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PhD Thesis

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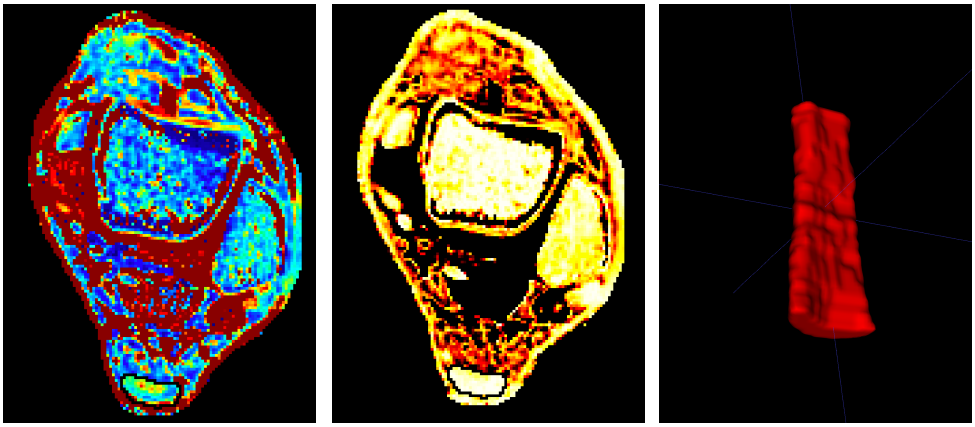
Characterization and treatment of early phase tendinopathy



Supervisor: Professor Michael Kjær
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PhD thesis

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Acknowledgements

I think my path towards this thesis resembles that of many others at the department. Sport has always been a big part of my life. Therefore, I was instinctively attracted to the department, and as a very young medical student I presented at Michaels office the first time and explained that I was interested in doing research. Soon after I was attached to a real-life research project with human subjects. Rie Nielsen who was the research leader on the project and Katja Heinemeier deserve a special thanks. You nourished my interest in research and those six months convinced me that I had to return. When I returned a few years later, I was fortunate enough to work close together with Rasmus Bechshøft and Lars Holm for a full year. I have a lot of good things to say about that year, but I think I'll focus on the one bad thing; it was too short!

Therefore, I didn't think twice when Michael offered me an opportunity to do a PhD, and I have not regretted my decision once since. Not only was I fortunate enough to have Michael as my principal supervisor, but I was also assigned Peter Magnusson as my primary co-supervisor. You have both been supreme support and you have a unique ability to deescalate the mandatory challenges that are inevitable during the process. Besides your great human skills, you both have a comprehensive knowledge and overview of the research field. Resultingly, I have had rare access to two distinguished tendon researchers during my PhD for which I am grateful. Additionally, you have consistently attracted great people to the department, that I have had the pleasure to be amongst. A lunch break with them can turn the greyest day around. I have to separately thank Anne-Sofie Agergaard. We have shared both frustrations and success along the way and the close collaboration with you have been a significant part of my time at the department. Also, a special thanks to René. I speak for many of us when I say: "without you it wouldn't have been possible". I have enjoyed working with you on the projects, but I have just as much enjoyed the fun and more adventurous parts including our one-day horse tendon biopsy trip to Paris. The weekly meetings in the tendon/cartilage group have always provided new insights and have served as an anchor in sometimes turbulent periods. Further the tendon group has been a constant source of support, joy and intelligent inputs.

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Resumé

Som bindeled mellem muskler og knogler, spiller kroppens sener en essentiel rolle for bevægelse. De består af tæt pakket bindevæv, hvis organiseringen og sammensætningen fordrer deres uovertrufne evne til at overføre kraft samt lagre og afgive elastisk energi. Dog kan den akkumulerede belastning i visse situationer overskride kapaciteten, hvilket kan igangsætte en kaskade af processer som i sidste ende kan føre til overbelastningsskader (tendinopati). Rækkefølgen af disse processer er imidlertid kun delvist beskrevet, dog antages det, at visse karakteristika er dominerende in den tidlige fase af tendinopati, hvorimod andre dominerer den mere kroniske fase. Herunder har man foreslået at, inflammation spiller en mere prominent rolle i den tidlige fase end de mere kroniske faser af tendinopati. Dette indikerer at inflammation muligvis spiller en central rolle, i de processer der i sidste ende kan føre til de vidtgående strukturelle forandringer, der undertiden kan observeres i sene vævet hos patienter med kronisk tendinopati. Derfor er det også nærliggende at foreslå en klinisk og fysiologisk effekt af anti-inflammatorisk behandling i tidlig tendinopati. Men undersøgelsen af de fysiologiske effekter er i nogen grad begrænset af de værktøjer der, for nuværende, er tilgængelige. Mest udtalt er dette i den tidlige fase af tendinopati. Derfor har der været stor interesse i at udvikle og udnytte nye metoder, der kan detektere de mere diskrete forandringer som må forventes i den tidlige fase af tendinopati, og som samtidig kan bruges til at detektere forandringer over tid i longitudinelle studier.

Billeddannende metoder har vakt særlig stor interesse, eftersom de giver mulighed for gentagne undersøgelser af hele senen uden brug af invasive procedurer. Konventionelle, klinisk tilgængelige, billedannende metoder har dog begrænset følsomhed over for mere diskrete forandringer i vævet, hvorfor nye mere følsomme metoder er blevet udviklet. Særligt interessante er nyere magnetisk resonans (MR) skannings sekvenser, som gør brug af exceptionelt korte ekko tider (UTE MR). I modsætning til konventionelle MR-sekvenser, kan man med disse sekvenser opfange et signal fra raskt sene væv. Ydermere kan man, ved at udføre gentagne skanninger og kombinere disse, udregne relaksations konstanter, herunder T2* (UTE T2* mapping) i sene vævet. T2* anses for at være udtryk for den strukturelle integritet, og er potentielt følsom overfor selv diskrete forandring i vævet.

De overordnede mål med denne afhandling var følgende:

- At undersøge hvorvidt UTE T2* mapping er i stand til at detektere diskrete strukturelle forandringer i tidlig tendinopati.
- At undersøge den kliniske og fysiologiske effekt, evalueret med kliniske og udvalgte fysiologiske udfald (inklusive UTE T2* mapping), af en initial kortvarig antiinflammatorisk behandling med et non-steroidt antiinflammatorisk middel (Naproxen) i tidlig tendinopati.

Først undersøgte vi reproducerbarheden af UTE T2* mapping hos patienter med tendinopati. Hvorved vi fandt at, metoden er tilstrækkeligt reproducerbar til brug i fremtidige studier på patienter med tendinopati. Dernæst undersøgte vi forskellen i T2* mellem patienter med tidlig tendinopati og raske kontroller. Vores resultater herfra viser at patienter med tidlig tendinopati har signifikant højere T2* værdier end de raske kontroller. Ydermere antyder vores data at de forhøjede værdier har funktionel betydning for senen. Endeligt undersøgte vi den kliniske og fysiologiske effekt af 7 dages behandling med et non-steroidt antiinflammatorisk middel i kombination med aflastning og en gængs træningsintervention. Deraf fandt vi ingen additiv effekt hverken på kliniske eller fysiologiske parametre i tidlig tendinopati. Selvom studiet ikke var designet til at undersøge den isolerede effekt af træningsinterventionen, antyder resultaterne en effekt på de kliniske symptomer. Ydermere skete den kliniske forbedring i fravær af ændringer af senens størrelse, blodkarforsyning og T2*.

Sammenfattende fandt vi at UTE T2* mapping er i stand til at detektere diskrete forskelle mellem patienter med tidlig tendinopati og raske kontroller. Samtidig observerede vi også at selvsamme metode ikke kunne detektere forandringer over tid, på trods af klinisk forbedring hos de undersøgte patienter. Endeligt antyder resultaterne at der var en effekt af aflastning og træning, men også at NSAID ikke tilføjede yderligere forbedringer. Slutteligt må det altså konstateres at, der kan stilles spørgsmålstegn ved den gængse brug af NSAID, som tillæg til aflastning og rehabilitering i tidlig tendinopati. Man bør derfor nøje overveje brugen af disse præparater i denne patientgruppe.

Summary

Tendons act as a link between muscle and bone, enabling locomotion of the body, and consist of a highly organized, fibril rich, and dense connective tissue. Its composition and structure allow for high amounts of force transmission and elastic energy storage, but when the cumulative load on the tendon is higher than physiologically tolerable, a cascade of events can lead to the development of clinical overload injury (tendinopathy).

The order of these events in development of overuse injury are sparsely described, but it is hypothesized that some features dominate at an early stage of disease whereas others dominate at a more chronic stage. Thus, inflammation has been suggested to be more pronounced at an early rather than a late stage of disease, which suggests that it may be a central part of the cascade leading to the severe structural changes observed in chronic tendinopathy. This implies that anti-inflammatory treatment may have a clinical and physiological effect in early-phase tendinopathy. However, the evaluation of physiological effects, especially in early tendinopathy, is somewhat limited by the tools available. Therefore, there has been a great interest in developing tools that can detect subtle changes at an early stage of disease and thus be used to track changes in longitudinal studies on patients. Imaging techniques with its non-invasive nature serve as ideal candidates that allow for repeated measures of the whole tendon in vivo. However, present conventional imaging modalities have some limitations in their ability to detect more subtle pathological changes in tendon. Relatively recently ultra-short time to echo magnetic resonance imaging (UTE MRI) has been introduced in tendon research, and in contrast to conventional MRI these sequences are able to obtain signal even from the healthy tendon. Further, the combination of repeated acquisitions allows extraction of relaxation constants, such as T2* relaxation (UTE T2* mapping). T2* can be considered a measure of structural integrity of the tissue and may be sensitive to subtle changes in early tendinopathy.

The aims of the thesis were to:

- Investigate the ability of UTE T2* mapping to detect subtle structural changes in early phase tendinopathy (symptom duration < 3months).
- Investigate the clinical and physiological effect of an initial short-term anti-inflammatory treatment with a non-steroidal anti-inflammatory drug (Naproxen), on clinical and selected physiological outcomes (including UTE T2* mapping) in early phase tendinopathy.

First, we evaluated the reproducibility of UTE T2* mapping in tendinopathic tendons and found that UTE T2* mapping is sufficiently reproducible for further investigations in patients with tendinopathy. Secondly, we investigated the difference in T2* between patients with early phase tendinopathy and healthy control subjects and demonstrated a significant difference in T2* between patients with early tendinopathy and healthy controls. Further, findings suggested that changes in T2* may have some moderate functional implications for the tendon. Lastly, we investigated the clinical and physiological effect of 7-days non-steroidal anti-inflammatory treatment added to a standard physical rehabilitation program including load management. And found that a short term NSAID treatment did not exert any additive clinical or physiological effect in early phase tendinopathy. Although the study was not designed to investigate the effect of the physical rehabilitation program the results indicate an improvement in patient symptoms, which occurred in the absence of changes in tendon dimension, vascularization and T2*.

In conclusion UTE T2* mapping is able to detect differences between patients with early phase tendinopathy and healthy controls. But the method was not able to detect changes over time despite clinical improvements. Further, patients with early-phase tendinopathy appear to improve clinically during standard physical rehabilitation, and NSAID does not demonstrate any additive effect to this. Therefore, the general use of NSAID as additive to standard rehabilitation and load management can be questioned. And careful consideration is advocated prior to the use of NSAID in early-phase tendinopathy.

List of abbreviations

COX	Cyclooxygenase
CSA	Cross sectional area
CV	Coefficient of variance
ECM	Extracellular matrix
HSR	Heavy slow resistance training
ICC	Intra class coefficient
LSC	Least significant change
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NRS	Numerical ranking scale
NSAID	Non-steroidal anti-inflammatory drug
PG	Proteoglycan
PGE2	Prostaglandin E2
PROMS	Patient reported outcome measures
T2*	T2* relaxation
SLRP	Small leucine rich proteoglycans
US	Ultrasound scan
UTE	Ultrashort time to echo
VISA-A	Victorian Institute of Sports Assessment-Achilles
VISA-P	Victorian Institute of Sports Assessment-Patella

Background

Tendon structure and function

Tendons act as a link between muscles and bones and enables locomotion of the human body. It is connected to the muscle via a specialized zone called the myotendinous junction, and to the bone via the enthesis. Each end possesses specific characteristics in regards of structure and function, and both are different to the midportion of the tendon[1–3]. Since neither the myotendinous junction nor the enthesis were investigated in the current thesis I will focus on the midportion of the tendon. The midportion of the tendon consists of highly organized dense connective tissue, and is, as a result of its unique structure, able to withstand a remarkable amount of repetitive load[4].

The primary constituent is collagen, which accounts for around 70 % of the dry mass, collagen type I is by far the most abundant form (~95%) whereas other types (III, V, XI, XII and XIV) exists at low levels. Collagen forms the fibrils that comprises the smallest component in the hierarchical build-up of the tendon (Diameter: ~30-200 nm), bundles of fibrils form fibres (~100 µm), which again forms the fascicles (diameter ~1 mm) that are grouped to form the tendon[5] (Figure 1). Each fascicle is separated by the synovium like endotenon and the whole tendon is surrounded by the epitenon, additionally the paratenon separates the tendon from its surroundings supports friction free sliding during locomotion[6].

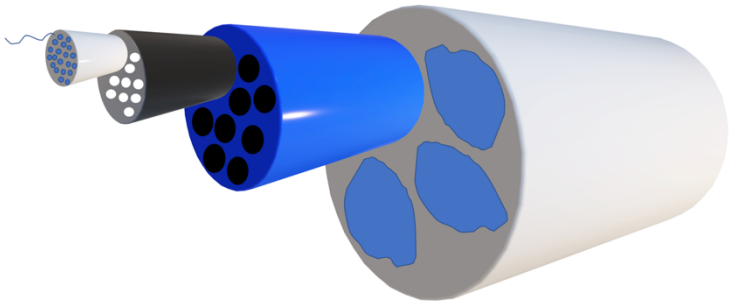

Figure 1 illustrating the hierarchical build-up of tendons. Blue cylinder: Fascicle, black cylinder: fibre, small white cylinder: fibril

Collagen is considered the main mechanical component of the tendon, however, the organization itself and or other constituents of the tendon also affect the mechanical properties[7–9].

The organization of collagen mainly takes place during maturation and includes enzymatic cross-linking, which stabilizes and affects the mechanical properties of collagen, this is reflected by the fact that the number of enzymatic cross-links relates to the function of the

tendon[10]. The amount of enzymatic cross links in mature tendon is thought to be more or less stable after maturation, on the contrary non-enzymatic cross-linking occurs throughout the lifespan, and although our understanding of non-enzymatic cross-links function and potential detrimental effects is incomplete, they are known to increase with age, affect the mechanical properties and the collagen structure, and potentially affect cell function[5].

Other constituents include proteoglycans (small leucine rich (SLRP) and large aggregating PGs), which support fibril assembly, serves as a reservoir for growth factors and due to the anionic hydrophilic glycosaminoglycan (GAG) side chains attracts and retains water[11,12].
The remaining part of the ECM not accounted for by collagen and PGs, consists of various glycoproteins, elastin and inorganic components[13].

Lastly the elongated tendon fibroblasts, which is the primary cell type in tendons are interspersed between the collagen fibres and act to maintain homeostasis in the tissue, an extensive crosstalk takes place between the cells and the extra cellular matrix (ECM), by which tendon fibroblasts sense the deformation and biochemical changes in the tissue[14,15]. Other cell types co-exist in the tendons including immunocompetent cells, here among resident macrophages, t-cells and mast-cells, thought to take part in the response to injury and to regulate inflammatory processes [16].
The unique build-up makes the tendon resilient to high loads, and allows energy storage and return, making locomotion energy efficient.

Gross anatomy

The studies included in the current thesis are based on work in human Achilles and patellar tendons. Therefore, the anatomy of both will be briefly introduced in the following. The Achilles tendon measures ~0.5 cm^2 in cross sectional area (CSA), and ~10 cm in length[17] (Figure 2). It originates from the triceps surae muscle, which is divided in the superficial gastrocnemius originating from the femoral condyles and the more profound soleus muscle originating from the posterior part of the tibial and fibular bone, the aponeurosis from the two parts of the triceps surae muscle fuses just distal to the middle of the lower leg to form the Achilles tendon. Distally it inserts on calcaneal tubercle in a fan like shape[18]. Between the proximal and distal ends the Achilles tendons is twisted laterally, which might influence the local load distribution within the tendon[19]. A retro calcaneal bursa is placed in the angle between the calcaneal bone and the tendon, posterior to the tendon at the distal end lies the subcutaneous bursa, and anterior to the

free tendon lies Kagers fat pad[20,21]. The triceps surae muscle is innervated by the tibial nerve whereas the tendon itself is partly innervated by the tibial nerve and partly by subcutaneous nerves from the sural nerve[22]. The blood supply originates from the posterior tibial and the peroneal arteries, a hypo vascular zone exists in the midportion which might partly explain a high occurrence rate of tendinopathies in this area[23].

The origin of the patellar tendon is the quadriceps muscle (Figure 2). The aponeurosis from the four heads of the quadriceps muscle, vastus lateralis, intermedius, mediales and rectus femoris, fuses to create the quadriceps tendon, part of the quadriceps tendon inserts into the cranial surface of the sesamoid patellar bone, whereas part of it continues distally to form the patellar tendon [24]. The patellar tendon is placed between the patellar bone and the tibial tuberosity where it has its insertion. It measures ~4.5 cm in length and ~1 cm^2 in CSA[25,26]. Three bursae relate to the patellar tendon; the prepatellar bursa anterior to the patellar bone and the proximal part of the patellar tendon, the superficial infrapatellar bursa anterior to the distal end of the patellar tendon and the deep infrapatellar bursa posterior to the distal patellar tendon[27]. Hoffas' fat pad is placed posterior to the tendon an act as a cushion between the tendon and the more profound bone[28]. The blood supply originates from the femoral, tibial and popliteal arteries wheras the nerve supply stems from the fibular and peroneal nerve[29,30].

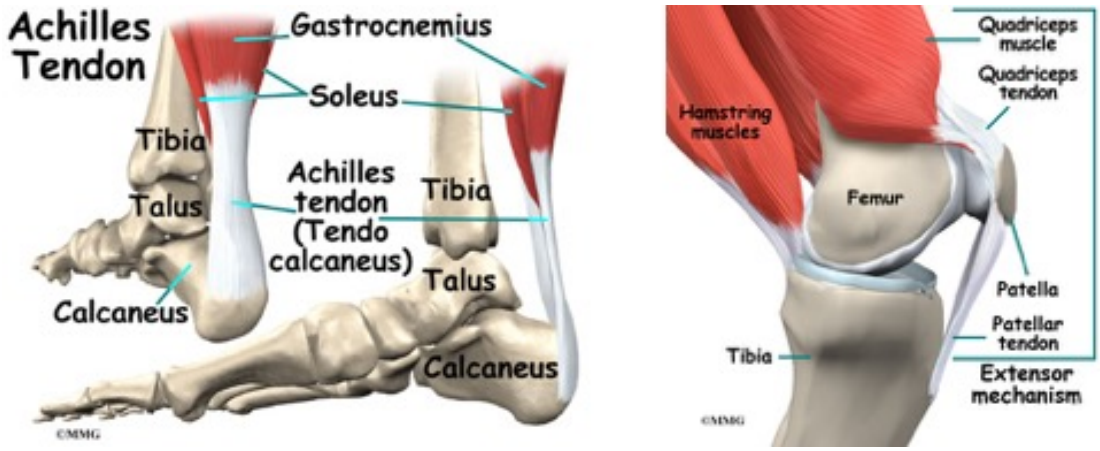

Figure 2 illustration of the human Achilles (left) and patellar tendon. Source: www.eorthopod.com

Tendinopathy

Clinical signs

The condition tendinopathy is characterized by pain, swelling and loss of function in the affected tendon [31]. It is a clinical diagnosis reliant on patient history and clinical examination, and can have long term impact on physical activity and function [32].

Patients often present with a set of common clinical features. Generally, symptoms develop gradually and include morning stiffness and stiffness after prolonged inactivity, activity related pain and an ability to reduce pain after warm-up. Eventually pain may become more permanent and can also be present between training bouts. Typically rest will lead to an improvement in symptoms. However, without treatment, symptoms will often resurface when the loading is reintroduced. Lastly, perceived performance and decreased function has been reported [33–35].

Clinical examination often but not always reveals visible swelling, whereas stretching and palpation of the tendon provokes the recognizable pain [34].

Ultrasound scans (US) can confirm the diagnosis and to some extend help to exclude differential diagnosis. Alterations on ultrasound include hypo echogenicity, increased thickness and hypervascularization. However, findings can be present in the absence of symptoms and therefore results from US should always be interpreted in conjunction with clinical findings [36–38]. Magnetic resonance imaging (MRI) is also occasionally used. Conventional MRI allows precise measurement of tendon dimensions[39,40], and is thus able to detect a change in size, also the more chronic alteration can be observed as hyperintense areas [41]. However conventional MRI appears to have little added clinical diagnostic value in tendinopathy [42]. US and MRI will be discussed separately in the section “tools to investigate tendons”.

Aetiology and pathogenesis

Repetitive overload is regarded the main component of the aetiology [43]. However, there is a huge variation in the amount of load necessary to develop symptoms, which suggests a multifactorial aetiology. And indeed, multiple intrinsic and extrinsic factors have been suggested to take part, including genetic factors, previous injuries, high age, high BMI, training in cold weather and male gender however relatively small cohort studies have repeatedly lead to few cases, which have produced a number of conflicting and insignificant results [44,45].

No single comprehensive description of the pathogenesis exists but several processes take part. Tendon biopsies from patients with chronic tendinopathy reveal

degenerative changes, rounding of tendon fibroblasts and an expansion of the cellular pool. These findings suggest that disruption of the structure occurs at some point towards chronic tendinopathy. But the link between mechanical overload and tendinopathy remains elusive. Some suggest that micro ruptures is the initial event[46], but studies investigating the mechanical properties has shown that the strain at failure of the individual fibril exceeds that of strain in tendons in vivo, which challenges this theory[47,48]. Studies on animal tendons have demonstrated that, repetitive sub-failure strains lead to the formation of kinks on the fibrils which may be speculated to alter the mechanical microenvironment[49]. Accumulation of these damages could be the initial event in tendinopathy, and thus represent the link between overload and subsequent events in tendinopathy. However, it remains largely unknown and many other factors have been suggested to take part including release of substances from the collagen during loading [50], hypoxia [51] and direct cell response to overload[52]. Most likely the initiation is caused by numerous additive and or synergistic events, which together leads to tendinopathy.

The overloading of the tendon initiates a cascade of events, including inflammation, degeneration, neo vascularization and ingrowth of nerves, but the sequence and significance of each of these events remains elusive. In long term chronic tendinopathy degeneration has been proposed to be dominating [53 54 55 56], although inflammation has been observed in chronic tendinopathy, when compared to healthy tendon [57,58 59]. Furthermore, angiogenesis and ingrowth of free nerve endings are thought, at least to some extent, to be responsible for the sensation of pain in chronic tendinopathy [60]. In agreement with this, increased vascularity has been observed in chronic tendinopathy patients using US power doppler measurements [38]. However, contradictory the vascularity does not appear to correlate with pain [61], which indicates that, other peripheral mechanism and or regulation in the central nervous system might also influence the sensation of pain in tendinopathic patients.

To understand the pathogenesis of tendinopathy in humans, there has been a growing interest in early phase tendinopathy. In a traditional sense this includes patients with duration of symptoms for less than 3 months [34], but studies have also included sub-clinical stages to get a more comprehensive picture, and observations on the response to load in healthy subjects have further advanced the understanding of the pathogenesis. In contrast to the more chronic phase, inflammation seems to have a more pronounced role in early phase tendinopathy [62–64]. Inflammation was originally defined clinically, an is characterized by the four well known cardinal signs; redness, heat, pain and swelling. However, the modern description involves the physiological responses including angiogenesis, permeabilization of vessels and increased

metabolism. Further inflammation can be characterized by the cell types involved e.g. fibroblasts, macrophages, mast cells, lymphocytes and neurons, and their actions including proliferation aggregation, migration, phagocytosis and apoptosis[65].

Even further, inflammation can be described at the molecular level. Herein a distinction can be made between the factors that induce and the factors that mediates inflammation. The list of these factors is growing, and so is the knowledge about the complex interplay. Consequently, the complexity has grown, and the definition widened[66]. According to the modern definition inflammation is an important part of tendon homeostasis in healthy tendons. Meanwhile it also appears to have a role in the diseased state at all stages of disease [57,62–64,67]. Additionally, the action of some mediators are context dependent, thus separating a healthy response from a destructive one can be extremely challenging[68]. Further some studies suggest that once the tendon fibroblast have been subjected to pro-inflammatory mediators in sufficient amounts, they enter an activated state and become hypersensitive to future inflammatory stimulation[57]. Lastly, it should be recognized that the mediators exert their effects in a complex environment influenced by the coexistence of other mediators, and the interplay between these is poorly understood. Nevertheless, several studies on human being have advanced our understanding of inflammation in tendinopathy.

In a model first described in 2008 [69] the subscapularis tendon is sampled from patients with a supraspinatus tear undergoing surgery. Importantly, the subscapularis tendons were only included if they did not show signs of tendinopathy on MRI and during arthroscopy. In this model the subscapularis tendon represents overloaded tendon with no signs of symptoms, hence a sub-clinical overloaded tendon. From this model an increase in inflammatory and apoptosis markers has been observed in early tendinopathy compared to healthy subscapularis tendon [70 63 71 72 73], potentially initiated by a rise in alarmins [64].

In another model torn supraspinatus tendon biopsies, representing an advanced stage of disease, were sampled during surgery, and intact but symptomatic supraspinatus tendon biopsies were sampled using a percutaneous needle technique, representing an earlier stage of disease [62]. Although the latter does not represent early tendinopathy in a traditional sense, the model provides insight into various stages of disease. From this model it was also found that the earlier stage of disease shows an upregulation of inflammatory markers compared to healthy and ruptured tendon, and also that resolution markers were higher [62]. Lastly in the early tendinopathy model that has been used in our group, and is used in study II and III in the current thesis, no overexpression of inflammatory mRNA markers could be found in patients with early patellar

tendinopathy, but a rice in substance-P was observed [74]. Despite being traditionally regarded as a neurotransmitter substance-P can also act as a pro-inflammatory mediator and might take part in the inflammatory response [75,76]. It should be mentioned that only a handful of selected inflammation markers were analysed, and an upregulation of others cannot be excluded.

Numerous studies have investigated how healthy human tendons and tendon cells respond to mechanical stimuli, from which insight can be gained. A technique called micro dialysis has allowed minimal invasive measurements of substances in the very near periphery of tendons. This serves as a proxy marker for events in the tissue of interest [77]. Using this method an acute increase in the pro-inflammatory prostaglandin E2 (PGE2), was observed immediately after load, and furthermore it was observed that dampening of this response using a non-steroidal anti-inflammatory drug (NSAID) lead to a decreased exercise induced blood flow in the tendon [78]. Another study using the same model found that collagen turnover was increased 72 hours after exercise, and this could again be dampened by NSAID. Thus, it was suggested that inflammatory mechanisms influenced the exercise induced collagen turnover [79]. Contradictory, a study using the percutaneous biopsy technique, found that cyclooxygenases (COX) mRNA expression was unaffected after exercise in healthy tendon [80]. COXs are enzymes responsible for the synthesis of prostaglandins which are potent pro-inflammatory lipid mediators. However, COXs were found to be expressed even in the resting state, hence the physiological increase in PGE2 could be facilitated by constitutively expressed COXs [80]. Using tendon biopsies and stable isotope labelling a rise in collagen synthesis could be found in response to loading [81]. However, others have produced conflicting results, and did not find an increase in collagen synthesis in response to exercise [82], but collagen mRNA was upregulated in the same study. This suggests that the loading induced increases in collagen turnover that was previously suggested by the early micro dialysis studies, might not translate into functional proteins incorporated in the tendon tissue. Nevertheless, data from the exercise induced changes in healthy tendons suggest that inflammation plays a role in the physiological response to load, and thus held together with the observations from studies on tendinopathic tendons it is reasonable to believe that a dysregulation of the inflammatory response could be a key player in the development of tendinopathy.

Collectively present data shows that both inflammation, degeneration, neo vascularization and ingrowth of free nerve endings can be observed at various stages of disease. Inflammation appears to be more pronounced in the early phases of tendinopathy and is part of an important

physiological response to load. This indicates that inflammation has a crucial role in the initial steps towards tendinopathy, and it can be speculated that the increased inflammation eventually leads to the more pronounced degenerative changes that can be observed in chronic tendinopathy. Lastly, it appears most likely that, even at the chronic stage of disease, inflammation is also present to some extent.

Treatment

Multiple treatments have been suggested in the treatment of tendinopathy, ranging from conservative treatment options to surgery. Common for all of these is that they do not always produce satisfactory treatment responses, but they appear to be superior to a wait and see strategy [83,84]. Treatment options include external manipulation therapies such as extra-corporal shock wave therapy, therapeutic ultrasound and laser therapy, however the role of these remains uncertain in midportion tendinopathies [85,86]. Also, a variety of injection therapies exists, some of which targets vascularization and some that targets inflammation. Some studies have shown promising results [87–89], but others have not [90,91], and the overall picture remains inconclusive [92]. Surgery serves, as a last resort in chronic recalcitrant tendinopathies, and can in some cases be a reasonable option [93]. However, this option should be reserved for patient that do not respond to less invasive treatments [94].

The most accepted and widely studied treatment modality is physical rehabilitation. A substantial number of studies have investigated the effect of a variety of exercise modalities [95–99]. Overall an active physical rehabilitation approach appears more effective than a wait and see strategy [83]. However, the type of contractions used in the rehabilitation program does not seem to significantly alter the effect of the treatment [97,98], thus heavy slow resistance training (HSR) and eccentric exercise seems to be equally efficient. The role of patient education remains elusive, but a substantial amount of the effect could be assumed to arise from this [84]. Despite being generally accepted as a well-established treatment, the effect of physical rehabilitation has been questioned [100] [101] [99]. Nevertheless, physical rehabilitation remains to be the first line treatment for tendinopathy, but a noticeable fraction of the patients do not obtain a satisfactory treatment response [101].

Lastly, a few pharmacological therapies exist, including glycerol trinitrate patches and non-steroidal anti-inflammatory drugs (NSAIDs), which can be administered orally or locally. And while some evidence supports the use of glycerol trinitrate patches [102] studies on NSAID treatment in human tendinopathy have showed limited effect on pain relief and long term clinical

outcomes [52,103–109]. NSAIDs work through inhibition of Cyclooxygenase enzymes (COX) and are usually described according to their effects on the two main isotypes COX-1 and COX-2. COX-1 is the constitutive expressed form and COX-2 is the inducible or reactive form [110]. Naproxen, which was used in paper III in this thesis, is an arachidonic acid derivate and a non-selective NSAID that inhibits both isoforms. The anti-inflammatory and pain reducing effects of Naproxen is supposedly mainly caused by the inhibition of COX-2 leading to a reduction in prostaglandin E2 (PGE2) production [111]. In addition to the clinical effects the cell response to NSAID in vivo has been investigated. Effects can be observed in the peritendinous tissue where it leads to a decrease in PGE2 concentration and collagen breakdown product PINP [79 78] in healthy tendons. But almost no effects were observed in the tendon core of tendinopathic Achilles tendons [52 106]. Further, in the study by Heinemeier et. al. the expression of two target genes ANGPLT4 and ATF3 that are highly sensitive to NSAID exposure were not affected by the NSAID treatment, which could indicate that very little of the drug actually reaches the tendon core in patients with Achilles tendinopathy[112]. In healthy human subjects the collagen synthesis of patellar tendons was significantly reduced by 2 weeks unloading, but NSAID did not affect this [113]. However, a small effect was observed on the mRNA expression of matrix metalloproteinase – 2 (MMP2), which suggests a reduced breakdown of collagen during unloading. It could be argued that, to some extent, unloading often occurs during tendinopathy, and thus the collagen sparring could potentially have an effect in tendinopathy, although this remains speculative.

In vitro cell studies on human fibroblasts have suggested that NSAIDs can reduce proliferation and proteoglycan production in these cells [114 115], and finally, animal studies have suggested both beneficial and detrimental effects on tendon healing [116 117]. Additionally one study found that injection of PGE2 in rat tendon may even improve the mechanical properties of the tendon[118].

Collectively, the effect of NSAID on tendons seems neglectable in humans in vivo, at least at a chronic stage of disease. Further, in vitro and animal studies does not provide unequivocal answers regarding the direct effect on the cells. Nevertheless, despite the gastrointestinal and cardiovascular side-effects, NSAIDs are still recommended in order to provide pain relieve in the early phase of tendinopathy [119,120], and NSAIDs are widely used in sports, as illustrated by a recent popular science report, based on questionnaires in German football [121].

No studies so far have specifically investigated the effect in early phase tendinopathy.

As described above a significant number of treatments exists, which reflects the sometimes-recalcitrant nature of tendinopathy. Most of them are considered as adjuvants to physical rehabilitation, that remains to be the first line treatment for tendinopathy. Future introduction of more targeted treatments will hopefully lead to a better treatment response, and extensive work is ongoing in this field [122–124].

Investigation tools in tendon research

There is a long list of tools that can be used in human tendon research, and whereas some are able to directly investigate biological mechanisms others aim to address clinical aspects. Despite the wide array of tools available many questions remain unanswered.

