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MAGNETIC RESONANCE IMAGING IN THE EVALUATION OF CHRONIC ACHILLES TENDINOSIS

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MAGNETIC RESONANCE IMAGING IN THE EVALUATION OF CHRONIC ACHILLES TENDINOSIS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

The aim of the thesis was to evaluate magnetic resonance imaging (MRI) as an outcome in the evaluation of treatment in mid-portion Achilles tendinosis. Conventional MRI sequences, dynamic contrast enhanced MRI and measurement of transversal relaxation time, using an ultrashort time to echo sequence, has been evaluated.

In **study I** intratendinous signal and tendon volume was evaluated in five different MR-sequences, using a computerized data program, in patients with unilateral chronic Achilles tendinopathy. Increased intratendinous signal on MRI correlated to increased severity of pain and functional impairment. A significant difference of intratendinous signal between the symptomatic and the contralateral asymptomatic tendons was found in all sequences except in T2-weighted images. Contrast enhanced T1-WI did not contribute to an improved correlation to clinical score compared to other sequences. The symptomatic Achilles tendons had a higher mean volume compared to the contralateral asymptomatic tendons but the volume did not correlate to clinical scores.

In **study II** symptoms and MRI findings were evaluated four to five years after eccentric calf-muscle treatment in patients with chronic Achilles tendinosis. Semi-quantitative evaluation showed decreased intratendinous signal in the symptomatic treated tendons after 4.2 years both compared to inclusion and after 3 months of eccentric calf-muscle training. The clinical scores were significantly improved both compared to inclusion and at 3 months. The decrease in tendon volume was not statistically significant.

In **study III** dynamic contrast-enhancement in tendon and in fat ventrally of the tendon in patients with Achilles tendinosis was evaluated before and after three months of eccentric calf-muscle training. In the fat ventrally of the tendon there was a significantly increased contrast enhancement in the symptomatic side compared to the contralateral non-symptomatic side before treatment that disappeared after three months of training. However there was no significant change of enhancement before compared to after training and there was no correlation of dynamic contrast enhancement to symptoms.

In **study IV** the short-term repeatability of the UTE sequence was evaluated and T2* in patients with chronic Achilles tendinopathy was compared to healthy controls. There was a significant difference in mean T2* between the 20 symptomatic tendons and the 20 control tendons. In the 13 patients with unilateral symptoms a significant difference in T2* was found between the symptomatic tendons and their contralateral asymptomatic tendon. The short-term repeatability of the UTE-sequence showed a CV of 35% and intra-class correlation with an average consistency of 0.98. The least significant change was 98%. There was no significant correlation between VISA-A and T2*.

Conclusions: Increased intratendinous signal on MRI correlate to clinical scoring in patients with chronic mid-portion Achilles tendinopathy but the arbitrary scaling of intratendinous signal makes the computerized method unreliable as an outcome measure.

The significantly better clinical outcome and decreased intratendinous signal on MRI after four to five years compared to directly after the eccentric training program indicates that the

long-term prognosis in chronic Achilles tendinosis is good. A remaining high volume may be a remnant of a previous disorder.

We could not show any additional value of dynamic contrast enhanced MRI compared to MRI without contrast media.

T2* relaxation obtained with a UTE sequence is able to differentiate between chronic Achilles tendinosis and healthy controls but it was not associated with the clinical index. There was a low reproducibility of the method limiting future evaluation of treatment effect to the group level.

LIST OF SCIENTIFIC PAPERS

I. Magnetic resonance signal, rather than tendon volume, correlates to pain and functional impairment in chronic Achilles tendinopathy.
Gärdin A, Bruno J, Movin T, Kristoffersen-Wiberg M, Shalabi A
Acta Radiol 47:718-24, 2006.

II. The long-term clinical and MRI results following eccentric calf muscle training in chronic Achilles tendinosis.
Gärdin A, Movin T, Svensson L, Shalabi A
Skeletal Radiology 39: 435–442, 2010.

III. Dynamic contrast enhanced magnetic resonance imaging in chronic Achilles tendinosis.
Gärdin A,Brismar TB, Movin T, Shalabi A
BMC medical imaging 13:39, 2013.

IV. T2* relaxation time in Achilles tendinosis and its correlation to clinical score - a comparison of patients and controls.
Gärdin A, Rasinski P, Berglund J, Shalabi A, Shulte H, Brismar TB
Manuskript.

CONTENTS

LIST OF ABBREVIATIONS

AUC	Area under the curve
CME	Contrast medium enhanced
CT	Computed tomography
CV	Coefficient of variation
CP	Coil preamplifier
2D	Two dimensional
3D	Three dimensional
DEMRI	Dynamic enhanced magnetic resonance imaging
FLASH	Fast low angle shot
FOV	Field of view
GE	Gradient echo
ICC	Intra class correlation coefficient
LSC	Least significant change
MR	Magnetic resonance
MRI	Magnetic resonance imaging
ms	milliseconds
NSF	Nephrogenic systemic sclerosis
OSR	Off-resonance saturation ratio
PACS	Picture archiving and communication system
PD	Proton density
ROI	Region of interest
SD	Standard deviation
SI	Signal intensity
T1	Longitudinal relaxation time
T2	Transversal relaxation time
TE	Time to echo
TR	Time to repetition
UTE	Ultrashort time to echo
VAS	Visual analog scale
VEGF	Vascular endothelial growth factor
VISA-A	Victorian institute of sports assessment Achilles
WI	Weighted image

1 INTRODUCTION

1.1 BACKGROUND

Chronic Achilles tendinopathy is common and may especially appear in athletes but also in non- athletes (Kvist 1991, Åström 1996, Rolf 1997). In the management of chronic Achilles tendinopathy a variety of treatments have been evaluated (Magnussen 2009, Roche 2013, Zweiers 2014). There is lack of evidence of the efficacy of the treatments because of few randomized controlled studies, heterogenic treatment groups and interventions and a wide range of outcome measurements used with little consistency between different studies. The most common outcome measurements are questionnaires and pain scores which are not fully objective since the treatment most often cannot be blinded.

The goal of this thesis is to evaluate magnetic resonance imaging (MRI) as an outcome in the evaluation of treatment of mid-portion Achilles tendinopathy. MRI of Achilles tendinopathy typically demonstrates a thickening of the tendon and a focal or diffusely raised intratendinous signal (Quinn 1987, Bergquist 1990, Åström 1996). The signal alterations have been shown to correlate to pathological changes in histological samples (Åström 1996). However there are varying degrees of overlap between clinically symptomatic and non-symptomatic tendons at MR imaging. (Soila 1999, Haims 2000) During the last decade an ultra-short time to echo pulse sequence (UTE-sequence) has become more commonly used in tendons (Robsson 2004, Filho 2009, Juras 2012, Grosse 2013). With UTE-sequences early tendon changes can be detected and quantified by different quantitative parameters.

1.2 ANATOMY AND STRUCTURE OF THE ACHILLES TENDON

The Achilles tendon is the strongest and thickest tendon of the body (Soma 1994). It is a traction tendon, formed by the junction of the gastrocnemius and soleus muscles and inserts by an enthesis into the calcaneal bone (Drake 2005). As the Achilles tendon descends the tendon fibers rotate laterally approximately 90 degrees producing a concentrated pressure where the tendon bundles meet (Jozsa 1997). The tendon may be exposed to forces as high as 3.9 times of the bodyweight when walking and 7.7 times when running (Giddings 2000). There is great variation in mean thickness of normal Achilles tendons between different studies ranging from 4.2 to7.1 mm (Koivunen-Niemelä 1995). A positive correlation of age, height and body weight to tendon thickness has been found in the adult population.

The Achilles tendon is surrounded by the paratenon, a multi-layered non-synovial tissue that facilitates movement (Jozsa 1997). The tendon receives its blood supply from the paratenon and from the muscle-tendon and bone-tendon junctions (Carr 1989, Josza 1997). **Fig 1.**

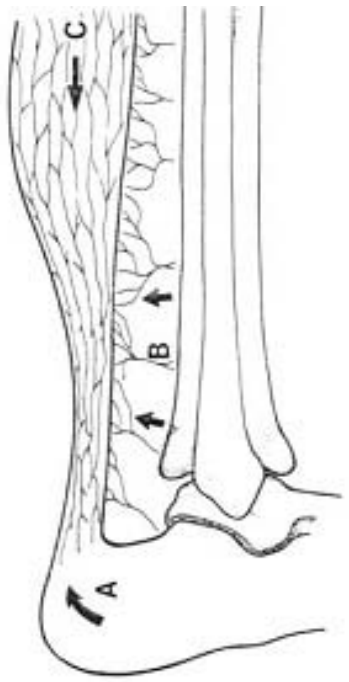


Fig 1. Illustration of the blood supply of the Achilles tendon from (A) the bone-tendon junction (B) the muscle-tendon junction and (C) the paratenon. From the thesis by Shalabi, Magnetic resonance imaging in chronic Achilles tendinopathy 2004, with permission.

Under the paratenon the tendon is surrounded by the epitenon which is contiguous with the endontenon that binds the collagen fiber together into primary, secondary and tertiary fiber bundles. A collagen fiber consists of varying number of fibrils that are made from collagen molecules arranged in a specific pattern (Maffulli 2005). **Fig 2.** The collagen molecules consist of three amino-acid chains, alpha-chains, and are in tendons primarily of type 1 collagen. In a resting state the collagen fibers show a wavy configuration that disappears if the tendon is stretched slightly (Rowe 1985). The collagen fibrils in traction tendons are orientated in the traction direction. The tendon react under increased mechanical strain with hypertrophy of the collagen fibrils (Michna 1984).

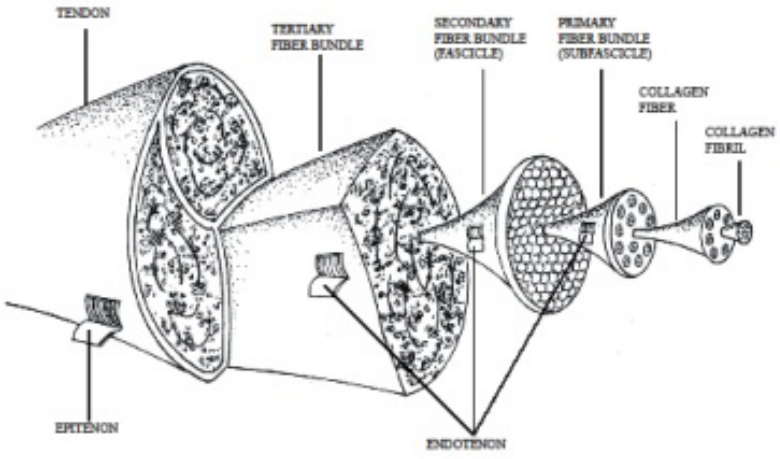


Fig 2. Schematic drawing of the tendon structure. From the thesis by Shalabi, Magnetic resonance imaging in chronic Achilles tendinopathy 2004, with permission.

80-90% of the dry weight of tendon is collagen and of the total weight of freshly dissected tendons 60% is water (Vogel 1999). The collagen fibrils are surrounded by ground substance containing proteoglukans and glycosaminoglukans that have a high water binding capacity (Jozsa 1997). The water in tissues can be divided into different compartments depending on its binding to other molecules (Peto 1990, Robsson 2003, Fullerton 2007). A suggested model for tendon is a three compartment model. One compartment is the interstitial water where the water molecules are tightly bound by two hydrogen bonds forming water bridges inside the collagen molecules. The second compartment is water molecules covering the collagen molecules like a water sheath. These water molecules are less tightly bound to the collagen molecules by positive and negative charges. The last fraction is located between the fibrils in the ground substance.

