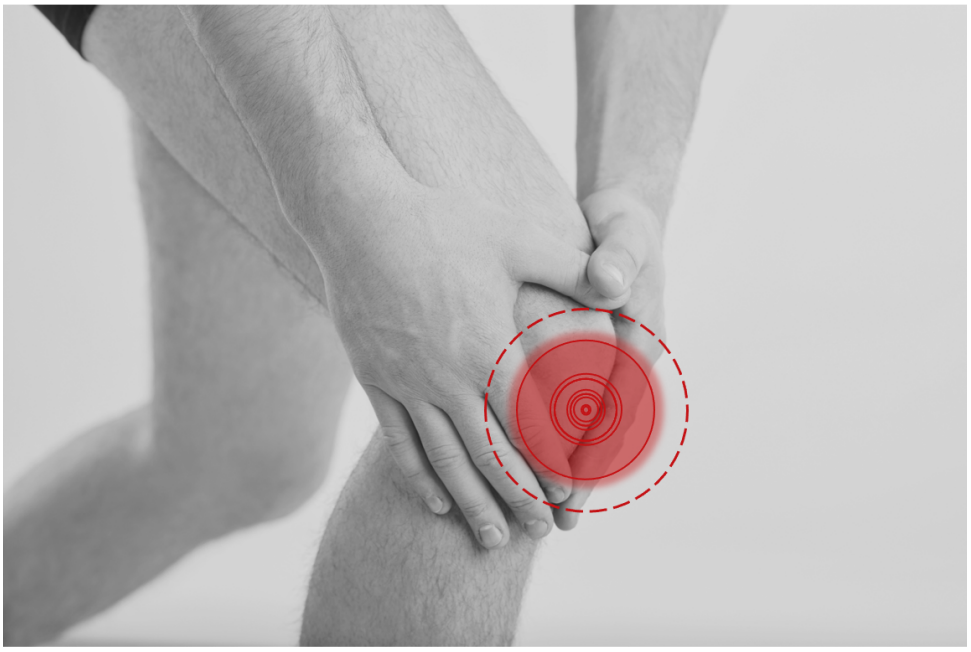
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

PhD Thesis

Anne-Sofie Agergaard

Load magnitude in exercise-based treatment of Patellar tendinopathy

Effect on clinical outcome, tendon structure and function



Supervisors: Peter Magnusson and Michael Kjær

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen November 8th, 2020

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1. PREFACE AND ACKNOWLEDGMENTS

This PhD thesis is based on results from three years work performed at the Institute of Sports Medicine Copenhagen (ISMC) and the Department of Physical and Occupational Therapy, Bispebjerg-Frederiksberg Hospital and the Faculty of Health Sciences, University of Copenhagen. The studies were funded by The Independent Research Fund Denmark, Medical and Health Sciences, the Danish Rheumatism Association, and Danish Society of Sports Physiotherapy.

First, I own a sincere gratitude to my principal supervisor Peter Magnusson for the trust placed in me conducting my PhD in your research group and for your endless and experienced support throughout the entire process. Thank you, for introducing me to tendon science and arouse my curiosity and be fascinated of the tendinous tissue. Your scientific work is inspiring and admirable. I also want to thank my supervisor Michael Kjær for sharing his extensive knowledge and for creating an inspiring work environment at ISMC. Also I wish to express a special and great acknowledgement to my co-supervisors; Christian Couppé for your work and unlimited assistance in the RCT study, and Rene Svensson, for always taking you time and for your ability to explain complicated theoretical and statistical issues in an understandable way. Your dedication to science and troubleshooting skills is admirable.

Thanks to all my colleagues and friends at the ISMC and the Research Unit at the Department of Physical and Occupational Therapy for contributing to an enjoyable work environment. In particular, thanks to the biomechanics group for inspirational scientific discussions and fun hours. To all the secretaries, especially Else Pedersen and Maria Bækgaard Kjær, your help with anything and everything has been highly appreciated. To my office mates, the “A-team”; Anne Gylling, Adam Jørgensen, Annesofie Thorup Olesen and Rikke Høffner, I appreciate all our scientific discussion and daily talks. Without you I would not have made it. I also own a special thanks to my PhD fellow Nikolaj Malmgaard-Clausen, for doing an amazing job with ultrasound examination during the project, collaboration in the MRI UTE adventure and for sharing frustrations of life as a PhD student. I admire your positive mindset and will miss your never-ending (bad) jokes.

I would also like to thank the Department of Radiology, Bispebjerg-Frederiksberg Hospital. In special Philip Hansen, Mikael Boesen and Janus Dam Nybing for your contribution with developing the MRI UTE method. It has been a pleasure to collaborate with you. To Nichlas Warming and Mikkel Holm Hjortshøj for an amazing job taking care of the supervised training in the RCT study. Furthermore, I greatly appreciated the assistance from the students who have taken part in the studies. To all the participants in the studies I also wish to express my great gratitude. Without you, it would not have been possible to conduct any research.

Finally, this would never been possible without the love and support from my family and friends. A special thanks to my parents and my sister for your belief and support in my sometimes ambitious projects and for always changing your plans and help out when there was not enough hours in the day. To my girls, Nanna and Ida, you were not a part of the original plan for this PhD project and you definitely did not make it easier. Yet, you make my lift outside science so much more fun and full of love, and I own you great thanks for helping me to retain focus on that there are other things in life than science. Last, but no mean least, I own my deepest gratitude to my husband Jakob, for always being there and your understanding throughout my entire PhD. Together, we stay on the right track to balance family life with scientific carriers. Your unconditional love means everything to me.

Anne-Sofie Agergaard, Copenhagen November 6th

2. LIST OF PAPERS

This thesis is based on the following three papers:

Paper I

Anne-Sofie Agergaard, Rene B. Svensson, Nikolaj M. Malmgaard-Clausen, Christian Couppé, Mikkel H. Hjortshoej, Simon Doessing, Michael Kjaer, S. Peter Magnusson, *Clinical outcome, structure and function improve with both heavy and moderate load in the treatment of patellar tendinopathy: A Randomized clinical trial*, Accepted for publication in AJSM

Paper II

Anne-Sofie Agergaard, Rene B. Svensson, Rikke Høffner, Philip Hansen, Christian Couppé, Michael Kjaer, Peter Magnusson, *Mechanical properties and UTE T2* relaxation time in Patellar tendinopathy: The effect of load magnitude in exercise-based treatment*, Submitted to Scand J Sci Sports (under review).

Paper III

Anne-Sofie Agergaard*, Nikolaj M. Malmgaard-Clausen*, Rene B. Svensson, Janus D. Nybing, Mikael Boesen, Michael Kjaer, S. Peter Magnusson, Philip Hansen,*Equal contributors, *UTE T2* mapping of tendinopathic patellar tendons – An MRI reproducibility study*, Published in Acta Radiologica 2020.

Paper III is also a part of Nikolaj M. Malmgaard-Clausen (NMM) PhD Thesis. Data in the article is based on a population of participants from the RCT study included in this PhD thesis by Anne-Sofie Agergaard (AA). Logistic work regarding the data collection was performed by AA. NMM and AA contributed equally to development the method setup, and analysis and interpretation of data. Further both authors, contributed equally to drafting the manuscript. The final manuscript preparation and submission was performed by NM

3. ABBREVIATION LIST

AP-thickness: Anterior-Posterior thickness
BMI: Body Mass Index
CMJ: Counter Movement Jump
COSMIN: COnsensus-based Standards for the selection of health Measurement INstruments
CSA: Cross Sectional Area
ECC: Eccentric exercising
GAG: Glycoaminoglycans
HSR: Heavy Slow Resistance exercising
KEM: Knee Extensor Moment
MCID: Minimal Clinical Important Difference
MRI: Magnetic Resonance Imaging
MRI UTE: Magnetic Resonance Imaging Ultra-Short Time to Echo
MSR: Moderate Slow Resistance exercising
MVC: Maximal Voluntary Contraction
NRS: Numeric Rating Scale
PG: Proteoglycan
PROM: Patient Reported Outcome Measure
RCT: Randomized Clinical Trial
RF-PULSE: Radio Frequency pulse
RM: Repetition Maximum
SLDS: Single Leg Decline Squat
SJ: Squat Jump
TE: Time to Echo
US-GS: Ultrasound Gray-scale
US-CD: Ultrasound Color Doppler
US-PD: Ultrasound Powe Doppler
VAS: Visual Analog Scale
VISA-P: Victorian Institute of Sport Assessment-Patella questionnaire

4. ENGLISH RESUME

Tendons play an essential role in transmitting force from muscle to bone and are thus designed to resist considerable loads during locomotion. Yet, repetitive use often results in overuse injuries such as tendinopathy, which accounts for 30-50% of all sports related injuries. This type of injury is a substantial clinical challenge, since it often causes long-term (months to years) impairment of function for the affected person. Tendinopathy is characterized by pain during activity, localized tenderness upon palpation, swelling of the tendon and impaired performance. The list of currently available interventions for tendinopathy is extensive. However, loading-based interventions have become the preferred treatment for tendinopathy, although, the optimal dose in regards to number of repetitions and sets, frequency, and load remains unknown. Thus, understanding how tissue structure, function and mechanical behavior in tendinopathic tendons adapt to varying loading configuration, and how this influence the clinical outcome is important and may contribute to optimizing the rehabilitation thereof.

The aim of the thesis was to bridge the gap in knowledge related to the effect of load magnitude on clinical outcome, tendon structure and function in exercise-based treatment of patellar tendinopathy. We conducted a randomized clinical trial on a population of male recreational athletes with chronic patellar tendinopathy. In the trial, we tested the hypothesis of a superior response to a 12-weeks exercise intervention with high load magnitude (90% of 1RM) compared to moderate load magnitude (55% of 1RM) when equalized to total exercise volume. The response was assed at short-term (12 week) and long-term (52 week) follow-up. Secondly, we aimed to investigate the reproducibility of the MRI Ultra-short time to echo (UTE) imaging with the T2* mapping method in tendinopathic tendons. We hypothesized that the method could be applied as a non-invasive marker for early internal tendon tissue material alterations.

The main finding showed, that in contrary to our hypothesis exercising with heavy load (HSR) was not superior to exercising with moderate load (MSR) in relation to clinical outcome, tendon structure and function. Importantly, both HSR and MSR treatment demonstrated clinical improvement after the 12-weeks intervention, which was maintained at one-year follow-up, although it did not reach the same level as asymptomatic tendons. Conversely, the mechanical, material and morphological properties of the tendon were unaltered in response to both HSR and MSR treatment. Finally, MRI UTE imaging with T2*

mapping was deemed to be a reproducible method to examine chronic tendinopathic patellar tendons.

In conclusion, when the total exercise volume was kept equal the effect of exercising with heavy load was not superior to moderate load in regards to clinically, structural, or functional improvements. This indicates that either of these loading regimes can be applied during treatment of tendinopathy. Both HSR and MSR yielded clinical improvements that were maintained after one year, although these did not reach normal values. On the mechanical, morphological or material tendon properties, no short-term effects of the interventions were detected. Finally, the MRI UTE-T2* method was shown to be reproducible in patellar tendons with chronic tendinopathy, but the applicability to detect internal material alteration and changes thereof need further investigation.

Future studies are needed to determine the reason for the protracted and incomplete clinical recovery and the lack of response to loading magnitude in mechanical, morphological and material properties. Furthermore, it remains to be established to what extent other factors like restitution time between session influences the clinical, structural and functional outcome.

5. DANSK RESUME

Under bevægelse er kroppens sener bindeleddet i kraftoverførslen fra muskel til knogle. Senerne er derfor designet til at kunne modstår stor kraftpåvirkning. Alligevel kan gentagne belastninger føre til overbelastningsskader af senerne, såsom tendinopati. Disse skader udgør 30-50% af alle sports relaterede skader. Tendinopati er karakteriseret ved; smerte under aktivitet, lokal ømhed ved palpation, hævelse og nedsat ydeevne. Skaderne udgør et alvorligt klinisk problem, idet det kan begrænse deltagelse i fysisk aktivitet i måneder, endda år for den berørte person. Listen over aktuelle tilgængelige behandlingsmetoder til tendinopati er omfattende, men den optimale behandling er fortsat ukendt. Behandling med forskellige træningsformer er blevet den typiske behandlingsstrategi. Dog er den optimale træningsdosis ift. sæt og repetitioner, træningshyppighed og belastningsstørrelse fortsat ukendt, specielt i relation til det langsigtede resultat. En øget forståelse af hvordan senens strukturelle forhold, mekaniske egenskaber og den kliniske effekt adaptere til forskellige grader af belastning kan på sigt medvirke til at optimere behandlingen af tendinopati.

Formålet med denne afhandling var derfor at undersøge den kliniske effekt (smertesymptomer), senestruktur og funktion ved forskellige belastningsstørrelser under træningsbaseret behandling af patellar tendinopati. For at undersøge dette gennemførte vi et randomiseret klinisk studie på en population af idrætsaktive mænd med kronisk patellar tendinopati. Gennem studiet testede vi hypotesen at 12 ugers genoptræning med høj belastning (90% af 1RM) ville være mere effektiv end genoptræning med moderat belastning (55% af 1RM), når træningsvolumen var den samme over interventionsperioden. Både den kortsigtede (12 uger) og den langsigtede (52 ugers) effekt af de forskellige belastningsgrader blev undersøgt. Yderligere, undersøgte vi reproducerbarheden af MRI ultra-kort tid til ekko (UTE) -billeddannelse med T2* analyse anvendt på tendinopatiske sener. Vores antagelse var at metoden kunne bruges som en ikke invasiv markør til at måle tidligere forandringer i senen på materiale niveau.

I modsætning til vores oprindelige hypotese, viste det overordnede resultat, at træning med tung belastning (90% af 1RM) ikke var bedre end træning med moderat belastning (55% af 1RM) i forhold til kliniske effekt, senestruktur og funktion. Der var en klinisk effekt for begge behandlingstilgange (moderat og høj belastningsgrad) på kort sigt (12 ugers opfølgning). Forbedringen fortsatte helt op til et års opfølgning, men nåede ikke et niveau svarende til

symptomfrie sener. Derimod var der ingen effekt på senens mekaniske, materielle eller morfologiske egenskaber efter træning med hverken moderat eller høj belastning. Endelig blev det vist at MR UTE-billeddannelse med T2* analyse er en reproducerbar metode til undersøgelse af kroniske tendinopatiske patellar sener.

Vi kan konkludere at den kliniske effekt (smertesymptomer), senestruktur og funktion ikke var bedre efter træning med tung belastning end med moderat belastning, når det samlede træningsvolumen var ens. Dette indikerer at belastningsregimet kan vælges frit. Begge programmer medførte kliniske forbedringer helt op til et år, men uden at nå et niveau svarende til symptomfrie sener. Derimod var der ingen effekt af træning på kort sigt for de mekaniske, morfologiske eller materielle senegenskaber uafhængigt af belastningsgraden. Endelig blev MR UTE-T2* metoden vist at være en reproducerbar metode til undersøgelse af patellar sener med kronisk tendinopati. I hvilken grad metoden kan anvendes til at detektere materiale forandring i senen og ændringer deraf kræver yderligere undersøgelse.

Fremtidig forskning er nødvendig for at klarlægge årsagen til at deltagerne ikke bliver raske efter et år og at de mekaniske, morfologiske og materielle sene egenskaber ikke påvirkes af træning. Derudover er det stadig uklart, i hvilket omfang andre faktorer, såsom restitutionstid mellem sessioner, påvirker det kliniske, strukturelle og funktionelle resultat.

6. INTRODUCTION

Tendon tissue play a key role as force-transducers transmitting force from bone to muscle, and therefore well-functioning tendons are crucial for optimal locomotion[41]. Despite tendons are designed to withstand considerable loads during locomotion, repetitive use often results in overuse injuries as tendinopathy[79], which account for 30-50% of all sports related injuries[136]. Specifically, patellar tendinopathy is a substantial clinical challenge, especially since it often causes long term (months to years) performance reduction for the affected person and among athletes often result in early retiring from sport[78]. Despite loading based treatment has been the preferred treatment for tendinopathy, the optimal dose in form of repetitions, set, frequency and load is still unknown[102]. To better understand tendinopathic tendon tissues adaptation and optimize the rehabilitation thereof it is necessary to investigate to what extent loading configuration during treatment influences the clinical outcome and the tendon structure, function and mechanical behavior of pathological tendons. It is my hope that the work of this thesis will contribute to some knowledge in answering that question.

7. BACKGROUND

7.1 Anatomy and structure of the patellar tendon

The patellar tendon connects the patella apex to the tuberosity tibia and is an extension of the quadriceps femoris muscle and quadriceps tendon[12]. It is a thick tendon (3-4 mm)[44,69] with a relative short length of approximately 5 cm, however, considerable variation occurs[37,72]. Furthermore, cross sectional area (CSA) of the patella tendon varies along its length by increasing from the proximal to the distal part[86].

Tendons are a densely packed connective tissue composed of triple-helical type I collagen molecules bound together by intermolecular crosslinks[76]. Organized in a strict hierarchical pattern as shown in Figure 1, the collagen molecules forming fibrils, fiber bundles, fascicles and hole tendon units with each structural level influencing the mechanical properties of the tendon. The fibers and fascicles are bound together and inverted by the endotenon that contains blood vessels, nerves and lymphatics supply to the tendon and finally a fine sheath,

the epitenon, enclose the entire tendon. The whole patellar tendon is further surrounded by a paratenon which ensure free movement of the tendon from the surrounding tissue.

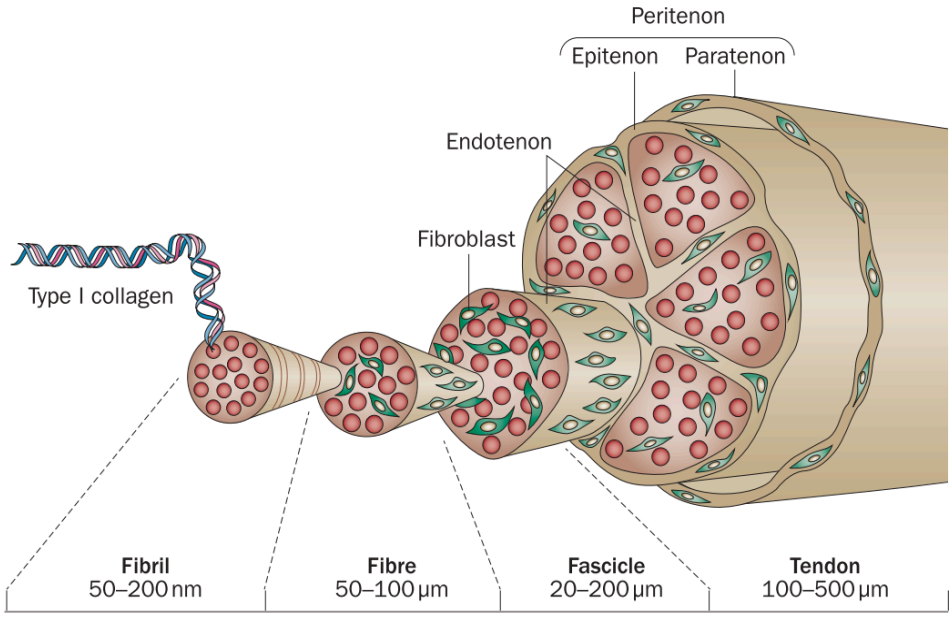

Figure 1: Hierarchical tendon structure (Nourissat et al. 2015[115])

The composition and structure of the tendon influence the mechanical properties resulting in function[82]. The fibrils is considered to be fundamental for the force-transmitting function of the tendon[122] and have recently been shown to run the hole tendon length in adults human patellar tendons[149]. Furthermore, region specific structural differences in form of greater fibril density and tendency to smaller fibril CSA in the posterior fascicles compared to anterior fascicles in the patellar tendon have been shown and indicating region specific material properties[56].

Collagen makes up 60-85% of the dry-weight of the tendon, with the majority being type I collagen (90%) and the remaining consisting among others of type III and type V[76,81]. The fibrillar collagen is embedded in an extracellular matrix, which in addition to collagen and water consists of a small amount of elastin (1-2%), proteoglycans (PG), glycosaminoglycans (GAGs), and glycoproteins. Like other connective tissues, water constitutes 55-70% of the total tendon and the amount of water is closely linked to the amount of proteoglycans in the extracellular matrix[81].

The dominant cell type in the tendon are the elongated shaped fibroblasts, placed between and in parallel with the collagen fibrils and in the paratenon[74,76]. During mechanical stretching the fibroblast undergo deformation and respond with expression of growth factors that work on the extracellular matrix components including synthesis of collagen[22]. Conversely, lack of mechanical loading (strain), might lead to degradation[8]. Therefore, mechanotransduction in the fibroblasts is important for both maintenance of matrix homeostasis and morphologic alterations in response to loading and unloading.

There is a large heterogenicity in the results of studies investigating the rate of tendon tissue turnover. An increase in collagen turnover rate in patellar tendons in response to acute exercising has been suggested in a study using a stable isotope infusion method[111]. Likewise, increased collagen protein synthesis in the peritendinous tissue of the Achilles tendon in response to both acute and long term exercising has been shown with microdialysis[91,92]. Conversely, a more recent study using the 14C bomb pule method has shown that the core of the human Achilles tendon is almost unaltered during adult life and only the periphery is replaced[60]. The differences have been suggested to be related to region specific alteration in the tendon tissue. However, a newly published study on healthy human patellar tendons from cadavers clearly show that turnover does not differ between the regions (length, width and thickness)[168]. Moreover, the data indicate that the turnover rate in the tissue do not change throughout life.

7.2 Biomechanics and function of the patellar tendon

The patellar tendon is designed to create and resist sizeable loads[76]. It transfers muscle generated force from the quadriceps muscle to the tibia bone to extend the knee and is essential for walking, running and jumping. It has been estimated that the patella tendon may be loaded up to 4 kN (~400 kg) during a single counter movement jump[41].

Previous the patella tendon has been considered as one force-transmitting structure. However, recent studies have shown different mechanical properties of collagen fascicles from the anterior part of the tendon (stronger) compared to the posterior part[56,57]. The region specific mechanical properties indicate that the different part of the patella tendon might operate more independently.

The mechanical properties of the individual tendon can be determined on the force-deformation curve (Figure 2, A) and requires simultaneously measurement of load and corresponding stretch of the tendon[74]. The initial part of the curve "the toe region" reflect that the crimped collagen fibers, existing in the resting state, are stretched out when load is applied. The following linear region of the curve initiates when deformation (mm) increases relative to linear response to force (N) and continues until the yield point where initial tissue damage occurs. After the yield point the tissue damage in form of fiber failure occurs until the plastic unreversible part of the curve ends with complete failure. The slope of the curve in the linear part represent the stiffness (N/m) of the tendon and have functional implication for the force transmission. Thus, tendon stiffness will change the force-length relationship of the muscle and has been shown to be associated with isometric muscle force output and dynamic muscle action measured as maximal jump hight[20,96].

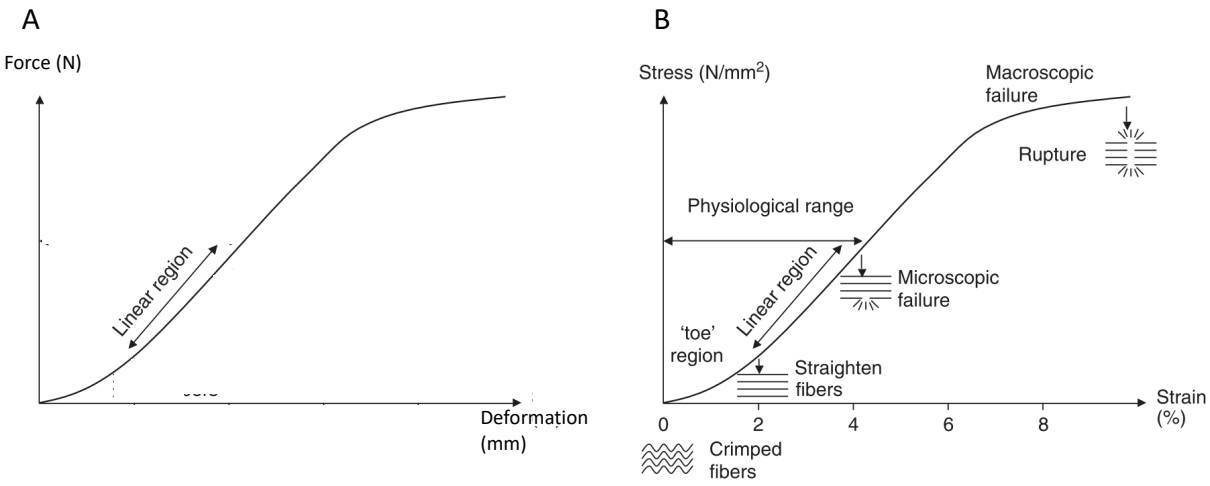

Figure 2: Force-deformation curve of the tendon (A), drawing with inspiration from Jozsa and Kannus 1997[77]; Stress-Strain curve of the tendon (B), duplicated from Wang 2006[158].

The dimension of the tendon (length and CSA) influence the structural properties, for which reason the force-elongation curve can be normalized dividing force by CSA and deformation by length giving the tendon stress (MPa) and strain (%), respectively[38,158]. The slope of the linear part of the normalized stress-strain curve (Figure 2, B) is then representing the material properties of the tissue (Young´s modulus, MPa), which enable tendon comparison. In vivo, human tendons operate during normal physical activity only on the elastic part of the curve (2-

4% strain) in which it returns to its original geometrical shape after the stress has been removed. Most of the energy storage in the tendon during stretching is returned on recoil. However, because tendons are viscoelastic tissue some energy is lost as heat (hysteresis)[74]. Further, a decline in force in response to ongoing deformation of the tendon (stress relaxation) or gradually increased deformation over time in response to constant load applied to the tendon (creep) may occur. Finally, the speed by which the tendon is loaded influence the mechanical properties because of the viscoelastic material[90].

7.3 Patellar tendinopathy- Prevalence, definition, and diagnosis

Overuse injuries account for 30-50% of all sports related injuries[136]. The incidence of lower extremity tendinopathies in Danish general practice has been reported to be as high as 7.9/1000 patients per year[125]. Among adolescents athletes patellar tendinopathy is the most common[42,157,169].

The majority of patellar tendinopathy occurs in jumping sports that requires high and repetitive loads on the knee extensors and mainly in men in the age group of 15-30 years[42,95]. The prevalence of patellar tendinopathy is high with 9% of non-elite athletes reporting symptoms[169]. In elite level athletes the overall prevalence is reported as high as 14%, and yet within explosive sport the prevalence can reach as high as 32% among basketball players and 45% among volleyball players[95]. Many athletes experienced recurrence of symptoms, substantial negative impact on sports performance (55%), and patellar tendinopathy pain is for more than 50% the reason for retiring early from sport[23,78]. Moreover, reduced work ability was reported as high as 16% among athletes with patellar tendinopathy[157].

Patellar tendinopathy (also called “Jumpers Knee“) is defined as a syndrome characterized by activity related pain at the inferior pole of the patella, impaired physical performance, swelling of the tendon and localized tenderness upon palpation[79,103]. The chronology of symptoms of tendinopathy can be classified into 3 stages were symptoms present for less than 6 weeks is characterized as “acute”, for 6 to 12 weeks as sub-acute and for more than 3 months as chronic[15].

Clinically, the diagnosis of patellar tendinopathy is usually determined based on patient history and physical examination[137]. Patients often present with a history of load-related pain in the patella tendon or the "warm-up phenomenon" with reduced pain after repeated loading, but reappearing pain the day after energy storing activities[103,129]. Further the clinical diagnosis is often stated on a specific test which causes tenderness upon palpation, however, the test despite of high sensitivity in reproducing symptoms show low specificity in identifying pathological structure[25]. Moreover, Fredberg et al[45] show a high rate of misdiagnosis for Achilles and patellar tendinopathy using only clinical examination. Therefore, it is recommended that clinical testing is verified by ultrasonographic pathological findings such as local anterior-posterior thickening of the tendon, hypo-echogenicity and increased vascularity[46,108,159]

7.4 Etiology and pathology of patellar tendinopathy

The exact etiology and mechanisms behind developing overuse injuries as tendinopathy remain elusive[115] and is likely multifactorial[147,164]. A resent systematic review of risk factors established several suggested risk factors with moderate to conflicting evidence; greater volume of strength training, better jumping performance, less normalized hip extension torque, decreased knee extension fatigue ratio and altered corticospinal excitability to the quadriceps[147]. However, there were a lack of strong evidence risk factors and they conclude that there is a need for lager-scaled prospective high quality studies in order to identify the exact risk factors for patellar tendinopathy.

It has been believed for many years that inflammation plays a role in tendinopathy and its time cause have been investigated and debated. However, it has been difficult to detect any signs of inflammation in long-term chronic tendinopathy[34] and a recent study of early tendinopathy corroborates the lack of inflammation also in early tendinopathy[151]. Furthermore, studies on anti-inflammatory medical treatment have not yielded successfully outcomes in either early (unpublished data from our lab) or prolonged tendinopathy[62,120]. However, to what extent inflammation play a role in some stages of tendinopathy or maybe prior symptoms occur is still not fully established. Even if it remains speculative an assumption

could be that inflammation occur in repeated but shorter periods, even though detection thereof is limited by the today available methods for detecting inflammation.

On a structural level, patellar tendinopathy is considered the result of repetitive isolated micro-ruptures of the fibrils[127]. It is well established that the collagen fibrils appears thinner and disorganized in the pathological tendon tissue[50,85,120]. Interestingly, in animal tendons it has been shown that even though cyclic loading result in buckling of the collagen fibrils leading to nanoscale molecular disorder, development of actual micro ruptures of the collagen fibrils is not initiated[65,153]. The lack of altered mechanical properties in early tendinopathy recently shown support this assumption that tendinopathic tendon maintain their microstructural pattern[151] with fibrils running all along the tendon length[149].

However, the today widely accepted model for chronic tendinopathy is still that overloading of the tendon might lead to a failed healing response of the micro-damages that is initiated and repaired well balanced in response to loading in healthy tendon tissue[101]. The insufficient repair process in the tissue lead to altered response of the fibroblast and thereof loss of tissue homeostasis. This assumption is supported of the well-established histopathology of the painful tendons presenting increased cellularity, increased content of proteoglycans (PG), Glycoaminoglycans (GAG´s) and water, hypervascularization and altered collagen synthesis[100]. The modified cellular and molecular response result in the pathological finding of thickening in addition to hypo-echogenicity and increased vascularity[108]. The pain in the tendinopathic tissue is suggested to be caused by ingrowth of sensory nerves prolonged with ingrowth of small capillary from the paratenon (neovascularization) which contribute to the hypervascularization[109]. Even if it remains speculative pain could also be caused just by increased pressure from the hypervascularization.

The pathological alteration in patellar tendinopathy is primarily presented in the proximal posterior portion of the tendon[72,80]. It has been speculated that region specific differences were the main reason why tendinopathy typically occur in specific regions of the tendons. However, due to the results of the newly published study by Zhang et al[168] on the human patellar tendon (clearly demonstrating lack of region specific differences in tissue turn-over) this cannot explain differences in the increased occurrence of tendinopathy in some parts of the tendon.

To what extent pathological alteration in the tendinopathic patellar tendon causes modification of the mechanical properties is still unclear[116]. Thus, patellar tendon stiffness has been shown to be both unaffected[30,85] and decreased[63,163] in response to tendinopathy. Importantly, due to the slow insidious onset of tendinopathy, the knowledge of etiology is built on pathological finding after onset of pain, which make it difficult to establish to what extent pathological tissue response is the cause or the result of tendinopathy. A major limitation for the current evidence on etiology of tendinopathy is the limited knowledge of the early stage of the disease primary due to the gradually increase of symptoms and there is a need of larger scaled studies following healthy tendons developing tendinopathy. Additionally, histopathological findings from all phases of tendinopathy is needed to extend the knowledge, however, the invasive approach of taking a biopsy, which might be the gold standard for this approach, is not feasible for repeated measures. Thus, it is of great interest to investigate the applicability of non-invasive imaging modalities which can be used for repeated measurement of both healthy and tendinopathic tendon tissue.

7.5 Exercise-based treatments of Patellar tendinopathy

The list of currently available treatment modalities for tendinopathy are broad and may include; exercise therapy, surgery, nonsteroidal anti-inflammatory drugs, steroid injection, platelet-rich plasma injection, therapeutic ultrasound, sclerosing therapy, extracorporeal shock wave therapy[4,39]. However, the dominant conservative treatment strategy for patellar tendinopathy have become loading-based interventions[93,102]. Yet, there is a lack of knowledge of the optimal exercise dose in terms of repetition, sets, frequency and load[102,166]. The present thesis will focus on the loading-based treatment paradigm, the development and the existing evidence of the effect of load on tendons which play a central role in the present PhD project.

Stanish and Curwin[148] were back in the 80´s the first to demand attention on loading-based exercise as a treatment of tendinopathy by introducing stretch-shortening exercise that included an eccentric component rapidly followed by a concentric component. A decade later, promising clinical results of isolating the eccentric contraction and eliminate the concentric component of a stretch-shortening cycle in treatment of Achilles tendinopathy was suggested by Alfredson and colleges[3]. This eccentric exercise paradigm (ECC) gained

considerable popularity and is today globally the commonly used treatment of tendinopathy despite limited and conflicting evidence that clinical outcomes is superior with eccentric loading compared to other loading programs in treatment of patellar tendinopathy[102]. Likewise, basic science does not rise evidence on superior effect of eccentric compared to concentric muscular contraction mode. Thus, in animal studies it has been shown that isometric, lengthening or shortening contraction mode do not yield different response on expression of collagen in the tendon tissue if exposed to similar force level[47]. Furthermore, a higher force production during the eccentric contraction compared to the concentric contraction did not induce increased collagen expression[59]. Moreover, in human tendons it has been show that Achilles tendon length change and peak tendon force is not significantly different during eccentric and concentric contractions[124] and lastly that patellar tendon hypotrophy in response to resistance training are similar irrespectively of contraction mode[40]. Collectively, these findings imply that given that the force applied is high enough to produce the required stain on the fibroblast, essential for mechanotransduction, there is no evidence to support isolating the eccentric component.

Although, the above described lack of evidence supporting the eccentric regime only a few loading-based exercise regimes such as; isolated concentric training[98], a combined program including eccentric-concentric progressing to eccentric training and faster eccentric-concentric[143], Heavy slow resistance training (HSR) including both the eccentric and concentric mode[84] and most recently isolated isometric exercise[67,128] have been suggested.
Reviewing the literature from the past two decades of studies investigating loading-based exercise regimes as treatment of patellar tendinopathy emphasized the need for high-quality Randomized Clinical Trials (RCT) investigating aspects of loading intervention as load magnitude before more systematic reviews pooling existing evidence is relevant. As shown in Table 1 the number of RCT studies in the period of 2000 to 2020 were sparse. Specifically, in the past 10 years there were only three studies[7,67,128] investigating the effect of isometric loading programs and with conflicting results. Interestingly, five systematic reviews[4,36,39,93,102] investigate the optimal loading-based treatment have been published within the same period including the same few above mentioned RCT studies, Table 1. Eccentric loading programs are recommended in nearly all of the published systematic reviews. However, 8 out of 11 RCT studies (Table 1),

which make up the body of evidence for the systematic reviews were carried out within the time-period were the eccentric loading approaches was the only available program which might questioned the recommendations. The fact that requirements for both RCT studies and systematic reviews have increased within the past 20 years might be a contributing factor that four out of the five reviews conclude that lager high-quality RCT studies is needed and that methodological issues and heterogenicity of the original research induce high risk of bias and is a limitation of the conclusions. Further, variability in follow-up duration[36,102], and various outcome scores among the available RCT studies [93] is stated as a limitation for recommendation of an evidence based treatment protocol. Beside these limitation, the review by Malliaras et al.[102] also stated that studies controlling for load intensity, time under tension, speed, and contraction type are needed. In summary, reviewing the literature indicate that the focus on the superiority of eccentric contraction mode and lack of high-quality RCT studies investigating more recently developed loading-based treatment protocols have overshadowed other relevant parameters influence on the loading program including the optimal load magnitude.

For both Achilles and patellar tendinopathy HSR and ECC loading have shown equivalent improvement on the clinical outcome Victorian Institute of Sport Assessment-Patella questionnaire (VISA-P) in short-term follow-up[13,84]. Moreover, HSR was superior on patient's subjective satisfaction both in short- and long-term follow-up. The HSR loading program are performed three times pr week in contrast to the ECC program consisting of two training session per day, seven days a week which may explain the variation in satisfaction. The superior patients satisfaction is a contributing factor why HSR loading regime have become the treatment of choice in the Sports medicine clinic at Bispebjerg Hospital and thus is the applied loading regime in the RCT study included in this PhD thesis.

The HSR program developed by Kongsgaard et al[84] is in addition to the above described prolonged restitution period between training sessions characterised by quadriceps resistance exercise carried out with high load magnitude (progression 15-6RM) and slow action (performed with 6s/repetition). The aspect of high load magnitude is supported by a meta-analysis by Bohm et al[19] showing that tendon are highly responsive to load and load magnitude in particular. The theories behind the HSR is further that it has been shown that healthy tendons were more responsive to a low number of loads of long duration than a high number of faster load[6]. Lastly, negative net balance between synthesis and degradation of collagen in

response to loading has been suggested to occur up to 36 hours after exercising[101], why three weekly sessions in the HSR program might ensure sufficient tendon tissue restitution time essential for healing.

In relation to loading-based rehabilitation the HSR loading regime has in addition to positive outcome with respect to improvement in tendon pain and function (VISA-P) in short-term also been shown to be beneficial in relation to long-term follow-up[84]. Noteworthy, the long-term component is generally rarely investigated in the literature[93,102]. Furthermore, HSR yields favorable structural composition (fibril diameter, fibril density), and biochemical composition (enzymatically derived cross-links) compared to ECC loading[84,85].

Although, the HSR loading programs has been shown to be beneficial, there was still a lack of full recovery and the VISA-P score, representing tendon pain and function, at half-year follow-up was only 86 points[84] out of the 100 points which represents an asymptomatic, fully performing individual[154]. This result point in direction of that it is relevant to investigate different components influencing the loading programs in an attempt to increase the rehabilitation success rate. In contrast to contraction mode, there is evidence from a clinical trial[84] and from basic science (will be outlined in the next paragraph) to suggest that load magnitude might be an import aspects in the attempt to optimize the treatment response. Thus, that more RCT studies have compared the effect of different loading programs, and repeated reviews have tried to establish the optimal treatment options based on these studies (Table 1), they rarely match the load or isolate specific parameters of exercise dose (repetition, set, frequency) or load magnitude[32]. Therefore, the optimal load magnitude in exercise-based treatment of tendinopathy is still unknown.

Table 1: Summary of Randomized Clinical Trials in the period 2000-2020 and Systematic Reviews in the period 2010-2020 investigating the effect of loading-based treatment of patellar tendinopathy.

RCT's 2000-2020	#No	Duration / Follow-up (wk)	Intervention	Outcome(s)	Systematic Reviews 2010-2020					
					Larsson 2012[96]	Malliaras 2013[105]	Everhart 2017[40]	Andriolo 2019[4]		Doelen 2020[37]
Cannell 2001[22]	10 9	12/6,12	1.ECC squat 2.Isotonic leg exten/ curl	VAS & return to sport	x					
Stasino-poulos 2004[152]	10 10 10	4/4,8,16	1.ECC 2.US 3.friction	Pain				x		
Jonsson 2005[75]	10 9	12/12	1.ECC squat on decline bord 2.CON squat on decline bord	VISA-P & VAS	x	x	x	x		
Visnes 2005[159]	13 16	12/6,12,30	1.ECC squat on decline bord 2.trained as usual	VISA-P, VAS & jump test	x	x				
Young 2005[171]	9 8	12/ 12, 52	1.ECC squat on decline bord 2.SLS on step	VISA-P & VAS,	x	x		x		
Bahr 2006[11]	20 20	12/12,26,5 2	1.ECC squat on decline 2.Surgery	VISA-A, Strength & jump test	x	x	x	x		
Frohm 2007[48]	11 9	12/12	1.ECC squat on decline 2. bilat ECC overload t	VISA-P, VAS, strength & jump test	x		x	x		
Kongsgaard 2009[87]	12 13 14	12/ 12,36	1.ECC squat on decline bord 2.HSR 3.CORT	VISA, VAS, strength, mek & biochemical	x	x	x	x		
Van Ark 2016[7]	8 11	4/Baseline + 4	1.Isometric 2.Isotonic	VISA-P, SLDS-test						x
Rio 2017[131]	10 10	4/ Baseline+4	1.Isometric 2.Isotonic	VISA-P, SLDS-test						x
Holden 2020[69]	10 11	4/ Baseline+4	1.Isometric 2.Isotonic	SLDS-test PPTs, Thickness						
Primary outcome					Assesses RCT's addressing the comparison of PT treatment	Evaluate the evidence in studies comparing loading programs in AT & PT	Compare the efficacy of invasive and non-invasive PT treatment strategies	Analyze the evidence of non-opr treatments and identify effective treatment		To determine the most effective non-surgical-treatments
Conclude in relation to loading-based treatment (recommended treatment regime)					**(ECC training)** Need for lager well-designed studies with sufficiently long-term follow-up	**(Little evidence for isolating the eccentric component)** Need for High quality studies	**(ECC for initial treatment)** Need for long-term follow-up	**(ECC in the short term)** Lack of high quality studies and heterogenicity of the literature.		**(Isometric and ECC for short-term pain relieving and ECC for long-term)**

RCT, Randomized clinical trial; #NO, number of participants included; Duration, Intervention duration; ECC, Eccentric exercise; HSR, Heavy slow resistance exercise; CORT, Corticosteroid injection treatment; VISA-P, Victorian Institute of Sport Assessment-Patella questionnaire; VAS, Pain score on a Visual analogue scale; PPT´s, Pressure pain thresholds

7.6 Effect of loading on tendons and tendinopathy

It is well established that tendon response to mechanical loading includes an adaptive response on both molecular, structural and mechanical level, however, the underling mechanisms are still not completely understood especially in relation to tendinopathic tendons[100].