In the previous sections two invasive methods were introduced; micro dialysis and tendon core biopsies, and whereas important insights have been gained from those, they also have limitations. As illustrated above the collagen turnover that was measured with the micro dialysis technique [79] does not necessarily lead to incorporation of new collagen in the tendon core [82] which illustrates its indirect nature. Using tendon core biopsies, we are able to make direct measurements of tendon biology, and recent methodological advances including single cell RNAseq and proteomics will further advance our understanding [125,126]. However, the use in longitudinal studies may be limited. In a study by Heinemeier et. al. the authors performed repeated biopsies, and observed that the first biopsy had a substantial effect on the second [127], thus effects of interventions cannot be separated from effects of the biopsy itself.

At the other end of the spectrum sits patient reported outcome measures. Being non-invasive and far from objective these tools are not able to answer mechanistical questions, but they are central in longitudinal studies investigating the clinical effect of various interventions in tendinopathy. The numerical ranking scale (NRS) and visual analogue scale (VAS) have been used in numerous studies. They give valuable easy interpretable information about the experience of pain in specific salutations at one point in time, but these scales are not disease specific and do not account for physical function [128]. Therefore, PROMs for Achilles and Patellar tendinopathy have been developed, the most widely used are the VISA-A and P questionnaires [129,130] that were developed around year 2000. These questionnaires have allowed standardized measurements of disease severity, it should however be mentioned that the validity have been questioned [131].

Imaging

Imaging provides an opportunity to observe the whole tendon in a non-invasive manner. Ultrasound (US) and magnetic resonance imaging (MRI), are both widely used in tendon research and in the clinic. The signal is generated in two very different ways, and thus the appearance of tendon tissue differs between US and MRI.

In US an ultrasound wave is generated by piezoelectric crystals in a transducer. The waves travel through the tissue and bounces of the structures. The structures have different abilities to reflect the ultrasound wave, and whereas some reflect most of it other will let more pass through. The degree of reflection will dictate its appearance on the B-mode ultrasound image. The image is generated when the transducer switches to receive mode, and the returned US waves are converted into electrical energy [132]. Further advances was made with the introduction of power doppler (PD) US, by which the detection of blood flow became possible [133,134], herein the shift in frequency that will arise from the reflection on a moving object is exploited, and thus a coloured overlay on the B-mode image, representing flow in vessels, is generated. Other US modalities have been introduced including ultrasonographic tissue characterisation (UTC) [135] and elastography [136]. Such modalities might hold great potential, since they are able to characterise tissue properties. However, they have only recently been introduced, and are currently not widely used in the clinic.

The most frequent outcomes reported from B-mode US is the dimensions and the grade of hypo echogenicity, whereas gradings or a direct measurement of the area of vessels have been reported from PD US. The healthy tendon appears regular and homogenous on B-mode US, with its hyperechoic fascicles aligned with the long axis of the tendon. Further vessels are normally sparse in the healthy tendon, which can be assessed using PD US. Together these findings reflect the highly organized avascular build-up of the tendon. In patients with tendinopathy the tendon will appear darker (hypoechoic) and with increased thickness on B-mode images. Further, neo vascularization can be visualized using PD US. Findings in chronic and early tendinopathy patients are similar, although changes in early tendinopathy appear to be less pronounced [38,74,89,97].

Thus, US is able to detect changes in tendinopathic tendons, also at an early stage of disease[74]. But whereas some studies have observed a relationship between the degree of changes and clinical outcome [137,138], others have not [139]. Further, a significant proportion of asymptomatic individuals will have abnormal findings on US, including hypoechogenic areas and detection of blood vessels in the tendons [140 141 36 142]. Collectively, US is a useful tool in tendon research and

in a clinical setting, but interpretation of findings should always be made in conjunction with clinical findings. Because of these uncertainties the potentials of MRI have been and are being explored. This is done in an attempt to investigate tendon tissue in a detailed, yet non-invasive, manner.

In MRI a strong magnetic field (B0) partly aligns the protons in the tissue. The protons always spin as an effect of the imbalance in the nucleus which creates a magnetic field. Further, the protons precess at a certain frequency dependent on the field strength. A radio frequency (RF) pulse with the precession frequency (Lamour frequency) is applied to flip the net magnetization vector into the transverse plane and synchronise the precession of the protons, making them spin in phase[143]. This process produces a quantifiable transverse magnetization vector that can be measured using an MRI coil[144]. This signal is then used to generate the image. When the RF pulse is turned off, the protons will seek back to the equilibrium in a process called relaxation. During relaxation the protons will begin to spin out of phase, and the signal in the transverse plain will decay exponentially (transverse relaxation) with a time constant called T2[145]. The time that passes between the application of the RF pulse to the recording of the signal is called the time to echo. The appearance of the structures in the body depend on the scan sequence and the properties of the tissue scanned.

In healthy tendons most of the protons are tightly bound to collagen and proteoglycans, which leads to a fast signal decay in the transverse plane (short T2). Because of this phenomenon tendons will appear black on conventional MRI sequences with relatively high TE[146]. Nevertheless, conventional MRI sequences produce excellent contrast between the tendon and adjacent tissue, which enables precise measurement of tendon dimensions[39,40]. Furthermore, in chronic tendinopathy, when structural changes in the tissue reaches a certain level it is visible as hyperintense areas in the tendon[41]. However, in heathy tendons and tendons with more subtle structural changes conventional MRI does not allow for quantification of the structure therefore ultrashort time to echo MRI (UTE MRI) has been introduced[147,148]. As a result of the very low TEs these sequences are able to obtain more signal from the tendon tissue. Further, when multiple UTE scans are combined, quantification of time constants in the tendon are possible[149]. A method that have gained ground in the previous years is UTE T2* mapping. Herein multiple scans with varying TE is used to calculate the time constant T2* [147]. This is done by plotting the signal intensity against the TE, which produces a curve that describes the transverse relaxation. T2* reflects the amount of unbound water and hereby becomes a proxy measure for structural alterations[149]. Several studies have quantified T2* in healthy and diseased Achilles and patellar

tendons, but a great variety exists in the build-up of the sequences. Thus, the lowest TE varies from below 0.1 ms to above 3ms [147,149–162]. Additionally, studies have used a wide array of fitting algorithms, and whereas some extract T2* values on a voxel by voxel basis by plotting the signal intensity in each voxel individually, others calculate mean T2* for a certain region of interest [153,163]. The great variety that exist between the different scan protocols and fitting algorithm is also reflected by the great variety that exists between T2* values obtained in different studies (healthy Achilles and patellar tendons ~0.5 – 3.5 ms [147,149–151,155–162]; diseased tendons from ~1.7-7.2 ms [152–155,159]). Therefore, no normal T2* value for healthy tendons exists. Nevertheless difference in T2* between healthy and chronic tendinopathic have been demonstrated[155], and such changes have been suggested to have a functional implication for the tendon[164]. Also, an ability to detect early changes in tendinopathy have been suggested, but no studies so far have specifically addressed this stage of disease with UTE T2* mapping. However, an ability to detect subtle structural changes in a non-invasive manner could prove valuable both in a clinical and in a research setting.

A great array of different tools exists in tendon research, and whereas some are able to answer mechanistical questions others provide useful information about the clinical outcomes and the tendon size and structure. A combination of these tools will advance our understanding of tendinopathy, and hopefully help reveal the complex pathogenesis of tendinopathy. Furthermore, the understanding of early events in tendinopathy will help target the treatment at an early stage of disease, which will hopefully lead to a better treatment response.

Aims

The current thesis aimed to investigate the ability of magnetic resonance imaging (MRI) UTE T2* mapping to detect subtle structural changes in early phase Achilles and Patellar tendinopathy, including an assessment of the reproducibility of UTE T2* mapping in tendinopathy. Further it aims to investigate the additive effect of a short term NSAID treatment, on clinical and physiological outcomes (including UTE T2* mapping) in early phase Achilles tendinopathy.

These common aims were pursued in three papers:

Paper I
Evaluated the test-retest reproducibility between two separate scans, further we evaluated the inter- and intra-observer reproducibility of UTE T2* mapping in chronic tendinopathic human patellar tendons.

Paper II

Investigated the difference in T2* values between healthy and tendinopathic Achilles and Patellar tendons in the early phase, and the relationship between clinical outcomes, tendon volume, mechanical properties and T2*.

Paper III
Investigated the additional effect of a short term NSAID treatment on clinical and physiological outcomes (including T2*) in early phase Achilles tendinopathy.

The overall hypothesis was, that UTE T2* mapping is able to detect the subtle structural changes that can be expected in early phase tendinopathy, and that it would prove useful in the monitoring of tendon tissue in a longitudinal study. Further, that the addition of an initial short term NSAID treatment to a physical rehabilitation intervention, would improve the clinical outcome after 3 months intervention. Which would be reflected in a normalization of structural alterations measured by UTE T2* MRI.

Methods

The papers included in the current thesis are derived from three separate study populations. Paper I is based on baseline measurements on a subset of patients with chronic patellar tendinopathy who participated in an intervention study. Paper II is based on data from a large cross-sectional study, which included patients with early Achilles and patellar tendinopathy and healthy controls. Paper III is based on an intervention study in early Achilles tendinopathy.

Study designs and populations

Paper I

Design

This study was designed as an observational reproducibility study. We performed two UTE T2* MRI scans on the same day (S1 and S2). Manual segmentation of the patellar tendon volume was performed by two blinded observers (O1 and O2). One observer (O1) segmented the first and the second scan (S1 and S2), in order to evaluate the test-retest reproducibility. Both observers (O1 and O2) segmented the first scan, to assess the inter-observer reproducibility. Lastly, in order to assess the intra-observer reproducibility one observer (O1) repeated the segmentation of the first scan (S1) after 2 weeks.

Population

A subset of 15 male sports active patients with chronic patellar tendinopathy were recruited from an ongoing intervention study. Clinical diagnosis was confirmed with ultrasound. Patients were included if they had symptoms for more that 3 months, and a confirmed diagnosis of patellar tendinopathy. They were excluded if they had symptom for more than 12 months, previous knee surgery, chronic systemic diseases that could affect the outcome, previous corticosteroid injection in or around the patellar tendon and if they were smoking.

Paper II

Design

The study is designed as a cross-sectional study. Data on clinical outcomes, conventional MRI, ultrasound, biomechanical properties and biochemistry (q-RT-PCR) has previously been published separately [74]. In this study we aimed to investigate differences in tendon T2* values between subjects with early Achilles and patellar tendinopathy, and also to correlate T2* values with clinical outcomes, tendon dimensions and biomechanical properties.

Population

The original study included 200 patients with early (symptoms < 3months) Achilles and patellar tendinopathy and 50 healthy controls. However, UTE T2* MRI scans were only performed in 65 tendinopathic and 25 healthy controls, these subjects were included in the current study. Patients were included if they had activity related pain and pain on palpation in the Achilles or Patellar tendon and a symptom duration <90 days. Patients were excluded if they have had previous injuries in the same location as the current injury or if they started treatment for the current injury.

Paper III

Design

This study was designed as a double blinded, placebo-controlled trial. At a screening visit a clinical examination, baseline questionnaires and ultrasound scans were performed. If the patients were considered eligible, they were included in the study. At baseline an MRI scan was performed as soon as possible after inclusion, and the intervention was initiated on the same day. Patients were randomized into 2 parallel groups; the Naproxen group (7 days Naproxen treatment) or the Placebo group (7 days placebo treatment). After the initial week, both groups received a 12-week standard rehabilitation program including load management. Follow-up was performed after the first week of treatment (1-week: questionnaires and US), after the

rehabilitation (13-weeks: questionnaires, US and MRI) and 1 year after inclusion (1-year: questionnaires).

The Naproxen group received 7 days Naproxen treatment (500 mg b.d.) and the Placebo group received 7 days placebo treatment (tablets in identical packaging; similar in size and colour to the naproxen tablets) (Figure 3)

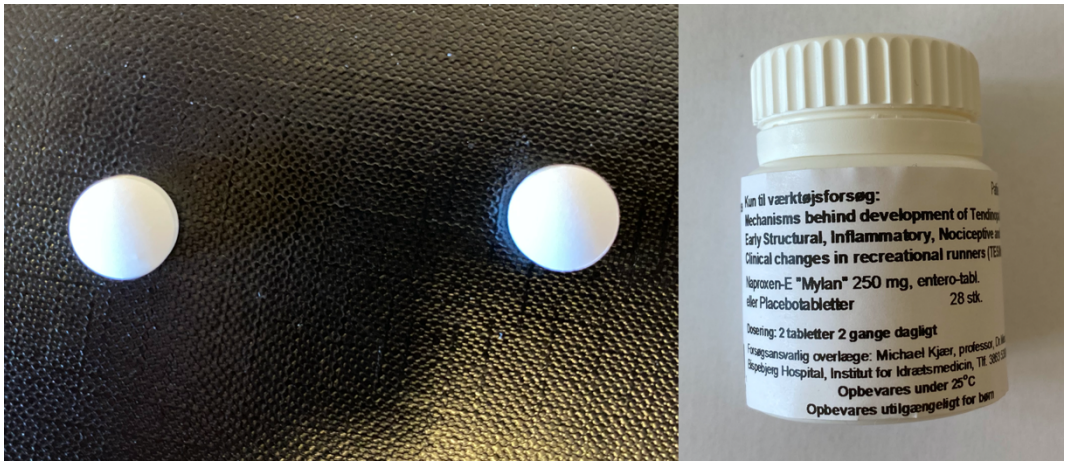

Figure 3 left image: the two tablets (Naproxen 250 mg and Placebo). Right image: the neutral packaging.

In a previous study it was speculated that the analgesic effect of NSAID could potentially lead to overload during treatment [106]. Therefore, patients were instructed to reduce the load on the Achilles tendon, and to abstain from activities such as sprinting and jumping. After the initial 7 days treatment patients received a 12 weeks physical rehabilitation program. This program consisted of homebased resistance training 3 times per week with 4 exercises in total and pain guided load management. Two exercises targeted the gastrocnemius and soleus muscles, and two targeted the hip abductors training program is provided in Table 1 and each exercise is illustrated in Figure 4–8.

Exercise	Week	Repetitions
Heel raises – knees straight	1-3	3x15
	4-12	3x10
Heel raises – knees bend	1-3	3x15
	4-12	3x10
Lateral band walk – (elastic band)	1-12	∞
Straight leg kick back – (elastic band)	1-12	3x15

Table 1 the home-based rehabilitation exercise program that was offered to both groups.

Figure 4 Heel raises – knees straight

Figure 5 Heel raises – knees bend

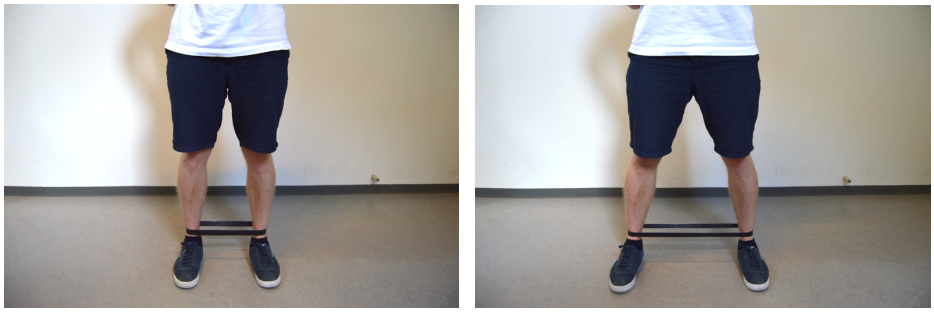

Figure 6 Lateral band walk

Figure 7 Straight leg kick back – (elastic band)

Meanwhile, patients were also instructed in load management. This included that activities with pain from 1-2 on an NRS scale (range [0-10]) were considered safe, 3-5 were acceptable and activities resulting in pain between 5-10 should be avoided.

Patients registered the training in a custom-made app (Injurymap Science, Injurymap ApS, C/O SUND Hub, Nørre Allé 41 Copenhagen). This enabled us to track the participation during the intervention. Furthermore, participants were able to access videos and documents with detailed descriptions of the training program through the app. The study flow is illustrated in Figure 8.

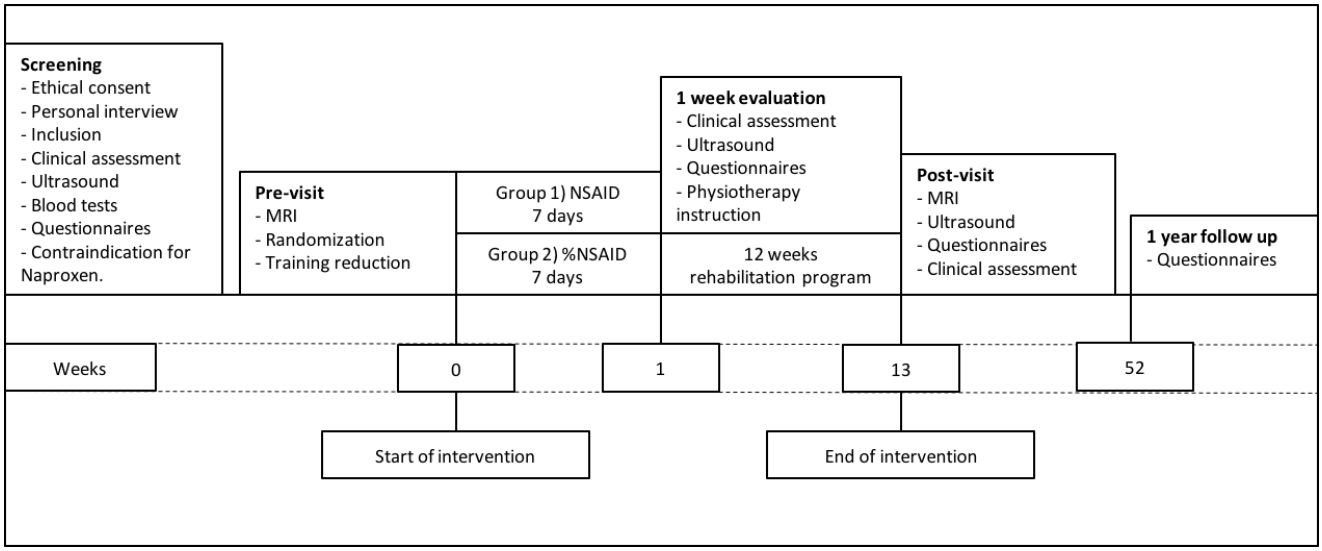

Figure 8 study flow chart for study III.

Participants

A total of 69 sports active participants with early phase Achilles tendinopathy were included. The inclusion criteria were age >18 years, pain in the Achilles tendon during loading, pain on palpation and onset of symptoms within the last 90 days. Patients were excluded if they had a previous injury in the ipsilateral Achilles tendon, recent infection in/around the Achilles tendon, enthesopathy, previous surgery in the Achilles tendon, contraindication for NSAID treatment, received NSAID treatment for the current injury or if they used medication with NSAID interaction.

Outcome measures

General remarks

The UTE MRI scan protocol and subsequent reconstruction of T2* maps are identical in paper **I II** and **III**. In paper **II** and **III** the Victorian Institute of Sports Assessment – Achilles and – patella (VISA-A and – P) questionnaires and the Numerical ranking scale were used to assess clinical symptoms.
In paper **II** an ultrasound-based method was used to investigate mechanical properties of the patellar tendon.
In paper **III** B-mode US was used to assess AP-thickness, and neovascularization was assessed with PD US, conventional MRI was used to measure tendon CSA and physical activity was assessed with questionnaires.

A description of each outcome measure is described in the following sections.

Questionnaires

The Victorian Institute of Sports Assessment-Achilles and Patella questionnaires (VISA-A and -P) (score from 0-100; 0 meaning lowest possible function of the Achilles tendon, 100 meaning full function of the Achilles tendon) were used to assess clinical symptoms. The total score is reported in paper II and III.

Questionnaires on pain and activity were made as a 1-week recall questionnaire. Patients reported their physical activity level, number of sessions and total hours of activity pr. week. Pain was reported using the NRS scale (range: [0-10]) Pain during activity, pain at rest, morning pain and worst pain experienced during the last week were reported. Further, an induced pain test was adapted from Silbernagel et. al. 2006 [35], and performed as part of the questionnaire, participants were asked to perform 25 vertical jumps on each leg and report the pain at the last jump.

Ultrasound

In paper II ultrasound recordings were used to assess the dimension and vascularization of the Achilles tendon. The same US scanner was used for all recordings (HI Vision Hitachi Ascendus (Hitachi Medical systems, Japan)) and settings were standardized. To account for the known interobserver-variability [165] the same observer performed pre and post scans within the same subject. Since the questionnaires included a jump test the US scans were always performed before the questionnaires.

First bilateral PD US was performed. Previous studies have demonstrated a reduction in doppler flow in stretched patellar tendons and Achilles tendon enthesis [166] [167], therefore patients were placed in a prone position and their feet in a relaxed position (Figure 9). A short linear transducer (EUP-L75, frequency 18-5 MHz, radius 38 mm, Hitachi Medical Systems, Japan) was used for PD recordings. The settings were standardized and adjusted in order to optimize sensitivity, but to allow for recordings free of flash artifacts in accordance with previous recommendations [168] (Doppler frequency: 10 MHz, pulse repetition frequency 250 Hz, doppler gain: 37, frame rate: 4 Hz (Table 2). Since transducer pressure can reduce PD flow[169] an abundant amount of gel was used in order to reduce transducer pressure. During examination the probe was held perpendicular to the Achilles tendon and in a stationary position to avoid flash artefacts. The short transducer could not cover the entire tendon; therefore, two recordings were made in extension. Each recording contained 20 frames.

Secondly B-mode recordings were performed, for this purpose we used a long linear transducer (EUP-L53L, frequency 10-5 MHz radius 92 mm, Hitachi Medical Systems, Japan) with standardized settings (Table 2). To allow simultaneous recording of the entire Achilles, the feet were placed flat against the wall. This ensured a stretch of the tendon with a minimal amount of load applied (Figure 9). Two longitudinal still frames were recorded on each side, with the transducer held perpendicular to the tendon.

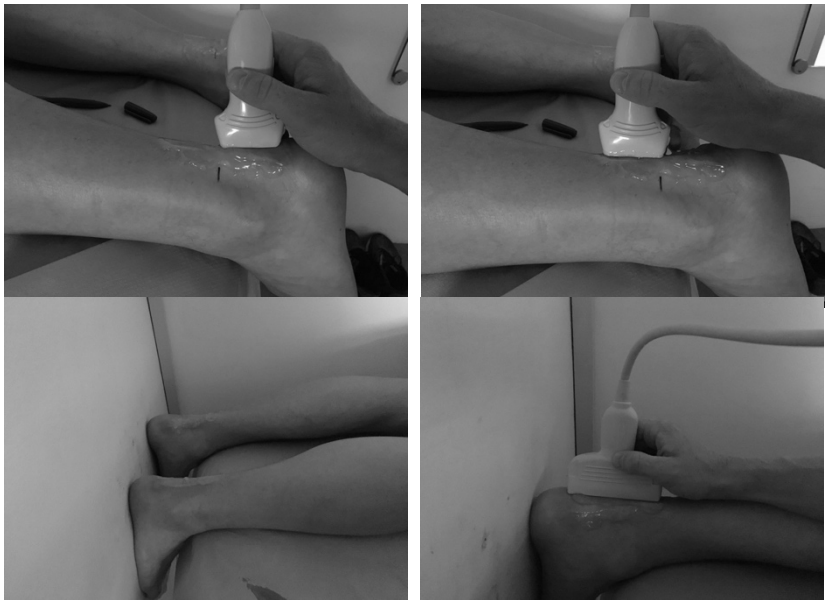

Figure 9 illustratin the ultrasound recordings. Top row: doppler US with the short linear transducer. Bottom row: Greyscale US with the long linear transducer.

	Doppler	Greyscale
Probe	Short linear transducer (EUP-L75)	Long linear transducer (EUP-L53L)
Depth	2 cm	4.5 cm
Dynamic range	70	70
Doppler frequency	10 MHz	-
Pulse repetition frequency	250 Hz	-
Gain	12	20
Doppler gain	37	-
Angle (range: 0-6)	6	6

Table 2 Full settings for US recordings.

US recording were analysed in FIJI image J (version 2.0.0-rc-68/1.52e). For PD recordings a customized macro was used. The area of coloured pixels was measured and served as a measure of vascularization (Figure 10). Before analysis recordings were manually checked for flash artefacts, which were removed using the polygon tool. Also, the polygon tool was used to outline the tendon, in order to record doppler area within the tendon. B-mode recording were analysed using the measurement tool. Thickness was measured 2 cm above the calcaneal bone (thickness) and at the thickest point (max thickness) (Figure 11).

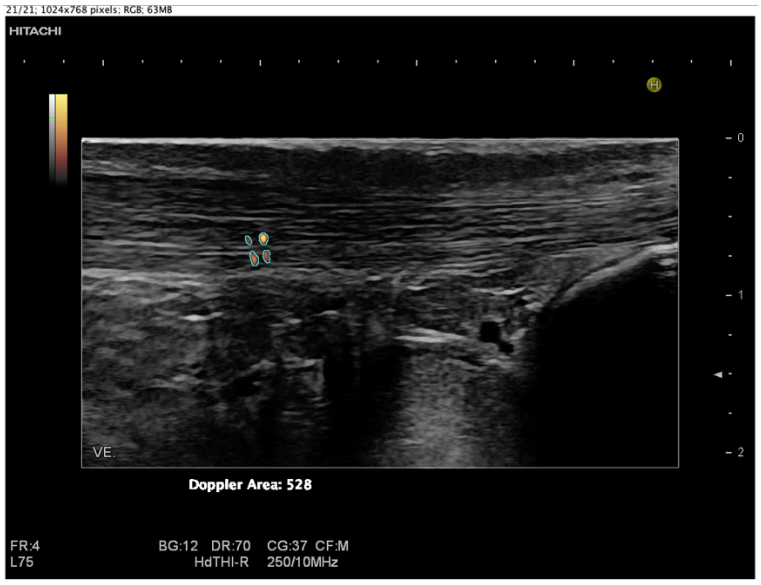

Figure 10 illustrates the quantification of doppler in FIJI image J. The macro would mark the coloured doppler area and return the area within this region.

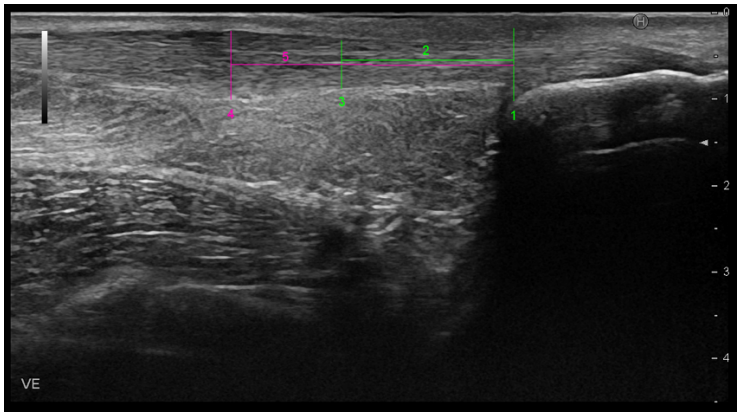

Figure 11 illustrates the thickness measurements performed on greyscale US. Line 3: indicates thickness 2 cm proximal to the calcaneal bone. Line 4: indicates max thickness.

Mechanical testing

In paper II patellar tendon mechanical properties are reported in patients with patellar tendinopathy. Data were obtained from the original work by Tran et. al. 2020 [74]. Herein the deformation was measured with B-mode US, which was combined with recordings of force during an isometric contraction. Before the test the subjects did a light warm-up on a cycle ergometer (Monark, Sweden). Thereafter, they were seated in a custom-made chair with the knees flexed at 90°. A cuff was fixated around the ankle and connected to a force transducer by a rigid lever arm. The US transducer (EUP-L75, frequency 18-5 MHz, radius 38 mm, Hitachi Medical Systems, Japan) was fixated in a holder to keep it in position during the contraction, and visualization of both the tibial tuberosity and the patella bone was ensured. The subjects were instructed to perform a ramped isometric knee extension over eight seconds. This was done to ensure a gradual increase of force, which was recorded during the contraction. A synchronized recording of the tendon with US was performed.

In order to measure deformation on the US recordings, the tendon origin on the patella bone and insertion on the tibial tuberosity was tracked in a custom-made Matlab script (Matlab R2016b, The MathWorks Inc, USA). From the coordinates the change in tendon length was calculated (deformation). Hereafter, the deformation data obtained from US was plotted against the synchronized force data in excel. To generate a force-deformation curve, the synchronized data were fitted to a second-order polynomial using Sigma Plot (Version 10.0, Systat Software, Germany). The predicted data points from this curve were exported to excel, and a linear regression was performed on the last 20 % of the curve, which represented the linear portion of the force deformation curve. The slope of this curve represents stiffness (N/mm). Furthermore, stress (Force/CSA) and strain (deformation/length) were calculated from the predicted data points on force and deformation. A linear regression was also performed on the last 20% of these data points, here the slope represents modulus (N/mm^2*strain (%)). In the current work, modulus was used as a measure of tissue properties, and the association with tendon T2* was investigated.

Conventional MRI

In paper III conventional T1 weighted MRI scans were used to measure Achilles tendon dimensions. All scans were performed in the same MRI scanner (Siemens Verio® (Siemens, Erlangen, Germany) – 3 Tesla). For practical reasons we only performed unilateral MRI scans at two time points (at baseline and 3 months follow up). Before the scan patients were placed in a supine position, and the foot fixated in a dedicated ankle coil using foam pads. Both an axial and a sagittal scan were performed. Laser guides and anatomical landmarks were used to ensure the same positioning between pre and post scans. The DICOM files were exported and the image analyses were performed in Horos (Horosproject.org, Nimble Co LLC d/b/a Purview in Annapolis, MD USA., V 4.0.0 RC3), which is a freeware DICOM viewer. The assessor was fully blinded during analyses, and all analyses were performed on the same computer, using a standardized set of values for contrast, zoom and brightness. First Sagittal and axial scans were opened in the same window (full screen, coupled split-screen view) (example in Figure 12), in this way the sagittal scan could be used to guide the positioning of the start and the end slice. The most proximal part of the free tendon was defined as the first slide without visible soleus muscle. The most distal part was defined as the last slide before insertion to calcaneus. Segmentation of the tendon was then performed on the axial scan in all slides of, the now defined, free tendon. The "polygon tool" was used for segmentation. And the "repulsor tool" was used to make corrections after visual inspection of the segmentation. Since the largest CSA might move between baseline and follow-up we chose to calculate and report the average CSA of the free Achilles tendon, expressed in cm^2.

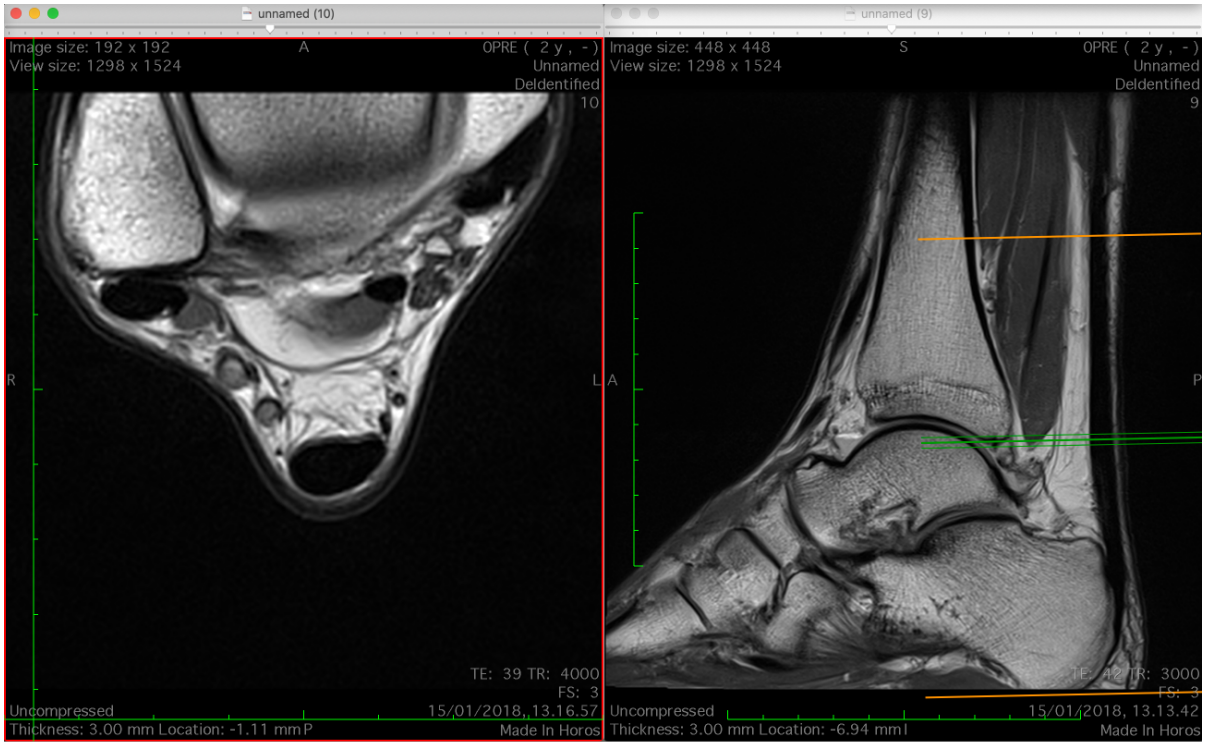

Figure 12 example of images from the conventional MRI scans with low homogenous signal in the tendon.

