specific diagnosis such as Achilles tendon rupture a condition-specific PROM is usually more appropriate (71). Many different PROMs have been used in relation to Achilles tendon rupture, such as the Leppilahti score (72) and the American Orthopedic Foot and Ankle Hindfoot score (73) among others, but only the Achilles tendon Total Rupture Score (ATRS) has been

validated as a condition-specific PROM (74, 75), and therefor this was the PROM we decided to apply in Study II and Study IIII as a secondary outcome.

Regarding Study II we considered, before writing the protocol, if the ATRS should be the primary outcome, but since the target of our intervention was to reduce the Achilles tendon length and improve the functional outcomes, we chose the heel-rise test, which can be measured reliably, and relates to function (76). There is a known correlation between heel-rise height and tendon elongation (39, 53), which also supported this decision. In Study II the patients were asked to report the ATRS score as they remembered their function a week before their Achilles tendon rupture. Consequently, it cannot be ruled out that recall bias affects this assessment. However, because randomization was applied and patients in both groups scored highly at this timepoint, it was expected that any potential recall bias did not have a substantial impact on the outcomes. In Study IIII the ATRS was likewise used as a secondary outcome and some of the same reflections contributed to the choice of not selecting it as primary outcome. The target of our examination in this study was to explore if the fascicle length could be changed by eccentric training and lead to better function and therefor the ultrasound measurements of fascicle length were found more appropriate.

The interpretation of patient reported outcomes can be challenged by the response shift phenomena, a concept that emphasizes cognitive appraisal processes (77). For example, when persons experience a health care change as an Achilles tendon rupture, they might change their mindset in relation to fatigue, participation in sports, and some of the constructs they are asked to self-report. Since effective coping with disability requires a regular reappraisal of a person's definition of participation, response shift is likely to be common in participation measurement (77) and probably also in the ATRS score.

The Physical Activity Scale (PAS) was another PROM that was used in both Study II and Study IIII. It was developed and validated to be a simple alternative to measuring physical activity by diary (78). PAS was used to estimate the total physical activity in 24 hours in sports, work, and leisure time on an average weekday. In Study II the first timepoint was, like the ATRS, reported as the patients remembered their function a week before their Achilles tendon rupture. Therefor the same cautions regarding recall bias apply here. Another important remark regarding physical activity is that during the recruitment period in Study II there were two major lock downs in Denmark due to Covid 19. Both times this included 3-4 months with no access to club sports or

gyms. For this reason, many patients reported that they had been less active than usual leading up to their injury.

Furthermore, a 5-point Likert scale was used to estimate general satisfaction with their injured ankle after the intervention was completed in Study II (52 weeks) and Study IIII (12 weeks). Two standardized questions were used: “How satisfied are you with the operated leg in your everyday life?” and the second question focused on sportive activities: “How satisfied are you with the operated leg during sportive activities including running and jumping?”

6.1.2 Functional evaluation

Force loading

Pedar force sensor insoles were used in Study I to quantify forefoot force. (Pedar®, Novel, München, Germany) Both the orthopedic boot on the right leg (Figure 5) and the standard sneaker on the left were fitted with insoles that measured force during gait. The insole was divided into a forefoot (sensors 34-99) and rearfoot (sensors 1-33) region. Peak and mean force within each of the two regions across all steps were extracted from the software. The system was calibrated before every new participant was tested (79).

Figure 5. *Pedar force sensor insole in an orthopedic boot.*

Electromyography

In Study I, surface electrodes (Ag/AgCl Blue-Sensor N-00-S, AMBU, Ballerup, Denmark) were applied to the skin over the medial gastrocnemius, soleus, and tibialis anterior muscles with a distance between the electrodes of 2 cm to obtain bipolar electromyographic (EMG) signals

(Figure 6). A reference electrode was placed on the tibial bone. The skin was shaved, abraded, and prepped with an alcohol wipe before electrode attachment to ensure optimal signals. The EMG signals were recorded at a sampling frequency of 1500 Hz using 16 bit analogue-to-digital resolution (Telemyo 2400T G2 Telemetry system, Noraxon USA Inc, Scottsdale, AZ) (79).

Participants performed a maximal voluntary contraction (MVC) with the plantar flexor muscles and the dorsi flexor muscles, which were used in the normalization procedure for the EMG data. The EMG recordings were exported from Noraxon to Matlab for additional processing to convert the raw signals to linear envelopes by digitally high-and low-pass filtering (Butterworth 4^{th} order zero-lag filter, cut-off frequencies: 20 and 500 Hz, respectively), full-wave rectification and finally low-pass filtering (cut-off frequency 15 Hz). The linear envelopes were normalized to the peak EMG amplitude of the linear envelopes obtained during the MVC (%MVC). For the tests, peak values were extracted in the same fashion, in addition the average EMG over the entire walk was also extracted (79).

Figure 6. *Placement of surface electrodes on the medial gastrocnemius, soleus, tibialis anterior muscles, and the tibial bone.*

Heel-rise test

A single leg standing heel-rise was used to evaluate calf muscle endurance and range of motion in Study II and Study IIII. It is a well-known functional test widely used in studies to evaluate heel-rise height and repetitions and has been found to be reliable and valid for testing patients with an Achilles tendon rupture (76). A number of 25 repetitions has been graded as normal in healthy individuals, however the range reported was rather wide with a minimum of 6 and a maximum of 70 repetition (80). The heel-rise test was performed on one leg at the time and the uninjured leg

was tested first. The patients should stand barefooted on a box with the ankle in neutral position and were allowed to place their fingertips, at shoulder height, against the wall for balance. We used a box with no incline, since we wanted to compare our results with a previous study performed in our department, where a box with a horizontal surface was used (38). The heel-rise test was performed with a cadence at 30 rises per minute, ensured by a metronome. The MuscleLab® (Ergotest Technology) computer software and a linear encoder censor was used for evaluation (Figure 7). The linear encoder was attached to the heel.

Figure 7. *The heel-rise test.*

The patients were instructed to raise the heel as heigh as possible on each repetition and perform as many heel-rises as possible. The test was terminated when the patient stopped, did not maintain the frequency, or did not perform a proper heel-rise (did not maintain full knee extension, used more support than just the fingertips). Maximum heel-rise height, number of repetitions, and the total work (the body weight times total distance) in joules was used for analyses. In Study II, the assessor was blinded in relation to intervention group and in both Study II and IIII, the assessor was not allowed to verbally encourage during the test to avoid some patients being more encouraged than others. However, persons have different pain thresholds and abilities to push oneself to the limit, which could have influenced the number and height of repetitions. Maybe if you focus on the height, you are not able to perform so many repetitions and vice versa.

Muscle strength

Both in Study II and Study IIII a dynamometer (Biodex Medical Systems, Shirley, New York) was used to measure muscle strength, but with two different test protocols. In Study II, to ensure that the tendon would not be exposed to excessive stresses that could result in a new injury, muscle strength was only measured at the final timepoint, a year following the injury. A previous described method (52) was used and first the uninjured and then the injured leg was tested. The patient was seated with the hip flexed in 70° and the knee in full extension (0°). As a warm-up and familiarization to the test, three incremental isometric plantar flexion contractions at 50%, 80%, and 100% of maximum strength were performed. Afterwards, the patient performed the test, comprising a series of maximal isometric plantar contractions (3-5 repetitions) at 20° of plantar flexion, 10° of plantar flexion, neutral (0°), 10° of dorsiflexion, and 20° of dorsiflexion. The test was continued as long as the patient's peak torque score increased; however, it was determined to complete a minimum of 3 and a maximum of 5 repetitions at each angle.

Study IIII's test protocol was based on a protocol that had previously been utilized in a comparable study (81). Both the uninjured and injured leg was tested in the order mentioned. First concentric and then eccentric strength in plantar flexion was determined isokinetically (Biodex Medical Systems, Shirley, New York) at 30°/s throughout the full ankle joint range (from 10° of dorsiflexion to 30° of plantarflexion) with the knee in flexion (40°) and thereafter with the knee in extension. Ten submaximal efforts were performed as warm up and familiarization to the test and thereafter 2 x 5 consecutive maximal plantar flexion contractions were completed.

In both Study II and Study IIII, verbal encouragement was given to the patients during the test to ensure that they gave their all. The patients had a variety of tests on the final test day (valid for both Study II and Study IIII), including an MRI scan, an ultrasound scan, a heel-rise test, and finally the muscle strength test, to reduce the number of test days. There was a 10-minute gap between the heel-rise test and the muscle strength test, which was used to complete questionnaires while seated and giving the muscles in the legs a rest. The outcome of the muscle strength test, however, might have been impacted by fatigue from the heel-rise endurance test. Since the tests were given to all patients in the same order and by the same assessor, all patients had the identical test course. To keep the test execution as uniform as possible, the same standardized test procedure was used at each test.

6.1.3 Muscle and tendon properties

Magnetic resonance imaging (MRI) – 3D and Dixon technique

We carried out some test scans on healthy persons without ankle injuries in preparation for the MRI protocol for Study II (Philips Ingenia Ambition 1.5T scanner, software version 5.6.1.2 (Eindhoven, Netherlands). We aimed for a standard setup with as identical an angle in both ankles as possible in relation to tendon length measurements. We performed two test scans with a person placed in a supine position and the feet placed in two distinct angles (30° and 90° of plantar flexion). The tendon length was then measured on the MRI images to determine if the position of the foot had an impact on the Achilles tendon length, and there was no difference between the two measurements in the two distinct positions, and the placement of the foot did not significantly affect the tendon measurements. Still, to make it easier to repeat the measurements in the same position, we tried to standardize the position in which the patients were lying during the scan as much as possible. For this reason, we also performed some test scans in which a plaster cast with a 30° plantarflexion angle was applied to the one leg, with both legs placed close together and the feet placed against a plastic foot plate with an incline resulting in approximately 30°of plantar flexion in the ankle joint, and a string around both forefeet. This was the setup we decided to use for the MRI protocol in Study II, so the legs were placed in a standard position with the injured leg placed in the same plaster cast that was applied just after the surgery at each timepoint (Figure 8). In Study IIII, no plaster cast was applied, but otherwise the same standard position was used.

Figure 8. *(A) MRI standard position and (B) Example of plaster cast that was used for each scan.*

We wanted to be able to measure independent elongation of the soleus part and the gastrocnemius part of the tendon as precise as possible and therefor a coronal isotropic 3D T1 weighted sequence of both ankle and calves was used. As described in a previous study (53), the 3D imaging allows reconstruction of the images to compensate for positional differences of the different parts of the tendons over time and thus reduce measurements errors. Besides measurements of tendon length, the MRI images were also used to measure the cross-sectional area (CSA) of the medial gastrocnemius, the lateral gastrocnemius, the soleus, and the flexor hallucis muscles in Study II and IIII.

Furthermore, in both Study II and IIII, an axial 6-point Dixon sequence was applied from the knee to the ankle joint of both calves to measure the free fat fraction of the soleus, medial gastrocnemius, lateral gastrocnemius, and flexor hallucis longus muscles. These sequences were more experimental and presented a few challenges in relation to artefacts and quality of the images and therefore resulted in a small number of not usable images.

Horos v3.3.6. open-source medical image viewer was used for the MRI tendon length and CSA measurements, and Fiji/ImageJ2 (Version 2.3.0) was used for Dixon free fat fraction quantitative analysis. Both the injured and uninjured leg were analyzed. Three landmark locations were established, by looking at the sagittal, horizontal, and coronal plane simultaneous, in each lower leg for the tendon length measurements as previously described (53).

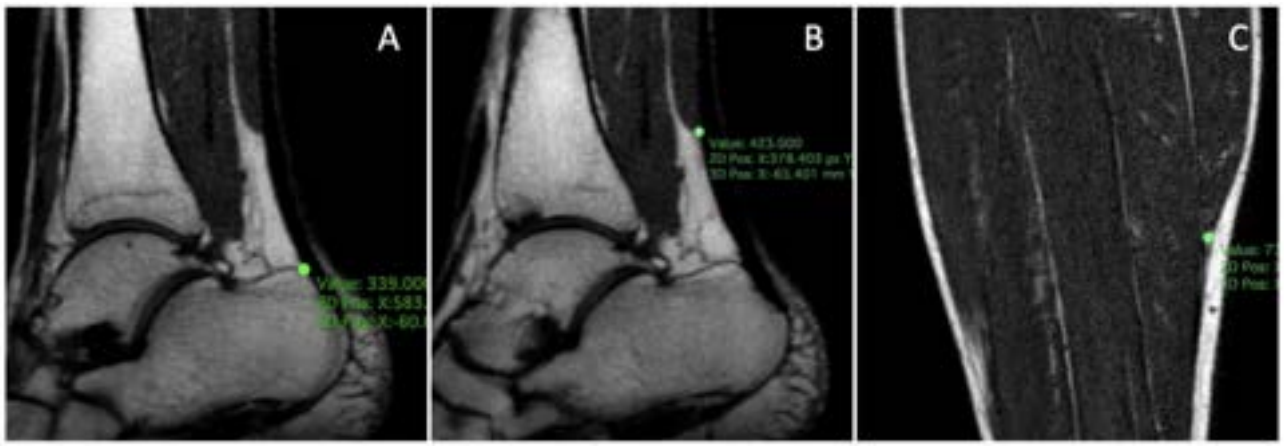

Figure 9. *Landmarks used to measure the Achilles tendon length. (A)The most proximal insertion of the Achilles tendon on the calcaneus. (B)The most distal part of the myotendinous junction (MTJ) between the soleus and the Achilles tendon. (C)The most distal part of the MTJ between the medial gastrocnemius and the Achilles tendon.*

Landmark 1: The most proximal point of insertion of the Achilles tendon on the calcaneus. Landmark 2: The most distal portion of the myotendinous junction (MTJ) that connects the soleus muscle to the Achilles tendon. Landmark 3: The most distal portion of the MTJ between the Achilles tendon and the medial gastrocnemius (Figure 9). The distance between the calcaneus and soleus MTJ (landmarks 1 and 2) was used to define soleus tendon length, while the distance between the calcaneus and medial gastrocnemius MTJ (landmark 1 and 3) was used to define gastrocnemius tendon length. In a study conducted previous in our department (53), using the same measurement procedures, the correlation coefficient and typical error percent were 0.98 and 5.1% for the injured soleus muscle, 0.99 and 4.6% for the uninjured soleus muscle, 0.94 and 3.1% for the injured gastrocnemius muscle, and 0.99 and 0.6% for the uninjured gastrocnemius muscle, demonstrating that repeated measurements were not subject to systematic bias. The tendon length was measured by two assessors in Study II, and to ensure measurement accuracy and interobserver reliability, the second assessor measured ten of the images that were already measured by the first person. The results were then compared, and the measurements were repeated until a satisfactory level of agreement between the ten measured images was reached. In Study IIII, with fewer participants and only two timepoint, all tendon length measurements were performed by the same assessor to reduce measurement bias.

