Ari Pajala

ACHILLES TENDON RUPTURE

COMPARISON OF TWO SURGICAL TECHNIQUES, EVALUATION OF OUTCOMES AFTER COMPLICATIONS AND BIOCHEMICAL AND HISTOLOGICAL ANALYSES OF COLLAGEN TYPE I AND III AND TENASCIN-C EXPRESSION IN THE ACHILLES TENDON
ARI PAJALA

ACHILLES TENDON RUPTURE
Comparison of two surgical techniques, evaluation of outcomes after complications and biochemical and histological analyses of collagen type I and III and tenascin-C expression in the Achilles tendon

Academic Dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in Auditorium 1 of Oulu University Hospital, on 8 May 2009, at 12 noon

OULUN YLIOPISTO, OULU 2009
Abstract

The Achilles tendon is the largest tendon in the human body and is affected by many diseases and is vulnerable to many forms of damage due to the heavy loads it must bear. Rupture of the Achilles tendon has become more common in recent times, with an almost four-fold increase in prevalence from 1979–1990 to 1991–2000 and a peak incidence of 19 ruptures per 100 000 of population in 1999 in our epidemiological assessment. The incidences of major complications, re-rupture and deep infection, increased along with primary ruptures, peaking in 1999. The results after successful primary repair are good in over 90% of cases, as we have shown in a randomized study and in a review of the literature, and the result after re-rupture is still good in about 70% of cases, but achieving good performance after deep infection is a highly random matter. Our retrospective survey did not identify any good results, but the deep infection cases in our randomized study showed good performance due to prompt action taken for their treatment.

The best method for treating a ruptured Achilles tendon has been under debate for almost 100 years, with surgery and conservative methods advocated to equal extents. We have advocated surgical treatment as the primary choice and conservative treatment is given for selected high risk patients, for example patients with diabetes, skin problems, systemic use of corticosteroids or severe other illness. The type of surgery technique is not a straightforward choice, either, and various forms of open surgery and percutaneous techniques exist. We compared an end-to-end simple suture with the same suture augmented with one central gastrocnemius turn-over flap in a randomized series of 60 patients and found no differences with respect to subjective complaints, calf muscle strength or tendon elongation with time. The end-to-end technique is simpler and is therefore justified as the primary method of choice for the surgical repair of fresh complete Achilles tendon ruptures.

The tissue composition has been shown to alter not only with time but also after repeated tearing of the tendon collagen fibres. A normal tendon is mainly composed of type I collagen, but the rupture areas express more type III collagen, which is thinner and withstands loads less effectively. Type III collagen accumulates slowly in the tendon, since its production does not increase very much, a situation that is indicative of microtrauma. Crosslinking of the fibres is important for collagen matrix properties, and we found that there is a change in the quality of crosslinking with age and that this may have role in the observed changes in tendon stiffness, as also noted in other studies.

We also studied the appearance of tenascin-C at the rupture site in the Achilles tendon and at two other sites in the same tendon, but found no difference in its expression. It has been proposed that tenascin-C may take part in the tendon’s reaction to loading, but its exact function remains unknown.

Keywords: Achilles tendon rupture, collagen type I, collagen type III, surgical repair, tenascin-C, tendon elongation
Acknowledgements

The present study was carried out at the Department of Surgery, Division of Orthopaedics, Department of Clinical Chemistry, the Department of Pathology and Department of Physical Medicine and Rehabilitation, Oulu University Hospital during the years 1998–2009. Oulu University Hospital has a well known tradition of Achilles tendon research.

