Stiffness of the healing human Achilles tendon

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Für Susanne
Table of Contents

1 List of studies .................................................................................................................. 9
2 Abstract ............................................................................................................................. 11
3 Populärvetenskaplig sammanfattning (summary in Swedish) .................................................. 13
4 Introduction ....................................................................................................................... 15
  4.1 Review of the literature ................................................................................................. 15
      4.1.1 Surgical or non-surgical treatment ..................................................................... 15
      4.1.2 Tendon healing and platelet-rich plasma ......................................................... 18
      4.1.3 Early loading ...................................................................................................... 19
  4.2 Mechanical properties ................................................................................................. 22
      4.2.1 Deformation ...................................................................................................... 22
      4.2.2 Stiffness ........................................................................................................... 22
      4.2.3 Strain ............................................................................................................... 22
      4.2.4 Modulus of elasticity ....................................................................................... 22
  4.3 Radiologic evaluation .................................................................................................. 22
      4.3.1 Radiostereophotogrammetric Analysis (RSA) .................................................. 22
      4.3.2 Computed tomography (CT) ........................................................................... 23
5 Aims, with background in brief ....................................................................................... 24
6 Methods ............................................................................................................................ 25
  6.1 Surgical and non-surgical treatment .......................................................................... 25
      6.1.1 Surgical treatment ........................................................................................... 25
      6.1.2 Non-surgical treatment .................................................................................... 26
      6.1.3 Platelet-rich plasma – preparation and injection ............................................. 27
  6.2 Postoperative treatment ............................................................................................... 28
  6.3 Rehabilitation ............................................................................................................. 30
  6.4 Radiologic measurements .......................................................................................... 30
      6.4.1 Radiostereophotogrammetric Analysis ............................................................ 30
      6.4.2 CT and Ultrasound ......................................................................................... 32
  6.5 Functional outcome .................................................................................................... 35
      6.5.1 Heel-Raise index ............................................................................................. 36
      6.5.2 Achilles tendon Total Rupture Score ................................................................. 36
7 Results in brief .................................................................................................................. 38
   A pilot study to find a method to measure mechanical properties of the healing human Achilles tendon...
   ….showing no difference between surgical and conservative treatment, but a greater variation in conservative treatment results...
   …and platelet injection did not improve mechanical properties...
   ….but tensile loading did improve…
   ….and radiodensity reflected the mechanical properties.
8 Discussion ......................................................................................................................... 40
9 Conclusions ..................................................................................................................... 48
10 Future research ............................................................................................................... 49
11 Acknowledgements ......................................................................................................... 50
12 References ..................................................................................................................... 52
13 Appendix ......................................................................................................................... 59
14 Papers I-V ......................................................................................................................... 61
1 List of studies

I
Mechanical properties during healing of Achilles tendon ruptures to predict final outcome: a pilot Roentgen stereophotogrammetric analysis in 10 patients.
Schepull T, Kvist J, Andersson C, Aspenberg P.
BMC Musculoskelet Disord. 2007 Nov 26;8:116.

II
Early E-modulus of healing Achilles tendons correlates with late function: similar results with or without surgery.
Schepull T, Kvist J, Aspenberg P.

III
Autologous platelets have no effect on the healing of human Achilles tendon ruptures: a randomized single-blind study.
Schepull T, Kvist J, Norrman H, Trinks M, Berlin G, Aspenberg P.

IV
Early, controlled tension improves the material properties of healing human Achilles tendons after rupture: a randomized trial.
Schepull T, Aspenberg P
Submitted manuscript

V
Healing of human Achilles tendon rupture: Radiodensity reflects mechanical properties.
Schepull T, Aspenberg P
Submitted manuscript
Abstract

Achilles tendon ruptures in humans are followed by a long period of immobilisation, rehabilitation and limitations of physical activity and sometimes work also. This prolonged period probably leaves a marginal for improvement in the management of this injury. Animal studies have shown that there are several possibilities to influence and improve tendon healing.

The aim of this thesis was to find a way to examine the mechanical properties of the healing human Achilles tendon and, by using that method, to gain a better understanding of the tissue properties and healing process in these tendons. It was also our aim to use our knowledge from animal studies in an attempt to improve tendon healing in humans.

We developed a radiological method using radiostereophotogrammetric analysis (RSA) and computed tomography (CT), which enabled us to measure the stiffness of the healing Achilles tendon. The results of these measurements, as early as 7 weeks after injury correlated with the late clinical results in all studies. Clinical results were measured using a heel-raise test comparing the injured with the non-injured tendon. We could not find a significant difference in stiffness between patients treated surgically or non-surgically. Neither could we demonstrate that platelet-rich plasma improved the mechanical properties of the healing tendon or the clinical outcome, within the limits of the statistical power. In contrast, patients following a specific training programme with early tension loading of the tendon twice a day developed stiffer tendon tissue later in the healing process.

Since RSA is unsuitable for routine clinical use, we evaluated the possibility to use radiodensity findings from CT as a proxy for healing and its correlation to mechanical and clinical results. Density and mechanical properties correlated strongly when analysing all time points together, but only weakly at each particular point in time. Density may still be useful in describing mechanical properties at a later stage of repair, but this remains to be seen.

In conclusion, these studies show that early mechanical properties correlate to late clinical outcome and that the early use of daily tension loading sessions leads to an improvement in the mechanical properties of the tendon tissue.
3 Populärvetenskaplig sammanfattning (summary in Swedish)

Orsaken till bristningar av hälsenan är hittills inte klar, men det verkar finnas flera faktorer av betydelse. De senaste åren har incidensen av bristningar av hälsenan stigit. Troligtvis beror det på att medelålders personer blir allt aktivare. Således borde trenden fortsätta.

En hälseneruptur är i och för sig inte svårbehandlad, oavsett om man väljer en operativ eller icke-operativ behandlingsstrategi, men oavsett behandlingen så följs skadan av en lång och besvärlig tid med immobilisering, rehabilitering och omfattande restriktioner. På grund av denna långa rehabiliteringsperiod skulle det vara av värde att finna möjligheter att förbättra läkningen och förkorta tiden tills patienten kan återgå till arbete eller fritidsaktiviteter.

Det finns en del litteratur om hur patienter med hälsenebristningar mår kliniskt och vilka behandlingsmetoder som leder till bättre resultat. Samtidigt finns det djurförsök, som visar att det är möjligt att påskynda senläkningen med olika metoder.

Syftet med den här avhandlingen har varit att försöka sammanfoga kunskapen från båda dessa delar, dvs. att försöka hitta en metod där man kan mäta mekaniska egenskaper hos läkande hälsenor i människor, få en bättre förståelse för hur senläkning i människor går till, och naturligtvis försöka påskynda senläkningen och följa tiden minska patientens besvär och lidande.

Vi har utvecklat en metod för att undersöka mekaniska egenskaper hos den läkande hälsenan och använt metoden för att studera olika behandlingsstrategier. Vi har även försökt att finna en metod som är mer användbar i klinisk rutin.

Vi har inte kunnat påvisa en skillnad mellan operativ eller icke-operativ behandling, och vi har inte sett att injektion av blodplättar har förbättrat senans mekaniska egenskaper, men däremot har tidig belastning lett till en förbättring.

I alla studierna har vi funnit att det finns ett samband mellan tidiga mekaniska egenskaper och kliniska resultat efter 1 år respektive 18 månader.

Även röntgentäthet, mätt med en CT-undersökning, har ett samband med mekaniska egenskaper hos den läkande senvävnaden.

Slutsatsen är att det verkar avgöras tidigt hur slutresultatet ska bli efter en hälsenebristning, eftersom de sena, kliniska resultaten har ett samband med våra tidiga mätningar. Vi har funnit stöd för att tidig, kontrollerad belastningsträning, som i tidigare kliniska studier har visat sig allmänt gynnsam, även förbättra senvävnadens egenskaper.
4 Introduction

Following the saga of the Greek mythological hero Achilles, “an Achilles heel” has become synonym for a significant weakness, often in terms of performance. Rupture of the Achilles tendon was fatal for Achilles, but in orthopaedic terms this is, of course, far from deadly. Indeed since it may heal easily through, for example, simple immobilisation, it is not even regarded as a severe injury. Nevertheless, for patients suffering from this kind of injury, it is an unpleasant and troubling condition since it leads to a long period of immobilisation and rehabilitation, often with only moderately good results. Due to this slow and prolonged healing process, alternative treatment possibilities for this injury are subject to broad research and discussion, since a speeding up of the healing process is desirable. At present there is no standard treatment for Achilles tendon rupture (Kearney and Costa 2012). Moreover, Achilles tendon rupture is an interesting field of research as it stands for 40% of all tendon operations (Jozsa, et al. 1989).