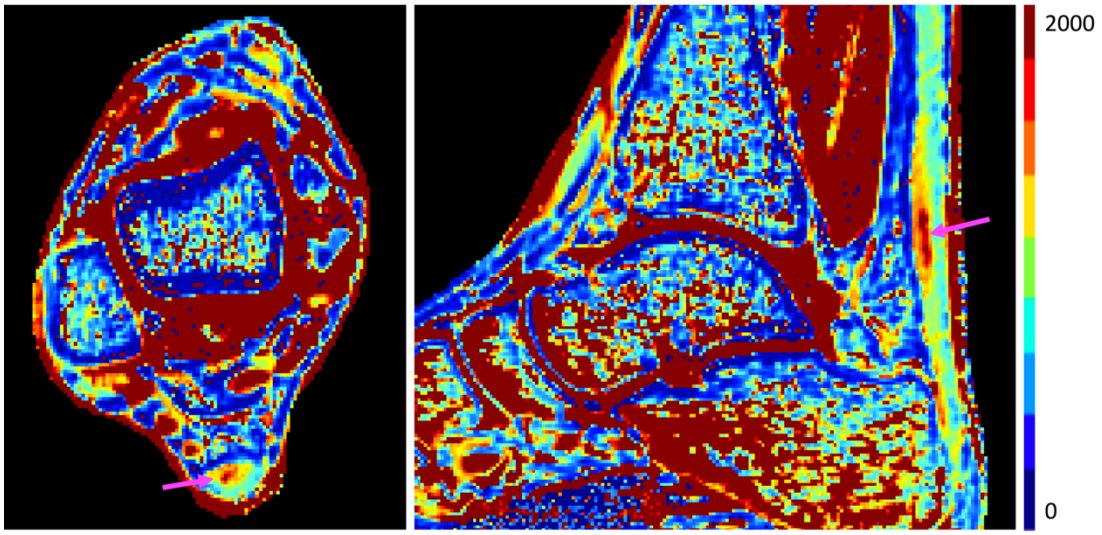

Figure 13 example of T2* map from the same patient as in figure 13. A distinct area with higher T2* values can be observed (marked with arrow).

UTE MRI

In paper I, II and III 3D UTE MRI scans were used to calculated tendon T2* relaxation times. For technical reasons only a subset of subjects in paper III had UTE scans performed. T2* values reflect the amount of bulk water in the tendon, and is suggested to be a proxy measure of collagen integrity.[155,164,170,171]

Acquisition

The UTE scans were performed together with the conventional MRI scans. Positioning of the patients was identical to the one described for the conventional MRI for Achilles tendons. For the patellar tendon the knee was placed in a dedicated knee coil. A gradient echo sequence with varying TE (0.07, 0.57, 1.07 and 1.57 ms) was used, and all other parameters were held constant (FOV 160 x 160 mm, matrix resolution 1.45 x 1.45 x 1.0 mm, TR 11 ms, FA 12 degrees, scan time 3m 14 s). The total scan time of the four scans was ~13 minutes.

T2* map reconstruction

DICOM files were exported and automatically loaded into a custom-made program written by X-Rai IVS, Copenhagen, Denmark, which was based on a MatLab algorithm written by Dr. Petros Martirosian, Section on Experimental Radiology, University Hospital, Tübingen, Germany. TE was read from the meta data and plotted against signal intensity on a voxel-by-voxel basis. In each voxel a mono-exponential fit incorporating noise was performed using the Levenberg-Marquardt method. The following formula was used, as described in[172]:

$$S = S_0 \cdot e^{\frac{-TE}{T2}} + c$$

From the fitting algorithm a map containing T2* values from each voxel in the full scan volume (T2* map) (Figure 13) and a map containing Pearson's correlation coefficients (r) (goodness of fit map) were obtained.

Manual segmentation

The result of the automated reconstruction was parameter maps of the full scan volume, but since we were only interested in the values within the tendon a manual segmentation was performed of the free tendons. The segmentation was performed on the scan with the longest TE (1.57 ms), since this scan had the best contrast for tendon segmentation. This scan was opened in ITK-SNAP version 3.6.0 for MAC OS (http://www.itksnap.org). Herein manual segmentation was performed on every 4th slide, for the Achilles tendon the distal and proximal ends of the free tendon were specified as previously described for the conventional MRI scans. For the patellar tendons the most proximal was the first slide of the segmentation where the patella bone was not visible, and the most proximal slide was the first slice where the corpus Hoffa fat pad deep to the tendon was not visible. After segmentation the interpolate label tool was used to calculate the total volume. The total volume was manually checked, to ensure correct segmentation in all slides. In general, a conservative approach was applied, in order to avoid inclusion of surrounding tissue. The segmentation volume was saved as a "nii.gz" – file, which was used for the extraction T2* values from the tendon.

Tendon T2* analyses

The reconstruction step and the segmentation step result in parameter maps containing T2* values and Pearson's correlation coefficients, and a segmentation of the tendon volume. These were combined in FIJI ImageJ (version 2.0.0-rc-68/1.52e) in order to extract parameters from the tendon from the reconstruction map. A custom-made macro was written in order to automatically generate the output file. The particle analyses function was used, and parameters were written to a .txt file. Mean T2* values of the full tendon volume were extracted, by combining the T2* map and the segmentation, further r-values were extracted, by combining goodness-of-fit maps and the segmentation. Also, the start and end slide and the full volume were extracted from the tendon segmentation. We observed some variation in r-values within the tendon, which was most pronounced in the chronic patellar tendinopathy patients. We considered fit curves with r-values >0.8 as sufficient for further analyses. By combining T2* - and Goodness-of-fit – maps and the tendon segmentation we extracted mean T2* values from the voxels which had sufficiently high r-values (>0.8), these are referred to as corrected T2* values. Also, to determine the volume that was excluded from the corrected T2* analyses we extracted the volume with $r > 0.8$.

Statistics

Paper I

Herein, an excel template was set up to assess bias. We used student's paired t-test to test for differences between the repeated scans (test-retest), and between the analysis by either two independent observers or within the same observer (intra- and inter-observer reproducibility). P-values ≤0.05 were considered significant. Furthermore, coefficient of variance (CV) was calculated. In SPSS we calculated intraclass correlation coefficients (ICC) with 95% confidence intervals. Appropriate ICC models where chosen for the different comparisons. For intra-observer reliability we used ICC model 3.1 (two-way mixed model, consistency type), and for inter-observer and test-retest we used ICC model 2.1 (two-way random model, absolute agreement type)[173]. Additionally, typical error percentages were used as a measure of the relative measurement error. Typical error percentages were calculated as $\frac{SD_{diff}}{\sqrt{2} \cdot \bar{x}} \cdot 100$ [174]. Data are presented as mean ± SD. We used Excel 2018 (Microsoft® Corporation, Redmind, WA) and SPSS (IBM®, Version 23, 64-bit edition) for statistical analysis.

Paper II

No formal sample size calculation was performed. Since this study sought to determine differences in T2* between patients with early phase tendinopathy and healthy controls, the sample size was determined by the number of participants undergoing UTE T2* MRI scans. Differences in subject characteristics were assessed using Students t-test for parametric outcomes and Fisher's exact test for categorical outcomes. In order to investigate differences in T2* between patients and controls, a generalized linear model was applied. T2* was set as the dependent variable whereas group (patient/control) and tendon (Achilles/Patella) were set as independent variables. An unadjusted crude analysis was performed and an analysis in which we adjusted for age, by including it as a random effect. We adjusted for age in order to account for the age difference that was present between the groups and also since tendon properties were associated with age in previous studies [175,176]. To investigate the correlation between T2* values, clinical outcomes (VISA-A and -P), tendon volume and biomechanical properties linear regressions were performed in the two patient groups separately. Results are presented as mean values ± SEM unless stated otherwise. An alpha level of 0.05 was used to test for significance. All statistical analyses were carried out in SAS studio (Release: 3.8 (Basic edition)).

Paper III

The required sample size to detect a 10 points difference in VISA-A between the groups with 80% power and an alpha level of 0.05 was estimated to 50. To account for potential dropouts, we aimed to include 70 patients. However, we were only able to include 69 subjects in total before the study drugs expired. Baseline differences between groups were tested using unpaired t-test for parametric outcomes, whereas Wilcoxon sign rank test was used to test non-parametric outcomes. Fisher's exact test was used for categorical baseline characteristics. Results are presented as means ± standard error, unless stated otherwise. A constrained linear mixed model was applied in order to investigate changes between repeated measures. Time and group were included as dependent variables. We assessed the effect of treatment by examining two-way interactions (time x group). In this way we got an estimate of the difference in time effect between the groups. Additionally, we performed an exploratory analysis to test whether symptom duration had any effect on clinical outcomes at 3 months and 1 year. Herein, time and symptom duration were included as dependent variables, and the effect of symptom duration was assessed by examining two-way interaction (time x symptom duration). An unstructured covariance matrix was applied to account for correlation in repeated measures. An alpha level=0.05 was used to test for significance. All statistical analyses were carried out in SAS studio (Release: 3.8 (Basic edition)).

Results and discussion

Paper I

Introduction

Since UTE T2* MRI has been suggested to be a sensitive measure that can potentially be used to observe changes within subjects in interventional studies, we found it relevant to formally investigate the reproducibility of the method. In this paper we included 15 patients with chronic patellar tendinopathy. We performed two repeated scans to investigate test-retest reproducibility. Further, two blinded assessors analysed the scans which allowed for investigation of inter-rater reproducibility. Lastly, analysis was repeated by one of the two assessors in order to investigate the intra-rater reproducibility. In addition to describing the reproducibility of the method, the results herein add to the limited data that exists on T2* in patellar tendons.

A substantial number of voxels had poor fits in this population of patients with chronic patellar tendinopathy, therefore we reported both mean values from the full volume (T2*) and mean values from the part of the tendon that yielded acceptable fits (Pearsons' correlation coefficient $r>0.8$) ($T2^*_{cor}$). This pattern is most likely due to the fact, that no signal decay was observed in the areas of the tendon were the tendinopathic changes are presumably most severe (mid-proximal part). These areas co-incited with voxels with poor fit. This is in contrast to the steep decay observed in areas within the tendon with better fit ($r>0.8$), this observation is illustrated with an example in Figure 14. Since a limited signal decay was observed in the areas with poor fit within the TE range applied in this study, an estimation of T2* within these areas becomes highly suspicious. Therefore, the exclusion of voxels with poor fit was essential for valid interpretation of the results. Meanwhile we acknowledge that the true average T2* of chronic tendinopathic patellar tendons is well above the values we extract in the current study, which is in line with results from subsequent studies on patients with chronic patellar tendinopathy[153,154]. To describe changes within the more severely affected areas, longer TEs may be necessary and will, most likely, make the method more resilient.

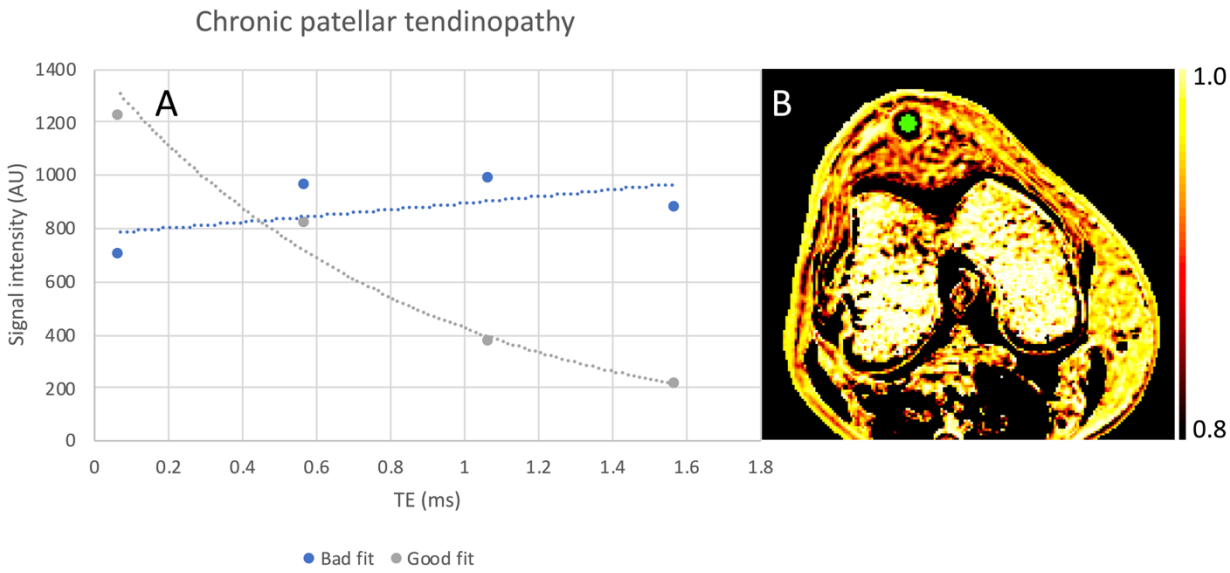

Figure 14 A) Signal intensity plotted against TE, for an area with good fit (r>0.8) (grey curve) and an area with bad fit (r<0.8) (blue curve) B) Goodness of fit map. The green dot indicates the area with bad fit in the mid proximal part of the tendon. (Pearsons' correlation coefficients range: [0.8-1.0]).

Results

Test-retest reproducibility

The results from the repeated scans analysed by observer 1 (O1) are presented in Table 3. In general, the typical error was smaller for the $T2^*_{cor}$ compared to T2*, which is most likely due to the high uncertainty in the values obtained from the uncorrected analyses. Overall ~20% of the total volume was excluded in the $T2^*_{cor}$ analyses.There was a significant increase between S1 and S2 for both T2* and $T2^*_{cor}$. However, the typical error was generally low, and substantial reproducibility was demonstrated by the ICC values.

For the volume measurements no significant differences were observed between the two scans, and the total average volume was almost identical between S1 and S2. Thus, no obvious relationship existed between changes in T2* and changes in tendon size. Also, for volume measurements ICC was generally high, but typical error was higher than for T2*.

Tendon part	S1 M1 O1	S2 M1 O1	Diff	P-values	TE %	ICC
Proximal						
T2* (ms)	2.92 ± 1.23	3.13 ± 1.21	0.21 ± 0.25	0.007	6.0	0.97 (0.81-0.99)
$T2^*_{cor}$ (ms)	1.61 ± 0.28	1.65 ± 0.27	0.04 ± 0.07	0.046	3.0	0.96 (0.87-0.99)
Volume (mm^3)	3193 ± 1153	3218 ± 1086	26 ± 209	0.639	4.6	0.98 (0.95-0.99)
$Volume_{cor}$ (mm^3)	2503 ± 696	2422 ± 692	81 ± 229	0.192	6.6	0.94 (0.84-0.98)
Distal						
T2* (ms)	2.65 ± 1.04	2.96 ± 1.05	0.30 ± 0.35	0.005	8.9	0.91 (0.55-0.97)
$T2^*_{cor}$ (ms)	1.75 ± 0.24	1.84 ± 0.20	0.09 ± 0.11	0.008	4.6	0.80 (0.33-0.94)
Volume (mm^3)	2600 ± 1056	2570 ± 966	30 ± 268	0.669	7.3	0.97 (0.90-0.99)
$Volume_{cor}$ (mm^3)	2104 ± 696	1956 ± 587	148 ± 298	0.076	10.4	0.88 (0.65-0.96)
Total						
T2* (ms)	2.84 ± 0.97	3.09 ± 0.94	0.25 ± 0.27	0.003	6.4	0.93 (0.58-0.98)
$T2^*_{cor}$ (ms)	1.67 ± 0.23	1.73 ± 0.21	0.06 ± 0.07	0.006	3.0	0.91 (0.58-0.98)
Volume (mm^3)	5793 ± 2160	5788 ± 2015	4 ± 420	0.969	5.1	0.98 (0.95-0.99)
$Volume_{cor}$ (mm^3)	4605 ± 1370	4376 ± 1260	228 ± 473	0.083	7.5	0.93 (0.78-0.98)

Table 3 Test-retest results. Abbreviations: S1& 2: Scanning one and two; M1: Measurement one, O1: Observer one; Diff: difference between the two measurements (Mean ± SD); TE %: Typical error percentage; ICC: Inter Class Coefficient (95% CI); Cor: corrected

Intra-observer reproducibility

The results from the repeated analysis by the same observer on the same scans are presented in Table 4. In general, the average differences approached zero for both T2* and $T2^*_{cor}$, whereas small differences in volume were observed between the two measurements. Since high T2* values were generally located to the mid tendon, small differences in the included volume in the periphery would not affect the average T2*, which explains this mismatch between the two. However, no significant differences were observed between the two measurements, and low typical errors and high ICC values were observed across all values.

Tendon part	S1 M1 O1	S1 M2 O1	Diff	P-values	TE %	ICC
Proximal						
T2* (ms)	2.92 ± 1.23	2.91 ± 1.24	0.01 ± 0.13	0.727	3.1	1.00 (0.94-1.00)
$T2^*_{cor}$ (ms)	1.61 ± 0.28	1.61 ± 0.28	0.00 ± 0.03	0.993	1.2	1.00 (0.99-1.00)
Volume (mm^3)	3193 ± 1153	3255 ± 1137	62 ± 198	0.246	4.3	0.99 (0.96-1.00)
$Volume_{cor}$ (mm^3)	2503 ± 696	2561 ± 668	58 ± 201	0.283	5.6	0.96 (0.88-0.99)
Distal						
T2* (ms)	2.65 ± 1.04	2.67 ± 1.08	0.01 ± 0.24	0.836	6.4	0.98 (0.93-0.99)
$T2^*_{cor}$ (ms)	1.75 ± 0.24	1.76 ± 0.26	0.01 ± 0.06	0.468	2.2	0.98 (0.93-0.99)
Volume (mm^3)	2600 ± 1055	2778 ± 1101	178 ± 254	0.017	6.7	0.97 (0.92-0.99)
$Volume_{cor}$ (mm^3)	2102 ± 696	2231 ± 660	129 ± 200	0.025	6.6	0.96 (0.88-0.99)
Total						
T2* (ms)	2.84 ± 0.97	2.83 ± 0.97	0.01 ± 0.14	0.837	3.5	0.99 (0.97-1.00)
$T2^*_{cor}$ (ms)	1.67 ± 0.23	1.67 ± 0.24	0.01 ± 0.03	0.493	1.3	0.99 (0.98-1.00)
Volume (mm^3)	5793 ± 2160	6033 ± 2194	240 ± 382	0.029	4.6	0.99 (0.96-1.00)
$Volume_{cor}$ (mm^3)	4605 ± 1370	4792 ± 1300	187 ± 330	0.045	5.0	0.97 (0.91-1.00)

Table 4 intra-observer results. Abbreviations: S1: Scanning one; M1&2: Measurement one and two, O1: Observer one; Diff: difference between the two measurements (Mean ± SD); TE %: Typical error percentage; ICC: Inter Class Coefficient (95% CI); Cor: corrected

Inter-observer reproducibility

The results from repeated analyses by two different observers is presented in Table 5. In general, larger and to some degree systematic differences were observed between the two observers. Even though no significant differences were observed in $T2^*_{cor}$ it showed a similar pattern as T2* which differed significantly between observers in the proximal part (4.8%) and in the total tendon (4.2%). Again, ICC values were generally high, and the typical error low for T2* values. For the volume the second observer consistently measured a larger volume than the first observer. When compared to the intra-observer reproducibility, this highlight the importance of having the same observer in studies that aims to find small effect of relatively short-term interventions.

Tendon part	S1 M1 O1	S1 M1 O2	Diff	P-values	TE %	ICC
Proximal						
T2* (ms)	2.92 ± 1.23	2.79 ± 1.19	0.14 ± 0.14	0.002	3.5	0.99 (0.89-1.00)
$T2^*_{cor}$ (ms)	1.61 ± 0.28	1.59 ± 0.27	0.02 ± 0.04	0.077	1.8	0.99 (0.96-1.00)
Volume (mm^3)	3193 ± 1153	3477 ± 1117	284 ± 185	<0.001	3.9	0.96 (0.28-1.00)
$Volume_{cor}$ (mm^3)	2503 ± 696	2795 ± 653	292 ± 189	<0.001	5.1	0.88 (0.04-0.97)
Distal						
T2* (ms)	2.65 ± 1.04	2.56 ± 0.95	0.09 ± 0.20	0.106	5.6	0.98 (0.93-0.99)
$T2^*_{cor}$ (ms)	1.75 ± 0.24	1.73 ± 0.25	0.02 ± 0.06	0.234	2.3	0.97 (0.92-0.99)
Volume (mm^3)	2600 ± 1055	2913 ± 1031	313 ± 338	0.003	8.7	0.91 (0.50-0.98)
$Volume_{cor}$ (mm^3)	2102 ± 696	2394 ± 709	292 ± 274	0.001	8.7	0.85 (0.23-0.96)
Total						
T2* (ms)	2.84 ± 0.97	2.72 ± 0.92	0.12 ± 0.15	0.008	3.8	0.98 (0.89-1.00)
$T2^*_{cor}$ (ms)	1.67 ± 0.23	1.65 ± 0.23	0.02 ± 0.03	0.052	1.3	0.99 (0.96-1.00)
Volume (mm^3)	5793 ± 2160	6390 ± 2095	597 ± 487	<0.001	5.7	0.94 (0.38-0.99)
$Volume_{cor}$ (mm^3)	4605 ± 1370	5189 ± 1350	584 ± 427	<0.001	6.2	0.87 (0.09-0.97)

Table 5 inter-observer results. Abbreviations: S1: Scanning one; M1: Measurement one, O1&2: Observer one and two; Diff: difference between the two measurements (Mean ± SD); TE %: Typical error percentage; ICC: Inter Class Coefficient (95% CI), Cor: corrected

Discussion

In the present study the test-retest data demonstrate a numerically small bias between recordings the two recordings, but reproducibility and typical error percentages were both within acceptable range. The method showed excellent intra- and inter-observer reproducibility, although significant differences were observed between the two observes.

UTE T2* mapping appears to be a sensitive measure of the amount of free water secondary to structural changes in the tendon tissue [177,178], and can differentiate between healthy and tendinopathic tissue [155,170,179]. The aim of this study was to investigate the reproducibility of the method, in order to compare this to the values that have been obtained in the literature. Herein we observed a typical error of ~3.0% between the two scans, this value is lower than the difference between healthy and tendinopathic tendon tissue reported in the literature [157,170,180,181], and generally our T2* values were lower compared to many other studies in tendinopathic tendons.

All together, we suggest that the method is a promising tool that can detect relevant changes in tendon T2*. Further, in contrast to tissue sampling which only allows investigation of small specific areas UTE T2* mapping is able to assess the full tendon volume.

The sequence that was applied in the current study enables us to describe the areas within the tendon that cannot usually be investigated using conventional MRI protocols. But as described above regions that were more severely affected were excluded based on the low r-values. However, it could be speculated that the regions with less severe alterations are also more susceptible to treatment, and thus relevant to describe in intervention studies.

Although small, a significant difference was observed between the two scans. This can however possibly be explained by prior loading of the patellar tendon before scanning. A previous study observed a decrease in T2* after physical activity[182], in the present study subjects were instructed to abstain from physical activity 24 hours before the scan, but the transport to the radiology department may have been enough to induce a small decrease in T2* that would affect the first scan. Thus, the difference between the two scans can be explained by a decrease due to prior physical activity before the first scan, which emphasizes strict standardization of the pre-scan regime. However, we did not test this in the current study and thus other reasons for the variation between the scans should be considered. In order to assess the isolated technical variation, we performed repeated scans on a set of $MnCl_2$ phantoms with varying concentrations (1, 2, 4, 8, 16 and 160 mM). There was a small technical variation at ~ 1%. This suggests that the technical

variation only accounts for a small part of the variation seen in the tendons. Another contributing factor could be the positioning of patients, since T2* is susceptible to inhomogeneities in the main magnetic field B_0. The positioning of the patients could be assessed since the center of the field of view was fixed to the iso center of the magnetic field, and thus changes in positioning of anatomical features on the images in the Z direction would be due to changes in positioning. However, we did not find a systematic difference in positioning between the two scans, and mean difference was within 0.5 cm. Thus, it is unlikely that the positioning caused the observed difference between the two scans.

Generally larger variation was observed in the volume measurements compared to the T2* values. We did apply a conservative approach when performing the segmentation, which might affect the reproducibility since the outer border of the tendon might be more poorly defined. This was however done to avoid inclusion of peritendinous tissue, which we suspected could affect the T2* values. And indeed T2* values appear to be largely unaffected by the differences in volume between segmentations. A possible solution could be to implement automized segmentation which have been attempted by others[151]. The present study and previous studies have mainly focused on chronic tendinopathic changes, therefore the ability to detect subtle structural changes at earlier stages of disease remains elusive at this point, however this will be addressed in paper II. One previous study has formally investigated the reproducibility of UTE T2* in chronic Achilles tendinopathy[183]. Therein a coefficient of variation of 18 % and a least significant change of 50 % was reported, the corresponding values based on data from the current study was a coefficient of variation of 3.9 % and a least significant change of 11% for the T2* corrected values in the whole tendon. However, in that study, only three slices were analyzed and more importantly all voxels within the segmentation were used in the analyses, if similar calculations were made on the uncorrected T2* value in the current study considerably higher variation was also observed (CV=8.5% and LSC=24%). Which underscores the importance of the goodness of fit correction, at least in patients with chronic tendinopathy, when applying a relatively small narrow of TEs.

In conclusion despite the numerical small bias that was observed between the two scans. However, we believe that the method is sufficiently reproducible for further investigation in tendon research. And lastly, that it might serve as an objective measure of relatively subtle structural changes in tendon tissue, that might occur in disease and as an effect of rehabilitation.

Paper II

Introduction

In paper I a satisfactory reproducibility of our UTE T2* mapping setup was demonstrated. However, whether the method is sensitive to the more subtle changes that we expect in the early phase of tendinopathy remained elusive. Therefore, in this paper we aimed to describe the difference in T2* using the same setup as in paper I. However, in this study both Achilles and patellar tendons were investigated. The MRI scans used herein were performed as part of a larger cross-sectional study, which aimed to investigate the sequence of events in early phase tendinopathy[74]. Also, in this study we excluded the voxels with poor fit ($r<0.8$), and herein only report the corrected values (T2* herein ($T2^*_{cor}$ in paper I)). However, as we expected only an average of 1.5% of the voxels were excluded overall across the groups, this is remarkably lower that the 20% that was excluded in paper I. The difference between the two studies is most likely explained by the fact that we observed a signal decay during our TE range for almost all voxels within the tendon segmentation in these patients with early phase tendinopathy and healthy subjects, in contrast to the patients with chronic tendinopathy.

Results

Table 6 summarizes baseline characteristics. Out of the 200 patients and 50 controls that were included in the original study a subset of 90 subjects were scanned using the UTE MRI protocol. Hereof 65 were patients (Achilles (n=45), patella (n=20)) and 25 were healthy controls (Achilles(n=15), patella (n=10)). The two groups were comparable on all parameters despite an approximately 10 years age difference ($p<0.0001$) between patients and healthy controls. Because of this difference and since an age effect on T2* was previously described[176] we adjusted for age in our analyses. The total VISA-P was missing for one subject with patellar tendinopathy and thus only 19 patellar patients are included for this outcome.

	Patients (n=65)	Healthy (n=25)	t-test
Age (y)	37.1 ± 1.3	28.5 ± 1.3	<0.0001
BMI (kg/m^2)	24.0 ± 0.3	23.6 ± 0.4	0.45
Training (h/week)	7.5 ± 0.5	6.3 ± 0.6	0.15
Sex (female/male) †	22/43	12/13	0.23
Pain (NRS)	5.0 ± 0.3	-	-
Symptom duration (days)	45.01 ± 2.9	-	-

Table 6 Baseline characteristics for paper II, p-values derived from unpaired t-test. †=Fishers exact test.

T2 in patients with early phase tendinopathy and healthy controls*

To allow for interpretation of main effects we tested for interaction between group and tendon. No interaction was observed in the unadjusted analyses (p=0.79) or the age adjusted analyses (p=0.62). Therefore, we continued with the interpretation of main effects. The main purpose of the current study was to investigate whether the method could detect differences between healthy tendons and tendons in patients with early tendinopathy and indeed a significant difference in T2* was observed between the healthy and tendinopathic group (Table 7) (Mean difference; Achilles: 349.2 ± 72.8 µs; Patella: 371.1 ± 92.1), in the age adjusted model. Results from the unadjusted model indicates a larger main effect of group, but the pattern is similar. Further, there was a significant main effect of age on T2* (16.9 ± 3.7 µs/year; p<0.0001). Estimates from the age adjusted and unadjusted analyses are presented in Table 7, and the calculated mean values are plotted in Figure 15.

	T2* (µs) listed as: Estimates (95%CI) and p-values	
	Group	Tendon
ΔT2*—unadjusted	342.8 (178.1–507.4) (<0.0001)	269.3 (112.9–425-7) (0.001)
ΔT2*—age adjusted	204.8 (44.5–365.0) (0.01)	356.3 (210.1–502.4) (p<0.0001)

Table 7 main results with results from the unadjusted and age adjusted analyses. Main effect of group (patient/control) and tendon (Achilles/patella) are presented. The healthy control and the Achilles tendon were used as reference group and reference tendon respectively.

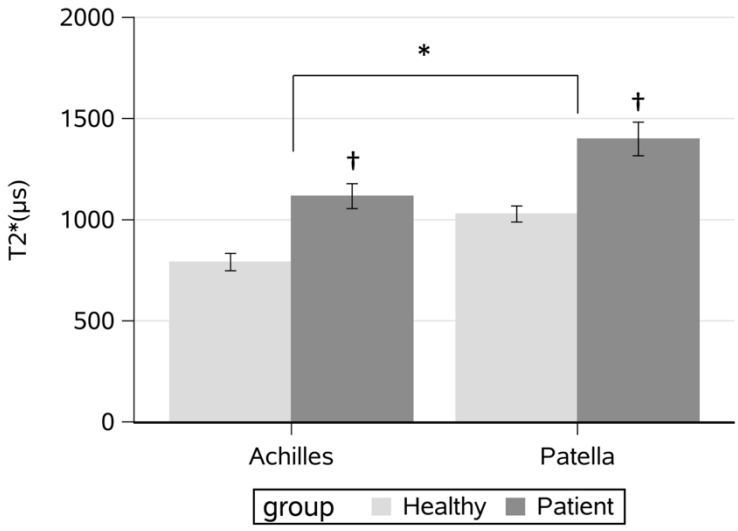

Figure 15 Mean T2* values (µs) are provided (Error bars: SEM) for Achilles and patellar tendons in healthy controls and patients with early phase tendinopathy. P-values are obtained from the age adjusted model. † = main effect of group (patient/healthy) (p=0.01). * = main effect of tendon (Achilles/Patella) (p<0.001).

Interestingly the age effect is almost identical to the results from a recent study by Loegering et. al.[176] in which the authors report a 60% difference between a group of young ~25 years and old ~65 years subjects. Herein, 40 years age difference would mean an increase in T2* of about 680 µs (17 µs/year * 40 years), and with healthy values for the relatively young control group at about 1000 µs this corresponds to 68 % increase, assuming a constant rate.

T2 vs. tendon size clinical outcomes and mechanical properties*

Tendon size was calculated as and average CSA from the tendon volume and correlated to T2* in order to investigate the association. Also clinical outcomes (VISA-A/P) and in vivo biomechanical outcomes were obtained in the original study we searched to test whether we could confirm the association to clinical outcomes that was observed in previous studies on human subjects[155,159], and the association with mechanical properties that was observed in one previous study on bovine tendon transplants [164].

There was a positive correlation between cross-sectional area and T2* for both Achilles tendons (r= 0.72; p<0.0001) and patellar tendons (r=0.53; p=0.02) (Figure 16). Mean CSA was 83.8 ± 3.2 mm^2 for the tendinopathic Achilles tendons and 127.2 ± 5.0 mm^2 for the tendinopathic patellar tendons. CSA was 73.4 ± 5.3 mm^2 and 110.1 ± 5.7 mm^2 for the healthy Achilles and patellar tendons respectively.

There was no significant correlation between VISA-A and T2* (r=-0.2; p=0.17) or

VISA- P and T2* (r=-0.5; p=0.0504) (Figure 16). However, a trend was observed in the patella patients. Mean VISA-A was 58 ± 2.7 and VISA-P 61.2 ± 3.4.