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AR</td>
<td>Augmented repair group</td>
</tr>
<tr>
<td>AT</td>
<td>Achilles tendon</td>
</tr>
<tr>
<td>CONT1</td>
<td>Control 1 site</td>
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<tr>
<td>CONT2</td>
<td>Control 2 site</td>
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<tr>
<td>DF</td>
<td>Dorsiflexion</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PINP</td>
<td>Aminoterminal polypeptide collagen type I</td>
</tr>
<tr>
<td>PICP</td>
<td>Carboxyterminal polypeptide collagen type I</td>
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<tr>
<td>PF</td>
<td>Plantar flexion</td>
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<tr>
<td>PT</td>
<td>Peak torque</td>
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<tr>
<td>PTA</td>
<td>Peak torque angle</td>
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<tr>
<td>PW</td>
<td>Peak work</td>
</tr>
<tr>
<td>RIA</td>
<td>Radio immunologic assay</td>
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<tr>
<td>RUPT</td>
<td>Rupture site</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEM</td>
<td>Standard error of measurement</td>
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<tr>
<td>SR</td>
<td>Simple repair group</td>
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<tr>
<td>TIMP</td>
<td>Tissue inhibitors of matrix metalloproteinases</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasonography</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogical scale</td>
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</tbody>
</table>
List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


Contents

Abstract
Acknowledgements
Abbreviations
List of original publications

Contents
1 Introduction 13
2 Review of the literature 15
  2.1 Normal Achilles Tendon (AT) ................................................................. 15
    2.1.1 Anatomy of the normal AT ................................................................. 15
    2.1.2 Biomechanics of the normal AT .......................................................... 16
    2.1.3 Histology of the normal AT ................................................................. 17
  2.2 Achilles tendon rupture ................................................................. 18
    2.2.1 Epidemiology of AT rupture ................................................................. 18
    2.2.2 Aetiology of AT rupture .................................................................... 19
    2.2.3 Diagnosis of AT rupture .................................................................... 20
  2.3 Surgical treatment ................................................................................... 21
    2.3.1 Surgical techniques ............................................................................ 21
    2.3.2 Non-surgical treatments .................................................................... 22
    2.3.3 Complications of surgical treatment .................................................. 23
    2.3.4 Complications of non-surgical treatment ............................................. 23
    2.3.5 Treatment of complications ............................................................... 24
  2.4 Tendon histology changes upon AT rupture ........................................... 25
    2.4.1 Collagen changes ............................................................................. 25
    2.4.2 Tenascin-C ....................................................................................... 26
3 Purpose of the research 27
4 Materials and methods 29
  4.1 Materials .................................................................................................. 29
  4.2 Methods ................................................................................................... 30
    4.2.1 Treatments .......................................................................................... 30
    4.2.2 Evaluation methods .......................................................................... 36
    4.2.3 Statistical methods (I, II, III, IV) ....................................................... 40
    4.2.4 Ethics (I, II, III, IV) ........................................................................... 41
5 Results 43

11
5.1 Augmented vs. non-augmented surgical repair of acute total AT rupture. A randomized prospective study (I) ........................................ 43

5.2 Re-rupture and deep infection following treatment of total AT rupture (II) ................................................................................................. 51

5.3 Increased type III collagen content at the human AT rupture site (III) .. 55

5.4 Tenascin-C and type I and III collagen expression in total AT rupture (IV) ............................................................................................. 60

6 Discussion 63

6.1 Augmented vs. non-augmented surgical repair of acute total AT rupture ................................................................................................. 63

6.2 Re-rupture and deep infection following treatment of total AT rupture. 65

6.3 Type I and III collagen expression in total AT rupture ......................... 68

6.4 Tenascin-C in Achilles tendon rupture ................................................ 69

7 Conclusions 71

8 Future prospects for Achilles tendon rupture research 73

References

Original publications
1 Introduction

It was the well-known French surgeon Ambroise Pare who first recorded an attempt to treat an Achilles tendon rupture, in 1575. He introduced a bandage dipped in wine and spices to be wrapped around the ankle to treat this injury (Ronel et al. 2004).