In view of the fact that research on Achilles tendon ruptures has been going on for a long time, it is surprising that there is no consensus on how to treat an Achilles tendon rupture. One of the first to describe an Achilles tendon rupture was probably the French barber surgeon Ambroise Pare in 1575, when a collection of his works was published. He tried to treat Achilles tendon ruptures using certain bandages with wine and spices (Quenu J 1929; Ronel, et al. 2004). In 1929 a study by Quenu and Stoianovich was published advocating surgical repair (Popovic and Lemaire 1999). Since then, many studies on the treatment of Achilles tendon rupture have been published, especially since the Eighties (Medline search for “Achilles Tendon Rupture” for the last 5 years gives approximately 500 results). In 1972 Lea and Smith (Lea and Smith 1972) stated that surgical treatment of a ruptured Achilles tendon is not necessary, and in 1981 Nistor et al. (Nistor 1981) showed no significant difference between surgical and non-surgical (conservative) treatment in a randomised trial.

The functional outcome of the treatment of Achilles tendon rupture in man has largely been evaluated using questionnaires (Suchak, et al. 2008) and scores involving movement of the ankle (Leppilahti, et al. 1998; Nilsson-Helander, et al. 2007; Thermann, et al. 1995), but not much is known about the mechanical properties of the healing tendon tissue in humans. Since this is an interesting and important field, several recent studies have addressed this question (Hansen, et al. 2013; Kongsgaard, et al. 2011b). There are, however, several animal studies that have provided information about tendon properties during healing (Andersson, et al. 2012; Krapf, et al. 2012; Obst, et al. 2013; Sahin, et al. 2012). The question arises; is it possible to measure tendon properties in humans, and if so, do these findings give us any useful information that may be used to improve tendon healing?

4.1 Review of the literature

4.1.1 Surgical or non-surgical treatment
The study published by Quenu and Stoianovich in 1929 “Les ruptures du tendon d’Achille” (Quenu J 1929) was probably the first article discussing the surgical and non-surgical treatment alternatives for this injury. It is interesting, that discussions regarding the pros and cons of surgical and non-surgical treatment are still going on.
Surgical treatment can be divided into open surgical repair and minimally invasive repair, first introduced by Ma et al. (Ma and Griffith 1977). Open repair seems to be the most common choice, while minimally invasive repair seems to be primarily chosen for cosmetic reasons (Wong, et al. 2002). It is not clear if minimally invasive repair can decrease the complication rate seen with open surgery, as various studies show different results (Gigante, et al. 2008; Khan, et al. 2005; Lansdaal, et al. 2007; Metz, et al. 2008). Non-surgical (conservative) treatment involves simple immobilisation in a plaster cast or orthosis, initially often in the equinus position and neutral later on.

Despite the fact that surgical repair might lead to improved functional results (Costa, et al. 2006) and seems to provide a more rapid return to sporting activities (Cetti, et al. 1993) and heavy work (Moller, et al. 2001), surgical and non-surgical treatments show comparable clinical outcomes (Bhandari, et al. 2002; Keating and Will 2011; Pajala, et al. 2002). This is a strong argument in favour of conservative treatment, as complications related to surgery are avoided and the cost of treatment seems to be lower. Surgical repair, however, has one argument that weighs heavily in favour; in almost all studies, surgery decreases the risk for rerupture of the tendon during the healing process. In a study by Möller et al. (Moller, et al. 2001) the risk for rerupture increases significantly from 1.7 % for operated patients to 20.8 % for non-operated patients. The risk for rerupture after conservative treatment varies greatly between studies (Ingvar, et al. 2005; Jacobs, et al. 1978; Moller, et al. 2001; Wallace, et al. 2004) but it is a strong argument against conservative treatment, since rerupture returns the patient back to the beginning of treatment, with loss of the immobilisation period and usually some weeks of rehabilitation too. In other words, the patient’s rerupture must be treated as a new tendon rupture. Moreover, the outcome after treatment of a rerupture is satisfactory but poorer than after successful primary repair (Pajala, et al. 2002). In the study by Ingvar et al from 2005, rerupture occurred between 0 and 140 days after cast removal, on average after 51 days. Four of the reruptures occurred within 13 days after removal of the orthosis, and the remaining 10 reruptures were evenly spread up to 140 days (Ingvar, et al. 2005).

Another argument for surgical treatment has been the fact that surgeons have recommended more aggressive rehabilitation (Yotsumoto, et al. 2010) after surgery than after conservative treatment. Earlier, more aggressive rehabilitation could lead to a sooner return to work and physical activity. Recent studies seem to show that patients being treated conservatively also have a better clinical outcome after early rehabilitation (Nilsson-Helander, et al. 2010). Moreover, it seems that early, functional rehabilitation decreases the risk for rerupture in non-surgically treated patients (Wallace, et al. 2004). So it is uncertain if the rerupture argument, favouring a surgical solution, is really correct. Moreover the advantage of conservative treatment is obviously the lack of complications related to surgery such as adhesions, disturbed sensibility, and deep or superficial wound infection (Bhandari, et al. 2002; Khan, et al. 2005).

In some cases, decisions on how to treat the patient are simple as there are absolute or relative contraindications to surgery. What, actually, are the indications for surgical repair of a total Achilles tendon rupture? It seems that there is great regional variation; where different hospitals try to establish their own model for how identify patients that should be operated on. Young and active people are usually considered to be appropriate patients for surgery (Wong, et al. 2002). The surgeon aims to get the patient back to work and activity as soon as possible (but does surgery really provide a sooner return to activity? – see above). Furthermore, the risk for complications, primarily infection, is lower in younger patients than in older ones.
(Leppilahti, et al. 1998). But so far there is no evidence showing that younger and active patients are the ones that benefit most from surgery. Table 1 shows a survey of review articles that have compared surgical and non-surgical treatment.

Table 1: Meta-analysis and reviews comparing surgical (OP) and non-surgical treatment (Non-OP).

<table>
<thead>
<tr>
<th>Author</th>
<th>Methods</th>
<th>Included n</th>
<th>Results</th>
<th>Authors conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wills 1986</td>
<td>Literature 1959-84</td>
<td>20 studies</td>
<td>Rerupture rate 1.5% (OP) vs. 17.7% (Non-OP)</td>
<td>Prefer OP: decreased rerupture rate; Non-OP for risk patients</td>
</tr>
<tr>
<td>Lo 1997</td>
<td>Medline (66-97) and Index Medicus (59-65); inclusion discussed by 4 authors</td>
<td>2 RCT, 17 case series</td>
<td>Rerupture rate higher in Non-OP; complications in OP 20 times higher;</td>
<td>Prefer Non-OP: offer healthy active patients both; individual treatment plan incl. patient “belief”;</td>
</tr>
<tr>
<td>Wong 2002</td>
<td>Medline search 1966-2000; articles graded using a validated score</td>
<td>125 articles</td>
<td>Rerupture rate highest in Non-OP + immobilised groups, complications highest in open repair groups</td>
<td>Open OP + early mobilisation, individual treatment plan, OP if necessary; percutaneous repair for cosmetic reasons; Non-OP for the elderly</td>
</tr>
<tr>
<td>Bhandari 2002</td>
<td>Medline, Grateful Med, Cochrane search (1969-2001) and major orthopaedic journals; rating following scale</td>
<td>6 RCT</td>
<td>OP: 68% reduction in relative risk or re-rupture, but 4.7% incidence of infections</td>
<td>Favouring OP (infections can be treated successfully + consequences of re-rupture more serious)</td>
</tr>
<tr>
<td>Lynch 2004</td>
<td>Medline, Embase, Cinahl, Cochrane, Google, bibl. search, hand search, internet journal search</td>
<td>5 RCT, 6 non-rand 5 reviews</td>
<td>OP: Better functional outcome; OP: Less re-rerupture; Early mobilisation: Better functional outcome; OP: Large incidence of other complications, but no effect on functional outcome</td>
<td>Prefer OP and early mobilisation; Non-OP if patients refuse or are “unfit”</td>
</tr>
<tr>
<td>Khan 2005</td>
<td>Cochrane, Medline, Embase, Cinahl and manual search</td>
<td>12 RCT</td>
<td>Open OP vs. Non-OP: Rerupture: 3.5% vs. 12.6 and infection 34.1% vs. 2.7; Open OP vs. percutaneous OP; Rerupture: 4.3% vs. 2.1 and infection 19.6% vs. 0.9; OP: Cast vs. functional bracing; Rerupture: 5% vs. 2.3% and infection 3.5% vs. 3.0; Non-OP Cast vs. functional bracing; Rerupture: 12.2% vs. 2.4%</td>
<td>Open OP reduces risk for re-rupture but higher risk for other complications. Complications reduced by percutaneous surgery. Postoperative functional brace reduces overall complication rate.</td>
</tr>
<tr>
<td>Soroceanu 2012</td>
<td>Cochrane, MEDLINE, Web of Science, and Embase (2005 – 2011)</td>
<td>10 RCT</td>
<td>Functional rehabilitation: Rerupture equal for OP vs. Non-OP (risk difference = 1.7%; p = 0.45); No functional rehabilitation: Risk difference 8.8% (p = 0.001 in favour of surgery). OP: Other complications 15.8%</td>
<td>Centres using functional rehabilitation: Non-OP, otherwise OP.</td>
</tr>
<tr>
<td>Wilkins 2012</td>
<td>MEDLINE, Cochrane, ACP Journal Club; Review Database</td>
<td>7 RCT</td>
<td>Open OP vs. Non-OP: Lower re-rupture rate (3.6% vs. 8.8%; odds ratio 0.4)</td>
<td>Future studies: Focus on testing strength (functional and reproducible) instead of isokinetic testing</td>
</tr>
</tbody>
</table>