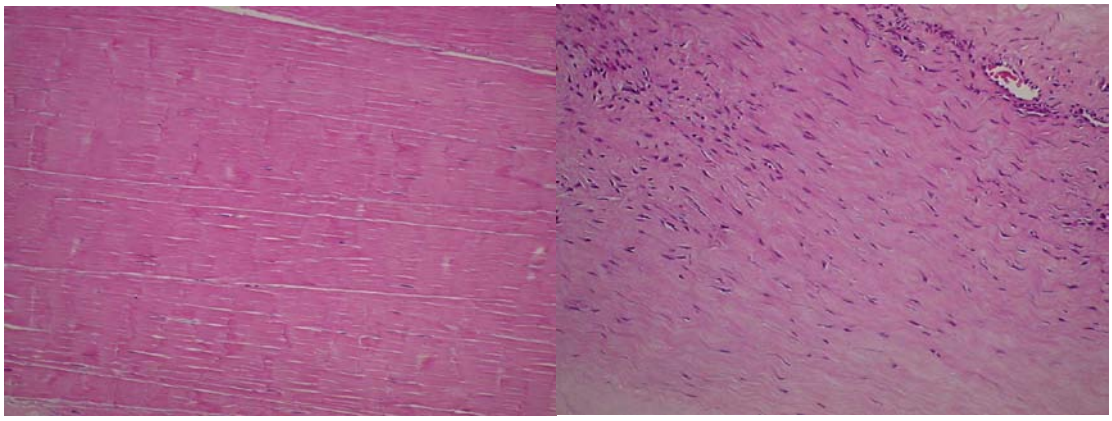
1.3 ACHILLES TENDINOSIS

1.3.1 Ethiology and pathology

Excessive physical activity is thought to be a major triggering factor of Achilles tendinosis (Kvist 1991). Several other intrinsic and extrinsic risk factors have been reported such as age, gender, genetic vulnerability, systemic and metabolic diseases, drugs and training errors but the exact etiological pathway is still unclear even though most agree that it is usually a result from a combination of different pathways (Järvinen 1992, Åström 1995, van der Linden 2002, Maffulli 2003, Riley 2004, Gaida 2009, Ribbans 2013, Magnan 2014).

Macroscopically tendinosis is seen as poorly demarcated regions with loss of tendon structure (Åström 1996, Paavola 2002). Typical histopathological findings are irregular fiber structure

with loss of parallel arrangement and decreased collagen stainability, increased non-collagen extracellular matrix, regional variations in cellularity, rounded nuclei of cells and areas of hyper vascularity (Åström 1996, Movin1997). **Fig 3.** Several subcategories such as mucoid, hypoxic, fibrinoid, lipomatous and calcific have also been identified. These types overlap and are probably a result of different etiologies (Maffulli 2003). Higher age correlate to more abnormal histopathological findings (Åström 1996).



A **B**

Figure 3. Histopathological images from (A) a normal tendon and from (B) a tendon with tendinosis.

It has been hypothesized that a reduced vascularity in the mid-portion of the Achilles tendon would be of significance in the etiology of Achilles tendinosis (Carr 1989, Pufe 2005). This area is also where most ruptures occur (Jozsa 1997). One hypothesis has been that a force reapplied before recovery from injury would lead to tendinosis promoted by delayed healing in the hypovascular tendon tissue (Leadbetter 1992). This hypo-vascular zone was described in cadavers originally 1958 by Lagergren and Lindholm and later by others (Carr 1989, Stein 2000). But other studies in vivo with laser Doppler technique have shown low vascularity rather in the distal part of the tendon and not in the middle part (Åström 1994). Biopsies and laser or ultrasound Doppler flow examinations in patients with Achilles tendinosis have shown areas of increased blood vessels and blood flow in the degenerative tendons (Åström 1994, Movin 1997, Öhberg 2001, Alfredsson 2003, Knoblosh 2006). Neo-vessels may be part of a reparative process at least in an acute phase. During remodeling of tendon auto-grafts angiogenesis contributes to repair of the graft but may also cause reduced mechanical strength (Petersen 2003, Pufe 2005). Angiogenesis is controlled by several stimulatory and inhibitory proteins necessary for the development and maintenance of tissues (Neufeld 1999). One of the most important angiogenic factors is the VEGF. Higher concentrations of VEGF have been detected in degenerative tendon tissue compared to controls (Pufe 2001).

Biopsies have shown nerve structures in close relation to the neo-vessels in Achilles tendinosis (Alfredson 2003). Neuropeptides such as substance P and calcitonin gene-related peptide have been detected in and around tendons (Ljung 1999, Ackermann 1999). There is a neurogenic hypothesis of overuse injury suggesting that nerve endings and mast cells mediate an adaptive response to mechanical strain (Hart 2005). Excessive load may lead to release of neuropeptides such as substance P and calcitonin gene-related peptide that stimulate the degranulation of mast cells, releasing agents that module cell activities resulting in pathological tendon changes.

Glutamate, a potent pain mediator, and glutamate receptors have been found in tendons, with a higher concentration of glutamate in patients compared to controls (Alfredson 1999, 2001). This may be an explanation to the experienced pain in Achilles tendinosis.
But tendinopathy may also be asymptomatic and it has been suggested that pain is only the top of an iceberg in the pathological process of Achilles tendinopathy (Kannus 1991, Magnan 2014).

1.3.2 Diagnosis

Mid-portion Achilles tendinopathy is diagnosed clinically as pain and swelling of the mid-portion of the tendon and impaired performance (van Dijk 2011). Insertional Achilles tendinopathy is located at the insertion of the Achilles tendon into the calcaneus. (Jozsa 1997) Some of the differential diagnoses of Achilles tendinopathy are partial tears, proximal pain of the myotendinous junction, paratendinopathy with inflammation or degeneration of the parathenon and retro- or superficial calcaneal bursitis (Jozsa 1997, Maffulli 1998, Krishna 2005, van Dijk 2011).

1.3.3 Epidemiology

The prevalence of overuse injuries has increased dramatically during the last decades due to an increased participation in sports activities (Jozsa 1997). The exact incidence and prevalence of Achilles tendinosis in the total population is difficult to measure. Studies of sports injuries are often based on reports from different kinds of caregivers with different population and are often non-uniform in methodology with different definition of injury and level of sports participation (Maffulli 2003, Sobhani 2013). In a cohort of Dutch GP registered patients the incidence of non-insertional Achilles tendinosis was 1.85 per 1000 person years (de Jonge 2011). The actual incidence is probably higher since a person with a complaint may contact other forms of care in the first place. In a population with overuse injuries from running 60% were males but women under the age of 30 are at greatest risk (Maffulli 2003). In a population of elite gymnasts there were Achilles tendon symptoms in 17.5% of the females and 12.5% of the males compared to none of the controls (Emerson 2010).

1.3.4 Treatment

The treatment of Achilles tendinosis is initially non-surgical. Only if other treatments have failed surgery is sometimes indicated (Zveiers 2014, Paavola 2002). Eccentric exercise has been recommended in the treatment of Achilles tendinosis since the mid-1980s and was first developed in 1979 (Stanish 1985, 1986). Of non-operative treatments eccentric calf muscle training has the best evidence and extracorporeal shockwave therapy also have some evidence (Mafi 2001, Rompe 2007, Rasmussen 2008, Rompe 2009, Magnussen 2009, Rowe 2012, Roche 2013, Zweiers 2014). Other examples of treatments that have been evaluated are injections of various substances such as a sclerosing agent near the ventral part of the tendon (Alfredson 2005, van Sterkenberg 2010) or platelet-rich plasma in the degenerative area of the tendon (de Vos 2010, de Jonge 2011) or splinting (de Vos 2007,de Jonge 2010). A common problem is that in many studies Achilles tendinosis of the mid-portion is not differentiated from distal tendinosis even though this may affect the outcome (Jonsson 2008, Rasmussen 2008, Magnussen 2009, van Dijk 2011, Roche 2013).

The mechanism behind the results of eccentric training is not fully understood. In a continuum model of tendinopathy three different stages are described with reactive tendinopathy, tendon dysrepair and degenerative tendinopathy (Cook 2009). Adding or removing load drives the tendon forward or back along the continuum.

In another model four stages of tendon healing have been described, the first three stages are proliferative and the last stage formative (Stanish 1985). In the final stage tension is important for the collagen fibers to reorient.

During the first phases of healing there is an ingrowth of nerves inside the tendons (Ackermann 2002). In experiments with rats increased physical activity accelerated healing of Achilles tendon rupture and disappearance of sensory nerves from the healing tendon (Bring 2007). Tendon cells have the potential to communicate through extended cell processes and gap junctions forming a network throughout the extracellular matrix allowing coordination and response to loading and damage (McNeilly 1996).

1.4 MAGNETIC RESONANCE IMAGING

1.4.1 Technique

MRI is based on the magnetic properties of the protons of the body. The use of a magnetic field and radio waves allows detailed imaging of the body with excellent soft tissue contrasts.

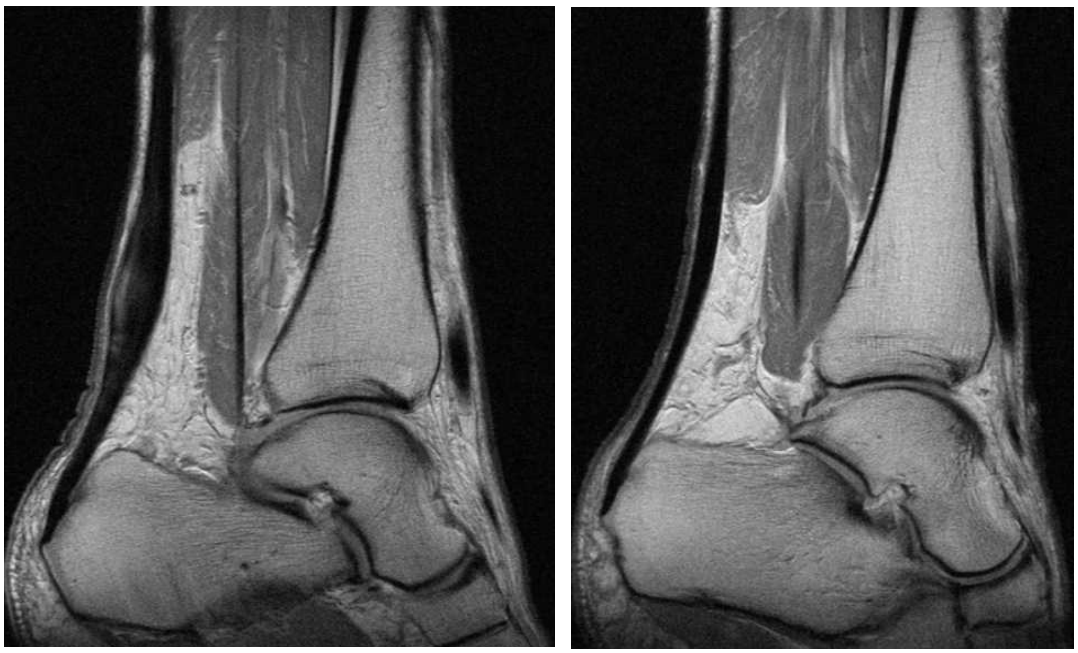
When a patient is put into the MR scanner a small abundance of the protons of the body are lined up parallel to the main magnetic field, creating a net magnetic vector. This is called the longitudinal magnetization (Schild 1990). When a radio frequency pulse is sent in some protons pick up the energy and turn upside down and also start to spin synchronized leading to a decrease of the longitudinal magnetization and an increase of the transversal magnetization. When the radio frequency pulse is switched off the protons relax so that the transverse magnetization disappears and the longitudinal magnetization returns to its original size. The time for this relaxation is called transversal relaxation time (T2) and longitudinal relaxation time (T1) respectively. T2 in all tissues is influenced by inhomogeneity of the local magnetic field from the molecules of the tissue.