The CSA of the soleus, medial, and lateral gastrocnemius muscles were measured manually at one third of the length of the triceps surae muscle proximal to landmark 3, while CSA of the flexor hallucis longus muscle was measured 4 cm proximal to landmark 1. To account for a potential muscle shortening due to a tendon elongation, we chose the methodology as stated for the triceps surae rather than only a point, for example, 30 cm proximal to landmark 1. Thus, we assumed to perform the measurements closer to the same area in the muscle at the different timepoints. Due to the very time-consuming measurements of the CSA, just two slices were measured rather than multiple slices, which would have resulted in the CSA for a larger section of the muscle. The free fat fraction was analyzed on the Dixon images by use of the identical ROI´s (Regions of interest) as measured on the 3D images used for CSA.

Ultrasound – greyscale and power Doppler

It is well known that ultrasound measurements are highly assessor dependent, so it was essential to ensure the same person conducted all the scans to reduce measurement bias. Therefore, out of

the 192 ultrasound examinations conducted in Study II, the 189 were conducted by the same assessor and the few exceptions were due to illness. Furthermore, the same ultrasound machine (Hitachi HI VISION Ascendus ultrasound system) was used for all the examinations at all timepoints. To ensure a consistent test technique, a standardized protocol was developed, along with a template for the greyscale and power Doppler settings. A long transducer was used to visualize the largest portion of the muscle. Muscle fascicle length, thickness, and pennation angle were measured with B-mode ultrasound. During the test the patient was lying prone, with the knee in 0^0 (stretched leg). The ultrasound scans were performed in two positions (*Free* and *Supported*), starting with the healthy leg in both positions. Position 1 (*Free*, scanned at week 2, 12, 26 and 52): With their feet and ankles lying free of the patient bed in a relaxed position. Position 2 (*Supported*, scanned at week 12, 26 and 52): With their feet and ankles supported in plantarflexion by a foam block, to reproduce the same degree of plantarflexion that was measured at week 2, where the plaster cast had just been removed, on the injured side in the *Free* position. Note that the uninjured side was also placed at the angle corresponding to the injured side at 2 weeks to enable comparison at equal plantarflexion angles between legs. A protocol based on previously described techniques was used (82, 83). At the beginning of each ultrasound test, two landmarks were identified and marked on the skin with a pen. The first landmark corresponded to the muscle-tendon junction of the medial gastrocnemius muscle and the Achilles tendon, and the second landmark corresponded to the medial knee joint line. Additionally, the distance from the muscle-tendon junction to the most distal part of the heel was measured in cm and noted in the data sheet. For fascicle length measurements of the medial gastrocnemius, the probe was aligned perpendicular to the skin and parallel to the tibia. The probe was then positioned halfway between the two landmarks = area 1, then 2 cm laterally = area 2, then 2 cm further lateral = area 3.

All measurements and quantitative analysis were performed using Fiji/ImageJ2 (Version 2.3.0). A previously published plugin (Simple Muscle Architecture Analysis, SMA) (84) was utilized to investigate fascicle length, pennation angle, and muscle thickness to reduce assessor influence. We made two modifications to the plugin: 1) Changed the data import method to support our file format. 2) Replaced the automated detection of aponeuroses with manual outlining due to difficulty establishing automated parameters that worked consistently across all recordings.

Power Doppler measurements were performed at weeks 12, 26, and 52, but not at week 2 because we wanted to prevent suture area infection and the patients had just had the skin stitches removed. The patient was lying in a prone position, with the feet and ankles just free of the patient bed in a relaxed position without any load on the Achilles tendon. The transducer was placed perpendicular to the tendon and to verify that the scan was performed in the location of the rupture the sutures in the tendon were identified, thereafter the transducer was adjusted to find the area with maximum Doppler activity. Only a minimum transducer pressure was applied during the scanning to demonstrate as much flow as possible. The scan was recorded as a 5 second sine loop (20 frames) in the sagittal plane with the highest power Doppler activity. From each sine loop, the image with the highest amount of Doppler activity was selected and used in the analyses. A short linear transducer and a project template was used.

Fiji/ImageJ2 (Version 2.3.0) with a customized plugin was used to quantify power Doppler flow by automatically detecting the frame with most Doppler signal within the tendon, after manual removal of noise artifacts. Analyses was performed by persons blinded to intervention allocation and side.

In Study IIII, the same two ultrasound templates for greyscale and power Doppler were used, but since treatment with a plaster cast was not a part of this study, only position 1 (Free) was used. All ultrasound examinations in Study IIII were conducted by the same assessor.

Achilles Tendon Resting Angle (ATRA)

The ATRA are often used as an indirect measure of the tendon length in the clinic and was thus relevant to measure in Study II and IIII. The participant was lying prone, with the knee flexed in 90°. The participant was asked to relax the ankle and foot. A standard goniometer with 2° increments was pointed with one arm of the goniometer along the shaft of the fibula, directed towards the center of the fibula head, the axis at the tip of the fibula and other arm centered on the head of the fifth metatarsal. The center of the head of the fibula was palpated to make the placement of the goniometer as accurate as possible (85). As we believe a bent knee better targets the soleus part of the tendon, the same measurements were performed with the knee extended to target the gastrocnemius part of the tendon more efficiently. In both positions, the injured was measured after the uninjured.

Muscle architecture

In study III, fascicle length, sarcomere length, and serial sarcomere number was investigated using a dissecting microscope and transmitted light microscopy. We developed a standardized dissection protocol to ensure uniform handling of the muscle samples. First an image of the entire medial gastrocnemius muscle was obtained, and a cut was made as indicated by the blue dotted line on Figure 10.

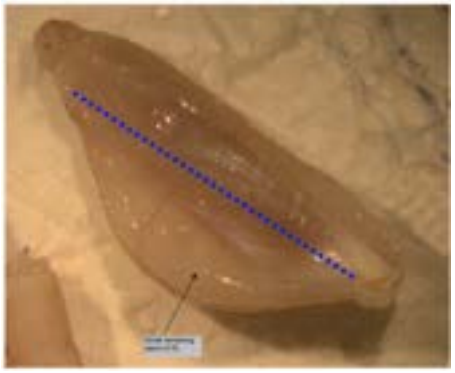

Figure 10. *The medial gastrocnemius muscle. The blue dotted line indicates where the cut was made.*

By cutting along the direction of the aponeurosis fibers, using the customized tool consisting of two knife blades (Figure 11), we wanted to minimize the number of muscle fibers that were transected, when taking the muscle sample. The cut was performed on the concave side of the muscle. The removed muscle sample was placed in 25% nitric acid (HNO_3) and put in an oven at 37 degrees Celsius for 4 hours to loosen the intramuscular connective tissue and enable easier fiber extraction.

Figure 11: *The customized tool consisting of two knife blades.*

The extraction was performed by use of a dissecting microscope using fine tipped tweezers and scissors. A bundle of fibers was extracted from the distal, middle, and proximal part of the muscle sample. Again, an image was taken of the length (along the fibers). To assure intactness of the

fibers the presence of connective tissue at both ends of each bundle was ensured. Obvious broken fibers were removed, and a new image was taken of a small bundle of 8-10 fibers. Lastly, 5 intact fibers were identified and placed parallel on a microscope slide with a coverslip carefully placed on the top as shown on Figure 12. Transmitted light microscopy (40 times magnification) was then used for imaging and 4 images of 5 fibers at the distal, middle, and proximal site were obtained.

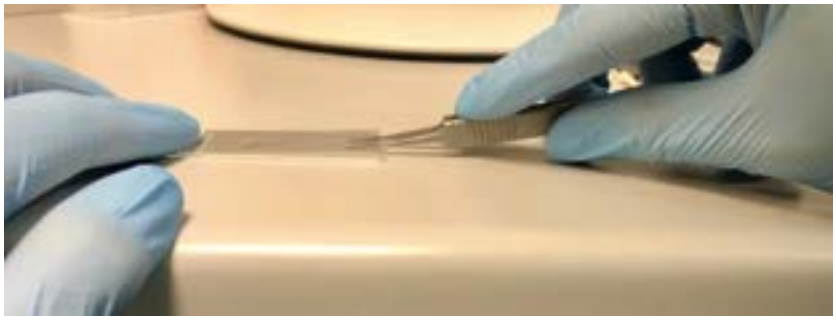

Figure 12. *Five intact fibers were placed parallel on a microscope slide with a coverslip carefully placed on the top.*

6.2 Randomizing

Study II: To ensure balance in the two groups at any time during the trial we decided to use block randomization (86). This choice was made based on knowledge gained from earlier research (38) conducted in our department, in which there were significant seasonal recruitment pauses. This was before we knew anything about Covid-19 lock-downs, which resulted in two major lock-down periods during our recruitment. Considering this, using block randomization was probably a wise choice, as it allows us to be certain that we recruited patients for both groups (Early and Delayed) during the whole recruitment period that ended up being longer than expected. The randomization procedure was performed using a computer-generated block randomization so that for every block of eight, four would be allocated in each arm of the trial.

In Study III, the rats were randomly assigned following simple randomization procedures to one of the two treatment groups.

6.3. Blinding

Study II: Because this was an “open label” trial, the physiotherapists providing the interventions and the patients could not be blinded to treatment allocation. Outcome assessors were blinded to

treatment allocation, and participants were requested not to disclose their allocation when outcomes were assessed. Furthermore, all data were analyzed blinded.

6.4 Intervention

In Study II, we wanted to compare current clinical practice with delayed loading, so the control group (Early) reflected the rehabilitation protocol already used after an Achilles tendon repair at the hospital where the study was conducted, and in the intervention group (Delayed) the initial loading was delayed by 6 weeks. The two different rehabilitation protocols are schematic presented in table 2.

	EARLY	**DELAYED**
Plaster cast (30° plantar flex.)	Week 0-2	Week 0-2
Walker orthosis	Week 3-6	Week 3-12
3 heel wedges -remove from:	Week 4 -one peer week	Week 6 -one every second week
No weightbearing	Week 0-2	Week 0-6
Partial weightbearing	From week 3	From week 7
Full weightbearing	From week 7	From week 13
ROM exercises (5 times a day)	From week 3	From week 3
Ergometer bike / Swimming	From week 9/9	From week 13/15
Standing heel-rise	From week 15	From week 21
Jogging	6 months after surgery	8 months after surgery
Return to contact sport	8-9 months after surgery	12 months after surgery

Table 2: *Schematic comparison of the two interventions.*

In both groups, open repair surgery was performed. Ideally, we would have only had one surgeon to operate to ensure uniformity but due to the surgeons working hours and other duties, it was practically best to have more than one and thus, three experienced surgeons performed the operations. Before the intervention started, we had a couple of meetings were all three surgeons participated to discuss and develop standardized guidelines for the surgery procedures. (See paper II for a more detailed description). We compared and analyzed the tendon length measurements obtained by MRI at one week after the operation, distributed among the three surgeons, to assess the consistency of the surgeons' performance and found no significant difference between the surgeons. The three surgeons followed the same pattern and showed good agreement, which indicated that they had followed the standardized guidelines.

Following to the operation, a below-knee plaster cast with the ankle in 30° of plantar flexion was applied to the lower limb, and after two weeks without any weightbearing allowed, the cast was replaced by an orthosis (AirSelect^{TM} Standard Aircast Walker) that restricted movements of the ankle joint. Thereafter, the patients followed their assigned rehabilitation group, which was one of the two following:

Early rehabilitation: The orthosis was used for 4 weeks. No weight-bearing was allowed the first two weeks after surgery. Partial weight bearing from week 3 and full weight bearing from week 7 was allowed. From week 3 the patient was told to remove the orthosis five times a day and in a seated position perform 20 repetitions of active ankle joint range of motion exercises (not allowed dorsiflexion past 0 degrees) with the knee flexed and extended. (Figure 13A & 13B) Swimming and ergometer biking were allowed from week 9, standing heel-rise from week 15, jogging 6 months after surgery and return to contact sports 8-9 months after surgery.

Delayed rehabilitation: The orthosis was used for 10 weeks. No weight-bearing was allowed the first 6 weeks after surgery. Partial weight bearing with pressure on the heel only from week 7 and full weight bearing from week 13 was allowed. The patients were told to remove the orthosis five times a day and in a seated position perform 20 repetitions of active ankle joint range of motion exercises (not allowed dorsiflexion past 0 degrees) with the knee flexed and extended. Ergometer biking was allowed from week 13, swimming from week 15, standing heel-rise from week 21, jogging 8 months after surgery and return to contact sports 12 months after surgery. Patients in both groups attended four consultations (week 2, 6, 12, 18) with a physiotherapist to ensure that each group received equal attention and care. To guarantee that the patients received

the same accurate instructions, a skilled physiotherapist performed all the consultations in the project.

Figure 13. *Active ankle joint range of motion exercises (not allowed dorsiflexion past 0 degrees) with the knee (A) flexed and (B) extended.*

In Study IIII, the training protocol was designed to be similar to a training protocol described in a previous study (87) that included healthy persons with no Achilles tendon injuries. In the aforementioned study, 12 weeks of training resulted in muscle architecture alterations in terms of increased fascicle length and muscle thickness. By using the same design, we knew that the increased fascicle length that we wanted to achieve in the injured participants could be achieved in healthy participants with this protocol. The isokinetic eccentric training program was performed for 12 weeks in a dynamometer (Biodex Medical Systems, Shirley, New York). The program included three 4-week mesocycles and consisted of two weekly training sessions corresponding to a total of 24 training sessions with minimum 48 hours restitution between consecutive sessions. Throughout the first four weeks, the exercise volume was steadily increased and comprised 3 × 10 repetitions with the knee flexed and 3 x 10 repetitions with the knee extended. In week 5-8 the participants performed 4 x 10 repetitions each with the knee in flexion and extension and in week 9–12 they performed 5 x 10 repetitions with the knee in flexion and extension. Only the injured leg was trained while the healthy served as a control. Each training session started with a 10 min warm up on a cycle ergometer followed by a specific warm up comprising ten submaximal eccentric contractions at $30^{o}\ s^{-1}$ throughout the full ankle joint range from 10° of dorsiflexion to 30° of plantarflexion in the isokinetic dynamometer. The participant was seated with the hip flexed at 70°. Training was performed with the knee in flexion (40°) first and thereafter with the knee in extension (0°). During training participants were instructed to resist the dorsiflexion motion generated by the dynamometer using maximal effort contractions with the same range and speed as specified above. To ensure maximal effort, verbal

encouragement was provided during each training session. Participants only performed eccentric contractions during the training program. All repetitions were completed continuously, with 1-minute breaks between sets.