The adaptation in morphological, material and structural properties of healthy tendon in response to loading has been investigated in several studies[162]. In relation to tendon morphology, there is evidence to support that tendons can hypertrophy in response to long term training. Endurance runners have been shown to have lager Achilles tendon CSA suggesting that years of training lead to tendon grow[33,87,99,133]. Likewise, the long duration of morphological alteration is supported by a study on elite fencers and badmintons player[29]. In this study, were the athletes due to an asymmetric loading pattern of the lower extremities for years had been loading the lead leg more than the non-lead leg, they showed a 20% increase in patellar tendon CSA on the lead extremity. Conversely, shorter duration (months) of resistance training has shown unchanged[104] or a more moderate CSA increase[5,86,140]. Noteworthy, the tendon hypotrophy is region specific being more pronounced at the insertions in the studies on both endurance and resistance training, respectively. These findings further support the assumption that the tendon growth is linked to loading dose.

The underling mechanism of increased CSA as material changes in terms of increased amount of collagen in still unknown, however limited evidence from both animal and human studies imply that fibrils are unaffected by loading[100]. The evidence of the influence of loading on tendinopathic tendons are very limited. However, one study show both abnormal fibril morphology in tendinopathic patellar tendons and alteration toward normal fibril density and area in response to exercising with heavy load[85]. Likewise, heavy load exercising has been shown to influence the composition of crosslink in tendinopathic tendons[84].

A recent systematic review by Weisinger et al.[162] shows that tendon structure response to both short- and long term mechanical loading in healthy tendons, however, there is substantial variation in the detected stiffness (varies between -26% to 84.3%). In tendinopathic tendons, stiffness has been shown to decrease in response to exercise [84,85,94].

On the cellular and molecular level, the fibroblast in healthy tendon tissue response to loading (thus strain) by initiating synthesis of collagen and other extracellular matrix component important for maintenance of tissue homeostasis and tendon growth as

outlined in a previous paragraph. Furthermore, that tendinopathic tendons have decreased cell number and increased anabolic and catabolic activity in responses to loading. In vivo cell culture studies suggested that increased dynamic strain might lead to increased response of the fibroblast[158,165]. However, the optimal dose and if there is an upper limit were load begin resulting in degradation is still unknown.

Altought, tendon adaptation seems to be related to mechanical loading, no dose-relationship could be detected based on the existing evidens on healthy tendons in vivo[116,162]. However, in human a study of Arampatzis et al.[5] showed that with equal exercise volume, exercising with greater load (90% of MVC) (and thus strains) yield increased stiffness and increased cross section compared to moderate load (55% of MVC) in healthy Achilles tendons. Likewise, for the patellar tendon in asymptomatic humans, it was shown that changes in stiffness was related to load intensity[104].

The above outlined effect of loading on healthy tendons is supported by a recent systematic review and meta-analysis by Bohm et al.[19]. They conclude that tendons are highly responsive to loading and suggest that loading magnitude play an essential role for the tendon response. Importantly, reviewing the literature it still remains unknown to what extend load magnitude also play a key role for tendinopathic tendons adaptation.

7.7 Tendon imaging

The two commonly used imaging modalities to investigate structural change in tendons are Ultrasound (US) and Magnetic resonance imaging (MRI) in both scientific research and clinical evaluation[35,137]. As described in a previous paragraph it is recommended that clinically diagnosed tendinopathy is verified by imaging and US assessment is increasingly used in both diagnosing and monitoring pathology in tendons[45,141]. The tendinopathic tendon appears hypoechoic and thickened on gray-scale-ultrasound (GS-US) imaging in response to degenerative changes leading to increased water and swelling of the tendon and disorganized fibrils. Additionally, power-doppler-ultrasound (PD-US) provides information on neovascularization, which is likewise a response of pathology in the tendinopathic tendon.

GS-US has been shown to be more accurate than MRI in diagnosing patellar tendinopathy[159] and, used in combination with color-doppler-ultrasound (US-CD), which have

shown good ability to confirm an asymptomatic tendon, the accuracy of US imaging is further strengthened. Importantly, US detected abnormalities in tendons without corresponding presentation of pain has been reported within ~20% of asymptomatic patellar tendons[24,43]. Conventional MRI is more time-consuming and costly[46] but eliminates some of the main disadvantages of using US; high degree of operator dependency and high intra-observer variation[17]. Reliable assessment of tendon structure is crucial for monitoring changes in response to treatment and consequently MRI might be of value for repeated imaging of smaller structural changes in a research setting despite limited applicability in diagnosing tendinopathy.

Yet, neither US nor conventional MRI provide detailed material or quantitative structural information of the tendon tissue[118,152]. Tendon biopsies are the gold standard in relation to methods raising evidence on structural and biochemical composition[85]. However, the invasive nature of biopsy sampling makes it practically and ethically challenging to use for repeated measurements. Moreover, it has recently be shown that the trauma caused by biopsy sampling in healthy human patellar tendons lead to upregulation of cell activity making repeated measures infeasible[58]. An alternative non-invasive method suggested for quantification of detailed structural information of tendon tissue is MRI ultra-short time to echo (UTE) imaging with T2* mapping[48,130].

MRI imaging are based on detection of protons in the tissue[131]. When a person is placed in a MRI scanner all protons of the body will align in the same direction and application of a radiofrequency pulse (RF-pulse) will flip the protons to a transversal plan. The time it takes before the protons fall back to their transversal resting state, the transversal relaxation time (T2*) depends on the tissue density. The dense tendon tissue consists mainly of short T2* components due to strongly bound water to collagen and proteoglycans leading to a rapid decay of signal (T2* relaxation time of 1-2 ms) after a RF-pulse application during the MRI scan[130]. Thus, by the TE´s (time between RF pulse applied and the detection of signal) used in conventional clinical MRI for investigation of tendon dimensions (8-20 ms) the tendon will appear dark. This provides no information on tendon tissue quality or possible alterations taking place before micro structural alteration might be visible.

In contrast, UTE sequences with TE< 1ms have been developed and used in connective tissue such as tendons[131]. The UTE MRI sequences make it possible to obtain

sufficient signal from tendons[130] and subsequently perform T2* mapping analysis[48]. Figure 3 show an example of UTE sequences and T2* analysis.

Furthermore, longer T2* relaxation time of tendinopathic tendons compared to healthy tendons have been suggested to be related to loosely bound or free water content increased in tendinopathic tissue as a result of increased amount of proteoglycans and collagen disruption[10,21,52,75]. However, the reproducibility and monitoring applicability in tendinopathic tendons remain unknown.

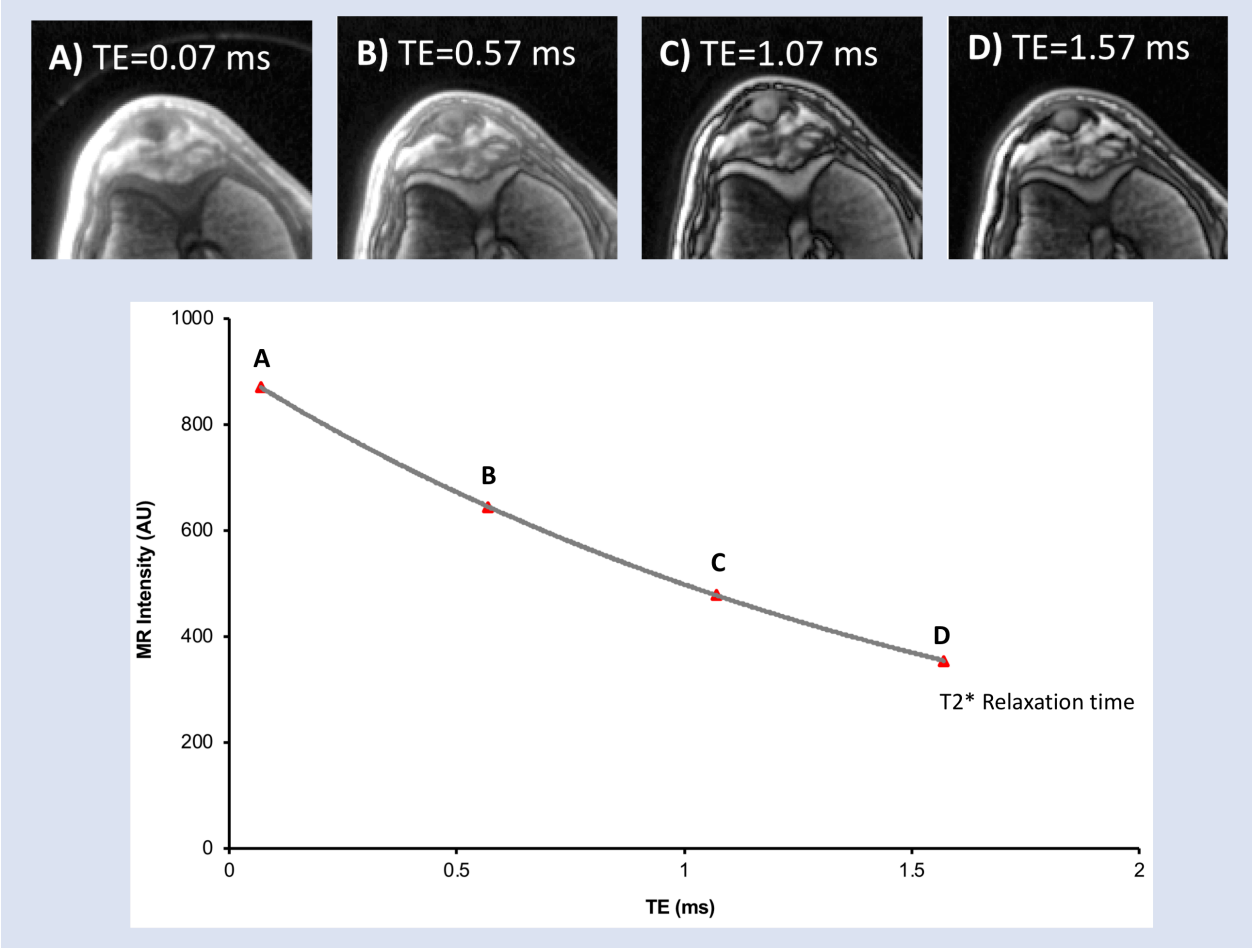

Figure 3: Representative images from UTE MRI sequences (mid tendon) for increasing TE (A to D) with decreasing signal intensity (upper part of the figure); Representative plot from the mono-exponential fitting procedure including values from the four TE's (A, B, C and D), the T2* analysis (lower part of the figure).

8. AIMS AND HYPOTHESIS

The overall aim of the thesis was to examine the effect of load magnitude in exercise-based management of patellar tendinopathy in recreational adult male athletes on clinical outcome, and tendon structure and function. Furthermore, the reproducibility of the MRI ultra-short time to echo imaging with T2* mapping method recently applied on tendons were investigated.

Specific aims Study 1:

To investigate how 12 weeks of exercise intervention, with high (90% of 1RM) or moderate (55% of 1RM) load magnitude at equalized volume, applied to the tendinopathic patellar tendon:

- influence the clinical, structural, and functional outcomes when assessment on the short term (12 seeks) and the long term (52 weeks) **(paper I).**

- influence on the mechanical, material and morphological properties. Furthermore, to explore if T2* relaxation time obtained with MRI UTE could be used as a non-invasive marker for internal alterations on a material level to quantify early changes in response to intervention **(paper II).**

It was hypothesized that a superior response to an intervention of high load magnitude (90% of 1RM) compared to moderate load magnitude (55% of 1RM) would be seen, when total exercise volume was equal among the two interventions.

Specific aims Study 2:

To evaluate the test-retest reproducibility, and the inter- and intra-observer reproducibility of MRI UTE-T2* mapping in tendinopathic human patellar tendon **(paper III).**

9. METHODOLOGICAL CONSIDERATIONS

In this section methodological considerations and challenges of applied methods in this thesis will be presented. Further methodological details can be found in the material and method section of each paper at the end of the thesis. An overview of studies, timeline, included participants and the generated papers is shown in the flowchart, Figure 4.

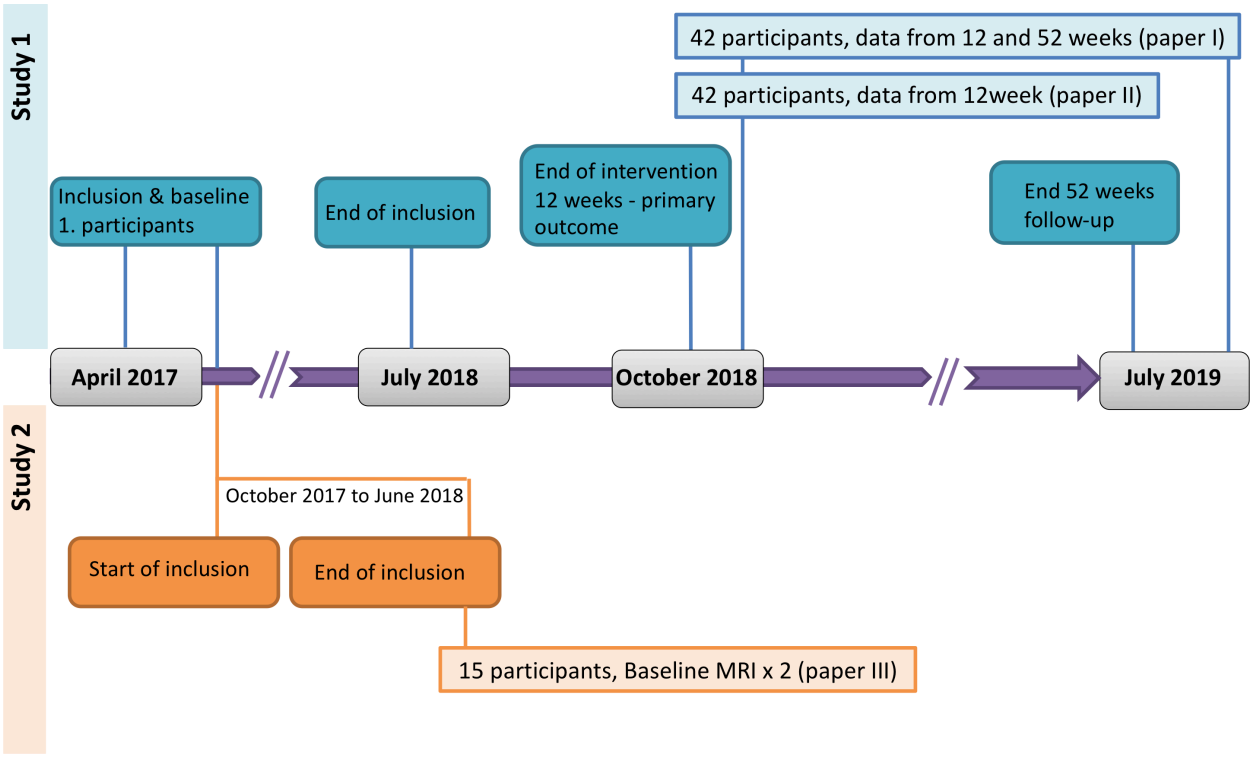

Figure 4: Flowchart showing overview of the two studies, timeline, included participants and the generated papers. Blue colors indicates Study 1 (the treatment study). Orange colors indicates Study 2 (The method study).

9.1 DESIGN AND PATICIPANTS

9.1.1 Study 1– Treatment study (paper I and II)

The study was a prospective randomized controlled, single-blinded, superiority trial, designed with a two-group parallel design comparing interventions of high load magnitude (HSR) with intervention of moderate load magnitude (MSR). The study was designed with a primary endpoint after a 12-week intervention period. Given that tendon tissue has a relatively slow turnover, and long-term follow-up are rarely investigated, a 52-week follow-up period was additionally included. We could have chosen 52-weeks as primary outcome in the present study

to account for the general slow tendon tissue turnover. However, it was not feasible to control the participants behavior or sufficiently measure the adherence following cessation of the intervention period.

We decided not to include a non-exercise control group due to ethical reasons. Two previous studies on Achilles[132] and patellar tendinopathy[155], respectively, have compared exercise to wait-and-see treatment and suggested no or little effect of wait-and-see on clinical outcomes. Thus, we did not expect an effect on clinical outcomes of a control group in the present study. It cannot be ruled out that some improvements have occurred over time regardless of treatment, however, we expect this influence to be equal in both treatment groups.

Between April 2017 and July 2018 participants were recruited from the Sports Clinic at Bispebjerg Hospital and via advertisements on social media and the internet based on the inclusion and exclusion criteria listed in Table 2. In total, 44 participants with chronic patellar tendinopathy were enrolled in the study and randomly allocated to one of two intervention group; Moderate slow resistance group (MSR) or the heavy slow resistance group (HSR). The number of included participants were based on a sample size calculation performed a priori to detect a between group difference of 13 points on Victorian Institute of Sports Assessment-Patella questionnaire (VISA-P) score (minimal clinical important difference[64]) with 80% power and an alpha level of 0.05. The used standard deviation was 13 points based on previous data of 0-12 weeks VISA-P improvement[84]. We estimated that a sample of 18 participants were needed in each group and 44 participants were included in total to account for a 20% drop out rate.

Table 2: Eexclusion and inclusion criteria for participants

Inclusion criteria	Exclusion criteria
• Male athletes • Age 20-45 years • BMI 18.5-30 • Uni- or bilateral patellar tendinopathy • Symptoms >3 months • Ultrasonographical tendon swelling • Ultrasonographical hypo-echoic area with power doppler	• Patellar tendinopathy >12 month • Previous knee surgery • Confounding diagnosis to the knee joint • Diabetes or arthritis • Previous corticosteroid injection for patellar tendinopathy • Smoking • Being elite volley ball player

Taken the relative small number of participants in each group into account a computer-generated minimization randomization procedure (MinimPy version 0.3, Python Software Foundation, Beaverton, OR, USA)[134] stratified according to pre-injury physical activity level, pain level and symptoms duration was applied. The advantages of the minimization compared to simple randomization is the ability making small groups closely similar in relation to participants characteristics and thereby decreasing the risk of bias[112].

Limiting the inclusion criteria; sex, age and symptom duration for the study population decreases the extern validity of the results. However, the present study, was designed to answer the question about influence of load magnitude, which is why we attempt to keep other confounding parameters as stable as possible. It is well established that tendons response to loading differs in men and women[119] and that tendons undergoes aging-related changes[100]. Therefore, eligible age were restricted to 20-45 years and only men were included in the present study. A pain duration of above three months were used to ensure a chronic condition of patellar tendinopathy. It has been suggested that longer duration of tendinopathy might result in a stages of degrative tendinopathy, where pathological changes have little or no capacity for reversibility[27]. To exclude participants in this potential end stage of tendinopathy, and only include reversible stages where the primary stimulus for tendon tissue alteration still might be 'load', symptom duration was limited to 12 months.

Diagnosing patellar tendinopathy using only clinical examination have shown a high rate of misdiagnosis[45]. Therefore, the clinical diagnosis in the present study was confirmed by ultrasonographic (US) pathological findings; local anterior-posterior(AP) thickening of the tendon of at least 1 mm compared with the mid-tendon level, a hypo-echoic area and presence of power doppler (PD) signal. Magnetic resonance imaging (MRI) have been suggested to be more accurate than US in differential diagnostic of tendinopathy[35] and were therefore used for examination of confounding diagnosis to the knee joint, which was an exclusion criteria in the study. However, MRI was conducted at baseline examination. Thus, the MRI-description resulting in exclusion of two participants was first received after the randomization. Retrospectively, inscribing in the study protocol the opportunity for replacing excluded participants after randomization might have been relevant.

In the study a two week "wash-out" period from any previous treatment was included after inclusion and prior to baseline assessment. The length of two weeks might be

minimal and potentially insufficient time to control for previous treatment. However, in relation to e.g. NSAID treatment two weeks might be sufficient, and corticosteroid injection treatment (which have documented high impact on treatment effect) was an exclusion criteria. Moreover, if the participants previously had performed exercise-based treatment it would require a much longer "wash-out" period, which was not feasible in the design of the present study. Furthermore, we speculated that a longer "wash-out" period could result in some eligible participants refraining to participate. Taken into account that any previous exercise-based treatment would have made it even less likely that they improved over time we decided to use the duration of two weeks.

Designing the study in a PhD setting carrying out the RCT within three years lead to some pragmatically decisions about the study population. We decided to include both proximal and distal patellar tendinopathy thus we expected this could increase the possibility of including sufficient participants within the limited time course. Based on the study results, only including one distal tendinopathy and the fact that the response might depend on location of the tendinopathy[146] it might retrospectively been relevant only to included proximal tendinopathy. However, in fact only one case of distal tendinopathy was included which has likely not influenced the result. Moreover, the participant with distal tendinopathy were excluded in the per protocol analysis. Due to the same time aspect we included participants with both uni- and bilateral tendinopathy. For the participants with bilateral tendinopathy the leg with the most severe symptoms was included in the analysis to allow for unpaired statistical analysis. However, due to the inherent limitation of comparing injured versus contralateral asymptomatic leg in participants with bilateral symptoms, it was only possible to carry out between leg comparison within a reduced study population.

Both primary and secondary outcomes of the study were pre-registered on clinicaTrials.gov which reduce the risk of bias. Pre-registration increase the transparency of the study and eliminate selective reporting of outcomes in trails[112]. Within the time span of the present study the popularity of publishing the RCT trial protocols have increased. Publishing the study protocol may have increased the transparency of the present study even further. Yet, this opportunity might be more relevant for lager-scaled multicenter study with more than one stake-holders and a wider intervention protocol.

9.1.2 Study 2 - Method study (paper III)

The study was an observational reproducibility study designed to evaluate the test-retest and intra- and inter-observer reproducibility of the MRI UTE-T2* mapping method in tendinopathic patellar tendons. An important factor regarding developing new measurement methods is that two different operators using the same method or the same operator repeating the method have the same results. The reproducibility of repeated measures is essential for tracking changes between assessment with a minimal risk of measurement error [70,160].

We included 15 consecutively enrolled participants from Study 1 between October 2017 and June 2018 (Figure 4). As the specific MRI UTE-T2* mapping method used in the study was tested for the first time, a sample size calculation was not performed due to the lack of references values. Instead, the sample size was based on feasibility. MRI is costly and we decided that a sample of 15 was acceptable to detect a differenced within and between observes, although the included number of participants in the study might be in the low end. Prior to the study a standardized protocol for evaluation was developed and practiced among the observers, which is a strength of the design because it decreases the risk of bias.

Retrospectively, choosing to validate the method only in the study population from the RCT only answers if it is applicable in this specific population. However, tissue alteration differ between stages of tendinopathy and changes might occur during an intervention or follow-up period. Therefore, it might have been relevant to investigate the reproducibility of the method in different stages of tendinopathy. Likewise, day to day reproducibility of the method need to be investigated since the utilization of the method is measuring tissue alterations over a period of time.

9.2 INTERVENTION (Study 1)

Heavy slow resistance training (HSR) have shown favorable outcomes, especially in the long term, and is an effective treatment for patellar tendinopathy[84]. The HSR program used by the participants in the present study was based on that applied in the previous study and the somewhat modified HSR program used by the clinical sports physiotherapists at our department. Importantly, the number of applied exercises was changed from three in the original program to two in the present study. Identical programs in respect to exercise choice

between the HSR and MSR groups in the present study was preferable and adding one extra exercise would have made the MSR program even more time consuming to perform. Furthermore, two quadriceps loading exercise have been applied in the program used at our department within the last 10 years with beneficial results. Therefore, we decided to include one bilateral leg-press and one unilateral knee extension exercise performed separately with both leg (Figure 5).

Figure 5: Depiction of applied exercises. A: Leg press, performed bilaterally; B: Knee extension performed unilaterally. *Figure are duplicated from supplement paper I.*

The premise for including the applied loading approach (90% and 55% of 1RM) in the present study came from a study on healthy Achilles tendon by Arampatzis and colleges[5]. Based on the data from this study we hypothesized that the same superior effect of a high load compared to moderate load magnitude could be obtained in tendinopathic tendons when total exercise volume was equal in both groups.

Designing the loading protocol to obtain equivalized total work produced some challenges and was therefore based upon different methodological consideration. We designed the present study to test the influence of load magnitude per se., and it was not possible to exactly match all parameters completely accurately. In addition to equalized volume, slow contraction mode (6s/repetition) was applied in both groups and sets (3-5) and sessions (3 weekly) were matched. However, this was at the cost of repetitions and total time under tension differing (~30%) between groups. The final intervention protocol for the two groups, HSR and MSR are shown in Table 3.

Table 3: Intervention protocol the two treatment groups

Exercise protokol								
	Week	**1**	**2**	**3**	**4**	**5**	**6**	**7-12**
	Sets	3	3	3	4	4	4	5
HSR	% of 1RM	55	65	70	75	80	85	**90**
	Repetitions	15	12	10	8	6	5	4
MSR	% of 1RM	55	55	55	55	55	55	**55**
	Repetitions	15	14	13	11	9	8	7

HSR, heavy slow resistance group; MSR, moderate slow resistance group; RM, repetition maximum. *Table are duplicated from paper I & II*

For both groups, one of the three weekly sessions were carried out under supervision. This approach was chosen to ensure high adherence rate, specifically in relation to how the exercises were performed and that the prescribed load was applied. Moreover, every second week the supervision included a sub-maximal test (5RM test) in order to estimate progression in strength and adjust training load accordingly. The participants were given a maximum of five attempt to determine 5RM, and were allowed to perform the exercise in a self-selected speed. The measurement was standardized and equal among each test session and a two-minute rest-period was applied between each test trial to avoid muscle fatigue. The percentage of 1RM and thereof the training load was calculated based on the 5RM test. To ensure agreement between the physiotherapists performing the supervision and the sub-maximal test a protocol was developed. However, it might have been relevant to use the template for Intervention Description and Replication (TIDieR) and the SPIRIT statement to increase standardization and transparency of the intervention protocol[66,71].

Pain

Pain during exercises was accepted, similarly to previous studies including participants with tendinopathy. The pain monitoring model developed by Thomee[150] and more recently modified by Silbernagel[144] was applied in both intervention groups for controlling pain. Pain during exercises was allowed to reach up to five on the numeric rating scale (NRS). Yet, pain was not allowed to increase after the end of exercise and had to subside side 3-4 hours after, otherwise load was adjusted. Furthermore, the participants were carefully informed that pain during the controlled loading programs was a normal tendon tissue response.

No effect of loading-based treatment for patellar tendinopathy have previously been shown among elite volleyball players allowed to continue training and compete during the intervention period[155]. However, reducing activity by only allowing leisure-time activity performed within a pain threshold of 50, on the visual analogue scale (VAS), has been applied successfully in the management of Achilles tendinopathy[143]. Based on these results both groups in the present study were allowed to perform sporting activities throughout the 12-weeks intervention period introducing a load management model where activity related pain up to a maximal NRS score of three was allowed. A maximal allowed pain of three was chosen to reduce risk of relapse. Furthermore, the participants were encouraged to not change their duration of weekly sport participation during the intervention period.

Compliance

A training diary using a smartphone app was used in the study. The training records ensured close registration of training intensity (repetitions, sets and load) and attendance and further pain during training (NRS) and information of deviation from the planed intervention protocol were collected.

9.3 PROCEDURE AND TESTING ORDER

Study 1

An overview of the procedure for each participant are shown in Figure 6. Outcome measurements were obtained at baseline, mid-intervention (6 weeks), post-intervention (12 weeks) and one-year follow-up (52 weeks). The examination order was identical for all follow-up evaluation and the participants were instructed to abstain from physical activity 24 hour prior to examination day one and two, respectively. At test day two the US examination were followed by a 5-minute warm-up on a bicycle-ergometer prior to further examinations.

Study 2

The first MRI scan included in this study was the baseline MRI recording from Study 1. A consecutive MRI recording was obtained at the same day (Figure 6) by the same technician

separated by a 45-minute break were the participants were seated in the waiting room without performing any physical activity.

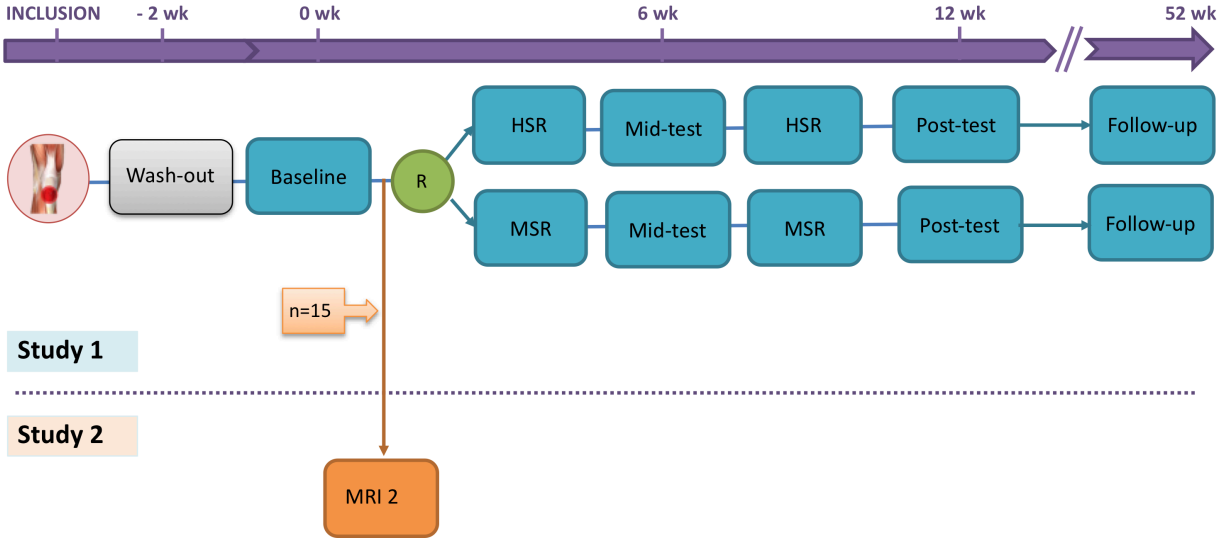

Figure 6: Overview of the testing procedure for each participants. HSR, heavy slow resistance intervention; MSR, moderate slow resistance intervention; R, Randomization.

9.3.1 Blinding

Study 1

When planning a Randomized clinical trial (RCT) blinding is important to minimize the risk of bias[112]. The present study was a single blinded study thus it was not possible to blind participants and the physiotherapists for the treatment intervention. Lack of blinding might influence the participant compliance with the allocated intervention[112]. In an attempt to reduce this risk of bias, the physiotherapists performing the supervision in the present study were instructed to state to the participants that both intervention programs could potentially provide beneficial results.

Designing the study pragmatically in relation to how many members of the research-team it was feasible to blind results in some limitation related to blinding. The primary outcome was blinded, and furthermore we prioritize blinding of subjective outcomes as ultrasound examination. However, it was not possible systematically to blind assessment of all outcomes and consequently risk of bias cannot be ruled out. Based on the fact that all baseline measurements were collected before randomization and that all data analyzes were performed

blinded, we find it unlikely that there would be a high risk of bias influencing the results of the present study.

Study 2

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

9.4 OUTCOMES

An overview of outcomes included in the papers and when obtained are shown in Table 4.

Table 4: Outcomes and when obtained

	Study 1				Study 2
	Paper I		Paper II		Paper III
	Baseline	6 weeks	12 weeks	52 weeks	Baseline + 1h
Participant reported outcomes					
VISA-P	●	●	●	●	
NRS -running	●	●	●	●	
NRS -squat	●	●	●	●	
NRS -preferred sport	●	●	●	●	
Weekly sport participating	●	●	●	●	
Participants satisfaction			●	●	
Imaging					
Ultrasound PD	●	●	●	●	
Ultrasound Thickness	●	●	●	●	
MRI conventional (tendon CSA)	● ●		● ●		
MRI UTE (tendon material)	●		●		●
Function					
Strength MVC (isometric)	●		●		
Jump-test (SJ & CMJ)	●		●		
SLDS-test	●	●	●	●	
Mechanical testing (tendon properties)	●		●		

VISA-P, Victorian Institute of Sport Assessment-Patella questionnaire; NRS, Pain score on a numeric rating scale; PD, Power doppler; MRI, Magnetic resonance imaging; MRI UTE, Magnetic resonance imaging ultra-short time to echo; MVC, Maximal voluntary contraction; SJ, Squat Jump; CMJ, Counter movement jump; SLDS-test, Single leg decline squat test.

9.4.1 Clinical evaluation

The focus of including patient reported outcome measures (PROM) have increased during the last years and PROM's are now highly recommended as primary outcome in RCT studies[14,112]. When we designed the present RCT (Study 1) we therefore decided to use a PROM as primary outcome and the choice of VISA-P questionnaire was based on different considerations. First, the VISA-P questionnaire assess both symptoms, function and the ability to participate in sports[154]. Using VISA-P compared to only monitoring pain is relevant because patellar tendinopathy is a syndrome characterized by activity related pain and impaired physical performance. Secondly, VISA-P has been shown to be valid for monitoring severity and reliable for repeated outcome measure in patients with patellar tendinopathy[110]. The VISA-P questionnaire is translated into several languages and acceptable cross-cultural validity have been shown repeatably[117]. A cross-cultural adaptation was recently performed and a Danish VISA-P-DK version is developed but not published. Yet, we requested the VISA-P-DK from the author and applied this version in the present study. However, the VISA-P questionnaire have a limitation given that the relative change score depends on the baseline score[64]. Moreover, we experienced limitation related to other aspect of validity like structural validity in the danish version of VISA-P questionnaire and in general the validation of the questionnaire is limited in relation to more of the domains (reliability, validity and responsiveness) in the COnsensus-based Standards for the selection of health Measurement INstruments (COMIN) taxonomy developed to evaluate measurement properties of health status measurement instruments[113].

Based on consideration of that VISA-P, despite its limitations, was the best available population specific questionnaire we decided to apply this in the present study. Moreover, VISA-P is applied in nearly all RCT studies investigating patellar tendinopathy within the last two decades[36,93], which make comparison of result from the present study to previous studies possible. That minimal clinical important difference (MCID) for the VISA-P in athletes with patellar tendinopathy have been investigated and estimated to be 13 points[64] is another reason why VISA-P can be applied with advantages.

The questionnaire was completed without assistance from the investigator in order to minimize bias. The questionnaire was completed after the ultrasound examination and the participants perception of the results might therefore have affected their answers.

Retrospectively it may have been relevant to complete the questionnaire prior to ultrasound examination. Importantly, the order was equal at al timepoints.

Secondary clinical outcome included evaluation of maximal tendon pain where participants were asked to rate pain during preferred sporting, squat and running on a 0 to 10 NRS scale (10 being the worst imaginable pain and 0 denoting no pain). Furthermore, self-reported evaluation of treatment satisfaction was included at the end of the intervention and in the long-term follow-up.

To be able to control for altered activity level as a confounder for potential effects of load magnitude we decided to monitor weekly sport participations during the intervention period. Therefore, the participants were asked to rate weekly sport participation in hours during the preceding week at every visit.

9.4.2 Functional evaluation

Strength measurements

To monitor if both applied intervention protocols result in knee extensor strength gains, maximal peak force and KEM were obtained during an approximately eight seconds isometric contraction as previous described[20]. Four maximal isometric contractions were conducted, with a 2-minute rest-period between each test. The first trial was considered as familiarization to the test and maximal peak force and knee extensor moment (KEM) among the remaining three were used for analysis. Normally, the strength test is continued until no further improvement occurred. We decided to apply four trials, because further outcomes were assessed after the strength-test. However, the same approach was applied at both baseline and 12-weeks follow-up, and should thus not influence the time effect investigation.

Single leg decline squat test

The single leg decline squat (SLDS) test is the only published clinical test for patellar tendinopathy, which assess participants subjective reported pain on a Numeric rating scale (NRS) upon maximal eccentric loading of the patellar tendon[110]. The test has shown good reliability[123] and has been successfully used as clinical assessment tools in a previous study[170], and therefore we decided to use it to assess the efficacy of the treatment over time. However,

the test has limitations that we considered. First, the participants expectation of pain might influence the performance and pain score during the test. Secondly, pain improvement after repeated loading is characteristic for patellar tendinopathy, and thus a decrease in pain among the practice trial and the first test (and test trial one and two) might influence the pain score. In an attempt to standardize the warm-up effect, which potentially might be most pronounced in the beginning of the study due to more severe symptoms, we decided that the SLDS test should be performed after the 5-minute warm-up on bicycle-ergometer. Finally, the evaluation of pain using a NRS scale is an inherent limitation due to the ordinal nature of the scale and given that increasing pain by one is not as easy in the high as in the low end of the scale. Although, a two-point difference on the NRS is considered to be a MCID[135], MCID specifically for the SLDS test or in a population of patients with patellar tendinopathy is unknown. Furthermore, the speed of the loading test is self-selected by the participants, thus it cannot be ruled out that the score of pain might be influenced by the speed or the time under tension. To reduce bias in the present study, participants were not allowed to know the score from their previous test before the test trial so the rating would not be affected by the previous result. In fact, the test was carried out at baseline, 6 weeks, 12 weeks and one-year follow-up it cannot be excluded that the data obtained at the end of the study may have been influenced by a learning effect.

Jump test

In the study we chose to include the two jump tests; Squat jump (SJ) and Counter Movement Jump (CMJ) to assess if patellar tendinopathy caused functional deficits. Based on the high prevalence of patellar tendinopathy in jumping sports we hypothesized that the jumping ability were affected in tendinopathy and might respond to the loading-based treatment. Since assessment of jumping ability was secondary outcome in the study, and extending the time of the total test program was not feasible, we decided to perform the jump tests on a portable contact-mat. From this devices and associated software vertical jump height was estimated from recorded flight time. Conversely, carrying out the jump tests on a force-plate would have added information on movement of the knees, hip and trunk influencing the results, specifically in relation to the SJ which is technically difficult to perform. To avoid any counter movement in the starting positioning of SJ, which might be a painful position in the study population we included an adjustable steel frame with an attached sound sensor on the top to the test setup.

This custom-made equipment provided auditive feedback if the participant squatted to deeply. In addition, we decided that it was allowed to provide verbal feedback to the participants with respect to critical performance points (starting and ending positions, not bending the knee in the air prior to landing, movement of trunk, hips and knees) between both the practical trials and the test trials. The test was continued until three technically corrected jumps were recorded and the mean jump height was used for further analysis. The effect of pain on the jumping performance was another consideration thus it might change during baseline and post-test. Therefore, participants were reporting pain on a NRS scale upon the jump tests.

9.4.3 Mechanical properties

Patellar tendon mechanical properties were examined by a previous described method[55,151] consisting of measuring patellar tendon elongation with ultrasound during isometric knee extension. The method was applied in the present study, since it has been shown to be accurate and reproducibly[55]. Yet, in vivo testing of tendon mechanics is challenging and the method has some inherent limitation covered by Seynnes and colleges[139] in a recently published critical evaluation of the method. We tried to eliminate issues based on these recommendations, thus only one investigator performed all tests, and the preconditioning (warm-up, equal examination order and 1-3 practical-trials) was equal at pre- and post-test. Furthermore, we tried to optimized the method previously used at our department by recording the US directly on the machine to gain a better quality (Figure 7, A). In addition, the way of determine the Patellar tendon deformation was optimized by using a more recent custom-made Matlab scrip (Matlab R2016b, The MathWorks Inc, USA) based on a cross-correlation algorithm to track the tendon insertions on patella and tibia[151]. Figure 7 B, show an example of tracking the patellar insertion movement by the Matlab script, placing around 10 tracking nodes on apex patellar. The tracking was performed 2-3 time at apex patella and tuberositas tibia, respectively, and the calculated tendon deformations were then correlated with associated force measurements in a costom-made excel template replacing the previous applied analysis in SigmaPlot. An example of a generated force-deformation curve, fitted to a second-order polynomial is shown in Figure 7, C. Three investigators conducted the tracking analysis in a blinded fashion. Re-analysis of five participants during the training phase of the

method show a typical error below 7% for the intra- and inter-observer measurements. However, to eliminate risk of bias the pre- and post-scan for each participant were analyzed by the same investigator.

Mechanical and material properties were extracted from each curve at maximum force. Because we wanted to investigate the differences between the two groups all curves were additionally cut to the lowest common force across all participants and timepoints, which ensure that a potential effect was not only related to different force levels between the two groups. In analysis of differences between injured and asymptomatic tendons, all curves were cut to the lowest common force across each leg and timepoint, because we wanted to investigate differences between legs and change over time within the same participants.