It seems clear that conservative treatment, with a plaster cast or orthosis, leads to a significantly higher risk for reruptures, but that non-surgical treatment combined with early, functional rehabilitation decreases the risk for rerupture to a level similar to that after surgical repair (Soroceanu, et al. 2012). Therefore, since rerupture is still seen to be the main complication and problem in the management of Achilles tendon ruptures, recent studies...
recommend non-surgical treatment in centres where early, functional rehabilitation is available, whereas in other centres surgical treatment is preferable (Soroceanu, et al. 2012).

4.1.2 Tendon healing and platelet-rich plasma

Histologically, tendon tissue is composed of collagen fibres arranged in parallel. The fibres mainly consist of collagen (98% collagen Type I) but also elastin and proteoglycans. Why Achilles tendons rupture remains unclear, but there are several factors that might play an important role. Histological examinations of ruptured Achilles tendon tissue have shown degenerative changes in the tissue (Lynch 2004; Tallon, et al. 2001), where density and diameter of the fibrils are reduced compared to normal tendons and Type I collagen is partially replaced by Type III collagen in injured areas (Leadbetter 1992; Liu, et al. 1995). Other tissues are found between the fibres as well as microfractures within the tendon (Jozsa and Kannus 1997). Tendons are poorly vascularised but there are blood vessels usually running parallel to the collagen fibres. Vascularisation decreases with age (Carr and Norris 1989). Moreover, in the typical rupture site, vascularisation is often poorer at the narrowest point of the tendon (Ahmed, et al. 1998). Consequently, vascularisation might play an important role in rupture of the Achilles tendon.

Whether or not poor vascularisation is also a reason for the slowness of the healing process is unclear (Fernandez-Sarmiento, et al. 2013), but the stages of repair in tendons, as in other tissues, are inflammation, followed by proliferation and remodelling. All stages are signified and accompanied by different gene expressions. An important part of the healing process is the release of a broad cocktail of different growth factors from activated platelets (Molloy, et al. 2003) probably leading to increased cellular migration, proliferation, angiogenesis and matrix deposition (Baksh, et al. 2013; McCarrel and Fortier 2009).

Many studies have been performed showing that PRP has a positive effect on bone, wound, and soft tissue healing (Baksh, et al. 2013). Moreover, in vivo animal studies have tested the effect of platelet-rich plasma on the mechanical strength of tendon tissue and showed an increase in load to failure (Lyras, et al. 2009; Spang, et al. 2011; Virchenko and Aspenberg 2006). This has lead to the speculation that platelet-rich plasma could help human patients with tendon injuries to recover faster and go back to work or activity sooner. While various animal studies concur that platelet-rich plasma has a positive effect on tendon healing (Aspenberg and Virchenko 2004; Fernandez-Sarmiento, et al. 2013), results conflict when it comes to the healing of human tendon tissue (Foster, et al. 2009; Mei-Dan, et al. 2010b). While Sanchez et al. (Sanchez, et al. 2007) showed a convincing positive effect, when using platelet-rich plasma, others were not able to demonstrate a positive effect of platelet treatment on healing tendons in humans (de Jonge, et al. 2011; de Vos, et al. 2010; Schepull, et al. 2011).

One reason why it would be so convenient to use platelet-rich plasma, is the fact that it is easy, fast and safe to produce from the patient’s own blood using a simple centrifuge. Unfortunately, this has also led to commercial interest, since all of the sudden there is a market for centrifuges.
4.1.3 Early loading

For many years, research and discussions have focused on the topic whether surgical or non-surgical treatment should be chosen in patients with an Achilles tendon rupture. In later years, research has focused more on trying to improve rehabilitation, regardless of whether surgical or non-surgical treatment is chosen.

One track that researchers have been and still are following is trying to find ways to improve healing of the tendon tissue by pharmacological or biological means. Animal studies have been able to show that pharmacological treatment such as the use of COX-inhibitors has the potential to affect healing tendons (Carlstedt, et al. 1987; Forslund, et al. 2003; Virchenko, et al. 2004). Furthermore, biological manipulation such as the use of platelet concentrate, has been shown to affect the healing process (Aspenberg 2007; Aspenberg and Virchenko 2004; Baksh, et al. 2013; Fernandez-Sarmiento, et al. 2013; Forslund and Aspenberg 2003; Mei-Dan, et al. 2010a; Virchenko and Aspenberg 2006; Virchenko, et al. 2006). But so far there hasn’t been a breakthrough when it comes to treatment of tendon ruptures in humans.

Research nowadays is focusing much on the use of early functional treatment after Achilles tendon rupture, already described by Marti and Weber in 1974 (Marti and Weber 1974), instead of total immobilisation using a plaster cast. An important reason for this is that extracellular matrix turnover is influenced by physical activity (Kjaer, et al. 2005) and the positive effect of mechanical stimulation has been well described (Aspenberg 2007). Already in the 80’s (Enwemeka, et al. 1988; Mabit, et al. 1986) the effect of early functional treatment on tendon tissue was documented, and in 1992 Saleh et al. (Saleh, et al. 1992) published a randomised, controlled trial favouring early mobilisation versus plaster cast treatment. Many studies have followed, showing that early functional rehabilitation improves early patient satisfaction without increasing the risk for rerupture in operated patients. Early functional rehabilitation may even decrease the rate of rerupture, at least in conservatively treated patients (Soroceanu, et al. 2012; Twaddle and Poon 2007; Wallace, et al. 2004), at the same time as it does not increase the rate of other complications (Suchak, et al. 2006) such as infection or nerve damage. It is still not clear if early functional treatment really leads to a significant improvement in the final outcome (Kangas, et al. 2007), but early gains have been shown, and this plays an important role in increasing patient satisfaction as they are able to return to work and physical activity sooner.

The main problem with early, functional treatment is the fact that we are not certain what this form of management actually involves. Functional rehabilitation is often a combination of range of movement training, without overstretching the healing tendon, and weight-bearing (Kearney, et al. 2012b). The extent, to which movement training and weight-bearing are allowed, if at all, varies greatly between countries, regions or even hospitals (see Table 2).

Moreover, it is still unclear what the relationship is between weight-bearing and the forces affecting the Achilles tendon, as it may be possible to put a load on the foot without causing any tension forces within the tendon tissue, i.e. increase in weight-bearing might not necessarily lead to a greater mechanical load on the healing tendon.

There is a great variation in different treatment regimes in the literature, and no clear manual for the treatment of Achilles tendon rupture is identifiable. Attempts are being made to solve this problem, as larger groups such as the American Academy of Orthopedic Surgeons have published guidelines for treatment (Chiodo, et al. 2010). Moreover there are guidelines from
various universities e.g. University of Wisconsin: Rehabilitation Guidelines for Achilles Tendon Repair, or even hospitals. But a glimpse at recent publications shows that these guidelines are not followed in detail. Table 2 is a summary of important publications on early functional rehabilitation after Achilles tendon rupture, showing the differences in range of motion and weight-bearing.