6.5 Statistics

6.5.1 Study I

Descriptive characteristics were reported as means and standard deviations (SD). The effects of ambulation type and wedge count were analyzed using 2-way ANOVA with repeated measures. Post-hoc analyses were made with Sidak's correction for multiple comparisons. The statistical significance level was set to $p < 0.05$ for all analyses (79).

6.5.2 Study II

Sample size: Based on previous data from a study conducted at our department (38) where the heel-rise height (primary outcome) on the injured side was 76% of the uninjured side at 12 months. We aimed to achieve a side-to-side difference of 90% and consequently calculated the sample size to be n=21 with a power of 80% (u_A=76 %, u_B=90 %, σ=16%). To account for possible drop-outs, a sample of 24 in each arm were recruited.

All data were analyzed using GraphPad Prism 9.4.1 following a predefined statistical analysis plan. Results were reported as mean $\pm$ standard error of the mean (SEM). Delta values were used and referred to as difference values (injured minus uninjured side), in the different outcome measures. Demographic baseline data were analyzed by use of the unpaired Students *t* test. A two-way mixed model (group x time) that included all timepoints was used to analyze the difference values in the outcome parameters. For isometric strength measurements, only a single timepoint (52 weeks) was measured and therefore a two-way mixed model (group x ankle angle) was used to analyze the percentage difference values. All analyses were performed as intention to treat. The significant level for all tests was set to $p< 0.05$.

6.5.3 Study III

Sample size: Based on previous sarcomere length data (88) a sample of n=10 was required to detect a difference of 20% with a power of 80%.

The statistical analyses were performed using GraphPad Prism 9.4.1. Unpaired Students *t* tests were used to analyze the differences in tendon and muscle length between the operated leg in group A and B. Paired Students *t* test were used to analyze the differences in tendon length, muscle length, and muscle mass between the operated leg and the unoperated leg. For comparison of the fibers and sarcomeres a mixed-effect analysis was performed with side (ATT, CTRL) and region (distal, middle, proximal) as factors (both repeated) using Šídák's multiple comparisons test. The level of significance was $p < 0.05$ for all analyses.

6.5.4 Study IIII

Sample size: Based on previous data (87) a sample size of n=15 was required to detect a 13% increase in fascicle length.

All data were examined using two-way ANOVA (side x time) with repeated measures, except the ATRS score and tendon length measurements, which were analyzed with a paired Students *t* test. An alpha-level of 0.05 was considered significant. All statistical analyses were completed in GraphPad Prism 9.4.1. Data was reported as 95% CI and as mean ± SEM in the figures.

7. SUMMARY OF THE RESULTS

In the following section a summary of the results from the four studies are presented. A more thorough description of the results from each study can be found in the associated papers in chapter 12.

7.1 Study I

The main findings of this study were that peak forefoot force showed a main effect of the number of wedges, with three wedges resulting in a lower load than one wedge (115 N, *p*= 0.0006). The same was valid for the relative peak force on the forefoot that showed a main effect with a significant decrease when using three wedges compared to one wedge across all three ambulation types (19.1%, *p*= 0.0009) (Figure 14) (79).

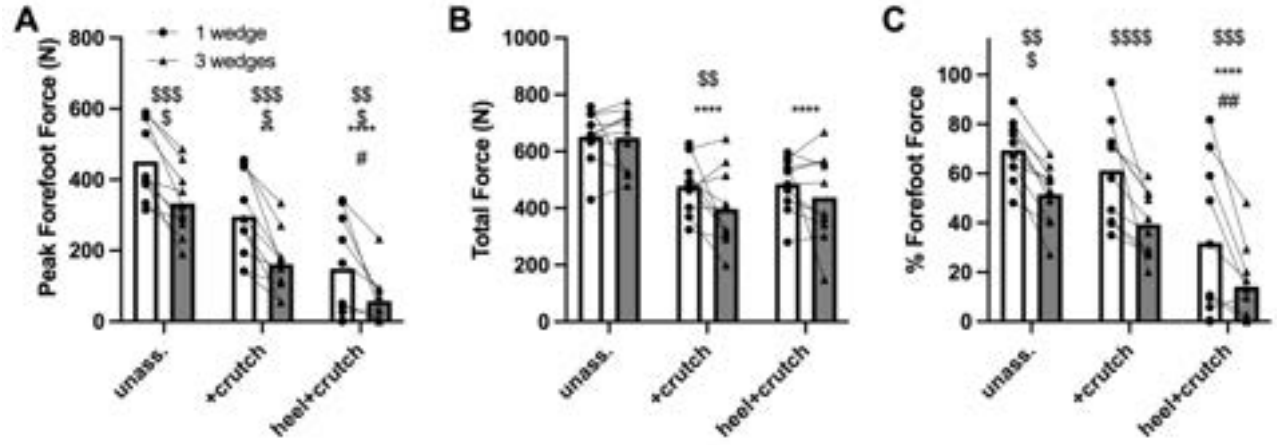

Figure 14 *(A) Change in peak forefoot force with numbers of wedges and the different ambulation types. (B) Change in total force with numbers of wedges and the different ambulation types. (C) Change in relative peak forefoot force with numbers of wedges and the different ambulation types.*
**: Indicates significant different from unass., #: Indicates significant different from +crutch, $: Indicates significant difference between 1 and 3 wedges. (Hoeffner et al. 2020 (79))*

Peak EMG of the medial gastrocnemius muscle showed a main effect of the numbers of wedges across ambulation types, decreasing with three wedges (6.5%, *p*= 0.01). For the soleus muscle there was a significant interaction presenting a decrease in peak EMG only for +crutch (9.3%, *p*= 0.003). For the tibialis anterior muscle, a main effect of wedge number was observed with an increased peak EMG (7.9%, *p*= 0.008) when using three wedges (Figure 15) (79).

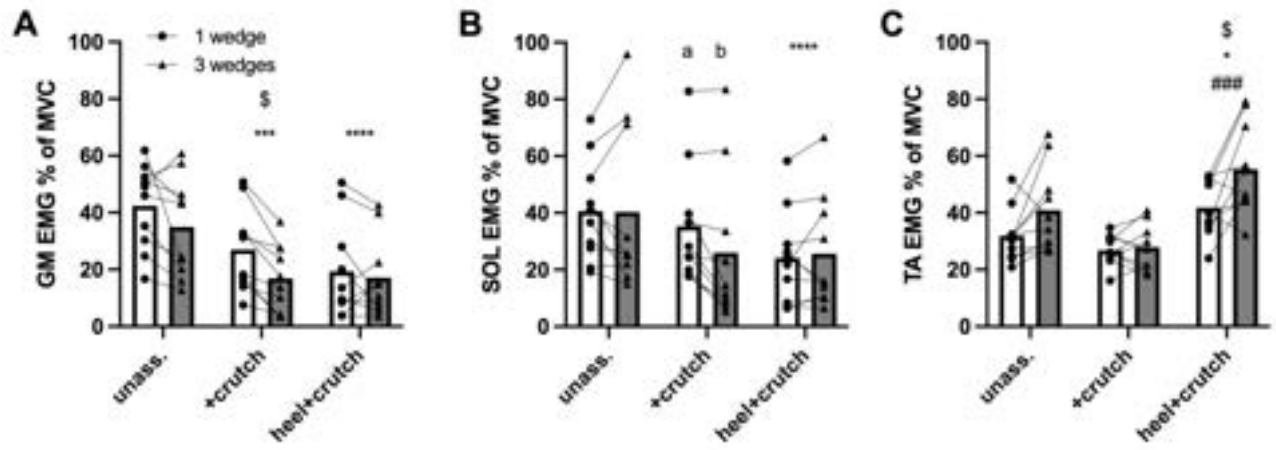

Figure 15. *Change in peak EMG with numbers of wedges and the different ambulation types. Normalized EMG to the participants peak MVC (A) GM; Gastrocnemius medialis muscle (B) SOL; Soleus muscle. (C) TA; Tibialis anterior muscle.*
**: Indicates significant different from unass., #: Indicates significant different from +crutch, $: Indicates significant difference between 1 and 3 wedges, a = significant difference between unassisted and +crutch (3 wedges), b = significant difference between +crutch and heel+crutch (1 wedge).*

Overall, the study demonstrated that adding heel wedges to an orthopedic boot and using crutches while walking can lower the force on the forefoot and subsequently the tensile load on the Achilles tendon.

7.2 Study II

There were no significant differences between the two groups (Early and Delayed) at baseline regarding sex, age, height, BMI, preinjury (recall one week before injury) ATRS score, and preinjury (recall one week before injury) PAS score, as shown in Table 3.

Characteristics	**Early** n=24	**Delayed** n=24
Male sex, n (%)	17 (71%)	18 (75%)
Age, years	36 ± 10	37 ± 9
Height, cm	179 ± 8	179 ± 11
BMI, kg/m^2	25 ± 3	26 ± 3
Injured side right/left n	10/14	12/12
ATRS (recall 1 w), point	99 ± 3	99 ± 3
PAS (recall 1 w), MET-hours	43.6±7	47.6±8

Table 3. *Baseline characteristics, Mean ± SD.*

The primary outcome in Study II was heel-rise height difference between injured and uninjured side at 12 months. A significant effect of time (p<0.0001), but no group or interaction effect was found with respect to heel-rise height difference at 6 months (Early= -37.0 mm, Delayed= -39.6 mm, p= 0.682) and the same was valid at 12 months (Early= -22.4 mm, Delayed= -21.0 mm, p= 0.719): the 95% CI of the group difference was [-8.4 to 11.1 mm] (Figure 16).

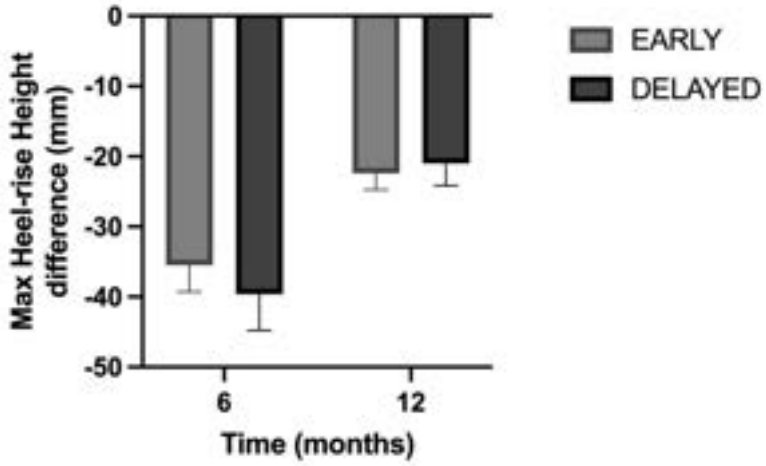

Figure 16*. Heel-rise height difference between injured and uninjured side in the Early and Delayed group at 6 and 12 months. Mean ± SEM, ⊿-values (Figure is duplicated from Study II).*

The analyses of tendon length, one of the secondary outcomes, showed that the soleus (free) part of the tendon was significantly elongated already at one week after surgery in both groups without a difference between the Early and Delayed group. The side-to-side difference in the Early group was 16.3 mm and in the Delayed group it was 17.5 mm (p=0.997). The side-to-side difference in soleus tendon length corresponded to a 35% elongation on the injured side at week 1, 30% at week 12, 41% at week 26, and 42% at week 52. In contrast, the gastrocnemius tendon length was unchanged at 1 week, but significantly elongated over time albeit without a between-group difference. The side-to-side difference at 12 months in the Early group was 10.5 mm and in the Delayed group it was 13.0 mm (p=0.899). The side-to-side difference in gastrocnemius tendon length corresponded to a 1% elongation on the injured side at week 1, 4% at week 12, 5% at week 26, and 6% at week 52 (Figure 17A & 17B).

ATRA revealed no significant group or interaction effect but a significantly effect of time (p<0.0001) was seen from week 2 to the other timepoints. This was true for the relative ATRA with the knees in 90° and the relative ATRA with the knees in 0° in both the Early and the Delayed group showing a larger angle on the injured side.

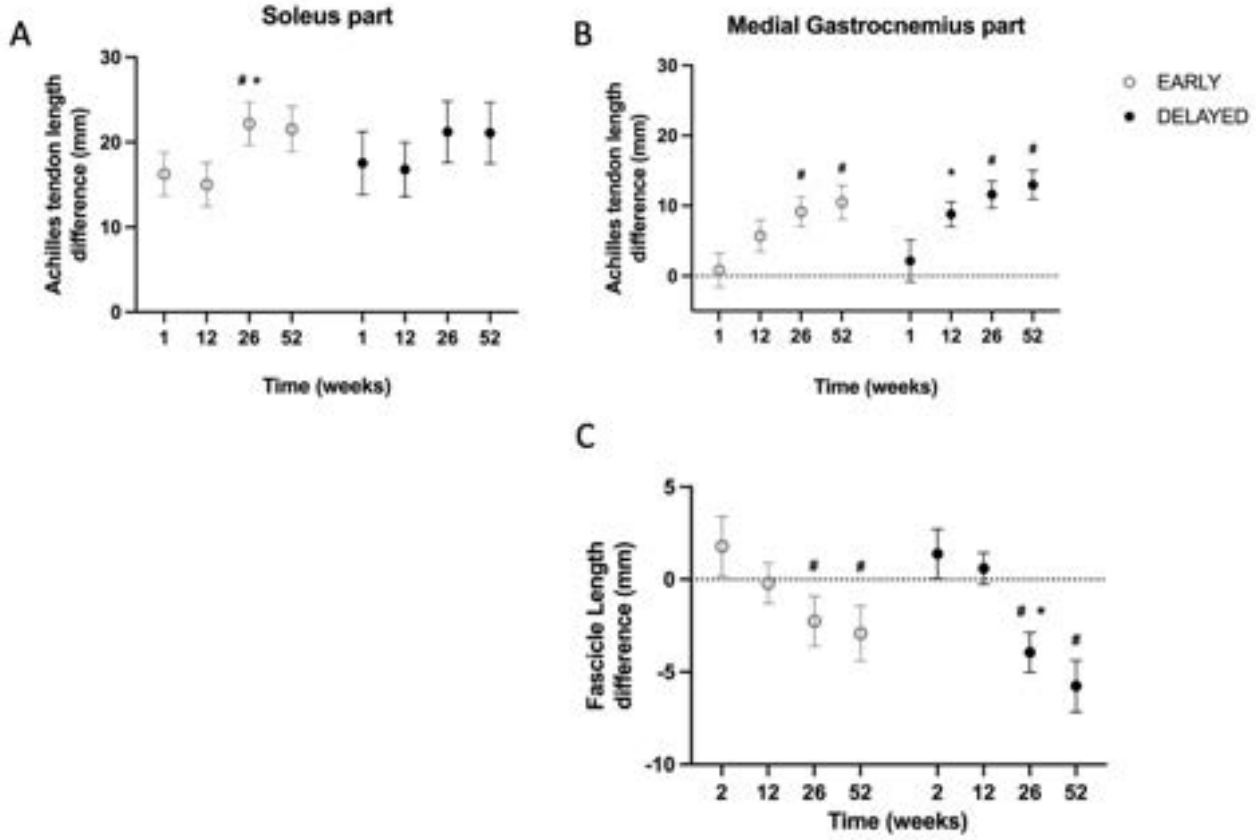

Figure 17. *The Achilles tendon length difference between injured and uninjured side at different timepoints in the Early and Delayed group, (A) Soleus part. (B) Medial gastrocnemius part, (C) Fascicle length (medial gastrocnemius muscle) difference between injured and uninjured side at different timepoints.*
** different from preceding timepoint, # different from 1 week. Mean ± SEM, Δ-values. (Figure is duplicated from Study II.)*

Regarding the muscle dimensions no significant group or interaction effect was found, but there was a significant effect of time (p<0.0001) with reduced gastrocnemius medialis muscle thickness, fascicle shortening (Figure 17C), and increased pennation after 6 months. The CSA of the soleus, gastrocnemius medial, and gastrocnemius lateralis were significant smaller (p<0.0001) at the injured side compared to the uninjured side at all timepoints. However, for CSA of the soleus, gastrocnemius medialis, and gastrocnemius lateralis no group or interaction effect was found, but a significant effect of time (p<0.0001, Figure 18). At week 1, week 12, week 26, and week 52, the CSA of the soleus muscle was 6%, 21%, 21%, and 20% smaller on the injured side. CSA of the gastrocnemius medialis muscle was 16%, 27%, 14%, and 13% smaller on the injured side at the different timepoints and CSA of the gastrocnemius lateralis was 18%, 26%, 10%, and 4% smaller on the injured side at the different timepoints.