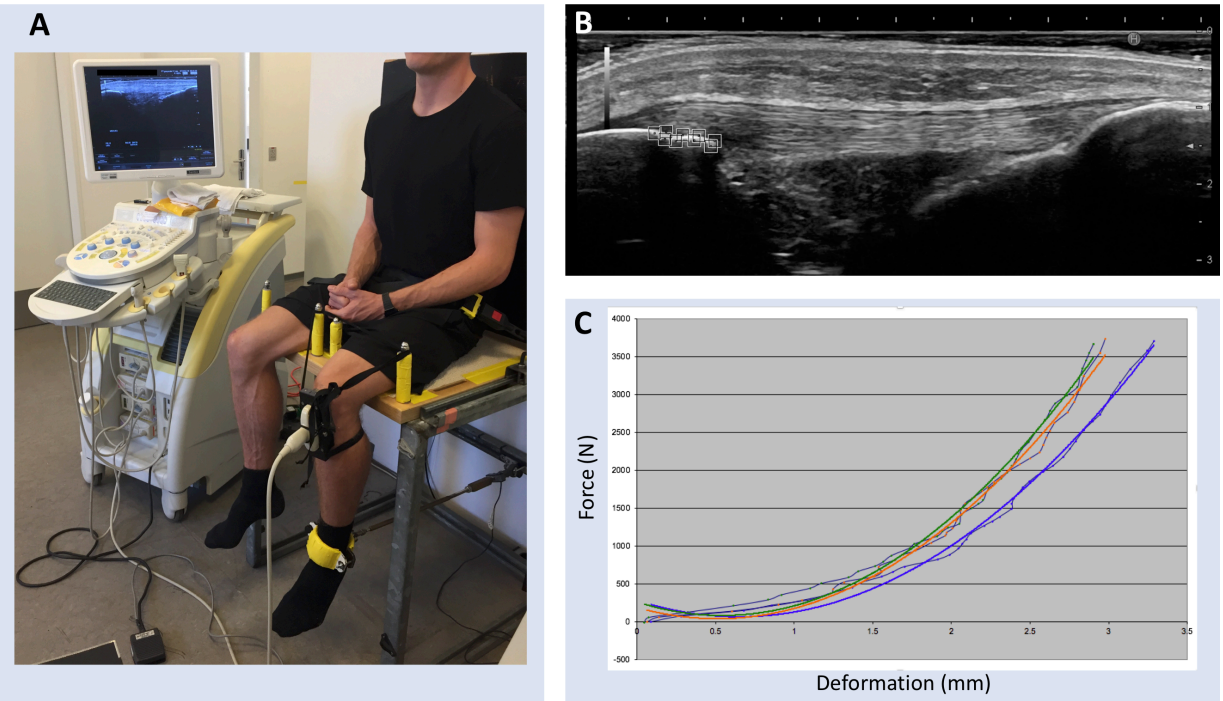

Figure 7: A: Experimental test setup for synchronous recording of force during a ramped isometric contraction and ultrasound recording directly on the machine; B: Example of a tracking of the patellar insertion movement by the Matlab script, placing around 10 tracking nodes; C: Example of a generated force-deformation curves, fitted to a second-order polynomial

Tendon cross sectional area (CSA) is an important factor in calculation of the patellar tendon stress component. The reports of conflicting tendon CSA in tendinopathic patellar tendons in the literature likely due to differences in measurements location and applied methods (US and MRI) might explain a similar large variation in detected modulus[162]. Based on the assumption

that CSA in the study may be influenced by the intervention we decided to use the average (proximal, mid, distal) CSA of the whole tendon determined from the conventional MRI. The choice of applying MRI for determining tendon CSA and length although MRI is more time-consuming and costly to asses was the superiority of MRI compared to US in relation to repeated measures[89]. Obtaining MRI recordings bilaterally was not feasibly due to the total scan time of the MRI protocol including both conventional and UTE MRI. This implies that only stiffness and not modulus could be compared between the injured leg and the contralateral asymptomatic leg in participants with unilateral symptoms.

9.4.4 Tendon imaging

Ultrasound grey scale and power doppler assessment

Gray-scale-ultrasound (GS-US) and power-doppler-ultrasound (PD-US) assessment was applied to confirm the clinical diagnosis of patellar tendinopathy and as an objective method for monitoring the effect of the treatment. The applied methods and settings of GS and PD were identical for confirming the diagnosis, baseline assessment and all follow-up examinations in the study.

One of the disadvantages of US is a high degree of operator dependency and high intra-observer variation[17]. To avoid bias during the repeated measures and among investigators a standardized protocol was developed. Furthermore, a template for GS and PD settings, respectively, was applied and the same machine use for all examinations. In addition, the ultrasound examination was performed by the same two assessors and if possible all scan for each participant were conducted by the same assessor.

Measurement by GS-US is a widespread and established parameter for detecting the level of degeneration (thickness and hypo-echoic areas) in the patellar tendon[45,105]. Although, the method has been shown to be valid[44], the use in the present study was based on different considerations. Because of the repeated nature of measurements in the present study we included anterior-posterior (AP)-thickness from a longitudinal scan (Figure 8, A) as a measure of tendon thickness as recommended by Fredberg et al.[44]. Including both a longitudinal and transversal scan is preferable[44] and might have been optimal to elucidate influence of angling of the probe. However, we tried to eliminate angulation of the transducer

by standardizing the scan protocol. Furthermore, we used a long transducer, which gave the opportunity to visualize the whole tendon in one image and all GS examination recordings were obtained with the participant seated and with 90 degree of knee flexion to standardize knee angle and tendon stress as.

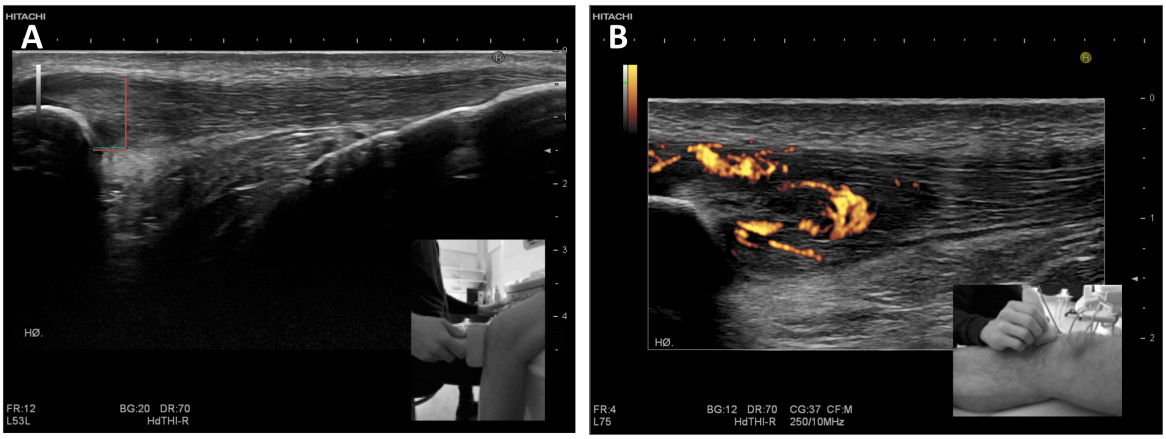

Figure 8: A: Ultrasonography gray-scale assessment using a long transducer. Red lines illustrate measuring site for Anterior-Posterior tendon thickness. B: Ultrasonography assessment of Power Doppler activity using a short transducer.

A reliability study on healthy patellar tendon show considerable variation in tendon thickness throughout the full range of the patellar tendon[69]. Even though the study is on healthy tendons it underlines the importance of standardizing the measurement. In the present study, the specific measuring site 0.5 cm distally from the patellar apex was performed as previous described[84] and based on the typical location of the degenerative changes. However, to optimize accuracy of the measurement, recorded images were exported to the FIJI/ImageJ program where the analyses were carried out on pixel level by one assessor blinded for group, timepoint and scan number. The max AP-thickness of two recorded scans at each timepoint were used for analysis. Previously the mean of three AP-thickness measurements were used for analysis[84]. However, Skou and Aalkjær[145] found no further improvement on intra- and interrater reliability or measurement precision when using a mean of three measurements compared to two in the evaluation of patellar tendon thickness.

As previously described, the neovascularization is another response of pathology in the tendinopathic tendon, which was evaluated by PD-US, Figure 8, B. We chose to use PD because of the increased sensitivity reduced dependence on the transducer angle compared to color-doppler ultrasound (CD-US)[17]. Although, using PD might limit the opportunity to compare

our results to previous studies, the technological improvement on the imaging quality and sensitivity within the last decades[138] might anyway lead to incomparable results for neovascularization. To avoid bias in the measured amount of perfusion care was taken to avoid artifacts in the study; scan was performed on stretch leg in a relaxed position (reduced strain on the patella tendon) and compression reduced by applying a thin layer of gel. Further participants were instructed to abstain from hard physical activity 24 hours prior to the examination, because this might lead to increased flow[83].

An inherent limitation of the measurement is that it was not possible to cover the whole tendon in one recording by the shorter transducer used for PD assessment. We tried to standardize the region of interest by adjusting the position to ensure that the apex patella was "exactly" in the left side of the image and the color box fill out the entire image. However, in participants with doppler more widespread in the tendon it might have underestimated the total amount of PD.

Another artifact in the PD recordings is noise[17]. Firstly, we try to eliminate noise in the recordings by optimizing the scan parameters. Moreover, noise was taken into account in the post process analysis of the recorded videos after they were imported to the FIJI/ ImageJ program. The custom-made macro was set to only include areas >20 combined pixel as areas <20 pixel were considered as noise. Additionally, all detected PD areas were manually evaluated and if established as noise not included in the analysis. Furthermore, PD was only included if it was localized within the tendon.

Two investigators conducted all analyses in a blinded fashion. A small intra-rater reliability study was performed on the PD analysis method by a master student on 10 random selected series showing a typical error percentage of 1.64%. Additionally, another small inter-rater reliability test at our department showed a typical error of 4.71% when the analysis method was applied on Achilles tendon. Therefore, all scans for each participant were analyzed by the same investigator.

Magnetic resonance imaging – tendon dimensions

Conventional MRI was used to provide detailed information on dimensions of the injured patellar tendon. Although, MRI is more time-consuming and costly it eliminate some of the main disadvantages of using US; high degree of operator dependency and high intra-observer

variation[17], which is why we chose to use it in estimation of CSA in the present study. The measurement method applied by Kongsgaard et al.[86] was applied in the present study since it has been shown to be informative of region specific dimension and alteration following resistance training. To avoid CSA underestimation, which has been reported when measuring only on gray-scale images[33], a NIH color-scale were additionally applied during the measurement, Figure 9. This approaches has been shown to reduce the underestimation of CSA by 2.8% compared to using only grey scale[33]. To further optimize accuracy of the measurement the same investigator carried out all measurements and a mean of three measurements at each location was used for analysis. Prior to measurement of tendon dimension in the study, a reliability test of the method was performed on a small random sample showing a typical error percentage of approximately three percent for the investigator who performed the final analysis.

The patella tendon length was also determined from MRI. This consideration was based on the 3D nature of the MRI compared to the 2D nature of the grey scale US recordings. The 3D imaging eliminates assessment errors of the US transducer related to angle and movement[44].

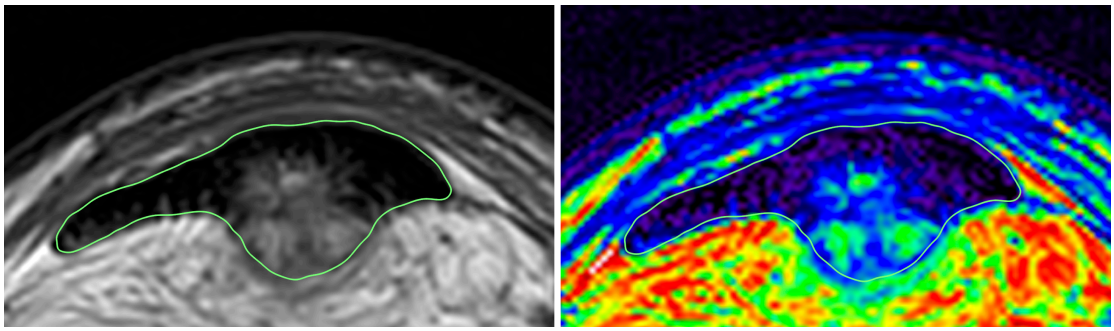

Figure 9: MRI axial grey scale (A) and National Institute of health (NIH) colour scale (B) of the patellar tendinopathic tendon. In both pictures green line indicates tendon outline.

Ultra-short time to echo magnetic resonance imaging with T2* mapping

We hypothesized that the MRI ultra-short time to echo (UTE)-T2* mapping method would be a relevant method to apply in Study 1 as a non-invasive marker for tendon material alterations based on the existing evidence (described in the background section). The work with setting up the method and further developing the analysis was a considerable part of this PhD, because

the method was not previously applied in our department and included several collaborators and failed attempts prior to the final solution applied in Study 1 and Study 2. Furthermore, since the applicability of UTE-T2* as a tool for monitoring of tendinopathy was not established we further decided to set up the reproducibility study (Study 2).

Detailed description of the final developed and applied UTE-T2* protocol as well as pros and cons of the method are covered in paper III. The T2* value of each voxel included in the T2* fitting analysis will determine the outcome (mean T2* relaxation time(ms)), thus the selection of voxel included was an important part of the development of the method, why the methodological considerations thereof will be elaborated below.

The mono-exponential fitting procedure was made in all voxels for the whole tendon and presented in a T2* map (Figure 10, A). A corresponding goodness of fit map presented the Person correlation r-values in all voxels (Figure 10, B). In many previous studies[51–53,75], they included al voxels within the tendon in the analysis of T2* values without adjusting for voxels with poor fitting. We decided to replicate this approach and a mean T2* including all voxels within the segmented tendon volume was calculated. However, because we on the goodness of fits maps consistently observed an area with poor fit in the posterior medial tendon part we decided to account for this.

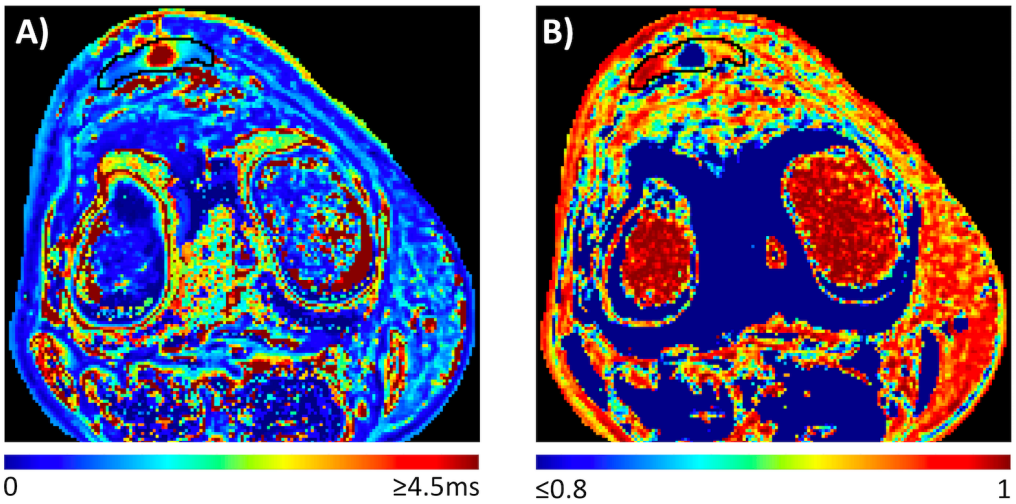

Figure 10: A: Representative T2* map, scale bar values ≥4.5 ms are red. B: Corresponding goodness of fit map (Pearson correlation r-values), scale bar values ≤0.8 are blue. In both pictures black line indicates tendon outline. (*Figure are duplicated from paper III, Agergaard et al. 2020[1]).*

Therefore, T2* values for corrected values where calculated only including voxels with goodness of fit > 0.8 (corrected values). Voxels with a poor fit coincide with high T2* values, thus excluding these voxels result in a lower mean T2*, which mean that the T2*corrected values end up lower than T2* including all voxels, Figure 11. The corrected T2* values are those which will be presented in the thesis as T2* values, because we found it most relevant to present values based on voxels where an exponential fit actually was possible.

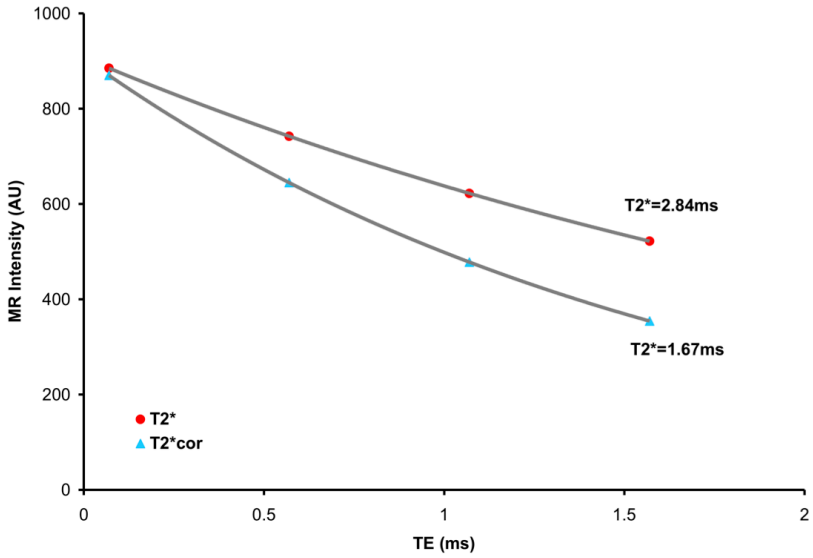

Figure 11: Representative plot from the mono-exponential fitting procedure, which was made in all voxels for the whole tendon volume. T2*: fitting procedure including all voxels; $T2^*_{cor}$: fitting procedure including only voxels with r>0.8. *(Figure are duplicated from Paper III, Agergaard et al 2020[1])*

Tendinopathic tissue have a less dense structure and therefor an inherently higher T2 value. To describe these areas with severe tendinopathy, longer TE than used in our protocol might be required and for this reason the most server part of the tendinopathic tendon might have had worse fitting and therefore not be included in the corrected T2* values. However, the possibility to address this limitation of the method in relation to different disease stages and different tendon parts in same investigation need further investigation.

9.5 STATISTICS

In Study 1 statistical analysis was performed in in GraphPad Prism (version 8.2.1 for macOS, GraphPad Prism Software, California) whereas the statistical analysis of Study 2 was carried out in Excel 2018 (MicrosoftVR Corporation, Redmond, WA, USA) and SPSS (IBMVR, Version 23, 64-bit edition). Prior to analysis all data was evaluated for normality by visual inspection of residual plots. All demographic baseline data were analyzed by use of unpaired Students t-test and reported as mean ± standard deviation. All other results are presented as mean error for continuous variables and mean (95%CI) for log-transformed values unless otherwise noted.
In both studies, the statistical significance level was set to 5%.

Study 1

We employed unpaired Students t-test to determine whether the treatment groups (HSR and MSR) were statistically different with respect to demographic baseline data and participants compliance. Two-way analyzes of variance (time x intervention) with Bonferroni post hoc analyzes were used to analyze differences in other outcome parameters.

In **paper I** Fisher exact tests were used to compare the treatment groups with respect to participants satisfaction at 12 and 52 weeks. Furthermore, correlation of changes in VISA-P score and PD from baseline to 12-week and baseline to 52-weeks were analyzed with Persons correlation

In **paper II** for individuals with unilateral involvement, Two-way analyses of variance were used to calculate difference between mechanical properties of the injured and contralateral asymptomatic tendons with time and side as factors. Furthermore, correlation of material properties (T2* relaxation time) with mechanical properties (stiffness and modulus) at baseline were analyzed with Persons correlation.

In Study 1, all analyzes were performed as intention to treat, with the last observation carried forward. For all outcomes, per-protocol analyzes including participants that fulfilled at least 75% of the prescribed training sessions were also carried out but did not change any of the

conclusions in paper I or paper II. Therefore, in the thesis only intention to treat data will be presented.

Study 2

Paired Students t-test was used to assess differences between repeated measures (test–retest) and within and between observers (intra- and inter- observer reproducibility).

To evaluate reliability, intraclass correlation coefficient (ICC) with 95% confidence intervals was calculated. Furthermore, typical error percentages were used as a measure of the relative measurement error and Bland-Altman plots for visualization of the relative difference between the two tests.

10. RESULTS AND DISCUSSION

In the following section, the main findings from the two studies will be presented, explained and discussed in relation to the existing literature. Complete results sections and more detailed discussions can be found in the three papers at the end of the thesis.

10.1 Summary of key findings Study 1

A total of 44 participants were enrolled in the study and randomized into one of the two groups. Due to baseline findings on MRI two participants did not receive the allocated intervention and were removed from the analysis. Characteristics for the 42 participants included in the analysis are shown in Table 5 and the two groups were well balanced at baseline.

Table 5: Participants demographics and baseline characteristics

Variable	**HSR** (n=21)	**MSR** (n=21)
Age (y)	28.8 ± 5.1 (20-38)	32.3 ± 4.9 (23-41)
Height (cm)	185.8 ± 7.1	180.7 ± 7.2
Weight (kg)	86.7 ± 9.3	82.2 ± 9.2
BMI (kg/m^2)	25.1 ± 2.4	25.2 ± 2.6
Symptom duration (months)	6.9 ± 2.4 (3-12)	7.3 ± 2.9 (3-12)
Weekly sport participation (hours)	9.0 ± 4.8 (1-21)	7.0 ± 3.8 (1-14)
Pain during activity (NRS)	4.7 ± 2.2 (1-8)	5.2 ± 2.0 (2-9)
Unilateral/bilateral (n)	14:7	13:8
Proximal/distal injury (n)	21:0	20:1

Values are expressed as mean ± SD (range). HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. There were no differences between the two groups for any parameters at baseline. *Table are duplicated from paper I & II*

Paper I

In the study both groups received a loading-based intervention. The prescribed exercises were performed with either moderate loads (55% of 1RM) or high loads (90% of 1RM). Regardless of loading regimes a significant improvement of clinical outcomes was observed after 12 weeks intervention, and these were maintained after one-year. Thus, HSR was not superior to MSR loading as measured by self-reported pain and function in VISA-P (primary outcome), NRS-preferred sport and SLDS test, Figure 12.

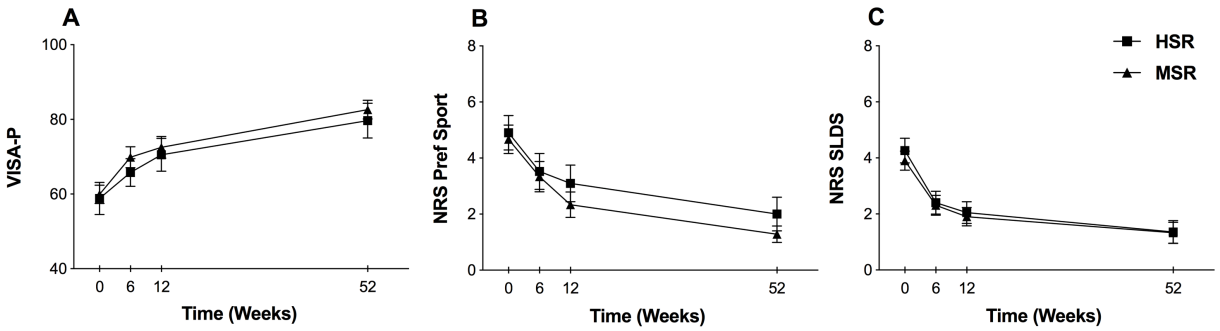

Figure 12: Clinical improvement in VISA-P (A), NRS-preferred sport (B) and SLDS test (C). Values are presented as mean±SEM at baseline (0 weeks), mid the intervention (6weeks), after the intervention (12 weeks) and the one-year follow-up (52 weeks) for the two intervention groups. VISA-P, Victorian Institute of Sports Assessment-Patella; NRS pref Sport, pain during preferred sport measured on a numeric rating scale; SLDS, Single leg decline squat test. HSR, Heavy slow resistance group (n=21); MSR, Moderate slow resistance group(n=21).
Two-way analysis of variance was conducted with group and time as main factors. VISA-P: P-values group (0.57), time (<0.0001) and interaction (0.89). NRS pref sport: P-values group (0.48), time (<0.0001) and interaction (0.73). SLDS: P-values group (0.73), time (<0.0001) and interaction (0.93).

Similarly, there were no significant differences between HSR and MSR in regards to changes in US measures (A-P thickness and PD area), and there were no significant time effects at 12 weeks, Figure 13. However, neovascularization decreased significantly from baseline to 52 weeks in both groups whereas no effect of time was observed in A-P patellar tendon thickness.

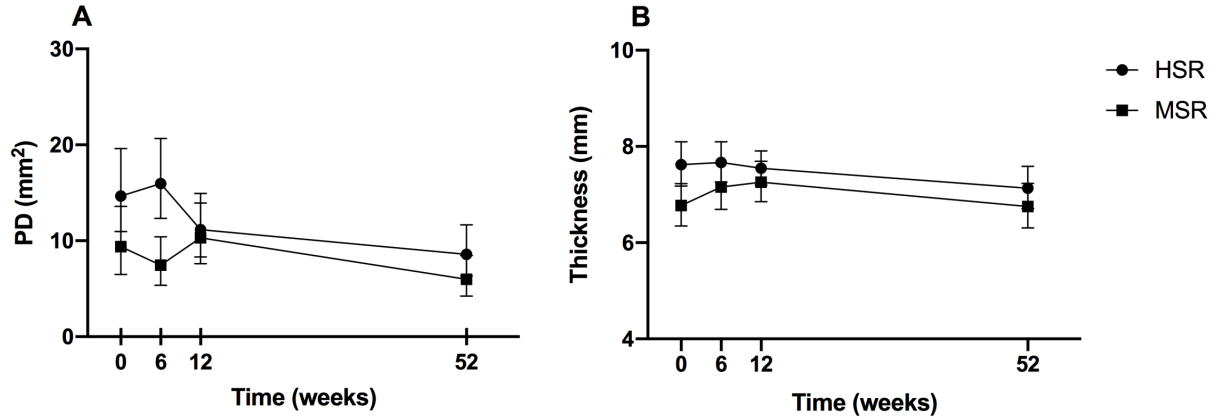

Figure 13: Changes in power doppler (PD) area (A) and AP-tendon thickness (B). Values are presented as mean±95%CI at baseline (0 weeks), mid the intervention (6weeks), after the intervention (12 weeks) and the one-year follow-up (52 weeks) for the two intervention groups. HSR, Heavy slow resistance group (n=21); MSR, Moderate slow resistance group(n=21). Two-way analysis of variance was conducted with group and time as main factors. Doppler: P-values group (0.30), time (0.01), and interaction (0.22); Thickness: P-values group (0.37), time (0.10) and interaction (0.58).

Paper II

Both 12 weeks exercise with moderate (55% of 1RM) and high load (90% of 1RM) resulted in significant strength gains (Figure 14, A) in participants with patellar tendinopathy, but there were no effects on stiffness (mechanical properties)(Figure 14, B) and T2* relaxation time (material tissue structure), Figure 15.

For the participants with unilateral symptoms, stiffness did not differ significantly between the injured and contralateral asymptomatic tendon and there was no effect of time, Figure 16.

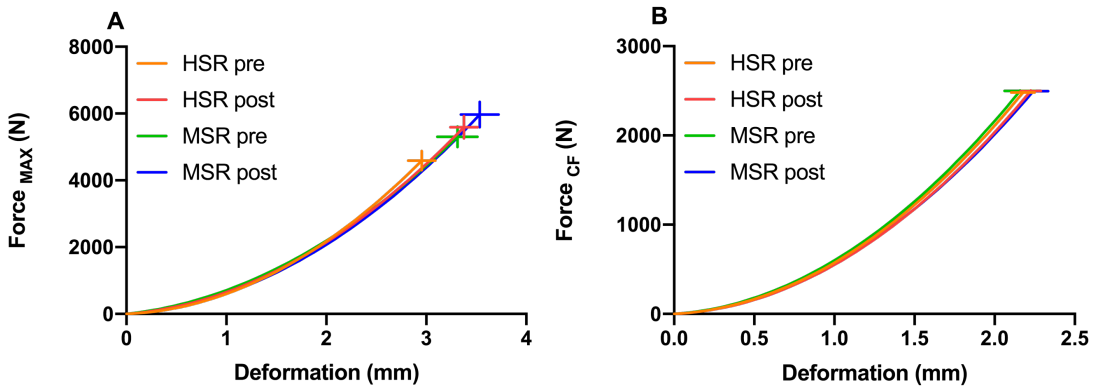

Figure 14: Pre and post force-deformation curves for maximum force (MAX)(A) and for lowest common force (CF) across all participants and timepoints (B) for the two loading regimes. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Pre, baseline 0 weeks; Post, after the treatment intervention (12 weeks). Peak points are displayed as mean±SEM. Two-way analysis of variance was conducted with group and time as main factors. Peak $Force_{Max}$: P-values group (0.21), time (<0.0001) and interaction (0.32). Peak $Stiffness_{CF}$: P-values group (0.85), time (0.43) and interaction (0.68).

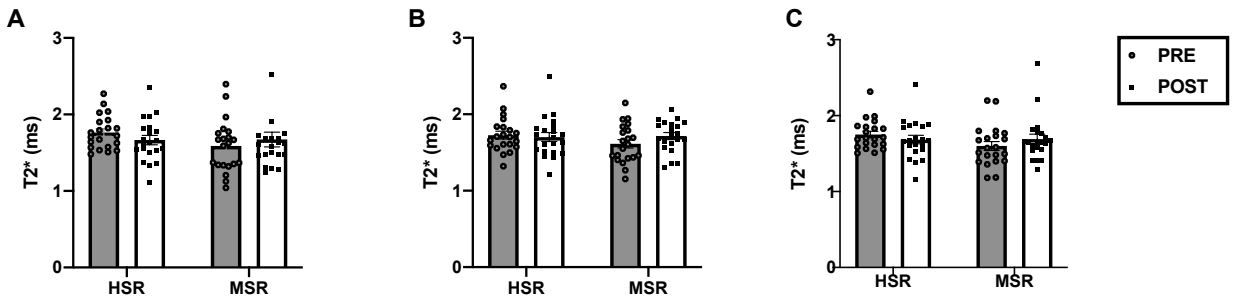

Figure 15: Proximal (A), distal (B) and total (C) tendon T2* values of the injured Patellar tendon at baseline 0 weeks (PRE) and after 12 weeks intervention (POST). Values are presented as mean±SEM. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. Two-way analysis of variance was conducted with group and time as main factors. T2* Proximal: P-values group (0.35), time (0.88) and interaction (0.07). T2* Distal: P-values group (0.45), time (0.29) and interaction (0.08). T2* Total: P-values group (0.29), time (0.07) and interaction (0.07). *Figure are duplicated from Paper II*

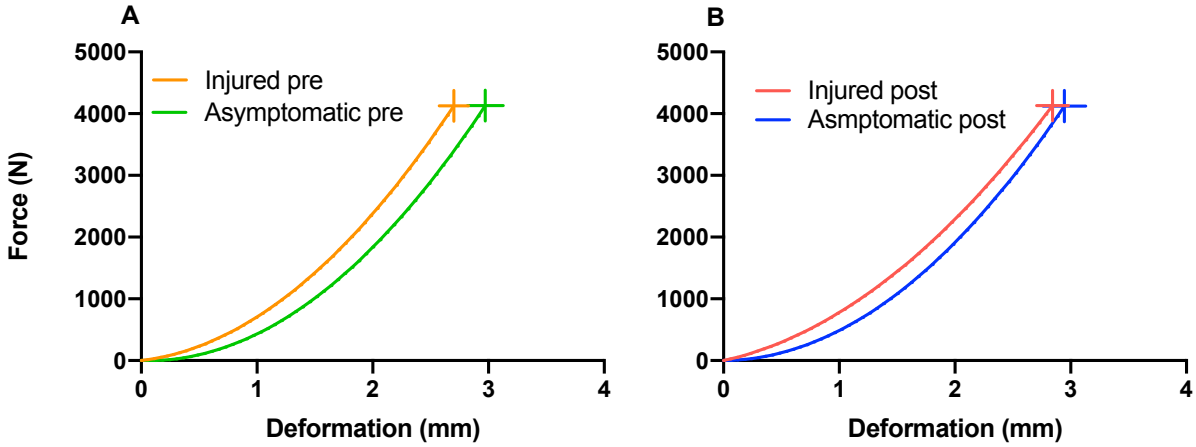

Figure 16: Force-deformation curves for lowest common force across each leg and timepoint within each participants at baseline (PRE) (A) and after 12 weeks intervention (POST) (B). Iinjured leg and contralateral asymptomatic leg for participants with unilateral symptoms (HSR and MSR together, n=26). Peak points are displayed as mean±SE. Two-way analysis of variance was conducted with leg and time as main factors. Stiffness: P-values between leg (0.08), time (0.65) and interaction (0.22).

10.2 Discussion of the main results Study 1

In the following section, the key findings from Study 1 will be explained and compared with findings from other studies. Further, additional data on changes in strength and load during the intervention period for all participants and data from ultrasonography in participants with unilateral symptoms will be presented. These are not presented in the papers but will be included in the discussion below. Finally, the implication of applying different load magnitude in exercise-based treatment programs will be covered.

10.2.1 The effect of load magnitude on clinical outcomes (paper I)

The present study demonstrated clinically improvements whit respect to pain and function (Figure 12) from baseline to 12 weeks, and the magnitude of these were in line with improvements that have been reported in previous studies on loading-based rehabilitation in patients with patellar tendinopathy[32,93,102]. Specifically for VISA-P the baseline values were comparable with those that have previously been reported in a populations with chronic patellar tendinopathy[67,84]. Furthermore, at 12-weeks the changes in VISA-P approached the suggested clinically relevant change (13 points) [64] in both groups (HSR 11.7 points and MSR 12.6 points).

The lack of superiority of HSR compared to MSR at the primary endpoint (12-week follow-up) was surprisingly. However, this may be explained by the relationship between load magnitude and clinical improvements. Thus, theoretically clinical improvements might increase up to a certain amount of load and then levels off, so increasing the load above this point would not further enhance the clinical response to the treatment. The data from the present study certainly support this notion. The explanation for the proposed relationship between load magnitude and clinical improvements remains speculative. However, the results in the present study suggest that the training volume may be more important than the load magnitude. Thus, a high volume of more moderate loads may be sufficient to induce sufficient strain on the fibroblast in order to stimulate the synthesis of collagen[49,73], which is essential for tendon healing. Two previous studies[13,84] have compared HSR and ECC training. They showed similar improvements in VISA-P and VAS in both groups. Although, they did not match the groups in regard to load or volume in these studies, it might be reasonable to assume that the HSR group received higher load magnitude compared to the ECC group who trained with their own body weight as resistance. This support the finding from the present study, and emphases that very high load is not necessary to induce short term clinical effect.

In the present study, we included a six-weeks follow-up, which enabled us to evaluate whether the improvements primarily occurred during the familiarization period or in the period where high load magnitude was applied. In the familiarization period from baseline to six weeks the VISA-P score improved by 7 and 10 points (HSR and MSR respectively), but with no significant interaction between the groups, which supports that a substantial amount of the improvements occurred in this period. The data from the present study thereby further support that very high load is not necessary to induce short term clinical effect. Thus, high load magnitude was first applied in the HSR group the last six weeks of the intervention period. However, the minimal threshold for initiating improvements in tendons remains elusive. In skeletal muscle strength gains have been demonstrated in response to both heavy (70% of 1RM) and very light load (15% of 1RM) at equalized total work[68]. Thus, it could be speculated that the minimal threshold for clinically improvement might be as low in tendon.

When we designed the study, we were concerned that the time performing the MSR program due to the extensive amount of reps and set performed in slow actions would result in reduced compliance. However, there were no difference in compliance rate between

the two treatment groups in relation to session performed of aim (HSR 78% and MSR 86%). Another aspect that could have influenced the results of no superiority of HSR was if the participants were not loaded with the prescribed load. As shown in Figure 17 the load changes were substantially different between the two treatment groups in all three exercises from baseline to six-weeks follow-up, but not from seven to 12-weeks follow-up, which supports that the two loading intervention differed in load magnitude, as prescribed. Additionally, the improvement in load for the HSR group was a result of both increased load intensity (55-85%) and gain in strength, whereas the improvement in the MSR group where it only reflects gain in strength (unaltered load magnitude at 55%). This further supports a difference in load magnitude between the two groups.

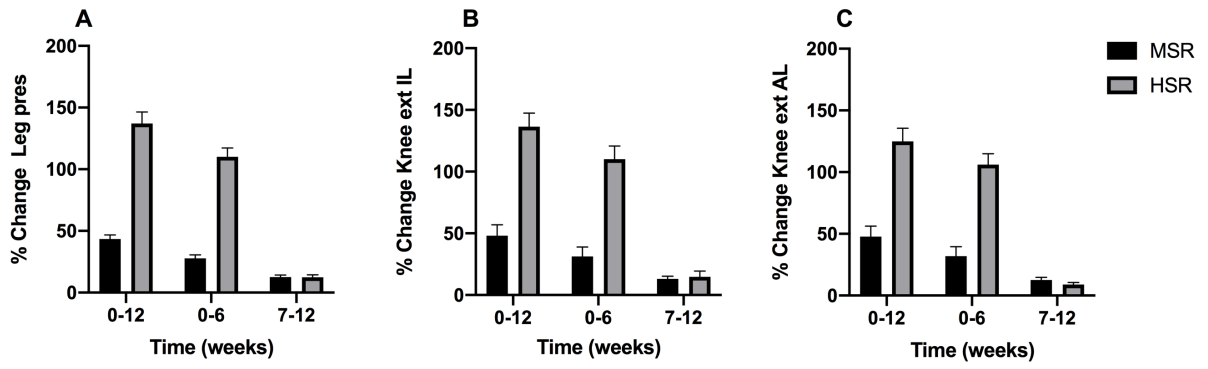

Figure 17: Change in load % for the three exercises; Leg press (A), Knee extension injured leg (B), Knee extension contralateral asymptomatic leg (C) during the total intervention period (0-12 weeks), from baseline to mid intervention (0-6 weeks) and from mid to after intervention (6-12 weeks) for the two intervention groups. HSR, Heavy slow resistance group (n=21); MSR, Moderate slow resistance group (n=21). Values are presented as mean±SEM.

Moreover, as shown in Figure 18 there were no markedly differences between the groups for any of the exercises in actual strength gains (1RM) from baseline to 12-weeks follow-up, which supports equivalized total volume despite different load magnitude applied. Comparing the relative change in load with the mean MVC in the injured leg at baseline (HSR: 193±11Nm; MSR: 194±10 Nm) there were no significant differences (P=0.95) between groups. This further strengthen the assumption that the volume was equivalized and not a confounding factor for the obtained results in the present study.

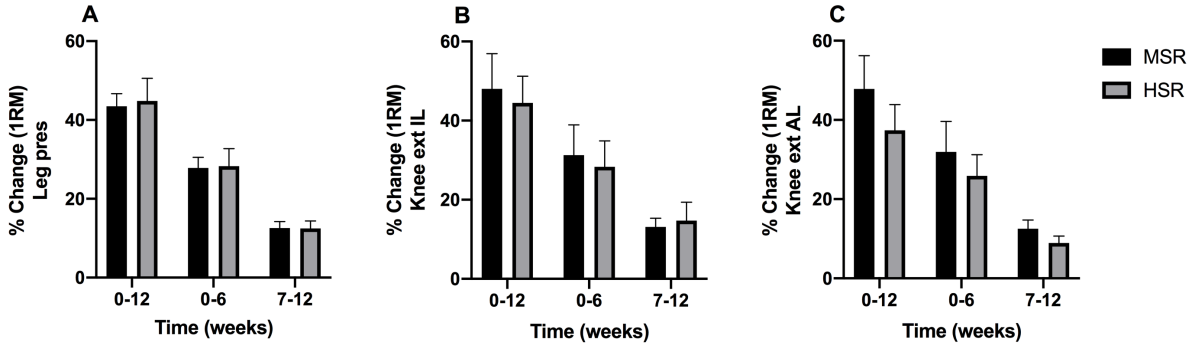

Figure 18: Change in strength % for the three exercises; Leg press (A), Knee extension injured leg (B), Knee extension contralateral asymptomatic leg (C) during the total intervention period (0-12 weeks), from baseline to mid intervention (0-6 weeks) and from mid to after intervention (6-12 weeks) for the two intervention groups. HSR, Heavy slow resistance group (n=21); MSR, Moderate slow resistance group(n=21). Values are presented as mean±SEM.

The duration of the intervention could also be an explanation of the results in the present study. Thus, it could be speculated that a longer intervention period could have revealed superior effects of HSR compared to MSR. Indeed the effect of relatively short loading interventions (typically 12-weeks) have been questioned[102], and Bohm it al.[19] conclude in a systematic review that durations >12 weeks of loading interventions is more effective inducing tendon tissue alterations. However, after 12-weeks intervention the two groups performed almost identical on all parameters, which makes further improvements highly unlikely. Furthermore, due to the inherent limitation in keeping all other parameters than load magnitude stable, an effect of differences in time under tension and reps between the two loading programs cannot be ruled out. Finally, an inherent risk of type two error exists. However, the relatively small within participants variation in VISA-P score at 12-week, which is maintained at 52-week follow-up supports that no real differences exist. However, it cannot be ruled out that the statistical power in the analysis was too low.