Time to echo (TE) is the time from when the radio frequency pulse is switched off to when a signal, the spin echo, is collected.

1.4.2 MRI of tendons

The nuclear magnetic resonance properties of tissues are not only affected by the chemical and macromolecular structure but also by the physical organization of the macromolecules (Henkelman 1994). Normal Achilles tendons are slim and appear dark on conventional MR sequences (Quinn 1987). In the highly organized tendons the transversal signal disappears quickly since a large fraction of the water in tendon tissue is immobilized by tight dipolar coupling inside the collagen molecules. This gives a short transversal relaxation time, a short T2, of normal tendon tissues (Peto 1990).

In MRI of Achilles tendinopathy there is thickening of the tendon and a focal or diffusely increased intratendinous signal (Åström 1996, Bergquist 1990, Quinn1987). **Fig 4.** There are varying degrees of overlap of MR imaging between clinically symptomatic and non-symptomatic tendons. Asymptomatic Achilles tendons frequently demonstrate mild intratendinous signal but symptomatic tendons often have more obvious intratendinous signal. The subtle increase in signal found in controls may represent normal fascial anatomy, magic angle effects from oblique distal fibers or possibly small vessels (Soila 1999, Haims 2000, Schweitzer 2000).



A **B**

Figure 4. PD-weighted images from a 45 year old man. The symptomatic right tendon (A) show increased intratendinous signal and increased volume, the contralateral asymptomatic left tendon (B) is dark and slim.

The increased signal seen in tendinopathy is caused by the loss of order in collagen structures and increase of hydrophilic ground substance which leads to a decrease of short T2 component and an increase of long T2 component. Reduction in signal from short T2 components may also result from a decrease into the extremely short T2 range or an increase in T1 (Robson 2003). All tissues contain short T2 relaxation components, but most tissues only have a small short T2 component. Tissues such as cortical bone, tendons, ligaments and menisci contain a majority of short T2 components.

In the same way that the water or protons of tendons can be divided into compartments, as described above, the T2 of tendons can be divided into components. The shortest component of T2 arises from the collagen protons and from the protons of water fixed by two hydrogen bonds inside the collagen molecules between the alpha-chains. Their relaxation is dependent on the orientation of the magnetic field (Peto 1990). The second T2 compartment is water weakly bound outside the collagen molecules. These water molecules are much more mobile than interstitial water but they still feel the influence of macromolecules. Its relaxation is determined by interference of the interstitial water on the collagen molecule since there is fast exchange of spin state between the immobilized water molecules and the less immobilized surface water (Henkelman 2001). The first of these two compartments is the largest with 89% of the volume (Fullerton 2007). The last T2 component is from water in the ground substance. This component has a relaxation comparable to the relaxation of a protein solution.

The movement of protons in tendons is also dependent on the orientation of the collagen fibers. When the collagen fibers are oriented 55 ˚ to the magnetic field the dipolar interactions are minimized and T2 is increased, the so called the magic angle effect (Peto 1990, Henkelman 1994). The magic angle effect tends to be greater for short T2 components than for longer T2 components. Usually the magic angle effect is regarded as an artifact and the finding of increased signal intensity in tendon on sequences with low TE such as T1-, PD- and GRE sequences is sometimes not considered significant if the increased signal is not also found in a sequence with long TE (Schweitzer 2000, Fullerton 2007). But by deliberately placing tendons near the magic angle tissue changes that are not otherwise apparent may be detected (Bydder 2007). In the magic angle signal from normal tendon may increase as much as 600% and is usually higher than signal from pathologic tendon tissue in MRI sequences that are sensitive to the magic angle effect (Fullerton 2007).

1.4.3 Contrast agents and dynamic enhancement

Gadolinium is a metal with unpaired electrons causing paramagnetism (Brash 1992). Gadolinium based contrast agents act in low concentrations by shortening T1 of nearby protons and thereby increasing the signal best seen in T1-weighted images. Paradoxically in high concentrations the shortening of T2 dominates which gives a low signal. Gadopentetate dimeglumine (Magnevist®, Schering) was the first MRI contrast agent to be approved for clinical use in North America. Another gadolinium based contrast agent that has been widely used is gadodiamid (Omniscan ®, Nycomed Amersham). After intravenous infusion these contrast agents rapidly diffuses from the vascular compartment to equilibrate with the extracellular space except from in organs with special blood barriers. The distribution half-life of these agents is approximately 4 minutes and the elimination half-life is approximately 70 minutes (Abraham 2008). The plasma concentration of gadopentetate dimeglumine decreases with 70% within 5 minutes after intravenous injection (Brasch 1992). During the first pass of the capillary bed 50% of gadopentetate dimeglumine diffuses from the blood into the extravascular compartment. Dynamic contrast-enhanced MR imaging may therefore give information about tissue vascularity, perfusion and capillary permeability by calculating the slopes from time-intensity curves (Verstrate 1994).

Since 2000 nephrogenic systemic fibrosis (NSF) has been described as an adverse effect of gadolinium based contrast agents in patients with renal failure (Grobner 2006, Kuo 2007). NSF is a systemic disorder characterized by widespread tissue fibrosis. Gadopentetate dimeglumine and gadodiamid have a high risk of NSF but there are other gadolinium based contrast agents with low risk of NSF such as gadoterateme glumine (Dotarem ®,Gothia medical) (Läkemedlsverket).

1.4.4 UTE sequences

Tendons in healthy controls have shown a mean T2 of 1.5-3.3 milliseconds (ms) at 3 Tesla (Juras 2013, Grosse 2015). This means that the signal has already disappeared when the echo signal is collected with conventional sequences such as T1 weighted sequences with TE 8–

10 ms or T2 weighted sequences with TE 50–100 ms. The lack of signal with conventional sequences is useful to provide a dark background against which a high signal can be recognized but they are not sensitive to tissue changes of different types or degrees because there has been little or no signal available to manipulate with (Gatehouse 2003).

Sequences with ultra-short TE (UTE) use TEs as low as 0.05-0.5 ms (Gold 1995, Robson 2003, Filho 2009). With UTE-sequences early tendon changes can be detected and quantified by measuring parameters such as T1, T2 and T2* (Robson 2004, Juras 2012).

T2 is the transversal relaxation time when the protons are re-phased by 180˚pulses during the T2 signal curve (Schild 1990). T2 is a fundamental property of a tissue at specific conditions such as field strength and temperature and is largely independent of the measurement technique (Robsson 2003). T2* relaxation has a more rapid signal loss than T2. With fast sequences such as UTE-sequences there is no time for the 180˚pulses used in spin echo sequences and the signal disappears much faster. Instead the UTE sequence is a type of gradient echo (Gatehouse 2003, Robson 2003). T2* depend on both T2 relaxation and dephasing effects that arise from spins within a voxel and this requires knowledge of voxel size and field inhomogeneity within it. But when using UTE sequences the difference between T2 and T2* is indistinguishable (Robsson 2003). Local magnetic field inhomogeneity is increased with stronger external magnetic field. This affects the T2* in tendons with lower values at 7 Tesla compared to 3 Tesla (Juras 2012). The transversal relaxation in tendons decays multi-exponentially with a four-component of T2 (Peto 1990). But T2* is often reported as a single component value or a bi-component value with a short and long component (Filho 2009, Robson 2004, Juras 2012).

A disadvantage of the UTE sequences is the lower signal to noise ratio caused by the shorter data collection time and higher bandwidths compared to conventional sequences (Gatehouse 2003). UTE sequences are also especially sensitive to the magic angle because of the short TE (Bydder 2007, Gatehouse 2003). It is therefore important that the tendon is placed parallel to the magnetic field when using UTE sequences.

1.4.5 Effect of physical activity on tendons

It may be important to consider the physical activity level of patients in studies with MRI of Achilles tendons since some studies have shown that physical activity affects the intrateninous signal and volume.

T2* values of the Achilles tendon have been found to be significantly longer in healthy recreational long-distance runners than in controls especially in the mid portion where the mean difference was 29%. This is considered to be attributed to adaptations of the tendon microstructure and water content (Grosse 2015). Examination before and within 72 hours after 15 min of rope skipping and 6.6 km cross-country running in healthy males has shown an increased off-resonance saturation ratio (an indirect measurement of tissue hydration using UTE sequences) and decreased tendon volume indicating a loss of free water during

exercise (Syha 2014). In patients with Achilles tendinosis conventional MRI performed immediately after 30 minutes of eccentric calf-muscle training showed increased tendon volume and increased intratendinous signal indicating hyperemia or higher water content (Shalabi 2004). T2* has also been measured in tendons harvested from cadavers before and after 10 minutes of 1 kilogram tension. Despite that no change of single- or bi-component T2* could be found, a layering of exudates from the tendon during loading was visualized (Chang 2014).

1.5 ASSESSMENT OF PAIN AND FUNCTION

In reviews of treatment of Achilles tendinopathy most studies quantified pain by a wide variety of numeric pain scales. Another common outcome is return to activity (Magnussen 2009, Malliaras 2013, Rowe 2012). We have used a modified classification described by Curwin and Stanish (Rolf 1997) in the first three papers of this thesis where pain is categorized on a six-level scale and performance on a four-level scale (Table 2 in Methods). This questionnaire was commonly used when the collection of data to these papers started.

VISA-A is the first disease specific validated questionnaire (Robinson 2001). It was developed to measure the severity of Achilles tendinopathy containing eight questions covering pain, function and activity. Pain and function is reported on a visual analogue scale (VAS) and activity is measured using a categorical rating system. VISA-A has become a widely used tool for assessment of Achilles tendinopathy. In a review of conservative management of mid-portion Achilles tendinopathy VISA-A was used as an outcome measure in 29% of the reviewed papers (Rowe 2012). The VISA-A questionnaire has been adapted to Swedish and has been shown to be a reliable and valid instrument (Silbernagel 2005).

2 AIMS OF THE THESIS

The general aim of this thesis was to evaluate the value of different MRI sequences and methods as an outcome measurement in the evaluation of treatment in chronic Achilles tendinosis.

The specific aim of each study was as follows:

Study I

To correlate quantified intratendinous signal and tendon volume in five different MR-sequences with the clinical parameters, in terms of pain and functional impairment, in patients with chronic Achilles tendinopathy.

Study II

To evaluate the long-term symptoms and MRI findings, four to five years after treatment with eccentric calf-muscle training, in patients with chronic Achilles tendinosis.

Study III

To evaluate the dynamic contrast-enhancement in Achilles tendinosis and to examine if an altered dynamic contrast-enhancement is normalized after short-term eccentric calf-muscle training. We also evaluated if the dynamic contrast-enhancement correlates to the subjective symptoms.

Study IV

To investigate if T2* of the Achilles tendon obtained using the UTE sequence can discriminate between chronic Achilles tendinosis and healthy controls, to evaluate its short-term repeatability, estimate minimal detectable change and to evaluate if clinical symptoms correlate to T2*.