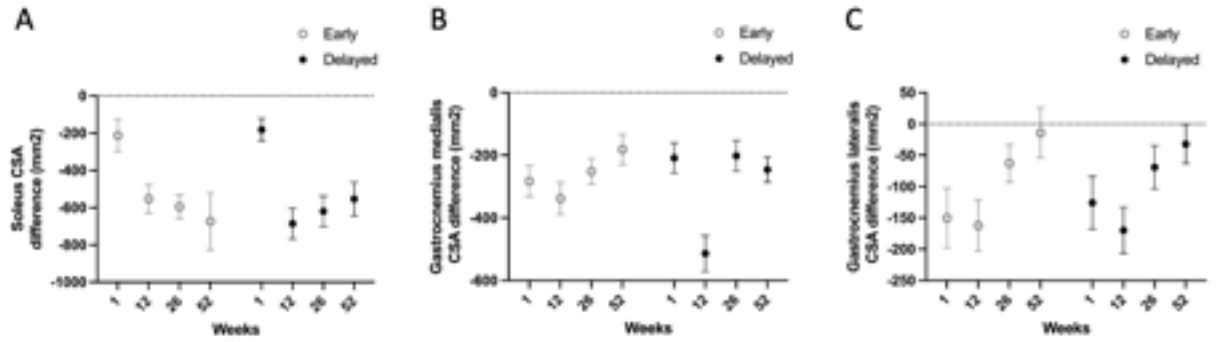

Figure 18. *Side-to-side CSA difference (mm^2) for the soleus, gastrocnemius medialis, and gastrocnemius lateralis muscles. Mean ± SEM, ∆-values. (Figure is duplicated from Study II.)*

The free fat fraction analysis showed no group or interaction effect, but a significant effect of time (p<0.0001, Figure 19) and at all timepoints the free fat fraction percentage was significantly greater (P<0.0001) at the injured side compared to the uninjured side for soleus, gastrocnemius medialis, and gastrocnemius lateralis. At week 1, week 12, week 26, and week 52, the soleus free fat fraction was 15 %, 46%, 58%, and 50% greater on the injured side. The gastrocnemius medialis free fat fraction was 21%, 29%, 31%, and 25% greater on the injured side at the different timepoints and the gastrocnemius lateralis free fat fraction was 8 %, 20%, 16%, and 18% greater on the injured side at the different timepoints.

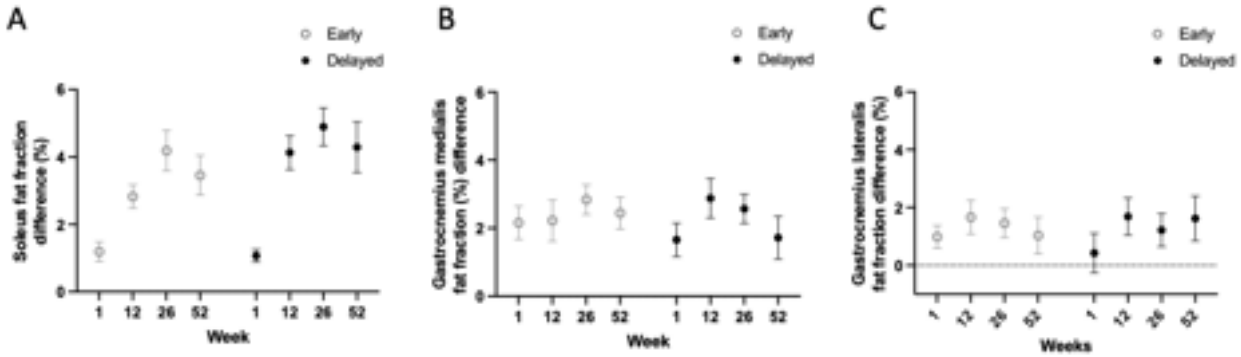

Figure 19. *Side-to-side fat fraction difference (%) for the soleus, gastrocnemius medialis, and gastrocnemius lateralis muscles. Mean ± SEM, ∆-values. (Figure is duplicated from Study II.)*

The power Doppler activity analyses revealed a significant interaction between time and group (p< 0.0001). At week 12 the Delayed group had significantly less Doppler area than the Early group (p= 0.006), while there was no difference between the groups at 26 and 52 weeks. A

significant decrease in Doppler area was seen in the Early group from 12 to 26 weeks (p= 0.0001) and from 26 to 52 weeks (p= 0.0006). From 12 to 26 weeks, the Doppler area in the Delayed group remained unchanged, but from 26 to 52 weeks, it significantly decreased (p= 0.0003) (Figure 20).

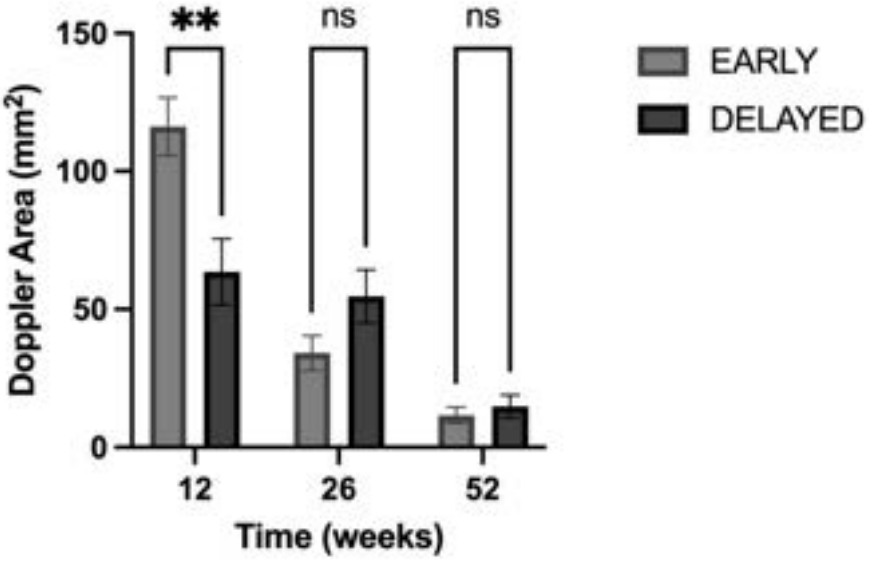

Figure 20. *Area with power Doppler in the injured Achilles tendon measured at different timepoints. Mean ± SEM. (Figure is duplicated from Study II.)*

Concerning ATRS, a significant interaction was observed between time and group (p= 0.021) and a significantly effect of time (p<0.0001). There was no significant difference between the Early (99±3) and Delayed (99±3) group at baseline (reported as recall 1 week prior to injury), but at 52 weeks a significant difference between Early (59.6±4.5 points) and Delayed (72.2±3.4 points) was found with a mean difference of 12.5±5.7, 95%CI [3.3 to 21.8], p= 0.053 (Figure 21).

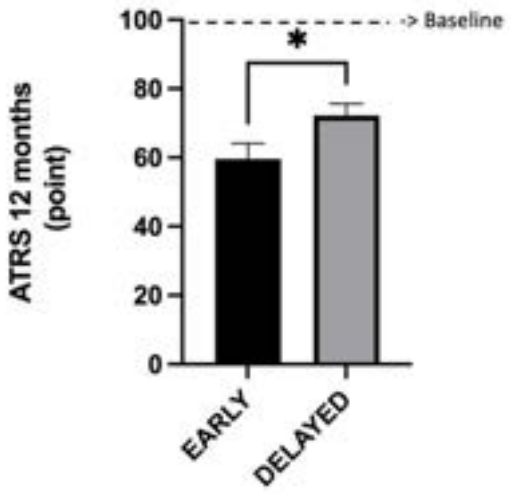

Figure 21. *ATRS score in the Early and Delayed group at 12 months. Mean ± SEM. (Figure is duplicated from Study II)*

The satisfaction score on a 5-point Likert scale (only measured at week 52) for everyday life was 4.3 for Early and 4.6 for Delayed, *p*= 0.076. For sports and activities, the score was 2.8 for Early and 3.4 for Delayed, *p*= 0.081.

Finally, there was no significant group, time, or interaction effect for PAS. Preinjury, the MET-hours for PAS for Early was 43.64±1.5 and for Delayed was 47.64±1.5 (*p*=0.0909) and at 12 months, Early was 45.65±1.3 and Delayed was 46.26±1.2 (*p*= 0.9429).

7.3 Study III

There was no difference in tendon and muscle length between the two surgery groups and they were therefor pooled and considered as one (ATT) in the further analyses and were compared to the intact legs (CTRL). The Achilles tendon was significantly longer (15.2%) on the ATT compared to CTRL side (*P*<0.0001) and GM muscle length was significantly shorter (8.2%) on the ATT compared to CTRL side (*P*<0.0001) (Figure 22).

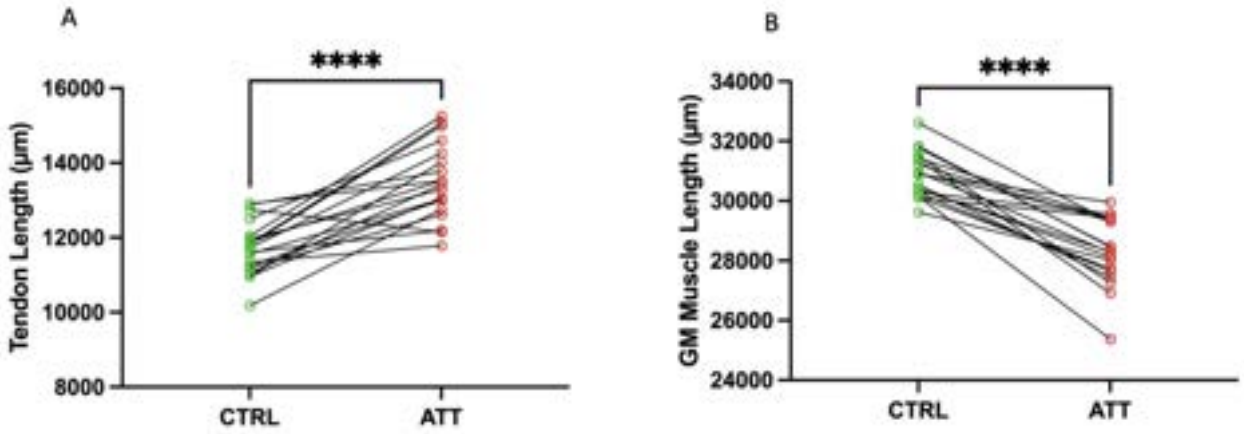

Figure 22. *(A) Achilles tendon length (B) Gastrocnemius Medialis muscle length. CTRL, Control. ATT, Achilles tendon transection. (Figure is duplicated from Study III.)*

For fascicle length there was a main effect of side and region, but no interaction. Fascicle length was shorter on the ATT side in all regions (*P*< 0.0001) and was also shorter in the proximal compared to both the mid (*P*= 0.015) and the distal (*P*= 0.0001) region.

For sarcomere length there was a significant interaction between side and region (*P*=0.0021). Sarcomere length was longer on the ATT side in the proximal (*P*< 0.0001) and mid (*P*< 0.005) but not the distal region and the difference between the ATT and CTRL side was significantly greater in the proximal region than in the distal (*P*= 0.014).

For sarcomere number there was a main effect of side, and region, but no significant interaction. In all regions there was a smaller number of sarcomeres on the ATT side (*P*< 0.0001) (Figure 23).

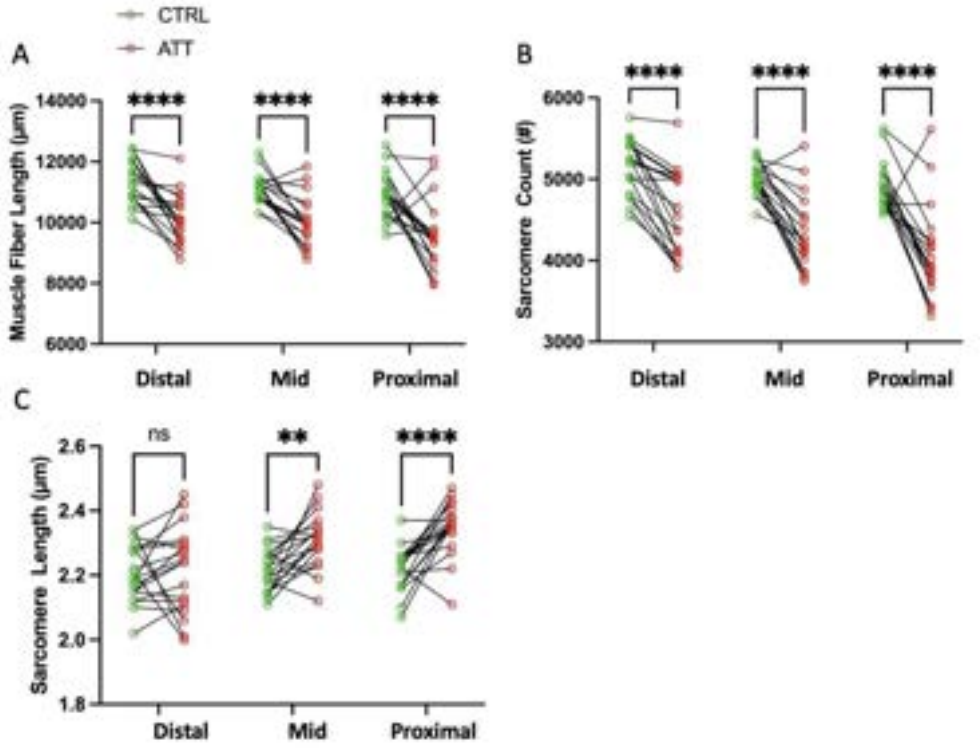

Figure 23. *(A) Muscle fiber length in the distal, middle, and proximal parts of the muscle samples. (B) Sarcomere numbers in the distal, middle, and proximal parts of the muscle samples. (C) Sarcomere length measured in the distal, middle, and proximal parts of the muscle samples. All samples from the Gastrocnemius Medialis muscle. CTRL, Control. ATT, Achilles tendon transection. (Figure is duplicated from Study III.)*

7.4 Study IIII

All participants completed the 12-week eccentric training program. Four people missed one session, five missed two, and five finished all 24 training sessions. Compliance was 95% overall.