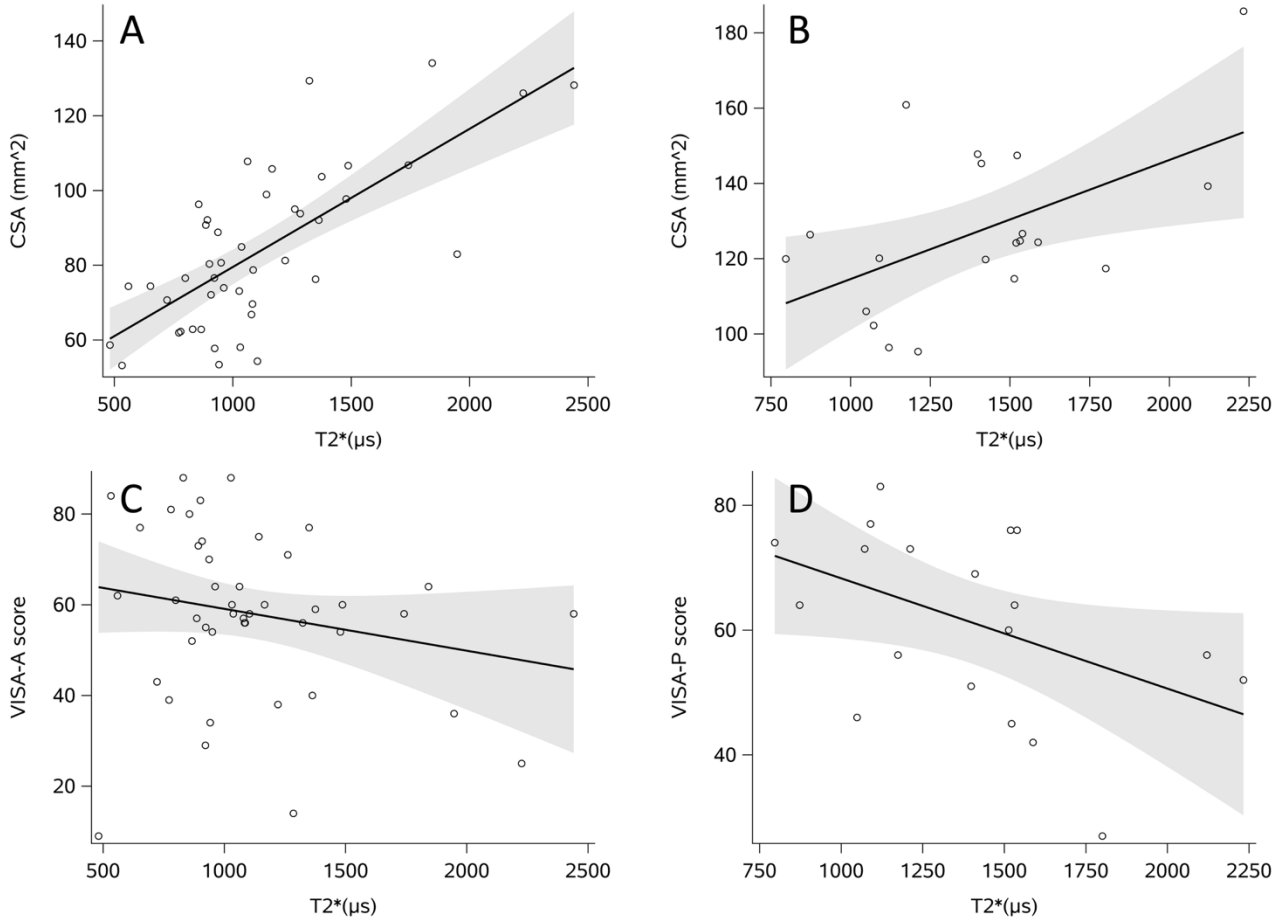

Figure 16 correlations with tendon size and clinical scores. Panel A: correlation between CSA and T2* within the Achilles group (Pearson's correlation coefficient (r)=0.72; p<0.0001).
Panel B: correlation between CSA and T2* within the Patellar group (r=0.53; p=0.02). Panel C: correlation between VISA-A and T2* (r=-0.2; p=0.17) Panel D: correlation between VISA-P and T2* (r=-0.5; P=0.0504).

Lastly both mechanical properties and UTE T2* scans were available in 18 patellar patients in total. There was a negative correlation between modulus and T2* (r=-0.51; p=0.03) (Figure 17). In a previous study by Bachmann et. al. stress was used instead of modulus, however max stress can clearly not be obtained by in vivo mechanical testing, since this would require pulling to failure. And thus, modulus was used instead.

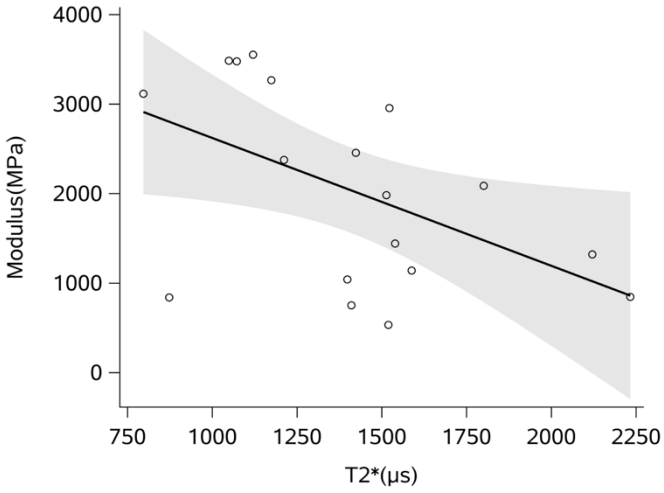

Figure 17 Correlation between mechanical properties (modulus) and T2* in patients with early phase patellar tendinopathy (n=18) (Pearson's correlation coefficient (r)=-0.5; p=0.03).

Discussion

In the current study we observed a significant difference in tendon T2* between patients with early phase tendinopathy and healthy controls. Estimated difference was ~20% between the patients and healthy controls. Which is considerably lower compared to studies comparing patients with chronic tendinopathy and healthy subjects in which two to four-fold differences have been observed [155,159]. However, this was expected since tendinopathic changes are most likely less pronounced at an early stage of disease. Nevertheless, we were able to detect a difference in T2* between patients with early tendinopathy and healthy controls, which supports its use as a sensitive tool to investigate subtle structural changes in tendons. Further, when we compare the results herein to studies using a similar setup our T2* values appears to be placed between healthy controls and patients with chronic tendinopathy, this observation will be further elaborated in the general discussion[152,159]. Since age differed between the patients and the healthy controls, we adjusted for age in our analyses of T2*, and we did indeed observe a main effect of age in our model. This indicates that tendon structure may be altered with increasing age. This is supported by recent findings, were a significant difference was observed between a young and old group of healthy subjects [176], and an increased macromolecular fraction was observed with age [175]. Although speculative, this may contribute to the increased risk of tendinopathy with increasing age.

To test whether the increase in unbound water measured with T2* could explain the variation in CSA in two patient groups we correlated T2* and tendon size. And herein, the correlations in the two patient groups showed an association between tendon size and T2*. Previous studies have

observed differences in tendon size between healthy and tendinopathic tendons [38], but whether this increase in size is caused by increases in ground substance, collagen or an accumulation of water is somewhat unexplored. Our data suggest that, at least in the early phase of tendinopathy, the increase in size was, to some extent, explained by an increase in unbound water. Since an association was present between the two, it was also tested whether detect differences between the two groups in tendon size. But in the corresponding age adjusted analyses we did not find a significant difference between the two groups in mean CSA (p=0.30), although mean differences were observed between the groups.

Also, we aimed to describe the association between T2* and clinical outcomes, which were assessed by two questionnaires; VISA-A -and VISA-P. And although a trend was observed in the patellar patients (p=0.0504) overall no clear association was present. This is in contrast to some [155,159] but not all studies in chronic Achilles tendinopathy [183]. However, the discrepancy between ours and previous findings may be described by differences in scanning protocols (number of TEs and TE range), but also in the patient population. Furthermore, in the study by Juras et. al. the Achilles tendon rupture score (ATRS) was used and not the VISA-A score, which might also contribute to the different observations between the two studies. Nevertheless, in the current study we did not observe a clear association, which indicates that other factors in the tendon tissue or in the central nervous system contribute to the clinical presentation.

Thus, we found a higher T2* in patients with early tendinopathy compared to healthy controls, and we suggest that the increase in size that is observed in tendinopathy, but not the changes in clinical outcomes, can to some extent be explained by an increase in T2*.

Additionally, we also sought to investigate whether these changes translated into functional properties of the tendon. To do this we correlated T2* with tendon modulus, which represents the intrinsic mechanical properties[7], and observed a negative correlation between the two. Thus, higher T2* lead to inferior mechanical properties. Although not directly comparable this finding is supported by a previous study in bovine tendon explants, in which an association between T2* and stress was observed [164].

It should however be acknowledged that the study had some limitations. The mono-exponential fitting algorithm that was used in the current study, might be an oversimplistic description of the individual voxel, since water exists in both unbound and bound states within the individual voxel, and even more than two pools exists in the tendon[171]. To account for the heterogeneity

within the individual voxels bi-exponential fitting algorithms have been used in several studies, and even more complex models that account for more than two pools have also been incorporated[153–155]. Bi-exponential fitting was not incorporated in the current study, since we considered the number of TEs insufficient and the range of TEs two small (0.07–1.57 ms). Nevertheless, the high r-values herein demonstrates that the mono-exponential fit might be appropriate for a population with early phase tendinopathy, but this may closest resemble the short component of bi-exponential model [153,155].

Further, we did not perform bilateral scans, and thus we did not have an internal control, in patients with unilateral symptoms. However, bilateral changes in patients with unilateral symptoms have been suggested by previous studies[152], and thus the asymptomatic side may not serve as an appropriate internal control.

From the observations herein we cannot conclude that there is a causal relationship changes in T2* and the development of tendinopathy, but it indicates that structural changes may occur very early in the disease process. However, whether this takes part in the initiation of tendinopathy or not remains elusive. Nevertheless, the apparent ability to detect subtle changes at an early stage of disease and the association with tendon size and mechanical properties makes UTE T2* MRI an interesting addition to the already extensive toolbox.

We did however not observe a clear relationship with symptom severity, which highlights the need for multiple approaches in tendinopathy research to fully understand the pathophysiology.

Paper III

Introduction

This paper aimed to investigate the additive effect of an initial short term NSAID treatment to a standard physical rehabilitation program including load management in patients with early phase Achilles tendinopathy. We use the same model for early tendinopathy that was used in paper II, which relies on clinical diagnosis. After all baseline measurements were performed. Patients received 1-week NSAID treatment or placebo treatment in conjunction with a marked reduction in activity, followed by a 12-week physical rehabilitation program. Follow-up was performed after 1 week, after 13 weeks and after 1 year. The primary outcome was changes in VISA-A score between 0-3 months. Secondary outcomes include VISA-A at other time points, NRS, physical activity (sessions and hours/week), US (vascularization and dimensions), conventional MRI and UTE T2* MRI. T2* was assessed using the same protocol that was used in paper I and II.

Results

In total 225 subjects were screened by phone 69 of them were included and randomized into two groups. Hereof 34 were allocated to the Naproxen group and 35 were allocated to the placebo group. 60 participants (Placebo (n=30) Naproxen (n=30)) completed the 3-month follow-up (primary end point) and 53 (Placebo (n=25) Naproxen (n=28)) completed the 1-year follow-up. Participant flow chart is provided in Figure 18 and baseline characteristics are presented in Table 8, which shows that no significant differences were observed between the two groups at baseline. Further the adherence to the initial drug intervention was similar in the two groups (Naproxen group: 92 %; Placebo group: 97 %) and no significant difference was present between the two groups (p=0.49). Likewise, adherence to the physical rehabilitation program was comparable in the two groups (Naproxen: 74 %; Placebo: 73 %) and no statistically significant difference was observed between the two (p=0.88). 70 % of the injuries were related to running which makes results most applicable in populations that to some extent incorporates running in their training. An almost identical proportion of the patients had symptoms in the dominant leg (51%) and the non-dominant leg, thus no preference was observed. Lastly 70 % had unilateral symptoms and no significant differences were observed between the two groups. Collectively the two groups were comparable at baseline, which forms the basis for further interpretation of the results.

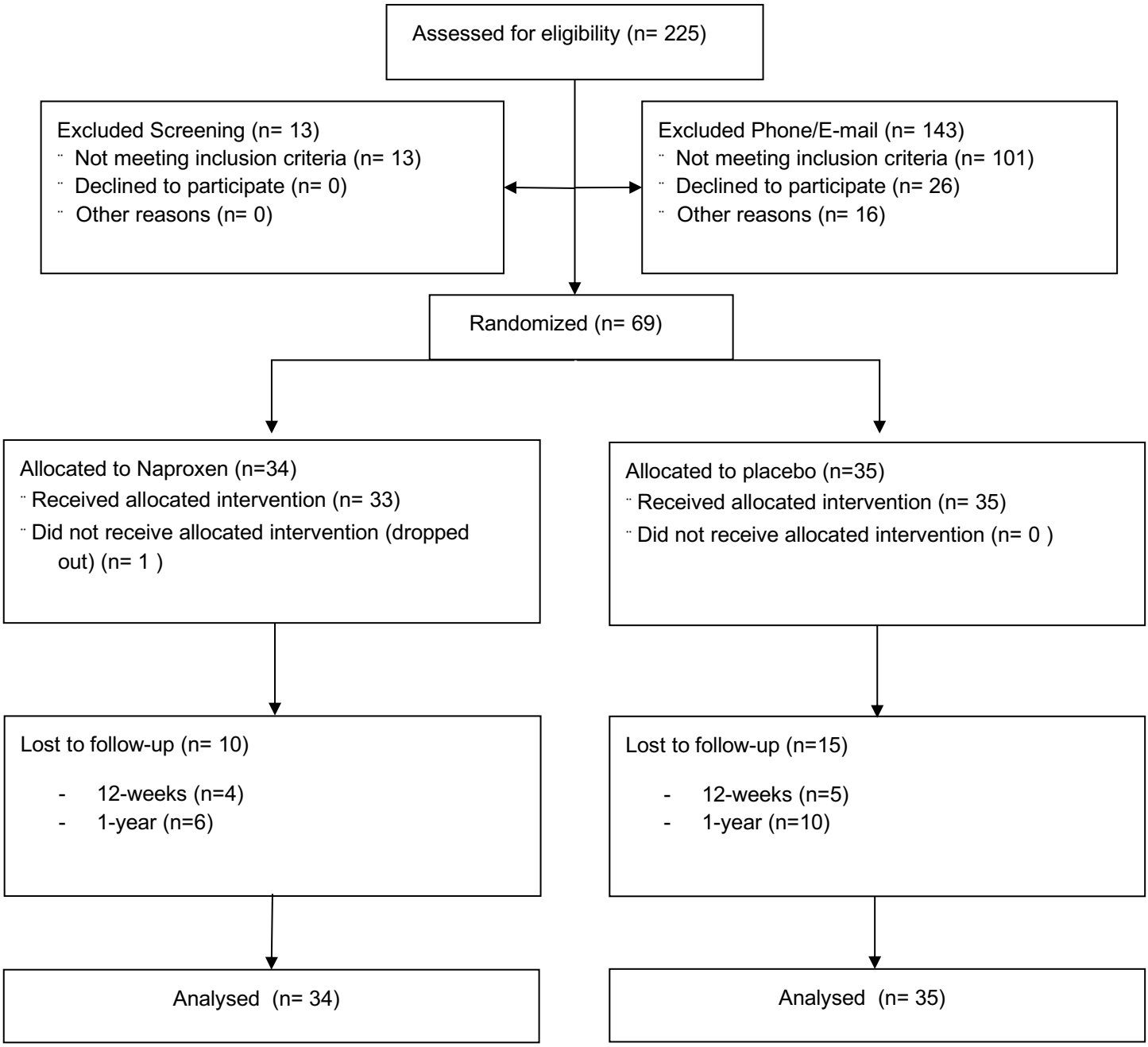

Figure 18 flowchart of participants

	Naproxen	Placebo	t-test
Age (y)	41 ± 2.1	40.7 ± 1.7	0.9
BMI (kg/m^2)	24.4 ± 0.5	25.1 ± 0.4	0.3
Duration (days)	43.4 ± 3.7	52.3 ± 3.5	0.1
Training (hrs/week)	7.4 ± 0.7	8.5 ± 0.9	0.3
NRS – during activity	3.8 ± 0.35	4.0 ± 0.3	0.7
Sex (%females) †	32.4	25.7	0.6

Table 8 Baseline characteristics. †=Fishers exact test.

Clinical outcomes and physical activity

Results from the questionnaires are presented in Table 9 and illustrated in Figure 19. We observed a significant increase in VISA-A score between baseline and 3-months follow-up in both groups (14.5 ± 2.8 $p<0.0001$) but no interaction was observed. Thus, both groups improved at a comparable rate during the intervention. Between 3-months and 1-year follow-up the VISA-A increased further (7.6 ± 3.3 $p<0.05$). No changes were observed between baseline and 1-week follow-up.

Additionally, we performed an exploratory analysis to see whether symptom duration at baseline affected the outcome after 3 months and 1 year. In order to do this the patients were divided into 3 groups, with duration of symptoms 0–1 month, 1–2 months and 2–3 months respectively. Herein we observed a significant interaction between symptom duration (0–1-month vs 2–3 months) and time at 3-months follow-up. Thus, VISA-A improved significantly more between baseline and 3-months follow-up in patients with symptom duration for 0–1 month compared to patients with symptom duration for 2–3 months (11.7 ± 4.2 $p<0.01$) (Figure 20). At 1-year follow-up no significant interaction was observed. Furthermore, we did also perform an analysis including symptom duration in days as a continuous variable, herein the same pattern was observed with a significant interaction between time and symptom duration (-0.20 ± 0.07 points/day; $p<0.01$) at 3 months. The size of these effects is considerable when compared to the overall increase in VISA-A of 14.5 points.

Data from the NRS questionnaire showed a similar pattern. Thus, between baseline and 3-months follow-up a significant decrease was observed (Figure 19 and Table 9), and at 1-year follow-up morning pain (-0.6 ± 0.3 $p<0.05$) and the induced pain test were further significantly reduced compared to 3 months (-1.0 ± 0.3 $p<0.05$), a numerical, yet not significant decrease was observed for all other NRS items at 1-year follow-up. At 1-week follow-up only NRS in the

morning and maximum pain during the last week were significantly decreased in both groups. No interactions were observed between the groups at any time point for any NRS item (Table 9). When compared to pre-injury levels, obtained from recall questionnaires at inclusion, the physical activity was significantly reduced (-2.7 ± 0.6 h/week) (Figure 19), and remained significantly lower at 3-months and 1-year follow-up compared to pre-injury levels. Furthermore, a significant decrease in overall activity level was observed between baseline and 1-week follow-up (-1.2 ± 0.3 h/week; $p<0.05$). This was however expected since the patients were instructed to decrease their physical activity during the first week of intervention.

		Preinjury	Baseline	1 week	13 weeks	52 weeks
VISA-A – score *, †	Naproxen	-	67.3 ± 2.1	72.1 ± 1.8	84.0 ± 2.3	88.7 ± 3.3
	Placebo	-	70.5 ± 2.4	72.0 ± 2.1	83.2 ± 2.4	90.6 ± 2.1
NRS-during activity *	Naproxen	-	3.8 ± 0.4	3.2 ± 0.4	1.4 ± 0.3	1.0 ± 0.4
	Placebo	-	4.0 ± 0.3	3.4 ± 0.4	1.8 ± 0.3	1.2 ± 0.3
NRS - at rest *	Naproxen	-	0.9 ± 0.2	0.6 ± 0.2	0.1 ± 0.1	0.4 ± 0.3
	Placebo	-	0.9 ± 0.2	0.8 ± 0.2	0.4 ± 0.2	0.0 ± 0.0
NRS – morning ‡, *, †	Naproxen	-	2.8 ± 0.4	1.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.2
	Placebo	-	3.2 ± 0.3	2.1 ± 0.3	1.2 ± 0.3	0.5 ± 0.2
NRS - max pain last week ‡, *	Naproxen	-	4.6 ± 0.4	3.2 ± 0.3	1.7 ± 0.3	1.5 ± 0.4
	Placebo	-	4.9 ± 0.4	3.6 ± 0.3	2.0 ± 0.4	1.1 ± 0.3
NRS - jump test *, †	Naproxen	-	3.5 ± 0.4	2.2 ± 0.3	0.6 ± 0.2	0.6 ± 0.3
	Placebo	-	3.3 ± 0.4	2.9 ± 0.4	1.3 ± 0.3	0.3 ± 0.1
Activity (sessions/week) #	Naproxen	5.4 ± 0.4	3.8 ± 0.4	2.8 ± 0.3	4.0 ± 0.6	3.9 ± 0.5
	Placebo	6.2 ± 0.6	4.0 ± 0.5	3.7 ± 0.6	3.6 ± 0.4	4.0 ± 0.5
Activity (hrs/week) ‡, #	Naproxen	7.4 ± 0.7	4.4 ± 0.6	3.3 ± 0.5	5.4 ± 0.8	4.8 ± 0.7
	Placebo	8.5 ± 0.9	6.0 ± 0.9	4.6 ± 0.7	5.1 ± 0.7	5.2 ± 0.9

Table 9 Result overview — Clinical outcomes and physical activity. Presented as mean values ± SEM. ‡ = significant time effect between baseline and 1 week. * = significant time effect between baseline and 13 weeks. † = significant time effect between 13 weeks and 1 year. # = significant time effect between pre-injury and baseline.

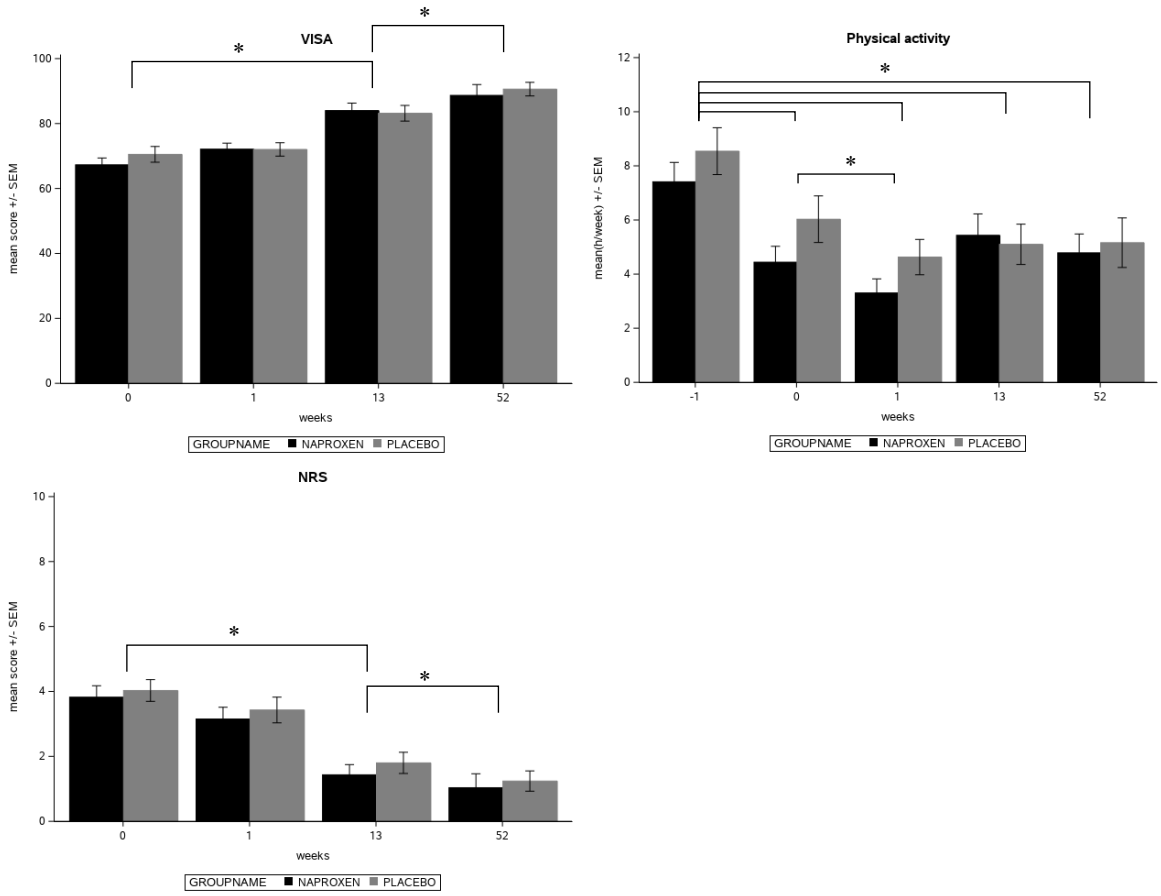

Figure 19 VISA-A, NRS during activity and weekly activity level by group. Time points as follows; Week -1: pre-injury, week 0: baseline, week 1: 1-week follow-up (last day of Naproxen treatment), week 13: 3-months follow-up (end of physical rehabilitation period), week 52: 1-year follow-up. * = significant time effect ($p<0.05$) between marked timepoints.

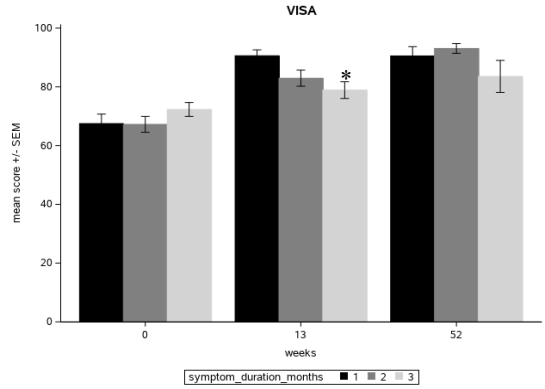

Figure 20 VISA-A score by duration of symptoms at baseline. Time points as follows; week 0: baseline, week 1: 1-week follow-up (last day of Naproxen treatment), week 13: 3-months follow-up (end of physical rehabilitation period), week 52: 1-year follow-up. * = significant interaction (reference weeks=0; symptom_duration_months=1)

Imaging

To characterise our patient population and to compare with a previous study using the same model of early tendinopathy[74] we tested whether the two sides differed at baseline in patients with unilateral symptoms (n=49). Herein, we observed that the symptomatic side was significantly thicker (0.12 ± 0.03 cm $p<0.0001$) and had a significantly larger Doppler area (0.3 ± 0.1 cm^2 $p<0.005$)) compared to the healthy side. Hereafter we proceeded to look at changes over time on the symptomatic side. No significant changes were observed between baseline and 3-month follow-up for neither Doppler area nor thickness (Table 10). MRI mean area showed no significant difference and were almost identical between the two time points in both groups (+0.01 ± 0.06 and 0.03 ± 0.05 cm^2, for naproxen and placebo respectively). Results are presented in Table 10 and illustrated in Figure 21.

In paper II we observed a difference in T2* between patients with early tendinopathy and healthy controls. But whether the method would be useful as a sensitive tool to track progress during a short-term intervention remained unanswered. Therefore, we attempted to apply the method in the current study. Unfortunately, due to technical issues, not all patients were scanned using the UTE MRI protocol. In total of 74 unilateral UTE T2* MRI scans were performed. Hereof 44 were baseline scans (Naproxen (n=19); Placebo (n=25)) and 30 were at 3-months follow-up scans (Naproxen (n=14); Placebo (n=16)). We did not observe a significant difference between baseline and 3-months follow-up. However, a numerical decrease was observed in the Naproxen group(ΔT2*: -286.4 (CI: -48.9–621.6) μs) which was not observed in the placebo group (ΔT2*: 0.7 (CI: -272.1-270.8) μs). Results are presented in Table 10 and illustrated in Figure 21.

		Preinjury	Baseline	1 week	13 weeks	52 weeks
USPD (cm^2)	Naproxen	-	0.43 ±	0.37 ±	0.48 ±	-
	Placebo	-	0.27 ±	0.26 ±	0.24 ±	-
Max Thickness (cm)	Naproxen	-	0.77 ±	0.74 ±	0.74 ±	-
	Placebo	-	0.70 ±	0.67 ±	0.69 ±	-
Thickness (cm)	Naproxen	-	0.67 ±	0.63 ±	0.62 ±	-
	Placebo	-	0.56 ±	0.55 ±	0.56 ±	-
MRI area mean (cm^2)	Naproxen	-	0.86 ±	-	0.87 ±	-
	Placebo	-	0.78 ±	-	0.81 ±	-
T2* (ms)	Naproxen	-	1.45 ±	-	1.17 ±	-
	Placebo	-	1.21 ±	-	1.21 ±	-

Table 10 Result overview — imaging. Presented as mean values ± SEM. ‡ = significant time effect between baseline and 1 week. * = significant time effect between baseline and 13 weeks. † = significant time effect between 13 weeks and 1 year. # = significant time effect between pre-injury and baseline.

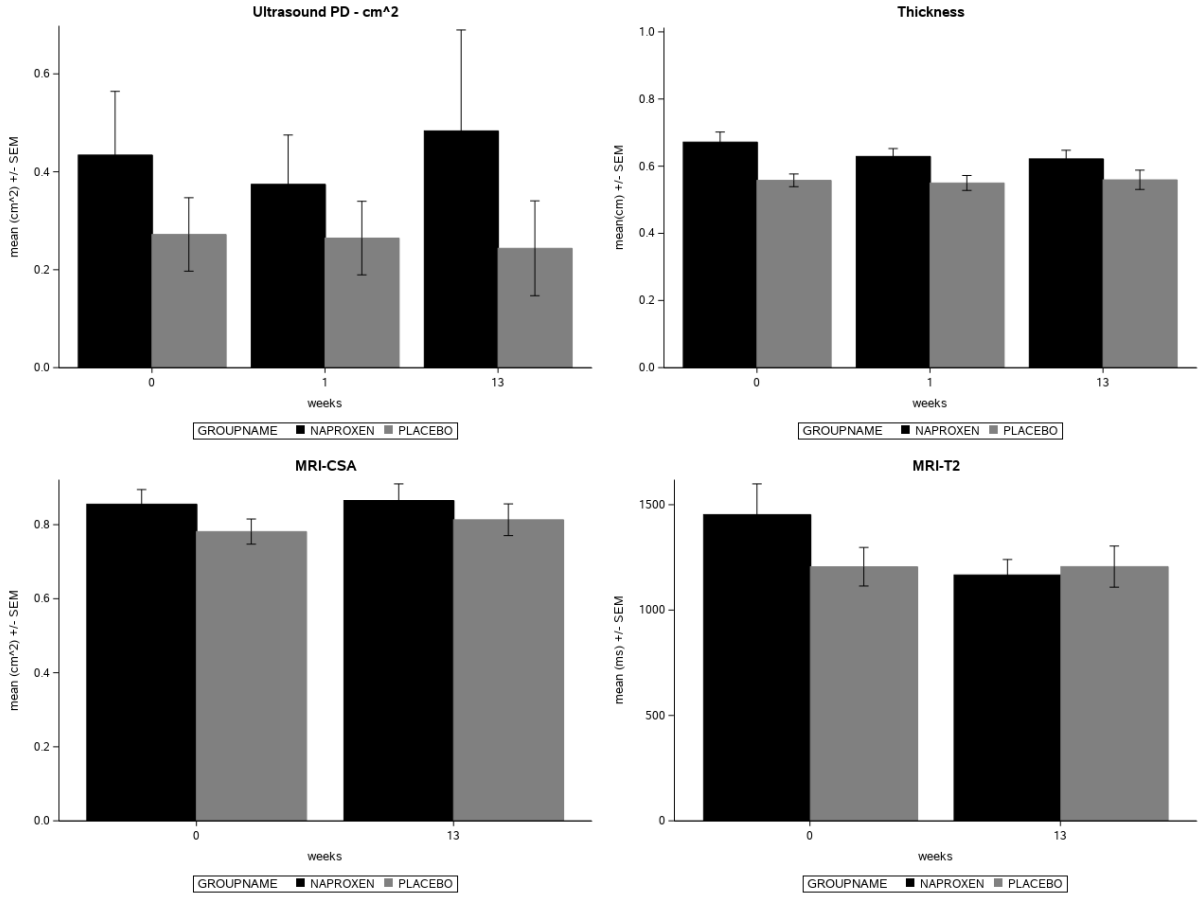

Figure 21 Power doppler ultrasound (US), US thickness, cross-sectional area (MRI) and T2* values by group. Time points as follows; week 0: baseline, week 1: 1-week follow-up (last day of Naproxen treatment), week 13: 3-months follow-up (end of physical rehabilitation period).

Discussion

The current study was designed to investigate the effect of an initial short-term NSAID treatment in early phase Achilles tendinopathy. This resembles common practice as NSAID is often prescribed in the early phase of tendinopathy by general practitioners. And since NSAID is available as over the counter medication the rate of self-administration is high. However, we were not able to observe an additive effect of NSAID, which is in agreement with previous studies that have investigated the clinical effect in more chronic tendinopathy [103,184]. Further, we did not observe an analgesic effect at 1-week follow-up. At this time point subjects consumed the last dose in the morning, and thus this finding was somewhat surprising. However, in a previous study on patients with chronic Achilles tendinopathy running induced pain was almost identical between a group who received NSAID and a group who received placebo[185]. And also no clear effect of 1-week NSAID was observed in another study on chronic tendinopathy[112]. Although we do acknowledge that we cannot separate the effect of the unloading in the initial week of treatment from the effect of NSAID this strongly indicates that the analgesic effect of NSAID in early tendinopathy is minor. In further support of this, patients that were in the active group were not able to guess that they received Naproxen (Table 11). Additionally, this suggests that the blinding of participants was successful.

	Group		Fisher exact test
	Naproxen	Placebo	p-value
Drug perception (true/total)	9/31	25/27	<0.0001

Table 11 The proportion of patients that were able to guess which group they were in. Almost all participants that were asked the question 47/58, guessed that they received the placebo treatment.

It has recently become increasingly evident that inflammation plays a role in tendinopathy[57,62–64,67], and it appears to more pronounced in early phase tendinopathy compared to more chronic disease [62–64]. Thus, an inhibition of inflammation seems reasonable at this stage of disease. Herein, we attempted to inhibit inflammation using an anti-inflammatory drug in order to attempt to affect the clinical outcome but did not see any effects of the medication. This may however be explained by several factors. First even though NSAID is considered an anti-inflammatory drug in vitro studies suggest that they can also inhibit anti-inflammatory cytokines [186,187] and thus potentially have pro-inflammatory effects.