Most studies on humans have understandably focused on measuring the clinical outcome after functional rehabilitation and weight-bearing, but not much is known about the changes in mechanical properties of the healing human Achilles tendon caused by range of movement training and loading. On the other hand, animal studies have shown that loading leads to up-regulation of gene-expression and production of collagen, increasing callus thickness and tensile strength of the healing tendon (Eliasson, et al. 2012; Kjaer, et al. 2005; Yang, et al. 2004). At present it is unknown if weight-bearing and functional treatment in humans does in fact lead to stress in the tendon, thereby improving the healing process. Patients would definitely benefit from early functional rehabilitation if it increases tendon strength, as this should reduce the risk for rerupture.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>0-2 weeks</th>
<th>2-4 weeks</th>
<th>4-6 weeks</th>
<th>6-8 weeks</th>
<th>8-10 weeks</th>
<th>10-12 weeks</th>
<th>&gt;12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortensen</td>
<td>1999</td>
<td>No</td>
<td>No</td>
<td>As tolerated with crutches + heel stretch</td>
<td>Boot + crutches + heel stretch</td>
<td>Full</td>
<td>Full</td>
<td>Full</td>
</tr>
<tr>
<td>Maffulli (group 1)</td>
<td>2003</td>
<td>Full on tip toes as tolerated with crutches</td>
<td>Full as tolerated with crutches</td>
<td>Synthetic slab + exercise several times a day</td>
<td>Synthetic slab + exercise several times a day</td>
<td>ROM</td>
<td>ROM</td>
<td>ROM</td>
</tr>
<tr>
<td>Costa</td>
<td>2006</td>
<td>Full as tolerated with crutches + heel stretch</td>
<td>Full as tolerated with crutches + heel stretch</td>
<td>Full as tolerated with crutches + heel stretch</td>
<td>Full as tolerated with crutches + heel stretch</td>
<td>wean crutches</td>
<td>Full</td>
<td>Full</td>
</tr>
<tr>
<td>Saleh</td>
<td>1992</td>
<td>Equinus cast</td>
<td>Equinus cast</td>
<td>Brace (neutral to 30° flexion with elastic band)</td>
<td>Brace (neutral position + active, unloaded)</td>
<td>ROM</td>
<td>ROM + stretching</td>
<td>ROM + stretching</td>
</tr>
<tr>
<td>Lansdaal</td>
<td>2007</td>
<td>Full after one week plaster cast, then tape/soft cast</td>
<td>Full</td>
<td>Full</td>
<td>Full</td>
<td>Full</td>
<td>Full</td>
<td></td>
</tr>
<tr>
<td>Suchak</td>
<td>2008</td>
<td>Equinus splint</td>
<td>Equinus splint</td>
<td>Orthosis 20° flexion gradually reduced; ROM twice/day</td>
<td>Orthosis 90°, ROM twice/day</td>
<td>Full</td>
<td>Full</td>
<td>Full</td>
</tr>
<tr>
<td>Nilsson-Helander</td>
<td>2010</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>As tolerated</td>
<td>Full + heel-lift</td>
<td>Full + heel-lift</td>
<td>Full + heel-lift removed after 16 weeks</td>
</tr>
<tr>
<td>Willis</td>
<td>2010</td>
<td>No</td>
<td>Full with crutches</td>
<td>As tolerated</td>
<td>Full</td>
<td>Full</td>
<td>Increased dynamic</td>
<td></td>
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4.2 Mechanical properties of tendon tissue

As rerupture is the main problem during rehabilitation after Achilles tendon rupture, it is useful to have basic knowledge about deformation and consequences of deformation on mechanical tissue properties. Applied forces lead to stress and strain of tissues, and when forces become too high the tissue fails. In our patients, failure means rerupture followed by a prolonged healing process. The deformation of a tissue is based on intrinsic factors such as material properties and structural factors such as size (i.e. cross sectional area) and shape.

4.2.1 Deformation
Deformation describes a change in shape of tissue when exposed to a force. There are different kinds of deformation: elastic deformation is reversible, meaning that the tissue returns to its former shape when the force is removed; plastic deformation is irreversible, meaning the tissue remains deformed when the force is removed. Viscoelastic deformation is a combination of elastic deformation and viscous behaviour of a body, where viscosity is a measure for the “thickness” of the fluid part of the material.

4.2.2 Stiffness
Stiffness describes the resistance of a tissue to elastic deformation.

4.2.3 Strain
Strain is the deformation of tissue due to an externally applied force. The strain, described in this thesis is longitudinal only, meaning that the force leads to elongation of the tendon tissue.

4.2.4 Modulus of elasticity
In these studies we have assumed that the Achilles tendon is deformed elastically. Therefore, the modulus of elasticity seemed to be an ideal variable to describe the material properties of the healing tendon, combining strain and cross-sectional area.

Modulus of elasticity in mechanics is a characteristic value describing the relation between stress and deformation for a certain material, under the assumption that the material is elastic. Modulus of elasticity is often referred to as elastic modulus, or as its abbreviation e-modulus or also as Young’s modulus. But Young’s modulus is in fact only one type of elastic modulus, namely the tensile elasticity that was used in these studies. Bulk modulus and shear modulus are also contained within the group of e-moduli.

4.3 Radiologic evaluation

4.3.1 Radiostereophotogrammetric Analysis (RSA)
RSA was further developed in 1972 by Selvik in Lund, Sweden (Selvik 1990). It is a method in radiology, where two x-ray pictures of a region where tantalum markers (beads) are inserted are taken simultaneously. At the same time, a calibration cage is used, containing tantalum beads as reference markers. By analysis of both x-ray pictures in a computer system, the position of the tantalum beads inserted into the tissue (in our studies the healing Achilles tendon) can be converted into a three-dimensional system with high accuracy. This technique is usually used with rigid bodies to measure displacement of implants or between different parts of the body. In our case it provided a simple means to measure the distance between two
points on either side of the Achilles rupture and thus to ascertain the strain-per-force during the healing process.

RSA has been used as an examination technique in many different research fields, e.g. examination of growth zone changes, scoliosis measurements, implant displacement of joint replacements, fusion stability, and kinematics of different joints (Bragdon 2003).

4.3.2 Computed tomography (CT)
CT is an imaging technique in radiology, showing cross-sectional pictures of the scanned object. In comparison with conventional x-ray pictures, it offers the possibility to evaluate soft tissues as well, and it provides much better contrast between different tissues. In the healing tendon, it clearly shows demarcation of the tendon tissue against the surrounding soft tissues, allowing cross-sectional area measurement of the tendon. Moreover, the computer programme allows the measurement of tissue density, with a standard deviation, the latter giving information about the variation within the region of measurement.

CT was invented in 1972 by Sir Godfrey Hounsfield (Hounsfield 1980), based on a mathematical theory by Johann Radon. For his invention of the CT scan, Hounsfield later received the Nobel Prize.
### 5 Aims, with background in brief

<table>
<thead>
<tr>
<th><strong>Background</strong></th>
<th><strong>Aim</strong></th>
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<tbody>
<tr>
<td>Tendon healing in humans has not been described in terms of tissue properties. Most methods of evaluation are based on clinical findings.</td>
<td>To find a technique to examine the mechanical properties of the healing, human Achilles tendon after rupture.</td>
</tr>
<tr>
<td>There is still no consensus on whether surgical or non-surgical treatment is to be preferred.</td>
<td>To see if surgery improves tendon tissue properties during the healing process compared to conservative management.</td>
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<tr>
<td>Platelet-rich plasma improves healing tendon tissue in animals due to a variety of growth factors. Moreover, the first published clinical study in humans showed a positive effect.</td>
<td>To see if the use of platelet concentrate improves human Achilles tendon healing after rupture.</td>
</tr>
<tr>
<td>Loading improves the mechanical properties of the healing Achilles tendon in animals, and the clinical results in humans.</td>
<td>To examine the effect of early tension loading on the mechanical properties of the healing Achilles tendon in humans.</td>
</tr>
<tr>
<td>There is at present no quantitative radiological technique to measure tendon healing. As tendon tissue can be roughly described as consisting of collagen and water, changes in radiodensity might reflect the healing process.</td>
<td>To see if radiodensity measurements from CT can describe and measure the healing process after Achilles tendon rupture.</td>
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</table>
6 Methods

Patients with a total rupture of the Achilles tendon after a sports trauma were included. Patients with direct trauma, such as cutting were not included. Patients had to be between 18 and 60 years of age, with no history of rheumatic, lung-, heart- or malignant disease, diabetes mellitus or other disease affecting the locomotor system. Patients with a previous history of Achilles tendon rupture on either side were also excluded. Patients had to be included in the studies within 5 days after injury. Table 3 is an overview of the number of patients included and examined at different time points.

Table 3: Synopsis showing number of patients in the studies and at different measurements time points (e-mod = Modulus of elasticity; HU = Hounsfield Units; HRI = Heel-Raise index % compared to other side; ATRS = Achilles tendon Total Rupture Score; strain/force = strain per force).