3 MATERIALS AND METHODS

3.1 PATIENTS

The patients of each four studies suffered from pain and local tenderness in the mid-portion of the Achilles tendon, 2-7 cm proximal to the calcaneal insertion. The ethics committee at Karolinska Institutet has approved the studies and written informed consent has been obtained from each study participant.

Totally 59 patients and 10 healthy controls were examined in this thesis. In study I, II and III a total of 39 patients were included. They had originally been referred to the orthopedic clinic at our hospital. Some of these patients only appeared in one study and some of the patients appeared in all three studies. **Table 1.** In study IV 20 patients were recruited by advertisements and 10 healthy controls were recruited among colleagues.

Study	Number of patients
I	10
I+II	3
I+III	3
I+II+III	9
II	6
II+III	6
III	2
Total	39

Table1. Overlap of patients appearing in study I, II and III.

In study I all patients had unilateral symptoms and in study II, III and IV the patients had uni- or bilateral symptoms. In case of bilateral symptoms the most symptomatic side at inclusion was considered as the symptomatic tendon.

Study I: Twenty-five patients, 16 men and 9 women were included. Median age was 50 years (range 37 to 71). The median duration of symptoms at inclusion was 35 months (range 4-144 months). The level of physical activity ranged from recreational running or heavy labour in 10, walking or moderate work in 8 to deskwork in 7 patients.

Study II: A total of 24 patients, 16 men and 8 women, were included in the study. Median age was 49 years (range 33–75). The median duration of symptoms at the time of inclusion was 12 months (range 6–120). Nine of the patients participated in sport activities on a regular basis at the time of the long term follow-up. Nine of the patients had or had had symptoms in the contralateral Achilles tendon. Two of these patients had had an operation on the contralateral Achilles tendon 10 and 3 years ago, respectively. 20 of the patients had previously participated in a heavy-loaded eccentric calf-muscle training program and had no other treatment during the follow-up period of 4.2 years (range 29–58) except acupuncture in one patient.

These patients had previously been included in a study by Shalabi et al. where MRI was performed on 25 patients with chronic Achilles tendinopathy before and immediately after three months of eccentric calf-muscle training (Shalabi 2004). Of these 25 patients two patients had an operation in the Achilles tendon of interest since the previous study and were therefore excluded and three patients were not interested in participating, or could not be contacted.

Four patients (three males and one female), with a median age of 48 years (range 36–56), were unable to participate in the eccentric training program for different reasons (diabetes mellitus, mild dementia, and, in two instances, illness in the family) and they did not get any other specific treatment for their Achilles tendon problems and were re-examined clinically and by MR after 14 months, thus forming a control group of untreated patients.

Study III: 20 patients, 14 men and 6 women were included. Median age was 51 years (range 28 to 70). Median duration of symptoms at inclusion was 31 months (range 6 to 120 months). In 9 of the 20 patients the Achilles tendon symptoms were sports related; at the time when symptoms appeared 7 patients practiced recreational running and one of them also practiced skiing and another also basketball. One patient practiced golf and one patient related the symptoms to snowboard skiing. 14 of the 20 patients had unilateral symptoms whereas 6 of the patients had a history of symptoms in the contralateral Achilles tendon. One of the 20 patients had had an operation in the contralateral tendon 5 years previous to this study. None of the other patients had had any previous treatment other than pain medication.

This study consists of a subgroup of a previously published patient material where MRI was performed before and immediately after three months of heavy loaded eccentric calf-muscle training (Shalabi 2004). 18 of the patients in this study also appear in study I or study II in this thesis. **Table 1.** Because of problems when performing the dynamic enhanced MRI (DEMRI) (three cases of incorrect contrast injection timing, one with extravasation and in one case intolerance of the contrast agent) only 20 patients of the original 25 patients participated in this study.

Study IV: A total of 30 subjects were included in this study, 20 patients and 10 healthy controls. The 20 patients, recruited by advertisements, 10 males and 10 females had a median age of 42.5 years (range 24 to 66 years). Median duration of symptoms was 12 months (range 6 to 300 months). 13 of the patients had unilateral symptoms but of these patients 5 had a history of symptoms in the contralateral Achilles tendon. 7 of the patients had bilateral symptoms at the time of inclusion. 10 controls, 2 males and 8 females without history of Achilles tendon pain or ankle pain were recruited. The median age of the controls was 51 years (range 26 to 57 years).

3.2 METHODS

3.2.1 Clinical outcome

In case of bilateral Achilles tendon symptoms the patients were instructed to evaluate the most severe side at inclusion. At follow up they were instructed to evaluate the same tendon that was evaluated at inclusion.

Study I, II and III: The clinical outcome was evaluated by a modified classification described by Curwin and Stanish (Rolf 1997), where pain was categorized on a six-level scale and performance on a four-level scale. **Table 2.**

Study IV: The clinical outcome was measured using the VISA-A questionnaire. The results range from 0 to 100 where 100 represent no symptoms at all. (Robinson 2001) We used the VISA-A-S version, a cross-cultural adaptation of the VISA-A into Swedish (Silbernagel 2005**).** VISA-A-S is shown in page 19-20.

Intensity	Level	Pain	Level	Performance
Mild	1	None	1	Normal
	2	With extreme exertion only, not intense	1	Normal
Moderate	3	Starts with activity, lasts for 1-2 hours after activity	2	Performance may be affected
	4	With any athletic activity, increase during activity	3	Performance level significantly decreased
Severe	5	Immediately upon any activity involving tendon. Sudden increase in pain if activity is continued, lasts for 12-24 hours	3	Performance markedly curtailed or prevented
	6	During daily activities	4	Unable to participate

Table 2. Classification of level of pain and functional impairment, modified from Curwin&Stanish, 1984 (Rolf 1997).

VISA-A-S FRÅGEFORMULÄR – utvärdering av hälsenebesvär

Namn:_____ **Datum:**_____ **Skadad hälsena: Höger / Vänster**

I DETTA FRÅGEFORMULÄR SYFTAR ORDET *SMÄRTA* SPECIFIKT PÅ SMÄRTA I HÄLSENAN.

1. När Du stiger upp på morgonen, under hur många minuter upplever du då stelhet i hälsenan?

100 min | 100 min | 90 min | 80 min | 70 min | 60 min | 50 min | 40 min | 30 min | 20 min | 10 min | 0 min | **0 min**

0 1 2 3 4 5 6 7 8 9 10

Poäng

2. När du väl är igång under dagen, har du då smärta när du stretchar hälsenan maximalt över en trappkant? (med sträckt knä)

kraftig/ svår smärta | | | | | | | | | | | | **ingen smärta**

0 1 2 3 4 5 6 7 8 9 10

Poäng

3. Om du går på plant underlag i 30 minuter, får du då ont i hälsenan inom de närmaste 2 timmarna? (Om du på grund av smärta inte kan gå på plant underlag i 30 minuter, sätt 0 på denna fråga).

kraftig/ svår smärta | | | | | | | | | | | | **ingen smärta**

0 1 2 3 4 5 6 7 8 9 10

Poäng

4. Får du ont i hälsenan vid normal gång nedför en trappa?

kraftig/ svår smärta | | | | | | | | | | | | **ingen smärta**

0 1 2 3 4 5 6 7 8 9 10

Poäng

5. Om Du gör 10 tåhävningar (på ett ben) på plant underlag, får du då ont i hälsenan under tiden eller direkt efter?

kraftig/ svår smärta | | | | | | | | | | | | **ingen smärta**

0 1 2 3 4 5 6 7 8 9 10

Poäng

6. Hur många hopp på ett ben kan du göra utan att få ont i hälsenan?

0 | | | | | | | | | | | **10**

0 1 2 3 4 5 6 7 8 9 10

Poäng

VISA-A-S questionnaire from; Silbernagel KG, Thomeé R, Karlsson J Cross-cultural adaptation of the VISA-A questionnaire, an index of clinical severity for patients with Achilles tendinopathy, with reliability, validity and structure evaluations. BMC Musculoskeletal Disorders 2005, 6:12 (6 March 2005) *Published with permission from Silbernagel KG.*

7. Utövar du för närvarande någon idrott eller annan fysisk aktivitet?

0	☐	Inte alls	Poäng
4	☐	Anpassad/begränsad träning och/eller anpassad/begränsad tävling	
7	☐	Tränar och/eller tävlar för fullt, men inte på samma nivå som innan hälsenebesvären började.	
10	☐	Tävlar på samma nivå eller högre nivå som innan hälsenebesvären började.	

8. Besvara antingen A, B eller C i denna fråga.

- Om du inte har någon smärta under aktivitet som belastar hälsenan, besvara endast **fråga A.**
- Om du har smärta under aktivitet som belastar hälsenan, men smärtan hindrar dig inte från att fullfölja aktiviteten, besvara endast **fråga B.**
- Om du har smärta som hindrar dig från att slutföra aktivitet som belastar hälsenan, besvara endast **fråga C.**

A. Om du inte har någon smärta under aktivitet som belastar hälsenan, hur länge kan du då delta i aktiviteten?

0 min	1-10 min	11-20 min	21-30 min	>30 min	Poäng
☐	☐	☐	☐	☐	
0	7	14	21	30	

ELLER

B. Om du har smärta under aktivitet som belastar hälsenan, men smärtan hindrar dig inte från att fullfölja aktiviteten, hur länge kan du då delta i aktiviteten?

0 min	1-10 min	11-20 min	21-30 min	>30 min	Poäng
☐	☐	☐	☐	☐	
0	4	10	14	20	

Eller

C. Om du har smärta som hindrar dig från att slutföra aktivitet som belastar hälsenan, hur länge kan du då delta i aktiviteten?

0 min	1-10 min	11-20 min	21-30 min	>30 min	Poäng
☐	☐	☐	☐	☐	
0	2	5	7	10	

VISA-A-S questionnaire from; Silbernagel KG, Thomeé R, Karlsson J Cross-cultural adaptation of the VISA-A questionnaire, an index of clinical severity for patients with Achilles tendinopathy, with reliability, validity and structure evaluations. BMC Musculoskeletal Disorders 2005, 6:12 (6 March 2005) *Published with permission from Silbernagel KG.*

3.2.2 Eccentric training

In study II and III MRI and clinical scoring were performed before and after participation in an eccentric exercise program following a model developed by Alfredson et al. (Alfredson 1998). Eccentric loading of the calf were done in two ways; with the knee straight and with the knee bent. The eccentric loading was performed with all body weight on the symptomatic side.Each of the two exercises was done with 15 repetitions in 3 sets (3x15 repetitions) twice a day for 12 weeks. The load was increased successively as tolerated by adding weights to a backpack. The exercise was done at home and the patients saw a physiotherapist for instructions shortly before the treatment started and at 12 weeks for a final follow-up. The patients kept a training diary and they were also contacted on telephone at 6 weeks.

3.2.3 MRI acquisition

In study I, II and III the MRI examinations were performed on a 1.5 Tesla Magnetom Vision (Siemens, Erlangen, Germany) In study IV a 3TeslaMagnetom Trio (Siemens Healthcare, Erlangen, Germany) was used.

In all four studies both Achilles tendons were examined simultaneously using a commercially available CP-flexible 21×52 cm coil centred over the Achilles tendons with the patients in a feet-first supine position and with the feet fixed in about 15 degrees plantar flexion. The Achilles tendons were placed parallel to the magnetic field and in the iso-centre of the bore.

Study I and II: Five sagittal MR sequences were used in study I. **Table 3.** The same sagittal T1-weighted and PD-weighted images used in study I was also used in study II. All sagittal MR sequences were obtained with a slice thickness of 3 mm and a 0.3- mm gap.