Also, potentially desired responses to loading such as increased collagen and proteoglycan synthesis may be inhibited by NSAID[79,114]. Another explanation could be that NSAID simply never reached the tendon fibroblast. This has been indirectly studied in human Achilles tendon and rat tail tendon. In human Achilles tendons, mRNA results from tendon core biopsies revealed that genes that were normally affected treatment were not affected by 1-week NSAID treatment[112]. In rat tail tendon NSAID markedly reduced the accumulation of macrophages and neutrophils in a collagenase induced tendinopathy model in the peritendinous tissue, but no effects were observed in the tendon core [188]. In support of this the existence of a blood-tendon barrier have been suggested. A barrier impermeable to larger molecules, but still permitting passage of water[189]. This may partly explain the lack of effect.

We acknowledge that we did not include a control group that did not receive the physical rehabilitation intervention, and thus cannot conclude on effects of the physical rehabilitation. But we did observe a clear and clinical relevant improvement in symptoms (NRS>2 points)[190] and VISA-A (>10 points)[191] after 3 months and 1 year. This indicates that the physical rehabilitation may have a positive effect similar to the effect observed in chronic tendinopathy.[97] This is reinforced by the fact that early tendinopathy in many ways resembles a more chronic stage of disease and thus the effective treatments may be similar[74]. We did try to test this by correlating training adherence with clinical improvement but did not find a relationship between the two. This could indicate that the most important part of the intervention at an early stage of disease is the patient education in load management, the role of this in tendinopathy in general has however not been thoroughly investigated as highlighted in a recent review[84].

To explore whether the clinical outcome depended on the duration of symptoms at baseline we performed an exploratory analysis. Herein we observed that patients with symptom duration <1 month improved significantly more in VISA-A score between baseline and 3-months follow-up compared to patients with symptom duration >2 months. No significant difference was observed at baseline between the two. Additionally, we observed numerical differences in global assessment score. Specifically, patients were asked if they had experienced an overall improvement in symptoms. Herein, all patients with symptom duration <1 answered ‘yes’ whereas 6 patients with symptoms >2 month answered ‘no’. Thus, it may be suggested that patients benefit from intervention as early as possible. However, it could also be argued that the symptoms is less severe and more transient in the patients with symptom duration for less that 1 month. However, a very recent study including 100 runners with onset of symptoms within the last month found persisting symptoms in a substantial proportion after 1 year[192]. Therein, runners

were asked to fill in a questionnaire at a big running event, and the diagnosis of Achilles tendinopathy was based on this questionnaire. Hence, in contrast to the current study they did not all seek medical attention and may thus represent a less severe patient population. However, this remain speculative. Nevertheless, these results indicate that despite short symptom duration a large proportion will develop persisting symptoms.

All together across the groups we observed a clinical improvement, and thus wanted to investigate potential changes in physiological outcomes. However, we did not observe any changes on US or MRI. Changes in response to physical rehabilitation have been observed on tendon vascularisation and tendon dimension in chronic tendinopathy[95,193,194]. We could however not reproduce these results in the current study. This might be due to the less pronounced changes on US and MRI at baseline. Which could be speculated to make it harder to detect changes. To our knowledge UTE T2* MRI has not been used in longitudinal studies. But differences between healthy subjects and patients with early tendinopathy was observed in paper II, and thus we expected this method to be sensitive enough to detect the small changes that may occur in response to the intervention. But no significant changes were observed in the current study. This implies that clinical changes happen in the absence of structural changes, or at least structural changes happens at a slower rate than clinical improvements. However, it may also be speculated that the methods used herein to detect structural alterations may not be sensitive enough to detect changes over such a short time span.

Since there was an improvement in clinical symptoms, we also expected an increase in physical activity. But, the habitual level of physical activity remained constant between baseline and 1-year follow-up, and at a level that was significantly lower than before the injury. This finding is supported by the results of a 1-year follow-up study on runners with early phase Achilles tendinopathy. Therein physical activity was also affected after one year[192]. The reason for this mismatch between physical activity and clinical symptoms remains elusive, but it could be speculated that patients are overcautious when returning to sports. This phenomenon could be addressed by post-rehabilitation interventions, which were proposed to optimize return to sports[33]. Even though we did not approach this systematically in the current study patients were encouraged to return to prior sports. Nonetheless physical activity remained at a stable low level (compared to pre-injury levels) throughout the study period.

Collectively the main finding of the current study was, that we did not observe a significant effect of anti-inflammatory treatment on clinical outcomes in early phase Achilles tendinopathy. Surprisingly, neither was an analgesic effect observed, which questions the rationale behind NSAID treatment in early tendinopathy. Additionally, NSAIDs carry a number of potential side effects, which should be taken into consideration before use of these drugs[120]. We do acknowledge that the study was not designed to investigate the effect of our physical rehabilitation program. However, we did observe substantial improvements in clinical symptoms over time, which was dependent on the symptom duration at inclusion. Thus, we suggest that targeted interventions should be initiated as early as possible. Lastly clinical improvements occurred in the absence of structural changes. Which indicated that the clinical improvements do not depend on structural alterations, and vice versa clinical improvements does not necessarily mean that the tendon tissue has regain its normal healthy structure.

General discussion and conclusion

The current thesis includes three papers based on studies with different designs and separate populations. However, all three studies share common methods which enables comparisons across. A wide spectrum of tendons at various stages of disease has been presented herein — ranging from healthy to chronic tendinopathic tendons. The overall goal of characterizing early tendon disease is to invent and optimize treatment in order to avoid the more permanent damage which is observed in chronic tendinopathy. Herein, we attempted to make a detailed non-invasive characterization of early tendinopathic tendons using UTE T2* MRI. Further, on the basis of clinical experience and previous observations in early tendon disease, we investigated the effect of a short-term anti-inflammatory treatment in early phase Achilles tendinopathy. Which included an evaluation of the effect on tendon T2* relaxation.

The UTE T2* MRI setup we used in the studies is not an established clinically used method. Therefore, we sought to investigate the reproducibility of the method itself in order to guide the interpretation of the results obtained in subsequent studies. In paper I we found a small systematic difference between two scans, which was potentially caused by prolonged inactivity before the second scan. This finding emphasises the need for strict standardization of the pre-scanning regime. We cannot exclude that this might have affected the results in study II and III. However, at least in study II the magnitude of the differences we observe exceeds the magnitude of the bias we observed in study I, which indicates that changes should still be detectable. A study on the reproducibility in early tendinopathy would off course have been preferred, but since voxels with poor fit and very long T2* were excluded in paper I we concern the two comparable. If anything, we would expect even better reproducibility in patients with early tendinopathy. These findings form the basis of subsequent interpretation.

As the title emphasises one purpose of the thesis was to characterize early tendinopathy, this was pursued by performing T2* mapping in patients with early tendinopathy and healthy controls. In paper II we observed a significant difference between the two groups and concludes that UTE T2* mapping can detect subtle changes in early tendinopathy. Differences between areas with different disease severity within the same tendon has been observed previously [159]. However, it remained elusive from paper II whether our group of patients with early tendinopathy was a separate population between healthy tendon and chronic tendinopathic tendons. In an attempt to answer this question, data from paper I and II was combined in order to see the difference between the 3 groups. Specifically, data from healthy and early tendinopathy patellar tendons was obtained from paper II and data from chronic

tendinopathic patellar tendons was obtained from paper I. By combining the two not only could we confirm the difference between healthy and early tendinopathic tendons, but we did also observe a significantly higher T2* in chronic compared to early tendinopathic patellar tendons (Figure 22). Thus, it appears that our population with early tendinopathy can be distinguished from patients with chronic tendinopathy by UTE T2*. Whereas the clinical scores (VISA-A and VISA-P) are comparable to the values from previous studies on chronic tendinopathy [97,98]. Thus, it could be speculated that UTE T2* could help predict the outcome in patient with otherwise similar clinical presentations. This does however remain elusive.

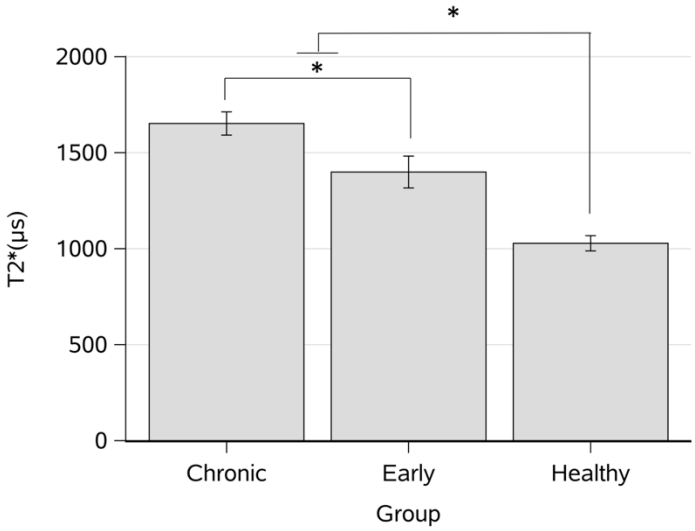

Figure 22 When data from paper I and II was combined a significant difference in T2* was observed between the two patients' groups and the healthy control group in patellar tendons was observed (Chronic vs healthy p<0.000, estimate=624 µs1; Early vs healthy p=0.0020, estimate=371 µs). Also, a significant difference was observed between the early and the chronic group (p=0.01, estimate=253 µs)

Since T2* differs between early and chronic tendinopathic tendons, whereas symptom severity appears to be somewhat similar, it was not surprising that T2* was not associated with clinical symptoms in paper II. This finding was also confirmed by correlating T2* with VISA-A in study III, which showed no association between the two. Thus, it might seem that the patient is able to sense an alteration in the tendon tissue, but the extent is not reflected by the symptom severity. This might also partly explain the relatively high recurrence rate of tendinopathy[195]. Assuming that improvements in symptoms does not necessarily lead to structural changes, the tendon might be more susceptible to re-injury despite clinical improvements. In paper III we attempted to correlate clinical changes with baseline T2* but did not find any association between the two. Thus, there is no indication in our data that T2* is able to predict the clinical outcome, which is not surprising considering the lack of relationship between baseline T2* and clinical outcomes.

The absence of relationship between VISA questionnaires and T2* may however, to some extent, be explained by shortcomings of the questionnaires. Hence the validity of those have been questioned [131]. Meanwhile more simple measures like the NRS which is easy interpretable may be more appropriate. But NRS may be highly affected by changes in physical activity. Thus, if a decrease in NRS is observed in a patient who decrease the physical activity markedly it might not be an actual change in the severity of tendinopathy, but solely a change in physical activity that drives the change.

In order to explore whether the decrease of NRS was driven by a decrease in physical activity a composite score was calculated (NRS/weekly physical activity) in the data from paper III, thus pain was expressed relative to physical activity. This score could be considered a simple easy interpretable alternative to the VISA-A score that includes both symptoms and physical activity level. This did not change the overall conclusion of the study, but it may be a more appropriate outcome to report than the NRS score. However, it would probably only be useful in sports active populations and the validity of such a score is obviously unknown.

In the combined analyses of data from paper I and II we observed a significant difference between early and chronic phase tendinopathy. Further previous studies found differences in inflammatory activity between early phase and chronic tendinopathies [62–64]. Thus, even though previous studies have failed to show an effect of NSAID in chronic tendinopathy, we hypothesized that it could have an additive effect in a distinctive population of patients with early phase tendinopathy. Which would also support the widespread use of NSAIDs. However, we did not observe any detectable clinical or physiological effect of NSAID in early phase tendinopathy, and even short-term analgesic effects were absent. Contrary we suggest that the physical rehabilitation program may be effective and should be employed as soon as possible after symptom onset. Although we do acknowledge that we did not include a non-training control group and thus cannot conclude on the isolated effect of the physical rehabilitation program. We do not know the natural course of disease in this particular group of patients, and whether it differs from the course of disease in our patient group in paper I. This is probably also the greatest Achilles heel of our early tendinopathy model, since we do not know if a patient will develop chronic tendinopathy if left untreated. However, our findings in patients with early tendinopathy are similar to findings in patients with chronic tendinopathy although less pronounced [74]. And the tendon fibroblasts appears to be less heavily affected than it has previously been observed in chronic stage disease[196]. Thus, there might be a window of

opportunity in regard to treatment in patients with early phase tendinopathy defined by the duration of symptoms.

Collectively the results presented in the current thesis indicates that UTE T2* mapping is a reproducible and sensitive technique that can detect subtle changes in the early phase of tendinopathy and distinguish between various stages of disease. Furthermore, it appears likely that patients with early phase tendinopathy benefits from a standard physical rehabilitation program including load management, and the rate of improvement is related to duration of symptoms at baseline. The clinical improvement that was observed after 3 months happened in the absence of detectable structural changes. Lastly, NSAID did not have any clinical or physiological additive effect on early phase tendinopathy, and thus careful consideration is stressed before use of these drugs.

Perspectives

In paper II we observed differences in T2* between patients with early phase tendinopathy and healthy controls. However, in paper III there were no changes in T2* despite clinical improvements. It could however be speculated that structural changes halts after clinical changes, and therefore spacing between baseline and follow-up was below the detection limit. It would be interesting to perform long-term follow-up studies in order to detect the structural changes, that would be expected to happen at a slow pace. Since structural changes seems to persist even after clinical improvements, in my view, it would be an important part of such studies to track the reoccurrence rate of tendinopathy.

Further, even though UTE T2* MRI has gained momentum as a sensitive method to describe structural changes in tendon tissue, there would at some point have to be made strict comparisons with US and conventional MRI, in order to uncover the differences that most likely exists. However, before such studies can be made the UTE scan protocols and also post processing algorithms should be further optimized and maybe even tailored to different disease stages. First, we will have to consider whether other parameters from the scans could be more useful to distinguish between patient groups. In the data from paper I and II, I have attempted to define an upper limit for healthy tendon T2* (1500 µs). This allow for extraction of voxels with T2* above 1500 µs which is considered diseased in this case. In Figure 23 the relative values of this volume (volume with T2*>1500 µs/total volume) is plotted. This measure might be even better at separating healthy from diseased tendons.

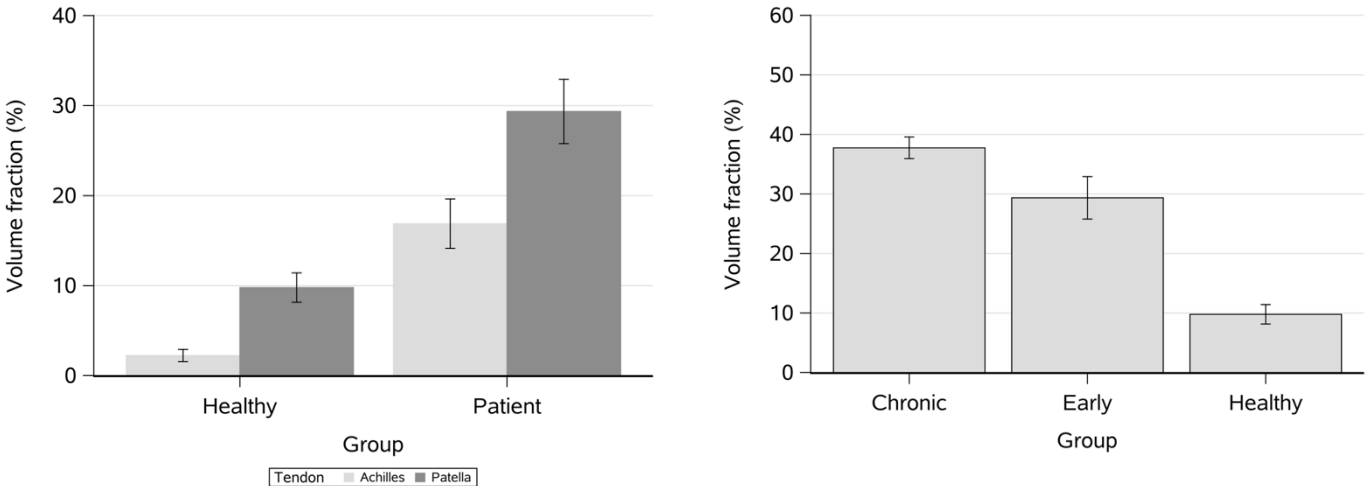

Figure 23 data from paper I and II expressed as volume fractions (volume of voxels with T2*>1500/total volume). Alternative to presentation of T2* values.

Furthermore, adding more TEs and especially including longer TEs would allow for a more comprehensive description of the tendon. As observed in previous studies this allows bi-exponential fitting algorithms to be performed [153,155], which might describe the tissue in a more comprehensive way. However, this will inevitably also increase scan times which makes the scanning less available in a clinical setting and increases the risk of movement artefacts. This can however possibly be avoided by the use of scanners with higher field strength[197]. Further, since no gold standard remains to describe tendon tissue there would still be a need to refer the results to other presumably even more sensitive methods such as tendon tissue biopsies. Therefore, we have initiated a new study in cooperation with Danish Research Centre for Magnetic Resonance (DRCMR), herein we are comparing data from MRI scans performed in a 7T scanner at DRCMR with scans performed on 3T and lastly with percutaneous patellar tendon biopsies. Furthermore, we include both healthy subjects, patients with early phase patellar tendinopathy and patients with chronic patellar tendinopathy, in order to directly compare these.

Hopefully such detailed studies at various stages of disease will advance our understanding of the pathogenesis in tendinopathy and aid future more targeted treatment. In paper III we did not find any effects of a common member of one of the most frequently used drug groups in the world (NSAIDs). We did however observe indices that patients may benefit from early targeted intervention, but studies designed to investigate this would have to be performed before any firm conclusions can be made. One approach would be to randomize patients into two groups; one groups that receives immediate treatment and one that waits for a fixed amount of time before the same intervention is initiated. This would be similar to the approach used for muscle strain injuries where an effect of early intervention has been observed[198]. However, in tendinopathy the effect of physical rehabilitation is well established and a recent review underscores that an active intervention is better than a wait-and see approach[84]. Thus, the ethical aspects of such studies could be discussed. In the development of new drug interventions in early tendinopathy a detailed description of the pathogeneses will be key, however cross-sectional studies will always struggle to find causal relationships and large cohort studies of healthy subjects would require a monstrous number of participants to be included to generate enough cases. Thus, translational research implementing detailed in vitro models that closely mimics in vivo conditions[199], will likely help reveal the pathogenesis of tendinopathy, which will aid the development of new treatments in early phase tendinopathy.

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Papers

Paper I:

Title: UTE T2* mapping of tendinopathic patellar tendons: an MRI reproducibility study

Status: Published in Acta Radiologica

Paper II

Title: Magnetic resonance T2* is increased in patients with early Achilles and Patellar tendinopathy

Status: Manuscript — not submitted

Paper III

Title: No additive clinical or physiological effect of short-term anti-inflammatory treatment to physical rehabilitation in the early phase of human Achilles tendinopathy: a randomized controlled trial

Status: Manuscript — submitted to American Journal of Sports Medicine (under review)

Paper I

Original Article

UTE T2* mapping of tendinopathic patellar tendons: an MRI reproducibility study

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Abstract

Background: There is currently a lack of imaging modalities that can be used as a sensitive measure in tendinopathy. Recent findings suggest the applicability of ultra-short echo time (UTE) magnetic resonance imaging (MRI) T2* mapping in tendons, but the reproducibility remains unknown.

Purpose: To evaluate test–retest reproducibility of UTE MRI T2* mapping of tendinopathic patellar tendons and to evaluate the intra- and inter-observer reproducibility of the measurement.

Material and Methods: Fifteen patients with chronic patellar tendinopathy were evaluated with UTE MRI twice in a 3.0-T scanner on the same day. Manual segmentation of the patellar tendon was performed by two blinded investigators and automated T2*map reconstruction was performed in custom-made software.

Results: There was a significant and numerically small difference in test–retest T2* values ($T2^*mean_{diff} = 0.06 \pm 0.07$ ms $\approx$ 3.7%; $P = 0.006$) with an ICC = 0.91 (95% confidence interval [CI] 0.58–0.98; typical error of 3.0%). The intra- and inter-observer reproducibility showed no significant bias ($P = 0.493$ and $P = 0.052$), and generally substantial reproducibility was demonstrated for T2* (intra-observer ICC = 0.99; 95% CI 0.98–1.00 and inter-observer ICC = 0.99; 95% CI 0.96–1.00, and typical error 1.3% and 1.3%, respectively).

Conclusion: These data demonstrate a small bias between repeated measurements for UTE T2*, but with a very low associated mean difference (3.7%) between the two tests. The high ICC values and low typical error % demonstrate reproducibility of repeated T2*-mapping sessions. Further, the method showed substantial intra- and inter-observer reproducibility for T2* values proving feasibility for use of UTE T2* mapping in research and clinical practice.

Keywords
Patellar tendon, tendinopathy, ultra-short echo time imaging, T2* mapping, reproducibility

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Introduction

Tendinopathy is a clinical condition affecting a large proportion of sports-active individuals, and characterized by symptoms include pain, swelling and morning stiffness which often leads to long lasting impaired performance (1). There is currently a lack of imaging modalities that can be used for early detection and objective monitoring of tendinopathy (2). Despite being a clinical diagnosis, imaging is often used to exclude differential diagnoses and to confirm diagnosis. The common modalities include magnetic resonance imaging (MRI) and ultrasound, and

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although both can be used to visualize the tendon, they cannot predict clinical outcomes and prognosis or provide detailed structural information (3,4).

Currently used clinical MRI protocols provide high-resolution multiplanar images suited for measuring tendon dimensions. However, the inherent properties of tendon tissue, with its abundant short T2 species, make clinical protocols with relatively long echo times (TE) insensitive to subtle structural changes that may take place within the tendon before macroscopic structural changes detectable by conventional MRI such as tendon thickening and hyperintensity on fluid-sensitive sequences. These severe tendinopathic changes are visualized at a late disease stage when treatment is often quite challenging and no quantitative information on disease severity can be obtained on standard MRI sequences (5). Quantitative mapping techniques, e.g. T2 mapping, have proven useful in structural assessment of connective tissue such as cartilage (6) and are increasingly implemented in clinical MRI systems, but scanning sequences with echo times (TE) of 8–20 ms are poorly suited for detailed assessment of tendon tissue, which has T2-relaxation times of 1–2 ms. Therefore, ultra-short echo time (UTE) protocols with TE $\leq$ 1 ms have been developed, which make it possible to obtain sufficient signal in tendons for quantitative purposes, and thus describe the tissue before severe pathological alterations (7). UTE-mapping techniques include T2* analysis, and it has been employed in tendons with encouraging results (8). Interestingly, UTE-T2* mapping has been reported to relate to clinical outcomes (9) and free water content secondary to changes in tendon proteoglycan abundance and collagen disruption in tendinopathy (9–11). However, in order for UTE-T2* to serve as a useful tool for monitoring of tendinopathy, the reproducibility of the imaging modality has to be established, which has never been investigated. The purpose of this study was to evaluate the test–retest reproducibility, and the inter- and intra-observer reproducibility of MRI UTE T2* mapping in tendinopathic human patellar tendon.

Material and Methods

Study design

The present study was designed as an observational reproducibility study. Study reporting is in accordance with the Guidelines for Reporting Reproducibility and Agreement studies GRRAS (12). Before the actual study commenced, a standardized protocol for the evaluation was developed and rehearsed in consensus between observers. Two MRI UTE T2* recordings (S1 and S2) were performed, and recordings were subsequently evaluated the first time (M1) by two observers (O1 and O2). To investigate the test–retest reproducibility, evaluation of the repeated MRI recordings (S1 and S2) were performed by one observer (O1). To investigate inter-observer reproducibility, recording S1 was evaluated by observer O1 and O2, respectively. Additionally, to investigate intra-observer reproducibility O1 conducted a re-evaluation of S1 after two weeks (M2).

Observers

The two observers performing the tendon segmentation (AA and NMM) both had prior experience in tendon and muscle segmentation on MRI images. An experienced musculoskeletal radiologist with particular expertise in tendon evaluation (PH) supervised the preparation and training phase. All MRI recordings were anonymized and randomized before evaluation. All segmentations were performed in a fully blinded fashion and no communication between the observers was allowed during the study phase.

Participants

The study group comprised 15 male athletes with chronic ($>$ 3 months) patellar tendinopathy (mean age = 31 $\pm$ 4.9 years, body mass index [BMI] = 25.6 $\pm$ 2.0 kg/m^2, tendinopathy duration = 7.9 $\pm$ 2.6 months). The clinical diagnosis was confirmed by ultrasonography in the form of tendon swelling and hypoechoic appearance with pathological power doppler activity within the tendon. The exclusion criteria were patellar tendinopathy $>$12 months, previous knee surgery, confounding diagnoses to the knee joint, diabetes or arthritis, previous corticosteroid injection for patellar tendinopathy, and smoking. Due to the lack of existing data on T2* values in tendinopathic patellar tendons, the sample size was based on feasibility. The patients were consecutively recruited from a lager ongoing training study registered at ClinicalTrials.gov (ID: NCT03096067) investigating treatment of patellar tendinopathy and the influence of load magnitude on clinical outcome, tendon structure, and function. The 15 consecutively enrolled patients were asked to undergo an additional MRI scan identical to the one planned in the main project; all scans were obtained before intervention. All individuals gave written informed consent to participate in the study and ethical approval was obtained from the Regional Scientific Ethics Committee (H-15017806).

MRI procedure

All MRI recordings were obtained between October 2017 and June 2018 by three experienced MRI technicians. Two consecutive recordings (S1 and S2) were performed on the same day by the same technician separated by an interval of 45 min. During the interval,

participants were seated in the waiting room until next scanning. Only the tendinopathic patellar tendon was examined, and in patients with bilateral symptoms, the side with the most severe symptoms was chosen. All participants were instructed to abstain from physical activity 24 h before the examination. All MRI scans were performed in a Siemens Verio® (Siemens, Erlangen, Germany) 3-T scanner. The participants were scanned in a supine position using a dedicated 15-channel send/receive knee coil. The exact same positioning of the knee was obtained by using the scanner laser guides and anatomical landmarks. This ensured identical positioning of each slide between S1 and S2. The following MRI protocol was used: gradient echo (GRE) scout, slice thickness (ST) = 6 mm; field of view (FOV) = 280 × 280 mm; echo time (TE) = 3.67 ms; repetition time (TR) = 7.7 ms; scan time = 29 s; flip angle (FA) = 20°; transversal UTE T2* sequence. A slab of 160 slices was scanned four times with a varying TE: 0.07 ms; 0.57 ms; 1.07 ms; and 1.57 ms (Fig. 1) (FOV = 160 × 160 mm, matrix resolution = 1.45 × 1.45 × 1.0 mm, TR = 11 ms, FA = 12°, scan time = 3 m 14 s). The center of the FOV was fixed to the isocenter to avoid field inhomogeneity issues.

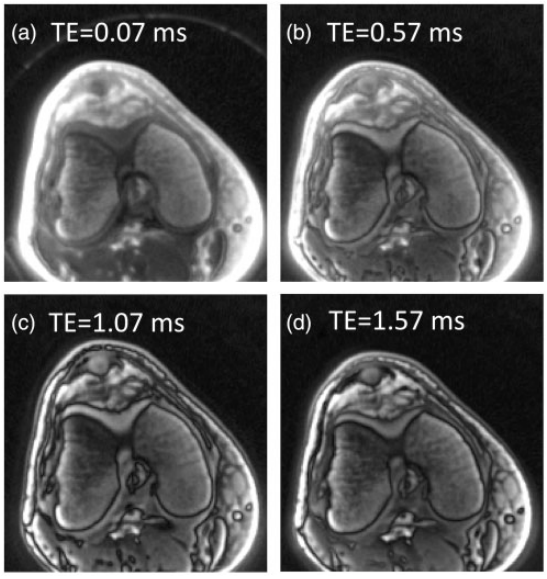

Fig. 1. Representative UTE MRI images in the mid-tendon, with increasing TE (a–d) (0.57–1.57 ms) and decreasing signal intensity in the tendon.

MRI analysis

Reconstruction of T2 maps.* DICOM files from the UTE recordings were automatically loaded into a custom-made software developed by X-Rai (X-Rai IVS, Copenhagen, Denmark). The software was built around a MatLab algorithm derived from Dr. Petros Martirosian, Section on Experimental Radiology, University Hospital, Tübingen, Germany. TE was plotted against the signal intensity on a voxel-by-voxel basis for the whole volume. Mono-exponential fitting incorporating noise correction and using the Levenberg–Marquardt method, was performed to reconstruct T2* maps. The following equation was used (13): $S = S_0 \cdot e^{\frac{-TE}{T2}} + C$

where S is intensity values from the recordings, TE is the corresponding echo times, and S_0, T2, and c were the parameters to be fitted. A lower bound of 0 ms was applied to all parameters. From the fitting procedure T2* maps containing T2* values for each voxel (Fig. 2a) and goodness-of-fit maps (Fig. 2b) containing r-values for each voxel were reconstructed.

ITK-SNAP segmentation

The open source software ITK-SNAP version 3.6.0 for MAC OS (http://www.itksnap.org) was used for segmentation of the patellar tendon volume used for T2* analysis. The segmentation was performed on the sequence with the longest TE (TE = 1.57 ms) (Fig. 1d). The patellar tendon volume was segmented by manually outlining the tendon in the axial plane of every fourth slice, using the polygon tool in ITK-SNAP (polygon segment length = 8). A conservative approach was purposely applied in selecting the tendon outline to avoid including peritendinous tissue. The starting slice was defined as the first proximal slide without the patellar bone visible (to avoid any effects of partial volume phenomena) and the final slice was defined as the first slice where the corpus Hoffa fat pad deep to the tendon was no longer visible. All images were identically contrast calibrated (linear contrast range 0–2000) before segmentation. After manual segmentation the tendon volume was calculated using the interpolate labels tool in ITK-SNAP (Fig. 3).

T2* fitting analysis

The tendon volume segmentation was exported from ITK-SNAP in the ".nii.gz" format and imported to FIJI/ImageJ (version 1.52, National Institutes of Health, Bethesda, MD, USA) for quantitative analyses. A macro was set up to extract data from the T2* and goodness of fit maps within the tendon segmentation, using the particle analysis function. Mean values of T2* were determined in the total tendon volume as well as the proximal and distal half of the tendon. In the goodness of fit map, an area in the proximal mid part of the tendon was consistently observed to have a poor fit to the curve, potentially introducing a source of error. To account for those voxels with poor exponential fits, mean value was also calculated solely in voxels with goodness of fit > 0.8 subsequently denoted corrected (cor) values. The reported outcomes are the T2* mean (ms), (including all voxels within the

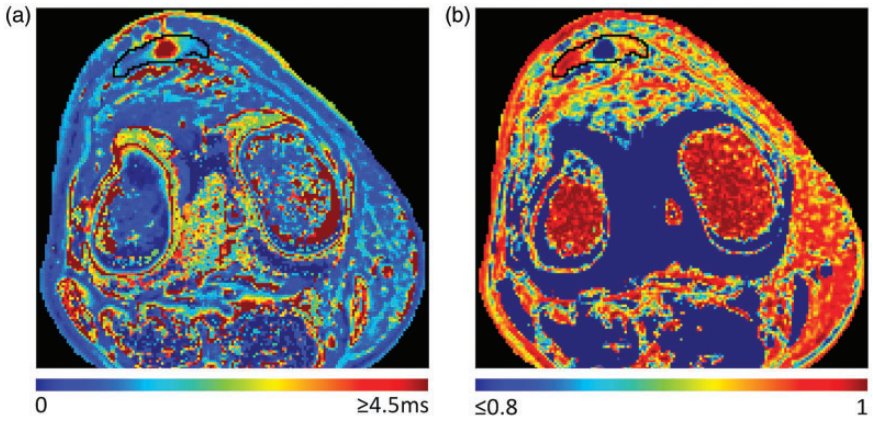

Fig. 2. (a) Representative T2* map, with scale bar values ≥4.5 ms in red, black line indicates tendon outline. (b) Corresponding goodness of fit map (Pearson correlation r-values) with scale bar values ≤0.8 in blue; black line indicates tendon outline.

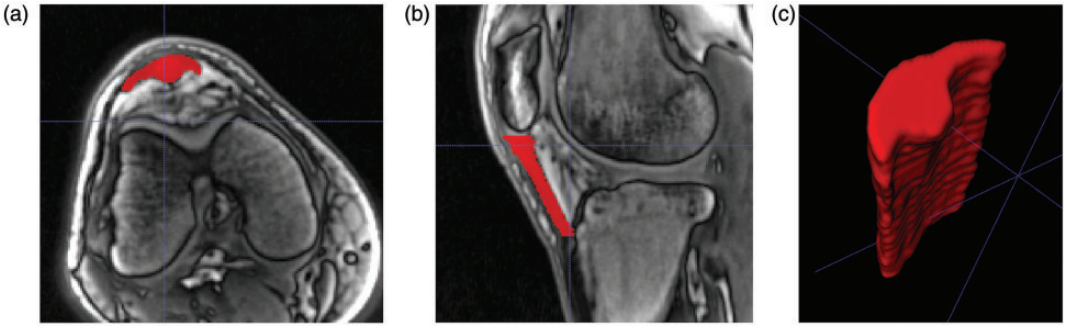

Fig. 3. (a) Representative axial mid-tendon section (TE = 1.57 ms), with tendon segmentation overlaid. (b) Sagittal view (c) 3D model for visual inspection of irregularities after interpolating segmentation.

segmentation), $T2^*_{cor}$ mean (ms) (only including voxels in the segmentation with $r > 0.8$), volume of the segmentation (mm^3), and $volume_{cor}$ (mm^3) (only including voxels in the segmentation with $r > 0.8$). Representative plot for T2* mean and $T2^*_{cor}$ mean is shown in Fig. 4.