<table>
<thead>
<tr>
<th>Study</th>
<th>Incl. (n)</th>
<th>Mechanical exam. 1</th>
<th>Mechanical exam. 2</th>
<th>Mechanical exam. 3</th>
<th>Mechanical exam. 4</th>
<th>Functional exam.</th>
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<td>e-mod</td>
<td>e-mod</td>
<td>e-mod</td>
<td>HRI</td>
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<td></td>
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<td>-</td>
<td>e-mod</td>
<td>strain/force</td>
<td>HRI+ATRS</td>
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<tr>
<td>Time</td>
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<td>e-mod</td>
<td>e-mod</td>
<td>HRI+ATRS</td>
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<tr>
<td>Time</td>
<td>7 weeks</td>
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<td>-</td>
<td>e-mod</td>
<td>e-mod</td>
<td>HRI+ATRS</td>
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<td>Time</td>
<td>7 weeks</td>
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<tr>
<td>Study V</td>
<td></td>
<td>HU+e-mod</td>
<td>-</td>
<td>HU+e-mod</td>
<td>HU+e-mod</td>
<td>HRI+ATRS</td>
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<td>Time</td>
<td>7 weeks</td>
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6.1 Surgical and non-surgical treatment

6.1.1 Surgical treatment
All patients were operated on, except the 15 patients randomised to non-surgical (conservative) treatment in Study II. The surgical procedure was the same for all patients in all studies. All patients were operated on under local anaesthesia. To decrease the risk for nerve injury, a dorso-medial approach was chosen. The tendon stumps were adapted with a thick absorbable suture using a single loop Kessler suture technique (Figure 1). In some cases approximation of the tendon stumps was enhanced with a few simple, single, absorbable sutures. Vicryl® was used both as main suture and as complementary suture since Vicryl
holds its tensile strength for a limited number of weeks only and is totally absorbed by hydrolysis at approximately 60 days. The choice of suture material was made in order to be sure that only the tensile properties of the tendon tissue were tested and not those of the suture used during surgery. According to our own measurements (not reported) the e-modulus of Vicryl Size 0 is about 10 MPa, compared to about 100 MPa in the healing tendon at 7 weeks. The load at rupture was 100 – 120 N for the double suture. In a study with in vivo incubation of suture material, Vicryl showed no remaining structure after 6 weeks (Greenwald, et al. 1994). In summary, the thread may have kept the stumps adapted at rest, but must have been unable to take the loads applied during the training programme.

After suture of the tendon tissue, 4 tantalum beads with a diameter of 0.8 mm were implanted in the tendon tissue. Two beads were placed in the proximal and two in the distal tendon stump. The paratenon was then closed thoroughly, the subcutaneous tissue was adapted and the skin closed with an intracutaneous, absorbable suture. The patients were provided with a short leg plaster cast in the equinus position.

6.1.2 Non-surgical treatment
In Study II, approximately half of the patients were randomised to non-surgical treatment. After examination in the emergency room, the two tendon stumps were outlined on the skin with a permanent marker pen (Figure 2).
While still in the emergency room, the patients were immediately provided with a short leg plaster cast, with the foot in the equinus position (just the same as the patients that were operated on). After 3.5 weeks the cast was changed to a short leg cast with the foot in the neutral position (Figure 3).

![Figure 3: Cast with foot in equinus position (red) and in neutral position (blue).](image)

During the cast change, 4 tantalum beads were implanted into the healing tendon. The permanent pen marks, previously sketched onto the skin in the emergency room, were used as reference points to place two beads in the proximal and two in the distal part of the tendon. The tantalum beads were implanted in the healing tendon tissue through small incisions under local anaesthesia. After 3.5 weeks with the foot in the neutral position, the cast was removed.

6.1.3 Platelet-rich plasma – preparation and injection

All patients in Study III were sent to the Department of Transfusion Medicine the day before surgery. A unit of whole blood was collected from the patients, and platelet-rich plasma was extracted. After this process, the platelet-rich plasma was stored until the day of surgery and the platelet concentration was measured.

On the day of surgery, the platelet-rich plasma was picked up at the Department of Transfusion Medicine just prior to surgery. During surgery, after the tendon stumps had been sutured, the patients were randomised to platelet-rich plasma treatment or a control group. In the treatment group, the platelet-rich plasma was injected directly into the tendon tissue during surgery (Figure 4).
6.2 Postoperative treatment

All operated patients, except the patients in the loading group in Study IV, were treated postoperatively with a plaster cast in the equinus position for 3.5 weeks, and in the neutral position for a further 3.5 weeks. Weight-bearing, as tolerated, was allowed from the beginning.

Patients, who were postoperatively randomised to the loading group in Study IV, were provided with a short leg cast in the equinus position, similar to the control group. The cast was removed after 2 weeks and replaced by a foam walker (Figure 5). The foam walker was used with heel wedge inlays (Figure 5) to obtain an equinus position of the ankle joint.
The patients used the foam walker for 5 weeks resulting in a total of 7 weeks immobilisation (similar to the control group). The heel wedge inlays were gradually removed. Weight-bearing, as tolerated, was allowed from the beginning. Moreover, patients in the loading group were instructed to remove the foam walker twice a day and use a training pedal. The training pedal was constructed so that pressure was put on the forefoot, leading to controlled loading of the healing Achilles tendon. The load was increased each week (Figure 6).
6.3 Rehabilitation

After cast removal all patients were treated by a physiotherapist following a standard rehabilitation programme. There were no differences between the treatment and control groups in the three randomised studies (Studies II, III and IV) as regards the rehabilitation programme after cast removal. Patients in the loading group (Study IV) were allowed to progress more rapidly in the rehabilitation programme since they had already trained the ankle during the immobilisation period with the foam walker. Some patients in the loading group skipped the first week of the rehabilitation programme and went directly to the second week.

6.4 Radiologic measurements

6.4.1 Radiostereophotogrammetric Analysis (RSA)

RSA was used at different points in time after cast removal in order to measure strain on the healing tendon under controlled loading. A pedal was applied to the forefoot and the forefoot had to resist a force, keeping the foot in one position (Figure 7).

![Figure 7: Frame used to keep foot in position during RSA; loading of the forefoot using strings.](image)

The height of the foot could be adjusted so that the patient felt that the point of pressure of the pedal fitted their foot size (Figure 8).
As this change in height affected the lever arm and thereby force on the tendon, it was included in the calculation of the lever arm (see below). To begin with, a force of 25 N was applied as a baseline instead of no force at all, assuming that 25 N would be enough to lead to relaxation of the dorsal flexor muscles. Thereafter the main force was applied (Study I 200 N and in Studies II and III 150 N) (Figure 10). Each force was applied for 15 seconds before the RSA picture was taken. There was a pause of 3 minutes between loadings. This procedure was repeated in Study I, II and III (double measurements to control method).
After one year a slightly different protocol was used, because the tendon at 1 year is stiffer and a higher load is necessary to achieve a strain that can be measured with sufficient precision. Therefore, a sequence of 25, 125, 225, 335 and 425 N was performed, also 15 seconds of loading with a 3-minute pause between. To minimize radiation, the 125 and 335 N x-rays were skipped from Study 3.

Using the UmRSA® system, the distance between the tantalum beads on both sides of the rupture was measured. Having values for baseline (25 N) and for the experimental forces (see above), it was possible to calculate the strain of the healing tendon under a defined force (expressed as strain-per-force) from the regression lines of each sequence.

In Studies I, II and III baseline and applied force examinations were performed twice, in order to analyse reproducibility. Moreover, the first pair of forces served as a standard preconditioning exercise for all patients in order to achieve similar starting conditions prior to the second sequence. In order to decrease the number of X-ray exposures, the repeated RSA examinations were skipped in Study IV. However, even in study IV the patients went through the same sequence of loadings for preconditioning, but no x-rays were taken during the first sequence.

6.4.2 CT and Ultrasound

CT measurements were primarily done to determine the correct position of the tantalum beads in Studies I and II. In later studies it was also used for cross-sectional area measurements. Using the cross sectional CT pictures, the healing tendon tissue could be followed and tantalum beads positioned outside the tendon could be eliminated from the calculations (Figure 11).

In order to be able to calculate strain-per-force on the tendon, at least one tantalum bead on each side of the rupture site had to be correctly positioned in the tendon. The four beads were numbered 1 to 4, where bead 1 was the most proximal bead and bead 4 the most distal bead.
This means that beads 1 and 2 were implanted above the rupture and beads 3 and 4 below the rupture (Figure 12).

Ultrasound was used to measure the cross-sectional area of the tendon in Studies I and II. A correlation was found between the ultrasound results and the CT results, but it was not certain if ultrasound magnified the cross-sectional area or scaled it down compared to CT. Moreover, as CT was required to inspect the correct position of the beads, and as our access to ultrasound and radiologist was restricted, the decision was made to do all cross-section measurements using CT in Studies III and IV (Figure 13). This decision was approved by the hospital radiation safety committee. All ultrasound measurements were performed by one experienced radiologist. All measurements of cross-sectional area using CT were performed by the author. The region of measurement chosen was the middle section between beads 2 and 3.