Sequence	TR/TE (ms)	Acquisition	Time of Acquisition (TA)/min	Field of view (FOV)/mm	Matrix
T1-WI	**550/20**	**2**	**4.45**	**180**	**256x512**
T2-WI	**35007119**	**1**	**4.48**	**180**	**410x512**
PD-WI	**3500/17**	**1**	**4.48**	**180**	**410x512**
GRE-WI	**460/10**	**2**	**6.19**	**180**	**410x512**
CME T1-WI	**550/20**	**2**	**4.45**	**180**	**256x512**

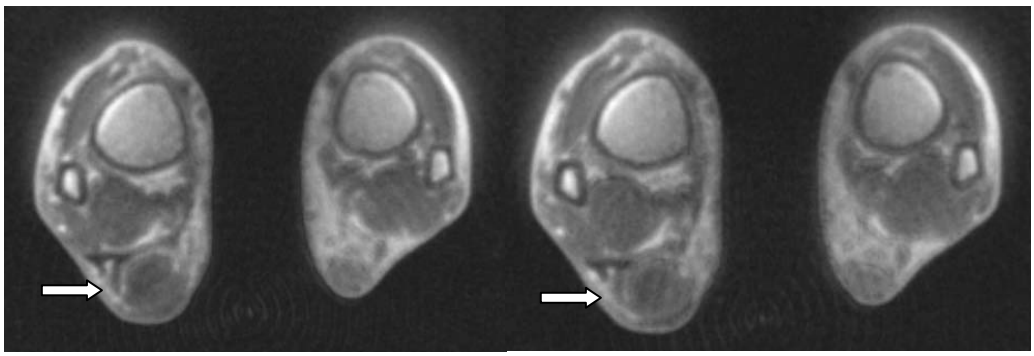
Table 3. Parameters of the five sagittal MR sequences that were used,T1-weighted spin echo (T1-WI), PD/T2- weighted turbo spin-echo (T2-WI, PD-WI), Flash 2 D-weighted (GRE-WI) and T1-weighted spin-echo after intravenous contrast agent (CME T1-WI).

The contrast agent, 0.2 mmol/kg body weight of gadopentetate dimeglumine (Magnevist, Schering, **Sweden**) or gadiodamide (Omniscan, Nycomed, Amersham, **Sweden**), was injected as a bolus within 20 seconds. Immediately after the injection, the catheter was flushed with saline. The T1-weighted sequence after gadolinium (Gd) contrast agent enhancement (CME) was performed within 10 min.

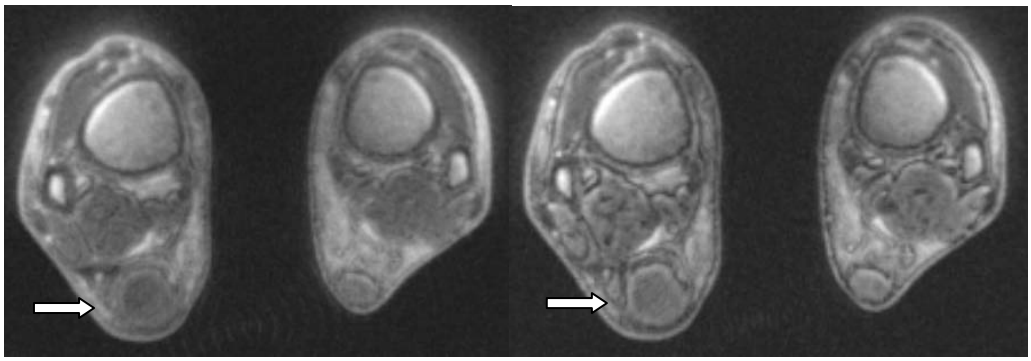
Study III: The sequence used for the dynamic MRI series was a sagittal Flash 2D with 3 mm slice thickness, TR/TE 50/9 ms, flip angle 40°, FOV 180x180 mm, matrix 128x256 mm, time of acquisition 31 seconds. The contrast agent (gadopentetate dimeglumine, Magnevist®, Schering or gadodiamid, Omniscan ®, Nycomed Amersham), 0.2 mmol/kg body weight, was injected by an auto-injector, 2 ml/second, via a catheter in the cubital vein followed by 20 ml of saline. The acquisition of the Dynamic MR series was started at the same time as the contrast injection. The Dynamic MR series lasted 5 minutes and 20 seconds, consisting of 5 sets with an interval of 40-seconds followed by two sets with an interval of 80 seconds. Every set had four sagittal slices, two from the symptomatic tendon and two from the contralateral tendon.

Study IV: The UTE sequence used was a 3D Flash with 0.78 mm slice thickness, TR 6 ms, a flip angle of 10°, field of view200x200 mm, voxel size 0.8x0.8x0.8 mm. Five different TE were applied: 0.07, 0.17, 0.37, 0.67 and 0.97 ms, resulting in an acquisition time of 5x3 minutes. Before the MR examinations the patients sat down and rested for 30 minutes. Both Achilles tendons were examined simultaneously using a commercially available CP-flexible 21×52 cm coil centred over the Achilles tendons. The patients were examined in a feet-first supine position with the feet fixed in about 15 degrees plantar flexion using a T-shaped vacuum cushion placed partly inside the coil. The Achilles tendons were placed parallel to the magnetic field and in the isocenter of the bore. **Figure 5.**

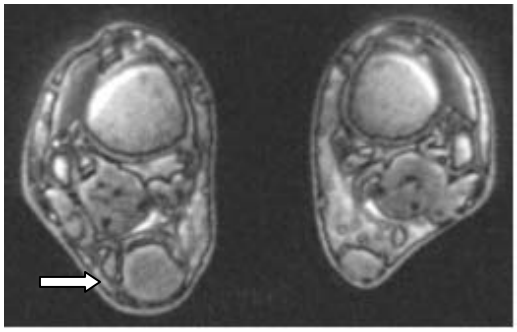
The UTE-sequence was acquired twice to test repeatability after repositioning. Repositioning was defined as being released from the coil and standing up on the floor for a few seconds.



TE 0.07 ms **TE 0.17 ms**



TE 0.37 ms **TE 0.67 ms**



TE 0.97 ms

Figure 5. UTE images obtained with the five different TE in a 66 year old man with chronic Achilles tendinosis in his right tendon (arrow).

3.2.4 MRI evaluation

Study I: The volume and intratendinous signal of the Achilles tendon was evaluated with a data program called computerized 3-D seed growing technique previously described by Shalabi et al (Shalabi 2005). The tendons were evaluated 2-12 cm proximal to the tendon insertion. Seven sagittal slices were used to cover each tendon. The pixel size was 0.35 x 0.35 mm^2 and the slice-to-slice distance 3.3 mm, giving a voxel volume of 0.35 x 0.35 x 3.3 mm^3. The 10 cm interval volume of each tendon was masked to give a 3D-region of interest. A seed growing algorithm semi-automatically marked the tendon volume. The volume of the tendon was calculated by summing all voxels within the tendon and multiplying with the size

of the voxel volume. The mean signals of all tendons were also estimated from the masked volume. **Figure 6.**

A B

Figure 6. A computerized 3-D seed growing technique used on PD-MR images of the asymptomatic Achilles tendon (A) and the symptomatic right Achilles tendon (B). The tendon volume and the mean intratendinous signal 2-12 cm proximal to tendon insertion were calculated using the 3-D seed growing technique.

Study II: Sagittal T1-weighted MR images were used to evaluate tendon volume and PD-weighted MR images were used to evaluate intratendinous signal. The volume of the Achilles tendon was evaluated with the same method as in study I. Intratendinous signal was evaluated visually using a four-point semi-quantitative grading scale, a modified classification described by Shalabi et al(Shalabi 2005). **Table 4.**

Grade	
0	No elevation of intratendinous signal
1	Mildly elevated intratendinous signal
2	Moderately elevated intratendinous signal
3	Severely elevated intratendinous signal

Table 4. Four-point semi-quantitative grading scale for evaluation of intratendinous signal.

We used T1-weighted images to measure tendon volume as tendon borders were best demarcated on the T1-weighted sequence. PD-weighted images were used to evaluate intratendinous signal since it is sensitive in revealing tendinopathy (Shalabi 2001).

Intra- and inter-observer tests were performed to evaluate the reliability of the semi quantitative categorization. Two observers graded MR I (PD sequences) of 48 tendons. The MRI images were shown on a PACS workstation and all patient and examination data was hidden. The patient's two tendons were shown in a random order for the observers. The replicate tests were identically performed after 7 days.

Study III: The Dynamic MRI series of the symptomatic and the contralateral tendons, before and after three months of eccentric training, were evaluated on a Hermes workstation (Nuclear Diagnostics, Stockholm, Sweden). From the two sagittal slices obtained from each Achilles tendon in every set, the most centred one was chosen by visual estimation. A circular region of interest (ROI) with a radius of 2 mm was placed in the thickest part of the tendon, and in the horizontal direction the area with the highest signal was chosen. In the case of no visible signal increase the ROI was placed in the centre of the thickest part of the tendon. The ROI´s distance from the upper posterior margin of the calcaneal bone was noted to ensure correct placement of the ROI at the follow up examination. One ROI with a radius of 2 mm was placed in a vessel ventrally of the tendon. One ROI with a radius of 5 mm was placed in the fat ventrally of the tendon. **Figure 7.** The three ROI:s placed in the first image of the dynamic series were then copied and pasted into the following images of the dynamic series. The signal intensity (SI) of each ROI was then plotted as a function of time, reflecting the contrast enhancement. Since signal intensity is a relative value and changes proportionally, the signal intensity at each point of the dynamic series was normalized by dividing the signal intensity of all subsequent intensity values with that obtained at the start of contrast injection (SI_0) (i.e. so that $SI_0 = 1$). The area under the curve (AUC) was then calculated for each ROI from the start of contrast injection until 320 seconds later, using the trapezoid method (Aschton 1984).

$$AUC = (SI_0 + SI_1) \times 40 \text{ seconds}/2 + (SI_1 + SI_2) \times 40 \text{ seconds}/2 + (SI_2 + SI_3) \times 40 \text{ seconds}/2 + (SI_3 + SI_4) \times 40 \text{ seconds}/2 + (SI_4 + SI_5) \times 80 \text{ seconds}/2 + (SI_5 + SI_6) \times 80 \text{ seconds}/2 - SI_0 \times 320 \text{ seconds}$$

In addition to AUC, time to peak (i.e. the time from start of contrast injection until maximum signal), SI/s (i.e. the increase of signal intensity per second until maximum signal) and SI% (i.e. the increase in signal between start and peak, expressed in percent) was calculated for each ROI.

Fig 7. Proton density weighted turbo spin-echo image prior to eccentric training in a 45-year-old man with chronic Achilles tendinosis. There is a fusiform thickening of the mid-portion of the Achilles tendon with increased intratendinous signal. The measurement regions of interest were placed in: A = the tendon, B = the fat ventrally of the tendon and C = a vessel.

Study IV: T2* maps were calculated by linear least squares fitting of a mono-exponential model to the logarithm of the signal magnitude at all echo times. The axial slice where the tendon appeared the thickest in the anterior-posterior direction was selected and a ROI was manually delineated along the border of the tendon placed on the border of the tendon. **Figure 8.** The distance from the most distal part of the tendon insertion was noted and used at repeated examinations. Measurements were also made 1cm above and 1 cm below this level and the mean T2* was then calculated. In case of uniform tendons the estimated middle part of the tendon was used.