Only a few studies[11,84,167] have investigated the long-term effect of loading-based treatment in patellar tendinopathy. Likewise, studies investigating long-term effect of treatment with loading protocols in Achilles tendinopathy is lacking[114]. However, since HSR have shown superior long-term clinically effect compared to ECC training in a previous study on a similarly population with chronic patellar tendinopathy[84], we would have expected a superior clinical effect of HSR compered to MSR on the long-term follow-up. The discrepancies between the two studies may be explained by differences in follow-up length (52 weeks in the present

study and 36 weeks in the previous study). In support of the finding in the present study a previous study[13] have shown similar clinical improvement of HSR and ECC training at one-year follow-up in patients with Achilles tendinopathy.

Noteworthy, despite a difference in follow-up duration there was a lack of full recovery in VISA-P in both studies (20% in the present study (Figure 12) and 15% in the study by Kongsgaard et al.[84]), which suggests that deficit might extend beyond one year. In support hereof, persistent pain and impaired function for up to 5 year have been shown in patients with Achilles tendinopathy[121,142]. An explanation for the lack of full recovery might be that tendinopathy may lead to some kind of unreassurable damages in the tendon tissue as suggested by Cook et al.[27,28] in their continuum model for tendinopathy. This model includes different stages of disease, whereof the last and most severe stage of degenerative tendinopathy had little capacity for reversibility of pathological changes. Another key aspect could be that an almost complete absent of tissue turnover in the core of adults Achilles tendons has been shown[60]. However, it has also been suggested that the turnover is increased in response to tendinopathy, at least in a smaller pool of the tissue[61] although, the amount, location and involvement of other components of the extracellular matrix than collagen remains elusive. Even though it remains speculative this could explain why the patients do not regain full function even after one year. Moreover, it indicates that a longer intervention duration might be needed, in order to regain full function. Finally, another aspect explaining lack of full clinical recovery at one-year follow-up could be fear-avoidance. Psychological variables have been suggested to be associated with outcomes from treatment in tendinopathy, however, the body of evidence within this area is conflicting and high quality of evidence is lacking[107]. Noteworthy participants in the present study were asked about their fear of return of pain and symptoms (data not included in papers) at one-year follow-up. At that point 54% answered that they were afraid of relapse of pain and symptoms related to patellar tendinopathy and 10% that the symptoms were persistent. Moreover, the data on weekly sports participation at one-year follow-up was significantly different from pre-injury level in both groups, Table 6. This might support that the lack of full recovery one year after loading-based treatment is reduced due to persistent pain or fear of pain. On the other hand, it could also reflect a period of overloading prior to symptoms and thus more a contributing factor developing the patellar tendinopathy.

Table 6: Weekly sport participation

Sport participation (hours/ week)	**HSR** (n=21)	**MSR** (n=21)
Pre Injury	9 ± 1 (1-21)	7 ± 1 (1-14)
Week 0	6± 1 (1-10)	5 ± 1 (0-15)
Week 6	4 ± 1 (1-18)	5 ± 1 (0-20)
Week 12	4 ± 1 (0-18)	5 ± 1 (0-16)
Week 52	6 ± 1 (0-18)	5 ± 1 (0-17)
Δ Pre injury-week 0	-3± 1 (-4.6, -1.8)*	-2 ± 1 (-3.8, -0.9)*
Δ Pre injury-week 52	-3 ± 1 (-4.5, -0.4)*	-2 ± 1 (-4.3, -0.2)*
Δ 0-12 week	-1 ± 1 (-3.7, 1.1)	1 ± 1 (-1.8, 3.0)
Δ 12-52 week	2 ± 1 (-0.3, 4.5)	-1 ± 1 (-2.9, 1.9)
Δ 0-52 week	1 ± 1 (-1.6, 3.1)	1 ± 1 (-2.3, 2.5)

Data are obtained as recall at pre examination (pre injury) and as self-reported at week 0, 6, 12 and 52. Data are reported as Mean±SEM (range) and Δ values are expressed as Mean±SEM (95% CI). There were no effect of time (p= 0.439), group (p= 0.75) or Interaction (p=0.13) from baseline to 52-weeks follow-up but participating hours at 52 weeks were significantly different (P=<0.05) from Pre injury level. *Table are duplicated from paper I*

10.2.2 Effect of load magnitude on ultrasonographical alteration

The results from the present study demonstrated reduced power-doppler (PD) from baseline to one-year follow-up in response to 12 weeks of loading-based exercise (Figure 13, A), which are in line with previous studies in Achilles[13] and patellar tendopathy[84]. It is noteworthy that we were not able to reproduce the results from the previous study in patellar tendinopathy[84] in relation to the magnitude of decline in PD at 12 weeks. Thus, the previous shown decline in PD at 12 weeks (45% reduction) was first reached at one-year follow-up (HSR 41% and MSR 36%) in the present study. An explanation of faster reduction in vascularization could be a response to the biopsies taken at both baseline and 12 weeks in the previous study. Biopsy sampling of healthy patellar tendons have recently been shown to result in increased tendon cell activity[58] and in addition, dry needling has shown positive effect as tendinopathy treatment[88]. Thus, the biopsies themselves may contribute to the healing response. Another explanation could be between study variation in sensitivity in relation to the applied US protocol and analysis of PD area. Simply, the technological improvement on the imaging quality and sensitivity within the last decays[138] may also explain the discrepancy.

Indeed, there is a reduction in doppler area from baseline to one-year follow-up (HSR 41% and MSR 36%), still a considerable amount of doppler exist one-year after initiation of treatment (Figure 13, A), which previous only has been shown for Achilles tendinopathy[13].

This could suggest that ingrown capillaries[109] do not disappear within a year, but rather decrease in size or flatten out in response to the treatment and therefore some degree of doppler activity is present even after one year.

We expected a correlation between improvements in pain and changes in PD area since nerve ingrowth along with the increased neovascularization have been suggested to be the cause of pain in tendinopathy[2,109]. However, no correlations were detected between change in VISA-P and PD area from baseline to 12 weeks (r=-0.28, 95%CI [-0.34, 0.2]) or baseline to 52 weeks (r=-1.9, 95%CI [-0.46, 0.13]) in the present study. This finding is in line with a study investigating badminton players were no association between intratendinous flow and pain could be detected[16] and similarly results of no correlation between PD and clinical severity has been shown in Achilles tendinopathy[118]. However, in contrast Cook et al.[26] showed that the presence of neovascularization in abnormal tendons was associated with greater tendon pain. The fact that increased doppler flow represents both physiological response in response to training and a pathophysiological response might explain the variation between studies. Increased flow has been shown in healthy tendon after exercise[18,83,106] and decreased response in doppler flow to activity (running) has been suggested as a risk of developing tendinopathy[161]. In relation to pathological tendons, the existence of doppler has recently been demonstrated in the early phase (<3 months symptom duration) of tendinopathy[151]. However, it remains unknown if doppler is the cause of or the response to tendinopathy. It has been demonstrated that tendons presenting with doppler activity are not necessarily painful, but the presence of hypervascularization in asymptomatic athletes at rest represent a risk factor for developing tendinopathy [24,46,156].

In the present study we did not observe presence of PD signal on the contralateral asymptomatic site for participants with unilateral chronic tendinopathy (Figure 19, B), which strengthen the notion that presence of PD is linked to localized pathological alterations. Moreover, the present data show that prolonged loading might reduce but not remove doppler in tendinopathic tendons (Figure 13, A & Figure 19, B) and importantly it does not induces doppler in the contralateral asymptomatic tendons (Figure 19, B), which to the best of our knowledge has not been shown previously. However, the immediately physiological flow in response to training in tendinopathic tendons is still unknown. The fact that no PD on the asymptomatic site could be detected in the present study further supports that using

contralateral asymptomatic tendon might be the best comparison when investigating e.g. altered mechanical properties in response to tendinopathy due to the inherent effect of limiting confounding variables.

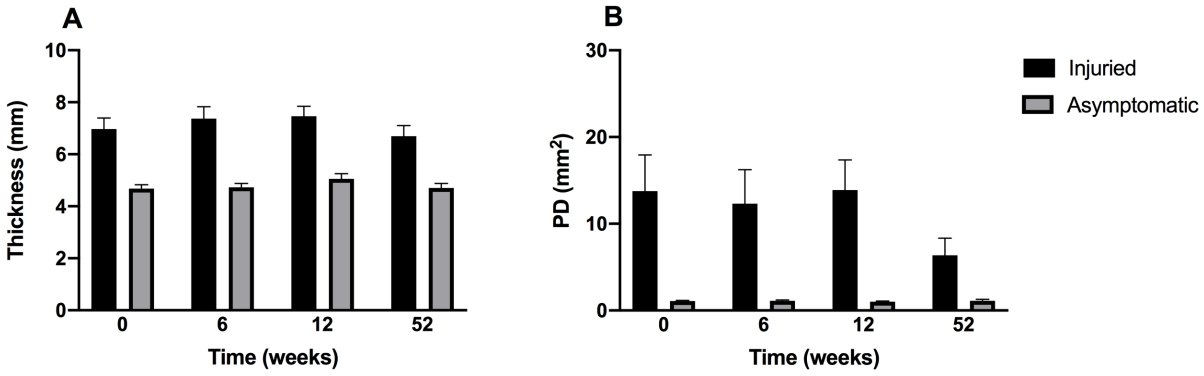

Figure 19: Power doppler area (PD) (A) and AP-thickness of the tendon (B). Data presented are for participants with unilateral symptoms (HSR and MSR together, n=27). Values are presented as mean±95%CI at baseline (0 weeks), mid the intervention (6weeks), after the intervention (12 weeks) and the one-year follow-up (52 weeks) for injured and asymptomatic contralateral leg. Two-way analysis of variance was conducted with leg and time as main factors. Thickness: P-values Leg (<0.0001), time (0.01) and interaction (0.50). PD: P-values leg (<0.001), time (0.01) and interaction (0.001).

Lastly it should be mentioned that the mean A-P-thickness for participants with unilateral symptoms at baseline was 6.96mm (95%CI: [7.4, 6.6]), which was significantly more than the contralateral asymptomatic tendons, mean 4.68mm (95%CI: [4.83, 4.54]) (Figure 19, A) and in line with previous results[44]. Notable, the patellar tendon A-P thickness did not accompany the detected decrease in PD from baseline to one-year follow-up. However, in thickened patellar tendons pain has been demonstrated in absence of doppler flow[105]. Thus, it could be speculated that increased pressure and not the nerve ingrowth might be the cause of pain in tendinopathy. It is well established that increased water content along with hypervascularization occur in tendinopathic tendons[126], which highlights that increased water and associated increased pressure could cause the pain if it is unaffected by reduced hypervascularization. The data from the present study certainly support this notion, however the mechanisms remain elusive.

10.2.3 The effect of load magnitude on mechanical properties and UTE-T2* (paper II)

In the present study baseline values for tendon stiffness (HSR: 2189±89N/mm; MSR: 2198±100N/mm) correspond to values from previous studies in tendinopathic patellar tendons[84,85,94,163]. However, in contrast to previous studies[84,85,94] showing decreased stiffness in response to exercise in tendinopathic tendons there was no effect of 12 weeks exercising of either HSR or MSR on the tendon stiffness in the present study (Figure 14). Thus, the discrepancies between detected stiffness change in the present and previous studies could be explained by a difference in pain induced reduction in loading relative to the pre-injury level caused by tendinopathy. It is well established that patellar tendons mechanical stiffness albeit with sizable variation, increase in response to training in healthy tendons[162] and conversely more rapidly decrease in response to unloading[31], which highlights tendons capacity to adapt to unloading. In the present study sports participation per week in hours for both groups during the intervention period was unchanged (Table 6), which might have contributed to the unaltered stiffness.

Variation of the HSR program applied in the previous study by Kongsgaard et al[84] and the present study could also have influenced the results. It could be speculated that number of exercise and variation in load magnitude progression and thereof varied total volume and time under tension could have caused the discrepancy in detected stiffness between the two studies. Indeed, high training compliance (>78%) and gain in strength (HSR 18% and MSR 11%) confirm that both groups were loading there tendons as prescribed in the present study and because Heinemeier et al.[60] have shown relative low turnover in tendon in general makes insufficient time to change the mechanical properties most likely.

The T2* values obtained with MRI UTE did not change over the course of 12-weeks in the present study. Indeed, T2* relaxation time have been suggested to reflect the amount of unbound water in the tendons[130], the T2* values obtained in the present study (Figure 15) indicating unaltered material properties in the tendon. Thus, stiffness (reflecting the tensile bearing component of the tissue) did not correlate with the T2* values (Figure 20, A) but stiffness and modulus (influenced by both CSA and water content) did correlate (Figure 20, B). Indirectly, because CSA is unchanged in the present study these correlations suggest that the method might be applicable for detecting material alterations before they are transferred to

mechanical alterations. However, this needs to be confirmed in response to longer interventions were alterations in mechanical properties might occur.

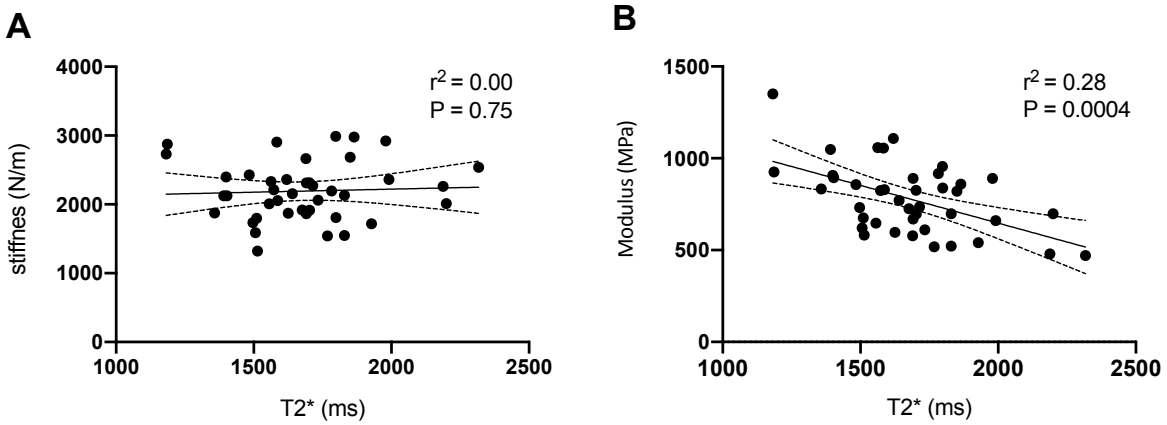

Figure 20: Data presented are baseline values merged for the HSR and MSR groups (n=41). Figure A: Scatter plots between T2* and Stiffness and figure B: Scatter plots between modulus and T2* at baseline. *Figure are duplicated from Paper II*

It has been established that pathological alteration in patellar tendinopathy most frequently involves the proximal posterior portion of the tendon[72,80]. In the present study values for T2* in both the proximal and the distal tendon regions are increased compared to values from our department (unpublished) in healthy patellar tendons (~1ms). Indirectly, the results support that internal alteration on a material level occurs in the whole tendon region in response to tendinopathy, which suggest more widespread tissue alteration than previously assumed. Moreover, the present data show unaffected tendon stiffness in tendinopathic patellar tendon compared to contralateral asymptomatic tendons among participants with unilateral symptoms (Figure 16). Thus, the discrepancy in the values for T2* between tendinopathic and healthy tendons could suggest that some material alterations might occur in tendinopathic tissue but that they might be too small to be detected as alterations in the mechanical properties. This indicates that the method could be used as a non-invasive marker for internal material alterations, however, this need further validation.

The expectation of intensity specific changes in the mechanical properties was based on findings from previous studies on healthy tendons[5,104]. The pathology of the tendon could have influenced the results. Thus, the lack of response to loading indirectly supports that no accretion of collagen to the fibrils occurs, which would have resulted in altered mechanical properties. In fact, it was beyond the scope of the precent study to obtain biopsies precise

information of the collagen concentration and other factors (e.g. crosslinks) influence the mechanical properties is elusive. However, the duration of the intervention could also have influenced the results. The present data show no difference in stiffness between injured and contralateral asymptomatic tendon among participants with unilateral symptoms in response to 12 weeks of exercising (Figure 16), which suggest that mechanical response might require a longer duration (properly due to the relatively low turnover in tendons) more than a different response to loading among tendinopathic and healthy tendons.

10.2.4 Implication of applying different load magnitude in the exercise-based treatment programs

We were surprised to observe that mean training session compliance was high in both intervention groups (HSR 78% and MSR 86%) and with no between-group difference,Table 7. Still, we would have expected that it could be difficult to achieve equally high compliance in both groups. In the MSR group, participants were instructed to maintain the magnitude at 55% throughout the intervention period and further in the beginning of the intervention period had to perform a sizable number of repetitions in a slow manner. It has previously been speculated that similar interventions (sizable number of slow reps) may lead to lower satisfaction compared to the HSR program [13,84], why we thought it could influence the compliance in the MSR group. Further, the physiotherapists performing the supervision reported of pain in the end range in the knee extension machine applied in the commercial fitness center specially for the HSR group, which could have reduced compliance of the performed absolute volume percent of aim in that exercise. Yet, the data from the present study show that participants completed both the moderate and high load exercise program with a high percent of the total absolute volume prescribed for both exercises; leg press and knee extension, Table 7. Moreover, equal gains in strength after the intervention period (15% HSR and 10% MSR) were observed, which confirms that the variation in form of repetition, set, frequency and load appears less important at least when the total volume is kept sufficiently high. Furthermore, the present study demonstrated that participants treatment satisfaction did not differ significantly between the two treatment groups at 12-weeks ($P>0.99$) or at 52-weeks follow-up ($P=0.33$). Thus, at 12-week follow-up 95% (18/19) in the HSR and 95% (20/21) in the MSR group

was satisfied with the clinical outcome. Almost similarly, 84% (16/19) in the HSR and 95% (20/21) in the MSR group were satisfied at 52-weeks follow-up. Altogether, the demonstrated high compliance with both intervention programs and the lack of superiority of satisfaction with the HSR program indicates that both loading regimes can be applied in a clinical setting why the exercise regime that best fits the patient preferences can be freely chosen.

Table 7: Compliance of prescribed interventions 0-12 weeks

	HSR (n=21)	**MSR** (n=21)	**P- value**
Session % of Aim	78.2 ± 4.3	85.8 ± 2.18	0.13
Supervision % of Aim	81.3 ± 4.5	85.7 ± 2.3	0.39
Load % of Aim			
Leg press	97.6 ± 0.9	99.8 ± 0.2	0.02
Knee ext IL	93.6 ± 1.3	95.9 ± 0.9	0.16
Knee ext AL	93.5± 1.7	95.7 ± 0.8	0.25
Reps % of Aim			
Leg press	97.9 ± 1.6	99.7 ± 0.3	0.29
Knee ext IL	97.7 ± 1.2	97.7 ± 0.6	0.97
Knee ext AL	96.5 ± 1.4	98.0 ± 0.6	0.36
Volume % of Aim			
Leg press	97.6 ± 3.5	87.8 ± 1.5	0.16
Knee ext IL	77.8 ± 3.3	83.2 ± 2.1	0.18
Knee ext AL	77.5 ± 3.8	83.3 ± 2.0	0.19
Absolut volume % of Aim			
Leg press	76.8 ± 4.2	84.8 ± 2.2	0.10
Knee ext IL	72.9 ± 4.3	80.3 ± 2.7	0.15
Knee ext AL	71.7 ± 4.7	80.4 ± 2.6	0.12
Absolut TUT % of Aim			
Leg press	79.0 ± 3.9	85.3 ± 2.1	0.17
Knee ext IL	77.1 ± 3.9	83.4 ± 2.4	0.18
Knee ext AL	75.0 ± 4.2	83.6 ± 2.3	0.09

Values are presented as mean±SEM and expressed as % of Aim. Load, reps and volume demonstrating compliance during the training session. Absolut volume and TUT percent of aim demonstrate the total compliance, including missing training session and adjusted for progression in prescribed load during the intervention period. Knee ext IL: Knee extension exercise on injured leg; Knee ext AL, knee extension exercise contralateral asymptomatic leg; HSR: Heavy slow resistance group; MSR: Moderate slow resistance group. TUT: time under tension. *Table are modified from table in supplement in paper I&II.*

In the present study, the absolute training volume (adjusted for missing training session and progression in prescribed load during the intervention period) was high and no significant difference were observed between the groups, Table 7. Finally, training was performed both supervised and unsupervised, in a commercial fitness center in training machines (leg-press and

knee-extension) that are very common. Because, the participants missed training sessions due to work, school, illness etc. the intervention setting is comparable to treatment of patients with patellar tendinopathy in a clinically setting, which increases the generalizability of the results from the present study.

10.3 Summary of key findings Study 2

In Study 2 (paper III), we evaluated the reproducibility of multi-slice UTE-T2* mapping of human tendinopathic patellar tendons. The method was overall shown to be feasibly for use in a research setting and clinical practice. The test-retest evaluation demonstrated a small bias but with a very low associated mean difference (3.7%) between the two recordings and with an excellent reproducibility (ICC $\geq$ 0.08) and low typical error (<4.6%) in all three tendon regions; proximal, distal and total, Table 8.

Table 8: Test-retest reproducibility of UTE-T2* analysis

Tendon part	S1 M1 O1	S2 M1 O1	Diff	P-values	TE %	ICC (95% CI)
Proximal						
T2*(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)	2503 ± 696	2422 ± 692	81 ± 229	0.192	6.6	0.94 (0.84-0.98)
Distal						
T2*(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)	2104 ± 696	1956 ± 587	148 ± 298	0.076	10.4	0.88 (0.65-0.96)
Total						
T2*(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)	4605 ± 1370	4376 ± 1260	228 ± 473	0.083	7.5	0.93 (0.78-0.98)

Values are presented as Mean ± SD. T2* relaxation time (ms) from monoexponentially fitting of UTE Images of the patellar tendon. Values are presented for the proximal, distal and total tendon region. S1& 2: Scanning one and two; M1: Measurement one, O1: Observer one; Diff: difference between the two measurements; TE %: Typical error percentage; ICC: Inter Class Coefficient. *Table are modified from table presented in paper III, Agergaard et al 2020[1].*

Similarly, excellent reproducibility was shown for intra- and inter-observer evaluation (ICC ≥ 0.98 and ICC ≥ 0.97, respectively) of the method in all regions followed by a low typical error percentages for intra-observer (<2.2%) and inter-observer (<2.3%), respectively, Table 9 and Table 10.

Table 9: Intra-observer reproducibility of UTE-T2* analysis

Tendon part	S1 M1 O1	S1 M2 O1	Diff	P-values	TE %	ICC (95%)
Proximal						
T2*(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)	2503 ± 696	2561 ± 668	58 ± 201	0.283	5.6	0.96 (0.88-0.99)
Distal						
T2* (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)	2102 ± 696	2231 ± 660	129 ± 200	0.025	6.6	0.96 (0.88-0.99)
Total						
T2* (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)	4605 ± 1370	4792 ± 1300	187 ± 330	0.045	5.0	0.97 (0.91-1.00)

Values are presented as Mean ± SD. T2*relaxation time (ms) from monoexponentially fitting of UTE Images of the patellar tendon. Values are presented for the proximal, distal and total tendon. S1: Scanning one; M1 & M2: Measurement one and two, O1: Observer one; Diff: difference between the two measurements; TE %: Typical error percentage; ICC: Inter Class Coefficient. *Table are modified from table presented in paper III, Agergaard et al 2020[1].*

Table 10: Inter-observer reproducibility of UTE-T2* analysis

Tendon part	S1 M1 O1	S1 M1 O2	Diff	P-values	TE %	ICC (95%CI)
Proximal						
T2* (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)	2503 ± 696	2795 ± 653	292 ± 189	<0.001	5.1	0.88 (0.04-0.97)
Distal						
T2* (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)	2102 ± 696	2394 ± 709	292 ± 274	0.001	8.7	0.85 (0.23-0.96)
Total						
T2* (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)	4605 ± 1370	5189 ± 1350	584 ± 427	<0.001	6.2	0.87 (0.09-0.97)

Values are presented as Mean ± SD. T2* relaxation time (ms) from monoexponentially fitting of UTE Images of the patellar tendon. Values are presented for the proximal, distal and total tendon. S1: Scanning one; M1: Measurement one, O1 & O2: Observer one and observer two; Diff: difference between the two measurements; TE %: Typical error percentage; ICC: Inter Class Coefficient. *Table are modified from table presented in paper III, Agergaard et al 2020[1].*

10.4 Discussion of the main results Study 2

In the following section I will compare the main findings from Study 2 with findings from other studies and discuss the results in relation to the COSMIN taxonomy[113] of relationship of measurement properties.

Detection of alterations in the microstructural composition of tendon tissue is of great interest in order to establish the pathology of tendinopathy and monitoring the effect of treatment.

Therefore, it was advantageous to investigate if the UTE-T2* method was a valid non-invasive tool for monitoring of tendinopathy. To the best of our knowledge the present study is the first to formally evaluate test-retest and intra- and inter-observer reproducibility in tendinopathic patellar tendons in vivo. Only one other study by Gärdin et al.[53] have quantified the reproducibility of UTE-T2* in human tendons in vivo. They show a test-retest coefficient of variation (CV) of 18% in Achilles tendons, and a mean intra- and inter-observer CV of 22% and 64%, respectively. They additionally observed a 40% difference in T2* values between symptomatic and non-symptomatic tendons and conclude that the reproducibility of the method was low and therefore not applicable on the individual level or in clinical trials with small number of participants included. Conversely, the data from the present study showed a considerably more promising reproducibility of the T2* quantification using UTE. Firstly, the measurement seems valid in relation to the measurement variation indeed the demonstrated typical error for test-retest of 3% (proximal tendon) far exceeds the differences in T2* values between healthy and tendinopathic tendons, which are reported between 40-190% in the literature [9,51,53,97]. Further, unpublished values from investigations in our department have shown mean T2* values in healthy patellar tendons around 1 ms and around 1.4 ms in a population of patients with early patellar tendinopathy (<3 month). Thus, compared with the detected T2* values in the present study at 1.67 ms, it suggests that the method is able to distinguish between different stages of tendinopathy, which strengthens the validity of the method.

The ability of a method to detect changes over time is another important aspect of the overall validity. We showed unchanged T2* values in response to 12 weeks exercise-based treatment in Study 1. However, it remains unknown if this lack of response was due to poor responsiveness of the UTE-T2* method. Moreover, it could be a true unaltered tissue response, which need further investigation. Likewise, the day to day reproducibility of the method needs to be investigated since the utilization of the method primarily is to monitor tissue alterations over a period of time. Additionally, there was no correlation of T2* to VISA-P at baseline (r=-0.01; 95%CI [-0.31, 0.30]; P=0.97) in Study 1, which is in line with some [53] but not all other studies[52,54,75]. However, this relationship needs further investigations in order to establish the responsiveness of the method.

In addition, the criterion validity of the method needs to be established. Tendon biopsies is the gold standard in relation to quantification of structural and biochemical composition and changes thereof [85]. Therefore, it might be relevant to investigate criterion validity of the UTE-T2* method by comparing it with biopsy findings, which is an ongoing project in our department.

The present data show a small systematic increase in T2* values from the first to the second recording. As discussed in paper III this variation might be explained by different factors, such as higher free water content at the second recording compared to the first recording and an inherent technical variation of the MRI method. Likewise, between study variation in these factors might explain the discrepancy in results between the precent study and the previous reproducibility study by Gärdin et al[53]. Further, it cannot be ruled out that differences between Achilles and patellar tendons might contribute to the discrepancy between the two studies. In fact, the value of the method further depends on the ability to detect changes in tendon structure over longer duration this also need to be investigated in future studies.

Sufficiently Intra- and interobserver reproducibility are other important factors for the overall validity of a measurement[113]. The present data show an excellent intra- and inter-observer reproducibility of the applied UTE-T2* method. However, similar to the study by Gärdin et al[53] higher absolute values for intra-observer reproducibility (Table 9) compared to those obtained for the inter-observer reproducibility (Table 10) were detected in the present study, which highlights that reduced variation can be achieved if the same investigator perform the evaluation.

Altogether, the UTE-T2* protocol, which was applied in the present study demonstrated feasibility for use in clinical practice and science for detecting early alteration and monitoring alteration in tendons. Importantly as outlined above, other validity aspects need to be covered and lastly, the interpretability and minimal clinical important difference for the measurement need to be established in future studies.

11. CONCLUSIONS

Overall, this thesis demonstrated that there is no statistically superior effect of exercising with heavy load (90% of 1RM) compared to moderate load (55% of 1RM) in relation to clinical outcome, tendon structure and function in management of chronic patellar tendinopathy as long as the total exercise volume are equal.

In a clinical perspective we can conclude that both heavy slow resistance training (HSR) and moderate slow resistance training (MSR) show equally improvements in clinical outcome, tendon structure and function in the short-term. Improvements in clinical outcome and neovascularization continued in the long-term, but surprisingly did not reach values for healthy tendons indicating that it takes long time to fully recover patellar tendinopathy. In relation to mechanical, material and morphological properties of the tendon there were no significantly improvement of either HSR or MSR treatments. Additionally, stiffness was unaffected in the tendinopathic tendons compared to contralateral asymptomatic tendons among participants with unilateral symptoms. Lastly, non-invasive MRI ultra-short time to echo (UTE) imaging with T2* mapping of tendons was demonstrated to be a reproducible method and seems to be applicable for detecting early alterations in response to interventions. However, this needs further investigation.

12. PERSPECTIVES AND CLINICAL IMPLICATIONS

This PhD thesis contributes with important knowledge regarding to what extent loading configurations during treatments influence the clinical outcome, the tendon structure, and function and mechanical behavior of tendinopathic patellar tendons.

The lack of superiority of exercising with heavy load compared to moderate load is important when treating patients with patellar tendinopathy. This indicates that the exercise regime can be chosen on basis of patient preferences. Furthermore, the results from Study 1 indicate a protracted long-term recovery. This might be important to keep in mind when treating and inform patients with tendinopathy in clinical practice, but also when designing and performing clinical trials. Future studies need to investigate the reason for this lack of long-term full recovery to establish a more efficient treatment of tendinopathy. Additionally, the relatively

low turnover of tendon tissue in general[60] support the importance of an extended duration of treatment intervention and likewise follow-up durations in future studies. Examining the participants from the current study years after end of intervention would add important information on the long-term recovery. Even though we not systematically record what they have done in the intervening years. This could elucidate to what extent the tissue alterations are becoming degenerative as believed by some research groups within the field of tendinopathy.

Even though we detected a clinical improvement after 12 weeks intervention it was not associated with significant alterations in mechanical, material or morphological changes. Further research should focus on the mechanisms actually driving this improvement, and to what extent clinical improvements are linked to e.g. material alteration occurring prior to altered mechanical properties and altered tendon morphology. The UTE-T2* mapping method show promising results as a non-invasive marker for early alterations. Although, the validity of the method needs to be further investigated it might be relevant to apply the method in future studies. In fact this might provide important knowledge of internal material alterations and is an applicably method to obtain repeated measures.

Finally, the current study was designed to test the influence of load magnitude in treatment of patellar tendinopathy. However, influence of other factors like restitution time between session remains unknown and need to be investigated in future studies in an attempt to improve outcome and time to complete recovery.

"The More You Know – The More You Realize You Don´t Know"

Aristotle

13. REFERENCES

1. Agergaard A-S, Malmgaard-Clausen NM, Svensson RB, et al. UTE T2* mapping of tendinopathic patellar tendons: an MRI reproducibility study. *Acta Radiol*. April 2020:284185120918807. doi:10.1177/0284185120918807.
2. Alfredson H, Ohberg L, Forsgren S. Is vasculo-neural ingrowth the cause of pain in chronic Achilles tendinosis? An investigation using ultrasonography and colour Doppler, immunohistochemistry, and diagnostic injections. *Knee Surg Sports Traumatol Arthrosc*. 2003;11(5):334-338. doi:10.1007/s00167-003-0391-6.
3. Alfredson H, Pietilä T, Jonsson P, Lorentzon R. Heavy-load eccentric calf muscle training for the treatment of chronic Achilles tendinosis. *Am J Sports Med*. 1998;26(3):360-366. doi:10.1177/03635465980260030301.
4. Andriolo L, Altamura SA, Reale D, Candrian C, Zaffagnini S, Filardo G. Nonsurgical Treatments of Patellar Tendinopathy: Multiple Injections of Platelet-Rich Plasma Are a Suitable Option: A Systematic Review and Meta-analysis. *Am J Sports Med*. 2019;47(4):1001-1018. doi:10.1177/0363546518759674.
5. Arampatzis A, Karamanidis K, Albracht K. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J Exp Biol*. 2007;210(15):2743-2753. doi:10.1242/jeb.003814.
6. Arampatzis A, Peper A, Bierbaum S, Albracht K. Plasticity of human Achilles tendon mechanical and morphological properties in response to cyclic strain. *J Biomech*. 2010;43(16):3073-3079. doi:10.1016/j.jbiomech.2010.08.014.
7. van Ark M, Cook JL, Docking SI, et al. Do isometric and isotonic exercise programs reduce pain in athletes with patellar tendinopathy in-season? A randomised clinical trial. *J Sci Med Sport*. 2016;19(9):702-706. doi:10.1016/j.jsams.2015.11.006.
8. Arnoczky SP, Lavagnino M, Egerbacher M. The mechanobiological aetiopathogenesis of tendinopathy: Is it the over-stimulation or the under-stimulation of tendon cells? *Int J Exp Pathol*. 2007;88(4):217-226. doi:10.1111/j.1365-2613.2007.00548.x.
9. Bachmann E, Rosskopf AB, Götschi T, et al. T1- and T2*-Mapping for Assessment of Tendon Tissue Biophysical Properties: A Phantom MRI Study. *Invest Radiol*. 2019;54(4):212-220. doi:10.1097/RLI.0000000000000532.
10. Bachmann E, Rosskopf AB, Götschi T, et al. T1-and T2∗-Mapping for Assessment of

Tendon Tissue Biophysical Properties: A Phantom MRI Study. *Invest Radiol*. 2019;54(4):212-220. doi:10.1097/RLI.0000000000000532.

11. Bahr R, Fossan B, Løken S, Engebretsen L. Surgical treatment compared with eccentric training for patellar tendinopathy (Jumper's Knee). A randomized, controlled trial. *J Bone Joint Surg Am*. 2006;88(8):1689-1698. doi:10.2106/JBJS.E.01181.
12. Basso O, Johnson DP, Amis AA. The anatomy of the patellar tendon. *Knee Surgery, Sport Traumatol Arthrosc*. 2001;9(1):2-5. doi:10.1007/s001670000133.
13. Beyer R, Kongsgaard M, Hougs Kjær B, Øhlenschlæger T, Kjær M, Magnusson SP. Heavy slow resistance versus eccentric training as treatment for achilles tendinopathy: A randomized controlled trial. *Am J Sports Med*. 2015;43(7):1704-1711. doi:10.1177/0363546515584760.
14. Black N. Patient reported outcome measures could help transform healthcare. *BMJ*. 2013;346(7896):1-5. doi:10.1136/bmj.f167.
15. Blazina ME, Kerlan RK, Jobe FW, Carter VS, Carlson GJ. Jumper's knee. *Orthop Clin North Am*. 1973;4(3):665-678.
16. Boesen AP, Boesen MI, Torp-Pedersen S, et al. Associations between abnormal ultrasound color doppler measures and tendon pain symptoms in badminton players during a season: A prospective cohort study. *Am J Sports Med*. 2012;40(3):548-555. doi:10.1177/0363546511435478.
17. Boesen MI, Boesen M, Langberg H, et al. Musculoskeletal colour/power Doppler in sports medicine: Image parameters, artefacts, image interpretation and therapy. *Clin Exp Rheumatol*. 2010;28(1):103-113.
18. Boesen MI, Koenig MJ, Torp-Pedersen S, Bliddal H, Langberg H. Tendinopathy and Doppler activity: The vascular response of the achilles tendon to exercise. *Scand J Med Sci Sport*. 2006;16(6):463-469. doi:10.1111/j.1600-0838.2005.00512.x.
19. Bohm S, Mersmann F, Arampatzis A. Human tendon adaptation in response to mechanical loading: a systematic review and meta-analysis of exercise intervention studies on healthy adults. *Sport Med - Open*. 2015;1(1). doi:10.1186/s40798-015-0009-9.
20. Bojsen-Møller J, Magnusson SP, Rasmussen LR, Kjaer M, Aagaard P. Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. *J Appl Physiol*. 2005;99(3):986-994.

doi:10.1152/japplphysiol.01305.2004.

21. Breda SJ, Poot DHJ, Papp D, et al. Tissue-Specific T2* Biomarkers in Patellar Tendinopathy by Subregional Quantification Using 3D Ultrashort Echo Time MRI. *J Magn Reson Imaging*. 2020:1-11. doi:10.1002/jmri.27108.
22. Chiquet M, Gelman L, Lutz R, Maier S. From mechanotransduction to extracellular matrix gene expression in fibroblasts. *Biochim Biophys Acta*. 2009;1793(5):911-920. doi:10.1016/j.bbamcr.2009.01.012.
23. Cook JL. A cross sectional study of 100 athletes with juniper's knee managed conservatively and surgically. *Br J Sports Med*. 1997;31(4):332-336. doi:10.1136/bjsm.31.4.332.
24. Cook JL, Khan KM, Harcourt PR, et al. Patellar tendon ultrasonography in asymptomatic active athletes reveals hypoechoic regions: a study of 320 tendons. Victorian Institute of Sport Tendon Study Group. *Clin J Sport Med*. 1998;8(2):73-77. doi:10.1097/00042752-199804000-00001.
25. Cook JL, Khan KM, Kiss ZS, Purdam CR, Griffiths L. Reproducibility and clinical utility of tendon palpation to detect patellar tendinopathy in young basketball players. Victorian Institute of Sport tendon study group. *Br J Sports Med*. 2001;35(1):65-69. doi:10.1136/bjsm.35.1.65.
26. Cook JL, Malliaras P, De Luca J, Ptasznik R, Morris ME, Goldie P. Neovascularization and pain in abnormal patellar tendons of active jumping athletes. *Clin J Sport Med*. 2004;14(5):296-299. doi:10.1097/00042752-200409000-00008.
27. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med*. 2009;43(6):409-416. doi:10.1136/bjsm.2008.051193.
28. Cook JL, Rio E, Purdam CR, Docking SI. Revisiting the continuum model of tendon pathology: What is its merit in clinical practice and research? *Br J Sports Med*. 2016;50(19):1187-1191. doi:10.1136/bjsports-2015-095422.
29. Couppe C, Kongsgaard M, Aagaard P, et al. Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. *J Appl Physiol*. 2008;105(3):805-810. doi:10.1152/japplphysiol.90361.2008.
30. Couppé C, Kongsgaard M, Aagaard P, et al. Differences in tendon properties in elite

badminton players with or without patellar tendinopathy. *Scand J Med Sci Sports*. 2013;23(2):e89-95. doi:10.1111/sms.12023.

31. Couppé C, Suetta C, Kongsgaard M, et al. The effects of immobilization on the mechanical properties of the patellar tendon in younger and older men. *Clin Biomech*. 2012;27(9):949-954. doi:10.1016/j.clinbiomech.2012.06.003.
32. Couppé C, Svensson RB, Silbernagel KG, Langberg H, Magnusson SP. Eccentric or Concentric Exercises for the Treatment of Tendinopathies? *J Orthop Sport Phys Ther*. 2015;45(11):853-863. doi:10.2519/jospt.2015.5910.
33. Couppé C, Svensson RB, Sødring-Elbrønd V, Hansen P, Kjaer M, Magnusson SP. Accuracy of MRI technique in measuring tendon cross-sectional area. *Clin Physiol Funct Imaging*. 2014;34(3):237-241. doi:10.1111/cpf.12086.
34. Dakin SG, Martinez FO, Yapp C, et al. Inflammation activation and resolution in human tendon disease. *Sci Transl Med*. 2015;7(311):1-14. doi:10.1126/scitranslmed.aac4269.
35. Docking SI, Ooi CC, Connell D. Tendinopathy: Is Imaging Telling Us the Entire Story? *J Orthop Sports Phys Ther*. 2015;45(11):842-852. doi:10.2519/jospt.2015.5880.
36. Vander Doelen T, Jelley W. Non-surgical treatment of patellar tendinopathy: A systematic review of randomized controlled trials. *J Sci Med Sport*. 2020;23(2):118-124. doi:10.1016/j.jsams.2019.09.008.
37. Edama M, Kageyama I, Nakamura M, et al. Anatomical study of the inferior patellar pole and patellar tendon. *Scand J Med Sci Sport*. 2017;27(12):1681-1687. doi:10.1111/sms.12858.
38. Edwards PK, Ebert JR, Littlewood C, Ackland T, Wang A. A Systematic Review of Electromyography Studies in Normal Shoulders to Inform Postoperative Rehabilitation Following Rotator Cuff Repair. *J Orthop Sports Phys Ther*. 2017;47(12):931-944. doi:10.2519/jospt.2017.7271.
39. Everhart JS, Cole D, Sojka JH, et al. Treatment Options for Patellar Tendinopathy: A Systematic Review. *Arthrosc - J Arthrosc Relat Surg*. 2017;33(4):861-872. doi:10.1016/j.arthro.2016.11.007.
40. Farup J, Rahbek SK, Vendelbo MH, et al. Whey protein hydrolysate augments tendon and muscle hypertrophy independent of resistance exercise contraction mode. *Scand J Med Sci Sport*. 2014;24(5):788-798. doi:10.1111/sms.12083.