For fascicle length of the medial gastrocnemius muscle there was no interaction, but a significant main effect of side (*P*< 0.0001) and time (*P*= 0.0179) with shorter fascicle length on the injured side and increased fascicle length at 12 weeks (Figure 24).

The tendon length analyses revealed that at week 0, both the soleus part (mean diff.= 44.0 mm, 95%CI [31.7 – 56.3 mm], *P*< 0.0001) and the gastrocnemius part (mean diff.= 32.0

mm, 95%CI [21.4 – 42.6 mm], *P*< 0001) of the Achilles tendon on the injured leg were significantly longer than on the uninjured leg. (Figure 25).

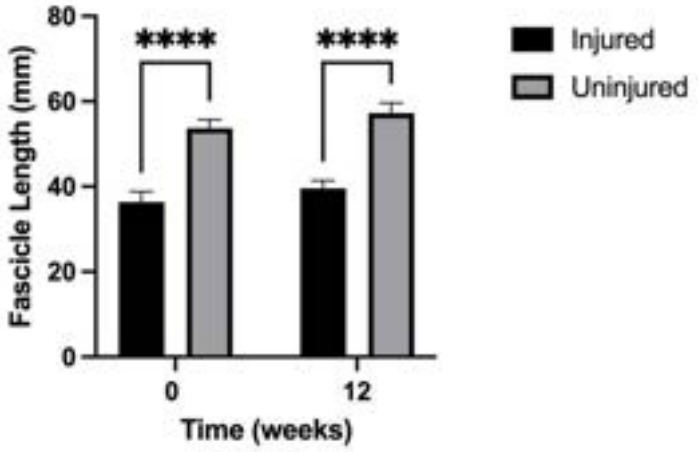

Figure 24. *Medial gastrocnemius muscle fascicle length on the injured and uninjured side. A significant difference (p<0.0001) between injured and uninjured side was found at both timepoints and there was a main effect showing an increase with time (p=0.0179). Mean±SEM (Figure is duplicated from Study IIII.)*

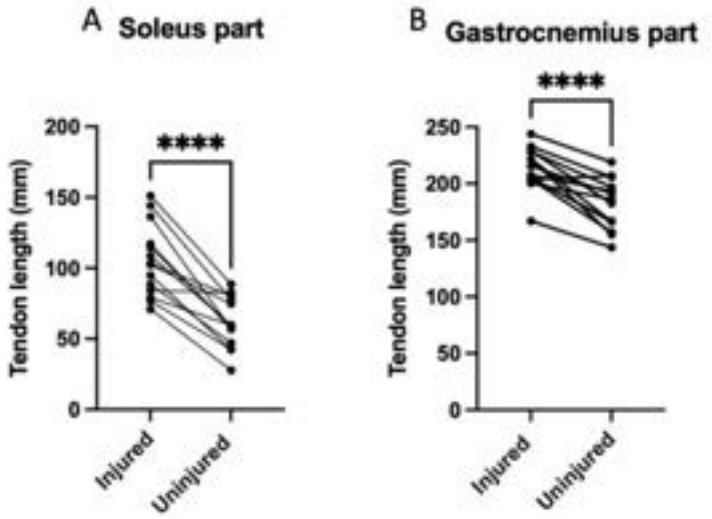

Figure 25. *Achilles tendon length on the injured and uninjured side. (A) The soleus part and (B) the gastrocnemius part were both significantly longer on the injured side than the uninjured side at week 0 (P<0001). (Figure is duplicated from Study IIII.)*

For heel-rise height no interaction or time effect was found, but a significant effect of side (*P*< 0.0001) with a lower mean heel-rise height on the injured side.

In terms of heel-rise count, there was a significant interaction effect (*P*= 0.0065) and side effect (*P*< 0.0001), with an increase in mean count on the injured side over time, resulting in a significantly smaller difference between the sides at 12 weeks (Figure 26).

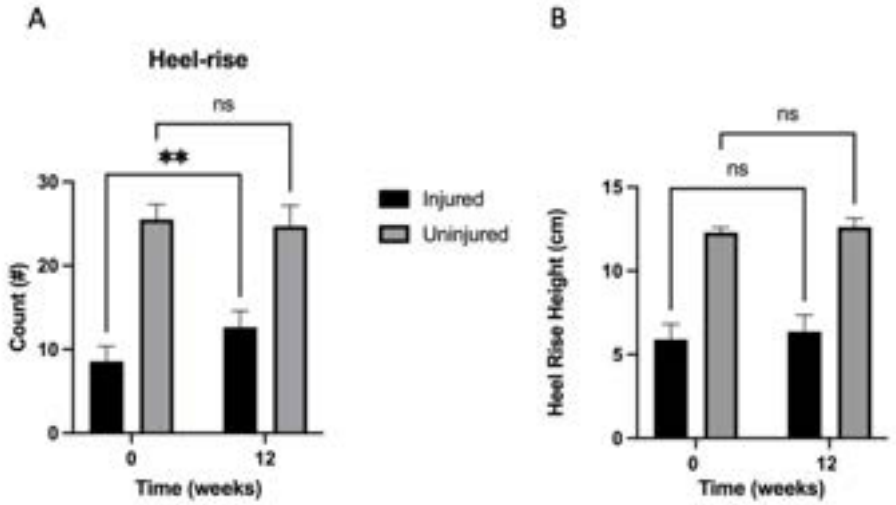

Figure 26. *(A) Heel-rise count displayed a significant difference (p<0.0001) between injured and uninjured side and an interaction between side and time (P=0.0065). (B) Heel-rise height displayed a significant difference (p<0.0001) between injured and uninjured side at both timepoints. Mean±SEM (Figure is duplicated from Study IIII.)*

For plantarflexion peak torque, both eccentric and concentric, performed with the knee extended there was a significant interaction effect (eccentric: *P*= 0.0074 and concentric: *P*= 0.0187) and a significant effect of side (eccentric: *P*= 0.0002 and concentric: *P* < 0.0001) and time (eccentric *P*= 0.0179 and concentric *P*= 0.0093) displaying a greater increase in peak torque on the injured side over time, resulting in a smaller difference between sides at 12 weeks (Figure 27).

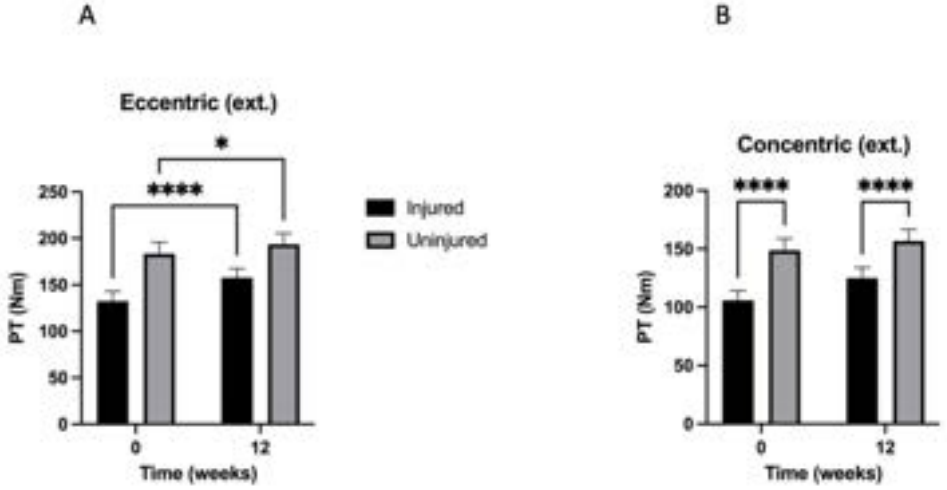

Figure 27. *(A) Eccentric plantarflexion peak torque with the knee extended. (B) Concentric plantarflexion peak torque with the knee extended. A significant difference (p<0.0001) between injured and uninjured side was found at both timepoints. Mean±SEM (Figure is duplicated from Study IIII)*

There was no interaction or time effect for the CSA and fat fraction of the medial gastrocnemius and soleus muscles (Table 3), but there was a significant side effect, *P*< 0.0001,

with a smaller CSA and a larger proportion of fat on the injured side. There was no interaction or side effect for the CSA of the lateral gastrocnemius, however there was a significant increase over time, *P* = 0.0272. No interaction or time effect was seen in the lateral gastrocnemius fat fraction, but there was a significant side effect, *P* =0.0316, with a larger percentage of fat on the injured side. There were no significant effects for the flexor hallucis longus muscle.

	Week 0		Week 12		P-value		
	Injured	**Uninjured**	**Injured**	**Uninjured**	**Time**	**Side**	**Time x Side**
CSA, GL (cm^2)	7.3±0.8	8.0±0.7	7.8±0.8	8.9±0.7	0.0272	0.0709	0.5292
CSA, GM (cm^2)	12.0±0.9	16.1±1.2	12.3±1.0	16.3±1.3	0.5014	<0.0001	0.7793
CSA, SOL (cm^2)	20.8±1.5	30.0±2.0	21.1±1.7	30.0±2.2	0.7624	<0.0001	0.6339
CSA, HAL (cm^2)	3.6±0.3	3.9±0.3	3.7±0.4	4.0±0.3	0.3486	0.2418	0.7767
FF, GL (%)	11.5±1.6	8.0±0.6	11.3±1.8	7.0±0.8	0.5652	0.0316	0.8675
FF, GM (%)	13.8±1.3	7.8±0.8	14.0±1.4	7.5±1.0	0.5353	<0.0001	0.5435
FF, SOL (%)	14.5±1.5	7.0±0.6	14.5±1.6	7.0±0.7	0.9670	<0.0001	0.3233
FF, HAL (%)	11.1±0.8	11.5±0.9	11.7±1.5	10.0±0.9	0.3597	0.3621	0.0581
Thick, GM (mm)	15.9±0.7	20.5±0.9	16.5±0.6	20.2±0.9	0.7249	<0.0001	0.1869
Penn, GM (°)	30.2±1.2	23.7±0.9	28.4±1.1	22.5±0.8	0.0072	0.0002	0.5057
PT, flex. C (Nm)	90.5±9.1	138.0±8.0	98.4±8.1	136.6±9.4	0.5976	<0.0001	0.2461
PT, flex. E (Nm)	115.6±12.4	173.4±12.9	125.6±9.0	166.5±10.6	0.8303	<0.0001	0.0480

Table 4. *Cross sectional area (CSA), Fat fractions (FF), Thickness (Thick), Pennation angle (Penn), and Peak torque (PT) measured at week 1 and 12. GL, Gastrocnemius lateralis muscle. GM, Gastrocnemius medialis muscle. SOL, Soleus muscle. HAL, Flexor Hallucis longus muscle. Flex, knee in flexion. C, concentric. E, eccentric. Data are presented as mean ± SEM.*

8. DISCUSSION

This PhD thesis aimed to improve the understanding of the healing processes of tendon after an acute Achilles tendon rupture, and to identify a loading pattern of the tendon during the rehabilitation period that would ensure a good recovery of the tendon, muscle structures and overall function.

The four studies of the thesis investigated (1) how the use of one or three wedges in an orthosis affects muscle activity in the lower leg and the Achilles tendon load, (2) if delayed loading following surgically treated Achilles tendon rupture, influences the functional, clinical, and structural outcomes for muscle and tendon during one year after surgery, (3) how a muscle adapts to an elongated tendon in an animal model, and (4) if a muscle that has undergone shortening secondary to elongation of the tendon can increase in fascicle length, and improve function following 12 weeks of eccentric training.

8.1 Unloading the tendon initially after an Achilles tendon rupture

It is standard procedure to unload the tendon initially after an Achilles tendon rupture by use of a cast or orthosis coupled with crutches, although the degree of weightbearing allowed varies a great deal. We demonstrated that increasing the wedge height resulted in a reduction in forefoot loading, which serves as an indirect indicator of the tensile load on the Achilles tendon. The overall load on the Achilles tendon and the maximum load that the sutures can resist must be considered as the mechanical strength of the surgical repair has been estimated to be between 250-500 N (89-92). The estimated peak loads on the Achilles tendon that we found in Study I indicate that walking without crutches, whether using one (828 N) or three wedges (600 N), would result in an Achilles tendon load greater than the surgical suture strength. The tensile load did not fall below the suture strength until crutches were used together with three wedges (390 N), and walking with the weight placed on the heel reduced the load further both by using one (237 N) and three wedges (94 N). The same pattern with reduced tensile load on the Achilles tendon with increased wedges has likewise been reported in other studies (93-95). Based on this information, the load in the intervention group (Delayed) in Study II were modified and gradually increased in the first twelve weeks. Thus, the number of heel wedges in the orthosis was gradually reduced from three at first to one in the final week of its usage and no weight-bearing was allowed the first 6 weeks

after surgery. Partial weight bearing with pressure on the heel only from week 7 and full weight bearing from week 13 was allowed.

In study II, one week after surgery, the gastrocnemius muscles revealed a length equivalent to the muscles on the uninjured side, whereas the soleus muscle had dramatically retracted. As there was no load on the tendon before the MRI, it seems doubtful that this retraction happened within the first postoperative week because of suture creep or failure. A plaster cast was used to immobilize the leg and foot, and weight bearing was prohibited so it seems more likely that normal length of the soleus tendon component of the Achilles tendon was not obtained at the time of surgery. Surgery was performed by three surgeons who produced comparable outcomes with the Kessler suturing technique, which is the norm in most hospitals in Denmark. Several descriptive studies (96-98) have shown complex rupture patterns with distinct tendon component separation, but to the best of our knowledge, this is the first time a differential tendon elongation has been documented following surgical repair.