To test reliability the image analysis was performed by two individual raters, one experienced musculoskeletal radiologist (rater 1) and one radiologist in training (rater 2). Each of the two raters performed the visual image analysis on two occasions, with a time span of minimum 2 and maximum 4 weeks between the two analyses. Both raters were instructed in how to perform the analysis directly prior to the first occasion (Measurement 1), but did not get any instructions prior to the second occasion (Measurement 2). The two raters were blinded to which images belonged to the patient group and which to the control group. Both were also blinded to each other's results.

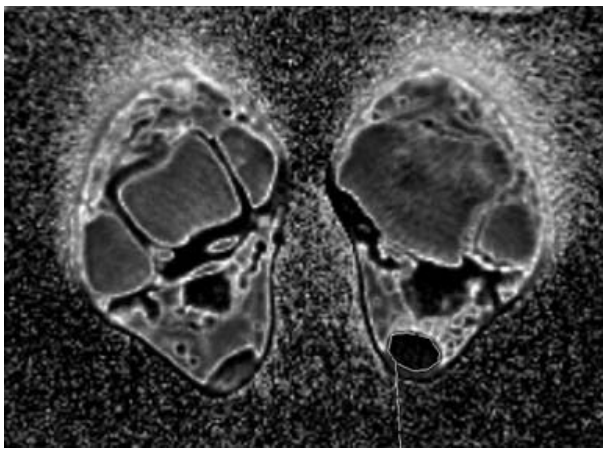


Figure 8. 1/T2* map with a ROI in the symptomatic left Achilles tendon of a 60 year old woman.

3.2.5 Statistical analyses

Statistical analyses were carried out on the SAS system for Widows in study I, II and III. In study IV the IBM SPSS statistics 20 was used. The 5, 1 and 0.1% levels of significance were considered. In the case of a statistically significant result the probability value (p-value) has been given. Variables of continuous and ordinal types are presented as mean and standard deviation or median and range (minimum-maximum).

The statistical tests used in each study are as follows:

Study I: The non-parametric Spearman rank correlation coefficient, r, was used in order to test independence between the intratendinous signal and the clinical variables, in terms of pain and functional impairment. The non-parametric Wilcoxon Signed Rank test was used to compare the differences between the symptomatic and asymptomatic contra-lateral Achilles tendon.

Study II: Statistical comparisons in order to test differences between two groups were made by using the Mann-Whitney's U-test or the Student's t test for uncorrelated means, after validation for normal distribution by use of the Shapiro Wilk test. The within group analysis was made using pair wise Student's t-test for correlated means. Multiple comparisons of continuous data were made by analysis of variance. The procedure proposed by Fisher was used to control for multiplicity. In order to evaluate hypotheses of variables in contingency tables, the Chi-square test was used. The inter- and intra-observer reliability of the semi-quantitative signal grading was analyzed with Cohen's kappa.

Study III: Statistical comparisons in order to test differences between independent or dependent groups were made by use of paired Student's t-test for correlated variables. The Spearman rank order correlation was used in order to test independence between variables.

Study IV: Statistical comparisons in order to test differences between independent or dependent groups were made by use of Wilcoxon rank sum test. To test short-term repeatability of the UTE sequence, intra-rater and inter-rater reliability the intra-class correlation coefficient, coefficient of variation (CV) and least significant change (LSC) were calculated. The LSC was calculated as 2.8 x CV% (El Maghraoui 2006). The Spearman rank

order correlation was used in order to test independence between variables. When calculating the short-term repeatability of the UTE-sequence, differences between groups and independence between variablesmeasurement 1 by rater 2 was used. In case of bilateral symptoms the most symptomatic tendon was considered as the symptomatic tendon.

4 RESULTS

Study I: The severity of pain and functional impairment showed a significant correlation to the mean intratendinous signal in all five MR-sequences. **Table 5.**

A significant difference of mean intratendinous signal between the symptomatic and the contralateral asymptomatic tendons was found in all sequences except in T2-weighted images. **Table 6.**

The symptomatic Achilles tendons had a higher mean volume than the contralateral asymptomatic tendons, mean 8.1 (4.8-16.8) cm^3 and 5.9 (4.4-7.8) cm^3 ($P<0.001$), but the volume of the painful Achilles tendon did not correlate with pain or functional impairment.

Sequence	**Intratendinous signal**			**Performance**		**Pain**	
	Median	**Range**	**SD**	**Spearman [r]**	**p-value**	**Spearman [r]**	**p-value**
T1-WI	71	31-127	21	0.49	0.001	0.30	0.05
T2-WI	81	59-108	10	0.29	0.05	0.36	0.01
PD-WI	208	79-330	60	0.44	0.001	0.38	0.01
GRE-WI	206	68-375	75	0.48	0.001	0.43	0.002
CME T1-WI	104	38-195	43	0.49	0.001	0.28	0.06

Table 5: The mean intratendinous signal of symptomatic tendons in five different sequences, with correlation to functional impairment and pain in 25 patients with unilateral symptoms.

Sequence	Symptomatic tendon	Asymptomatic tendon	Median Δ	Median Δ%	
T1-WI	71 (31-127)	68 (43-103)	5 (18-55)	7%	P< 0.02
T2-WI	81 (59-108)	80 (62-106)	-1.3 (-20-17)	-2%	P= 0.63
PD-WI	208 (79-330)	175 (99-255)	27 (-61-186)	18%	P< 0.0001
GRE-WI	206 (68-375)	148 (85-206)	52 (-68-227)	34%	P< 0.0001
CME T1-WI	104 (38-195)	78 (47-165)	15 (-29-125)	18%	P< 0.0001

Table 6: The median (range) intratendinous signal in the symptomatic and the contralateral asymptomatic tendon in five different MRI sequences.

Study II: The semi-quantitative evaluation showed decreased intratendinous signal in the symptomatic treated tendons at a mean time follow-up of 4.2 years both compared to time of inclusion and 3 months. **Table 7.**

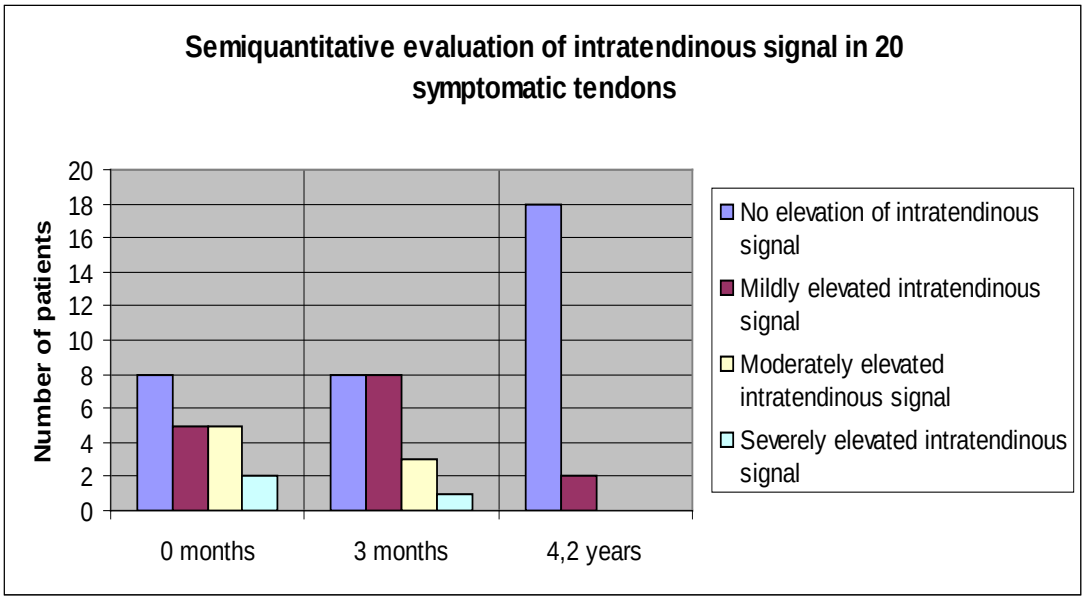


Table 7.

The symptomatic non-treated tendons had remaining intratendinous signal elevation 14 months after time of inclusion. **Table 8.**

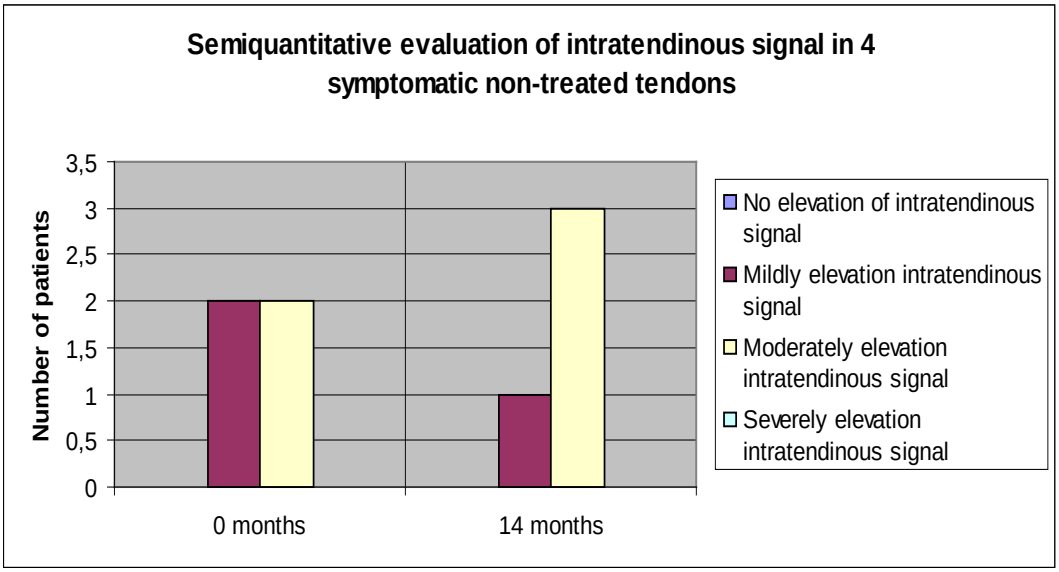


Table 8.

The kappa value for the inter-observer reliability of semi-quantitative evaluation of intratendinous signal was 0.71 and intra-observer test–retest reliability 0.58. Kappa reliability values of 0.6–0.7 are considered to correspond to satisfactory agreement (Cohen 1960).

The 20 symptomatic treated tendons had a mean tendon volume of 6.7 (SD 2.0) cm^3 at mean 4.2 years follow-up, 6.8 (SD 2.8) cm^3at 3 months and 6.4 (SD 2.0) cm^3 at time of inclusion. The change in tendon volume between inclusion and after 4.2 years was not significant (p=0.18).

At the 4.2-year follow up, the pain had decreased in 19 of 20 of the symptomatic treated patients and 13 patients experienced none or only mild pain. The performance had improved in 17 of the 20 patients and 12 of the patients experienced normal performance. The decrease of pain and improvement of performance was significant both compared to inclusion and immediately after the eccentric calf-muscle training period (pain: $p<0.001$ and <0.01) (performance: $p<0.001$ and <0.05).**Table 9.**

	Level of pain		Level of performance	
	Median	Range	Median	Range
0 months	4.5	2-6	4	3-4
3 months	3.5	1-6	3	1-4
4.2 years	1.5	1-4	1	1-4

Table 9. Median level of pain and functional performance in 20 patients with chronic Achilles tendinosis according to the classification modified from Curwin&Stanish, 1984. (Rolf 1997)

Study III:

Comparison between symptomatic and contralateral ankle in the 14 patients with unilateral symptoms: In the fat ventrally of the symptomatic tendon, AUC, SI/s and SI% was significantly higher than that of the asymptomatic side before, but not after, eccentric training. **Table 10.** In the tendons there was no statistically significant difference of contrast enhancement between the symptomatic and the contralateral asymptomatic tendons before or after eccentric training.