41. Finni T, Komi P V, Lepola V. In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. *Eur J Appl Physiol*. 2000;83(4-5):416-426. doi:10.1007/s004210000289.
42. Florit D, Pedret C, Casals M, Malliaras P, Sugimoto D, Rodas G. Incidence of Tendinopathy in Team Sports in a Multidisciplinary Sports Club Over 8 Seasons. *J Sports Sci Med*. 2019;18(4):780-788.
43. Fredberg U, Bolvig L. Significance of ultrasonographically detected asymptomatic tendinosis in the patellar and Achilles tendons of elite soccer players: A longitudinal study. *Am J Sports Med*. 2002;30(4):488-491. doi:10.1177/03635465020300040701.
44. Fredberg U, Bolvig L, Andersen NT, Stengaard-Pedersen K. Ultrasonography in evaluation of Achilles and patella tendon thickness. *Ultraschall Med*. 2008;29(1):60-65. doi:10.1055/s-2007-963027.
45. Fredberg U, Bolvig L, Pfeiffer-Jensen M, Clemmensen D, Jakobsen BW, Stengaard-Pedersen K. Ultrasonography as a tool for diagnosis, guidance of local steroid injection and, together with pressure algometry, monitoring of the treatment of athletes with chronic jumper's knee and Achilles tendinitis: A randomized, double-blind, placebo-controlled . *Scand J Rheumatol*. 2004;33(2):94-101. doi:10.1080/03009740310004126.
46. Fredberg U, Stengaard-Pedersen K. Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation: Review. *Scand J Med Sci Sport*. 2008;18(1):3-15. doi:10.1111/j.1600-0838.2007.00746.x.
47. Garma T, Kobayashi C, Haddad F, Adams GR, Bodell PW, Baldwin KM. Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise. *J Appl Physiol*. 2007;102(1):135-143. doi:10.1152/japplphysiol.00776.2006.
48. Gatehouse PD, Bydder GM. Magnetic resonance imaging of short T2 components in tissue. *Clin Radiol*. 2003;58(1):1-19. doi:10.1053/crad.2003.1157.
49. Gauvin R, Parenteau-Bareil R, Larouche D, et al. Dynamic mechanical stimulations induce anisotropy and improve the tensile properties of engineered tissues produced without exogenous scaffolding. *Acta Biomater*. 2011;7(9):3294-3301. doi:10.1016/j.actbio.2011.05.034.
50. Glazebrook MA, Wright JR, Langman M, Stanish WD, Lee JM. Histological analysis of

Achilles tendons in an overuse rat model. *J Orthop Res*. 2008;26(6):840-846. doi:10.1002/jor.20546.

51. Grosse U, Springer F, Hein T, et al. Influence of physical activity on T1 and T2* relaxation times of healthy Achilles tendons at 3T. *J Magn Reson Imaging*. 2015;41(1):193-201. doi:10.1002/jmri.24525.
52. Grosse U, Syha R, Hein T, et al. Diagnostic value of T1and T2∗ relaxation times and off-resonance saturation effects in the evaluation of achilles tendinopathy by MRI at 3T. *J Magn Reson Imaging*. 2015;41(4):964-973. doi:10.1002/jmri.24657.
53. Gärdin A, Rasinski P, Berglund J, Shalabi A, Schulte H, Brismar TB. T2∗ relaxation time in Achilles tendinosis and controls and its correlation with clinical score. *J Magn Reson Imaging*. 2016;43(6):1417-1422. doi:10.1002/jmri.25104.
54. Hager B, Walzer SM, Deligianni X, et al. Orientation dependence and decay characteristics of T2 * relaxation in the human meniscus studied with 7 Tesla MR microscopy and compared to histology. *Magn Reson Med*. 2018;(June):1-13. doi:10.1002/mrm.27443.
55. Hansen P, Bojsen-Moller J, Aagaard P, Kjaer M, Magnusson SP. Mechanical properties of the human patellar tendon, in vivo. *Clin Biomech (Bristol, Avon)*. 2006;21(1):54-58. doi:10.1016/j.clinbiomech.2005.07.008.
56. Hansen P, Haraldsson BT, Aagaard P, et al. Lower strength of the human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. *J Appl Physiol*. 2010;108(1):47-52. doi:10.1152/japplphysiol.00944.2009.
57. Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, Magnusson SP. Region-specific mechanical properties of the human patella tendon. *J Appl Physiol*. 2005;98(3):1006-1012. doi:10.1152/japplphysiol.00482.2004.
58. Heinemeier KM, Lorentzen MP, Jensen JK, et al. Local trauma in human patellar tendon leads to widespread changes in the tendon gene expression. *J Appl Physiol*. 2016;120(9):1000-1010. doi:10.1152/japplphysiol.00870.2015.
59. Heinemeier KM, Olesen JL, Haddad F, et al. Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. *J Physiol*. 2007;582(3):1303-1316. doi:10.1113/jphysiol.2007.127639.
60. Heinemeier KM, Schjerling P, Heinemeier J, Magnusson SP, Kjaer M. Lack of tissue

renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. *FASEB J*. 2013;27(5):2074-2079. doi:10.1096/fj.12-225599.

61. Heinemeier KM, Schjerling P, Øhlenschlæger TF, Eismark C, Olsen J, Kjær M. Carbon-14 bomb pulse dating shows that tendinopathy is preceded by years of abnormally high collagen turnover. *FASEB J*. 2018:fj.201701569R. doi:10.1096/fj.201701569R.
62. Heinemeier KM, Øhlenschlæger TF, Mikkelsen UR, et al. Effects of anti-inflammatory (NSAID) treatment on human tendinopathic tissue. *J Appl Physiol*. 2017;(7):jap.00281.2017. doi:10.1152/japplphysiol.00281.2017.
63. Helland C, Bojsen-Møller J, Raastad T, et al. Mechanical properties of the patellar tendon in elite volleyball players with and without patellar tendinopathy. *Br J Sports Med*. 2013;47(13):862-868. doi:10.1136/bjsports-2013-092275.
64. Hernandez-Sanchez S, Hidalgo MD, Gomez A. Responsiveness of the VISA-P scale for patellar tendinopathy in athletes. *Br J Sports Med*. 2014;48(6):453-457. doi:10.1136/bjsports-2012-091163.
65. Herod TW, Veres SP. Development of overuse tendinopathy: A new descriptive model for the initiation of tendon damage during cyclic loading. *J Orthop Res*. 2018;36(1):467-476. doi:10.1002/jor.23629.
66. Hoffmann TC, Glasziou PP, Boutron I, et al. Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide. *BMJ*. 2014;348(3):g1687. doi:10.1136/bmj.g1687.
67. Holden S, Lyng K, Graven-Nielsen T, et al. Isometric exercise and pain in patellar tendinopathy: A randomized crossover trial. *J Sci Med Sport*. 2020;23(3):208-214. doi:10.1016/j.jsams.2019.09.015.
68. Holm L, Reitelseder S, Pedersen TG, et al. Changes in muscle size and MHC composition in response to resistance exercise with heavy and light loading intensity. *J Appl Physiol*. 2008;105(5):1454-1461. doi:10.1152/japplphysiol.90538.2008.
69. Holm PM, Skou ST, Olesen JL, et al. Ultrasonographic assessment of patellar tendon thickness at 16 clinically relevant measurement sites – A study of intra- and interrater reliability. *J Bodyw Mov Ther*. 2019;23(2):344-351. doi:10.1016/j.jbmt.2019.02.004.
70. Hopkins WG, Schabort EJ, Hawley JA. Reliability of power in physical performance tests. *Sports Med*. 2001;31(3):211-234.

71. Hro A, Mann H, Dickersin K, et al. Research and Reporting Methods Annals of Internal Medicine SPIRIT 2013 Statement : Defining Standard Protocol Items for Clinical Trials. *Ann Intern Med*. 2015;1(3):200-207. doi:10.7326/0003-4819-158-3-201302050-00583.Requests.

72. Johnson DP, Wakeley CJ, Watt I. Magnetic resonance imaging of patellar tendonitis. *J Bone Jt Surg - Ser B*. 1996;78(3):452-457. doi:10.1302/0301-620x.78b3.0780452.

73. Joshi SD, Webb K. Variation of cyclic strain parameters regulates development of elastic modulus in fibroblast/substrate constructs. *J Orthop Res*. 2008;26(8):1105-1113. doi:10.1002/jor.20626.

74. Józsa LG, Kannus P. *Human Tendons: Anatomy, Physiology, and Pathology*. Champaign, Ill.: Human Kinetics; 1997.

75. Juras V, Apprich S, Szomolanyi P, Bieri O, Deligianni X, Trattnig S. Bi-exponential T2 analysis of healthy and diseased Achilles tendons: an in vivo preliminary magnetic resonance study and correlation with clinical score. *Eur Radiol*. 2013;23(10):2814-2822. doi:10.1007/s00330-013-2897-8.

76. Kannus P. Structure of the tendon connective tissue. *Scand J Med Sci Sports*. 2000;10(6):312-320.

77. Kannus P, Józsa L, Natri A, Järvinen M. Effects of training, immobilization and remobilization on tendons. *Scand J Med Sci Sports*. 1997;7(2):67-71. doi:10.1111/j.1600-0838.1997.tb00121.x.

78. Kettunen JA, Kvist M, Alanen E, Kujala UM. Long-term prognosis for jumper's knee in male athletes. A prospective follow-up study. *Am J Sports Med*. 2002;30(5):689-692. doi:10.1177/03635465020300051001.

79. Khan K, Cook J. The painful nonruptured tendon: clinical aspects. *Clin Sports Med*. 2003;22(4):711-725. doi:10.1016/S0278-5919(03)00035-8.

80. Khan KM, Bonar F, Desmond PM, et al. Patellar tendinosis (jumper's knee): Findings at histopathologic examination, US, and MR imaging. *Radiology*. 1996;200(3):821-827. doi:10.1148/radiology.200.3.8756939.

81. Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev*. 2004;84(2):649-698. doi:10.1152/physrev.00031.2003.

82. Kjær M, Langberg H, Heinemeier K, et al. From mechanical loading to collagen synthesis,

structural changes and function in human tendon. *Scand J Med Sci Sport*. 2009;19(4):500-510. doi:10.1111/j.1600-0838.2009.00986.x.

83. Koenig MJ, Torp-Pedersen S, Boesen MI, Holm CC, Bliddal H. Doppler ultrasonography of the anterior knee tendons in elite badminton players: Colour fraction before and after match. *Br J Sports Med*. 2010;44(2):134-139. doi:10.1136/bjsm.2007.039743.
84. Kongsgaard M, Kovanen V, Aagaard P, et al. Corticosteroid injections, eccentric decline squat training and heavy slow resistance training in patellar tendinopathy. *Scand J Med Sci Sport*. 2009;19(6):790-802. doi:10.1111/j.1600-0838.2009.00949.x.
85. Kongsgaard M, Qvortrup K, Larsen J, et al. Fibril Morphology and Tendon Mechanical Properties in Patellar Tendinopathy. *Am J Sports Med*. 2010;38(4):749-756. doi:10.1177/0363546509350915.
86. Kongsgaard M, Reitelseder S, Pedersen TG, et al. Region specific patellar tendon hypertrophy in humans following resistance training. *Acta Physiol*. 2007;191(2):111-121. doi:10.1111/j.1748-1716.2007.01714.x.
87. Kongsgaard M, Aagaard P, Kjaer M, Magnusson SP. Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. *J Appl Physiol*. 2005;99(5):1965-1971. doi:10.1152/japplphysiol.00384.2005.
88. Krey D, Borchers J, McCamey K. Tendon needling for treatment of tendinopathy: A systematic review. *Phys Sportsmed*. 2015;43(1):80-86. doi:10.1080/00913847.2015.1004296.
89. Kruse A, Stafilidis S, Tilp M. Ultrasound and magnetic resonance imaging are not interchangeable to assess the Achilles tendon cross-sectional-area. *Eur J Appl Physiol*. 2017;117(1):73-82. doi:10.1007/s00421-016-3500-1.
90. Kösters A, Wiesinger HP, Bojsen-Møller J, Müller E, Seynnes OR. Influence of loading rate on patellar tendon mechanical properties in vivo. *Clin Biomech*. 2014;29(3):323-329. doi:10.1016/j.clinbiomech.2013.12.010.
91. Langberg H, Rosendal L, Kjær M. Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. *J Physiol*. 2001;534(1):297-302. doi:10.1111/j.1469-7793.2001.00297.x.
92. Langberg H, Skovgaard D, Petersen LJ, Bulow J, Kjaer M. Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in

humans. *J Physiol*. 1999;521 Pt 1:299-306.

93. Larsson MEH, Käll I, Nilsson-Helander K. Treatment of patellar tendinopathy--a systematic review of randomized controlled trials. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(8):1632-1646. doi:10.1007/s00167-011-1825-1.
94. Lee W-C, Ng GY-F, Zhang Z-J, Malliaras P, Masci L, Fu S-N. Changes on Tendon Stiffness and Clinical Outcomes in Athletes Are Associated With Patellar Tendinopathy After Eccentric Exercise. *Clin J Sport Med*. 2020;30(1):25-32. doi:10.1097/JSM.0000000000000562.
95. Lian OB, Engebretsen L, Bahr R. Prevalence of jumper's knee among elite athletes from different sports: a cross-sectional study. *Am J Sports Med*. 2005;33(4):561-567. doi:10.1177/0363546504270454.
96. Lichtwark GA, Barclay CJ. The influence of tendon compliance on muscle power output and efficiency during cyclic contractions. *J Exp Biol*. 2010;213(5):707-714. doi:10.1242/jeb.038026.
97. Liu F, Kijowski R. Assessment of different fitting methods for in-vivo bi-component T2* analysis of human patellar tendon in magnetic resonance imaging. *Muscles Ligaments Tendons J*. 2017;7(1):163-172. doi:10.11138/mltj/2017.7.1.163.
98. Mafi N, Lorentzon R, Alfredson H. Superior short-term results with eccentric calf muscle training compared to concentric training in a randomized prospective multicenter study on patients with chronic Achilles tendinosis. *Knee Surg Sports Traumatol Arthrosc*. 2001;9(1):42-47. doi:10.1007/s001670000148.
99. Magnusson SP, Kjaer M. Region-specific differences in Achilles tendon cross-sectional area in runners and non-runners. *Eur J Appl Physiol*. 2003;90(5-6):549-553. doi:10.1007/s00421-003-0865-8.
100. Magnusson SP, Kjaer M. The impact of loading, unloading, ageing and injury on the human tendon. *J Physiol*. 2018;0:1-16. doi:10.1113/JP275450.
101. Magnusson SP, Langberg H, Kjaer M. The pathogenesis of tendinopathy: Balancing the response to loading. *Nat Rev Rheumatol*. 2010;6(5):262-268. doi:10.1038/nrrheum.2010.43.
102. Malliaras P, Barton CJ, Reeves ND, Langberg H. Achilles and patellar tendinopathy loading programmes: A systematic review comparing clinical outcomes and identifying potential

mechanisms for effectiveness. *Sport Med*. 2013;43(4):267-286. doi:10.1007/s40279-013-0019-z.

103. Malliaras P, Cook J, Purdam C, Rio E. Patellar tendinopathy: Clinical diagnosis, load management, and advice for challenging case presentations. *J Orthop Sports Phys Ther*. 2015;45(11):887-898. doi:10.2519/jospt.2015.5987.
104. Malliaras P, Kamal B, Nowell A, et al. Patellar tendon adaptation in relation to load-intensity and contraction type. *J Biomech*. 2013;46(11):1893-1899. doi:10.1016/j.jbiomech.2013.04.022.
105. Malliaras P, Purdam C, Maffulli N, Cook J. Temporal sequence of greyscale ultrasound changes and their relationship with neovascularity and pain in the patellar tendon. *Br J Sports Med*. 2010;44(13):944-947. doi:10.1136/bjsm.2008.054916.
106. Malliaras P, Richards PJ, Garau G, Maffulli N. Achilles tendon Doppler flow may be associated with mechanical loading among active athletes. *Am J Sports Med*. 2008;36(11):2210-2215. doi:10.1177/0363546508319052.
107. Mallows A, Debenham J, Walker T, Littlewood C. Association of psychological variables and outcome in tendinopathy: A systematic review. *Br J Sports Med*. 2017;51(9):743-748. doi:10.1136/bjsports-2016-096154.
108. Matthews W, Ellis R, Furness J, Hing W. Classification of Tendon Matrix Change Using Ultrasound Imaging: A Systematic Review and Meta-analysis. *Ultrasound Med Biol*. 2018;44(10):2059-2080. doi:10.1016/j.ultrasmedbio.2018.05.022.
109. Mead MP, Gumucio JP, Awan TM, Mendias CL, Sugg KB. Pathogenesis and Management of Tendinopathies in Sports Medicine. *Transl Sport Med*. 2018;1(1):5-13. doi:10.1002/tsm2.6.
110. Mendonça LDM, Ocarino JM, Bittencourt NFN, Fernandes LMO, Verhagen E, Fonseca ST. The Accuracy of the VISA-P Questionnaire, Single-Leg Decline Squat, and Tendon Pain History to Identify Patellar Tendon Abnormalities in Adult Athletes. *J Orthop Sports Phys Ther*. 2016;46(8):673-680. doi:10.2519/jospt.2016.6192.
111. Miller BF, Olesen JL, Hansen M, et al. Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol*. 2005;567(Pt 3):1021-1033. doi:10.1113/jphysiol.2005.093690.
112. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration:

Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2012;10(1):28-55. doi:10.1016/j.ijsu.2011.10.001.

113. Mokkink LB, Terwee CB, Patrick DL, et al. The COSMIN checklist for assessing the methodological quality of studies on measurement properties of health status measurement instruments: An international Delphi study. *Qual Life Res*. 2010;19(4):539-549. doi:10.1007/s11136-010-9606-8.
114. Murphy M, Travers M, Gibson W, et al. Rate of Improvement of Pain and Function in Mid-Portion Achilles Tendinopathy with Loading Protocols: A Systematic Review and Longitudinal Meta-Analysis. *Sport Med*. 2018;48(8):1875-1891. doi:10.1007/s40279-018-0932-2.
115. Nourissat G, Berenbaum F, Duprez D. Tendon injury: From biology to tendon repair. *Nat Rev Rheumatol*. 2015;11(4):223-233. doi:10.1038/nrrheum.2015.26.
116. Obst SJ, Heales LJ, Schrader BL, et al. Are the Mechanical or Material Properties of the Achilles and Patellar Tendons Altered in Tendinopathy? A Systematic Review with Meta-analysis The lack of evidence for a consistent effect of tendi- nopathy on the mechanical and/or material properties of t. *Sport Med*. 2018;48(0123456789):2179-2198. doi:10.1007/s40279-018-0956-7.
117. Palazón-Bru A, Tomás Rodríguez MI, Mares-García E, Hernández-Sánchez S, Carbonell-Torregrosa MÁ, Gil-Guillén VF. The Victorian Institute of Sport Assessment Scale for Patellar Tendinopathy (VISA-P): A Reliability Generalization Meta-analysis. *Clin J Sport Med*. 2020;00(00). doi:10.1097/JSM.0000000000000810.
118. Peers KHE, Brys PPM, Lysens RJJ. Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. *Int Orthop*. 2003;27(3):180-183. doi:10.1007/s00264-002-0426-5.
119. Peter Magnusson S, Hansen M, Langberg H, et al. The adaptability of tendon to loading differs in men and women. *Int J Exp Pathol*. 2007;88(4):237-240. doi:10.1111/j.1365-2613.2007.00551.x.
120. Pingel J, Wienecke J, Kongsgaard M, et al. Increased mast cell numbers in a calcaneal tendon overuse model. *Scand J Med Sci Sport*. 2013;23(6):e353-e360. doi:10.1111/sms.12089.
121. Van Der Plas A, De Jonge S, De Vos RJ, et al. A 5-year follow-up study of Alfredson's heel-

drop exercise programme in chronic midportion Achilles tendinopathy. *Br J Sports Med*. 2012;46(3):214-218. doi:10.1136/bjsports-2011-090035.

122. Provenzano PP, Vanderby R. Collagen fibril morphology and organization: Implications for force transmission in ligament and tendon. *Matrix Biol*. 2006;25(2):71-84. doi:10.1016/j.matbio.2005.09.005.
123. Purdam CR, Cook JL, Hopper DM, et al. Discriminative ability of functional loading tests for adolescent jumper's knee. *Phys Ther Sport*. 2003;4(1):3-9. doi:10.1016/S1466-853X(02)00069-X.
124. Rees JD, Lichtwark GA, Wolman RL, Wilson AM. The mechanism for efficacy of eccentric loading in Achilles tendon injury; an in vivo study in humans. *Rheumatology*. 2008;47(10):1493-1497. doi:10.1093/rheumatology/ken262.
125. Riel H, Lindstrøm CF, Rathleff MS, Jensen MB, Olesen JL. Prevalence and incidence rate of lower- extremity tendinopathies in a Danish general practice : a registry-based study. 2019:4-9.
126. Riley G. Tendinopathy - From basic science to treatment. *Nat Clin Pract Rheumatol*. 2008;4(2):82-89. doi:10.1038/ncprheum0700.
127. Riley G. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology*. 2004;43(2):131-142. doi:10.1093/rheumatology/keg448.
128. Rio E, van Ark M, Docking S, et al. Isometric Contractions Are More Analgesic Than Isotonic Contractions for Patellar Tendon Pain: An In-Season Randomized Clinical Trial. *Clin J Sport Med*. 2017;27(3):253-259. doi:10.1097/JSM.0000000000000364.
129. Rio E, Moseley L, Purdam C, et al. The pain of tendinopathy: Physiological or pathophysiological? *Sport Med*. 2014;44(1):9-23. doi:10.1007/s40279-013-0096-z.
130. Robson MD, Benjamin M, Gishen P, Bydder GM. Magnetic resonance imaging of the Achilles tendon using ultrashort TE (UTE) pulse sequences. *Clin Radiol*. 2004;59(8):727-735. doi:10.1016/j.crad.2003.11.021.
131. Robson MD, Gatehouse PD, Bydder M, Bydder GM. Magnetic resonance: an introduction to ultrashort TE (UTE) imaging. *J Comput Assist Tomogr*. 2003;27(6):825-846. doi:10.1097/00004728-200311000-00001.
132. Rompe JD, Nafe B, Furia JP, Maffulli N. Eccentric loading, shock-wave treatment, or a wait- and-see policy for tendinopathy of the main body of tendo Achillis: A randomized

controlled trial. *Am J Sports Med*. 2007;35(3):374-383. doi:10.1177/0363546506295940.

133. Rosager S, Aagaard P, Dyhre-Poulsen P, Neergaard K, Kjaer M, Magnusson SP. Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. *Scand J Med Sci Sports*. 2002;12(2):90-98.
134. Saghaei M, Saghaei S. Implementation of an open-source customizable minimization program for allocation of patients to parallel groups in clinical trials. *J Biomed Sci Eng*. 2011;04(11):734-739. doi:10.4236/jbise.2011.411090.
135. Salaffi F, Stancati A, Silvestri CA, Ciapetti A, Grassi W. Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. *Eur J Pain*. 2004;8(4):283-291. doi:10.1016/j.ejpain.2003.09.004.
136. Scott A, Ashe MC. Common tendinopathies in the upper and lower extremities. *Curr Sports Med Rep*. 2006;5(5):233-241. doi:10.1097/01.csmr.0000306421.85919.9c.
137. Scott A, Docking S, Vicenzino B, et al. Sports and exercise-related tendinopathies: A review of selected topical issues by participants of the second International Scientific Tendinopathy Symposium (ISTS) Vancouver 2012. *Br J Sports Med*. 2013;47(9):536-544. doi:10.1136/bjsports-2013-092329.
138. Seo J, Kim Y sun. Ultrasound imaging and beyond: recent advances in medical ultrasound. *Biomed Eng Lett*. 2017;7(2):57-58. doi:10.1007/s13534-017-0030-7.
139. Seynnes OR, Bojsen-Møller J, Albracht K, et al. Ultrasound-based testing of tendon mechanical properties: a critical evaluation. *J Appl Physiol*. 2015;118(2):133-141. doi:10.1152/japplphysiol.00849.2014.
140. Seynnes OR, Erskine RM, Maganaris CN, et al. Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. *J Appl Physiol*. 2009;107(2):523-530. doi:10.1152/japplphysiol.00213.2009.
141. Sharpe RE, Nazarian LN, Parker L, Rao VM, Levin DC. Dramatically increased musculoskeletal ultrasound utilization from 2000 to 2009, especially by podiatrists in private offices. *J Am Coll Radiol*. 2012;9(2):141-146. doi:10.1016/j.jacr.2011.09.008.
142. Silbernagel KG, Brorsson A, Lundberg M. The majority of patients with Achilles tendinopathy recover fully when treated with exercise alone: a 5-year follow-up. *Am J Sports Med*. 2011;39(3):607-613. doi:10.1177/0363546510384789.

143. Silbernagel KG, Thomeé R, Eriksson BI, Karlsson J. Continued sports activity, using a pain-monitoring model, during rehabilitation in patients with achilles tendinopathy: A randomized controlled study. *Am J Sports Med*. 2007;35(6):897-906. doi:10.1177/0363546506298279.
144. Silbernagel KG, Thomeé R, Thomeé P, Karlsson J. Eccentric overload training for patients with chronic Achilles tendon pain--a randomised controlled study with reliability testing of the evaluation methods. *Scand J Med Sci Sports*. 2001;11(4):197-206.
145. Skou ST, Aalkjaer JM. Ultrasonographic measurement of patellar tendon thickness-a study of intra- and interobserver reliability. *Clin Imaging*. 2013;37(5):934-937. doi:10.1016/j.clinimag.2013.01.007.
146. Sprague A, Epsley S, Silbernagel KG. Distinguishing Quadriceps Tendinopathy and Patellar Tendinopathy: Semantics or Significant? *J Orthop Sports Phys Ther*. 2019;49(9):627-630. doi:10.2519/jospt.2019.0611.
147. Sprague AL, Smith AH, Knox P, Pohlig RT, Grävare Silbernagel K. Modifiable risk factors for patellar tendinopathy in athletes: a systematic review and meta-analysis. *Br J Sports Med*. 2018:bjsports-2017-099000. doi:10.1136/bjsports-2017-099000.
148. Stanish WD, Rubinovich RM, Curwin S. Eccentric exercise in chronic tendinitis. *Clin Orthop Relat Res*. 1986;(208):65-68. doi:10.1097/00003086-198607000-00014.
149. Svensson RB, Herchenhan A, Starborg T, et al. Evidence of structurally continuous collagen fibrils in tendons. *Acta Biomater*. 2017;50:293-301. doi:10.1016/j.actbio.2017.01.006.
150. Thomeé R. A comprehensive treatment approach for patellofemoral pain syndrome in young women. *Phys Ther*. 1997;77(12):1690-1703.
151. Tran PHT, Malmgaard-Clausen NM, Puggaard RS, et al. Early development of tendinopathy in humans: Sequence of pathological changes in structure and tissue turnover signaling. *FASEB J*. 2020;34(1):776-788. doi:10.1096/fj.201901309R.
152. Tsehaie J, Poot DHJ, Oei EHG, Verhaar JAN, de Vos RJ. Value of quantitative MRI parameters in predicting and evaluating clinical outcome in conservatively treated patients with chronic midportion Achilles tendinopathy: A prospective study. *J Sci Med Sport*. 2017;20(7):633-637. doi:10.1016/j.jsams.2017.01.234.
153. Veres SP, Harrison JM, Lee JM. Repeated subrupture overload causes progression of

nanoscaled discrete plasticity damage in tendon collagen fibrils. *J Orthop Res*. 2013;31(5):731-737. doi:10.1002/jor.22292.

154. Visentini PJ, Khan KM, Cook JL, Kiss ZS, Harcourt PR, Wark JD. The VISA score: an index of severity of symptoms in patients with jumper's knee (patellar tendinosis). Victorian Institute of Sport Tendon Study Group. *J Sci Med Sport*. 1998;1(1):22-28.
155. Visnes H, Hoksrud A, Cook J, Bahr R. No effect of eccentric training on jumper's knee in volleyball players during the competitive season: a randomized clinical trial. *Clin J Sport Med*. 2005;15(4):227-234. doi:10.1097/01.jsm.0000168073.82121.20.
156. Visnes H, Tegnander A, Bahr R. Ultrasound characteristics of the patellar and quadriceps tendons among young elite athletes. *Scand J Med Sci Sport*. 2015;25(2):205-215. doi:10.1111/sms.12191.
157. De Vries AJ, Koolhaas W, Zwerver J, et al. The impact of patellar tendinopathy on sports and work performance in active athletes. *Res Sports Med*. 2017;25(3):253-265. doi:10.1080/15438627.2017.1314292.
158. Wang JH-C. Mechanobiology of tendon. *J Biomech*. 2006;39(9):1563-1582. doi:10.1016/j.jbiomech.2005.05.011.
159. Warden SJ, Kiss ZS, Malara FA, Ooi ABT, Cook JL, Crossley KM. Comparative accuracy of magnetic resonance imaging and ultrasonography in confirming clinically diagnosed patellar tendinopathy. *Am J Sports Med*. 2007;35(3):427-436. doi:10.1177/0363546506294858.
160. Watson PF, Petrie A. Method agreement analysis: A review of correct methodology. *Theriogenology*. 2010;73(9):1167-1179. doi:10.1016/j.theriogenology.2010.01.003.
161. Wezenbeek E, Willems T, Mahieu N, et al. The Role of the Vascular and Structural Response to Activity in the Development of Achilles Tendinopathy: A Prospective Study. *Am J Sports Med*. 2018;46(4):036354651775085. doi:10.1177/0363546517750854.
162. Wiesinger H-P, Kösters A, Müller E, Seynnes OR. Effects of Increased Loading on In Vivo Tendon Properties: A Systematic Review. *Med Sci Sports Exerc*. 2015;47(9):1885-1895. doi:10.1249/MSS.0000000000000603.
163. Wiesinger H-P, Seynnes OR, Kösters A, Müller E, Rieder F. Mechanical and Material Tendon Properties in Patients With Proximal Patellar Tendinopathy. *Front Physiol*. 2020;11(June):1-11. doi:10.3389/fphys.2020.00704.

164. Van Der Worp H, Van Ark M, Roerink S, Pepping GJ, Van Den Akker-Scheek I, Zwerver J. Risk factors for patellar tendinopathy: A systematic review of the literature. *Br J Sports Med*. 2011;45(5):446-452. doi:10.1136/bjsm.2011.084079.

165. Yang G, Crawford RC, Wang JHC. Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. *J Biomech*. 2004;37(10):1543-1550. doi:10.1016/j.jbiomech.2004.01.005.

166. Young JL, Rhon DI, Cleland JA, Snodgrass SJ. The Influence of Exercise Dosing on Outcomes in Patients With Knee Disorders: A Systematic Review. *J Orthop Sport Phys Ther*. 2018;48(3):1-47. doi:10.2519/jospt.2018.7637.

167. Young MA, Cook JL, Purdam CR, Kiss ZS, Alfredson H. Eccentric decline squat protocol offers superior results at 12 months compared with traditional eccentric protocol for patellar tendinopathy in volleyball players. *Br J Sports Med*. 2005;39(2):102-105. doi:10.1136/bjsm.2003.010587.

168. Zhang C, Couppé C, Scheijen JLJM, et al. Regional collagen turnover and composition of the human patellar tendon. *J Appl Physiol*. 2020;128(4):884-891. doi:10.1152/japplphysiol.00030.2020.

169. Zwerver J, Bredeweg SW, Van Den Akker-Scheek I. Prevalence of jumper's knee among nonelite athletes from different sports: A cross-sectional survey. *Am J Sports Med*. 2011;39(9):1984-1988. doi:10.1177/0363546511413370.

170. Zwerver J, Bredeweg SW, Hof AL. Biomechanical analysis of the single-leg decline squat. *Br J Sports Med*. 2007;41(4):264-268; discussion 268. doi:10.1136/bjsm.2006.032482.

14. Papers

PAPER I

Clinical outcome, structure and function improve with both heavy and moderate load in the treatment of patellar tendinopathy: A Randomized clinical trial

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Accepted for publication in AJSM but not published

Clinical outcome, structure and function improve with both heavy and moderate load in the treatment of patellar tendinopathy: A Randomized clinical trial

ABSTRACT

Background: Loading intervention has become a predominant treatment strategy for tendinopathy, and positive clinical outcomes and tendon tissue response may depend on the exercise dose and the load magnitude.

Purpose/ hypothesis: To investigate if the load magnitude influenced the effect of a 12-weeks loading intervention for patellar tendinopathy in the short-term (12 weeks) and long-term (52 weeks). We hypothesized that a greater load magnitude of 90% would yield a more positive clinical outcome, tendon structure and function compared to a lower load magnitude of 55% when the total exercise volume was kept equal in both groups.

Study Design: Randomized clinical trial

Methods: Forty-four adult participants with chronic patellar tendinopathy were included and randomized into a moderate slow resistance group (MSR, 55% of 1RM throughout the period), or a heavy slow resistance group (HSR, rising up to 90% of 1RM). Function and symptoms (VISA-P questionnaire), tendon pain during activity (NRS) and ultra-sound (tendon vascularization and swelling) were assessed before, after 6 and 12 weeks of intervention and at 52 weeks follow-up. Tendon function (functional tests) and tendon structure (ultrasound and MRI) were investigated before and after the intervention period.

Results: The HSR and MSR treatments both yielded significant clinical improvements in VISA-P, NRS-running, NRS-squat, NRS-preferred sport, Single-leg decline squat test and patient satisfaction after 12 weeks and these were maintained after 52 weeks. HSR was not superior to MSR loading for any of the measured clinical outcomes. Similarly, there were no differences in functional (strength and jumping ability) or structural (tendon thickness, Power-Doppler area and CSA) improvements between the HSR and MSR loading.

Conclusion: There is no superior effect of exercising with high load magnitude (HSR) compared to moderate load magnitude (MSR) for the clinical outcome, tendon structure or function in treatment of patellar tendinopathy in the short-term. Both HSR and MSR show, equally good,

continued improvement in outcomes in the long-term, but did not reach normal values for healthy tendons.

Clinical Relevance: Exercising with high or low load resistance can improve the clinical outcome, tendon function and structure following a patellar tendinopathy. Therefore, both Moderate Slow Resistance group (MSR) and Heavy Slow Resistance group (HSR) regimes can be applied in a clinical setting. Importantly, the current study indicates that the time for complete recovery in patellar tendinopathy exceeds one year. Future studies are needed to determine the reason for the protracted long-term recovery to establish the most efficient treatment recommendations.

Key terms: Patellar tendon, tendinopathy, loading based treatment, load magnitude

What is known about the subject: There has been considerable focus on the clinical effects of loading based exercise regimes as treatment for tendinopathy, but very little is known about the importance of changing specific loading parameters.

What this study adds to existing knowledge: Load magnitude per se in loading based treatment did not influenced the clinical outcome, tendon function or tissue response of the tendinopathic tendons. Improvement in clinical outcomes, structure and function continued up to 1 year after initiation of loading based treatment but did not reach normal values.

INTRODUCTION

The patellar tendon is designed to withstand considerable loads during locomotion, and may be loaded up to 4 kN during a single counter movement jump.[13] However, repetitive use often results in overuse injuries such as tendinopathy, which is a common clinical condition characterized by pain during activity, localized tenderness upon palpation, and impaired performance.[27,44] The histopathology includes increased cellularity, increased proteoglycans, glycosaminoglycans and water, along with hypervascularization and disorganized collagen.[33]

The prevalence of patellar tendinopathy has been reported as high as 14% in elite athletes and 9% among recreational athletes, especially within explosive sports.[32,50] Further, among elite male volleyball players, the prevalence can reach as high as 45%[32] and the symptoms as well as performance reduction may be long lasting (years).[26] However, despite tendinopathy being a common condition and a substantial clinical challenge, the exact pathology and optimal treatment modalities remain elusive.[18,31]

Loading interventions are the preferred treatment for tendinopathy, but the optimal dose in form of repetitions, sets, frequency and load is debated.[34,48] Optimal exercise dose and load magnitude during loading interventions may improve the clinical outcome[43] and lead to a positive tendon tissue response. [28,29] Nevertheless, while many clinical trials have compared the effect of different exercise programs, they rarely isolate specific parameters influencing these loading-based approaches e.g. to what extend load magnitude make a difference. Thus, the optimal load magnitude in patients with tendinopathy is still unknown.

Different eccentric muscle loading programs have become the treatment of choice for tendinopathy over the last decade.[16,31] However, a recent systematic review concludes that there is little clinical or mechanistic evidence that supports to isolate the eccentric component.[5,34] Further, for patellar tendinopathy one study has shown that heavy slow resistance training yields a superior long term response compared with an eccentric loading program.[28] Nevertheless, the focus on eccentric exercise may have overshadowed important aspects of loading intervention including optimal load magnitude, which has some support from both basic science and clinical trials.

The primary cells in the tendon are fibroblasts, which respond to mechanical strain.[25,46,47] Tissue loading results in strain on the fibroblasts and thus initiate synthesis of

collagen and other extracellular matrix components important for the tendon healing process. Conversely, lack of strain can lead to degeneration.[3] However, response of tendon, *in vivo*, to various exercise (dose/response) remains unknown. In healthy, non-symptomatic humans, a study by Arampatzis et al[2] showed that with equal exercise volume, the exercise with greater loads (and thus strains) yield increased tendon stiffness and increased cross sectional area in healthy human Achilles tendons. Importantly, it remains unknown if this load magnitude also is important in patients with tendinopathy.

Therefore, we designed a study to specifically examine if load magnitude influences the outcome of a 12-week loading intervention as treatment for patellar tendinopathy. Furthermore, we assessed the long term (52 weeks) response, which has only been examined in few previous studies.[4,28,49] Based on the above considerations, we hypothesized that a greater magnitude (90% of 1 repetition maximum (RM)) of loading would yield in a more positive clinical (primary outcome, VISA-P), functional and structural outcome compared to a lower magnitude of loading (55% of 1RM) in patients with patellar tendinopathy when total exercise volume is equal in both groups.

MATERIALS AND METHODS

This study was a prospective randomized controlled, single-blinded, superiority trial conducted in Copenhagen, Denmark. Ethical approval was obtained from the regional Ethics Committees for medical research (No. H-15017806) (original study protocol are in the supplement) and all participants provided written informed consent. The study was pre-registered on ClinicalTrials.gov (No. H-15017806) (before inclusion of the first participant) and the reporting of this trial follows the CONSORT guidelines.[36]

Participants

Participants were recruited through the Sports Clinic at Bispebjerg Hospital, Copenhagen, Denmark and via advertisements on social media and the internet. Inclusion criteria were: Male athletes, age 20-45 years, BMI 18.5-30, patellar tendon pain duration of 3-12 months, and clinical diagnosis of patellar tendinopathy by a sports physiotherapist or experienced sports physician

based on defined clinical findings. Further the clinical diagnosis required confirmation by ultrasonography: local anterior-posterior thickening of the tendon of at least 1 mm compared with the mid-tendon level, a hypo-echoic area and presence of power Doppler signal.[8] Exclusion criteria were: corticosteroid injection for patella tendinopathy, previous knee surgery, arthritis, diabetes, any confounding diagnosis to the knee joint, smoking, or being elite volleyball players. Based on previous data[28], we estimated that a sample of n=18 was needed in each group to detect a between group difference at 13 points difference on VISA-P score (minimal clinical important difference[22]) with an alpha level of 0.05 and a power/beta level of 0.80. Therefore, 44 participants were included in total to account for a 20% dropout rate.

Randomization

Participants underwent a phone screening and pre-examination to verify compliance with inclusion criteria. After inclusion and prior to baseline assessment, there was a 2-week "washout" period from any previous treatment and heavy resistance training. After baseline participants were randomly allocated to either the Moderate Slow Resistance group (MSR) or the Heavy Slow Resistance group (HSR). The randomization sequence was a computer-generated minimization randomization procedure[40] (MinimPy version 0.3, Python Software Foundation, Beaverton, OR, USA) with a 1:1 allocation ratio and participants were stratified according to pre-injury physical activity level, pain level and duration.

Blinding

Victorian Institute of Sport Assessment-Patella questionnaire (VISA-P) and other participant reported outcomes were obtained blinded for members of the research team. Furthermore, examiners conducting ultrasonography and Magnetic resonance imaging (MRI) were blinded to treatment allocation. The person conducting the other follow-up test was not systemically blinded, however, all baseline measures were collected before treatment allocation and all data was analyzed blinded. Participants and the physiotherapists providing the intervention could not be blinded, however, the physiotherapists were instructed to state that both intervention programs could potentially provide beneficial results, during supervision of the participants.