8.2 Delayed loading

In the main study of the PhD thesis, Study II, the randomized controlled trial investigated if delayed loading following surgically treated acute Achilles tendon rupture influenced the clinical outcome, function and muscle and tendon structure after one year. The data showed that the side-to-side difference in heel-rise height at one year following surgery was not improved by a delayed loading (-2.1 cm) rehabilitation protocol compared to an early (-2.2 cm) rehabilitation protocol at 12 months, which is consistent with earlier studies (38, 61, 99, 100).

As described in section 3.5, the healing response following surgical repair of an Achilles tendon rupture includes an inflammatory phase, a proliferative or repair phase, and a remodeling phase (31) and as vascularization may be a sign of a tendon healing process (101), we thus used power Doppler ultrasound to investigate the vascularization of the tendon indirectly. Our finding of increased vascularization on the injured side in comparison to the uninjured side after an Achilles tendon repair is consistent with the findings of earlier research (38) and consequently unsurprising. Increased power doppler activity and elevated metabolic activity postoperative after an Achilles tendon rupture has been described to persist for at least six months (102). Therefore, maybe the amount of power Doppler initially is an expression of natural reparative response. However, the Delayed group had significantly less vascularization in the

injured tendon than the Early group at 12 weeks. It has also been shown that there is an inverse relationship between metabolic activity and patient reported outcome at 6 months, which would indirectly indicate that prolonged inflammation may negatively influence outcome. In the present study, reduced loading and activity level postoperatively yielded less inflammation. However, this reduced power Doppler in the Delayed group after 12 weeks did not seem to affect the amount of power Doppler at 26 or 52 weeks or any of the other outcomes after 12 months. Hence, the implication of this observation is unclear and must be considered in future research.

Regarding the patient reported outcome measures, the Delayed group (72.2 points) had a significantly higher ATRS score than the Early group (59.6 points) at 52 weeks, where a 10-point difference is considered a clinically meaningful difference (74). The scores reported in both groups were below the average of the scores reported in previous studies (5), where the average scores reported were around 80 points one year after an Achilles tendon rupture. The relatively younger age of the patients in the study, who may have a larger expectation of a full recovery, could perhaps be an explanation for the lower scores. The Delayed group also tended to report higher levels of satisfaction in relation to everyday living and sporting activities. Collectively, these findings imply that the Delayed group did better, but as there were no functional or structural differences to account for this difference, further research is needed to explore this. It should be emphasized, nonetheless, that there were no complications in the Delayed group while there were four in the Early group (one DVT, one deep infection, and two sensory nerve disturbances). Thus, it would be fair to state that there were no obvious negative consequences of delayed loading.

8.3 Muscle-tendon unit changes

Interesting differences between the elongation of the soleus and gastrocnemius parts of the tendon were found in the assessments of tendon length in Study II. Already one week postoperatively, where the patients still had the plaster cast on and were not yet allowed weight bearing, the soleus part (but not the gastrocnemius part) of the tendon displayed an elongation of 35 % compared to the uninjured side. Over the course of the following 12 months, the soleus tendon continued to elongate slightly albeit not statistically significantly, while the gastrocnemius part of the tendon elongated significantly (6%). According to our knowledge this demonstration of tendon elongation (the soleus part) as early as one week postoperatively has not previously been described in the literature. Precise measurements of the soleus part, and especially at the early

timepoints, have been difficult and nearly impossible to perform with the measurement techniques formerly used to measure the length of the tendon, such as tantalum beads and x-ray, ultrasound, and conventional MRI (described in section 3.8). As a result, the previous reported elongations have mainly expressed the elongation of both parts of the tendon or possibly just the gastrocnemius part. We measured differential tendon length using 3D-Tw isotopic MRI, which enables accurate and reliable (53) reconstruction of the imaging data, even in the initial stages following surgery. Therefore, it is difficult to compare the elongation of the tendon reported in the present study to previous studies. However, in studies where ultrasound or MRI were used to measure the tendon length, elongations between 11.4-22 mm (39, 42, 68, 103) were reported, and the mean elongation of the tendon measured at one year follow-up in the present study is comparable to these numbers (Soleus part= 21 mm, Gastrocnemius part= 12 mm). Furthermore, the trend of the gastrocnemius tendon elongation over time mimics that of earlier findings (38).

The triceps surae muscle group showed some lasting alterations a year after the injury. The gastrocnemius medialis (16%) and lateralis (18%) CSA revealed pronounced atrophy already one week after surgery. The initial atrophy of the soleus was only 6%, but all three muscles continued to atrophy until week 12, when they displayed an atrophy of 21-27%. While the gastrocnemius muscle had almost recovered by week 52, the soleus muscle still showed a side-to-side difference in muscle CSA of 20%. This is in line with findings from earlier studies (38, 41) where a similar deficit in soleus muscle mass of 18% after 18 months was described (41). The reason for this persistent deficit in soleus muscle mass may be attributed to the elongation of the soleus part of the tendon, which may influence optimal muscular activation. It has been demonstrated that the loss of contractile tension causes an increase in intramuscular fat deposition (104, 105), and it is well known that the aging process is also linked to greater fat infiltration in the muscles (106, 107). Hence, the increased fat deposition in the current study may be caused by a decreased capacity to produce muscle contraction and tension on the tendon. In a previous study (108), investigating healthy persons and patients with Achilles tendon pain by the use of the MRI Dixon technique, no variations in free fat fractions of the tibialis anterior muscle between healthy persons and patients were seen compared to the gastrocnemius muscle where significantly more free fat was found in the patients. These findings suggest that tendon integrity is a more significant component in fatty muscle degeneration than age. Years after a tendon rupture, it has been demonstrated that the triceps surae muscle group has fat infiltration (109).

Here, we support those findings and demonstrate that the fat infiltration is muscle specific. We found that after 52 weeks the free fat fraction was 50%, 25%, and 18% greater on the injured side, relative to the uninjured side, in the soleus, medial gastrocnemius, and lateral gastrocnemius, respectively. Regarding muscle thickness an 8–10% side-to-side deficit in muscle thickness was found, and this muscle atrophy may be part of the reason for the continued muscle weakness that was also observed.

It is generally known that the total excursion of the muscle, which approximately correlates to joint range of motion, is related to the fiber length of the muscle (48, 49), and the loss in heel-rise height may be more affected by tendon and muscle fascicle length than a deficit in strength per se (39, 53-55). Isometric side-to-side strength deficits were seen in both groups after 52 weeks and this loss in muscle strength seems to be increased in the more plantarflexed position (deficit of 23%), which is consistent with earlier investigations that found end-range plantarflexion weakness as high as 28–34% (52, 110, 111). In Study II, the gastrocnemius muscle fascicles were shortened, and the soleus and gastrocnemius tendons were elongated after 52 weeks. If the reduction in muscle fascicle length is accompanied by an adaptation of sarcomeres in series remains unknown. In Study III, we therefore wanted to investigate if the medial gastrocnemius muscle would adapt when the Achilles tendon was elongated after Achilles tendon transection in a rat model. The tendon lengthened by 15%, while the soleus, medial gastrocnemius, and lateral gastrocnemius muscles clearly demonstrated atrophy (12.5%, 17.5%, and 18.1%, respectively) and decreased muscle length (10.5%, 8.2%, and 9.2%, respectively) four weeks after tendon transection. The reduced overall muscle length was accompanied by a reduced fascicle length and serial sarcomere number such that sarcomere length retained normal length in the distal portion of the fascicle but was unexpectedly even greater than normal in the mid and proximal portion of the fascicle. As previous mentioned, decreased range of motion during a heel-rise is associated with a reduced muscle fascicle length as measured by ultrasound (53), and in the current animal study we demonstrated that GM muscle and fascicle length was shortened as a result of a longer tendon. Based on animal studies, it is well acknowledged that serial sarcomere number can be changed over periods of immobilization in the lengthened or shortened position to enhance the ability of the muscle to generate force (112-115). Theoretically, if the serial sarcomere number was decreased to preserve the optimal actin-myosin overlap, the force-generating capability of the muscle fiber would still be maintained, but even with a normal sarcomere length, a shorter

muscle would still have less excursion. The results from Study III demonstrated that a longer tendon and shorter muscle resulted in fewer sarcomeres along the length of a fiber, restoring the sarcomere length to the same level on the transected as on the intact side in the distal region of the fiber. Surprisingly, in the mid and proximal region of the fiber, an even higher decrease in sarcomere number resulted in a significantly longer sarcomere length than on the intact side. In a previous rat study (114), it has been demonstrated that the sarcomere length decreased from 2.6 µm to 1.8 µm after tenotomy and that the length was regained after four weeks. After tendon transection, it would be fair to assume that a comparable decrease in sarcomere length would occur, and that some normalization would follow, which the data verified for the distal part but not the mid and proximal part. Perhaps the sarcomere length "overshoots" initially and adjusts to normal length at a later point. The ability to produce more force when the muscle is shortened during plantarflexion may be a possible advantage in such an overshoot. It is possible that this early "overshoot" is intended to make up for the reduced force produced by a shortened muscle with shorter sarcomere length and smaller excursion to improve the actin-myosin overlap and increase the capacity of the muscle to produce force during plantarflexion. Nevertheless, we cannot know if the alterations in sarcomeres would be different if assessed at an earlier or later time-point. Perhaps it helps to compensate for the reduced excursion, which is another conceivable reason since sarcomere length is crucial for obtaining the ideal force-length relationship (47).

8.4 Response to eccentric training in persons with persisting functional deficits

In Study IIII, the results showed that people who had an Achilles tendon rupture in the past (more than a year ago) and were dissatisfied with their physical function had an elongated Achilles tendon, a shorter medial gastrocnemius muscle, marked soleus and medial gastrocnemius muscle atrophy, decreased heel-rise height and count, decreased eccentric and concentric plantar flexion muscle strength, and more fat in the triceps surae muscle group on the affected side. Our hypothesis could not be confirmed since no increase in fascicle length of their medial gastrocnemius muscle was detected after 12 weeks of eccentric isokinetic training. However, plantarflexion toque, heel-rise count, and patient reported outcome increased significantly. Thus, no structural changes, but still functional and patient reported outcomes improved.

It has been demonstrated in previous studies (87, 116) that the fascicle length in healthy persons increased in response to eccentric training. The sizeable reduction in muscle CSA seen in our study may provide evidence that the participants were unable to produce enough force to serve as a suitable training stimulus for muscle adaptation, which may explain why there was no change in fascicle length. However, in line with other studies (52), we also found the greatest reduction in force generation capacity in the most plantarflexed positions. This end-range weakness may be related to the shortened fascicle length. Anecdotally, the participants verbally stated that they only began to feel their muscles activate after about eight weeks of training; hence, it cannot be completely ruled out that a longer training duration would have affected the fascicle length. "Delayed-onset muscular soreness" (DOMS) is a well-known side effect of eccentric strength exercise (117), and we anticipated that the participants would experience it. Surprisingly, they did not report any DOMS throughout the first weeks of the intervention. Nevertheless, after around eight weeks of training, several participants started to complain of cramping while exercising. Again, this could suggest that twelve weeks of training were insufficient to see improvements in the muscles.

The results of Study IIII revealed that the long-term effects of an Achilles tendon rupture led to a large increase in the fat percentage of the muscles on the affected side compared to the unaffected side in addition to a drastic reduction in the triceps surae muscle CSA. The soleus and medial gastrocnemius fat fractions on the affected side were, respectively, 52% and 43% higher compared to the unaffected side. These findings are consistent with previously reported data (41, 68, 109). As described in section 8.3 (Muscle-tendon unit changes), the increased fat deposition seen in this study is likely a result of a decreased capacity to generate tension secondary to the loss of contractile protein, which is supported by the reduced plantarflexion torque and CSA data also found in the study.

Finally, it is noteworthy that anecdotally the participants verbally reported improvements such as not needing to use a compression sock on the injured leg since it no longer became edematous as it did before the training period or that family and friends of the participants had commented that they had acquired a better gait pattern.

9. CONCLUSIONS

The findings from this PhD thesis make some contribution to a better understanding of the healing processes of tendon and sequela after an acute Achilles tendon rupture.

The thesis demonstrated that delayed loading was not superior compared to early loading in the primary endpoint of reducing the heel-rise height difference at one year after acute surgery following an Achilles tendon rupture. Persistent deficits were seen in both the Early and the Delayed group regarding ATRS, muscle strength, and heel-rise performance. In addition, 3D MRI imaging of the length of the Achilles tendon did also not reveal any group differences, but regardless of the rehabilitation protocol, all patients had a significant 35% soleus tendon elongation compared to the contralateral side already at one week, which increased to 40% over the course of 52 weeks after surgery. In contrast, the gastrocnemius tendon length was intact at one week but increased by 6% over the course of the same period. Furthermore, the Delayed group demonstrated less Doppler activity in the initial three months that could be contributed to differences in the rehabilitation, and presented a significantly higher ATRS at 12 months indicating a better patient reported outcome in this group, which needs to be verified in further studies.

The thesis also demonstrated in an animal model that Achilles tendon transection leads to elongation of the tendon and atrophy and shortening of the muscles. Moreover, a shorter medial gastrocnemius muscle leads to reduced fascicle length which in turn leads to adaptation by reduced serial sarcomere numbers. Surprisingly this adaptation appears to "overshoot" and lead to increased sarcomere length.

Finally, the thesis revealed that 12 weeks of eccentric isokinetic training did not alter the fascicle length of the medial gastrocnemius muscle in persons with a more than one-year-old Achilles tendon rupture who were dissatisfied with their physical function. However, plantarflexion toque, heel-rise count and, importantly, patient reported outcome improved. These persons had an elongated Achilles tendon, a shorter medial gastrocnemius muscle, marked soleus and medial gastrocnemius muscle atrophy, decreased heel-rise height and count, decreased eccentric and concentric plantar flexion muscle strength, and a higher triceps surae fat content on the affected side.

10. PERSPECTIVS AND CLINICAL IMPLICATIONS

Hippocrates provided the opening quote for my thesis, and he also provides the closing one: “Healing is a matter of time, but it is sometimes also a matter of opportunity.”

This PhD thesis adds new understanding about how the tendon is provided the best opportunities to heal after a rupture. Despite several attempts to find a solution over the years, the ideal treatment and rehabilitation strategy following an acute Achilles tendon rupture is still a mystery. The results of this thesis revealed an elongation of the soleus part of the tendon already one week after the operation, implying that the surgeon was unable to obtain the normal length of the soleus part of the Achilles tendon at the time of operation. To the best of our knowledge, this is the first time a differential tendon elongation following the surgical repair has been documented and this observation has led to a new study being started in collaboration with the surgeons to investigate whether the surgical techniques can be optimized. Hence, the idea with a future study is to measure whether a new technique of double row suturing of a ruptured Achilles tendon will result in a normalization of the length of the soleus part and of the gastrocnemius part of the tendon one-week post-operative.