The values for Hounsfield units in Study V were taken from the same region as the cross-sectional area measurements (Figure 13) and moreover, on various levels, in order to get a broad view of the density of the whole healing area of the tendon and to confirm that during the healing process density was similar along the complete region of interest (Figure 14).
Sagittal CT scans were used to determine the distance between the pivoting axis of the ankle joint and the middle of the tendon, related to the size of the foot, in order to determine the lever arm and in order to calculate the tension in the tendon, when the force on the forefoot was defined (Figure 15).
6.5 Functional outcome

Although our patients were included in a study, they were treated, as far as possible, as other patients with an Achilles tendon rupture at our department. Patients were followed by a physiotherapist, measuring different clinical outcome variables at different points in time. Most of the variables were measured and documented in Study I in order to get a broad view on the patient’s recovery after treatment, and to see any possible correlation between late measurable clinical values and early mechanical properties of the Achilles tendon. These measurements included range of motion, diameter of the calf muscle, heel-raising, pain, limp, sensitivity and gait pattern. In the later studies these examinations were performed for clinical purposes only. Nevertheless, a correlation between mechanical properties with functional outcome seemed interesting. A slightly modified heel-raise endurance measurement (Moller,
et al. 2005), called the Heel-Raise index, was chosen together with the validated Achilles tendon Total Rupture Score (ATRS) (Nilsson-Helander, et al. 2007) as our main clinical parameters.

6.5.1 Heel-Raise Index
The ability to perform a heel-raise appears to be an important early achievement and could possibly reflect the degree of healing (Olsson, et al. 2012). The heel-raise endurance test has been shown to have a good ability to find differences between the injured and non-injured Achilles tendon after rupture (Moller, et al. 2005). Moreover, it shows good validity (Silbernagel, et al. 2010). It is a product of the height of a heel-raise and the number of heel-raises possible.

A heel-raise endurance test was used, where the number of possible heel-raises of at least 5 cm elevation from the floor was counted. After a short break it was followed by one single, maximal heel-raise. The factor of height and number of repetitions was calculated (Figure 16) and "our" Heel-Raise index was then determined as a percentage, comparing the injured side with the non-injured side.

![Heel-Raise index; maximal height (h) multiplied by numbers of repetitions (n).](image)

6.5.2 Achilles Tendon Total Rupture Score (Appendix 1)
The Achilles tendon Total Rupture Score (ATRS) is a Score based on 10 questions. Five questions contain information about physical activity and five questions about symptoms after Achilles tendon rupture treatment. Each question is answered on an 11-grade scale. Consequently, it is supposed to reflect the patient’s restrictions when it comes to various types of symptoms and activity. It shows good reliability, validity and sensitivity after Achilles tendon rupture, and also shows internal consistency (Carmont, et al. 2012; Kearney, et al. 2012a; Nilsson-Helander, et al. 2007).
7 Results in brief

A pilot study to find a method to measure mechanical properties of the healing human Achilles tendon...

Study I
The transverse area of the healing Achilles tendon increased with time, while strain-per-force decreased. Modulus of elasticity increased with time, but the reason for this was probably the great variation between patients, where the e-modulus increased greatly in a few tendons. There was a negative correlation between e-modulus and transverse area, which implies that tendon callus either increases in size with decreased material properties or the other way around. Elongation of the tendon with time was seen. Modulus of elasticity correlated with the Heel-Raise index at one year. A comparison of the material properties outside the rupture site (beads 1 and 2 proximally and beads 3 and 4 distally) with the properties within the rupture (beads 2 and 3) showed no difference, indicating that the tendon in general and not only the rupture site behaves as one and can be regarded as homogeneous. Repeat measurements i.e. both the preconditioning and the experimental sequence were made and found to give similar results. Moreover, there was a significant correlation between measurements obtained at different points in time as regards the positions of the tantalum beads. Both these results support the assumption that there was no displacement of the metal beads within the tendon.

…. showing no difference between surgical and conservative treatment, but a greater variation in conservative treatment results …

Study II
There were no differences in mechanical properties between surgical and non-surgical treatments, but strain-per-force showed greater variation in patients treated conservatively at 7 and 19 weeks. Moreover, functional outcome, represented in our study as Heel-Raise index and ATRS, at 18 months did not show any difference between groups. Early modulus of elasticity and strain-per-force at 7 weeks correlated with the Heel-Raise index at 18 months. This correlation was greater than that seen in other studies comparing clinical results at 12 months. Elongation of the tendon between 7 and 19 weeks was observed but there was no difference between the groups. The method validation employed in Study I with repeat measurements and examination of the outer beads 1 to 4 and inner beads 2 to 3 was once again tested. Repeat measurements showed good correlation at 7 and 19 weeks, as did comparison of the outer and inner beads.

…and platelet injection did not improve mechanical properties…. 

Study III
There was no difference between the platelet treatment and control group regarding modulus of elasticity, strain-per-force and transverse area. Transverse area doubled between 7 and 19 weeks. Heel-Raise index at 12 months did not show any difference between the groups, while ATRS was lower in the platelet group, suggesting a negative effect. Early e-modulus and strain-per-force at 7 and 19 weeks correlated with the late Heel-Raise index. This study also showed elongation of the tendon between 7 and 19 weeks, but no difference between the groups. Repeat measurements showed good correlation at 7 and 19 weeks as did comparison of the outer and inner beads.
…but tensile loading did improve…

Study IV
Modulus of elasticity at 19 weeks and 12 months was significantly increased in the loading group compared to the control group. There were no other differences in mechanical properties between groups at any point in time. Transverse area doubled between 7 and 19 weeks. There were no differences regarding Heel-Raise index or ATRS at 12 months. Early modulus of elasticity and strain-per-force showed a correlation with late Heel-Raise index. Elongation occurred between 7 and 9 weeks, but there was no difference between the groups.

…and radiodensity reflected the mechanical properties seen in Studies III and IV.

Study V
Radiodensity was decreased by about 1/3 in the injured tendon compared to the uninjured tendon at 7 weeks. It remained so between 7 and 19 weeks but had returned to normal at one year. Radiodensity at 19 weeks and 52 weeks correlated with e-modulus at the same points in time and with the Heel-Raise index at 12 months.
8 Discussion

It is not clear why Achilles tendons rupture under physical activity, but it is known that there are several risk factors for rupture including decreased blood supply in the injured area (Ahmed, et al. 1998) and degenerative changes (Hess 2010; Tallon, et al. 2001) in the tendon tissue, leading to weakness of the tendon. Achilles tendons often seem to rupture at the end of a tough physical activity session, and previous studies have shown that untrained patients run a greater risk for this type of injury. Tired muscles have poor coordination (Kaalund, et al. 1989) and reduced ability to absorb energy. If the tendon attached is weak, and is exposed to sudden, unexpected dorsiflexion of the ankle, the energy overload may result in a rupture. This typically occurs during pushing off with a weight-bearing forefoot while extending the knee joint (Arner and Lindholm 1959; Maffulli 1998), as often seen in explosive sports such as badminton and squash.

Value of mechanical measurements

There is a lot of information about the mechanical properties of healing Achilles tendons in animals (Eliasson, et al. 2012; Eliasson, et al. 2007; Enwemeka 1992; Enwemeka, et al. 1988; Obst, et al. 2013) and there is also information about the clinical and qualitative radiological findings during the healing process in patients with this kind of injury (Moller, et al. 2002). Lately there have been studies published, describing methods used to measure mechanical properties in intact human tendon tissue (Hansen, et al. 2013; Klauser, et al. 2010; Kongsgaard, et al. 2011a), but we have little knowledge about the mechanical properties of the human Achilles tendon during the healing process. Such knowledge would be useful, since the most important complication of any treatment of this injury seems to be rerupturing during the rehabilitation period. A tendon with better mechanical properties should have a lower risk for rerupture. Moreover, reducing the risk for rerupture would enable us to shorten the period of immobilisation and allow patients to return to work and physical activity sooner. An accurate method to measure mechanical properties would give us the possibility to better assess the patient’s progress, and also to compare treatment regimens with the aim of improving tendon healing.