Interventions

The HSR program was started at 55% of 1RM and progressed toward 90% of 1RM. The MSR loading program likewise started at 55% of 1RM and maintained this throughout the intervention period. The total exercise volume was matched between groups and both groups performed three weekly sessions (all in a commercial fitness center), where one session was supervised and the last two were not supervised. Each session consisted of one bilateral leg press exercise and one unilateral knee extension exercise (*supplement) with* each repetition lasted six seconds with (three seconds for the concentric and eccentric phase, respectively) . Leg press was performed from complete extension to 90° of knee flexion. Knee extension was performed from 100° of knee flexion to 40° of knee flexion. Both groups completed one set of warm-up set before the exercise protocol with 2-3 min rest between sets (Table 1). Every second week, a 5RM sub-maximal test was performed in order to estimate 1RM and adjust training load accordingly. Pain during the exercises (numeric rating scale (NRS) <5) was accepted, but was not allowed to increase following cessation of the training session, and any training induced pain needed subside 3-4 hours after or else the load was adjusted.

TABLE 1: Intervention protocols

	Exercise protokol							
	Week	**1**	**2**	**3**	**4**	**5**	**6**	**7-12**
	Sets	**3**	**3**	**3**	**4**	**4**	**4**	**5**
HSR	% of 1 RM	55	65	70	75	80	85	**90**
	Repetitions	15	12	10	8	6	5	4
MSR	% of 1 RM	55	55	55	55	55	55	**55**
	Repetitions	15	14	13	11	9	8	7

HSR, heavy slow resistance group; MSR, moderate slow resistance group; RM, repetition maximum.

Participants were allowed to perform sporting activities throughout the 12-week intervention period if only causing light discomfort (NRS <3). A leisure-time activity pain threshold of 50, on the visual analog scale (VAS), has previously been applied successfully in the management of the Achilles tendinopathy.[43] The participants were encouraged to maintain a uniform leisure time sport participation during the 12-week intervention period.

All participants completed a training diary during the intervention using a smartphone application (Injurymap Science ApS, Copenhagen, Denmark). The training records

included training intensity (repetitions, sets and load), sessions, pain during training (NRS) and information about deviations from the planed intervention protocol.

When the intervention period ended after 12 weeks, the participants did not receive any further treatment but were encouraged to maintain their intervention and use the guidelines about pain management and training adjustment.

Follow-up evaluation

Outcome measurements were obtained at baseline, mid intervention (6 weeks), post intervention (12 weeks) and one-year follow-up (52 weeks). The primary outcome was VISA-P (change from baseline to 12 weeks). Mechanical properties and ultra-short echo time (UTE) MRI outcomes obtained will be published separately. Baseline and 12-week measurements were performed 3-4 days before and after the intervention period. The examination order was identical for all follow-up evaluations, always starting with MRI at day one and day two starting with ultrasound examination followed by a 5-minute warm-up on a bicycle-ergometer prior to further examinations.

Participant reported outcome

All participants completed an electronic VISA-P questionnaire to assess symptoms, function and ability to participate in sports. The VISA-P consists of 8 questions with a maximum score of 100 for an asymptomatic, fully performing individual, and lower scores indicating more symptoms and limitations of function and activity.[45] The VISA-P has been shown to be a valid and reliable outcome measure for patients with patellar tendinopathy.[35,45] The minimal clinical important difference for the VISA-P in athletes with patellar tendinopathy is considered to be 13 points.[22] In addition, maximal tendon pain during preferred sporting and function was evaluated on a 0 to 10 NRS with 10 being the worst imaginable pain and 0 denoting no pain. Participants completed the VISA-P and NRS questionnaire with no investigator assistance at baseline, after 6 weeks, 12 weeks and at one-year follow-up.

Weekly sport participation (hours) during the preceding week was evaluated: before injury (recall), at baseline, 6 weeks, 12 weeks and one-year follow-up. Participants evaluated their

satisfaction with the results at the end of the intervention (12 weeks) and at one-year follow-up, in the electronic written questionnaire.

Functional evaluation

Quadriceps muscle strength was measured during maximal voluntary isometric contractions (iMVC) as previous described[6] at baseline and 12 weeks. Briefly, participants were strapped to a custom-made rigid chair in a seated position with 90° hip and knee flexion. A rigid leg cuff was placed on the lower leg, approximately three cm above the medial malleolus, which was connected to a strain-gauge through a stiff steel rod. A wireless transmitter (8-channel, TeleMyo 2400T G2, Telemetry System, Noraxon Inc.) was used for force recording in Noraxon MR-XP 1.07 Master Edition. The distance from the cuff to the knee joint line (tibia length) was measured to evaluate the knee extensor moment (KEM, peak force * tibia length). Four maximal isometric contractions of approximately eight seconds, with a 2-minute rest period between each test, were conducted. The first contraction was considered as familiarization and the maximal peak force and KEM among the remaining three contractions were used for analysis.

A reliable patellar tendon pain provocation test, Single-leg decline squat (SLDS)[38], was used to examine pain during function at baseline, 6 weeks, 12 weeks and at one-year follow-up. Participants performed a decline squat on a 25° decline board and reported pain using the NRS pain scale upon completion. The participants were instructed to stand on one leg with their hands placed at the waist and to keep the trunk vertical and heels in contact with the board. A SLDS was performed until knee flexion of 50° and return to the starting position at self-determined speed.[9] One practice trial was performed before the two tests with a 1-minute rest period between the tests. The NRS pain was collected once at each test trail and the mean of the two were used for further analysis.

Furthermore, Squat jump (SJ) and Counter movement Jump (CMJ) were used to examine function at baseline and week 12. Jump tests were performed on a portable contact-mat (Chronojump-Boscosystem, Barcelona, Spain) and vertical jump height was estimated from flight time (*gravity / 8 * flight time squared).* The open-source system (Chronojump) has been demonstrated to be a valid and reliable tool for measuring jumping ability.[37] The SJ was performed from a starting position of 90° knee angle without counter movement. The CMJ started on straight

legs with a counter movement down to a knee angle of 90° before the jump. The subjects performed two-three practice trials prior to testing, and the mean jump height of three technically corrected jumps was used for further analysis.

Ultrasonography

Ultrasonography was performed on the patellar tendon using a HI Vision Hitachi Ascendus ultrasound machine (Hitachi Medical systems, Japan). Gray-scale (GS) and Power-Doppler (PD) setting were identical for all examinations. All participants were instructed to avoid strenuous physical activity 24 hours before examination. The ultrasound examination was performed at baseline, 6 weeks, 12 weeks and one-year follow-up by the same two experienced assessors.

GS examination was performed with the participant in a seated position with 90° hip and knee flexion. The GS examination was performed with a 10.5 long linear transducer, the depth fixed at 4.5 cm, a dynamic range of 70, and gain of 20. The transducer was placed at a 90° angle (not rotated along the tendon surface) and moved medially to laterally to find the place where the tendon was thickest. After imaging this position, the transducer was removed from the skin and the procedure repeated. Ultrasound recordings were imported to FIJI/ImageJ (version 1.52, National Institutes of Health, USA) for quantitative analysis. The A-P patellar tendon thickness was measured exactly 0.5 cm distally from the patella apex by the same blinded investigator. The specific measuring site and method was performed as previously described.[16] The max of the A-P thickness from the two recorded image was used for further statistically analysis.

PD examination was performed with the participants placed supine and with stretched and relaxed knee. The PD examination was performed with an 18.5 short linear transducer, the depth fixed at 2.0 cm, a dynamic range of 70, a color Doppler frequency of 10 MHz, a pulse repetition frequency of 250 and a color gain of 37. The investigator applied minimum transducer pressure during scanning. The transducer was placed at a 90° angle and moved medially to laterally, to locate the maximum Doppler signal. At this location, two 4-second sine loops were recorded in the sagittal plane (uncompressed AVI files each containing 16 frames). The ultrasound recordings were imported to FIJI/ImageJ (version 1.52, National Institutes of Health, USA) for quantitative analysis. A custom macro was set up to analyze the frame containing the largest area of Doppler activity in each series. Doppler was only included if it was localized within

the tendon and possible noise was excluded. The image with the largest Doppler area was used for further analysis. Two investigators conducted all analysis in a blinded fashion with the pre and post scan for each participant analyzed by the same investigator.

Tendon cross-sectional area

MRI was used to assess cross sectional area (CSA) of the Patellar tendon on the injured side at baseline and 12 weeks (Siemens, Erlangen, Germany, 3.0-T). The parameters used in the MRI scan were as follows: Transversal Proton density weighted; Slice thickness 3 mm, Field of View 80 x 80 mm, matrix resolution 0.42 x 0.42 x 3,00 mm, Echo Time 39 ms, Repetition Time 4000. Participants were scanned in the supine position using a dedicated 15-channel send/receive knee coil.

The open source software Horos v.3.3.5for MAC OS (https://horosproject.org) was used to analyze the MRI images. The Patellar tendon CSA was measured by manually outlining the tendon in the axial plane just distal to the patella, mid tendon level and just proximal to the tibia insertion as previous described.[30] The mean of three measurements at each location was used for further analysis. To optimize the measurement, both gray scale and NIH color scale were used during outlining. This procedure has been described in detail elsewhere and has been shown to reduce the underestimation of CSA by 2.8% compared with using only gray scale.[11]

Statistical analysis

Statistical analyzes was carried out in GraphPad Prism (version 8.2.1 for macOS, GraphPad Prism Software, California). Results are reported as mean ± standard error unless otherwise noted. Demographic baseline data and participant compliance were analyzed by use of unpaired Students t-test. Outcome parameters were analyzed by two-way analyzes of variance (time x intervention) with Bonferroni post hoc analyzes when appropriate. Participant satisfaction was analyzed with Fisher exact tests at 12 and 52 weeks. Correlation of changes in VISA-P score and PD from baseline to 12-week and baseline to 52-week were analyzed with Persons correlation. All analyzes were performed as intention to treat, with the last observation carried forward. Per-protocol analyzes (participants that fulfilled at least 75% of the prescribed training sessions) are shown in the supplement. Significance level for all tests was set to $P > 0.05$.

RESULTS

Participants

Between April 2017 and July 2018, 44 participants were enrolled in the study and randomized into one of the two groups. For details, see the flowchart (Figure 1). Two participants did not receive the allocated intervention due to MRI at baseline revealing confounding diagnoses and were removed from the analysis. There were no significant differences between the two intervention groups at baseline (Table 2). All participants were recreational athletes with a large number (n=13) conducting strength training (including Cross-Fit) and other sports: soccer (n=9), running (n=7), volleyball (n=4), badminton (n=3), basketball (n=3), gymnastics (n=1), swimming (N=1) and American football (n=1) as preferred sports.

TABLE 2. Participants demographics and baselines characteristics

Variable	**HSR** (n=21)	**MSR** (n=21)
Age (y)	28.8 ± 5.1 (20-38)	32.3 ± 4.9 (23-41)
Height (cm)	185.8 ± 7.1	180.7 ± 7.2
Weight (kg)	86.7 ± 9.3	82.2 ± 9.2
BMI (kg/m^2)	25.1 ± 2.4	25.2 ± 2.6
Symptoms duration (month)	6.9 ± 2.4 (3-12)	7.3 ± 2.9 (3-12)
Weekly sport participation (hours)	9.0 ± 4.8 (1-21)	7.0 ± 3.8 (1-14)
Pain during activity (NRS)	4.7 ± 2.2 (1-8)	5.2 ± 2.0 (2-9)
Unilateral/bilateral (n)	14:7	13:8
Proximal/distal injury (n)	21:0	20:1

Values are expressed as mean ± SD (range) unless otherwise noted. HSR, heavy slow resistance group; MSR, moderate slow resistance group. There were no differences between groups for any parameters at baseline.

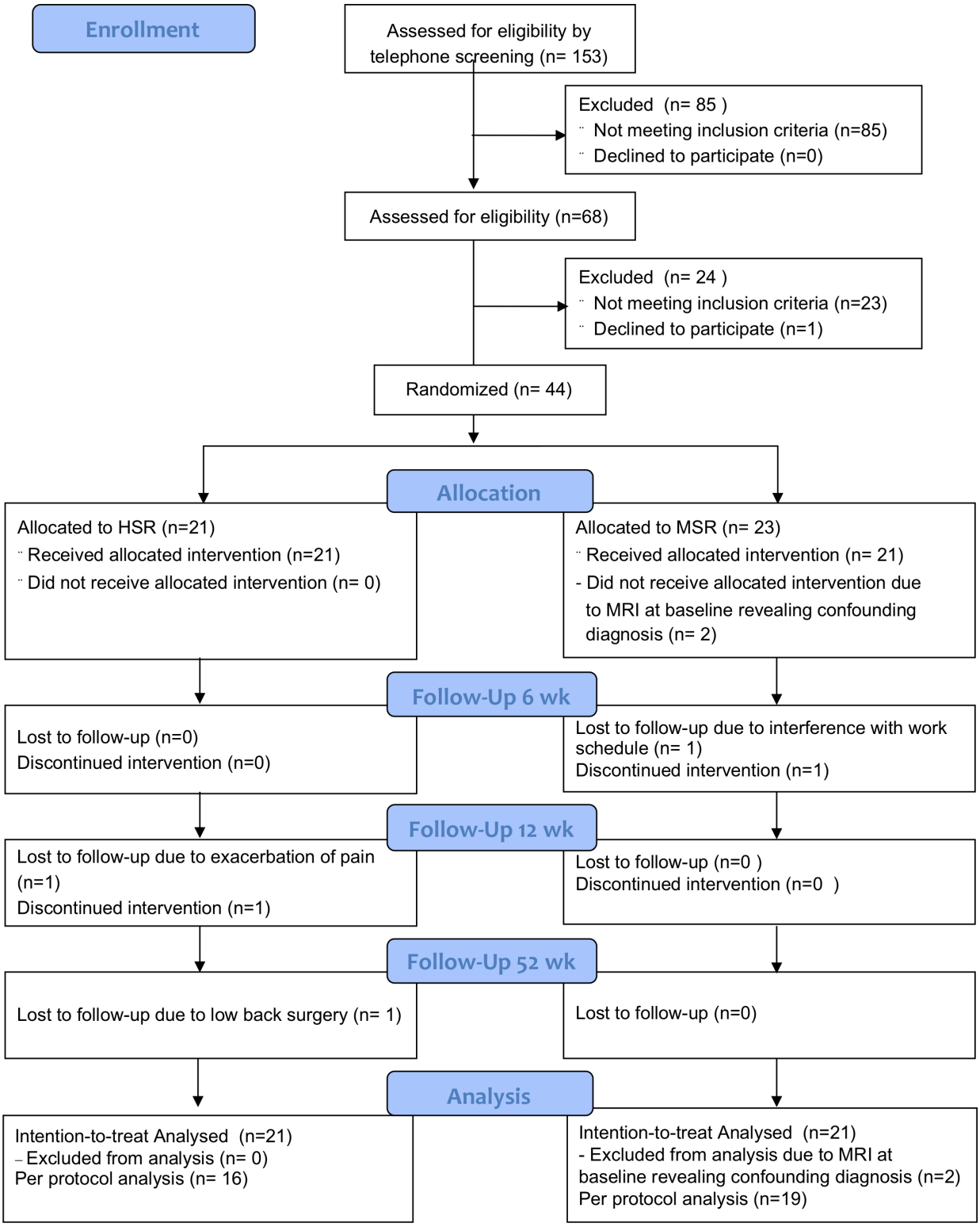

FIGURE 1: CONSORT (Consolidated Standards of Reporting Trials) flowchart for the primary outcome Victorian Institute of Sports Assessment- Patella (VISA-P). HSR, Heavy slow resistance group; MSR, Moderate slow resistance group

Compliance

Two participants dropped out; one due to exacerbation of pain, and one due to interference with his work schedule. Further, one participant was lost at the one-year follow-up due to low back surgery.

The mean training session compliance was 78±4% for the HSR and 86±2% for the MSR group, which was not significantly different (P=0. 12) (*supplement*). All data from per-protocol analysis (participants that fulfilled at least 75% of the prescribed training sessions) are shown in the supplement, and the overall conclusions were equal to the intention-to-treat analyses. The supervised session compliance was 81±4% for the HSR and 86±2% for the MSR (P=0.39).

The participants completed the leg press exercise with a mean of 76.8±4.2% and 84.8±2.2% of the calculated total absolute volume prescribed for HSR and MSR, respectively (P=0.10, *in supplement*). The knee extension exercise with the injured leg was performed with a mean of 72±4.3% for HSR and 80.3±2.7% for MSR of the calculated total absolute volume (P=0.15). Both exercises were performed with above 90% of the load prescribed for HSR and MSR, respectively. Similarly, no difference in the prescribed total time under tension was demonstrated between groups in leg press (HSR: 79±3.9%, MSR:85.3±2.1%, P=0.17) or knee extension on the injured leg (HSR:77.1±3.9%, MSR:83.4±2.4%, P=0.18). There was no interaction, group or time effect for the self-reported pain during both exercises (Table 3).

TABLE 3: Pain during activity measured on Numeric Rating Scale

	HSR (n=21)	**MSR** (n=21)	**P group**	**P time**	**P group x time**
Running (questionnaire)					
0 weeks	3.2 ± 0.4	4.0 ± 0.5	0.33	<0.0001	0.42
6 weeks	2.4 ± 0.4	3.0 ± 0.4			
12 weeks	1.8 ± 0.3	2.1 ± 0.4			
52 weeks	1.3 ± 0.4	1.3 ± 0.3			
Squat (questionnaire)					
0 weeks	4.0 ± 0.6	3.2 ± 0.5	0.07	<0.0001	0.96
6 weeks	2.5 ± 0.5	1.6 ± 0.2			
12 weeks	2.0 ± 0.5	1.2 ± 0.3			
52 weeks	1.8 ± 0.5	0.7 ± 0.2			
Pref sport (questionnaire)					
0 weeks	4.9 ± 0.6	4.7 ± 0.5	0.48	<0.0001	0.73
6 weeks	3.5± 0.6	3.3 ± 0.5			
12 weeks	3.1 ± 0.7	2.3 ± 0.5			
52 weeks	2.0 ± 0.6	1.3 ± 0.3			
Leg press (intervention)					
0-6 weeks	0.6 ± 0.1	0.5 ± 0.1	0.70	0.60	0.29
7-12 weeks	0.5 ± 0.1	0.5 ± 0.1			
Knee ext (intervention)					
0-6 weeks	0.8 ± 0.1	0.7 ± 0.1	0.69	0.32	0.44
7-12 weeks	0.7± 0.1	0.7 ± 0.1			
CMJ (examination)					
0 weeks	1.2 ± 0.3	1.3 ± 0.3	0.95	0.01	0.57
12 weeks	0.7 ± 0.3	0.6 ± 0.3			
SJ (examination)					
0 weeks	1.0 ± 0.3	0.8 ± 0.2	0.99	0.01	0.32
12 weeks	0.4 ± 0.2	0.6 ± 0.2			

Values are pain score measured on a numeric rating scale [NRS] and expressed as mean±SEM. Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Questionnaire, Patient reported outcome measured; Pref sport, Preferred sport; Intervention, Self-reported NRS pain expressed as averaged pr. session during week 0-6 and week 7-12; Knee ext, knee extension for injured leg; CMJ, Mean pain for the 3 counter movement jump; SJ, mean pain for the 3 Squat jump.

Clinical

There was no significant interaction (P=0.89) or group effect (P=0.57), but a significant effect of time (P <0.0001) for VISA-P (Table 4). VISA-P increased from baseline to 12 weeks and from 12 week to 52 weeks follow-up in both groups. For the NRS-running, NRS-squat, and NRS-preferred sport, there was a significant reduction with time (P <0.0001), but no significant interaction or difference between group (NRS-running: P=0.33 , NRS-squat: P=0.09, and NRS-preferred sport: P=0.50) (Table 3).

Similarly, for NRS pain score during the SLDS test, there was no interaction (P=0.93) or group effect (P=0.73), but a significant main effect of time (P < 0.0001). SLDS NRS decreased significant from baseline to 12 weeks for both groups (P< 0.0001) but not from 12 to 52 weeks (HSR, P=0.37; MSR, P=0.73) (Table 4).

The participants satisfaction with the clinical outcome at 12-week follow-up was similar for the HSR (95 %, 18/19) and MSR (95%, 20/21)(P > 0.99). At 52-weeks follow-up 84% (16 of 19) of the HSR and 95% (20 of 21) of the MSR group were satisfied with no group difference (P=0.33). For activity level, there was no significant effect of time (P=0.43), interaction (P=0.13) or difference between group (P=0.75) (Table 5). The mean activity level during the 12-weeks intervention period was not different from baseline level for any of the two groups. However, activity level at baseline and 52 weeks follow-up was significantly lower compared to pre-injury level.

TABLE 4: Clinical Results

	HSR (n=21)	**MSR** (n=21)
VISA-P, Point		
0 weeks	58.8 ± 4.3 (49.8, 67.8)	59.9 ± 2.5 (54.8, 65.0)
6 weeks	65.8 ± 3.7 (58.0,73.5)	69.9 ± 2.8 (64.0, 75.7)
12 weeks	70.5 ± 4.4 (61.3, 79.7)	72.5 ± 2.9 (66.5, 78.5)
52 weeks	79.7 ± 4.6 (70.0, 89.4)	82.6 ± 2.5 (77.4, 87.8)
Δ 0-12 weeks	11.71 ± 2.8 (4.1, 19.3)**	12.62 ± 2.8 (5.0, 20.2)**
Δ 12-52 weeks	9.1 ± 2.8 (1.6, 16.7)**	10.10 ±2.8 (2.5, 17.7)**
Δ 0-52 weeks	20.9 ± 2.8 (13.3, 28.4)***	22.7 ± 2.8 (15.1, 30.3)***
SLDS, NRS		
0 weeks	4.3 ± 0.4 (3.3, 5.2)	3.9 ± 0.3 (3.2, 4.6)
6 weeks	2.4 ± 0.4 (1.5, 3.3)	2.3 ± 0.4 (1.6, 3.0)
12 weeks	2.0 ± 0.4 (1.2, 2.9)	1.9 ± 0.3 (1.2, 2.6)
52 weeks	1.4 ± 0.4 (0.5, 2.2)	1.3 ± 0.4 (0.6, 2.1)
Δ 0-12 weeks	-2.2 ± 0.4 (-3.2, -1.2)***	-2.0 ± 0.4 (-3.0, -1.0)***
Δ 12-52 weeks	-0.7 ± 0.4 (-1.7, 0.3)	-0.6 ± 0.4 (-1.6, 0.4)
Δ 0-52 weeks	-2.9 ± 0.4 (-3.9, -1.9)***	-2.6 ± 0.4 (-3.6, -1.6)***

Values are presented as mean±SEM (95% CI). Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; VISA-P, Victorian Institute of Sports Assessment- Patella; SLDS, Single-leg Decline Squat test. 0 weeks, baseline; 6 weeks, mid intervention; 12 weeks, post intervention; 52weeks, 1-year follow-up. Δ, change in time interval. VISA-P: P-values group (0.57), interaction (0.89) and time <0.0001). SLDS: P-values group (0.73), interaction (0.93) and time (<0.0001) ** Significant effect of time, P <0.01 ***Significant effect of time, P<0.0001

TABLE 5: Weekly sport participation (hours). Pre injury level recall at pre examination and self-reported at week 0, 6,12 and 52.

Sport participation (hours/ week)	**HSR** (n=21)	**MSR** (n=21)
Pre Injury	9 ± 1 (1-21)	7 ± 1 (1-14)
Week 0	6± 1 (1-10)	5 ± 1 (0-15)
Week 6	4 ± 1 (1-18)	5 ± 1 (0-20)
Week 12	4 ± 1 (0-18)	5 ± 1 (0-16)
Week 52	6 ± 1 (0-18)	5 ± 1 (0-17)
Δ Pre injury-week 0	-3± 1 (-4.6, -1.8)*	-2 ± 1 (-3.8, -0.9)*
Δ Pre injury-week 52	-3 ± 1 (-4.5, -0.4)*	-2 ± 1 (-4.3, -0.2)*
Δ 0-12 week	-1 ± 1 (-3.7, 1.1)	1 ± 1 (-1.8, 3.0)
Δ 12-52 week	2 ± 1 (-0.3, 4.5)	-1 ± 1 (-2.9, 1.9)
Δ 0-52 week	1 ± 1 (-1.6, 3.1)	1 ± 1 (-2.3, 2.5)

Pre injury and week 0, 6, 12 and 52 are reported as Mean±SEM (range). All Δvalues are expressed as Mean±SEM (95% CI). Analyzed with 2way ANOVA (p< 0.05) show week 0,6,12 and 52 no effect of time (p= 0.439), treatment (p= 0.75) or Interaction (p=0.13).* Significant different from Pre Injury, P <0.05

Function

For the MVC, no significant interaction or group effect was detected, but there was a significant increase after 12 weeks (P < 0.0001) (Table 6). For jump height in both SJ and CMJ, there was no interaction, group or time effect (Table 6), but a significant main effect of time was found in pain (P=0.01) for CMJ and SJ, respectively (Table 3).

Ultrasonography and MRI

There was no significant effect of time (P=0.10), group (P=0.37) or interaction (P=0.58) for tendon A-P thickness. The PD area decreased significantly with time (P=0.01), but without any between group difference (P=0.30) or interaction (P=0.22) (Table 7). However, while there was an overall significant effect of time on PD area, the post hoc analysis within each group showed no significant effect for any of the periods baseline to 12 weeks, 12 to 52 weeks or baseline to 52 weeks, respectively. The change in PD area (HSR+MSR together) did not correlate significantly (r=- 0.28; P=0.22) with changes in VISA-P score over time (baseline to 12 weeks).

For the CSA of the proximal, mid and distal part of the patella tendon there was no group effect or difference from baseline to 12 week (Table 6).

TABLE 6: Structural and mechanical properties for Injured site

	HSR (n=21)	**MSR** (n=21)	**P group**	**P time**	**P g x t**
MVC (Nm)					
0 weeks	193 ± 11	194 ± 10	0.75	<0.0001	0.25
12 weeks	223 ± 11	213 ± 10			
CSA PROX (cm^2)					
0 weeks	1.53 ± 0.09	1.40 ± 0.08	0.15	0.72	0.76
12 weeks	1.55± 0.10	1.40 ± 0.08			
CSA MID (cm^2)					
0 weeks	1.13 ± 0.07	1.07 ± 0.07	0.39	0.17	0.26
12 weeks	1.13 ± 0.07	1.03 ± 0.05			
CSA DIST (cm^2)					
0 weeks	1.23 ± 0.01	1.08 ± 0.04	0.15	0.72	0.76
12 weeks	1.23 ± 0.08	1.10 ± 0.05			
CMJ Height (cm)					
0 weeks	32.49 ± 1.40	31.68 ± 1.26	0.51	0.08	0.20
12 weeks	33.46 ± 1.43	31.83 ± 1.21			
SJ Height (cm)					
0 weeks	28.24 ± 1.20	27.17 ± 1.42	0.77	0.65	0.43
12 weeks	27.17 ± 1.14	27.45 ± 1.01			

Values are presented as mean±SEM. Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. MVC, peak knee extension moment; CSA, Patella tendon cross-sectional area; CMJ, counter movement jump, SJ, Squat jump.

TABLE 7: Sonographic Results

	HSR (n=21)	**MSR** (n=21)
Doppler (mm^2)		
0 weeks	14.66 (26.91, 7.99)	9.38 (20.32, 4.33)
6 weeks	15.96 (27.33, 9.32)	7.46 (14.94, 3.73)
12 weeks	11.15 (20.55, 6.05)	10.3 (19.35, 5.49)
52 weeks	8.60 (16.28, 4.54)	5.99 (12.31, 2.92)
Δ % 0-12 weeks	-24 (-59, 41)	10 (-41, 103)
Δ % 12-52 weeks	-23 (-58, 43)	-42 (-69, 8)
Δ % 0-52 weeks	-41 (-68, 9)	-36 (-66, 18)
Thickness (mm)		
0 weeks	7.62 (8.64, 6.72)	6.77 (7.75, 5.92)
6 weeks	7.67 (8.59, 6.85)	7.16 (8.23, 6.22)
12 weeks	7.55 (8.32, 6.84)	7.26 (8.19, 6.43)
52 weeks	7.13 (8.11, 6.27)	6.75 (7.80, 5.85)
Δ % 0-12 weeks	-1 (-12, 11)	7 (-4, 20)
Δ % 12-52 weeks	-5 (-16, 6*)*	-7 (-17, 4)
Δ % 0-52 weeks	-6 (-16, 5)	0 (-11, 12)

Values are presented as mean (95% CI). Δ %, relative change in time interval. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Doppler, power doppler (total number of colored pixel converted to area in mm^2); Thickness, anterior-posterior thickness of the tendon. The data was log-transformed and two-way analysis of variance were conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. HSR.Doppler: P-values group (0.30), interaction (0.22) and time (0.01); Thickness: P-values group (0.37), interaction (0.58) and time (0.10).

Discussion

This randomized clinical trial investigated if load magnitude influenced the effect of a 12-week loading intervention for patellar tendinopathy in the short (12 weeks) and long-term (52 weeks). The main findings show that both a load magnitude of 90% (HSR) and 55% (MSR) of 1RM yielded significant clinical improvements: VISA-P, NRS -running, NRS -squat, NRS -preferred sport, SLDS and patient satisfaction after 12 weeks, and these were maintained one year later. However, contrary to our original hypothesis, HSR was not clinically superior to MSR loading, and there were no differences in improvements in function (strength and jumping ability) or tendon structure (A-P thickness, PD area and CSA) between the interventions.

Loading based rehabilitation approaches to patellar tendinopathy has proven clinically beneficial with respect to pain and function.[31,34] In the present study, the participants had baseline VISA-P values comparable with those previously reported in patients with chronic patellar tendinopathy,[23,28] and over the intervention period the VISA-P score improved by 11.7 (HSR) and 12.6 points (MSR), which approaches the clinically meaningful score of 13 points.[22] Furthermore, the pain improvement in the present study agrees well with the improvement reported in the literature.[10,31]

There has been considerable focus on the clinical effects of loading based exercise regimes as treatment for tendinopathy, but very little is known about the importance of changing specific loading parameters. Previous studies have solely focused on muscular contraction mode (concentric, eccentric or isometric) in an attempt to optimize outcomes.[34] However, clinical trials [5,28] and basic science [12,19,21,39] do not support a strategy based on a distinct muscular contraction mode. In fact, in animal models, collagen expression as a result of high forces appears to be independent of muscle contraction mode, i.e. isometric, eccentric or concentric contractions. [21] Furthermore, in healthy humans, resistance training associated tendon hypertrophy has also been shown to be independent of contraction mode.[12] On the cellular level it is well known that the fibroblasts in collagen rich tissue respond to loading (mechanical deformation) by stimulating the synthesis of collagen[20,25,47] and other extracellular components important for tendon growth.[7] In healthy humans, stiffness of tendon tissue increases in response to exercise loading at 90% but not at 55% of MVC [2], indicating that magnitude is an important factor for tendon adaptation, but this has never been investigated in the treatment of tendinopathy. Unexpectedly, the result of the

current study did not demonstrate a superior clinical effect of exercising with 90% compared to 55% of 1RM, which indicates that difference in load magnitude above 55% is inconsequential to the clinical outcome in tendinopathic tendons. Two previous studies[5,28] have compared HSR to eccentric training, which is commonly used as treatment for tendinopathy. The data suggest that the clinical improvement over 12 weeks is similar with the two approaches. These studies did not control for (or measure) training load per se, but it is likely that HSR achieved higher tendon loads than the eccentric group that largely used body weight for resistance. Indirectly, this support the findings of the present study that some magnitude of load is essential for the clinical picture to improve, but does not necessarily require extremely high loads. The minimal threshold to achieve such an improvement remains unknown.

Resistance training over 12 weeks will typically yield an increase in muscle strength by ~10-20% in healthy individuals.[41] The present study showed similarly gains with an increase in muscle strength by 15% (HSR) and 10% (MSR). It could be argued that the increase may be the result of a reduction in pain alone. However, it has been shown that a reduction in pain in patients with tendinopathy treated with corticosteroid did not result in increased strength.[28] The fact that previous studies have shown comparable gain in strength for HSR in both tendinopathic [28,29] and healthy patellar tendons [30] also supports that the increase in MVC in the present study was a response to the applied training protocol. It is noteworthy that both MSR and HSR yielded strength gains, however it is in accordance with previous data showing both heavy (70% of 1RM) and light load (15 % of 1RM) at equalized total work is sufficient to induce increase in strength.[24] Interestingly, the participants in the current study did not improve their jumping ability despite a decrease in pain and increased in strength, which may be because the gain was not large enough to transfer to improvements in jump height.

Few studies[4,28,49] have investigated the long term follow-up of loading based exercise. The present data show that the VISA-P score improved by ~13% from week 12 to 52 irrespective of loading regime (HSR or MSR), and the improvement from baseline to one year exceeded the clinically meaningful score. The reported pain during function displayed a similar pattern. The present study was not designed to answer if the effect of training with HSR and MSR was equally good, however, the data show that both are effective in the treatment of patellar tendinopathy. Further, no difference in patient satisfaction could be detected at 12 or 52 weeks.

Therefore, clinically it may be important to choose the exercise regime that best fit the patient's preferences in order to improve compliance. Notably, in both groups, none of the clinical outcomes or pre-injury weekly sport participation (hours) (lack around 30%) fully recovered after one year. Additionally, no normalization of A-P thickness or CSA was found after 12 week or even 52 weeks. Altogether, these findings indicate that time to recover may extend beyond one year, which is supported by a study on Achilles tendinopathy in which 20% of the patients had symptoms that persisted for up to 5 years.[42]

Ultrasonography with Doppler assessment is commonly used to evaluate vascularization in tendinopathy[14], and it has been suggested that increased neovascularization along with nerve ingrowth is the cause of pain.[1] In the literature, there is conflicting evidence regarding the coupling between the presence of doppler activity and symptoms.[15,17] In the present study, an improvement in VISA-P score improved while doppler activity decreased from baseline to 52 weeks. Reduced doppler in response to training has also been detected in previous studies both in the short term for patellar tendinopthy[28] and in the long term for Achilles tendinopathy[5], which is in agreement with the present data. The change in Doppler area after 12 weeks in the current study (HSR 24% decrease, MSR 10% increase) was less than the 45% reduction after 12 weeks of HSR shown previously[28], and this magnitude of decline was not reached until after one year (HSR 41%, MSR 36%). However, variation between studies in relation to applied US protocols and the way of measuring the Doppler area make it challenging to compare the absolute values.

This study has inherent limitations. First, a non-exercise control group was not included, and therefore, it cannot be ruled out that improvements would have occurred over time in the absence of either loading regime. Further, the wash-out period included to control for any previous treatment was relative short. Additionally, the current study was designed to test the influence of load magnitude, and optimally all other parameters should therefore be kept stable. However, it is not possible to exactly match all parameters and we decided to match the volume, rate of loading, sets and sessions at the cost of a group differences in repetitions and total time under tension over the intervention period. The role of these parameters could be important and will require additional research.

In conclusion, the current study demonstrates that there is no statistically superior effect of exercising with 90% compared to 55% of 1RM. Importantly, VISA-P, SLDS and PD

continued to improve up to one year, but did not reach normal values indicating that it takes a long recovery time after patellar tendinopathy. It remains unknown if other exercising variables e.g. frequency rate of loading have implications on the outcome and time to complete recovery.

REFERENCES

1. Alfredson H, Ohberg L, Forsgren S. Is vasculo-neural ingrowth the cause of pain in chronic Achilles tendinosis? An investigation using ultrasonography and colour Doppler, immunohistochemistry, and diagnostic injections. *Knee Surg Sports Traumatol Arthrosc*. 2003;11(5):334-338. doi:10.1007/s00167-003-0391-6.
2. Arampatzis A, Karamanidis K, Albracht K. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *J Exp Biol*. 2007;210(15):2743-2753. doi:10.1242/jeb.003814.
3. Arnoczky SP, Lavagnino M, Egerbacher M. The mechanobiological aetiopathogenesis of tendinopathy: Is it the over-stimulation or the under-stimulation of tendon cells? *Int J Exp Pathol*. 2007;88(4):217-226. doi:10.1111/j.1365-2613.2007.00548.x.
4. Bahr R, Fossan B, Løken S, Engebretsen L. Surgical treatment compared with eccentric training for patellar tendinopathy (Jumper's Knee). A randomized, controlled trial. *J Bone Joint Surg Am*. 2006;88(8):1689-1698. doi:10.2106/JBJS.E.01181.
5. Beyer R, Kongsgaard M, Hougs Kjær B, Øhlenschlæger T, Kjær M, Magnusson SP. Heavy slow resistance versus eccentric training as treatment for achilles tendinopathy: A randomized controlled trial. *Am J Sports Med*. 2015;43(7):1704-1711. doi:10.1177/0363546515584760.
6. Bojsen-Møller J, Magnusson SP, Rasmussen LR, Kjaer M, Aagaard P. Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. *J Appl Physiol*. 2005;99(3):986-994. doi:10.1152/japplphysiol.01305.2004.
7. Chiquet M, Gelman L, Lutz R, Maier S. From mechanotransduction to extracellular matrix gene expression in fibroblasts. *Biochim Biophys Acta*. 2009;1793(5):911-920. doi:10.1016/j.bbamcr.2009.01.012.
8. Cook JL, Khan KM, Kiss ZS, Purdam CR, Griffiths L. Reproducibility and clinical utility of tendon palpation to detect patellar tendinopathy in young basketball players. Victorian Institute of Sport tendon study group. *Br J Sports Med*. 2001;35(1):65-69. doi:10.1136/bjsm.35.1.65.
9. Cook JL, Purdam CR. The challenge of managing tendinopathy in competing athletes. *Br J Sports Med*. 2014;48(7):506-509. doi:10.1136/bjsports-2012-092078.

10. Couppé C, Svensson RB, Silbernagel KG, Langberg H, Magnusson SP. Eccentric or Concentric Exercises for the Treatment of Tendinopathies? *J Orthop Sport Phys Ther*. 2015;45(11):853-863. doi:10.2519/jospt.2015.5910.
11. Couppé C, Svensson RB, Sødring-Elbrønd V, Hansen P, Kjaer M, Magnusson SP. Accuracy of MRI technique in measuring tendon cross-sectional area. *Clin Physiol Funct Imaging*. 2014;34(3):237-241. doi:10.1111/cpf.12086.
12. Farup J, Rahbek SK, Vendelbo MH, et al. Whey protein hydrolysate augments tendon and muscle hypertrophy independent of resistance exercise contraction mode. *Scand J Med Sci Sport*. 2014;24(5):788-798. doi:10.1111/sms.12083.
13. Finni T, Komi P V, Lepola V. In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. *Eur J Appl Physiol*. 2000;83(4-5):416-426. doi:10.1007/s004210000289.
14. Finnoff JT. The Evolution of Diagnostic and Interventional Ultrasound in Sports Medicine. *PM&R*. 2016;8(3S):S133-S138. doi:10.1016/j.pmrj.2015.09.022.
15. Fredberg U, Bolvig L. Significance of ultrasonographically detected asymptomatic tendinosis in the patellar and Achilles tendons of elite soccer players: A longitudinal study. *Am J Sports Med*. 2002;30(4):488-491. doi:10.1177/03635465020300040701.
16. Fredberg U, Bolvig L, Andersen NT, Stengaard-Pedersen K. Ultrasonography in evaluation of Achilles and patella tendon thickness. *Ultraschall Med*. 2008;29(1):60-65. doi:10.1055/s-2007-963027.
17. Fredberg U, Bolvig L, Pfeiffer-Jensen M, Clemmensen D, Jakobsen BW, Stengaard-Pedersen K. Ultrasonography as a tool for diagnosis, guidance of local steroid injection and, together with pressure algometry, monitoring of the treatment of athletes with chronic jumper's knee and Achilles tendinitis: A randomized, double-blind, placebo-controlled . *Scand J Rheumatol*. 2004;33(2):94-101. doi:10.1080/03009740310004126.
18. Gaida JE, Cook J. Treatment options for patellar tendinopathy: Critical review. *Curr Sports Med Rep*. 2011;10(5):255-270. doi:10.1249/JSR.0b013e31822d4016.
19. Garma T, Kobayashi C, Haddad F, Adams GR, Bodell PW, Baldwin KM. Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise. *J Appl Physiol*. 2007;102(1):135-143.

doi:10.1152/japplphysiol.00776.2006.

20. Gauvin R, Parenteau-Bareil R, Larouche D, et al. Dynamic mechanical stimulations induce anisotropy and improve the tensile properties of engineered tissues produced without exogenous scaffolding. *Acta Biomater*. 2011;7(9):3294-3301. doi:10.1016/j.actbio.2011.05.034.
21. Heinemeier KM, Olesen JL, Haddad F, et al. Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. *J Physiol*. 2007;582(3):1303-1316. doi:10.1113/jphysiol.2007.127639.
22. Hernandez-Sanchez S, Hidalgo MD, Gomez A. Responsiveness of the VISA-P scale for patellar tendinopathy in athletes. *Br J Sports Med*. 2014;48(6):453-457. doi:10.1136/bjsports-2012-091163.
23. Holden S, Lyng K, Graven-Nielsen T, et al. Isometric exercise and pain in patellar tendinopathy: A randomized crossover trial. *J Sci Med Sport*. 2020;23(3):208-214. doi:10.1016/j.jsams.2019.09.015.
24. Holm L, Reitelseder S, Pedersen TG, et al. Changes in muscle size and MHC composition in response to resistance exercise with heavy and light loading intensity. *J Appl Physiol*. 2008;105(5):1454-1461. doi:10.1152/japplphysiol.90538.2008.
25. Joshi SD, Webb K. Variation of cyclic strain parameters regulates development of elastic modulus in fibroblast/substrate constructs. *J Orthop Res*. 2008;26(8):1105-1113. doi:10.1002/jor.20626.
26. Kettunen JA, Kvist M, Alanen E, Kujala UM. Long-term prognosis for jumper's knee in male athletes. A prospective follow-up study. *Am J Sports Med*. 2002;30(5):689-692. doi:10.1177/03635465020300051001.
27. Khan K, Cook J. The painful nonruptured tendon: clinical aspects. *Clin Sports Med*. 2003;22(4):711-725. doi:10.1016/S0278-5919(03)00035-8.
28. Kongsgaard M, Kovanen V, Aagaard P, et al. Corticosteroid injections, eccentric decline squat training and heavy slow resistance training in patellar tendinopathy. *Scand J Med Sci Sport*. 2009;19(6):790-802. doi:10.1111/j.1600-0838.2009.00949.x.
29. Kongsgaard M, Qvortrup K, Larsen J, et al. Fibril Morphology and Tendon Mechanical Properties in Patellar Tendinopathy. *Am J Sports Med*. 2010;38(4):749-756.

doi:10.1177/0363546509350915.