If delayed loading following a surgical treated Achilles tendon rupture provide better healing of the tendon is still not completely settled. A better patient reported outcome and less power Doppler was demonstrated in the group with delayed loading, so if the surgical techniques can be optimized, it would be interesting and necessary to replicate the study to investigate if better opportunities for tendon healing would occur.

With the eccentric training protocol, we observed certain improvements at the individual level that our statistical analyses of our small heterogeneous group did not detect. Consequently, it could be important to test the eccentric training protocol on a larger sample size. Possibly, some patients may benefit from performing eccentric training earlier in their rehabilitation process if they have significant functional impairments. Further research is required to learn more about this.

11. REFERENCES

1. Carden DG, Noble J, Chalmers J, Lunn P, Ellis J. Rupture of the calcaneal tendon. The early and late management. J Bone Joint Surg Br. 1987;69(3):416-20.
2. Ganestam A, Kallemose T, Troelsen A, Barfod KW. Increasing incidence of acute Achilles tendon rupture and a noticeable decline in surgical treatment from 1994 to 2013. A nationwide registry study of 33,160 patients. Knee Surg Sports Traumatol Arthrosc. 2016;24(12):3730-7.
3. Huttunen TT, Kannus P, Rolf C, Felländer-Tsai L, Mattila VM. Acute achilles tendon ruptures: incidence of injury and surgery in Sweden between 2001 and 2012. Am J Sports Med. 2014;42(10):2419-23.
4. Leino O, Keskinen H, Laaksonen I, Makela K, Loyttyniemi E, Ekman E. Incidence and Treatment Trends of Achilles Tendon Ruptures in Finland: A Nationwide Study. Orthopaedic journal of sports medicine. 2022;10(11):23259671221131536.
5. Hoeffner R, Svensson R, Bjerregaard N, Kjaer M, Magnusson S. Persistent Deficits after an Achilles Tendon Rupture: A Narrative Review. Translational Sports Medicine. 2022;2022:1-7.
6. Lerch TD, Schwinghammer A, Schmaranzer F, Anwander H, Ecker TM, Schmid T, et al. Return to Sport and Patient Satisfaction at 5-Year Follow-up After Nonoperative Treatment for Acute Achilles Tendon Rupture. Foot Ankle Int. 2020;41(7):784-92.
7. Doral MN, Alam M, Bozkurt M, Turhan E, Atay OA, Dönmez G, et al. Functional anatomy of the Achilles tendon. Knee Surg Sports Traumatol Arthrosc. 2010;18(5):638-43.
8. Maffulli N. Rupture of the Achilles tendon. J Bone Joint Surg Am. 1999;81(7):1019-36.
9. Bojsen-Møller J, Magnusson SP. Heterogeneous Loading of the Human Achilles Tendon In Vivo. Exerc Sport Sci Rev. 2015;43(4):190-7.
10. Edama M, Kubo M, Onishi H, Takabayashi T, Inai T, Yokoyama E, et al. The twisted structure of the human Achilles tendon. Scand J Med Sci Sports. 2015;25(5):e497-503.
11. Chen TM, Rozen WM, Pan WR, Ashton MW, Richardson MD, Taylor GI. The arterial anatomy of the Achilles tendon: anatomical study and clinical implications. Clin Anat. 2009;22(3):377-85.
12. Wang JH. Mechanobiology of tendon. J Biomech. 2006;39(9):1563-82.
13. Nourissat G, Berenbaum F, Duprez D. Tendon injury: from biology to tendon repair. Nat Rev Rheumatol. 2015;11(4):223-33.
14. Magnusson SP, Hansen P, Kjaer M. Tendon properties in relation to muscular activity and physical training. Scand J Med Sci Sports. 2003;13(4):211-23.
15. Komi PV. Relevance of in vivo force measurements to human biomechanics. J Biomech. 1990;23 Suppl 1:23-34.
16. Devaprakash D, Graham DF, Barrett RS, Lloyd DG, Obst SJ, Kennedy B, et al. Free Achilles tendon strain during selected rehabilitation, locomotor, jumping, and landing tasks. Journal of applied physiology (Bethesda, Md : 1985). 2022;132(4):956-65.
17. Hess GW. Achilles tendon rupture: a review of etiology, population, anatomy, risk factors, and injury prevention. Foot Ankle Spec. 2010;3(1):29-32.
18. Xergia SA, Tsarbou C, Liveris NI, Hadjithoma M, Tzanetakou IP. Risk factors for Achilles tendon rupture: an updated systematic review. Phys Sportsmed. 2022:1-11.

19. Arner O, Lindholm A. Subcutaneous rupture of the Achilles tendon; a study of 92 cases. Acta Chir Scand Suppl. 1959;116(Supp 239):1-51.
20. Pedowitz D, Kirwan G. Achilles tendon ruptures. Curr Rev Musculoskelet Med. 2013;6(4):285-93.
21. Chiodo CP, Glazebrook M, Bluman EM, Cohen BE, Femino JE, Giza E, et al. Diagnosis and treatment of acute Achilles tendon rupture. J Am Acad Orthop Surg. 2010;18(8):503-10.
22. Maffulli N. The clinical diagnosis of subcutaneous tear of the Achilles tendon. A prospective study in 174 patients. Am J Sports Med. 1998;26(2):266-70.
23. Myhrvold SB, Brouwer EF, Andresen TKM, Rydevik K, Amundsen M, Grün W, et al. Nonoperative or Surgical Treatment of Acute Achilles' Tendon Rupture. N Engl J Med. 2022;386(15):1409-20.
24. Fischer S, Colcuc C, Gramlich Y, Stein T, Abdulazim A, von Welck S, et al. Prospective randomized clinical trial of open operative, minimally invasive and conservative treatments of acute Achilles tendon tear. Arch Orthop Trauma Surg. 2020.
25. Nilsson-Helander K, Silbernagel KG, Thomee R, Faxen E, Olsson N, Eriksson BI, et al. Acute achilles tendon rupture: a randomized, controlled study comparing surgical and nonsurgical treatments using validated outcome measures. Am J Sports Med. 2010;38(11):2186-93.
26. Ochen Y, Beks RB, van Heijl M, Hietbrink F, Leenen LPH, van der Velde D, et al. Operative treatment versus nonoperative treatment of Achilles tendon ruptures: systematic review and meta-analysis. BMJ. 2019;364:k5120.
27. Reito A, Mattila V, Karjalainen T. Operative vs Nonoperative Treatment of Achilles Tendon Ruptures Using Early Functional Rehabilitation: Critical Analysis of Evidence. Foot Ankle Int. 2022;43(7):887-90.
28. Jiang N, Wang B, Chen A, Dong F, Yu B. Operative versus nonoperative treatment for acute Achilles tendon rupture: a meta-analysis based on current evidence. Int Orthop. 2012;36(4):765-73.
29. Khan RJ, Fick D, Keogh A, Crawford J, Brammar T, Parker M. Treatment of acute achilles tendon ruptures. A meta-analysis of randomized, controlled trials. J Bone Joint Surg Am. 2005;87(10):2202-10.
30. Wilkins R, Bisson LJ. Operative versus nonoperative management of acute Achilles tendon ruptures: a quantitative systematic review of randomized controlled trials. Am J Sports Med. 2012;40(9):2154-60.
31. Voleti PB, Buckley MR, Soslowsky LJ. Tendon healing: repair and regeneration. Annu Rev Biomed Eng. 2012;14:47-71.
32. Sharma P, Maffulli N. Biology of tendon injury: healing, modeling and remodeling. J Musculoskelet Neuronal Interact. 2006;6(2):181-90.
33. Müller SA, Todorov A, Heisterbach PE, Martin I, Majewski M. Tendon healing: an overview of physiology, biology, and pathology of tendon healing and systematic review of state of the art in tendon bioengineering. Knee Surg Sports Traumatol Arthrosc. 2015;23(7):2097-105.
34. Aspenberg P. Stimulation of tendon repair: mechanical loading, GDFs and platelets. A mini-review. Int Orthop. 2007;31(6):783-9.
35. Hammerman M, Dietrich-Zagonel F, Blomgran P, Eliasson P, Aspenberg P. Different mechanisms activated by mild versus strong loading in rat Achilles tendon healing. PLoS One. 2018;13(7):e0201211.

36. Notermans T, Hammerman H, Eliasson P, Isaksson H. Tendon mechanobiology in small-animal experiments during post-transection healing. Eur Cell Mater. 2021;42:375-91.
37. Khayyeri H, Hammerman M, Turunen MJ, Blomgran P, Notermans T, Guizar-Sicairos M, et al. Diminishing effects of mechanical loading over time during rat Achilles tendon healing. PLoS One. 2020;15(12):e0236681.
38. Eliasson P, Agergaard AS, Couppe C, Svensson R, Hoeffner R, Warming S, et al. The Ruptured Achilles Tendon Elongates for 6 Months After Surgical Repair Regardless of Early or Late Weightbearing in Combination With Ankle Mobilization: A Randomized Clinical Trial. Am J Sports Med. 2018;46(10):2492-502.
39. Silbernagel KG, Steele R, Manal K. Deficits in heel-rise height and achilles tendon elongation occur in patients recovering from an Achilles tendon rupture. Am J Sports Med. 2012;40(7):1564-71.
40. Rosso C, Vavken P, Polzer C, Buckland DM, Studler U, Weisskopf L, et al. Long-term outcomes of muscle volume and Achilles tendon length after Achilles tendon ruptures. Knee Surg Sports Traumatol Arthrosc. 2013;21(6):1369-77.
41. Heikkinen J, Lantto I, Flinkkila T, Ohtonen P, Niinimaki J, Siira P, et al. Soleus Atrophy Is Common After the Nonsurgical Treatment of Acute Achilles Tendon Ruptures: A Randomized Clinical Trial Comparing Surgical and Nonsurgical Functional Treatments. Am J Sports Med. 2017;45(6):1395-404.
42. Barfod KW, Hansen MS, Hölmich P, Kristensen MT, Troelsen A. Efficacy of early controlled motion of the ankle compared with immobilisation in non-operative treatment of patients with an acute Achilles tendon rupture: an assessor-blinded, randomised controlled trial. Br J Sports Med. 2020;54(12):719-24.
43. Nyström B, Holmlund D. Separation of tendon ends after suture of achilles tendon. Acta Orthop Scand. 1983;54(4):620-1.
44. Mortensen HM, Skov O, Jensen PE. Early motion of the ankle after operative treatment of a rupture of the Achilles tendon. A prospective, randomized clinical and radiographic study. J Bone Joint Surg Am. 1999;81(7):983-90.
45. Kangas J, Pajala A, Ohtonen P, Leppilahti J. Achilles tendon elongation after rupture repair: a randomized comparison of 2 postoperative regimens. Am J Sports Med. 2007;35(1):59-64.
46. Manegold S, Tsitsilonis S, Gehlen T, Kopf S, Duda GN, Agres AN. Alterations in structure of the muscle-tendon unit and gait pattern after percutaneous repair of Achilles tendon rupture with the Dresden instrument. Foot Ankle Surg. 2019;25(4):529-33.
47. Kruse A, Rivares C, Weide G, Tilp M, Jaspers RT. Stimuli for Adaptations in Muscle Length and the Length Range of Active Force Exertion-A Narrative Review. Front Physiol. 2021;12:742034.
48. Khair RM, Stenroth L, Cronin NJ, Reito A, Paloneva J, Finni T. Muscle-tendon morphomechanical properties of non-surgically treated Achilles tendon 1-year post-rupture. Clin Biomech (Bristol, Avon). 2022;92:105568.
49. Lieber RL, Roberts TJ, Blemker SS, Lee SSM, Herzog W. Skeletal muscle mechanics, energetics and plasticity. J Neuroeng Rehabil. 2017;14(1):108.
50. Zellers JA, Christensen M, Kjaer IL, Rathleff MS, Silbernagel KG. Defining Components of Early Functional Rehabilitation for Acute Achilles Tendon Rupture: A Systematic Review. Orthopaedic journal of sports medicine. 2019;7(11):2325967119884071.

51. de Boer MD, Seynnes OR, di Prampero PE, Pisot R, Mekjavić IB, Biolo G, et al. Effect of 5 weeks horizontal bed rest on human muscle thickness and architecture of weight bearing and non-weight bearing muscles. Eur J Appl Physiol. 2008;104(2):401-7.
52. Mullaney MJ, McHugh MP, Tyler TF, Nicholas SJ, Lee SJ. Weakness in end-range plantar flexion after Achilles tendon repair. Am J Sports Med. 2006;34(7):1120-5.
53. Svensson R, Couppé C, Agergaard AS, Josefsen C, Jensen M, Barfod K, et al. Persistent functional loss following ruptured Achilles tendon is associated with reduced gastrocnemius muscle fascicle length, elongated gastrocnemius and soleus tendon, and reduced muscle cross-sectional area. Translational Sports Medicine. 2019;2.
54. Peng WC, Chang YP, Chao YH, Fu SN, Rolf C, Shih TT, et al. Morphomechanical alterations in the medial gastrocnemius muscle in patients with a repaired Achilles tendon: Associations with outcome measures. Clin Biomech (Bristol, Avon). 2017;43:50-7.
55. Baxter JR, Hullfish TJ, Chao W. Functional deficits may be explained by plantarflexor remodeling following Achilles tendon rupture repair: Preliminary findings. J Biomech. 2018;79:238-42.
56. Huang J, Wang C, Ma X, Wang X, Zhang C, Chen L. Rehabilitation regimen after surgical treatment of acute Achilles tendon ruptures: a systematic review with meta-analysis. Am J Sports Med. 2015;43(4):1008-16.
57. Gould HP, Bano JM, Akman JL, Fillar AL. Postoperative Rehabilitation Following Achilles Tendon Repair: A Systematic Review. Sports Med Arthrosc Rev. 2021;29(2):130-45.
58. Maffulli N, Gougoulias N, Maffulli GD, Oliva F, Migliorini F. Slowed-Down Rehabilitation Following Percutaneous Repair of Achilles Tendon Rupture. Foot Ankle Int. 2022;43(2):244-52.
59. Schepull T, Kvist J, Andersson C, Aspenberg P. Mechanical properties during healing of Achilles tendon ruptures to predict final outcome: a pilot Roentgen stereophotogrammetric analysis in 10 patients. BMC Musculoskelet Disord. 2007;8:116.
60. Okoroha KR, Ussef N, Jildeh TR, Khalil LS, Hasan L, Bench C, et al. Comparison of Tendon Lengthening With Traditional Versus Accelerated Rehabilitation After Achilles Tendon Repair: A Prospective Randomized Controlled Trial. Am J Sports Med. 2020:363546520909389.
61. Aufwerber S, Edman G, Gravare Silbernagel K, Ackermann PW. Changes in Tendon Elongation and Muscle Atrophy Over Time After Achilles Tendon Rupture Repair: A Prospective Cohort Study on the Effects of Early Functional Mobilization. Am J Sports Med. 2020;48(13):3296-305.
62. Boesen AP, Boesen MI, Hansen R, Barfod KW, Lenskjold A, Malliaras P, et al. Effect of Platelet-Rich Plasma on Nonsurgically Treated Acute Achilles Tendon Ruptures: A Randomized, Double-Blinded Prospective Study. Am J Sports Med. 2020:363546520922541.
63. Powell HC, Silbernagel KG, Brorsson A, Tranberg R, Willy RW. Individuals Post Achilles Tendon Rupture Exhibit Asymmetrical Knee and Ankle Kinetics and Loading Rates During a Drop Countermovement Jump. J Orthop Sports Phys Ther. 2018;48(1):34-43.
64. Zellers JA, Cortes DH, Pohlig RT, Silbernagel KG. Tendon morphology and mechanical properties assessed by ultrasound show change early in recovery and potential prognostic ability for 6-month outcomes. Knee Surg Sports Traumatol Arthrosc. 2019;27(9):2831-9.