Statistical analysis

The statistical analysis was carried out in Excel 2018 (Microsoft® Corporation, Redmond, WA, USA) and SPSS (IBM®, Version 23, 64-bit edition). To assess bias between repeated analyses (test–retest, intra- and inter-observer reproducibility), Student's paired t-test were used. An alpha level of $P \leq 0.05$ was considered significant. Intraclass correlation coefficient (ICC) was calculated with 95% confidence intervals (CI) to evaluate reliability. For intra-observer reliability, ICC model 3.1 (two-way mixed model, consistency type) was used. For inter-observer and test–retest, ICC model 2.1 (two-way random model, absolute agreement type) was used. Additionally, typical error percentages were used as a measure of the relative measurement error. Typical error percentages were calculated as $\frac{SD_{diff}}{\sqrt{2}\cdot\bar{x}} \cdot 100$ (14). All descriptive data are presented as mean ± SD.

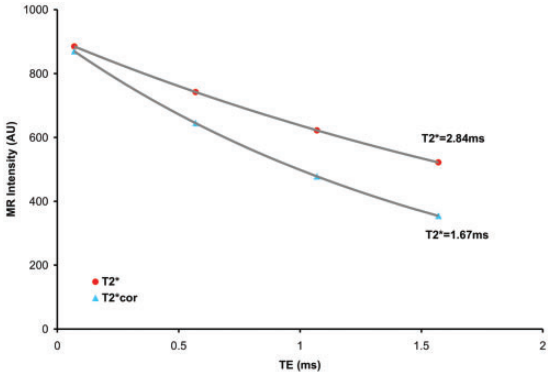

Fig. 4. Representative plot from the mono-exponential fitting procedure made in all voxels for the whole volume. T2*: all voxels; $T2^*_{cor}$: voxels with $r > 0.8$.

Results

The mean values and differences of T2* (ms), $T2^*_{cor}$ (ms), tendon volume (mm^3), and $tendon\ volume_{cor}$ (mm^3) for the proximal, distal, and total patellar tendon, are shown in Tables 1–3.

Table 1. Test–retest reproducibility.

Tendon part	S1 M1 O1	S2 M1 O1	Diff	*P*	TE %*	ICC (95% CI)
Proximal						
T2* (ms)	2.92 ± 1.23	3.13 ± 1.21	0.21 ± 0.25	0.007	6.0	0.97 (0.81–0.99)
$T2^*_{cor}$ (ms)	1.61 ± 0.28	1.65 ± 0.27	0.04 ± 0.07	0.046	3.0	0.96 (0.87–0.99)
Volume (mm^3)	3193 ± 1153	3218 ± 1086	26 ± 209	0.639	4.6	0.98 (0.95–0.99)
$Volume_{cor}$ (mm^3)	2503 ± 696	2422 ± 692	81 ± 229	0.192	6.6	0.94 (0.84–0.98)
Distal						
T2* (ms)	2.65 ± 1.04	2.96 ± 1.05	0.30 ± 0.35	0.005	8.9	0.91 (0.55–0.97)
$T2^*_{cor}$ (ms)	1.75 ± 0.24	1.84 ± 0.20	0.09 ± 0.11	0.008	4.6	0.80 (0.33–0.94)
Volume (mm^3)	2600 ± 1056	2570 ± 966	30 ± 268	0.669	7.3	0.97 (0.90–0.99)
$Volume_{cor}$ (mm^3)	2104 ± 696	1956 ± 587	148 ± 298	0.076	10.4	0.88 (0.65–0.96)
Total						
T2* (ms)	2.84 ± 0.97	3.09 ± 0.94	0.25 ± 0.27	0.003	6.4	0.93 (0.58–0.98)
$T2^*_{cor}$ (ms)	1.67 ± 0.23	1.73 ± 0.21	0.06 ± 0.07	0.006	3.0	0.91 (0.58–0.98)
Volume (mm^3)	5793 ± 2160	5788 ± 2015	4 ± 420	0.969	5.1	0.98 (0.95–0.99)
$Volume_{cor}$ (mm^3)	4605 ± 1370	4376 ± 1260	228 ± 473	0.083	7.5	0.93 (0.78–0.98)

Values are given as mean ± SD.
*Typical error percentage.
CI, confidence interval; Cor, corrected; Diff, difference between the two measurements (mean ± SD); ICC, interclass coefficient; M1, measurement 1; O1, observer 1; S1/2, scanning 1 and 2.

Table 2. Intra-observer reproducibility.

Tendon part	S1 M1 O1	S1 M2 O1	Diff	*P*	TE %*	ICC (95% CI)
Proximal						
T2* (ms)	2.92 ± 1.23	2.91 ± 1.24	0.01 ± 0.13	0.727	3.1	1.00 (0.94–1.00)
$T2^*_{cor}$ (ms)	1.61 ± 0.28	1.61 ± 0.28	0.00 ± 0.03	0.993	1.2	1.00 (0.99–1.00)
Volume (mm^3)	3193 ± 1153	3255 ± 1137	62 ± 198	0.246	4.3	0.99 (0.96–1.00)
$Volume_{cor}$ (mm^3)	2503 ± 696	2561 ± 668	58 ± 201	0.283	5.6	0.96 (0.88–0.99)
Distal						
T2* (ms)	2.65 ± 1.04	2.67 ± 1.08	0.01 ± 0.24	0.836	6.4	0.98 (0.93–0.99)
$T2^*_{cor}$ (ms)	1.75 ± 0.24	1.76 ± 0.26	0.01 ± 0.06	0.468	2.2	0.98 (0.93–0.99)
Volume (mm^3)	2600 ± 1055	2778 ± 1101	178 ± 254	0.017	6.7	0.97 (0.92–0.99)
$Volume_{cor}$ (mm^3)	2102 ± 696	2231 ± 660	129 ± 200	0.025	6.6	0.96 (0.88–0.99)
Total						
T2* (ms)	2.84 ± 0.97	2.83 ± 0.97	0.01 ± 0.14	0.837	3.5	0.99 (0.97–1.00)
$T2^*_{cor}$ (ms)	1.67 ± 0.23	1.67 ± 0.24	0.01 ± 0.03	0.493	1.3	0.99 (0.98–1.00)
Volume (mm^3)	5793 ± 2160	6033 ± 2194	240 ± 382	0.029	4.6	0.99 (0.96–1.00)
$Volume_{cor}$ (mm^3)	4605 ± 1370	4792 ± 1300	187 ± 330	0.045	5.0	0.97 (0.91–1.00)

Values are given as mean ± SD.
*Typical error percentage.
CI, confidence interval; Cor, corrected; Diff, difference between the two measurements (mean ± SD); ICC, interclass coefficient; M1, measurement 1; O1, observer 1; S1/2, scanning 1 and 2.

T2 and T2*cor*

Test–retest reproducibility. T2* increased significantly between S1 and S2 in both the proximal part (7.2%), the distal part (11.3%), and in the total tendon (8.8%). $T2^*_{cor}$ followed a similar pattern with a significant increase between S1 and S2 (proximal part = 2.5%, distal part = 5.1%, total tendon = 3.6%). ICC was ≥ 0.91 (T2*) and 0.80 ($T2^*_{cor}$) in all regions. Typical error was < 8.9% (T2*) and < 4.6% ($T2^*_{cor}$) in all regions. Data are presented in Table 1. Limits of agreement for proximal $T2^*_{cor}$ (95% limit of agreement [LOA] = –0.1 to 0.2) are shown in Fig. 5.

Intra-observer reproducibility. There were no significant differences in any of the regions between M1 and M2, neither in T2* or $T2^*_{cor}$ values. ICC was ≥0.99 (T2*) and 0.98 ($T2^*_{cor}$) in all regions. Typical error was < 6.4% (T2*) and < 2.2% ($T2^*_{cor}$) in all regions. Data are presented in Table 2.

Table 3. Inter-observer reproducibility.

Tendon part	S1 M1 O1	S1 M1 O2	Diff	*P*	TE %*	ICC (95% CI)
Proximal						
T2* (ms)	2.92 ± 1.23	2.79 ± 1.19	0.14 ± 0.14	0.002	3.5	0.99 (0.89–1.00)
$T2^*_{cor}$ (ms)	1.61 ± 0.28	1.59 ± 0.27	0.02 ± 0.04	0.077	1.8	0.99 (0.96–1.00)
Volume (mm^3)	3193 ± 1153	3477 ± 1117	284 ± 185	<0.001	3.9	0.96 (0.28–1.00)
$Volume_{cor}$ (mm^3)	2503 ± 696	2795 ± 653	292 ± 189	<0.001	5.1	0.88 (0.04–0.97)
Distal						
T2* (ms)	2.65 ± 1.04	2.56 ± 0.95	0.09 ± 0.20	0.106	5.6	0.98 (0.93–0.99)
$T2^*_{cor}$ (ms)	1.75 ± 0.24	1.73 ± 0.25	0.02 ± 0.06	0.234	2.3	0.97 (0.92–0.99)
Volume (mm^3)	2600 ± 1055	2913 ± 1031	313 ± 338	0.003	8.7	0.91 (0.50–0.98)
$Volume_{cor}$ (mm^3)	2102 ± 696	2394 ± 709	292 ± 274	0.001	8.7	0.85 (0.23–0.96)
Total						
T2* (ms)	2.84 ± 0.97	2.72 ± 0.92	0.12 ± 0.15	0.008	3.8	0.98 (0.89–1.00)
$T2^*_{cor}$ (ms)	1.67 ± 0.23	1.65 ± 0.23	0.02 ± 0.03	0.052	1.3	0.99 (0.96–1.00)
Volume (mm^3)	5793 ± 2160	6390 ± 2095	597 ± 487	<0.001	5.7	0.94 (0.38–0.99)
$Volume_{cor}$ (mm^3)	4605 ± 1370	5189 ± 1350	584 ± 427	<0.001	6.2	0.87 (0.09–0.97)

Values are given as mean ± SD.
*Typical error percentage.
CI, confidence interval; Cor, corrected; Diff, difference between the two measurements (mean ± SD); ICC, interclass coefficient; M1, measurement 1; O1, observer 1; S1/2, scanning 1 and 2.

Inter-observer reproducibility. T2* differed significantly between O1 and O2 in the proximal part (4.8%) and in the total tendon (4.2%); no significant difference was observed in the distal part. $T2^*_{cor}$ showed no significant differences between O1 and O2 in any of the regions. ICC was ≥0.98 (T2*) and 0.97 ($T2^*_{cor}$) in all regions. Typical error was < 5.6% (T2*) and < 2.3% ($T2^*_{cor}$) in all regions. Data are presented in Table 3.

Tendon volume

Test–retest reproducibility. There were no significant differences in any of the regions between S1 and S2, neither in volume or $volume_{cor}$. ICC was ≥ 0.97 (volume) and 0.88 ($volume_{cor}$) in all regions. Typical error was < 7.3% (volume) and < 10.4% ($volume_{cor}$) in all regions. Data are presented in Table 1.

Intra-observer reproducibility. In the distal part and total tendon, significant differences were observed between M1 and M2, in both volume (distal part = 6.8%, total tendon = 4.1%) and $volume_{cor}$ (distal part = 6.13%, total tendon = 4.1%). In the proximal part, no significant differences in volume or $volume_{cor}$ were observed. ICC was ≥0.97 (volume) and 0.96 ($volume_{cor}$) in all regions. Typical error was < 6.7% (volume) and < 6.6% ($volume_{cor}$) in all regions. Data are presented in Table 2.

Inter-observer reproducibility. Significant differences in both volume (proximal part = 8.9%, distal part = 12.0%, total tendon = 10.3%) and $volume_{cor}$ (proximal part = 11.7%, distal part = 13.9%, total tendon = 12.7%) were observed between O1 and O2 in all regions. ICC was ≥ 0.91 (volume) and 0.85 ($volume_{cor}$) in all regions. Typical error was < 8.7% (volume) and < 8.7% ($volume_{cor}$) in all regions. Data are presented in Table 3.

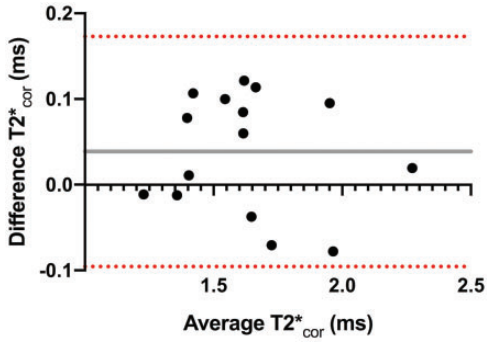

Fig. 5. Bland–Altman plot for test–retest proximal $T2^*_{cor}$. Gray line indicates bias. Red dotted lines indicate 95% limits of agreement.

Discussion

In the present study, we evaluated test–retest reproducibility and intra- and inter-observer reproducibility of multi-slice UTE T2* mapping of human tendinopathic patellar tendons. The test–retest data demonstrate a numerically small bias between recordings, but a substantial reproducibility and low typical error percentages between the two recordings. Furthermore, the method showed excellent intra- and inter-observer

reproducibility. Collectively, these data suggest that the method is sufficiently reproducible for use in future studies of tendinopathy.

To our knowledge, no other studies have investigated the reproducibility of UTE T2* mapping of tendinopathic human tendons. UTE T2* mapping appears to be a sensitive measure of collagen orientation and water content in the tendon tissue (7,15) and can differentiate between healthy and tendinopathic tissue (9,11,16). However, it is unknown whether the magnitude of the difference surpasses the inherent measurement variation of the method. In the present study, we observed a typical error of 3.0% for test–retest reproducibility, which far exceeds the difference between healthy and tendinopathic tendon tissue reported in the literature, which lies in the range of 96%–190% (10,11,17,18). Altogether, this indicates that the method is capable of detecting relevant differences in T2* values rendering the technique feasible for monitoring tendinopathy, and possibly also for evaluating the effect of various treatments. However, the ability to detect treatment effects needs further investigation.

It was observed that regions with long T2* times and poor goodness of fit coincided (Fig. 2). The tendinopathic areas with the most severe alterations of the tissue is less dense, and with a much higher inherent T2 time. Therefore, these areas may not be suitable for evaluation using the present UTE sequences since higher TE is probably required to observe a signal decay, which would explain low goodness of fit r-values in these areas (Fig. 2b). In the present study, a voxel-by-voxel analysis was applied, which enabled us to exclude voxels with low r-values making the derived $T2^*_{cor}$ values more robust (Fig. 4). This may be a more relevant measure than simply including the whole tendon volume without taking into consideration the quality of the fit. Thus, the most severely affected part of the tendon is not included in the $T2^*_{cor}$ values in the present study. To describe the areas with the most severe lesions, sequences with longer TE are likely required; to examine different parts of the tendon at different stages of disease would require protocols that are tailored to the severity of tendinopathy. This assumption should be addressed in future studies. With the current method we aimed to describe the parts of the tendon that are not usually visualized in clinical MRI protocols, i.e. the areas within the tendon in which the structural alterations are less severe and possibly most responsive to treatment. Therefore, the following discussion is based on the $T2^*_{cor}$ values unless stated otherwise.

The present data showed a small systematic increase in T2* values from the first to the second recording (mean difference $\sim$3.0%), which might relate to a higher free water content of the tendon after inactivity. It is well-known that dense connective tissue, such as tendons, mainly consists of short T2 components reflecting water molecules bound to collagen molecules and proteoglycans (15). However, tendon tissue also contains a small fraction of long T2 component in the form of unbound water and both the short and long T2 components influence the T2* values. Grosse et al. (19) showed that T2* values are decreased by prior physical activity. In the present study, the patients were inactive during the initial scan ($\sim$40 min) and for approximately 45 min between the scans; consequently, the increase in T2* could reflect a slightly higher free water content after a period of inactivity. Thus, a standardized pre-scan resting regime could possibly further improve the reproducibility of the method. In the present study, the patients were instructed to abstain from physical activity 24 h before the examination. However, the majority of the patients did load their tendon to some extent transportation themselves to the MRI facility, which might have resulted in a small drop in T2* values with a subsequent increase after inactivity. These findings underscore the need for strict standardization of the method in future studies, but also indicate that the method may be quite sensitive in detecting small changes in water content.

Another contributing factor to the systematic increase in T2* values over time could be inherent technical variation of the MRI method. To test the isolated technical variation, primarily thought to arise from differences in shimming, we performed test scans (data not shown) on repeated UTE T2* sequences of $MnCl_2$ phantoms (1, 2, 4, 8, 16, and 160 mM). There was a small technical variation at about 1% between two scans, which infers that the technical variation only accounts for a small part of the variation seen in the tendons.

Inhomogeneity in the B_0 field could also be a contributing factor to the test–retest bias, due to participant positioning variation between the two examinations. From visual inspection of our imaging dataset, we observed that participants were placed almost identically (within 0.5 cm) in the scanner between S1 and S2. Furthermore, no systematic difference in placement between S1 and S2 was found (data not shown). Based on this, we believe that the small variation in T2* values between the scans is unlikely explained by field inhomogeneity. Patient movement was minimized by careful knee fixation in a knee-coil and we did not observe signs of movement artefacts in our imaging data.

The present study showed excellent intra- and inter-observer reproducibility. Not unexpectedly, the intra-observer reproducibility was higher than the inter-observer, thus indicating that the introduction

of more than one observer will tend to increase measurement variability. Future optimization of the current method could include automated segmentation to reduce observer dependency inherent in manual segmentation.

The calculated T2* values in our study are generally lower than T2* values in the previous studies. Grosse et al. (17) studied T2* values of tendinopathic and healthy tendons and found a higher T2* (4.27 ms) in tendinopathic achilles tendons compared to our observations for total tendon in patellar tendinopathy (test–retest T2* = 2.84–3.09 ms; $T2^*_{cor}$ = 1.67–1.73 ms). For healthy controls, they showed lower T2* mean values at 1.47 ms. Other data in the literature, for example a study by Juras et al. (9) reported even higher mean T2* values of 3.35 ± 0.45 ms in healthy volunteers and 6.56 ± 1.70 ms in symptomatic patients. Likewise, Filho et al. (20) reported higher mean T2* values at 2.18 ± 0.30 ms in normal tendons. However, this study (20) was performed in cadaver samples, which may not adequately reflect in vivo values, in addition to the fact that freezing and thawing of samples could affect T2* values. Further, we cannot rule out that the difference between the patellar and Achilles tendon might be part of the observed variation. Only one previous study has investigated human patellar tendons (18) and reported T2* values of 2 ms for healthy tendons and 3.1 ms for tendinopathic tendons, which is comparable to the values of the present study.

In many of the previous studies (9,11,17), all voxels in a certain segmentation have been included in the analyses regardless of the quality of the fit, and the values are based on few selected slices. In contrast, $T2^*_{cor}$ calculated in the present study is based on the whole tendon volume rather than selected slices, and voxels with poor fitting were excluded from the analyses. As mentioned previously, voxels with a poor fit coincided with high T2* values, thus exclusion of those voxels contributes to the lower T2* values in the present work. Correspondingly on the test scan performed on a $MnCl_2$ phantom, the lowest concentration chamber (1 mM) with the longest inherent T2 displayed no signal decay across increasing TE, and thus yielded a poor fit to the curve. Altogether this confirms that sequences tailored to describe the most severe lesions in the tendinopathic tendons still need to be applied alongside UTE sequences to comprehensively describe the chronic tendinopathic tendon.

One limitation of the method in the present study is the relatively large variation of the volume measurements. A conservative approach was applied in manually outlining the tendon to avoid inclusion of peritendinous tissue, which may have led to some underestimation of the total tendon volume. However, by this conservative approach, we minimized the risk of including peritendinous tissue with a potential poor fitting and high confounding impact on the T2* values, and thereby we ensure that the values obtained primarily describe the tendon proper. Moreover, the variation in the volume measurements did not seem to bias the T2* values in the present study, which remained within a narrow range of values when comparing values for intra-observer, inter-observer, and test–retest measurements (Tables 1–3), thus the variation in the segmentation does not seem to influence the T2* values to a significant degree.

The present study focused on patellar tendons and included only chronic tendinopathies. Therefore, additional studies on healthy patellar tendons and early stages of patellar tendinopathy are needed to further expand our knowledge regarding UTE MRI derived T2* values in patellar tendons. However, we believe our results provide important new knowledge and data that support the feasibility of applying the method in future studies in tendons. The present study was conducted on a sample of 15 individuals, which is arguably on the low end. However, it is comparable to previous studies utilizing UTE T2* mapping, which have included an equivalent or smaller sample size (16–18).

The potential ability of the method to characterize tendon composition in more detail and detect subtle changes beyond what can be achieved by ultrasound and clinically available conventional MRI sequences may prove highly useful in the early detection and objective monitoring of tendinopathy in a clinical setting and in interventional studies. Although clinical MRI protocols have the ability to visualize structural changes in the tendon, only the most severe alterations can be detected. With UTE sequences, sufficient signals can be obtained from tissue with relatively low T2, which enables assessment of the tendon regions that are not visible with clinical sequences. This is expected to be of importance since these regions of the tendinopathic tendon are likely most susceptible to treatment compared to severely affected regions. The most commonly used modality to visualize tendon structure is ultrasound, and this method has the advantage of being applicable bed side in a clinical setting, but the sensitivity to minor alterations in tissue structure is low, it is highly dependent on the investigator and there is a poor correlations with prognosis and clinical outcomes (21–23). UTE T2* mapping serves as an addition to US and conventional MRI that should be considered when imaging tendon tissue in future studies, especially if interventions aim to alter and monitor early changes in structural integrity of the tissue. However, we acknowledge that it is too early to implement these sequences clinically, and also that they are

typically not readily available for clinical use. Nevertheless, we do believe that there is a need for more robust non-invasive measures of the tendon structural integrity and that UTE T2* may be an important future application.

In conclusion, we have demonstrated that UTE-T2* analyses show high levels of agreement and reliability. A small difference for test–retest values was observed, but with a very low associated mean difference (3.7%) between the two tests. The method also showed excellent intra- and inter-observer reproducibility. Collectively, the data suggest that the UTE-T2* protocol applied in the present study is sufficiently robust for use in research and clinical practice in early detection and objective monitoring of tendinopathy, potentially providing valuable information about tendon structure that cannot be obtained with current clinical MRI protocols.

Authors' note

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Paper II

Title: Magnetic resonance T2* is increased in patients with early Achilles and Patellar tendinopathy

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Running head: UTE T2* MRI – Healthy vs. tendinopathic tendons

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Abstract

Objectives: The aim of the current study was to investigate the difference in MRI T2* relaxation time between patients with early phase tendinopathy (Achilles and patellar tendons) and healthy controls. Further, we wanted to investigate the relationship between T2* and clinical outcomes (Victorian-institute of Sports Assessment- Achilles/ - Patella (VISA-A/VISA-P)), tendon size and mechanical properties, respectively.

Materials and Methods: 65 patients with Achilles and patellar tendinopathy and 25 healthy controls underwent an ultra-short time to echo (UTE) MRI scan with variable echo times (TE: 0.07,0.57,1.07,1.57 ms), and T2* values were extracted from the full tendon volume using a mono-exponential fitting algorithm. Clinical symptoms were evaluated using VISA-A in the Achilles patients and VISA-P in the patellar patients, and in vivo mechanical properties were measured using an ultrasound-based method in patellar patients. A generalized linear model adjusted for age was applied to investigate the difference between patients and controls. In the two patient groups linear regressions were applied to investigate the association between T2* and tendon size, VISA-A, VISA-P and biomechanical properties.

Results: We observed a significant difference between the patients and healthy controls (204.8 (95 % CI: 44.5–365.0) µs). There was a positive correlation between tendon size and T2* for both Achilles tendons ($r=-0.72$; $p<0.0001$) and patellar tendons ($r=0.53$; $p=0.02$). There was no significant correlation between VISA-A and T2* ($r=-0.2$; $p=0.17$) or VISA- P and T2* ($r=-0.5$; $p=0.0504$). Lastly, there was a negative correlation between modulus and T2* ($r=-0.51$; $p=0.03$) in patients with patellar tendinopathy.

Conclusions: In conclusion UTE T2* MRI is found to be elevated in the early phase of tendinopathy and correlated to increased tendon size and a more compliant tendon structure. This suggests that T2* can, detect subtle structural changes and, that translates to altered mechanical properties in early phase tendinopathy. Since we did not observe an association between T2* and clinical outcome scores, UTE T2* MRI cannot fully explain clinical changes in patients with early phase Achilles and patellar tendinopathy.

Introduction

Magnetic resonance imaging (MRI) is commonly used to study healthy and diseased tendinopathic tendons. The structural composition is, however, not easily quantified by conventional MRI[1], especially in healthy tendons and in the early phase of pathological tendon changes. Consequently, there has been a great interest in developing techniques that enables researchers and clinicians to assess the structure of tendon tissue in a detailed and yet non-invasive manner.

A major issue when trying to assess tendon structures with MRI is the very short T2 relaxation [2]. When tendinopathic structural changes become more pronounced conventional MRI can produce signal from the tendinopathic areas [3–5], whereas very little signal is obtained in healthy tendon tissue. Therefore, to study the structure of healthy tendons and tendons with more subtle structural changes, ultrashort time to echo (UTE) MRI has been introduced [6–8]. The short echo times in these sequences (<1 ms) generates more signal from the tendon and thus permits better analyses of the structure. Further, to allow quantification of time constants, that are dependent on the tissue structure, quantitative mapping techniques have been developed, and one such enables extraction of T2* relaxation times in the tendon by utilizing repeated acquisitions with varying echo times (TE) (UTE T2* MRI)[9–11].

T2* is considered a measure of unbound water secondary to structural changes [10–12]. Previous results suggest a considerable difference in T2* between chronic tendinopathic tendons and healthy tendons [10,11], but whether the subtle changes that we would expect in an early phase of tendinopathy can be detected remains unknown. Also, T2* appears to be related to clinical outcomes and thus, the symptom severity in tendinopathic patients [10 11]. Lastly, one previous study suggested an association between mechanical properties and T2*, in a chemically induced – tendinopathy – model on bovine tendon transplants [13], but this relationship has not been studied in humans.

In this cross-sectional study we measured T2* in healthy controls and patients with early phase tendinopathy using UTE T2* MRI. Further, we assessed clinical outcomes using the VISA-A and the VISA-P questionnaires and measured in vivo mechanical properties using an ultrasound-based method. The main objective was to investigate difference in T2* values between healthy and tendinopathic tendons (Achilles and patellar tendons) in the early

phase of disease (symptoms<3 months). Furthermore, we wanted to explore the relationship between clinical outcomes, tendon size and T2*. Lastly, we sought to reproduce previous findings, suggesting an association between mechanical properties and T2*.

We hypothesize that tendon T2* is higher in early phase tendinopathy patients compared to healthy controls. Furthermore, we hypothesize that T2* would correlate with symptom severity, tendon size and mechanical properties.

Methods

Study design

The current study was designed as a cross-sectional study. Data on clinical outcomes (VISA-score, NRS), conventional MRI, ultrasound, biomechanical properties and biochemistry (q-RT-PCR) has previously been published separately [14]. The original study included 200 patients with Achilles and patellar tendinopathy with symptoms for less than 3 months and 50 healthy controls. The overall aim of the original study was to investigate the effect of symptom duration on clinical, physiological and biochemical outcomes. In addition to the already reported outcomes, UTE T2* MRI scans were performed in 90 subjects (65 tendinopathic and 25 healthy controls), these subjects were included in the current study. Herein we investigate differences in T2* values between healthy and tendinopathic tendons and also correlate T2* values with clinical outcomes, tendon dimensions and biomechanical properties.

The study was performed in the outpatient clinic at the Institute of Sports Medicine Copenhagen and MRI scans were performed at the department of radiology, located at Bispebjerg and Frederiksberg Hospital. At the initial visit patient signed informed consent. The study was approved by the Danish local ethical committee (H-16019857) and was registered at clinical trials (NCT02797925) and the Danish data protection agency (BFH-2016-019, I-Suite nr.: 04519).

Subjects were included via social media, general practitioners and advertising in sports clubs. Patients were considered eligible if they had activity related pain and pain on palpation in the Achilles or Patellar tendon with symptom onset within the past 90 days. Patients were excluded if they had suffered previous injuries in the same location as the current injury or if they had started treatment for the current injury.

Outcome measures

At the initial visit clinical outcomes were recorded, and at a separate visit an MRI scan was performed, including the UTE T2* MRI scans. Finally, mechanical testing was performed on a separate day in patients with patellar tendinopathy.

Clinical outcomes

Function was assessed by the Victorian Institute of Sports Assessment-Achilles/Patella Questionnaire (VISA-A/P) (Range: [0-100]; 0 meaning lowest possible function of the tendon, 100 meaning full function of the tendon). Furthermore, pain intensity during training and at rest was assessed using the numerical ranking scale (range [0-10], with 0 meaning no pain and 10 meaning worst pain imaginable). Lastly, physical activity was assessed by recording weekly time consumption of physical activity.

UTE MRI

A unilateral MRI scan was performed using a 3D isotropic UTE (ultra-short time to echo) MRI sequence (FOV: 160 x 160 mm TR: 11 ms varying TE (0.07, 0.57, 1.07, 1.57 ms), matrix resolution: 1.45x1.45x1.00 mm Flip angle: 12°, scan time: 3 m 14 s). The varying echo time allows for quantification of T2* relaxation times. T2* reflects the amount of unbound water inside the tissue which changes secondary to structural changes [10,13,15].

The UTE scans were analysed according to the previously described protocol from our lab[16]. In brief, UTE recordings with varying TE were automatically loaded into a custom-made program, and all echo times were combined, and signal intensity was plotted against TE on a voxel-by-voxel basis. We used a mono-exponential fitting algorithm incorporating noise reduction to reconstruct T2* maps and goodness of fit maps (Pearson's correlation coefficients), and these maps were used for analyses. Manual segmentation of the tendon in the transverse plane was performed on every 4th slice in ITK snap (version 3.6.0) and the interpolate labels tool was used to segment the full tendon volume. For the Achilles tendon the most proximal slice of the free tendon was defined as the first slice without the soleus muscle, and the most distal slice was as the last slice in the proximal–distal orientation

where the calcaneal bone was not in contact with the tendon. For the patellar tendon the most proximal slice was defined as the first slice were the patellar bone was not visible and the most distal slice was defined where corpus Hoffa did not completely cover the posterior surface of the tendon. The segmentations, goodness of fit maps and T2* maps were then combined using a custom-made macro in FIJI ImageJ (version 2.0.0-rc-68/1.52e) to extract T2* values for voxels with r>0.8, volumes and Pearson's correlation coefficients (r-values) from the full volume of the free tendon. This was done to exclude voxels with poor fitting.

To account for differences in tendon length, mean cross-sectional area (CSA) was calculated by dividing the full volume with the number of slices included in the segmentation. Representative examples of a T2* map, goodness of fit map, and a tendon segmentation are presented in figure 1.

Mechanical testing

Mechanical properties were assessed in patellar tendinopathy patients, using ultrasound recordings (to track the deformation) combined with force recordings. The results from the mechanical testing have previously been published in the original study. A full description of the method can be found in the original article [14] and in previous studies from our lab [17,18]. In short, patients performed a light warm-up on a cycle ergometer (Monark, Sweden) prior to testing. Testing was performed in a custom-made chair at 90° knee flexion with a force transducer connected by a rigid lever arm to a cuff fixated around the ankle. A ramped isometric knee extension was performed over eight seconds, with synchronized recording of force and B-mode ultrasound video for measurement of the tendon deformation. in total four maximal contractions were performed on each side.

A custom Matlab script (Matlab R2016b, The MathWorks Inc, USA) was used to track the tendon insertions on the patella bone and tibia. Tendon deformation was correlated with force measurements using an excel template to generate a force-deformation curve. Data were fitted to a second-order polynomial using Sigma Plot (Version 10.0, Systat Software, Germany). Modulus was calculated from the slope in the final 20% of the stress strain curve and used herein as a measure of tissue mechanical properties.

Study size determination and statistical methods

The original study included 250 participants in total. However, for technical reasons only a subset underwent UTE T2* MRI, and thus 90 participants are included in the current study (Achilles: 45 patients and 15 healthy controls; patella: 20 patients and 10 healthy controls). To investigate differences in T2* between patients and controls a generalized linear model was applied with T2* as dependent variable and group (patient/control) and tendon (Achilles/Patella) as independent variables. Since tendon properties were associated with age in previous studies [19,20], and because age differed between the patients and controls we adjusted for age by including it as a random effect in the model. To investigate the correlation between T2* values, clinical outcomes (VISA-A -and -P), tendon mean CSA and biomechanical properties linear regressions were performed in the patient groups. Differences in subject characteristics were assessed using Students t-test for parametric outcomes and Fisher's exact test for categorical outcomes. Results are presented as mean values ± SEM unless stated otherwise. An alpha level of 0.05 was used to test for significance.

Results

Participants

Subject characteristics are listed in table 1. An age difference was observed between the healthy control group and the tendinopathy group ($p<0.0001$). No significant differences were observed on other characteristics. One subject did not fill in the VISA-P questionnaire, and therefore only 19 patellar patients were included in the correlation analyses for patellar tendons.