30. Kongsgaard M, Reitelseder S, Pedersen TG, et al. Region specific patellar tendon hypertrophy in humans following resistance training. *Acta Physiol*. 2007;191(2):111-121. doi:10.1111/j.1748-1716.2007.01714.x.
31. Larsson MEH, Käll I, Nilsson-Helander K. Treatment of patellar tendinopathy--a systematic review of randomized controlled trials. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(8):1632-1646. doi:10.1007/s00167-011-1825-1.
32. Lian OB, Engebretsen L, Bahr R. Prevalence of jumper's knee among elite athletes from different sports: a cross-sectional study. *Am J Sports Med*. 2005;33(4):561-567. doi:10.1177/0363546504270454.
33. Magnusson SP, Kjaer M. The impact of loading, unloading, ageing and injury on the human tendon. *J Physiol*. 2018;0:1-16. doi:10.1113/JP275450.
34. Malliaras P, Barton CJ, Reeves ND, Langberg H. Achilles and patellar tendinopathy loading programmes: A systematic review comparing clinical outcomes and identifying potential mechanisms for effectiveness. *Sport Med*. 2013;43(4):267-286. doi:10.1007/s40279-013-0019-z.
35. Mendonça LDM, Ocarino JM, Bittencourt NFN, Fernandes LMO, Verhagen E, Fonseca ST. The Accuracy of the VISA-P Questionnaire, Single-Leg Decline Squat, and Tendon Pain History to Identify Patellar Tendon Abnormalities in Adult Athletes. *J Orthop Sports Phys Ther*. 2016;46(8):673-680. doi:10.2519/jospt.2016.6192.
36. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2012;10(1):28-55. doi:10.1016/j.ijsu.2011.10.001.
37. Pueo B, Jimenez-Olmedo JM, Lipińska P, Buśko K, Penichet-Tomas A. Concurrent validity and reliability of proprietary and open-source jump mat systems for the assessment of vertical jumps in sport sciences. *Acta Bioeng Biomech*. 2018;20(4):51-57. doi:10.5277/ABB-01132-2018-02.
38. Purdam CR, Cook JL, Hopper DM, et al. Discriminative ability of functional loading tests for adolescent jumper's knee. *Phys Ther Sport*. 2003;4(1):3-9. doi:10.1016/S1466-853X(02)00069-X.

39. Rees JD, Lichtwark GA, Wolman RL, Wilson AM. The mechanism for efficacy of eccentric loading in Achilles tendon injury; an in vivo study in humans. *Rheumatology*. 2008;47(10):1493-1497. doi:10.1093/rheumatology/ken262.

40. Saghaei M, Saghaei S. Implementation of an open-source customizable minimization program for allocation of patients to parallel groups in clinical trials. *J Biomed Sci Eng*. 2011;04(11):734-739. doi:10.4236/jbise.2011.411090.

41. Schoenfeld BJ, Grgic J, Ogborn D, Krieger JW. Strength and hypertrophy adaptations between low- vs. High-load resistance training: A systematic review and meta-analysis. *J Strength Cond Res*. 2017;31(12):3508-3523. doi:10.1519/JSC.0000000000002200.

42. Silbernagel KG, Brorsson A, Lundberg M. The majority of patients with Achilles tendinopathy recover fully when treated with exercise alone: a 5-year follow-up. *Am J Sports Med*. 2011;39(3):607-613. doi:10.1177/0363546510384789.

43. Silbernagel KG, Thomeé R, Eriksson BI, Karlsson J. Continued sports activity, using a pain-monitoring model, during rehabilitation in patients with achilles tendinopathy: A randomized controlled study. *Am J Sports Med*. 2007;35(6):897-906. doi:10.1177/0363546506298279.

44. Sprague AL, Smith AH, Knox P, Pohlig RT, Grävare Silbernagel K. Modifiable risk factors for patellar tendinopathy in athletes: a systematic review and meta-analysis. *Br J Sports Med*. 2018:bjsports-2017-099000. doi:10.1136/bjsports-2017-099000.

45. Visentini PJ, Khan KM, Cook JL, Kiss ZS, Harcourt PR, Wark JD. The VISA score: an index of severity of symptoms in patients with jumper's knee (patellar tendinosis). Victorian Institute of Sport Tendon Study Group. *J Sci Med Sport*. 1998;1(1):22-28.

46. Wang JH-C. Mechanobiology of tendon. *J Biomech*. 2006;39(9):1563-1582. doi:10.1016/j.jbiomech.2005.05.011.

47. Webb K, Hitchcock RW, Smeal RM, Li W, Gray SD, Tresco PA. Cyclic strain increases fibroblast proliferation, matrix accumulation, and elastic modulus of fibroblast-seeded polyurethane constructs. *J Biomech*. 2006;39(6):1136-1144. doi:10.1016/j.jbiomech.2004.08.026.

48. Young JL, Rhon DI, Cleland JA, Snodgrass SJ. The Influence of Exercise Dosing on Outcomes in Patients With Knee Disorders: A Systematic Review. *J Orthop Sport Phys Ther*.

2018;48(3):1-47. doi:10.2519/jospt.2018.7637.

49. Young MA, Cook JL, Purdam CR, Kiss ZS, Alfredson H. Eccentric decline squat protocol offers superior results at 12 months compared with traditional eccentric protocol for patellar tendinopathy in volleyball players. *Br J Sports Med*. 2005;39(2):102-105. doi:10.1136/bjsm.2003.010587.

50. Zwerver J, Bredeweg SW, Van Den Akker-Scheek I. Prevalence of jumper's knee among nonelite athletes from different sports: A cross-sectional survey. *Am J Sports Med*. 2011;39(9):1984-1988. doi:10.1177/0363546511413370.

SUPPLEMENT

Figure S1: Depiction of applied exercises. A: Leg press, performed bilaterally; B: Knee extension performed unilateral.

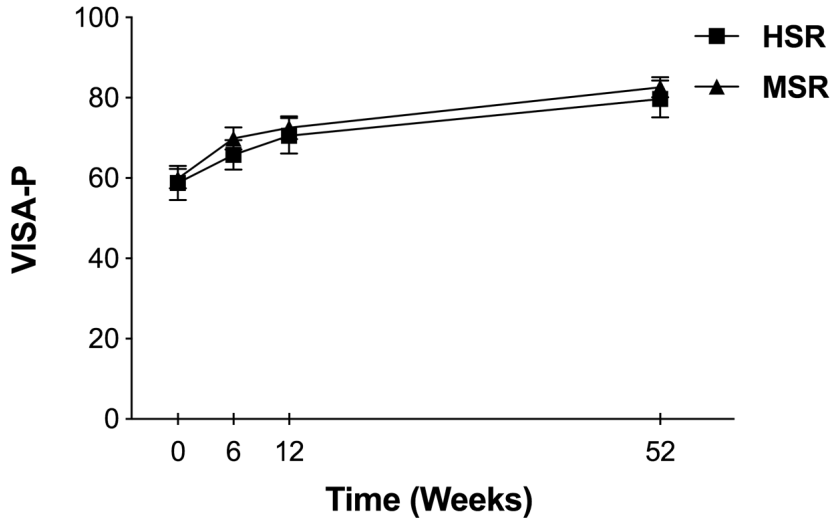

Figure S2: Intention to treat. VISA-P at baseline (0 weeks), mid the intervention (6 weeks), after the intervention (12 weeks) and at one-year follow-up (52 weeks) for the two intervention groups. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; VISA-P, Victorian Institute of Sports Assessment- Patella. Values are presented as mean±SEM.

TABLE S1: Compliance of prescribed interventions 0-12 weeks

	HSR (n=21)	MSR (n=21)	P- value
Session % of Aim	78.2 ± 4.3	85.8 ± 2.18	0.126
Supervision % of Aim	81.3 ± 4.5	85.7 ± 2.3	0.391
Load % of Aim			
Leg pres	97.6 ± 0.9	99.8 ± 0.2	0.019
Knee ext IL	93.6 ± 1.3	95.9 ± 0.9	0.157
Reps % of Aim			
Leg pres	97.9 ± 1.6	99.7 ± 0.3	0.292
Knee ext IL	97.7 ± 1.2	97.7 ± 0.6	0.973
Volume % of Aim			
Leg pres	97.6 ± 3.5	87.8 ± 1.5	0.159
Knee ext IL	77.8 ± 3.3	83.2 ± 2.1	0.177
Absolut volume % of Aim			
Leg pres	76.8 ± 4.2	84.8 ± 2.2	0.103
Knee ext IL	72.9 ± 4.3	80.3 ± 2.7	0.153
Absolut TUT % of Aim			
Leg pres	79.0 ± 3.9	85.3 ± 2.1	0.168
Knee ext IL	77.1 ± 3.9	83.4 ± 2.4	0.176

Intention to treat analysis. Values are expressed as % of Aim and presented as mean±SEM. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. Knee ext IL, knee extension exercise injured leg. Load, reps and volume demonstrating compliance during the training session. Absolut volume and TUT % of aim demonstrate the total compliance, including missing training session and adjusted for progression in prescribed load during the intervention period.

TABLE S2: Compliance of prescribed interventions 0-12 weeks

	HSR (n=16)	MSR (n=19)	P- value
Session % of Aim	86.8±1.7	88.1±1.7	0.570
Supervision % of Aim	87.0±2.9	86.8±2.4	0.971
Load % of Aim			
Leg pres	98.0±0.8	99.8±0.3	0.051
Knee ext IL	94.6±1.4	96.2±0.9	0.337
Reps % of Aim			
Leg pres	98.8±0.5	99.6±0.3	0.188
Knee ext IL	98.1±0.6	97.7±0.7	0.619
Volume % of Aim			
Leg pres	86.7±1.8	88.6±1.6	0.452
Knee ext IL	83.1±2.0	84.2±2.2	0.693
Absolut volume % of Aim			
Leg pres	84.8±1.9	87.2±1.7	0.368
Knee ext IL	81.3±2.2	82.8±2.3	0.635
Absolut TUT % of Aim			
Leg pres	86.3±1.6	87.4.±1.7	0.622
Knee ext IL	84.7±1.8	85.5±2.1	0.768

Per protocol analysis. Values are expressed as % of Aim and presented as mean±SEM. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. Knee ext IL, knee extension exercise injured leg. Load, reps and volume demonstrating compliance during the training session. Absolut volume and TUT % of aim demonstrate the total compliance, including missing training session and adjusted for progression I prescribed load during the intervention period.

TABLE S3: Weekly sport participation (hours). Pre injury level recall at pre examination and self-reported at week 0, 6,12 and 52.

Sport participation (hours/ week)	**HSR** (n=16)	**MSR** (n=19)
Pre Injury	8 ± 1 (1-14)	7 ± 1(1-14)
Week 0	5 ± 1 (1-10)	4 ± 1 (0-15)
Week 6	3 ± 1 (1-8)	4 ± 1 (0-20)
Week 12	4 ± 0 (0-10)	5 ± 1 (0-16)
Week 52	5 ± 1 (0-16)	5 ± 1 (0-17)
Δ Pre injury - week 0	-2 ± 1 (-3.8, -1.1)**	-2 ± 1 (-3.6, -1.1)**
Δ Pre injury - week 52	-2 ± 1 (-3.9, -0.1)*	-2 ± 1 (-3.8, -0.4)*
Δ 0-12 week	-1 ± 1 (-4.2, 1.2)	1 ± 1 (-1.9, 3.1)
Δ 12-52 week	2 ± 1 (-0.8, 4.5)	0 ± 1 (-2.8, 2.1)
Δ 0-52 week	0 ± 1 (-2.3, 3.1)	0 ± 1 (-2.2, 2.7)

Per protocol analysis. Pre injury and week 0, 6, 12 and 52 are reported as Mean±SEM (range). All Δvalues are expressed as Mean±SEM (95% CI). Analyzed with 2way ANOVA (p< 0.05) show week 0,6,12 and 52 no effect of time (p= 0.32), treatment (p= 0.82) or Interaction (p=0.20). * Significant different from Pre Injury, P <0.05. ** Significant different from Pre Injury, P <0.01

TABLE S4: Pain during activity measured on Numeric Rating Scale

	HSR (n=16)	**MSR** (n=19)	**P group**	**P time**	**P group x time**
Running (questionnaire)					
0 weeks	2.8 ± 0.4	4.1 ± 0.5	0.02	<0.0001	0.36
6 weeks	1.8 ± 0.3	3.2 ± 0.4			
12 weeks	1.3 ± 0.3	2.1 ± 0.3			
52 weeks	0.8 ± 0.2	1.3 ± 0.4			
Squat (questionnaire)					
0 weeks	3.3 ± 0.6	3.1 ± 0.5	0.32	<0.0001	0.75
6 weeks	1.8 ± 0.4	1.5 ± 0.2			
12 weeks	1.6 ± 0.4	1.1 ± 0.3			
52 weeks	1.3 ± 0.4	0.5 ± 0.2			
Pref sport (questionnaire)					
0 weeks	3.8 ± 0.5	4.7 ± 0.6	0.24	<0.0001	0.47
6 weeks	2.5± 0.4	3.5 ± 0.6			
12 weeks	2.0 ± 0.4	2.4 ± 0.5			
52 weeks	1.1 ± 0.3	1.2 ± 0.3			
Leg press (intervention)					
0-6 weeks	0.5 ± 0.1	0.5 ± 0.1	0.86	0.58	0.69
7-12 weeks	0.5 ± 0.1	0.5 ± 0.1			
Knee ext (intervention)					
0-6 weeks	0.7 ± 0.1	0.6 ± 0.1	0.69	0.65	0.52
7-12 weeks	0.6 ± 0.1	0.6 ± 0.1			
CMJ (examination)					
0 weeks	1.1 ± 0.3	1.3 ± 0.3	0.69	0.03	0.79
12 weeks	0.6 ± 0.3	0.7 ± 0.3			
SJ (examination)					
0 weeks	1.0 ± 0.3	0.8 ± 0.3	0.73	0.02	0.20
12 weeks	0.3 ± 0.1	0.6 ± 0.3			

Per protocol analysis. Values are pain score measured on a numeric rating scale [NRS] and expressed as mean±SEM. Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Questionnaire, Patient reported outcome measured; Pref sport, Preferred sport; Intervention, Self-reported NRS pain expressed as averaged pr. session during week 0-6 and week 7-12; Knee ext, knee extension for injured leg; CMJ, Mean pain for the 3 counter movement jump; SJ, mean pain for the 3 Squat jump.

TABLE S5: Clinical Results

	HSR (n=16)	**MSR** (n=19)
VISA-P, Point		
0 weeks	66.1 ± 3.9 (57.9, 74.4)	59.9 ± 2.7 (54.3, 65.5)
6 weeks	71.8 ± 2.4 (66.8, 76.8)	70.5 ± 2.9 (64.4, 76.6)
12 weeks	76.4 ± 3.5 (69.0, 83.9)	72.6 ± 3.1 (66.1, 79.0)
52 weeks	85.8 ± 3.0 (79.4, 92.3)	83.7 ± 2.6 (78.2, 89.2)
Δ 0-12 weeks	10.3 ± 3.2 (1.8, 18.8)**	12.7 ± 2.9 (4.9, 20.5)**
Δ 12-52 weeks	9.4 ± 3.2 (0.9, 17.9)*	11.1 ± 2.9 (3.3, 18.9)**
Δ 0-52 weeks	19.7 ± 3.2 (11.2, 28.2)***	23.8 ± 2.9 (16.0, 31.6)***
SLDS, NRS		
0 weeks	3.8 ± 0.5 (2.7, 4.8)	3.8 ± 0.4 (3.0, 4.5)
6 weeks	1.8 ± 0.3 (1.0, 2.5)	2.3 ± 0.3 (1.6, 3.0)
12 weeks	1.5 ± 0.4 (0.7, 2.3)	1.9 ± 0.4 (1.1, 2.6)
52 weeks	0.9 ± 0.3 (0.2, 1.6)	1.2 ± 0.4 (0.3, 2.0)
Δ 0-12 weeks	-2.2 ± 0.4 (-3.2, -1.2)***	-1.9 ± 0.4 (-3.0, -1.0)***
Δ 12-52 weeks	-0.7 ± 0.4 (-1.7, 0.3)	-0.7 ± 0.4 (-1.6, 0.4)
Δ 0-52 weeks	-2.9 ± 0.4 (-3.9, -1.9)***	-2.6 ± 0.4 (-3.6, -1.6)***

Per protocol analysis. Values are presented as mean±SEM (95% CI). Two-way analysis of variance was conducted with rehabilitation
regime (group) and time as main factors. Alpha level set at P <0.05. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; VISA-P, Victorian Institute of Sports Assessment- Patella; SLDS, Single-leg Decline Squat test. 0 weeks, baseline; 6 weeks, mid intervention; 12 weeks, post intervention; 52weeks, 1-year follow-up. Δ, change in time intervalVISA-P: P-values group (0.32), interaction (0.68) and time (<0.0001). SLDS: P-values group (0.45), interaction (0.86) and time (<0.0001)
* Significant different from Pre Injury, P <0.05
** Significant effect of time, P <0.01
***Significant effect of time, P<0.0001

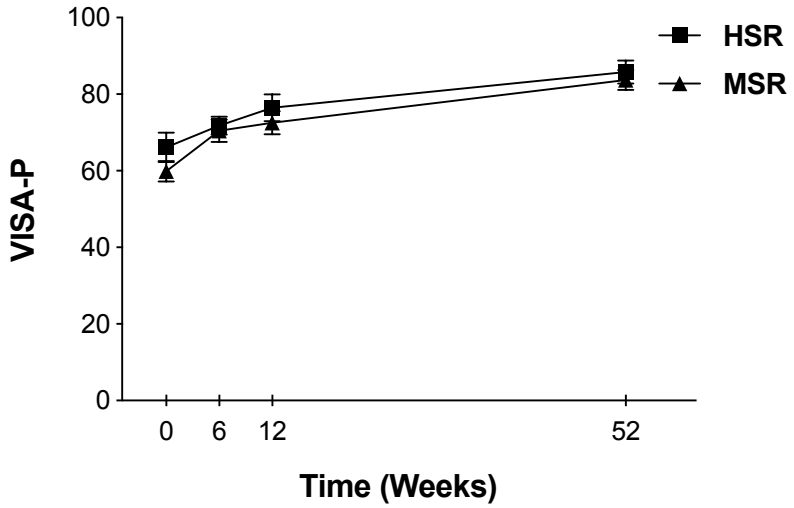

FIGURE S3: Per protocol analysis. VISA-P at baseline (0 weeks), mid the intervention (6weeks), after the intervention (12 weeks) and at one-year follow-up (52 weeks) for the two intervention groups. HSR, Heavy slow resistance group (n=16); MSR, Moderate slow resistance group(n=19); VISA-P, Victorian Institute of Sports Assessment- Patella. Values are presented as mean±SEM.

TABLE S6: Sonographic Results

	HSR (n=16)	**MSR** (n=19)
Doppler (mm^2)		
0 weeks	11.43 (24.47, 5.34)	10.54 (24.17, 4.60)
6 weeks	14.11 (27.87, 7.15)	8.29 (17.35, 3.96)
12 weeks	8.10 (16.89, 3.89)	10.98 (22.02, 5.47)
52 weeks	6.38 (13.76, 2.96)	6.03 (13.19, 2.76)
Δ % 0-12 weeks	-29 (-66, 47)	4 (-47, 103)
Δ % 12-52 weeks	-21 (-62, 63)	-45 (-72, 7)
Δ % 0-52 weeks	-44 (-73, 15)	-43 (-71, 11)
Thickness (mm)		
0 weeks	7.23 (8.41, 6.19)	6.84 (7.93, 5.90)
6 weeks	7.34 (8.46, 6.38)	7.26 (8.45, 6.24)
12 weeks	7.26 (8.20, 6.42)	7.38 (8.42, 6.47)
52 weeks	6.85 (8.08, 5.80)	6.86 (8.01, 5.87)
Δ % 0-12 weeks	-1 (-12, 16)	8 (-5, 23)
Δ % 12-52 weeks	-6 (-18, 9*)*	-7 (-18, 6)
Δ % 0-52 weeks	-5 (-18, 9)	0 (-12, 14)

Per protocol analysis. Values are presented as mean (95% CI). Δ %, relative change in time interval. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Doppler, power doppler (total number of colored pixel converted to area in mm^2); Thickness, anterior-posterior thickness of the tendon. The data was log-transformed and two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at $P < 0.05$. HSR.
Doppler: P-values group (0.84), interaction (0.16) and time (0.007)
Thickness: P-values group (0.90), interaction (0.79) and time (0.20)

TABLE S7: Structural and mechanical properties for Injured site

	HSR (n=16)	**MSR** (n=19)	**P group**	**P time**	**P group x time**
MVC (Nm)					
0 weeks	191 ± 13	197 ± 11	0.93	<0.0001	0.16
12 weeks	225 ± 13	216 ± 10			
CSA PROX (cm^2)					
0 weeks	1.49 ± 0.12	1.43 ± 0.08	0.56	0.29	0.46
12 weeks	1.53± 0.13	1.43 ± 0.09			
CSA MID (cm^2)					
0 weeks	1.13 ± 0.09	1.09 ± 0.08	0.39	0.17	0.26
12 weeks	1.14 ± 0.09	1.03 ± 0.06			
CSA DIST (cm^2)					
0 weeks	1.27 ± 0.12	1.08 ± 0.05	0.12	0.95	0.72
12 weeks	1.26 ± 0.10	1.10 ± 0.05			
CMJ Height (cm)					
0 weeks	32.03 ± 1.62	31.89 ± 1.38	0.78	0.10	0.25
12 weeks	33.06 ± 1.66	32.07 ± 1.32			
SJ Height (cm)					
0 weeks	28.02 ± 1.43	27.26 ± 1.26	0.91	0.33	0.29
12 weeks	26.20 ± 1.26	27.33 ± 1.12			

Per protocol analysis. Values are presented as mean±SEM. Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. MVC, peak knee extension moment; CSA, Patella tendon cross-sectional area; CMJ, counter movement jump, SJ, Squat jump.

PAPER II

Mechanical properties and UTE T2 relaxation time in Patellar tendinopathy: The effect of load magnitude in exercise-based treatment*

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Submitted to Scand J Sci Sports (under review).

Mechanical properties and UTE T2* relaxation time in Patellar tendinopathy: The effect of load magnitude in exercise-based treatment

ABSTRACT

Loading intervention is currently the preferred management of tendinopathy, but to what extent different loading regimes influence the mechanical response in tendons is scarcely investigated. Therefore, the purposes of the investigation were to examine the effect of exercise interventions with either high or low load magnitude applied to the tendinopathic patellar tendon and the influence on its mechanical, material and morphological properties. Forty-four men with chronic patellar tendinopathy were randomized to 12 weeks of exercising with either; 55% of 1RM throughout the period (MSR group) or 90% of 1RM (HSR group), and with equal total exercise volume in both groups. The outcomes were assessed at baseline and after 12 weeks of intervention. MRI with ultra-short echo times (UTE) and T2*-mapping was applied to explore if T2* relaxation time could be used as a noninvasive marker for internal material alteration and early change thereof in response to intervention. There was no effect of HSR or MSR on the mechanical (stiffness), material (T2* relaxation time) or morphological (CSA) properties, but both regimes resulted in significant strength gain. Further, the T2*-mapping MRI UTE method is a promising imaging technique for evaluating internal material alterations in chronic patellar tendinopathy. In conclusion, there were no statistically superior effect of exercising with high (90%) compared to low (55%) load magnitude on the mechanical, material or morphological properties.

KEYWORDS

Patellar tendon, tendinopathy, loading based treatment, load magnitude, mechanical properties, UTE T2*-mapping

INTRODUCTION

The patellar tendon plays an essential role in transmitting contractile force from the quadriceps muscle to produce knee extension[1]. However, repeated loading can cause patellar tendinopathy, which is a common overuse injury characterized by pain during activity, localized tenderness upon palpation and swelling of the tendon[2]. Patellar tendinopathy often results in long lasting decreased activity level, and impaired sports and work performance[3,4], and therefore restoration of normal structural and mechanical properties is essential.

Tendons consist primarily of fibroblasts and longitudinally aligned collagen type I embedded in an extracellular matrix consisting of water, proteoglycans and glycosaminoglycans[5,6]. Tendinopathic tendons typically display disorganization of the extracellular matrix, and the collagen fibrils along with hypervascularization and increased water content. Concomitantly, mechanical properties have been reported to decrease[7,8] or remain unchanged[9–11] in tendinopathic tendons. Therefore, maintaining or restoring the mechanical properties may be relevant in management of patellar tendinopathy.

Loading interventions are the preferred treatment for tendinopathy[12,13], but to what extend loading configurations influences the mechanical response in tendons is scarcely investigated[14]. In healthy human tendons, heavy load exercises yield an increase in stiffness[15]. Furthermore, it has been shown that with equal exercise volume, exercise with greater loads (and thus strains) yield increased tendon stiffness and increased cross sectional area compared to lower load, in healthy human Achilles tendons[16]. However, if these effects of load magnitude yield similar responses in tendinopathic tendons remains unknown.

Characterizing microstructural composition and mechanical behavior of the tendinopathic patellar tendon can improve our understanding of the pathology and enable us to better monitor progress. However, studies investigating both material, morphological and mechanical properties of patellar tendinopathic tendons is limited[17]. Magnetic resonance imaging (MRI) and ultrasound (US) are two commonly used imaging modalities to investigate structural changes in tendons although neither of them provide detailed material or structural information[18,19]. Tendon biopsies can provide useful structural and biochemical information, however, its invasive nature precludes repeated measurements[20]. Alternatively, non-invasive MRI ultra-short time to echo (UTE) imaging with T2* mapping of tendon is a reproducible method[21],

which has been proposed to correlate with collagen structure and water content in tendons[22,23]. MRI UTE-T2* mapping might therefore be a suitable non-invasive methodology to evaluate subtle changes in tendon over time.

The aim of this study, therefore, was to examine the effect of 12 weeks exercise intervention, with high (90% of 1RM) or low (55% of 1RM) load magnitude at equal volume, applied to the tendinopathic patellar tendon and the influence on its mechanical, material and morphological properties. We hypothesized a superior effect of the high load magnitude. A secondary aim was, exploratory, to investigate if T2* relaxation time obtained with MRI UTE could be used as a noninvasive marker for internal alteration on a material level to quantify early changes in response to intervention.

2. MATERIALS AND METHODS

2.1 study design

This study was a part of a prospective randomized controlled, single-blinded, superiority trial conducted in Copenhagen, Denmark. Ethical approval was obtained from the regional Ethics Committees for medical research (No. H-15017806) and all participants provided written informed consent. The original randomized controlled trial (paper I) was pre-registered on ClinicalTrials.gov (NCT03096067).

2.2. Participants

The study included 44 male athletes, age 20-45 years, BMI 18.5-30, patellar tendon pain duration for more than 3 month, and clinically diagnosed with patellar tendinopathy by a sports medicine physiotherapist or physician based on defined clinical findings and confirmation by ultrasonography. Exclusion criteria were corticosteroid injection for patella tendinopathy, previous knee surgery, arthritis, diabetes, any confounding diagnoses to the knee joint, smoking, patellar tendinopathy for more than 12 months. For participants with bilateral tendinopathy the tendon with most pronounced symptoms were selected for analysis of injured site. After a 2-week “wash-out” period from any previous treatment and resistance training and afterwards baseline assessment, the participants was randomly allocated to one of two intervention groups: Heavy

slow resistance (HSR) or Moderate slow resistance (MSR). For more details, please see the original study (Paper I).

2.3 Intervention

The HSR program was started at 55% of 1RM and progressed over the first 6 weeks toward 90% of 1RM which was then maintained for the rest of the intervention (Intervention protocols are shown in supplement). The MSR program likewise started at 55% of 1RM but maintained this throughout the intervention period. The total weekly exercise volume was matched between groups and both groups performed three weekly sessions, of which one was supervised. Each session consisted of one bilateral leg press exercise and one unilateral knee extension exercise with each repetition lasting six seconds (three seconds for the concentric and eccentric phase, respectively). Every second week a sub-maximal test was performed to estimate 1RM and adjust training load accordingly. Pain during exercises (numeric rating scale (NRS) <5) was accepted, however, pain and discomfort were not allowed to increase following cessation of training. Participants were allowed to perform sporting activities throughout the 12-week intervention period if only causing light discomfort (NRS <3).

All participants completed a training diary during the intervention using a smartphone application (Injurymap Science ApS, Copenhagen, Denmark). The training records included training intensity (repetitions, sets and load), sessions, pain during training (NRS) and information about deviations from the planed intervention protocol. For further details, please see the original study (paper I).

2.4 Follow-up

Mechanical properties and ultra-short echo time (UTE) MRI outcomes were obtained at baseline and post intervention (12 weeks), with measurements performed 3-4 days before and after the intervention period.

Outcome measurements in the original Randomized clinical trial (paper I) were; Victorian Institute of Sport Assessment-Patella questionnaire (VISA-P) (Function and symptoms), NRS (tendon pain during activity) and tendon vascularization and swelling (ultra-sound) assessed at baseline, 6 and 12 weeks of intervention and at 52 weeks follow-up. Isometric strength, Single-leg decline squat tests, and Jump test (tendon function) and ultrasound and conventional MRI

(tendon structure) were assessed before and after the intervention period. The previously published values for tendon cross sectional area (CSA) from conventional MRI at baseline and Post intervention (12 weeks) were used in the present study for calculation of mechanical properties.

The examination order was identical for all follow-up evaluations, always starting with MRI at day one, and day two starting with ultrasound examination followed by a 5-minute warm-up on a bicycle-ergometer prior to further examinations. The participants were instructed to abstain from physical activity 24 h before examination.

2.5 Mechanical Testing

A previously reported and validated method to measure patellar tendon elongation during isometric knee extension with ultrasound recordings was used[24,25]. In brief, participants were seated in a custom-made rigid chair with both knees and hips flexed to an angle of 90° (Experimental set up are shown in supplement). Ultrasound recordings were performed on a HI Vision Hitachi Ascendus ultrasound machine (Hitachi Medical systems, Japan) with a long linear transducer (EUP-L53L, Hitachi Medical systems, Japan), the depth fixed at 4.5 cm, a dynamic range of 70, and gain of 20. A leg cuff, connected to a force transducer (8-channel, TeleMyo 2400T G2, Telemetry System; Noraxon Inc) was placed on the leg just above the malleolus and trigged by the investigator to obtain force recordings synchronized with ultrasound recordings. The US recordings was recorded directly on the machine to gain the best quality. The participants performed ramped isometric knee extension over 8 seconds up to a maximum contraction on the injured leg. Four to five ramped contractions were performed with a 1-2 minute rest between the measurements. To determine tendon force, femur and tibia length were measured for estimation of the tendon moment arm and the knee extensor moment[26]. For participants with unilateral tendinopathy the measurement was also performed on the uninjured leg.

Patellar tendon deformation was defined as the change in distance between the patellar apex and the tibia. This distance was determined using a custom Matlab scrip (Matlab R2016b, The MathWorks Inc, USA) based on a cross-correlation algorithm to track the tendon insertions on patellar and tibia[25]. Around 10 tracking nodes were placed on either apex patellar or tuberositas tibia and the tracking was performed 2-3 times at each location. Tendon deformation was then correlated with force measurements using a custom-made excel template to generate a

force-deformation curve, which was fitted to a second-order polynomial. All curves were manually evaluated and curves with noise or lacking reproducibility between repeated trails were excluded. Patellar tendon stress was calculated by dividing tendon force with the average (proximal, mid, distal) CSA full tendon determined from the conventional MRI. Patellar tendon strain was calculated as the change in length relative to the initial patellar tendon length determined from 3D MRI UTE. It was assumed that tendon length does not change over time and therefore the same length (average of baseline and post) was used at both baseline and post intervention. Stiffness was calculated from the fit parameters at the peak point for each fitted curve. Max force, deformation, stiffness, strain and stress were extracted from each fitted curve and a mean of all included curves for each leg and time point were used for further analysis. To account for improved strength after the intervention period all curves were cut to the lowest common force across all participants and timepoints in the analysis of influence of load magnitude. In analysis of differences between injured and contralateral asymptomatic leg all curves were cut to the lowest common force between leg and timepoint within each participant. All curve selections and data analyses were performed blinded.

2.6 Magnetic resonance imaging

All MRI scans were performed in a Siemens Verio® (Siemens, Erlangen, Germany) 3 Tesla scanner. The subjects were scanned in a supine position using a dedicated 15-channel send/receive knee coil and only injured leg was examined.

2.6.1 UTE MRI T2 mapping*

The 3D UTE-T2* MRI sequences were performed using the following protocol; A slab of 160 slices was scanned 4 times with a varying Echo Time (TE): 0.07ms, 0.57ms, 1.07ms and 1.57ms (Field of View (FOV) 160 x 160 mm, matrix resolution 1.45 x 1.45 x 1.00 mm, Repetition Time (TR) 11 ms, Flip Angle (FA) 12 degrees, scan time 3m 14s. A detailed description of the experimental setup and measurement has been previously described[21]. In brief, the analysis was carried out in three steps. First, the DICOM files from the UTE recordings were automatically loaded into a custom-made software (X-Rai IVS, Copenhagen, Denmark). In the program TE was plotted against the signal intensity on a voxel-by-voxel basis for the whole volume and, based on a mono-exponential fitting

procedure T2* maps containing T2* values for each voxel and goodness-of-fit maps containing r-values for each voxel were reconstructed. Secondly, 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 patellar tendon volume was segmented by manually outlining the tendon in the axial plane of every 4th slice. The starting slice was defined as the first proximal slice displaying the patellar tendon immediately caudal to the patellar bone and the final slice was defined as the first slice adjacent to the most cranial tendon insertion on the tibial tuberosity defined where the corpus Hoffa's fat pad deep to the tendon was no longer visible. After manual segmentation the tendon volume was calculated using the interpolate labels tool in ITK-SNAP. Subsequently, the segmented volume from ITK-SNAP was imported to FIJI/ImageJ (version 1.52, National Institutes of Health, 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* (ms) and volume (mm^3), only including voxels in the segmentation with r>0.8, and volume (mm^3) for all voxels were determined in the total tendon volume as well as the proximal and distal half of the tendon.

2.6.2 Tendon dimension

Conventional MRI was used to assess cross sectional area (CSA) of the patellar tendon on the injured site at baseline and 12 weeks as previously described (Paper I). Briefly, the MRI scan was analyzed in the open source software Horos v.3.3.5for MAC OS (https://horosproject.org) and the patellar tendon CSA was measured by manually outlining the tendon in the axial plane just distal to the patella, mid tendon level and just proximal to the tibia insertion as previous described.[27] The procedure was performed 3 times, and a mean for each location was calculated. Average CSA full tendon was calculated as a mean of the three locations. Results of this analysis has previously been published (Paper I).

Initial patellar tendon length was determined from 3D MRI UTE baseline scan as the number of slices, from the previously described first proximal slide to the distal slice multiplied by the slice thickness (0.73mm).

2.7 Statistical analysis

Statistical analyzes was carried out in GraphPad Prism (version 8.2.1 for macOS, GraphPad Prism Software, California). Results are reported as mean ± standard error unless otherwise noted. Demographic baseline data and participant compliance were analyzed by use of unpaired Students t-test. Other outcome parameters were analyzed by two-way analyses of variance (time x intervention) with time as a repeated-measure and using Bonferroni post hoc analyzes when appropriate. In individuals with unilateral involvement, difference between mechanical properties of the injured and contralateral asymptomatic tendons was analyzed by two-way analyses of variance (time x side) with time as repeated measures. Correlation of material properties (T2*relaxation time) and modulus and mechanical properties (stiffness) at baseline were analyzed with Persons correlation. All analyzes were performed blinded as intention to treat with the last observation carried forward. Significance level for all tests was set to $P< 0.05$. Per-protocol analyzes (participants that fulfilled at least 75% of the prescribed training sessions) were also carried out but did not change any of the conclusions and are therefore not shown.

3. RESULTS

3.1 Participants and compliance

There were no significant differences in baseline characteristics between the two groups (Table 1). In addition to the two participants excluded from analysis in the original RCT study (Paper I) one participant was excluded from the mechanical analysis due to non-resolvable technical problems with the recorded baseline data. The mean total training session compliance (in supplement) was not significantly different ($P=0.12$) between the two groups with 78±4% for HSR and 86±2% for the MSR group. The supervised session compliance was 81±4% for the HSR and 86±2% for the MSR, likewise with no significant between group difference ($P=0.39$). The participants performed the leg press exercise with a mean above 76% of the calculated total absolute volume prescribed and without any between group difference ($P=0.10$). Likewise, the knee extension exercise was performed with a mean compliance above 73% for the injured leg and above 72% for the contralateral asymptomatic leg with no significant difference between groups for between injured ($P=0.15$) nor ($P=0.12$) contralateral asymptomatic leg.

TABLE 1: Baselines characteristics

Variable	**HSR** (n=21)	**MSR** (n=21)
Age (y)	28.8 ± 5.1 (20-38)	32.3 ± 4.9 (23-41)
Height (cm)	185.8 ± 7.1	180.7 ± 7.2
Weight (kg)	86.7 ± 9.3	82.2 ± 9.2
BMI (kg/m^2)	25.1 ± 2.4	25.2 ± 2.6
Symptom duration (month)	6.9 ± 2.4 (3-12)	7.3 ± 2.9 (3-12)
Weekly sport participation (hours)	9.0 ± 4.8 (1-21)	7.0 ± 3.8 (1-14)
Pain during activity (NRS)	4.7 ± 2.2 (1-8)	5.2 ± 2.0 (2-9)
Unilateral/bilateral (n)	14:7	13:8

Values are expressed as mean ± SD (range) unless otherwise noted. HSR, heavy slow resistance group; MSR, moderate slow resistance group.

3.2 Morphological properties

Patella tendon length did not differ (P=0.21) between the HSR (42.0±1.07 mm) and MSR (44.05±1.22 mm) group. Similarly, there was no difference in average CSA between groups (P=0.250) at baseline (HSR: 1.30±0.08 cm^2, MSR:1.19±0.06 cm^2) or 12 weeks (HSR: 1.30±0.08 cm^2, MSR:1.19±0.05 cm^2) and there was no effect of time (P=0.07) and no interaction (P=0.77).

3.3 Mechanical properties

Change in maximal peak force after intervention was 18% in the HSR group compared to the 11% in the MSR group, however there was no significant group effect (P=0.21). Likewise, there was no interaction (P=0.32), but a significant effect of time (P <0.0001) (Table 2). Force-elongation and stress-strain association remain unchanged after the intervention (Figure 1). Additionally, no group or time effect or interaction were detected for tendon deformation, stiffness, strain or stress when patellar tendon mechanical properties were analyzed at the highest common force between each participant's and timepoint (Table 2).

For participants with unilateral symptoms (HSR+MSR together; n=26), there was no difference in stiffness between legs (P= 0.08) at baseline (Injured: 2833±107 N/mm, contralateral asymptomatic 2494±102 N/mm) at baseline or 12 weeks (Injured: 2782±121 N/mm, contralateral asymptomatic 2604±121 N/mm) and there was no effect of time (P= 0.65) and no interaction (P=0.22).

TABLE 2: Patellar tendon mechanical properties injured leg

	HSR (n=21)		**MSR** (n=20)		P_{group}	P_{time}	$P_{g\,x\,t}$
	Pre	Post	Pre	Post			
Peak $Force_{Max}$, N	4589 ± 285	5590 ± 335	5303 ± 305	5968 ± 379	0.21	<0.0001	0.32
Peak $Force_{CF}$, N	2482 ± 6.7	2484 ± 4.3	2499 ± 6.5	2495 ± 3.6	0.02	0.87	0.54
Peak $Deformation_{CF}$, mm	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	0.99	0.35	0.81
Peak $Stiffness_{CF}$, N/mm	2189 ± 89	2162 ± 98	2198 ± 100	2111 ± 88	0.85	0.43	0.68
Peak $Strain_{CF}$, %	5.2 ± 0.2	5.4 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	0.27	0.28	0.83
Peak $Stress_{CF}$, MPa	20 ± 0.9	20 ± 0.9	22 ± 0.8	22 ± 0.8	0.22	0.97	0.86
Peak $Modulud_{CF}$, MPa	732 ± 37	721 ± 39	828 ± 45	804 ± 45	0.10	0.52	0.79

Values are presented as mean±SEM. Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. MAX, maximum force between participants and timepoints; CF, common force between participants and timepoint; HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Pre, baseline 0 weeks; Post, after the treatment intervention (12 weeks).