65. Zellers JA, Carmont MR, Silbernagel KG. Achilles Tendon Resting Angle Relates to Tendon Length and Function. Foot Ankle Int. 2018;39(3):343-8.
66. Hullfish TJ, O'Connor KM, Baxter JR. Medial gastrocnemius muscle remodeling correlates with reduced plantarflexor kinetics 14 weeks following Achilles tendon rupture. Journal of applied physiology (Bethesda, Md : 1985). 2019;127(4):1005-11.
67. Silbernagel KG, Shelley K, Powell S, Varrecchia S. Extended field of view ultrasound imaging to evaluate Achilles tendon length and thickness: a reliability and validity study. Muscles, ligaments and tendons journal. 2016;6(1):104-10.
68. Heikkinen J, Lantto I, Piilonen J, Flinkkilä T, Ohtonen P, Siira P, et al. Tendon Length, Calf Muscle Atrophy, and Strength Deficit After Acute Achilles Tendon Rupture: Long-Term Follow-up of Patients in a Previous Study. The Journal of Bone and Joint Surgery. 2017;99(18):1509-15.
69. McGee RG. How to Include Patient-Reported Outcome Measures in Clinical Trials. Curr Osteoporos Rep. 2020;18(5):480-5.
70. Krogsgaard MR, Brodersen J, Christensen KB, Siersma V, Kreiner S, Jensen J, et al. What is a PROM and why do we need it? Scand J Med Sci Sports. 2021;31(5):967-71.
71. Comins JD, Brodersen J, Siersma V, Jensen J, Hansen CF, Krogsgaard MR. Choosing the most appropriate PROM for clinical studies in sports medicine: Article two in a series of ten. Scand J Med Sci Sports. 2020.
72. Leppilahti J, Forsman K, Puranen J, Orava S. Outcome and prognostic factors of achilles rupture repair using a new scoring method. Clin Orthop Relat Res. 1998(346):152-61.
73. Kitaoka HB, Alexander IJ, Adelaar RS, Nunley JA, Myerson MS, Sanders M. Clinical rating systems for the ankle-hindfoot, midfoot, hallux, and lesser toes. Foot Ankle Int. 1994;15(7):349-53.
74. Nilsson-Helander K, Thomeé R, Silbernagel KG, Thomeé P, Faxén E, Eriksson BI, et al. The Achilles tendon Total Rupture Score (ATRS): development and validation. Am J Sports Med. 2007;35(3):421-6.
75. Ganestam A, Barfod K, Klit J, Troelsen A. Validity and reliability of the Achilles tendon total rupture score. J Foot Ankle Surg. 2013;52(6):736-9.
76. Silbernagel KG, Nilsson-Helander K, Thomee R, Eriksson BI, Karlsson J. A new measurement of heel-rise endurance with the ability to detect functional deficits in patients with Achilles tendon rupture. Knee Surg Sports Traumatol Arthrosc. 2010;18(2):258-64.
77. Schwartz CE. Applications of response shift theory and methods to participation measurement: a brief history of a young field. Arch Phys Med Rehabil. 2010;91(9 Suppl):S38-43.
78. Aadahl M, Jørgensen T. Validation of a new self-report instrument for measuring physical activity. Med Sci Sports Exerc. 2003;35(7):1196-202.
79. Hoeffner R, Agergaard AS, Funaro A, Bjerregaard N, Svensson RB, Alkjaer T, et al. The influence of an orthopaedic walker boot on forefoot force. Foot (Edinb). 2020;46:101739.
80. Lunsford BR, Perry J. The standing heel-rise test for ankle plantar flexion: criterion for normal. Phys Ther. 1995;75(8):694-8.
81. Geremia JM, Baroni BM, Bobbert MF, Bini RR, Lanferdini FJ, Vaz MA. Effects of high loading by eccentric triceps surae training on Achilles tendon properties in humans. Eur J Appl Physiol. 2018;118(8):1725-36.

82. Bolsterlee B, Gandevia SC, Herbert RD. Ultrasound imaging of the human medial gastrocnemius muscle: how to orient the transducer so that muscle fascicles lie in the image plane. J Biomech. 2016;49(7):1002-8.
83. Hullfish TJ, O'Connor KM, Baxter JR. Gastrocnemius fascicles are shorter and more pennate throughout the first month following acute Achilles tendon rupture. PeerJ. 2019;7:e6788.
84. Seynnes OR, Cronin NJ. Simple Muscle Architecture Analysis (SMA): An ImageJ macro tool to automate measurements in B-mode ultrasound scans. PLoS One. 2020;15(2):e0229034.
85. Carmont MR, Silbernagel KG, Mathy A, Mulji Y, Karlsson J, Maffulli N. Reliability of Achilles tendon resting angle and calf circumference measurement techniques. Foot Ankle Surg. 2013;19(4):245-9.
86. Moher D, Hopewell S, Schulz KF, Montori V, Gotzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. Int J Surg. 2012;10(1):28-55.
87. Geremia JM, Baroni BM, Bini RR, Lanferdini FJ, de Lima AR, Herzog W, et al. Triceps Surae Muscle Architecture Adaptations to Eccentric Training. Front Physiol. 2019;10:1456.
88. Mathewson MA, Chambers HG, Girard PJ, Tenenhaus M, Schwartz AK, Lieber RL. Stiff muscle fibers in calf muscles of patients with cerebral palsy lead to high passive muscle stiffness. J Orthop Res. 2014;32(12):1667-74.
89. Cottom JM, Baker JS, Richardson PE, Maker JM. Evaluation of a New Knotless Suture Anchor Repair in Acute Achilles Tendon Ruptures: A Biomechanical Comparison of Three Techniques. J Foot Ankle Surg. 2017;56(3):423-7.
90. Gebauer M, Beil FT, Beckmann J, Sárváry AM, Ueblacker P, Ruecker AH, et al. Mechanical evaluation of different techniques for Achilles tendon repair. Arch Orthop Trauma Surg. 2007;127(9):795-9.
91. Herbort M, Haber A, Zantop T, Gosheger G, Rosslenbroich S, Raschke MJ, et al. Biomechanical comparison of the primary stability of suturing Achilles tendon rupture: a cadaver study of Bunnell and Kessler techniques under cyclic loading conditions. Arch Orthop Trauma Surg. 2008;128(11):1273-7.
92. Ismail M, Karim A, Shulman R, Amis A, Calder J. The Achillon achilles tendon repair: is it strong enough? Foot Ankle Int. 2008;29(8):808-13.
93. Akizuki KH, Gartman EJ, Nisonson B, Ben-Avi S, McHugh MP. The relative stress on the Achilles tendon during ambulation in an ankle immobiliser: implications for rehabilitation after Achilles tendon repair. Br J Sports Med. 2001;35(5):329-33; discussion 33-4.
94. Zellers JA, Tucker LA, Higginson JS, Manal K, Gravare Silbernagel K. Changes in gait mechanics and muscle activity with wedge height in an orthopaedic boot. Gait Posture. 2019;70:59-64.
95. Aufwerber S, Heijne A, Gravare Silbernagel K, Ackermann PW. High Plantar Force Loading After Achilles Tendon Rupture Repair With Early Functional Mobilization. Am J Sports Med. 2019;47(4):894-900.
96. Douglas J, Kelly M, Blachut P. Clarification of the Simmonds-Thompson test for rupture of an Achilles tendon. Can J Surg. 2009;52(3):E40-1.
97. Dürig M, Schuppisser JP, Gauer EF, Müller W. Spontaneous rupture of the gastrocnemius muscle. Injury. 1977;9(2):143-5.

98. Robert S. Management of partial tears of the gastro-soleus complex. Clin Sports Med. 2008;27(1):219-29, x.
99. Nilsson L, Thorlund JB, Kjær IL, Kazlauskas A, Christensen M. Long-term follow-up after acute achilles tendon rupture - Does treatment strategy influence functional outcomes? Foot (Edinb). 2021;47:101769.
100. Nordenholm A, Nilsson N, Senorski EH, Helander KN, Westin O, Olsson N. Patients with chronic Achilles tendon rupture have persistent limitations in patient-reported function and calf muscle function one year after surgical treatment - a case series. J Exp Orthop. 2022;9(1):15.
101. Chianca V, Zappia M, Oliva F, Luca B, Maffulli N. Post-operative MRI and US appearance of the Achilles tendons. J Ultrasound. 2020;23(3):387-95.
102. Eliasson P, Couppé C, Lonsdale M, Svensson RB, Neergaard C, Kjær M, et al. Ruptured human Achilles tendon has elevated metabolic activity up to 1 year after repair. Eur J Nucl Med Mol Imaging. 2016;43(10):1868-77.
103. Stäudle B, Seynnes O, Laps G, Göll F, Brüggemann GP, Albracht K. Recovery from Achilles Tendon Repair: A Combination of Postsurgery Outcomes and Insufficient Remodeling of Muscle and Tendon. Med Sci Sports Exerc. 2020;Publish Ahead of Print.
104. Bayer ML, Hoegberget-Kalisz M, Svensson RB, Hjortshoej MH, Olesen JL, Nybing JD, et al. Chronic Sequelae After Muscle Strain Injuries: Influence of Heavy Resistance Training on Functional and Structural Characteristics in a Randomized Controlled Trial. Am J Sports Med. 2021;49(10):2783-94.
105. Gorgey AS, Mather KJ, Cupp HR, Gater DR. Effects of resistance training on adiposity and metabolism after spinal cord injury. Med Sci Sports Exerc. 2012;44(1):165-74.
106. Csapo R, Malis V, Sinha U, Du J, Sinha S. Age-associated differences in triceps surae muscle composition and strength - an MRI-based cross-sectional comparison of contractile, adipose and connective tissue. BMC Musculoskelet Disord. 2014;15:209.
107. Mikkelsen UR, Agergaard J, Couppé C, Grosset JF, Karlsen A, Magnusson SP, et al. Skeletal muscle morphology and regulatory signalling in endurance-trained and sedentary individuals: The influence of ageing. Exp Gerontol. 2017;93:54-67.
108. Fischer MA, Pfirrmann CW, Espinosa N, Raptis DA, Buck FM. Dixon-based MRI for assessment of muscle-fat content in phantoms, healthy volunteers and patients with achillodynia: comparison to visual assessment of calf muscle quality. Eur Radiol. 2014;24(6):1366-75.
109. Eken G, Misir A, Tangay C, Atici T, Demirhan N, Sener N. Effect of muscle atrophy and fatty infiltration on mid-term clinical, and functional outcomes after Achilles tendon repair. Foot Ankle Surg. 2020.
110. McHugh MP, Orishimo KF, Kremenic IJ, Adelman J, Nicholas SJ. Electromyographic Evidence of Excessive Achilles Tendon Elongation During Isometric Contractions After Achilles Tendon Repair. Orthopaedic journal of sports medicine. 2019;7(11):2325967119883357.
111. Pajala A, Kangas J, Siira P, Ohtonen P, Leppilahti J. Augmented compared with nonaugmented surgical repair of a fresh total Achilles tendon rupture. A prospective randomized study. J Bone Joint Surg Am. 2009;91(5):1092-100.
112. Williams PE, Goldspink G. The effect of immobilization on the longitudinal growth of striated muscle fibres. J Anat. 1973;116(Pt 1):45-55.
113. Williams PE, Goldspink G. Changes in sarcomere length and physiological properties in immobilized muscle. J Anat. 1978;127(Pt 3):459-68.

114. Baker JH, Hall-Craggs EC. Changes in length of sarcomeres following tenotomy of the rat soleus muscle. Anat Rec. 1978;192(1):55-8.
115. Heslinga JW, te Kronnie G, Huijing PA. Growth and immobilization effects on sarcomeres: a comparison between gastrocnemius and soleus muscles of the adult rat. Eur J Appl Physiol Occup Physiol. 1995;70(1):49-57.
116. Alonso-Fernandez D, Taboada-Iglesias Y, García-Remeseiro T, Gutiérrez-Sánchez Á. Effects of the Functional Heel Drop Exercise on the Muscle Architecture of the Gastrocnemius. J Sport Rehabil. 2020;29(8):1053-9.
117. Hody S, Croisier JL, Bury T, Rogister B, Leprince P. Eccentric Muscle Contractions: Risks and Benefits. Front Physiol. 2019;10:536.

12. PAPERS

Study I

The influence of an orthopaedic walker boot on forefoot force

Hoeffner R, Agergaard AS, Funaro A, Bjerregaard N, Svensson RB, Alkjaer T, Magnusson SP.

The Foot (Edinb). 2020;46:101739. https://doi.org/10.1016/j.foot.2020.101739

Study II

Tendon elongation and function following delayed or early loading of surgically repaired Achilles tendon ruptures – a randomized controlled trial

Hoeffner R, Agergaard AS, Svensson RB, Cullum C, Mikkelsen RK, Konradsen L, Krogsgaard MR, Boesen M, Kjaer M, Magnusson SP.

Manuscript draft.

Study III

Muscle fascicle and sarcomere adaptation in response to Achilles tendon elongation in an animal model

Hoeffner R, Svensson RB, Dietrich-Zagonel F, Schefte D, Kjær M, Eliasson P, Magnusson SP.

Manuscript in revision.

Study IV

Eccentric Training to restore persistent Functional Loss following Ruptured Achilles tendon: The role of Fascicle Length and Muscle Excursion

Hoeffner R, Svensson RB, Magnusson SP.

Submitted manuscript.