Overall mean Person's correlation coefficient (r) was 0.96 in the T2* maps across all groups including all voxels. On average 1.5 % of the segmentation volume was excluded from analyses after goodness-of fit correction (voxels with $r<0.8$ were excluded). Only the goodness of fit corrected T2* values are reported in the current study.

Main results

No interaction was observed between group (patient/control) and tendon (Achilles/Patella) (Crude analyses: $p=0.79$; Age adjusted analyses: $p=0.62$) which allowed for interpretation of main effects. A significant difference in T2* was observed between the healthy and tendinopathic group (table 2), (Mean difference; Achilles: 349.2 ± 72.8 µs; Patella: 371.1 ± 92.1), In the age adjusted model there was a significant main effect of age on T2* (16.9 ± 3.7 µs/year; $p<0.0001$), and a significant difference between patellar and Achilles tendons (356.3 ± 73.5 µs; $p<0.0001$ (patella>Achilles)). Lastly in the unadjusted model we also found a main effect of group and tendon. Estimates from the age adjusted and unadjusted analyses are presented in table 2, and the mean values are plotted in figure 1.

Correlations

There was a positive correlation between cross-sectional area and T2* for both Achilles tendons ($r= 0.72$; $p<0.0001$) and patellar tendons ($r=0.53$; $p=0.02$) (figure 3). Mean CSA was 83.8 ± 3.2 mm^2 for the tendinopathic Achilles tendons and 127.2 ± 5.0 mm^2 for the tendinopathic patellar tendons.

Further, there was no significant correlation between VISA-A and T2* ($r=-0.2$; $p=0.17$) or

VISA- P and T2* (r=-0.5; p=0.0504) (figure 3). Mean VISA-A was 58 ± 2.7 and VISA-P 61.2 ± 3.4.

Lastly data from both mechanical properties and UTE T2* scans were available in 18 patellar patients in total. There was a negative correlation between modulus and T2* (r=-0.51; p=0.03) (figure 4).

Discussion

The main findings in the current study was that T2* in tendinopathic tendons within the first 3 months after symptom onset was elevated. Furthermore, we did not observe an association between clinical outcomes (VISA-A -and -P) and T2* although a trend was observed in the patellar patients (p=0.0504). We observed a significant positive correlation between T2* and tendon cross-sectional area, which suggest that changes in tendon size might be caused by an increase in unbound water. Lastly, we found an association between tendon mechanical properties and T2*, suggesting functional implications of the differences in tissue structure we observed.

Published T2* values in healthy Achilles and patellar tendons ranges from ~0.5 – 3.5 ms [8–11,21–28] and in diseased tendons from ~1.7-7.2 ms [10,11,16,29,30], which shows the great variation that exists between published values, and that there is a substantial overlap between values from healthy and tendinopathic tendons. However, when comparing values obtained from protocols with similar TE range and spacing, and comparable fitting algorithms, a general pattern suggests higher values in tendinopathic compared to healthy tendons.

Few studies have directly compared T2* in healthy and chronic tendinopathic tendons in vivo. One study by Juras et. al. reported a two-fold difference between healthy and tendinopathic Achilles tendons [10]. Another study by Grosse et. al. reported an almost four-fold difference between symptomatic tendinopathic areas and asymptomatic non tendinopathic areas in the Achilles tendon [11]. The magnitude of these differences is considerably higher than the differences we observed in the current study. This was, however, expected since only early phase tendinopathy patients were included in the current study, and thus structural changes might be more subtle. Nevertheless, we did observe a difference between the healthy and early tendinopathic group, which supports the idea that UTE T2* MRI is a sensitive tool that can detect small changes in unbound water secondary to subtle structural changes in tendon tissue. Also compared to studies using similar sequences and fitting algorithms values from the current study on patients with early tendinopathy are in-between T2* in healthy controls and chronic tendinopathy patients [11,16].

Furthermore, we observed a main effect of age in our model, which suggests that T2* increases with increasing age. This observation is supported by a previous study on Achilles tendons, in which a difference in T2* was observed between young and old [20], and also a study on tibial tendons that observed lower macromolecular fraction measured by magnetization transfer UTE MRI in old compared to young [19].

Despite the differences that have been observed between healthy and tendinopathic tendons, to our knowledge few studies have directly investigated the relationship between T2* and symptom severity [10 11]. In the work by Juras et. al. a significant correlation was observed between Achilles tendon rupture score (ATRS (range [0-100])) and T2*. However, in the current study we did not observe any relationship between VISA-A score and T2* in tendinopathic Achilles tendons. It is possible that the discrepancy may partly reside in the different patient reported outcomes used, i.e., VISA-A and ATRS. There are also substantial differences between the scanning protocols. Most notably the lowest echo in the former study by Juras et. al was 0.8 ms, which is 10-fold higher than the lowest TE in the current study, which could potentially lead to higher T2* values caused by the scanning protocol. Also, in the current study only tendinopathy patients in the early phase were included, whereas symptom duration was not reported by Juras et. al. Should the patients have had symptoms longer than 3 months this could potentially lead to more pronounced structural changes. We did, however, observe a trend towards a relationship between T2* and VISA-P score in the patellar patients, which has not been shown previously. Collectively our results suggest that no clear association exists between T2* and symptom severity measured by VISA-A, whereas a possible association exists between VISA-P scores and T2* in the early phase of tendinopathy.

Previous studies have observed differences in tendon CSA between healthy and tendinopathic tendons [31], but whether this increase in size is caused by increases in ground substance, collagen or an accumulation of water is somewhat unexplored. Herein we observed an association between T2* and tendon size in both Achilles and Patellar patients, suggesting that size increases in early tendinopathy, to some extent may be explained by increases in unbound water measured by UTE T2* MRI.

To investigate the functional impact of the differences between healthy and tendinopathic tendons, we correlated T2* with tendon modulus, which represents the intrinsic mechanical properties of the tissue [32]. We observed a negative correlation between T2* and modulus,

309 which supports the previous findings in bovine tendon specimens [13], and thus indicates
310 functional implications of the structural changes.
311
312 The current study has some limitations. First, we performed a voxel wise mono-exponential
313 fitting, and thus did not account for signal heterogeneity in the individual voxel, which
314 consist of both short and long T2* components [29]. Also, we did not perform longer TE scans,
315 which resulted in a scan protocol with a narrow range of very short TEs. However, from the
316 high r-values obtained in the current study, it seems that most of the variation in our data
317 was explained by a mono-exponential decay, and thus could be considered comparable to
318 the short component T2* obtained from bi-exponential fitting methods [10,29]. We did not
319 attempt to perform bi-exponential fitting of our data since we did not have any long TEs to
320 describe the long T2* component and also considered the number of echoes insufficient in
321 the current study.
322 Further, we did not perform bilateral scans, and thus we did not have an internal control, in
323 patients with unilateral symptoms.
324
325 Clearly, the present study cannot explain any causality between an altered T2* signal and
326 the development of tendinopathy, but the coupling to tendon size and mechanical
327 properties together with its ability to detect subtle changes in the tendon tissue, makes the
328 use of T2* promising for future longitudinal studies on tendinopathy.
329
330 In conclusion, the data from the current study suggests that UTE T2* MRI obtained from a
331 simple variable 4-point echo time mono exponential fitting algorithm can be used to detect
332 subtle differences in unbound water secondary to structural changes in the early phase of
333 tendinopathy. Further we observed that these changes were associated with in vivo
334 mechanical properties, which suggest that the structural changes we observed have
335 functional implication. Although T2* contributes to the description of tendon pathology in
336 the early phase of disease, it is not clearly associated to symptom severity, and thus T2*
337 cannot currently fully explain clinical changes in early-phase tendinopathy patients.
338

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Tables

	Patients (n=65)	Healthy (n=25)	t-test
Age (y)	37.1 ± 1.3	28.5 ± 1.3	**<0.0001**
BMI (kg/m^2)	24.0 ± 0.3	23.6 ± 0.4	0.45
Training (h/week)	7.5 ± 0.5	6.3 ± 0.6	0.15
Sex (female/male) †	22/43	12/13	0.23
Pain (NRS)	5.0 ± 0.3	-	-
Symptom duration (days)	45.01 ± 2.9	-	-

Table 1 *Subject characteristics*
Age was significantly different between the patient group and the healthy control group. †= Fisher's exact test.

	T2* (µs) listed as: Estimates (95%CI) and p-values	
	Group	Tendon
ΔT2*—unadjusted	342.8 (178.1–507.4) (<0.0001)	269.3 (112.9–425-7) (0.001)
ΔT2*—age adjusted	204.8 (44.5–365.0) (0.01)	356.3 (210.1–502.4) (p<0.0001)

Table 2 *Difference in T2* between patients with early phase tendinopathy and healthy controls*
Main effect of group (patient/control) and tendon (Achilles/patella). Results from the crude analyses (unadjusted) and age adjusted analyses are displayed. The healthy control and the Achilles tendon were used as reference group and tendon respectively.

Figure legends

Figure 1 *Parametric maps and segmentation*
A) Example of an axial T2* map in the Achilles tendon, range: [0-2000 µs], colour scale-bar is displayed under the image (colourmap: JET – ITK snap). The tendon is marked with a pink outline and arrow. B) Goodness of fit map in the same Achilles tendon on the same slice, Pearson's correlation coefficient range: [0.8-1], colour scale bar under image (colourmap: HOT – ITK snap) The tendon is marked with a pink outline and arrow. C) Anterior-caudal view on the 3D segmentation of the corresponding Achilles tendon.

Figure 2 *Tendon T2* MRI in healthy controls and patients with early tendinopathy*
Mean T2* values (µs) are provided (Error bars: SEM) for Achilles and patellar tendons in healthy controls and patients with early phase tendinopathy.
P-values are obtained from the age adjusted model.
† = main effect of group (patient/healthy) (p=0.01)
* = main effect of tendon (Achilles/Patella) (p<0.001).

Figure 3 *T2* vs. tendon size and clinical tendinopathy score*
A) correlation between CSA and T2* within the Achilles group (Pearson's correlation coefficient (r)=0.72; p<0.0001) B) correlation between CSA and T2* within the Patellar group (r=0.53; p=0.02) C) correlation between VISA-A and T2* (r=-0.2; p=0.17) D) correlation between VISA-P and T2* (r=-0.5; P=0.0504).

Figure 4 *T2* and in vivo mechanical properties of the patellar tendon*
Correlation between tendon modulus and T2* in 18 patients with early phase patellar tendinopathy (Pearson's correlation coefficient (r)=-0.5; p=0.03).

FIGURE 1

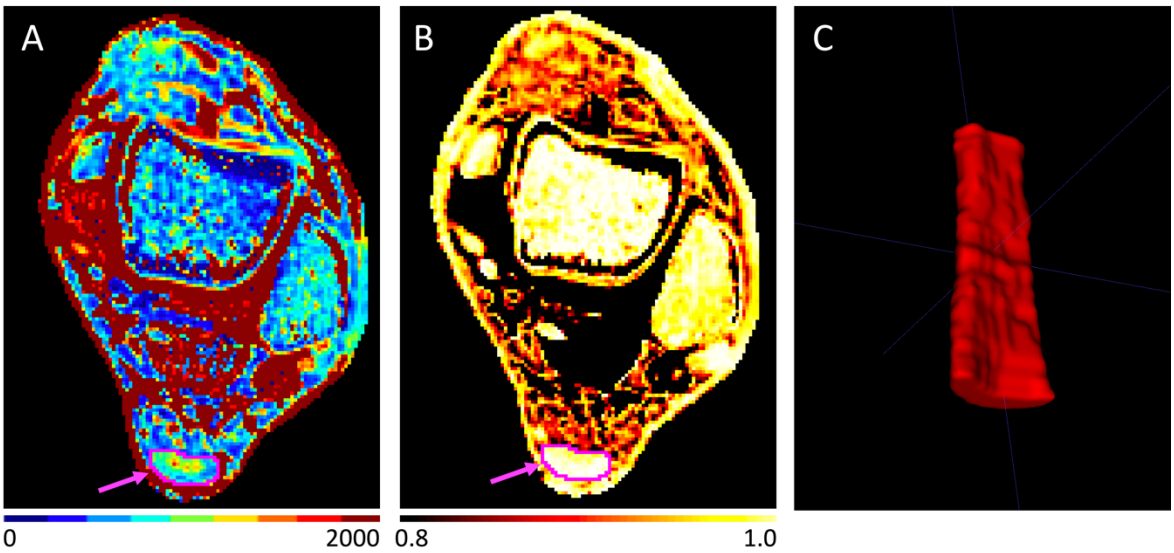


FIGURE 2

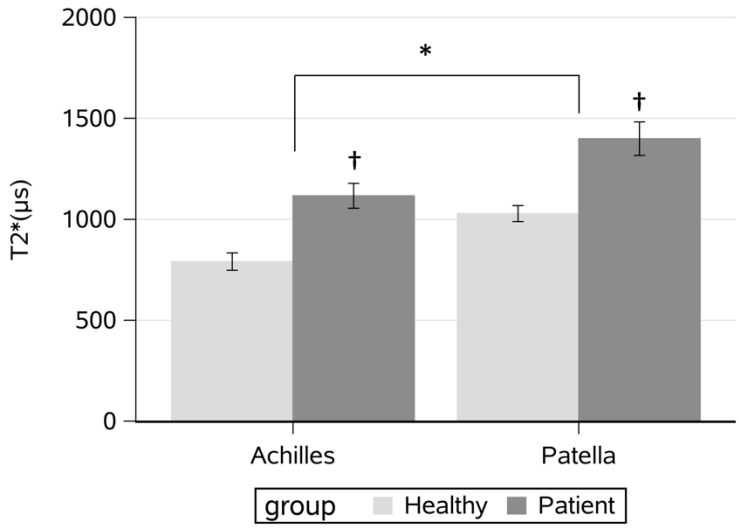


FIGURE 3

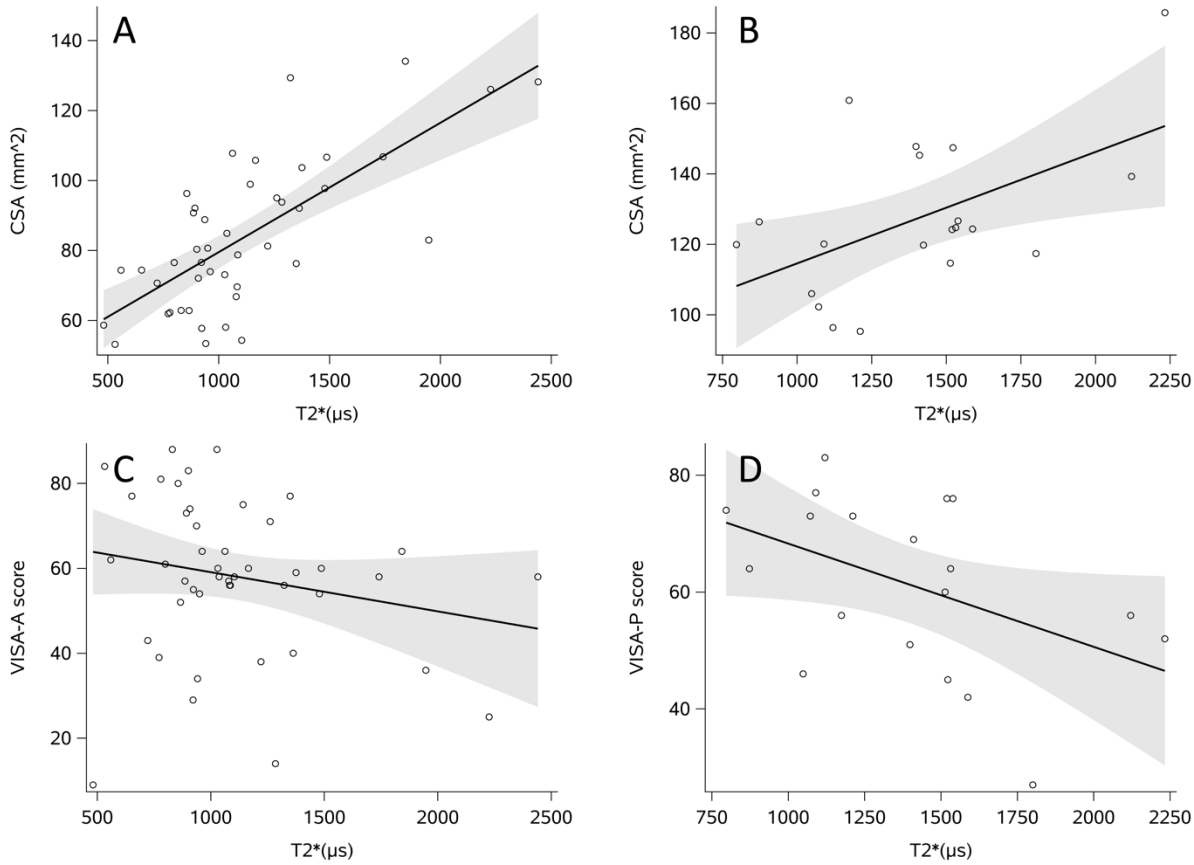
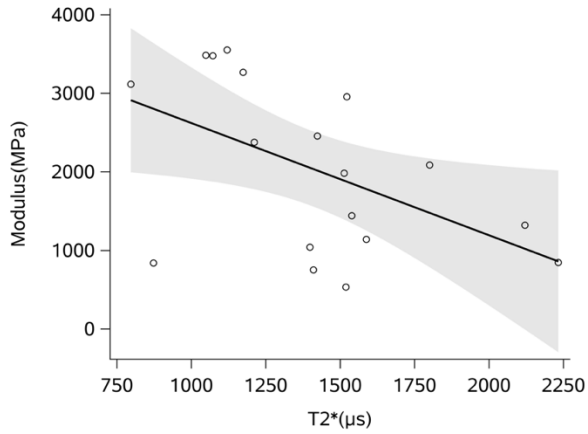


Figure 4



Paper III

No additive clinical or physiological effect of short-term anti-inflammatory treatment to physical rehabilitation in the early phase of human Achilles tendinopathy: a randomized controlled trial

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Abstract

Background: NSAIDs are commonly used in an attempt to dampen inflammation in the early phase of Achilles tendinopathy, but if this has an additive clinical effect during rehabilitation in early tendinopathy remains unknown.

Hypothesis/Purpose: To investigate the additive effect of an initial short-term NSAID treatment in the early phase of Achilles tendinopathy. We hypothesized that the combination of NSAID and physical rehabilitation is superior to the rehabilitation alone.

Study Design: Double blinded, placebo-controlled, parallel- group clinical trial.

Methods: 69 patients with early phase Achilles tendinopathy (lasting < 3 months) were randomly assigned to either a Naproxen group (7 days Naproxen treatment (500 mg b.d.)) (n=34) or a Placebo group (7 days placebo treatment) (n=35). Both groups received an identical 12-weeks physical rehabilitation program. The clinical outcome of the study was evaluated with questionnaires (VISA-A and NRS) and the physiological outcome was evaluated with ultrasonography (US), magnetic resonance imaging (MRI) and ultra-short time to echo T2* mapping MRI (UTE T2* MRI). Follow-up was performed after 1 week, 3 months and 1 year. Results are presented as (Mean ± SEM).

Results: VISA-A score increased significantly during rehabilitation in both groups (14.5±2.8 $p<0.0001$), and at 1-year follow-up additional improvements were observed in both groups (7.6 ± 3.3 $p<0.05$). Further, changes during rehabilitation in VISA-A score was greater in patients with very short (<1 month) symptom duration at inclusion compared to those with longer symptom

duration (>3 months) (11.7 ± 4.2 p<0.01). Despite clinical improvements total weekly physical activity (h/week) remained significantly lower compared to pre-injury levels in both groups (-3.4 ± 0.7 h/week p<0.0001). At baseline US showed increased thickness (0.12 ± 0.03 cm p<0.0001) and vascularity (0.3 ± 0.03 cm^2 p<0.005) on the tendinopathic side, but no changes over time were observed in US, MRI or UTE T2* MRI.

Conclusion: Clinical symptoms in early tendinopathy improves with physical rehabilitation but is not augmented with the addition of anti-inflammatory treatment. Furthermore, this clinical recovery occurs in the absence of measurable structural alterations. Finally, clinical improvements are greater in patients with very short symptom duration compared to those with longer symptom duration.

Key Terms: Clinical research, Achilles tendon, NSAIDS, Imaging, Diagnostic ultrasound, magnetic resonance, Physical therapy/rehabilitation.

What is known about the subject: Achilles tendinopathy is a common overuse injury. Current clinical practice includes initial short term NSAID treatment soon after onset of symptoms. However only few clinical trials have investigated the effect of NSAIDs in Achilles tendinopathy and to our knowledge no studies so far have specifically addressed the use in the early phase of the disease.

What this study adds to existing knowledge*:* Our study adds to the knowledge about the effect of NSAIDs in Achilles tendinopathy. Similar to previous studies performed in patients at a more chronic stage of disease we do not observe any effect of NSAID, not in clinical response measure by questionnaires, nor in physiological response measured by ultrasound, Magnetic resonance

66 imaging (both conventional, and ultra-short time to echo T2* mapping MRI). Also, analgesic effect
67 was absent when compared to placebo. Given the lack of effect and the known side effects of
68 NSAID use, we stress careful consideration before use of NSAID in early Achilles tendinopathy.

Introduction

Achilles tendinopathy is a common overuse injury in competitive and recreational athletes[13,21] which is characterized by pain, swelling and decreased function.[22,31] The treatment of chronic tendinopathy is often protracted and mainly includes physical rehabilitation programs,[7] whereas anti-inflammatory treatment in the form of non-steroidal anti-inflammatory drugs (NSAIDs) have very sparse effects on clinical outcomes at the chronic stage of disease.[4,8,17]

Despite the lack of evidence for the use of NSAIDs they are commonly used for the treatment of tendinopathy, especially in the early phase of disease.[3] Theoretically there is some support of this practice since inflammation be more pronounced in the early phase of tendinopathy compared to chronic state disease.[10,11,23] Although this time course is not comprehensively described in relation to Achilles tendinopathy, it could indicate a more likely effect of anti-inflammatory treatments in the early phase of the disease.

Some injuries like plantar fasciitis seems to benefit from an anti-inflammatory drug intervention in combination with a physical rehabilitation program including muscle strength training.[18] Thus, we suggest that an anti-inflammatory drug intervention with NSAID in the early phase of Achilles tendinopathy could be a beneficial additive to a physical rehabilitation program that includes moderate load reduction and strength training.

Despite the widespread use of NSAIDs, to our knowledge no studies so far have specifically investigated the additive effect of NSAIDs to physical rehabilitation in the early phase of tendinopathy. Therefore, the purpose of this study was to compare an initial short-term (one week) NSAID treatment plus a standard physical rehabilitation program over 3 months with rehabilitation alone, in patients with early Achilles tendinopathy (symptom duration <3 months).

The clinical outcome of the study was evaluated with questionnaires and the physiological outcome evaluated with ultrasonography (US) and magnetic resonance imaging (MRI). We hypothesize that NSAIDs in anti-inflammatory doses combined with rehabilitation is superior to the rehabilitation program alone.

Methods

This study was designed as a double blinded, placebo-controlled, parallel-group, clinical trial and reported in line with the CONSORT checklist. An automated minimization procedure was used to assign patients into 2 parallel groups. The protocol was approved by the Danish Regional Ethical Committees of the Capital Region (H-16019857) and registered at ClinicalTrials.gov (ID: NCT03401177) and the Danish Data Protection Agency (BFH-2016-019, I-Suite nr.: 04519). The full study protocol is attached as a supplementary file.

A total of 69 sports active participants were included, primarily from the capital region in Denmark. Most of the participants were recruited through advertising on social media and were encouraged to contact us if interested. Participants were scheduled for a screening visit in our specialized outpatient clinic. They were considered eligible if they were above 18 years old, had activity related pain in the Achilles tendon, palpation pain and onset of symptoms within the last 90 days. Patients were excluded if they had a previous injury in the ipsilateral Achilles tendon, recent infection in/around the Achilles tendon, enthesopathy, previous surgery in the Achilles tendon, contraindication for NSAID treatment, received NSAID treatment for the current injury or if they used medication with NSAID interaction.

The study was conducted between January 2018 – August 2019 (primary completion date), in the outpatient clinic at Institute of Sports Medicine Copenhagen, Bispebjerg Hospital. At the screening visit, patients were assessed for eligibility, informed consent was signed, and baseline questionnaires and ultrasound scans were performed. An MRI scan was performed as soon as possible after inclusion, and intervention was initiated at the same day. After 1 week the first

follow-up was performed, including questionnaires and ultrasound scans. A second follow-up was performed after 12 weeks including questionnaires, ultrasound and MRI. At 1-year patients completed the third and final follow-up only including questionnaires.

Patients were randomized to either the Naproxen group (7 days Naproxen treatment (500 mg b.d.)) or the Placebo group (7 days placebo treatment (tablets in identical packaging; similar in size and colour to the naproxen tablets)). Both groups then received an identical 12-week standard rehabilitation program. To avoid additional overload in the initial week, due to the potential pain reduction in the intervention group, patients were instructed to reduce the load on the Achilles tendon to about half their usual load, and further to completely abstain from activities exerting large forces on the Achilles tendon e.g. sprinting and jumping activities. The rehabilitation program consisted of 12 weeks homebased resistance training 3 times per week with 4 exercises in total; two directly targeting the gastrocnemius and soleus muscle (heel lifts with extended knees and heel lifts with flexed knees) and two elastic band exercises targeting the hip abductors (see full details on the training protocol in supplementary, table 1), in the rehabilitation period patients were further instructed to abstain from activities triggering and worsening symptoms, especially activities triggering morning stiffness and pain. Further to guide load management, the patients were introduced to the Numerical Ranking Scale for pain (NRS (range: 0-10). They were informed that activities with pain from 1-2 were considered safe, 3-5 were acceptable and activities resulting in pain between 5-10 should be avoided.

Patients were instructed to use a custom-made app (Injurymap Science, Injurymap ApS, C/O SUND Hub, Nørre Allé 41 Copenhagen) to register the rehabilitation training throughout the

intervention period, furthermore participants were able to access videos and documents with detailed descriptions of the training program through the app.

Outcome measures

Outcome measures included questionnaires, ultrasound (US) and MRI scans. MRI scans were performed at baseline and at 3-month follow-up. US was performed at baseline, 1-week, and 3-month follow-up. Questionnaires were performed at baseline, 1-week, 3-month and 1-year follow-up.

Primary outcome

VISA-A

Changes between baseline and 3 months follow-up in The Victorian Institute of Sports Assessment-Achilles Questionnaire (VISA-A) (score from 0-100; 0 meaning lowest possible function of the Achilles tendon, 100 meaning full function of the Achilles tendon) was chosen as the primary outcome measure in this study. Changes in VISA-A between other timepoints were considered secondary outcomes.

Secondary outcomes

US

Bilateral ultrasound recordings were performed at baseline, at the 1-week visit and at the post visit. All recordings were performed using the same US scanner (HI Vision Hitachi Ascendus (Hitachi Medical systems, Japan)) with a set of standardized setting for all recordings. Two observers performed the recordings, the same observer did pre and post recordings within the same patient. For bilateral power doppler recordings a short linear transducer (EUP-L75, frequency 18-5 MHz, radius 38 mm, Hitachi Medical Systems, Japan) was used (Doppler frequency: 10 MHz, pulse repetition frequency 250 Hz, doppler gain: 37, frame rate: 4 Hz (full settings attached in supplementary table 2)). Patients were placed in a prone position with their feet in a hanging relaxed outside the patient bed, a sufficient layer of acoustic gel was used to avoid unnecessary transducer pressure to be applied, and the probe was held perpendicular to the Achilles tendon in a stationary position during examination to avoid flash artefacts. One longitudinal recording (20 frames) was performed with the calcaneal bone visual in the distal part of the image, and one recording was performed just proximal to the first recording, to ensure coverage of the entire tendon (supplementary, figure 1A). For greyscale recording a long linear transducer (EUP-L53L, frequency 10-5 MHz radius 92 mm, Hitachi Medical Systems, Japan) was used (full settings attached in supplementary table 2). Patients were placed in a prone position with their feet outside the patient bed, in order to stretch the Achilles tendon with a minimum amount of load the feet were placed flat against the wall ensuring 90 degrees flexion of the ankle joint (supplementary, figure 1B). Acoustic gel was applied, and the transducer was held perpendicular to the Achilles tendon in the longitudinal direction. 2 still frames were acquired on each side.

Quantitative US analyses was performed in FIJI image J (version 2.0.0-rc-68/1.52e). Doppler recordings were analysed using a customized macro returning the area of coloured pixels as a measure for neovascularization. The polygon tool was used to remove potential flash artefacts. Further the polygon tool was used to outline the tendon. Doppler area within the tendon is reported. Greyscale images were analysed using the measurement tool, measuring thickness 2 cm above the most proximal part of the calcaneal bone (thickness) and at the thickest point (max thickness).

MRI

An MRI scan of the affected Achilles tendon was performed at baseline and at the 3-month follow-up. The scans were performed in a 3T Siemens MAGNETOM Verio scanner. Patients were scanned in supine position using a dedicated ankle receive coil, foam pads were used for fixation to avoid movement during scanning. An axial and a sagittal scan were performed with the following parameters; TE: 17; TR: 500; matrix: 512 × 512; FOV: 150 mm; Slice thickness: 3 mm. The image analyses were performed in a freeware DICOM viewer, Horos (Horosproject.org, Nimble Co LLC d/b/a Purview in Annapolis, MD USA., V 4.0.0 RC3). Before analyses, sagittal and axial scans were opened in the same window (full screen, coupled split-screen view) to guide placement of start and end slide. Pre-set values for contrast, zoom and brightness, were applied prior to analyses. A blinded assessor segmented the free Achilles tendon (between the most distal part of soleus and the insertion at calcaneus). Cross sectional area (CSA) on all axial sections was measured using the polygon tool in Horos, subsequent to segmentation a qualitative evaluation was performed, and adjustments of segmentations was made using the “Repulsor tool”. The mean CSA is reported.

UTE MRI

A subgroup of the participants was also scanned using a 3D isotropic UTE (ultra-short time to echo) MRI sequence (FOV: 160 x 160 mm TR: 11 ms varying TE (0.07, 0.57, 1.07, 1.57), matrix resolution: 1.45x1.45x1.00 mm Flip angle: 12°, scan time: 3 m 14 s) These scans were used to quantify T2* relaxation times in the tendon. T2* values reflect the amount of unbound water inside the tissue, and is regarded a proxy measure for collagen structure.[5,16,20]

The UTE scans were analysed according to a previously described protocol.[2] In short UTE recordings with varying TE were automatically loaded into a custom-made program, TE was plotted against signal intensity on a voxel-by-voxel basis, and a mono-exponential fitting incorporating noise reduction was applied to reconstruct T2* maps and goodness of fit maps used for analyses. Manual segmentation was subsequentially performed on every 4th slide in ITK snap (version 3.6.0) and the interpolate labels tool was used to calculate the full tendon volume. The most proximal slide of the free tendon was defined as the first slide were the soleus muscle was not visible, whereas the most distal slide was defined as the last slide were the calcaneal bone was not in contact with the tendon. The segmentations, goodness of fit maps and T2* maps were then combined using a custom-made macro in FIJI ImageJ (version 2.0.0-rc-68/1.52e) to extract T2* values from the full volume of the free tendon.

Questionnaires

Physical activity and NRS

Questionnaires on pain and activity were made as a 1-week recall questionnaire. Patients reported their physical activity level, number of sessions and total hours of activity pr. week are reported. Pain was reported using the NRS scale (range: [0-10]; 0 represents no pain, 10 represents the

worst pain that can be imagined). Pain during activity, pain at rest, morning pain and worst pain experienced during the last week were reported. Further, an induced pain test was performed as part of the questionnaire, participants were asked to perform 25 vertical jumps on each leg and report the pain at the last jump.

Statistics

All statistical analysis was carried out using SAS studio (Release: 3.8 (Basic edition)). Parametric testing on repeated measures was carried out using a constrained linear mixed model, including time and group as fixed effects. We assessed the effect of treatment by examining two-way interactions (time x group). Furthermore, an exploratory analysis was made to test whether symptom duration had any effect on clinical outcomes at 3 months and 1 year. To test this time and symptom duration were included as fixed effects, and the effect of symptom duration was assessed by examining two-way interaction (time x symptom duration). We applied an unstructured covariance matrix to account for correlation in repeated measures. An alpha=0.05 was used to test for significance. Baseline differences between groups were tested using unpaired t-test for parametric outcomes, whereas Wilcoxon sign rank test was used to test non-parametric outcomes. Non-parametric testing for categorical baseline characteristics where carried out using Fisher's exact test. Results are presented as means ± standard error, unless stated otherwise. The required sample size to detect a 10 points difference between the groups with 80% power and an alpha level of 0.05 was estimated to 50. To account for potential dropouts, we aimed to include 70 patients. However, we were only able to include 69 subjects total before the study drug expired.

Results

Participants were included between January 2018 and April 2019 (figure 1), and a total of 225 subjects were screened by phone. Of these, 69 were found eligible for participation and were randomized into two groups; 34 allocated to the Naproxen group and 35 allocated to the placebo group. In total 60 participants (Placebo (n=30) Naproxen (n=30)) completed the 3-month follow-up (primary end point) and 53 (Placebo (n=25) Naproxen (n=28)) participants completed the 1-year follow-up. There were no significant differences between the groups in baseline characteristics (table 1). Adherence to the drug intervention was 92 % for the naproxen group and 97 % for the placebo group with significant difference between the groups (p=0.49). Adherence to the exercise intervention (sessions attended/sessions planned x 100) was 74% (Naproxen group) and 73% (Placebo group) with no significant differences between the groups (p=0.88).