Comparison between before and after eccentric training in 20 patients: Mean AUC, SI/s, and SI% in tendon, fat and vessel did not change during eccentric training. Time to peak has been noted in **Table 11.**

Symptoms: The median level of pain decreased from 5 to 3 after training ($p < 0.01$ in all 20 patients and $p < 0.05$ in 14 patients with unilateral symptoms). The median level of performance improved from 4 to 3 after training ($p < 0.01$ in all 20 patients and $p < 0.001$ in 14 patients with unilateral symptoms).

Correlation of symptoms in the 20 symptomatic tendon sides: The dynamic contrast enhancement in tendons did not correlate to performance or pain before or after the exercise programme. In the fat ventrally of the tendon time to peak correlated to pain ($r= 0.61$, $p<0.01$) and performance ($r = 0.50$, $p<0.05$) after, but not before, training.

Dynamic contrast enhancement in tendon and in fat ventrally of tendon.				
	Symptomatic Before training	**Contralateral Before training**	**Symptomatic After training**	**Contralateral After training**
AUC tendon	80 (65) NS	66 (59)	76 (53) NS	52 (65)
SI/s 10^3 tendon	1.8 (1.5) NS	1.6 (0.8)	1.9 (1.2) NS	3.0 (4.7)
SI% tendon	41 (21) NS	38 (18)	38 (19) NS	33 (26)
AUC fat	31 (15) **	20 (11)	26 (15) NS	22 (13)
SI/s x 10^3 fat	0.44 (0.24)*	0.32 (0.13)	0.43 (0.20) NS	0.34 (0.16)
SI% fat	14.5 (7.4)**	9.5(4.9)	12.1 (6.3) NS	10.6 (5.4)

Table 10. Mean AUC, SI/s and SI% in the symptomatic and contralateral side measured in the tendon and in the fat ventrally of the tendon before and after eccentric training, in 14 patients with unilateral symptoms. One standard deviation is provided within parenthesis. The significance of mean difference between the symptomatic and contralateral side has been noted. ***=$p<0.001$, **=$p<0.01$, *=$p<0.05$, NS= Not Significant.

Mean time to peak contrast enhancement.				
	Symptomatic Before training	**Contralateral Before training**	**Symptomatic After training**	**Contralateral After training**
tendon	240 (89)	250 (78)	240 (100) NS	190 (110) *
vessel	260 (76)	270 (61)	240 (89) NS	270 (78) NS
fat	300 (64)	300 (57)	290 (53) NS	300 (42) NS

Table 11: Mean time, in seconds, to peak contrast enhancement in tendon, vessel and in the fat ventrally of the tendon in 20 patients. One standard deviation is provided within parenthesis. The p-value of the difference before compared to after three months of eccentric training has been noted. *=$p<0.05$, NS= Not Significant.

Study IV: The mean T2* relaxation times in different tendon categories are shown in **Table 12.**

20 symptomatic tendons	6.99ms (SD 7.43)
20 control tendons	1.85ms (SD 0.39)
13 symptomatic tendons in patients with unilateral symptoms	6.69ms (SD 5.74)
13 asymptomatic contralateral tendons	4.04ms (SD 3.88)
8 asymptomatic contralateral tendons with no history of tendon pain	3.08ms (SD 2.13)

Table 12. The mean T2* relaxation time in milliseconds (ms) and its standard deviation in different tendon categories.

There was a significant difference in mean T2* relaxation time between the 20 symptomatic tendons and the 20 control tendons ($p<0.0001$). In the 13 patients with unilateral symptoms a significant difference in T2* relaxation time was found between the symptomatic tendons and their contralateral asymptomatic tendon ($p<0.01$). There was no significant difference in T2* relaxation time between the 8 asymptomatic contralateral tendons with no history of tendon pain and the 20 control tendons ($p=0.12$).

In the 20 patients VISA-A-S mean was 57.9 (SD 19.0). There was no significant correlation between VISA-A-S and T2* relaxation time in the symptomatic tendons ($r = -0.30$, $p=0.19$). **Table 13.**

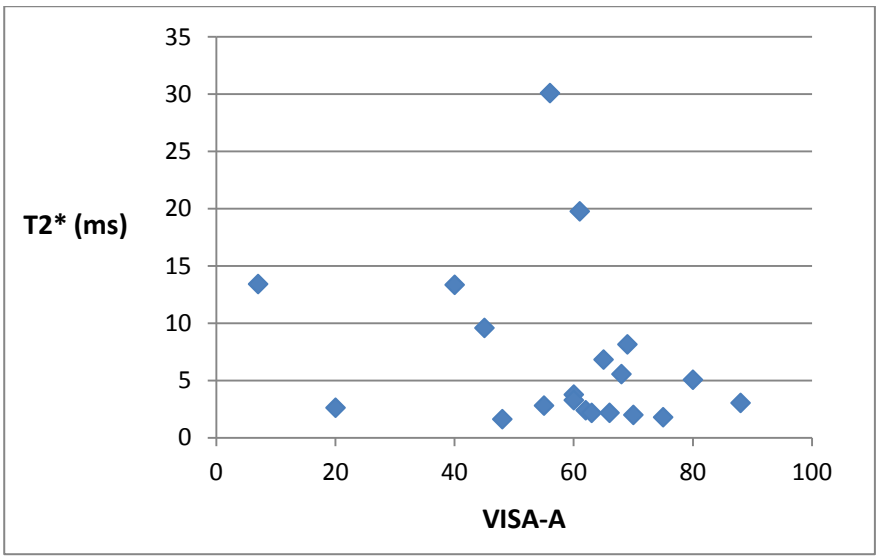


Table 13. Plot-diagram of VISA-A and T2* relaxation time of the symptomatic tendons in 20 patients.

The testing of short-term repeatability of the UTE-sequence showed a CV of 35% and LSC 98%. Intra-class correlation of T2* relaxation time between two short-term repetitions had an average consistency of 0.96 (confidence interval 0.93-0.98).

The results of the reliability test are presented in **Table 14 and 15.**

	ICC	CV(%)	LSC(%)
Rater 1	0.95	26	74
Rater 2	0.99	17	48

Table 14. Intra-rater reliability test.

	ICC	CV(%)	LSC(%)
Measurement 1	0.84	60	168
Measurement 2	0.82	67	187
Measurement 1+2	0.82	64	178

Table 15. Inter-rater reliability test.

5 DISCUSSION

5.1 GENERAL DISCUSSION INCLUDING STRENGTHS AND LIMIATIONS

In study I, II and III clinical scoring of Achilles symptoms was made by a slightly modified questionnaire by Curwin and Stanish (Rolf 1997). This questionnaire is simple and therefore easy to answer but a disadvantage is that higher scores means lower performance. This is confusing since higher scores on performance makes association to clinical improvement.
The modified questionnaire by Curwin and Stanish was commonly used when the collection of data to the first three papers started but has nowadays been replaced by other more specific clinical tests. In recent years the validated and disease specific VISA-A has become widely used (Robinson 2001). In order to enable comparisons with other studies we chose the VISA-A in study IV

A limitation of our and similar studies is that it may be difficult to differentiate symptoms from the right and left tendon when answering questionnaires. We have included patients both with unilateral and bilateral symptoms since it is difficult to find patients with symptoms only on one side. In case of bilateral symptoms the patients were supposed to answer the questionnaire according to the symptoms on the worst side at inclusion and on the treated side on follow up, but the contralateral symptoms may still influence the scores in both directions. This non-differential misclassification may hamper the results. Another limitation in common of all four studies is the heterogeneous material with both sports related and idiopathic tendinopathy.

Study I: The computerized 3-D seed growing technique extracts the mean value of the pixels of the imaged tendon (Shalabi 2005). The method was developed as a quantitative assessment of both focal intratendinous signal-changes and more diffuse intratendinous signal-change. The problem with this method is its arbitrary scaling since the signal can be affected by a number of patient- and devise-dependent factors. One example of such a factor is differences in coil loading which may vary not only between scanners but also between sessions (Deoni 2008). We know now that this kind of quantitative method is not reliable when comparing intratendionous signal between different occasions even when using the same MR camera, the same coil and the same sequences at every occasion.

Still, we consider the computerized 3-D seed growing technique to be reliable when evaluating tendon volume. Shalabi et al found the overall observer/replicate mean reliability of tendon volume measured in T1-WI to be 97.9% and coefficient of variation 4.9%. (Shalabi 2005)

The volume was 25% higher in the symptomatic tendons compared to the contralateral non-symptomatic tendons in our study but there was a lack of correlation to symptoms. This is supported by earlier studies where an increase in tendon thickness has been found in higher

ages (Koivunen 1995) or as a result of long lasting activity (Kvist 1991) or during and after a healing process (Karjalainen 1997, Möller 2001, Movin 1998).

Even though we no longer consider the computerized 3-D seed growing technique to be reliable in evaluating intratendinous signal the study revealed another interesting finding. Both PD-WI, GRE-WI, CM T1-WI and even T1-WI showed significant signal difference between symptomatic and contralateral asymptomatic tendons but the T2-WI did not reveal any significant difference. The T2 weighting is most sensitive when the TE is about the same as the tissue T2 (Robson 2003). Since normal tendons have low T2 and T2 is only slightly increased in tendinosis this explains why T2-WI did not reveal any significant difference between symptomatic and contralateral asymptomatic tendons. The advantage of T2-WI rests with the ability of identifying rupture within the tendon or other pathological conditions such as peritendinous abnormality or bursitis (Åström 1996, Shweitzer 2000).

The GRE-WI showed the highest difference of intratendinous signal between symptomatic and contralateral tendons and one of the strongest correlations to pain and functional impairment. But the GRE sequences are sensitive to the magic angle effect and may be a potential source of diagnostic misinterpretation. (Haims 2000, Schweitzer 2000) GRE sequences may reveal an intratendinous signal in 45% of the asymptomatic Achilles tendons (Soila 1999) and in up to 75% of the proximal patellar tendons without corresponding symptoms (Reiff 1995). Contrast enhanced T1-WI did not contribute to an improved correlation to clinical score compared to other sequences. The PD-WI was one of the most sensitive sequences in depicting intratendinous signal alterations and had one of the strongest correlations to pain and functional impairment in our study. PD weighted sequences do not detect a number of short T2 components but changes in proton density may be a greater source of contrast when imaging short T2 components than with long T2 components (Robson 2003). Consequently PD-WI could be recommended for asessment of the intratendinous signal.

Study II: This is the only long-term follow-up with MRI of Achilles tendinosis to this date that we know of. There are some other studies with long-term follow-up of Achilles tendinosis (Öhberg 2004, Silbernagel 2011, van der Plas 2012). Two of these use ultrasound as an objective outcome measurement. One is a 3.8-year follow-up showing normalized tendon structure and decreased tendon thickness (Öhberg 2004). The normalized tendon structure is in parity with our findings of normalized intratendinous signal on MRI. A 5-year follow up of mid-portion Achilles tendinosis with Doppler ultrasound showed despite significantly improved VISA-A score that 47% still had some degree of neovascularization compared to 59% at baseline (van der Plas 2012). Both our and their study groups had participated in a 12 week eccentric training program.