The ideal, of course, would be to be able to find patients with an increased risk for rerupture, so that these patients can be selected for surgical treatment. As patients treated conservatively seem to do as well as operated patients in the long run (Soroceanu, et al. 2012), we should be able to treat all other patients non-surgically, thus avoiding the complications caused by surgery. Furthermore, in times when global recession affects healthcare systems too, this is certainly an attractive economic alternative. However, if conservative treatment increases the time to return to activity and work, and the risk for rerupture, then the reduction in costs might not be significant (Wills, et al. 1986). Because we do not have the means to select patients with a higher risk for rerupture, we must continue looking for ways to improve tendon healing for all patients. Most important of all, we must find a method to measure the mechanical properties of the healing tendon quantitatively. Having access to an established laboratory for RSA, we began using this technique to find a way to describe the repair process of the healing tendon. As far as we know, this is the only method that has been used to compare different treatment methods by measuring the mechanical properties of healing tendons in humans. However, methods based on ultrasound for measurement of tissue elasticity, so called elastography, are currently being evaluated for use in tendon research (Klauser, et al. 2010).
Methodological considerations
A conservative postoperative regime was chosen, with the patients wearing a plaster cast for several weeks, since that was routine clinical practice in many departments at the time our studies started.

One of our main problems in Study I was to know how much loading the patient and the tendon tissue would tolerate while performing the RSA examinations, especially the examination directly after removal of the cast. On the one hand, the tendon should be loaded sufficiently to produce measurable strain, on the other hand too much loading might lead to permanent elongation of the tendon with an inferior end result (Kangas, et al. 2007; Silbernagel, et al. 2012), or even worse, traumatic rerupture. Patients were therefore asked to put pressure on a bathroom scale using the forefoot only, in order to get some idea of what pressure still felt comfortable (Figure 17).

Figure 17: Bathroom scale to get some idea of what force patients felt comfortable with.

This test showed that almost all patients loaded the scale with between 150 and 250 N. This is probably just a fraction of what is needed for rerupture or permanent elongation of the tendon. However, in Study I too many patients felt uncomfortable with 200 N at the first RSA examination, which is why the decision was made to decrease the load to 150 N from Study II onwards.

Another major methodological problem was the fact that the influence of antagonist muscles, i.e. the dorsal extensor muscles in our examinations, could not be determined. It is likely that they normally play an important role, thus making interpretation of the results difficult if the baseline pictures were to be taken with no load at all. The decision was therefore made to let the patients resist 25 N during the baseline examination, assuming that this would be enough to relax the extensor muscles.
Another methodological problem was that bead migration during an examination or between examinations at other points in time could not be excluded with all certainty. This was the reason, besides preconditioning, that repeat examinations were made in the first three studies. Moreover, these repeat examinations also allowed estimation of the error of measurement. The difference in e-modulus between the first and second measurements showed a normal distribution (Figure 18), with the exception of some extreme outliers. The error of measurement at 7 weeks, as estimated from these differences \((sd(\text{measure}_1 - \text{measure}_2) \times 2^{0.5})\), amounted to 18% of the mean of the second measurement. For 19 weeks, the error was 21%.

**Figure 18:** Difference in e-modulus between double measurements after 7 weeks and 19 weeks. One outlier has been removed at 7 weeks (value exceeded 3 SD). Two outliers have been removed at 19 weeks (values exceeded 2.75 SD).

Two of the randomised trials (Studies II and III) failed to show a significant difference. We therefore reported confidence intervals for the difference between group means. In Study II
the 95% CI excludes that surgery would improve the modulus of elasticity at 7 weeks by more than 23%. Considering that the modulus increased by about 200% between 7 weeks and 1 year in Studies III and IV, exclusion of an increase greater than 23% implies that there is no important effect.

Similarly, in Study III the 95% CI excludes that platelet-rich plasma improves the modulus of elasticity by more than 38% at 7 weeks.

The estimated modulus of elasticity at 7 weeks in the surgically treated control groups in all studies had a standard deviation of 33% of the mean value (Table 4). This meant that in order to have an 80% power to find a significant difference (α = 0.05) between two groups amounting to 33% of the control mean, we would need 16 patients in each group.

Table 4: Standard deviation of the mean value of modulus of elasticity at 7 weeks (33%) and 19 weeks (34%).

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<th>7 weeks</th>
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<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>Std.</td>
</tr>
<tr>
<td>Study II</td>
<td>13</td>
<td>82</td>
<td>30</td>
</tr>
<tr>
<td>Study III</td>
<td>14</td>
<td>80</td>
<td>22</td>
</tr>
<tr>
<td>Study IV</td>
<td>16</td>
<td>87</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>83</td>
<td>27(33%)</td>
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</tbody>
</table>

We wanted to exclude major bead migration during an examination. We therefore looked at the double examination in Studies I, II and III and found a strong correlation between the first measurement and the second measurement. Moreover, we wanted to exclude major bead migration over time. We therefore correlated the ratio of Gap 1 (beads 2 and 3) and Gap 2 (beads 1 and 4) in Studies II and III at 7 weeks with the same ratio at 19 weeks (Figure 19) and found a strong correlation with a $r^2$ value of 0.89. In other words, the ratio between the 2 distances remained constant, which implies that none of the beads had migrated.

We thus assumed that the great variation between patients found in our studies was a true variation and not a product of bead migration. We concluded that a great biological variation is positive, as it leaves room for improvement.
It also seemed important to measure the stiffness of the healing tendon without this being influenced by the strength of any remaining suture material. In tension, the load is mainly taken by the stiffest component of a construct. If we had chosen a stiff and slowly absorbable or non-absorbable suture, as did Yotsumoto in 2010 (Yotsumoto, et al. 2010), this would probably have influenced our results in two ways: first, we might have been measuring the properties of the suture instead of the tissue; second, the healing tissue might have become load-protected and thereby resistant to mechanical stimulation by loading. We therefore chose Vicryl, a rapidly absorbed suture material, that has a low e-modulus (Greenwald, et al. 1994).

**Surgical treatment versus non-surgical treatment**

Although there is a vast literature discussing surgical versus non-surgical treatment, it still seemed important to see if there are any differences between these two treatment options concerning the material properties of the healing tendon. Interestingly, we found a greater variation in early strain-per-force in the group that was treated conservatively. As there were also more negative outliers than in the surgical group, this could be the reason for a higher risk for rerupture after non-surgical treatment. However, as will be discussed below, this conclusion might not be valid: the one patient treated conservatively who suffered a rerupture in Study II actually had an unusually high modulus of elasticity at cast removal some weeks before his rerupture. This challenges our assumption that the reason for more reruptures in the non-surgical group is that more patients in this group have a low e-modulus. The greater variation in the conservatively treated compared to the surgically treated group could be the result of variation in the extent or type of initial injury. Conservative treatment may have less influence on this variation compared to surgical repair which might standardise healing conditions. Whatever, despite this difference in variation, no particular difference between the group means, favouring either surgical or non-surgical treatment, was found.

Due to the fact that surgical treatment showed more homogeneous results in Study II, the decision was made to use surgical repair in following studies in order to establish as similar and homogeneous conditions as possible between treatment and control groups.

**Platelet-rich plasma**

It seemed interesting to try to confirm the possibility to improve treatment of Achilles tendon ruptures using platelet-rich plasma, as several animal studies had suggested that such treatment would improve the mechanical properties of the healing tissue. A higher stiffness and higher load to failure was found for healing rat Achilles tendons treated with platelet concentrate (Aspenberg and Virchenko 2004). Apart from these animal studies, Sanchez et al. showed a faster return to sports after treatment with a single shot of PRP (Sanchez, et al. 2007). Unfortunately, we found no positive effect on mechanical properties or clinical findings after using platelet-rich plasma. The reason might be that we had different settings than Sanchez et al., but we were actually convinced that we applied a high concentration of viable platelets at the rupture site, probably with a high amount of growth factors and bioactive proteins. So why didn’t our injections of PRP lead to any favourable effect? Compared to animal studies, where the tendon ends have a clean transverse cut, there is a much bigger lesion in human ruptures, which might lead to a greater spontaneous accumulation of platelets at the rupture site from the beginning, making the injection of even more platelets superfluous. Our results do contrast with those of Sanchez et al., but on the other hand, the work of Sanchez et al. is a case series compared to historical controls, and therefore a possibility of bias and placebo effect exists. Another reason for our results might be that mechanical stimulation is necessary for platelets to have a positive effect. This appears
to be the case in the rat model (Virchenko and Aspenberg 2006). Our patients were locked in a cast for several weeks, and although there is not much known about loading of the Achilles tendon beneath the leg cast, there may have been a lack of mechanical stimulation, using the settings of our study.

In large tendons such as the human Achilles tendon, a single shot of platelet-rich plasma, which is sufficient to improve tendon healing in rats, might not be enough to achieve significant improvement in tendon healing. Repeated injections during the early phase of healing might have a better effect on the tendon healing process (Virchenko 2007).