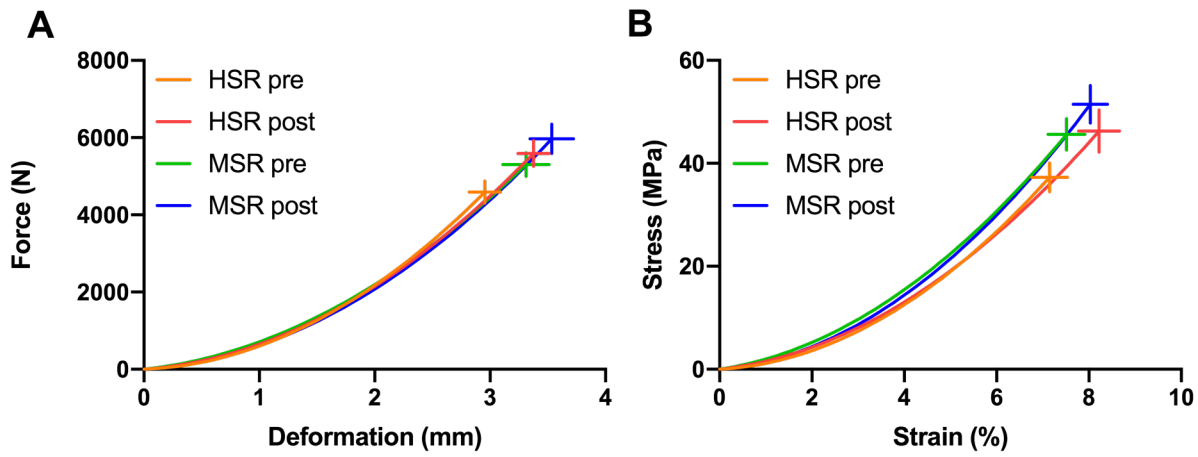

Figure 1: Pre and post force-elongation (A) and stress-strain (B) curves for maximum force for the two loading regimes. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Pre, baseline 0 weeks; Post, after the treatment intervention (12 weeks). Peak points are displayed as mean±SEM.

3.4 Material properties (UTE T2*)

The mean T2* relaxation time and tendon volume in the proximal, distal and total patellar tendon part for HSR and MSR, respectively, are shown in Table 3 and Figure 2. There was no significant effect of time or group an no interaction for T2* relaxation time or tendon volume of proximal, distal or total patellar tendon. On average around 20% of the voxels in the volume segmented were excluded due to a poor fit (Table 3). There was no significant group effect on the fraction of excluded voxels in the proximal, distal nor total tendon part, however, there was a significant time

effect for the distal (P= 0.02) and total (P=0.01) tendon part and an interaction was detected (P=0.04) for the proximal tendon part.

Table 3: T2* and volumes from UTE

Tendon part	HSR (n=21)		MSR (n=21)		P_{group}	P_{time}	$P_{g \times t}$
	Pre	Post	Pre	Post			
Proximal							
T2*(ms)	1.76 ± 0.05	1.67 ± 0.06	1.59 ± 0.08	1.67 ± 0.01	0.35	0.88	0.07
Volume (mm^3)	4025 ± 338	3873 ± 297	3726 ± 268	3623 ± 276	0.51	0.10	0.75
Volume $_{Exclud}$ (%)	22 ± 3	21 ± 3	15 ± 3	19 ± 3	0.33	0.08	0.04
Distal							
T2* (ms)	1.73 ± 0.05	1.70 ± 0.06	1.61 ± 0.05	1.71 ± 0.05	0.45	0.29	0.08
Volume (mm^3)	3296 ± 299	3261 ± 237	2927 ± 198	2958 ± 195	0.30	0.98	0.71
Volume $_{Exclud}$ (%)	19 ± 3	22 ± 3	15 ± 3	21 ± 3	0.44	0.02	0.23
Total							
T2*(ms)	1.75 ± 0.04	1.68 ± 0.06	1.60 ± 0.06	1.69 ± 0.07	0.29	0.77	0.07
Volume (mm^3)	7321 ± 621	7133 ± 513	6653 ± 449	6581 ± 445	0.39	0.40	0.71
Volume $_{Exclud}$(%)	21 ± 3	22 ± 3	15 ± 3	21 ± 3	0.36	0.01	0.06

Values are presented as mean±SEM. T2* relaxation time (ms) from monoexponentially fitting of UTE Images of the injured Patellar tendon, presented for Proximal, distal and total tendon. Volume; volume of segmentation; $Volume_{Exclud}$; Fraction of voxel from the segmentation with a fit value r < 0.08 presented as % of volume of segmentation. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Pre, baseline 0 weeks; Post, after the treatment intervention (12 weeks).

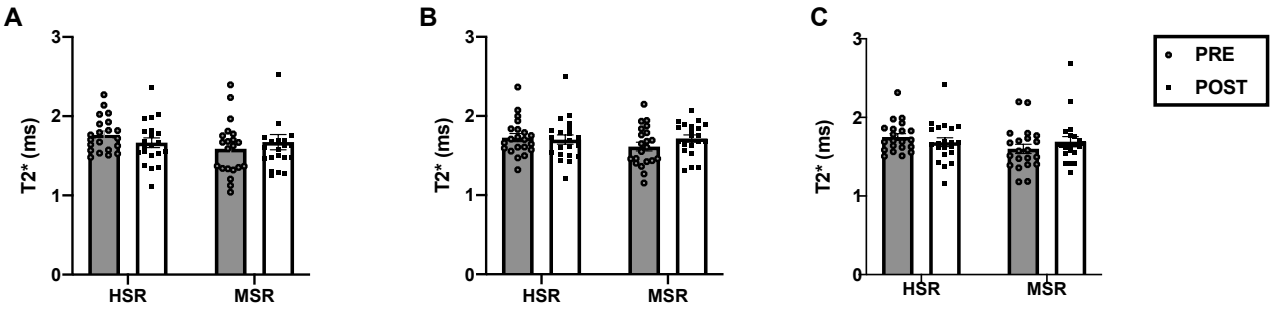

Figure 2: Values are presented as mean±SEM. T2* values from monoexponentially fitting of UTE Images of the injured Patellar tendon, presented for Proximal (A), distal (B) and total (C) tendon. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Pre, baseline 0 weeks; Post, after the treatment intervention (12 weeks).

3.5 Correlation between T2* and stiffness and modulus

There was a significant negative correlation between the T2* relaxation time material properties and modulus (r=-0.53, 95%CI [-0.72, -0.27]; P=0.0004) but not with stiffness(r=0.05, 95%CI [-0.26, 0.35]; P=0.75), Figure 3.

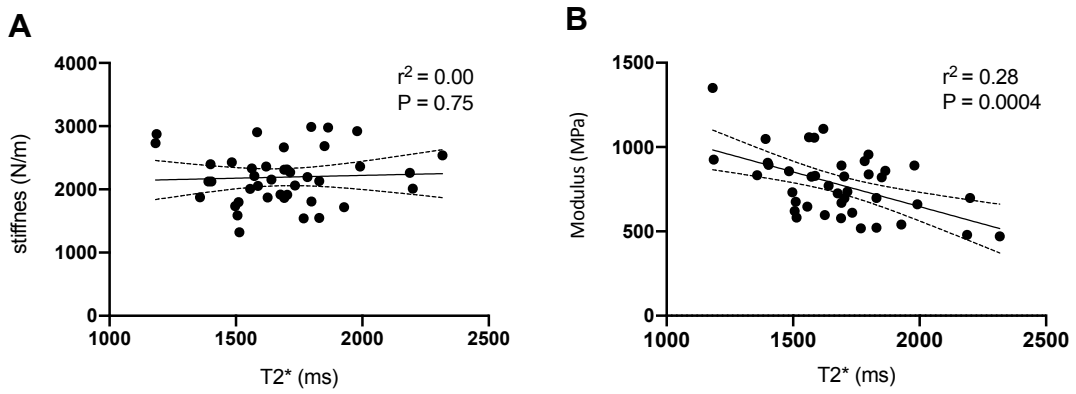

Figure 3: Scatter plots between T2* and Stiffness (A) and Modulus (B) at baseline. All data presented are merged values for HSR and MSR group (n=41)

4 DISCUSSION

The current study investigated the effect of a loading-based treatment on the mechanical, material and morphological properties of tendinopathic patellar tendons using 90% of 1RM (HSR) or 55% of 1RM (MSR). Both HSR and MSR resulted in significant strength gains. However, contrary to our hypothesis, there was no effect of 12 weeks of either HSR or MSR on the mechanical (stiffness), material (T2* relaxation time) or morphological (CSA) properties. For those participants with unilateral symptoms, stiffness did not differ in the tendinopathic tendons compared to contralateral asymptomatic tendons at baseline or at 12 weeks.

An increase in patellar tendon mechanical stiffness has been shown to occur in nearly all training studies of healthy persons, albeit with sizable variation in changes[17]. Yet, few investigations have explored this effects in tendinopathic tendons[14]. In the present study, there was no change in tendon mechanical stiffness for HSR or MSR despite training compliance of >78%, which yielded an increase in max strength (HSR:18%; MSR; 11%). In contrast, prior studies have shown a decrease in stiffness of the patellar tendon in response to exercise in tendinopathic tendons [9,11,28]. It is difficult to reconcile these differences. However, it is interesting that even

short periods of unloading leads to decrease in stiffness[29] and collagen synthesis rate[30]. Therefore, albeit speculative, a reduction in loading relative to the pre-injury level caused by tendinopathy related pain might have lead to a decline in the stiffness observed in previous studies. In the present study, the weekly sports participation during the 12-weeks intervention period was unchanged for both groups (data presented in the original paper (Paper I), which might have contributed to the unaltered mechanical properties. Furthermore, the results of unchanged mechanical properties, may simply reflect relatively low turnover tendon in general and that an adaptive mechanical response may require more than 12 weeks.

Previous work has shown that tendon adaptation is dependent on the magnitude of load in healthy persons [15,16]. Therefore, it was surprising that the data on mechanical properties was similar in response to MSR and HSR in the present study. The lack of difference underscores that injured tissue does not respond similarly to healthy tissue when loaded. Tendinopathic tissue typically displays disorganized extracellular matrix, including increased glycosaminoglycan content along with increased water content, hypervascularization and cellular content [31]. Collagen fibrils are the tensile load bearing structures [32,33], and appear to be continuous throughout the length of the patellar tendon [34]. However, the lack of change in mechanical properties over time indirectly suggest that there is not an accretion of collagen to the tensile load bearing fibrils, despite that the tendon was loaded up to 90% of the maximum quadriceps force.

Tendinopathy is associated with an increase in water content along with hypervascularization, which results in an increased CSA[31]. Although we did not obtain MRI bilaterally in the present study, our grey-scale ultrasonography data showed increased A-P diameter by 30% in the tendinopathic tendon compared to the contralateral asymptomatic tendons (unpublished data) indicating increased water content. In the present study the T2* values, which reflect the amount of unbound water in the tendon[35] ranged between 1.67-1.76 ms, which is consistent with the existing albeit limited literature on patellar tendon[36,37]. We have measured T2* values of 1.02 ms in healthy patellar tendons (unpublished data), indirectly indicating that the present T2* values represent pathology. Moreover, the CSA did not change significantly in response to different load magnitude (55% and 90% of 1RM) over the 12 week intervention period. This lack of change in CSA seem to be corroborated by the T2* values, which did also not change over the course of the 12 weeks, indicating an higher but unaltered water

content in tendinopathic tendon. The stiffness, which does not account for the size of the tendon and likely reflects the tensile bearing component, did not correlate with the T2* values (Figure 3A). In contrast, the modulus of the tendon, which accounts for the dimensions, including the CSA and water content, correlated with the T2* values (Figure3B), indicating a coupling between water content and material properties. The potential of T2* values serving as a biomarker for biochemical alteration has been investigated in vitro[38], but to the best of our knowledge this study is the first to show this on human in vivo tendons.

In line with previous studies[9,10] an unaffected tendon stiffness in the tendinopathic patellar tendons compared to contralateral asymptomatic tendons were shown in the present study among participants with unilateral symptoms. This further underscores that the changes in the tendinopathic tendon are related to increased water content more than immediate alteration in the tensile bearing components. In contrast, some studies[7,8] have shown decreased mechanical stiffness in tendinopathic tendons compared to healthy controls. Reduction in loading and pain or a combination might have led to the between-studies variation in stiffness. Activity related pain is characteristic for tendinopathy[2] and might have influenced the participants peak force in some studies. Lower peak force may preclude calculation of material properties on the linear part of the force-elongation curve resulting in a decreased stiffness compared to pain free tendons. Thus, the decreased stiffness detected in some studies might be confounded by pain. Furthermore, due to the progressive onset of tendinopathy, it cannot be ruled out to what extent the stiffness measured in tendinopathic tendon is the cause nor the result of decreased activity level (loading). However, in the present study there was no significant difference in maximum peak force between injured and asymptomatic contralateral tendons indicating that neither pain nor unloading caused by tendinopathy have influenced the peak force and therefore the calculated stiffness.

It should be noted that the T2* values in the present study were based only on voxels in the segmentation with a high quality of fit[21]. The tendinopathic tissue area with the most severe alterations displayed less dense tissue and an inherently higher T2* with poor fitting quality. Therefore, the T2* values in the present study do not represent the most severe lesion in the tendinopathic tendons and is therefore likely to underestimate the magnitude of tendon alterations. Metabolic turnover in the tendons takes place very slowly and alteration in the most

severe proximal tendon part may be slow or even unchangeable[39]. Clearly, the present study only examined short term effects (12 weeks) and therefore it cannot be excluded that T2* relaxation time response to load intensity may be altered on a longer time scale.

There are some inherent limitations to this study that need to be considered. First, in vivo testing of tendon mechanical properties is challenging. However, the method used is previously validated, and furthermore, we sought to optimize the method and eliminate issues based on recommendations from a recent critical evaluation of the method[40]. Additionally, it could be argued that using lowest between-participants common force might lead to material properties not being calculated on the linear part of the stress-strain curve for some participants. However, performing the analysis with lowest common force between timepoints within each participant (in supplement) did not change any of the conclusions presented in this paper. Further, we did not detect a response in tendon stiffness due to any of the loading regimes, but without a non-exercise control group we cannot determine if this is due to training or tendinopathy alone. Further, a quantification of the morphological and material alterations with MRI on the uninjured tendon was not possible in the present study. Finally, the most severe part of the tendinopathic tendon is not included in the calculated T2* values as result due to poor fitting in these areas. To describe these particular areas, sequences with longer TE are required. However, it was not feasible to visualize one areas with both long and short TE in the same sequences.

In conclusion, the current study demonstrates that there is no statistically superior effect of exercising with 90% compared to 55% of 1RM on the mechanical (stiffness), material (T2* relaxation time) or morphological (CSA) properties. Additionally, for participants with unilateral symptoms, stiffness did not differ in the tendinopathic tendons compared to contralateral asymptomatic tendons. Finally, the T2* relaxation time obtained with MRI UTE seems to be an applicable method for detecting early alteration in response to intervention, however longer intervention and values from comparable control tendons are needed to further explore the utility of the method used as a noninvasive biomarker of material alteration in tendons.

5. PERSPECTIVES

There is insufficient evidence in the literature for the influence of tendinopathy on the mechanical, material and morphological properties of patellar tendon[14]. However, maintaining or restoring the mechanical properties may be relevant in the management of patellar tendinopathy to regain optimal force transduction from muscle to bone and secure optimal function.

A previous study[11] focusing on the mechanistic response of patellar tendinopathy to different treatment modalities showed positive effects on mechanical properties with loading based treatment. Even though especially the HSR regime has been adapted in clinical practice for treatment of tendinopathy, the optimal magnitude of loading is still unknown. The present study has compared high load (90% of 1RM) to low load (55% of 1RM) and demonstrated that there is no superior effect of high load in relation to mechanical, structural or morphological tendon properties when maintain the total exercise volume equal. We believe this information is clinically important because the finding indicates that the exercise regime that best fits the patient's preferences can be freely chosen. Furthermore, the applicability of UTE T2* mapping as an early marker for non-invasive quantification of alterations in tendon tissue has been addressed. However, this method as well as questions regarding the optimal frequency of loading and whether the findings persist beyond the 12 weeks intervention, require further research.

6. REFERENCES

1. Finni T, Komi P V, Lepola V. In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. Eur J Appl Physiol 2000;83:416–26.
2. Khan K, Cook J. The painful nonruptured tendon: clinical aspects. Clin Sports Med 2003;22:711–25.
3. De Vries AJ, Koolhaas W, Zwerver J, Diercks RL, Nieuwenhuis K, Van Der Worp H, Brouwer S, Van Den Akker-Scheek I. The impact of patellar tendinopathy on sports and work performance in active athletes. Res Sports Med 2017;25:253–265.
4. Kettunen JA, Kvist M, Alanen E, Kujala UM. Long-term prognosis for jumper's knee in male athletes. A prospective follow-up study. Am J Sports Med 2002;30:689–92.
5. Magnusson SP, Kjaer M. The impact of loading, unloading, ageing and injury on the human tendon. J Physiol 2018;0:1–16.
6. Riley G. The pathogenesis of tendinopathy. A molecular perspective. Rheumatology 2004;43:131–142.
7. Helland C, Bojsen-Møller J, Raastad T, Seynnes OR, Moltubakk MM, Jakobsen V, Visnes H, Bahr R. Mechanical properties of the patellar tendon in elite volleyball players with and without patellar tendinopathy. Br J Sports Med 2013;47:862–8.
8. Wiesinger HP, Seynnes OR, Kösters A, Müller E, Rieder F. Mechanical and Material Tendon Properties in Patients With Proximal Patellar Tendinopathy. Front Physiol 2020;11:1–11.
9. Kongsgaard M, Qvortrup K, Larsen J, Aagaard P, Doessing S, Hansen P, Kjaer M, Magnusson SP. Fibril Morphology and Tendon Mechanical Properties in Patellar Tendinopathy. Am J Sports Med 2010;38:749–756.
10. Couppé C, Kongsgaard M, Aagaard P, Vinther A, Boesen M, Kjaer M, Magnusson SP. Differences in tendon properties in elite badminton players with or without patellar tendinopathy. Scand J Med Sci Sports 2013;23:e89-95.
11. Kongsgaard M, Kovanen V, Aagaard P, Doessing S, Hansen P, Laursen AH, Kaldau NC, Kjaer M, Magnusson SP. Corticosteroid injections, eccentric decline squat training and heavy slow resistance training in patellar tendinopathy. Scand J Med Sci Sport 2009;19:790–802.
12. Malliaras P, Barton CJ, Reeves ND, Langberg H. Achilles and patellar tendinopathy loading programmes: A systematic review comparing clinical outcomes and identifying potential

mechanisms for effectiveness. Sport Med 2013;43:267–286.

13. Young JL, Rhon DI, Cleland JA, Snodgrass SJ. The Influence of Exercise Dosing on Outcomes in Patients With Knee Disorders: A Systematic Review. J Orthop Sport Phys Ther 2018;48:1–47.
14. Obst SJ, Heales LJ, Schrader BL, Davis SA, Dodd KA, Holzberger CJ, Beavis LB, Barrett RS, Obst sobst SJ. Are the Mechanical or Material Properties of the Achilles and Patellar Tendons Altered in Tendinopathy? A Systematic Review with Meta-analysis The lack of evidence for a consistent effect of tendi- nopathy on the mechanical and/or material properties of t. Sport Med 2018;48:2179–2198.
15. Malliaras P, Kamal B, Nowell A, Farley T, Dhamu H, Simpson V, Morrissey D, Langberg H, Maffulli N, Reeves ND. Patellar tendon adaptation in relation to load-intensity and contraction type. J Biomech 2013;46:1893–9.
16. Arampatzis A, Karamanidis K, Albracht K. Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. J Exp Biol 2007;210:2743–2753.
17. Wiesinger HP, Kösters A, Müller E, Seynnes OR. Effects of Increased Loading on In Vivo Tendon Properties: A Systematic Review. Med Sci Sports Exerc 2015;47:1885–95.
18. Tsehaie J, Poot DHJ, Oei EHG, Verhaar JAN, de Vos RJ. Value of quantitative MRI parameters in predicting and evaluating clinical outcome in conservatively treated patients with chronic midportion Achilles tendinopathy: A prospective study. J Sci Med Sport 2017;20:633–637.
19. Peers KHE, Brys PPM, Lysens RJJ. Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. Int Orthop 2003;27:180–183.
20. Heinemeier KM, Lorentzen MP, Jensen JK, Schjerling P, Seynnes OR, Narici M V., Kjaer M. Local trauma in human patellar tendon leads to widespread changes in the tendon gene expression. J Appl Physiol 2016;120:1000–10.
21. Agergaard AS, Malmgaard-Clausen NM, Svensson RB, Nybing JD, Boesen M, Kjaer M, Magnusson SP, Hansen P. UTE T2* mapping of tendinopathic patellar tendons: an MRI reproducibility study. Acta Radiol 2020;284185120918807.
22. Juras V, Apprich S, Szomolanyi P, Bieri O, Deligianni X, Trattnig S. Bi-exponential T2 analysis of healthy and diseased Achilles tendons: an in vivo preliminary magnetic resonance study

and correlation with clinical score. Eur Radiol 2013;23:2814–22.

23. Filho GH, Du J, Pak BC, Statum S, Znamorowski R, Haghighi P, Bydder G, Chung CB. Quantitative characterization of the Achilles tendon in cadaveric specimens: T1 and T2* measurements using ultrashort-TE MRI at 3 T. AJR Am J Roentgenol 2009;192:W117-24.
24. Hansen P, Bojsen-Moller J, Aagaard P, Kjaer M, Magnusson SP. Mechanical properties of the human patellar tendon, in vivo. Clin Biomech (Bristol, Avon) 2006;21:54–8.
25. Tran PHT, Malmgaard-Clausen NM, Puggaard RS, Svensson RB, Nybing JD, Hansen P, Schjerling P, Zinglersen AH, Couppé C, Boesen M, Magnusson SP, Kjaer M. Early development of tendinopathy in humans: Sequence of pathological changes in structure and tissue turnover signaling. FASEB J 2020;34:776–788.
26. Visser JJ, Hoogkamer JE, Bobbert MF, Huijing PA. Length and moment arm of human leg muscles as a function of knee and hip-joint angles. Eur J Appl Physiol Occup Physiol 1990;61:453–460.
27. Kongsgaard M, Reitelseder S, Pedersen TG, Holm L, Aagaard P, Kjaer M, Magnusson SP. Region specific patellar tendon hypertrophy in humans following resistance training. Acta Physiol 2007;191:111–121.
28. Lee WC, Ng GYF, Zhang ZJ, Malliaras P, Masci L, Fu SN. Changes on Tendon Stiffness and Clinical Outcomes in Athletes Are Associated With Patellar Tendinopathy After Eccentric Exercise. Clin J Sport Med 2020;30:25–32.
29. Couppé C, Suetta C, Kongsgaard M, Justesen L, Hvid LG, Aagaard P, Kjær M, Magnusson SP. The effects of immobilization on the mechanical properties of the patellar tendon in younger and older men. Clin Biomech 2012;27:949–954.
30. de Boer MD, Selby A, Atherton P, Smith K, Seynnes OR, Maganaris CN, Maffulli N, Movin T, Narici M V., Rennie MJ. The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. J Physiol 2007;585:241–251.
31. Riley G. Tendinopathy - From basic science to treatment. Nat Clin Pract Rheumatol 2008;4:82–89.
32. Provenzano PP, Vanderby R. Collagen fibril morphology and organization: Implications for force transmission in ligament and tendon. Matrix Biol 2006;25:71–84.

33. Kannus P. Structure of the tendon connective tissue. Scand J Med Sci Sports 2000;10:312–20.
34. Svensson RB, Herchenhan A, Starborg T, Larsen M, Kadler KE, Qvortrup K, Magnusson SP. Evidence of structurally continuous collagen fibrils in tendons. Acta Biomater 2017;50:293–301.
35. Robson MD, Benjamin M, Gishen P, Bydder GM. Magnetic resonance imaging of the Achilles tendon using ultrashort TE (UTE) pulse sequences. Clin Radiol 2004;59:727–35.
36. Breda SJ, Poot DHJ, Papp D, de Vries BA, Kotek G, Krestin GP, Hernández-Tamames JA, de Vos RJ, Oei EHG. Tissue-Specific T2* Biomarkers in Patellar Tendinopathy by Subregional Quantification Using 3D Ultrashort Echo Time MRI. J Magn Reson Imaging 2020;1–11.
37. Liu F, Kijowski R. Assessment of different fitting methods for in-vivo bi-component T2* analysis of human patellar tendon in magnetic resonance imaging. Muscles Ligaments Tendons J 2017;7:163–172.
38. Bachmann E, Rosskopf AB, Götschi T, Klarhöfer M, Deligianni X, Hilbe M, Pfirrmann CWA, Snedeker JG, Fischer MA. T1- and T2*-Mapping for Assessment of Tendon Tissue Biophysical Properties: A Phantom MRI Study. Invest Radiol 2019;54:212–220.
39. Heinemeier KM, Schjerling P, Øhlenschlæger TF, Eismark C, Olsen J, Kjær M. Carbon-14 bomb pulse dating shows that tendinopathy is preceded by years of abnormally high collagen turnover. FASEB J 2018;fj.201701569R.
40. Seynnes OR, Bojsen-Møller J, Albracht K, Arndt A, Cronin NJ, Finni T, Magnusson SP. Ultrasound-based testing of tendon mechanical properties: a critical evaluation. J Appl Physiol 2015;118:133–141.
41. Eliasson P, Agergaard AS, Couppé C, Svensson R, Hoeffner R, Warming S, Warming N, Holm C, Jensen MH, Krogsgaard M, Kjaer M, Magnusson SP. The Ruptured Achilles Tendon Elongates for 6 Months After Surgical Repair Regardless of Early or Late Weightbearing in Combination With Ankle Mobilization: A Randomized Clinical Trial. Am J Sports Med 2018;46:2492–2502.

SUPPLEMENT

TABLE S1: Intervention protocols

	Exercise protokol							
	Week	**1**	**2**	**3**	**4**	**5**	**6**	**7-12**
	Sets	**3**	**3**	**3**	**4**	**4**	**4**	**5**
HSR	% of 1RM	55	65	70	75	80	85	**90**
	Repetitions	15	12	10	8	6	5	4
MSR	% of 1RM	55	55	55	55	55	55	**55**
	Repetitions	15	14	13	11	9	8	7

HSR, heavy slow resistance group; MSR, moderate slow resistance group; RM, repetition maximum.

TABLE S2: Compliance of prescribed interventions 0-12 weeks

	HSR (n=21)	MSR (n=21)	P- value
Session % of Aim	78.2 ± 4.3	85.8 ± 2.18	0.13
Supervision % of Aim	81.3 ± 4.5	85.7 ± 2.3	0.39
Load % of Aim			
Leg pres	97.6 ± 0.9	99.8 ± 0.2	0.02
Knee ext TL	93.6 ± 1.3	95.9 ± 0.9	0.16
Knee ext AL	93.5± 1.7	95.7 ± 0.8	0.25
Reps % of Aim			
Leg pres	97.9 ± 1.6	99.7 ± 0.3	0.29
Knee ext TL	97.7 ± 1.2	97.7 ± 0.6	0.97
Knee ext AL	96.5 ± 1.4	98.0 ± 0.6	0.36
Volume % of Aim			
Leg pres	97.6 ± 3.5	87.8 ± 1.5	0.16
Knee ext TL	77.8 ± 3.3	83.2 ± 2.1	0.18
Knee ext AL	77.5 ± 3.8	83.3 ± 2.0	0.19
Absolut volume % of Aim			
Leg pres	76.8 ± 4.2	84.8 ± 2.2	0.10
Knee ext TL	72.9 ± 4.3	80.3 ± 2.7	0.15
Knee ext AL	71.7 ± 4.7	80.4 ± 2.6	0.12
Absolut TUT % of Aim			
Leg pres	79.0 ± 3.9	85.3 ± 2.1	0.17
Knee ext TL	77.1 ± 3.9	83.4 ± 2.4	0.18
Knee ext AL	75.0 ± 4.2	83.6 ± 2.3	0.09

Values are expressed as % of Aim and presented as mean±SEM. HSR, Heavy slow resistance group; MSR, Moderate slow resistance group. Knee ext TL, knee extension exercise injured leg; Knee ext AL, knee extension exercise contralateral asymptomatic tendon. Load, reps and volume demonstrating compliance during the training session. Absolut volume and TUT percent of aim demonstrate the total compliance, including missing training session and adjusted for progression I prescribed load during the intervention period.

TABLE S3: Patellar tendon mechanical properties injured leg - Within participants common force

	HSR (n=21)		**MSR** (n=20)		P_{group}	P_{time}	$P_{g \times t}$
	Pre	Post	Pre	Post			
Peak $Force_{Max}$, N	4589 ± 285	5590 ± 335	5303 ± 305	5968 ± 379	0.21	<0.0001	0.32
Peak $Force_{CF}$, N	4245 ± 283	4239 ± 281	4807 ± 290	4845 ± 296	0.16	0.04	0.01
Peak $Deformation_{CF}$, mm	2.8 ± 0.13	2.9 ± 0.13	3.1 ± 0.20	3.2 ± 0.18	0.22	0.46	0.77
Peak $Stiffness_{CF}$, N/mm	2899 ± 157	2788 ± 160	2824 ± 121	2966 ± 125	0.79	0.80	0.04
Peak $Strain_{CF}$, %	6.8 ± 0.39	7.1 ± 0.41	7.1 ± 0.39	7.2 ± 0.37	0.76	0.34	0.74
Peak $Stress_{CF}$, MPa	35 ± 2.75	34 ± 2.74	41 ± 2.49	42 ± 2.82	0.07	0.56	0.37
Peak $Modulud_{CF}$, MPa	966 ± 54	928 ± 58	1057 ± 46	1117 ± 53	0.05	0.66	0-06

Values are presented as mean±SEM. Two-way analysis of variance was conducted with rehabilitation regime (group) and time as main factors. Alpha level set at P <0.05. CF, common force (within participants); HSR, Heavy slow resistance group; MSR, Moderate slow resistance group; Pre, baseline 0 weeks; Post, after the treatment intervention (12 weeks).

PAPER III

UTE T2 mapping of tendinopathic patellar tendons – An MRI reproducibility study*

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Publiced in Acta Radiologica 2020

Acta Radiologica
0(0) 1–10

DOI: 10.1177/0284185120918807
journals.sagepub.com/home/acr

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

Date received: 13 August 2019; accepted: 12 March 2020

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.

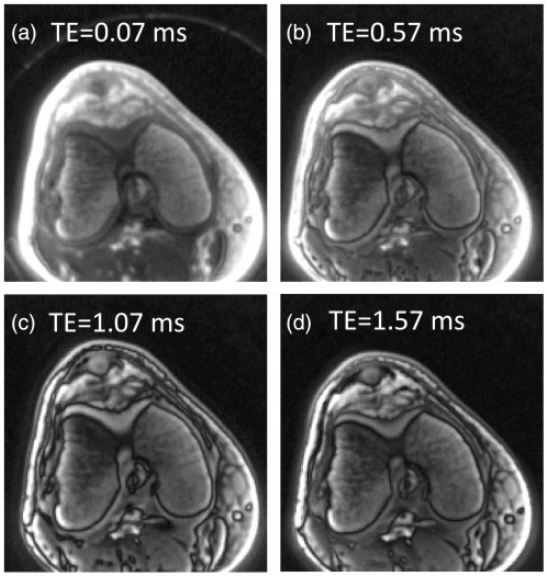

Fig. 1. Representative UTE MRI images in the mid-tendon, with increasing TE (a–d) (0.57–1.57 ms) and decreasing signal intensity in the tendon.

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

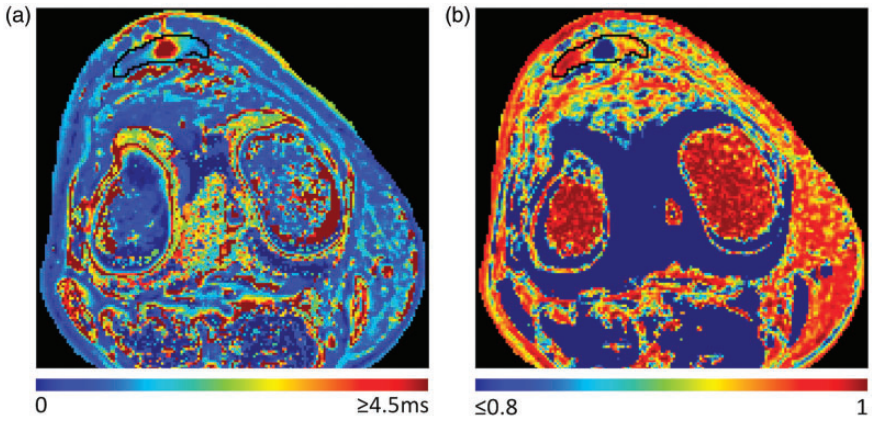

Fig. 2. (a) Representative T2* map, with scale bar values ≥4.5 ms in red, black line indicates tendon outline. (b) Corresponding goodness of fit map (Pearson correlation r-values) with scale bar values ≤0.8 in blue; black line indicates tendon outline.

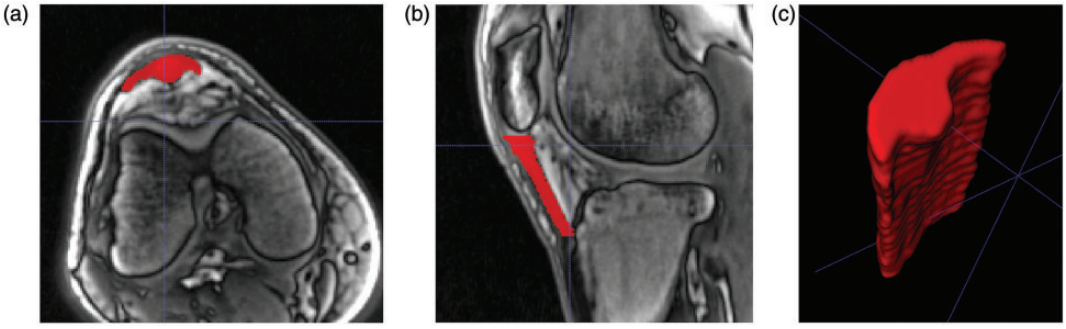

Fig. 3. (a) Representative axial mid-tendon section (TE = 1.57 ms), with tendon segmentation overlaid. (b) Sagittal view (c) 3D model for visual inspection of irregularities after interpolating segmentation.

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.

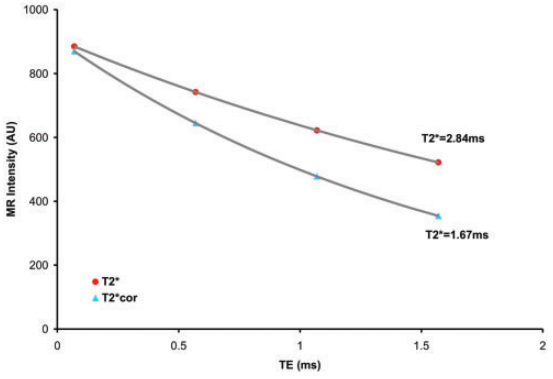

Fig. 4. Representative plot from the mono-exponential fitting procedure made in all voxels for the whole volume. T2*: all voxels; $T2^*_{cor}$: voxels with $r > 0.8$.

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.

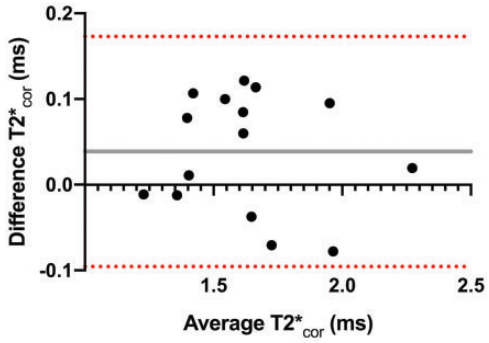

Fig. 5. Bland–Altman plot for test–retest proximal $T2^*_{cor}$. Gray line indicates bias. Red dotted lines indicate 95% limits of agreement.

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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Acknowledgements

The authors thank the participating patients and the staff at the Department of Radiology, Bispebjerg and Frederiksberg Hospital Copenhagen for their valuable help in obtaining the imaging material. They also thank Petros Martirosian, Section on Experimental Radiology, University Hospital, Tübingen for methodological support and feedback on the UTE MRI T2* mapping analysis. In addition, they thank Kasper Marstal, X-Rai IVS, Copenhagen, Denmark for developing the imaging analysis software and providing technical assistance during the preparation of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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References

1. Fredberg U, Stengaard-Pedersen K. Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. Scand J Med Sci Sports 2008;18:3–15.
2. Wright P, Jellus V, McGonagle D, et al. Comparison of two ultrashort echo time sequences for the quantification of T1 within phantom and human Achilles tendon at 3 T. Magn Reson Med 2012;68:1279–1284.
3. Peers KHE, Brys PPM, Lysens RJJ. Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. Int Orthop 2003;27:180–183.
4. Tsehaie J, Poot DHJ, Oei EHG, et al. Value of quantitative MRI parameters in predicting and evaluating clinical outcome in conservatively treated patients with chronic midportion Achilles tendinopathy: A prospective study. J Sci Med Sport 2017;20:633–637.
5. Weinreb JH, Sheth C, Apostolakos J, et al. Tendon structure, disease, and imaging. Muscles Ligaments Tendons J 2014;4:66–73.
6. Chu CR, Williams AA, West R V., et al. Quantitative Magnetic resonance imaging UTE-T2* mapping of cartilage and meniscus healing after anatomic anterior cruciate ligament reconstruction. Am J Sports Med 2014;42:1847–1856.
7. Robson MD, Benjamin M, Gishen P, et al. Magnetic resonance imaging of the Achilles tendon using ultrashort TE (UTE) pulse sequences. Clin Radiol 2004;59:727–735.
8. Gatehouse PD, Bydder GM. Magnetic resonance imaging of short T2components in tissue. Clin Radiol 2003;58:1–19.
9. Juras V, Apprich S, Szomolanyi P, et al. Bi-exponential T2*analysis of healthy and diseased Achilles tendons: An in vivo preliminary magnetic resonance study and correlation with clinical score. Eur Radiol 2013;23:2814–2822.
10. Bachmann E, Rosskopf AB, Götschi T, et al. T1- and T2*-mapping for assessment of tendon tissue biophysical properties: a phantom MRI study. Invest Radiol 2019;54:212–220.
11. Grosse U, Syha R, Hein T, et al. Diagnostic value of T1 and T2 * relaxation times and off-resonance saturation effects in the evaluation of Achilles tendinopathy by MRI at 3T. J Magn Reson Imaging 2015;41:964–973.
12. Kottner J, Audige L, Brorson S, et al. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. Int J Nurs Stud 2011;48:661–671.
13. Springer F, Martirosian P, Schwenzer NF, et al. Three-dimensional ultrashort echo time imaging of solid polymers on a 3-Tesla whole-body MRI scanner. Invest Radiol 2008;43:802–808.
14. Hopkins WG. Measures of reliability in sports medicine and science. Sports Med 2000;30:1–15.
15. Robson MD, Gatehouse PD, Bydder M, et al. Magnetic resonance: an introduction to ultrashort TE (UTE) imaging. J Comput Assist Tomogr 2003;27:825–846.
16. Juras V, Zbyn S, Pressl C, et al. Regional variations of T_2* in healthy and pathologic achilles tendon in vivo at 7 Tesla: preliminary results. Magn Reson Med 2012;68:1607–1613.
17. Grosse U, Springer F, Hein T, et al. Influence of physical activity on T1 and T2* relaxation times of healthy Achilles tendons at 3T. J Magn Reson Imaging 2015;41:193–201.

18. Liu F, Kijowski R. Assessment of different fitting methods for in-vivo bi-component T2* analysis of human patellar tendon in magnetic resonance imaging. Muscles Ligaments Tendons J 2017;7:163–172.
19. Grosse U, Syha R, Gatidis S, et al. MR-based in vivo follow-up study of Achilles tendon volume and hydration state after ankle-loading activity. Scand J Med Sci Sports 2016;26:1200–1208.
20. Filho GH, Du J, Pak BC, et al. Quantitative characterization of the achilles tendon in cadaveric specimens: Tl and T2* measurements using ultrashort-TE MRI at 3 T. Am J Roentgenol 2009;192:117–124.
21. Khan KM, Forster BB, Robinson J, et al. Are ultrasound and magnetic resonance imaging of value in assessment of Achilles tendon disorders? A two year prospective study. Br J Sports Med 2003;37:149–153.
22. van Snellenberg W, Wiley JP, Brunet G. Achilles tendon pain intensity and level of neovascularization in athletes as determined by color Doppler ultrasound. Scand J Med Sci Sports 2007;17:530–534.
23. Peers KHE, Brys PPM, Lysens RJJ. Correlation between power Doppler ultrasonography and clinical severity in Achilles tendinopathy. Int Orthop 2003;27:180–183.