The study is limited by the fact that it lacks a randomized control group and that our four drop out “controls” were only followed for 14 months and not for 4.2 years. We do not know if our long term clinical- and MRI-findings are results of the eccentric training program or due to other factors.

The statistical results may also have been affected by the two patients that were excluded because of an operation in the Achilles tendon of interest since the first study and also by the three patients who were not interested in participating, or could not be contacted.

The 20 patients who participated in the long-term follow-up did not undergo any other active treatments than the initial eccentric training program and their improvement must either be an effect of the performed treatment or self-healing. Arguments giving credit for the long-term effect of the eccentric training regimen is that the patients had had long-standing symptoms prior to the treatment.

The normal anatomy of the asymptomatic Achilles tendon is variable with distal stripes or punctate foci (Soila 1999). This may be a potential source of misinterpretation in semi-quantitative evaluation of intratendinous signal. When evaluating the intra-tendinous signal visually focal changes are easily detected while a more diffuse intra-tendinous signal-change is more difficult to evaluate.

The intra- and inter observer reliability of the semi-quantitative evaluation of intratendinous signal in this study was found to be satisfactory or near satisfactory. The inter-observer reliability was higher than the intra-observer test–retest reliability and this may have been influenced by the fact that the two observers agreed on a consensus rating shortly before the rating. The intra-observer re-test were performed after only 7 days, which is a bit short with risk of recall bias.

Study III: Intratendinous signal alterations are often better revealed on CME T1-WI sequences, and the contrast agent increases the depicted size of the tendon lesion (Movin 1998). An increased vascularity mainly inside but also ventrally of the symptomatic tendon has previously been shown (Öhberg 2001). Therefore, in theory, evaluation of dynamic contrast enhanced MRI should be ideal to quantify the histopathological changes that have been observed in chronic Achilles tendinosis.

We found an increased contrast enhancement in the fat ventrally of the tendon in the symptomatic side before treatment but not after three months of treatment. The observed increased contrast enhancement before treatment may be due to an increased vascularity. But the reports of the significance of neo-vessels in Achilles tendinosis are contradictive. Increased vascularity has been found in symptomatic Achilles tendinosis (Öhberg 2001, Reiter 2004). But other studies have shown that symptoms are not invariably associated with vascularity (Richards 2010, van der Plas 2012, Peers 2003). Evaluation of MRI has shown that pre-Achilles edema was even more common in controls than in patients (Haims 2000). In a resent systematic review the evidence of association between improved symptoms and imaging parameters after eccentric exercise was low (Drew2014). This is in line with the lack of correlation with symptoms in our study.

We could not show any difference of dynamic contrast enhancement in the symptomatic tendon compared to the contralateral tendon before or after the training programme. This finding is in disagreement with two previous studies that both could show an increased AUC

in the symptomatic tendons (Shalabi 2002, Richards 2010). This difference might be due to differences in duration of symptoms. The patients in Shalabi's study had median symptom duration of 12 months compared to 31 months in our study. In the study by Richards et al. we do not know the duration of symptoms. Perhaps the AUC had already returned to baseline due to the relatively long duration of the symptoms at the time of inclusion in our study.

There was no significant change of contrast enhancement in tendon or fat before compared to after 3 months of eccentric training. Even though we could not show a change in contrast enhancement, perhaps our result could be due to lack of statistical power or maybe another method of measuring the dynamic contrast enhancement would have given other results.

No intra- and inter-observer tests were performed to evaluate the reliability of the measurements. The reliability of the dynamic enhancement in vessels is probably not so good since the image quality did not allow differentiation of arteries from veins and the vessels were located in different areas in the fat ventrally of the Achilles tendons and with different size and orientation.

Study IV: There was a significant difference of T2* relaxation between symptomatic tendons and control tendons and also between symptomatic tendons and contralateral asymptomatic tendons in patients with unilateral symptoms.

The repeatability of T2* has earlier been quantified on phantoms, cadavers, trabecular bone and in cartilage (Brismar 2000, Fhilo 2009, Juras 2012, Qian 2013, Chang 2014) but to our knowledge there are no previously published results on human tendons in vivo. Only the repeatability of OSR (off-resonance saturation ratio), another tissue specific MR parameter, has been tested in human in vivo tendons as we know (Syha 2014). CV% in trabecular bone has been estimated to 3-6% (Brismar 2000) and in cartilage 1.3 to 20% (Qian 2013) depending on the area studied. In our study the obtained CV% was 35% resulting in a LSC of 98%. These are considerably high values indicating that detection of changes might be difficult, especially when they are put in relation to the observed 43% difference between symptomatic and non-symptomatic tendon. This means that T2* measurements using UTE cannot be used to monitor treatment effect on the individual level and that fairly large studies will be needed in clinical trials. The reason for the low reproducibility at MRI is that relaxometric measurements are influenced from sources such as patient movements, temperature, hydration, position in the magnet, magic angle effect and signal to noise ratio (Bydder 2007, Diaz 2012, Biswas 2012). However, the excellent intra-rater (0.95 and 0.99) and good inter-rater (0.84 and 0.82) ICC, show a low proportion of random error in relation to the total variance. This means that UTE can be used to correctly classify patients with Achillodynia from those without.

In accordance with our results a significant difference of T2* relaxation time has been shown by Juras et al between 10 patients with Achilles tendinosis and 10 healthy controls (Juras 2013). In another study Grosse et al came to similar results when evaluating the diagnostic value of T1 and T2* relaxation times and off resonance saturation ratios in 21 controls and 11 patients even though they have divided their patients differently with

tendonopathic and non-tendinopathic tendon areas according to findings on conventional MRI when comparing patients to controls (Grosse 2014).

To our knowledge these two studies are the only two previously published studies using UTE sequences comparing T2* in Achilles tendinosis and controls with correlation to clinical score. Juras et al found a strong correlation of T2* to Achilles tendon rupture score and Grosse et al found a strong correlation of T2* to VISA-A in symptomatic patients. In contrast we found no correlation between T2* and VISA-A in our 20 symptomatic patients. However, the studied patient groups are slightly different between the three studies. In the study by Grosse et al and in our study patients with both uni- and bilateral symptoms were included but while all symptomatic tendons were considered as symptomatic by Grosse et al only the most symptomatic tendon was considered as the symptomatic tendon in case of bilateral symptoms in our study. In the study by Juras et al the patients had "a symotomatic tendon", in other words all patients had unilateral symptoms. Also, our patient group had symptoms only in the mid-portion of the Achilles tendon while the patients in the study by Grosse had "posterior heel pain" and the patients in the study by Juras et al had "painful Achilles tendon". Since VISA-A is disease specific and we have a more homogenized patient group from that aspect this should in theory give better correlation in our patients. However, our patient group had duration of symptoms for at least 6 months while the patients of Grosse et al reported duration of symptoms for at least 4 weeks, indicating that their patients had more acute symptoms. Even though the VISA-A score of the symptomatic patients were similar (mean 57.9 in our study and 52.5 in their study) there is a possibility that more acute symptoms correlate better to T2* relaxation time than chronic symptoms. A continuum model of tendinpathy with three different stages of severity has been proposed (Cook 2008). At early tendinopathy there is inflammatory cell infiltration (Millar 2010), and this should perhaps be considered when comparing populations with different disease duration. It might be that T2* might better discriminates between a more acute phase and a later phase of Achillodynia than VISA-A does. The relation between changes in VISA-A and T2* following with recovery from Achillodynia needs further studies in order to better understand how to best evaluate different treatment strategies.

In the eight patients with unilateral tendon pain and without history of pain in that tendon the T2* relaxation time seemed to be increased compared to healthy controls. Although this was not statistically significant the etiological causes that affect one tendon may also affect the contralateral tendon in the same individual even though it is painless.

We have used a mono-exponential calculation of T2* relaxation time. Juras et al found a more significant difference of short T2* relaxation component between patients and controls and a stronger correlation of short T2* to Achilles tendon rupture score compared to the mono-exponentially calculated T2* (Juras 2013). However, the bi-exponential calculation is technically more difficult to implement due to the larger number of echoes required for a robust calculation and higher sensitivity to movements and the magic angle. The monoexponential calculation might therefore be better suited for analysis in a clinical setting (Grosse 2014).

5.2 CONCLUSIONS

Study I: Increased intratendinous signal on MRI rather than the tendon volume, correlated to increased severity of pain and functional impairment in patients with chronic mid-portion Achilles tendinopathy. T2-WI correlated less to increased pain than any other sequence and T2-WI was unable to reveal a difference in mean intratendinous signal between the symptomatic and contralateral asymptomatic tendon. Contrast enhanced T1-WI did not contribute to an improved correlation to clinical score compared to other sequences. The PD-WI was one of the most sensitive sequences in depicting intratendinous signal alterations; consequently it could be recommended for asessment of the intratendinous signal. However the arbitrary scaling of intratendinous signal makes the 3-D computerized method unreliable as an outcome measure.

Study II: The significantly better clinical outcome and decreased intratendinous signal on MRI after four to five years compared to directly after the eccentric training program indicates that the long-term prognosis in chronic Achilles tendinosis is good. The decrease in tendon volume was not statistically significant. A remaining high volume may be a remnant of a previous disorder.

Study III: In the fat ventrally of the tendon there was a significantly increased contrast enhancement in the symptomatic side compared to the contralateral non-symptomatic side before treatment that disappeared after three months of training. However there was no significant change of enhancement before compared to after training and there was no correlation to symptoms. This study did not show any additional value of dynamic contrast enhanced MRI compared to MRI without contrast.

Study IV:T2* relaxation time obtained with UTE sequence seems to be able to differentiate between chronic Achilles tendinosis and healthy controls but it was not associated with the clinical index. There was also a low reproducibility of the method limiting future evaluation of treatment effect to a group level.

5.3 FUTURE ASPECTS

We are currently collecting data in a follow-up study with MR UTE sequenses and VISA-A at 3 and 12 months after treatment of chronic Achilles tendinosis. The patients have been randomized to either eccentric training or shock-wave theraphy. The aim is to investigate if there is a change of T2* relaxation time after treatment and if a change of T2* can be correlated to a change of VISA-A during follow-up.

There are other techniques and modalities that remain to be more investigated;
We have used a mono-exponential calculation of T2* relaxation time in study IV. Juras et al found a more significant difference of short T2* relaxation component between patients and controls and a stronger correlation of T2* to Achilles tendon rupture score compared the mono-exponential T2*. But the bi-exponential calculation is technically more difficult to implement (Juras 2013, Grosse 2014).

With UTE images information about short T2 components can be calculated directly. By using magnetization transfer this information can be derived indirectly by saturating the short T2 components in the bound water compartment and determining the effect this has on the longer T2 components in the compartment of less tightly bound water (Gatehouse 2007). Magnetization transfer with off-resonance saturation is used to quantitatively measure the off-resonance saturation ratio (OSR) (Grosse 2013, Syha) OSR has been shown to have better sensitivity and specificity for differentiating mild and severe stages of tendinopathy from healthy controls compared to mean T2* (Grosse 2014).

Diffusion tensor imaging and tendon fiber tracking has been used in a retrospective study of patients with Achilles tendon rupture to assess the postoperative tendon microstructure. Diffusion tensor imaging might also be a valuable method to assess chronic tendinosis and response to treatment (Sarman 2015). Dual-energy computed tomography (CT) of Achilles tendinosis with partial tear has also been described (Mallinson 2013). Structural detail of tendons is limited with conventional CT images but with dual-energy CT additional information is provided (Nicolaou 2012). By using two different energy levels the chemical composition may be calculated from the two different x-ray attenuations.

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