**Early Rehabilitation**

Compared to platelet-rich plasma, early rehabilitation is a more accepted way to improve tendon healing. Many studies of early rehabilitation have reported positive results. However, the literature is confusing when it comes to the definition of early rehabilitation. Early rehabilitation includes one or both of two components, namely weight-bearing and range of movement training (Kearney and Costa 2012). Which of these components actually improves the clinical outcome is not clear. With the lower leg in a cast, it is obvious that training of range of movement is impossible, but it is not known if weight-bearing beneath a cast leads to mechanical stimulation of the tendon. Indeed, it is fully possible to walk with full weight-bearing with a cast and no Achilles tendon at all.

Results in studies on humans almost always focus on clinical findings, important for the patient, but do not have much to say about the impact of weight-bearing and range of movement training on the material properties of the healing tendon tissue. It is known from animal studies that loading of the tendon leads to better mechanical properties in a way that is at least partially “dose-dependent” (Andersson, et al. 2012).

When deciding on the loads in Study IV, two previous findings were used as guidelines. First, we knew from our bathroom scale measurements that at the time of cast removal patients found 150 - 250 N to be the maximum loading tolerated, so the pedal was constructed so that loading could be increased approximately to this level by 7 weeks. On the other hand, it was known from rat experiments that a few, short, daily loading cycles were enough to improve healing, without risking permanent elongation of the tendon (Andersson, et al. 2009). The questions were: can this be translated to humans; does a human have to train more often than a rat; and how much loading is required to achieve better results without compromising the final clinical results (e.g. by tendon elongation)? As most patients in Study IV had no problem in completing all the loading cycles, the training programme, in retrospect, might have been too soft. It is possible that a tougher training programme would have resulted in better mechanical properties and an even more dramatic difference between treatment and control groups. It thus remains unclear how to structure the training programme as regards loading forces, number of cycles, and number of sessions per day.

The patients in Study IV were not allowed to begin active training for the first two weeks because of the increased risk for wound infections if training is started before complete skin closure (Thomas, et al. 2009). However, omitting active rehabilitation during these first two weeks might be disadvantageous, and the risk for skin infection overrated. Recent rat studies have shown a positive effect on healing by loading already during the early inflammatory phase of healing (Eliasson, et al. 2012). There is a growing consensus that conservative treatment with early rehabilitation is as good as surgical repair. It is important, therefore, to
examine the effects of early rehabilitation on the mechanical properties of the healing Achilles tendon treated non-surgically. In a recent review by Soroceanu it is recommended that surgical treatment should be applied in hospitals where resources for early rehabilitation are lacking (Soroceanu, et al. 2012).

**Hounsfield**

The results of Study V indicate that the radiodensity of the healing tendon reflects its mechanical properties. It remains to be seen to what extent Hounsfield units can be used to compare different treatment protocols or even to follow single patients. If it were possible to use this method, it would definitively simplify follow-up. The technique is well-established and available all over the world and takes only a couple of minutes. It is also relatively affordable compared to other costs in research projects and would definitely be a useful instrument.

Density can only reflect the ratio between collagen and water. It does not describe the level of tissue maturation, as it cannot measure the organisation of the matrix, notably collagen orientation and cross-linking, and tissue organisation is important for the mechanical properties of the healing tendon. In Studies III and IV this effect became apparent as the correlation between density and mechanical properties was less pronounced in the loading study. The reason might be that loading led to better tissue organisation not detectable using Hounsfield units.

Studies in rats have shown that a high stiffness of healing tendons is associated with a high tension load to failure. We have confirmed this by re-analysing the data from a study on transected sheep Achilles tendons (Virchenko, et al. 2008). We therefore assumed that patients with a high early e-modulus would also show a lower risk for rerupture (especially as a high early modulus is associated with a better clinical outcome). One could also assume the opposite; that patients with low early e-modulus may be identified as being at risk for rerupture and treated accordingly. As all patients were examined at the time of cast removal, we have e-modulus values for the patients who subsequently suffered a rerupture of the tendon. Altogether there were three cases of rerupture amongst our study patients, and surprisingly, these patients were not the ones with a low modulus of elasticity but rather were amongst those with the highest values. Indeed, they had an e-modulus more than two standard deviations higher than the weighted mean of all our studies. This would suggest that patients with a high e-modulus have a good clinical outcome but also run a greater risk for rerupture. It could be that a stiff tendon gives the patient a feeling of having regained normal function, which makes him take higher risks. However, if the high modulus risk is related to structural weaknesses, this poses the question of how to interpret high e-modulus values. Is a high e-modulus really desirable? The risk for rerupture may be related to the ability of the tendon to rapidly absorb mechanical energy. As energy uptake is related to force and strain, the high e-modulus during early healing might be associated with a reduced load to failure, e.g. increased brittleness. Moreover, the time-deformation aspect needs to be taken into account. Not only must the tissue be able to tolerate strain, it must also tolerate a high strain rate. It would therefore be interesting to study the strain rate in healing Achilles tendons under standardised conditions.

It was also surprising that early mechanical properties did not correlate with the late Achilles tendon Total Rupture Score (ATRS is a validated score), whereas they correlated with the Heel-Raise index at one year in all four RSA studies. This may, at least in part, be due to a
ceiling effect of the ARTS: 38 of 55 patients had an ATRS score of 80 or more after one year (Studies III and IV), and 17 of 18 at 18 months (Study II). In contrast, the Heel-Raise index ranged between 17 and 120 at one year (Studies III and IV) and between 17 and 108 after 18 months (Study II). It appears that the ATRS is suited to study patients during the early phases of healing, whereas the Heel-Raise index might be more suitable for evaluation of results after one year or later.
9 Conclusions

1. It is possible to measure early mechanical properties of the healing human Achilles tendon using a method involving RSA. These properties show dramatic inter-individual variation.

2. There is a significant, but unexplained correlation between early material properties and late clinical results in terms of a combination of heel-raise height and heel-raise endurance.

3. No differences in mechanical properties between surgical and non-surgical cases could be shown, and the 95% CI for modulus of elasticity excludes a difference exceeding 1 standard deviation.

4. No effect of platelet-rich plasma on the healing process could be shown, indeed, a secondary but important variable, the ATRS score, indicated a negative effect.

5. Early exercises specifically designed to expose the healing Achilles tendon to traction, subsequently led to a higher modulus of elasticity at later time points, including 1 year.

6. Radiodensity as measured by CT was reduced in healing tendons until week 19, and had reached a normal level at one year. There was almost no overlap between density values at 19 weeks and at one year.
10 Future research

It would be interesting to see if it is possible to improve mechanical properties of the healing human Achilles tendon in conservatively managed patients, to levels similar to the operated patients in Study IV.

Using early rehabilitation in further studies, the amount of training occasions and also the severity of strain should be evaluated. More training sessions or tougher loads could lead to an even better outcome.

Although early rehabilitation seems to be the only reasonable option when treating patients with Achilles tendon ruptures, further studies are needed to investigate the effects on the mechanical properties of tendon tissues in cases where immobilisation is inevitable. So far there is very little information known about the effects of immobilisation in humans.

Furthermore, it would be interesting to further characterise tendon deformation in response to load. In all studies in this thesis the assumption was applied that tendon elongation occurs in a linear elastic manner. From the multiple examinations at one year, however, it appears that this is not completely true, as there is a measurable plastic component (data not shown).

Despite the fact that platelet-rich plasma didn’t show any effect in Study III, it shouldn’t be ruled out completely. Animal studies have shown that platelets can influence tendon healing and strength, but mainly if combined with mechanical loading.

The possibility of using CT for follow-up should be further explored, especially by analysing the course of normalisation during the period between 19 weeks and one year.
11 Acknowledgements

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Yang, G., R. C. Crawford, and J. H. Wang
Yotsumoto, T., W. Miyamoto, and Y. Uchio
### Appendix

**Appendix:** The Achilles tendon Total Rupture Score (ATRS) (in English)

0 = *major limitations/symptoms*  
10 = *no limitations/symptoms*

1. Are you limited due to decreased strength in the calf/Achilles tendon/foot?

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2. Are you limited due to fatigue in the calf/Achilles tendon/foot?

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3. Are you limited due to stiffness in the calf/Achilles tendon/foot?

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4. Are you limited due to pain in the calf/Achilles tendon/foot?

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5. Are you limited during activities of daily living?

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6. Are you limited when walking on uneven surfaces?

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7. Are you limited when walking quickly up the stairs or up a hill?

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8. Are you limited during activities that include running?

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9. Are you limited during activities that include jumping?

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10. Are you limited in performing hard physical labour?

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