The most common injury triggering activity was running, which accounted for 70% of the injuries in the study population, 51% suffered an injury in the dominant leg, and 70% had unilateral pain at inclusion with no significant differences between the groups.

Questionnaires

At 3-months follow-up there was a significant increase in VISA-A score in both groups (14.5 ± 2.8 $p<0.0001$) (figure 2 and table 2) compared to baseline with no interaction between groups. At 1-year the VISA-A score was further increased compared to 3-month follow-up (7.6 ± 3.3 $p<0.05$). No significant time effect or interactions in VISA-A score were found between baseline and 1-week follow-up. Interestingly, after 3 months VISA-A improved significantly more in patients with symptom duration <1-month at inclusion compared to patients with symptom duration >2 months

(11.7 ± 4.2 $p<0.01$) (figure 3). After 1 year there was no significant effect of symptom duration at inclusion (-6.9 ± 5.3 $p=0.2$).

After 3 months all NRS items were significantly decreased compared to baseline (figure 2 and table 2). At 1-week follow-up only NRS in the morning and maximum pain during the last week were significantly decreased in both groups with no interaction between the groups (table 2). At 1-year follow-up morning pain (-0.6 ± 0.3 $p<0.05$) and the induced pain test were significantly reduced compared to 3 months (-1.0 ± 0.3 $p<0.05$), all other NRS items where not significantly reduced, but did numerically decrease.

Weekly physical activity at baseline was significantly reduced compared to pre-injury levels (-2.7 ± 0.6 h/week) (figure2), and furthermore a significant decrease in overall activity level was observed between baseline and 1-week follow-up (-1.2 ± 0.3 h/week; $p<0.05$). At 3-months physical activity had returned to baseline levels but was still significantly lower compared to pre-injury levels (-3.4 ± 0.7 h/week; $p<0.0001$)). Weekly physical activity remained lower compared to pre-injury level and was not significantly different between 3-months and 1-year follow-up (-0.1 ± 0.7 h/week; $p=0.86$).

Ultrasound and MRI

At baseline patients with unilateral symptoms (n=49) had significantly thicker tendons determined by US in the symptomatic side compared with their own asymptomatic contralateral side (0.12 ± 0.03 cm $p<0.0001$), also the symptomatic side showed significantly higher Doppler area (0.3 ± 0.1 cm^2 $p<0.005$)) than on the healthy non-symptomatic side. No significant changes were observed between baseline and 3-month follow-up for neither Doppler area nor thickness (figure 4). MRI mean area showed no significant difference between baseline and 3-months in either of the groups (+0.01 ± 0.06 and 0.03 ± 0.05 cm^2, for naproxen and placebo respectively) (figure 4).

UTE MRI

A total of 74 unilateral UTE T2* MRI scans were performed and analysed, hereof 44 were baseline scans and 30 were at 3-months follow-up (Naproxen pre: n=19 post: n=14; Placebo pre: n=25 post: n=16). No significant differences were observed between baseline and 3-months in either of the groups (figure 4).

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Discussion

The results of this study demonstrate that anti-inflammatory treatment with NSAID does not have any additive short-or long-term effect on the clinical outcomes in the early phase of tendinopathy. Neither does addition of NSAID add to the treatment of early Achilles tendinopathy in regard to physiological parameters determined by ultrasonography and MRI. To our knowledge, this is the first study to specifically investigate the additive effect of an anti-inflammatory treatment to regeneration in early phase tendinopathy.

In contrast to our findings, a few clinical studies and some animal studies have previously suggested beneficial effects of NSAIDs on chronic tendinopathy,[1,14,34] our results though agree with newer clinical studies in chronic Achilles tendinopathy that were not able to detect an effect of NSAID[4,17] This is noteworthy given the widespread use of NSAIDs for soft tissue injuries seen among sports-active individuals. Also, a growing body of evidence suggests a substantial contribution of inflammation in tendinopathy.[10–12,23,24] This inflammatory component is more evident the early phase of tendinopathy compared to the chronic phase.[11,23] and may therefore be involved in the pathogenesis of tendinopathy.[10] These observations support the use of anti-inflammatory drugs specifically in early tendinopathy; however, the data herein does not demonstrate any measurable effect.

While NSAIDs have classically been described as anti-inflammatory drugs, subsequent results have demonstrated, seemingly contradictory, potentially pro inflammatory effects in vitro.[15] Further, in humans the load induced collagen production in healthy tendon can be blunted by NSAID,[9] and NSAIDs are able to inhibit proliferation and proteoglycan synthesis in vitro,[29] implying a weaker repair response. However, the results of the current study show that any possible effect of early oral anti-inflammatory treatment was not measurable or clinically

neglectable. The reason for this may be that oral NSAIDs does not reach the tendon fibroblasts in the first place; in a previous study on chronic Achilles tendinopathy, mRNA targets that are normally highly susceptible to NSAID exposure were not altered by high dose NSAID treatment,[17] and also high dose NSAID was not able to affect COX-2 expression in either healthy or diseased Achilles tendon tissue.[28] In addition to the effects on inflammation, NSAIDs also work as analgesics, which is a frequent argument for use in tendon disorders. However, surprisingly though we did not see any effect on pain at one-week follow-up, at rest or when provoked. Although, previous results have indicated that pain during running was not affected in chronic Achilles tendinopathy.[28] we expected morning pain and pain during the day to be lower, at least in the resting state. To ensure that the two groups were comparable we also markedly reduce the load on the Achilles tendon during NSAID treatment, which might have precluded a potential analgesic effect. Nonetheless, any potential pain-relief seems marginal at this stage of disease.

The present study was not designed to investigate the effect of physical rehabilitation in early tendinopathy, since we did not include a non-training control group. We did however see pronounced time effects on pain and function both after 3 months and 1 year, exceeding minimal clinical important difference for both NRS (>2 points)[30] and VISA-A (>10 points)[25] at both time points, and after 1 year, these values approach normal values. Thus, an effect of early physical rehabilitation and load management is suggested and in agreement with the effect observed in chronic tendinopathy.[7] Additionally, early tendinopathy shares a set of common features with chronic tendinopathy. The clinical presentation is somewhat similar, although symptoms are less severe, and patients present with less severe biochemical changes.[33] Considering the similarities, and the well-established effect of training in chronic tendinopathy, we believe that physical

rehabilitation from an ethical standpoint should be offered to all Achilles tendinopathy patients, also at an early stage of disease.

Interestingly, we found that clinical improvement at 3 months was dependent on the duration of symptoms at inclusion. Specifically, we found that patients with a symptom duration <1 month compared to patients with symptom duration >2 months had a significantly better treatment response as measured by VISA-A after 3 months. This was the case despite that the two groups had a similar VISA-A level at inclusion in the study. Further, in a global assessment of symptoms, all patients with symptom duration <1 month reported an improvement of symptoms after the treatment whereas 6 patients with symptoms >2 month did not. These findings indicate that patients might benefit from targeted interventions as early as possible after symptom onset.

Despite clinical improvements were observed over time, no changes were observed in tendon thickness or vascularisation measured with ultrasound, cross-sectional area measured with MRI or structure evaluated using UTE T2* MRI. Thus, clinical improvements occurred in the absence of any detectable structural alterations. Some studies have found changes in tendon vascularisation and dimensions when investigating chronic tendinopathy after prolonged physical rehabilitation[19,26,27] However, since alterations on ultrasound and MRI are less pronounced in early tendinopathy, clearly a decrease in thickness and or vascularization could be more difficult to detect. No longitudinal studies so far have evaluated changes in T2* values obtained from UTE MRI in early or late tendinopathic tendons, but higher values have been observed in chronic tendinopathy compared to healthy tendon, and these have been suggested to correlate with clinical outcomes.[20] In our data we did not see any significant changes in T2* values over the 3 months intervention period, and thus any structural alterations might happen at a slower rate

than clinical improvement. Alternatively, early tendinopathy simply does not cause any marked change in T2* values, and as we did not directly compare the symptomatic and the asymptomatic leg, it remains open whether T2* is increased in early tendinopathy. However, we did observe a side-to-side difference in doppler signal and tendon thickness on ultrasound in patients with unilateral symptoms, suggesting structural alterations.

As clinical symptoms improve over time, we would also expect the habitual level of physical activity in the patients to increase again. However, we did not see any significant changes between baseline and 1-year follow-up, and weekly activity remained significantly lower compared to pre-injury levels at all time points. It can be speculated that after injury and rehabilitation, patients might be more conscious about the tendon and potential pain therein and may thus be overcautious when returning to sports. To address this, extended interventions after a typical 3 months physical rehabilitation program, aiming to aid patients in return to pre-injury activity levels have been proposed,[32] and such interventions could potentially have optimized return to sports, but were not implemented in the current study. We did however encourage patients to return to sports and they were instructed in pain guided training progression. Nevertheless, we observed that despite the clinical improvements, patient did not within a year after injury return to pre-injury levels of activity.

The lack of a non-training control group is a limitation in the current study, and excludes any conclusion of rehabilitation effect upon early tendinopathy per se. Taken together, we included patients in regard to clinical symptoms and findings, and thus we cannot rule out that pathological changes found in the tendon tissue might have been present at an even earlier stage of disease,

than the symptoms. However, our patient population clearly represents a less clinically severe tendinopathic population compared to a more chronic tendinopathic population. Further we did not perform any tissue biopsies, which excludes us from any direct measure of inflammation in the tissue, and thus we only had indirect markers for inflammation. Still, despite these limitations we believe the study was an important contribution due to the widespread use and recommendation of NSAID use in the early phase of tendinopathy.

In conclusion a short-term anti-inflammatory treatment in the early phase of tendinopathy (lasting < 3 months) had no additive effect upon clinical or physiological outcomes 3 month or 1 year after onset of clinical symptoms. Further, we were unable to demonstrate any short-term analgesic effect of NSAID in tendinopathy. In conjunction with the well-known side effects of NSAIDs,[6] careful consideration before use in early tendinopathy should be stressed. Although the study was not designed to investigate the effect of physical rehabilitation, we did see a significant time effect on pain and clinical symptoms in the absence of any detectable structural alterations in the tissue. In spite of symptom improvements there was a long-lasting decrease in the physical activity level of the patients. Finally, we also observed that longer symptom duration was associated with inferior return to pre-injury functional levels. Thus, our results suggest that targeted physical rehabilitation in terms of load management and resistance training should be employed as soon as possible after symptom debut to facilitate fast recovery. And that anti-inflammatory medication does not add to that process.

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	Naproxen	Placebo	t-test
Age (y)	41 ± 2.1	40.7 ± 1.7	0.9
BMI (kg/m^2)	24.4 ± 0.5	25.1 ± 0.4	0.3
Duration (days)	43.4 ± 3.7	52.3 ± 3.5	0.1
Training (hrs/week)	7.4 ± 0.7	8.5 ± 0.9	0.3
NRS – during activity	3.8 ± 0.35	4.0 ± 0.3	0.7
Sex (%females) †	32.4	25.7	0.6

Table 1 Baseline characteristics. No differences were observed between the two intervention groups. Abbreviations: BMI, body mass index, NRS, numerical ranking scale.
†: Fishers' exact test.

		Preinjury	Baseline	1 week	13 weeks	52 weeks
VISA-A – score *, †	Naproxen	-	67.3 ± 2.1	72.1 ± 1.8	84.0 ± 2.3	88.7 ± 3.3
	Placebo	-	70.5 ± 2.4	72.0 ± 2.1	83.2 ± 2.4	90.6 ± 2.1
NRS-during activity *	Naproxen	-	3.8 ± 0.4	3.2 ± 0.4	1.4 ± 0.3	1.0 ± 0.4
	Placebo	-	4.0 ± 0.3	3.4 ± 0.4	1.8 ± 0.3	1.2 ± 0.3
NRS - at rest *	Naproxen	-	0.9 ± 0.2	0.6 ± 0.2	0.1 ± 0.1	0.4 ± 0.3
	Placebo	-	0.9 ± 0.2	0.8 ± 0.2	0.4 ± 0.2	0.0 ± 0.0
NRS – morning ‡, *, †	Naproxen	-	2.8 ± 0.4	1.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.2
	Placebo	-	3.2 ± 0.3	2.1 ± 0.3	1.2 ± 0.3	0.5 ± 0.2
NRS - max pain last week ‡, *	Naproxen	-	4.6 ± 0.4	3.2 ± 0.3	1.7 ± 0.3	1.5 ± 0.4
	Placebo	-	4.9 ± 0.4	3.6 ± 0.3	2.0 ± 0.4	1.1 ± 0.3
NRS - jump test *, †	Naproxen	-	3.5 ± 0.4	2.2 ± 0.3	0.6 ± 0.2	0.6 ± 0.3
	Placebo	-	3.3 ± 0.4	2.9 ± 0.4	1.3 ± 0.3	0.3 ± 0.1
Activity (sessions/week) #	Naproxen	5.4 ± 0.4	3.8 ± 0.4	2.8 ± 0.3	4.0 ± 0.6	3.9 ± 0.5
	Placebo	6.2 ± 0.6	4.0 ± 0.5	3.7 ± 0.6	3.6 ± 0.4	4.0 ± 0.5
Activity (hrs/week) ‡, #	Naproxen	7.4 ± 0.7	4.4 ± 0.6	3.3 ± 0.5	5.4 ± 0.8	4.8 ± 0.7
	Placebo	8.5 ± 0.9	6.0 ± 0.9	4.6 ± 0.7	5.1 ± 0.7	5.2 ± 0.9
USPD (cm^2)	Naproxen	-	0.43 ± 0.13	0.37 ± 0.10	0.48 ± 0.21	-
	Placebo	-	0.27 ± 0.08	0.26 ± 0.08	0.24 ± 0.10	-
Max Thickness (cm)	Naproxen	-	0.77 ± 0.03	0.74 ± 0.03	0.74 ± 0.03	-
	Placebo	-	0.70 ± 0.03	0.67 ± 0.03	0.69 ± 0.04	-
Thickness (cm)	Naproxen	-	0.67 ± 0.03	0.63 ± 0.02	0.62 ± 0.03	-
	Placebo	-	0.56 ± 0.02	0.55 ± 0.02	0.56 ± 0.03	-
MRI area mean (cm^2)	Naproxen	-	0.86 ± 0.04	-	0.87 ± 0.04	-
	Placebo	-	0.78 ± 0.03	-	0.81 ± 0.04	-
T2* (ms)	Naproxen	-	1.45 ± 0.15	-	1.17 ± 0.07	-
	Placebo	-	1.21 ± 0.09	-	1.21 ± 0.10	-

Table 2 Result overview. Clinical and paraclinical endpoints at all time points. Presented as mean values ± SEM.
‡ = significant time effect between baseline and 1 week,
* = significant time effect between baseline and 13 weeks
† = significant time effect between 13 weeks and 1 year.
# = significant time effect between pre-injury and baseline.

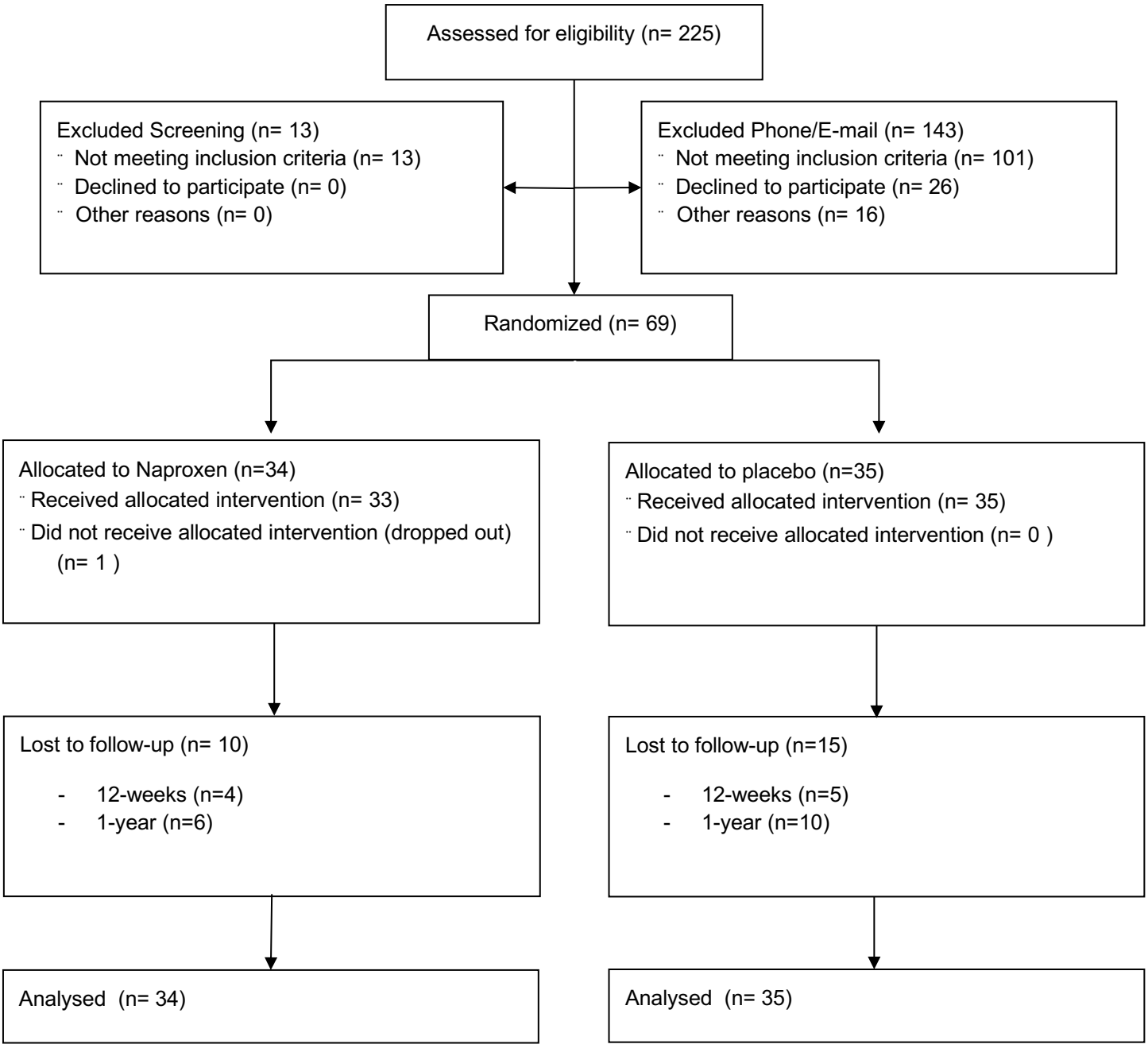


Figure 1
Flowchart of participants.

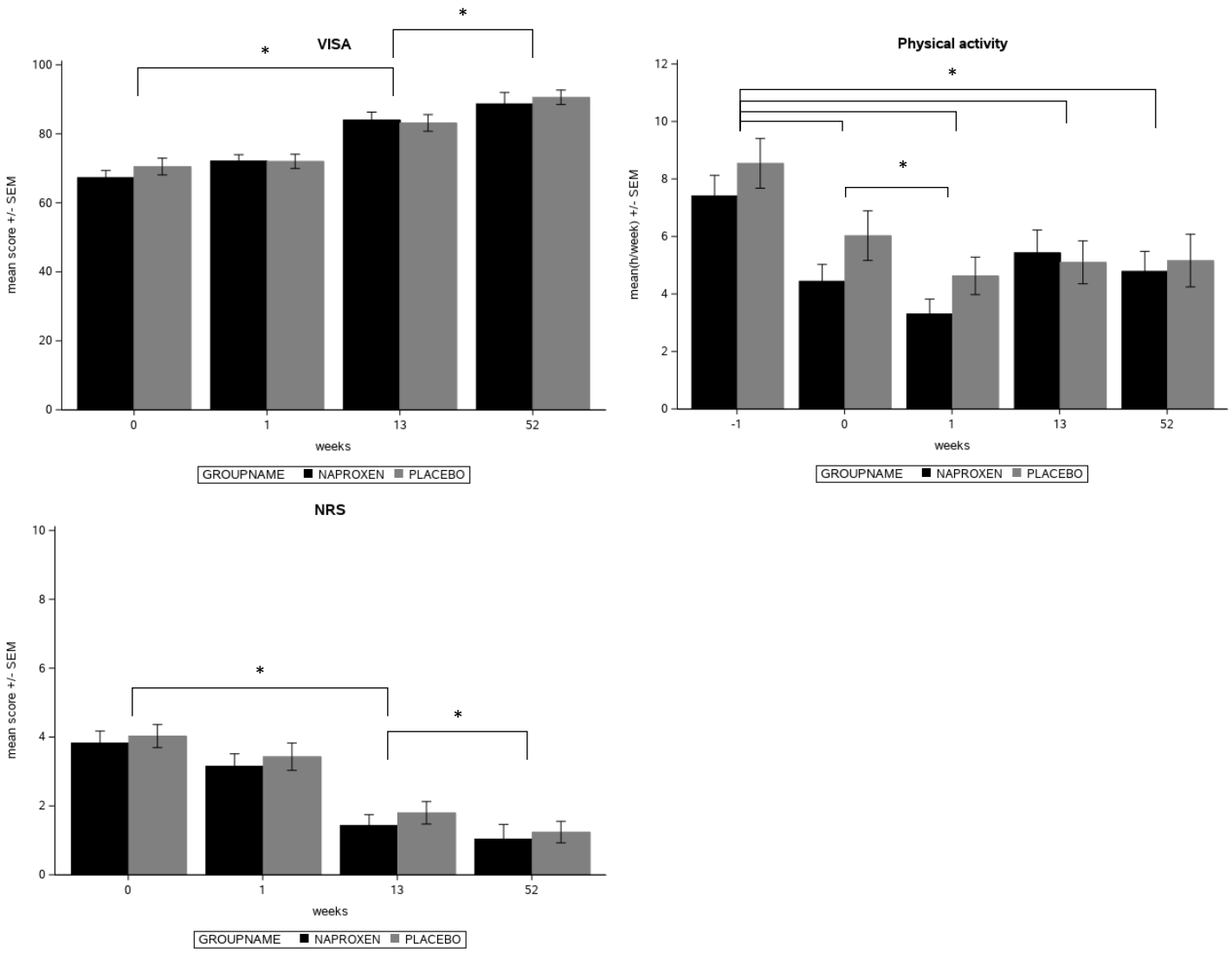


Figure 2

VISA-A, NRS during activity and weekly activity level by group. Week -1 indicates pre-injury, week 0 indicates baseline, week 1 indicates 1-week follow-up (last day of Naproxen treatment), week 13 indicates 3-months follow-up (end of physical rehabilitation period), week 52 indicates 1-year follow-up. (*) indicates significant time effect ($p<0.05$) between marked timepoints

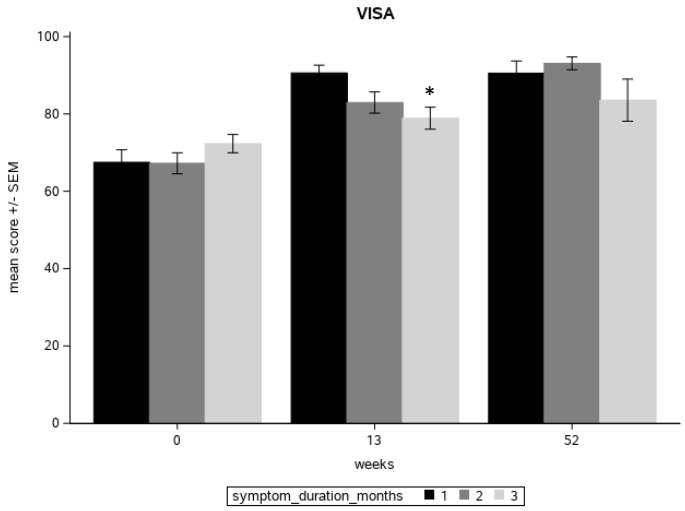


Figure 3

VISA-A score by duration of symptoms at baseline. week 0 indicates baseline, week 13 indicates 3-months follow-up (end of physical rehabilitation period), week 52 indicates 1-year follow-up. (*) indicates a significant interaction (reference weeks=0; symptom_duration_months=1)

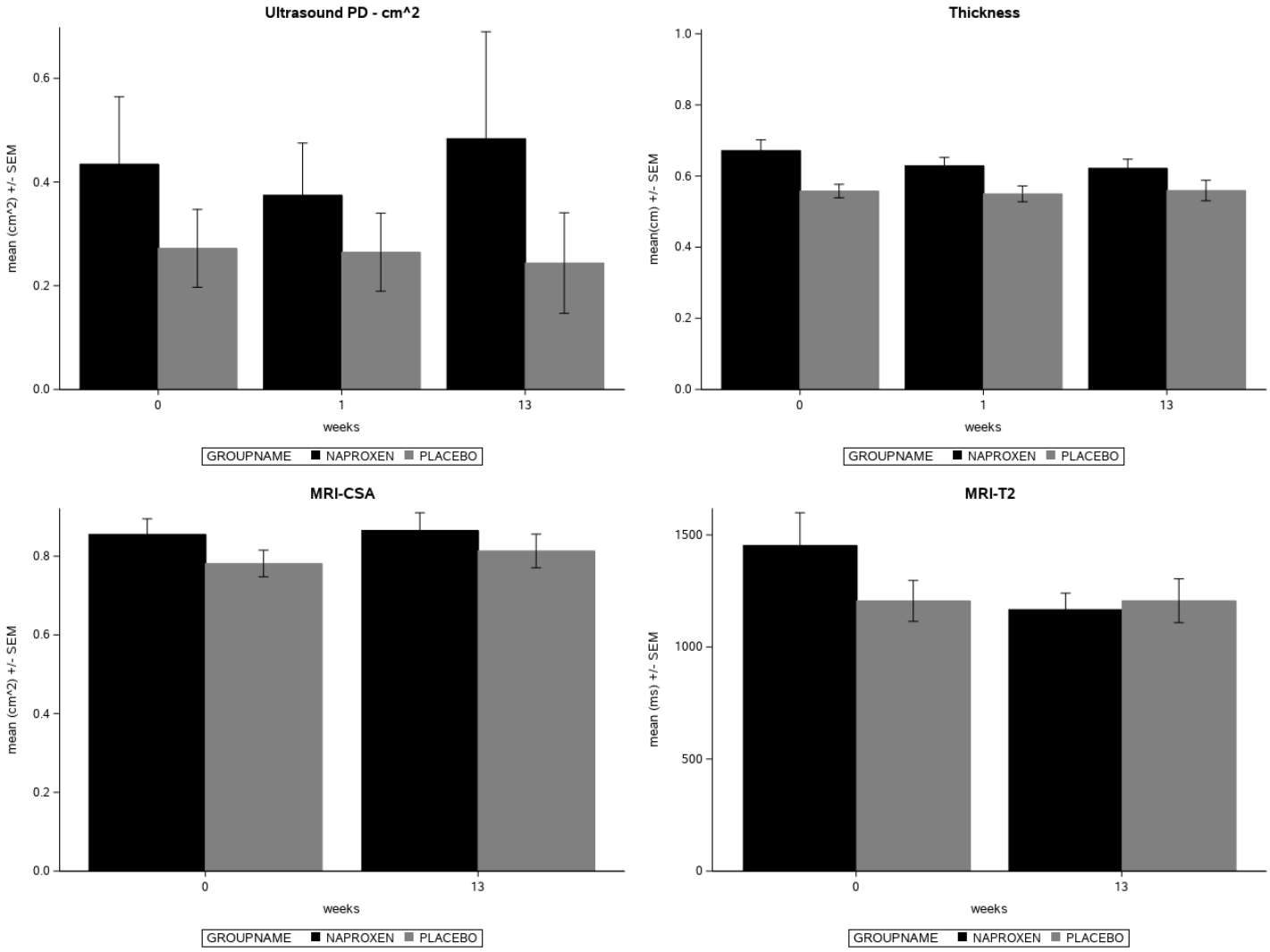


Figure 4

Power doppler ultrasound (US), US thickness, cross-sectional area (MRI) and T2 values. Week 0 indicates baseline, week 1 indicates 1-week follow-up (last day of Naproxen treatment), week 13 indicates 3-months follow-up (end of physical rehabilitation period).*

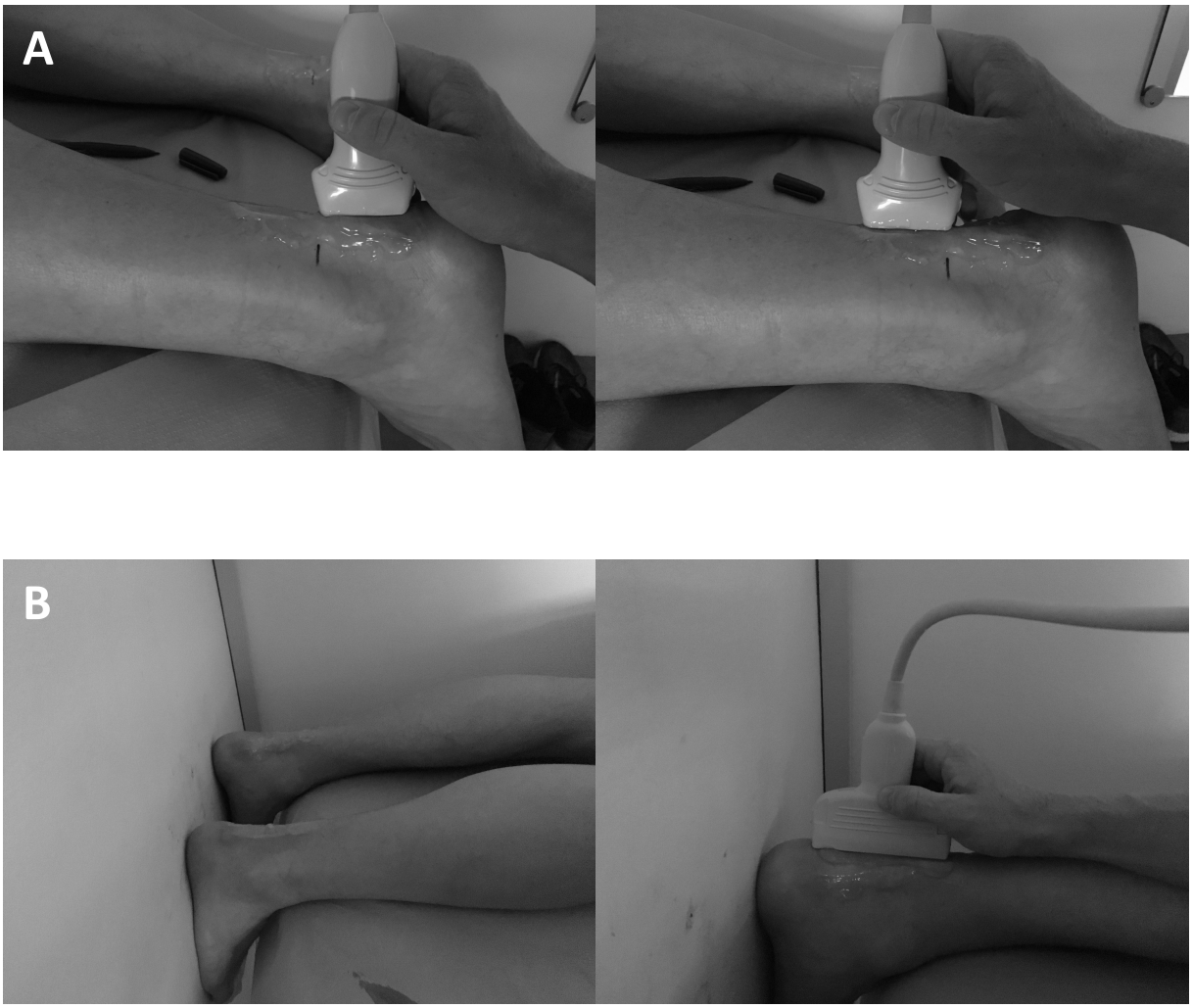
Exercise	Week	Repetitions
Heel raises – knees straight	1-3	3x15
	4-12	3x10
Heel raises – knees bend	1-3	3x15
	4-12	3x10
Lateral band walk – (elastic band) †	1-12	∞
Straight leg kick back – (elastic band) †	1-12	3x15

Supplementary table 1
12 weeks home-based heavy slow resistance training program. Patients were instructed in load progression for the 12-week period.
†: exercises activating gluteal muscles.
∞: performed until exhaustion.

	Doppler	Greyscale
Probe	Short linear transducer (EUP-L75)	Long linear transducer (EUP-L53L)
Depth	2 cm	4.5 cm
Dynamic range	70	70
Doppler frequency	10 MHz	-
Pulse repetition frequency	250 Hz	-
Gain	12	20
Doppler gain	37	-
Angle (range: 0-6)	6	6

Supplementary table 2

Settings for power doppler and greyscale ultrasound scans.



Supplementary figure 1 Ultrasound setup.

A: For power doppler ultrasound (US) one recording (20 frames) was performed distally (calcaneal bone visible in the distal part of the image) and one recording was performed just proximal from the first recording (a surgical marker was used to mark the proximal edge of the first recording).

B: For greyscale US patients foot soles were gently placed against the wall in order to stretch the Achilles tendon with a minimum amount of load. 2 still frames were recorded on each side (the probe was lifted from the skin between each recording).