Prior to the 20th century, Achilles tendon ruptures were most often treated non-operatively. Various means of immobilization for varying periods are described in the literature (Wills et al. 1986). Since 1929, however, surgery has been proposed as the treatment of choice (Ronel et al. 2004). The first recorded works by Christensen (1954) and Arner et al. (1958/59) compared patients treated operatively and non-operatively and found better results in the former group. Based on their findings, the surgical treatment of total Achilles tendon rupture became popular, although along with it came various complications of this surgery. A final consensus regarding the most effective management with least complications is still lacking.

Rupture of the Achilles tendon is often accidental in nature, but debilitating histological alterations in the tendon tissue have been suggested as the primary cause (Josza et al. 1989a). The mechanical and degenerative theories do not totally exclude one another, however, and much research has been done in order to clarify more closely histological and biomechanical backgrounds to total Achilles tendon rupture.

Our research focused on the outcomes of two surgical techniques, reporting the results after re-ruptures and infections and comparing the biochemical and histological changes at the rupture site with those at two other sites in the same Achilles tendon.
1 Review of the literature

1.1 Normal Achilles Tendon (AT)

1.1.1 Anatomy of the normal AT

The Achilles tendon, formed of tendinous parts of the gastrocnemius and soleus muscles, is the largest tendon in the human body. The gastrocnemius muscle has its origin on the femur above the knee joint, while the soleus muscle originates below the knee joint, at the proximal posterior tibia and fibula. The gastrocnemius muscle fibres extend 11–26 cm above the heel bone and those of the soleus 3–11 cm (Cummins et al. 1946). The AT is formed by three broad, flat aponeuroses of the gastrocnemius and soleus muscles. The tendon is circular in shape at its midpoint and widens out at the insertion into the heel bone. As the AT runs down towards the heel bone, its fibres rotate 90° so that the posterior gastrocnemius tendon fibres are turned anterolaterally and the anterior soleus fibres posteromedially (Cummins et al. 1946, Stein et al. 2000) (Figure 1). The shape and cross-sectional dimensions of the AT vary from 80 to 140 mm$^2$ along the course of the tendon (O’Brien 1992). The AT receives its blood circulation at the muscle and heel bone insertions and at the endotenon and peritenon. At its midpoint, 4–7 cm from the heel bone insertion, arterial nutrition is very limited and takes place through anterior vessels running through the fatty tissue into the peritenon. The number of mid-tendon blood vessels is very low, as is their area relative cross-sectional area (Carr & Norris 1989). The AT is covered by a thin epitenon and paratenon and does not have a true sheath. The paratenon forms several thin membranes on the dorsomedialateral side which can glide in relation to each other, while on the ventral side it consists of fatty tissue, blood vessels and connective tissue in thin septal structures.
1.1.2 Biomechanics of the normal AT

The AT transmits the tension generated by the gastrocnemius and soleus muscles to the heel bone. These two tendons are capable of resisting high tensile forces with no marked elongation (Best & Garrett 1994). There are high forces in the AT during normal active daily living, ranging from 600N in cycling up to 9kN (11kN/cm²) in running at a speed of 6m/sec (Komi et al. 1992). The AT also has the ability to deform and recover its original length. Its unloaded collagen fibres assume a wavy configuration, but this will disappear when the tendon is stretched by about 2%. Loading the tendon tissue gives a linear stress-strain curve at less than 4% strain as the collagen fibres deform within their capacity. After loading of tendon at less than 4% strain all the fibres regain their wavy configuration and no damage occurs, but strain above 4% starts to destroy the intermolecular collagen crosslinking and the stress-strain curve starts to become more horizontal. Loading at this level causes microscopic damage to the tendon tissue and a repair process will start. Strain over 8% will cause macroscopic damage (rupture) to tendon tissue (O'Brien 1992). The tensile strength of tendons is about 50 N/mm² in vitro (Jozsa...
& Kannus 1997), and a tendon such as the AT, with a thickness of 1 cm (78.5mm²), is capable of supporting a weight of 500 to 1000kg. The diameter of the AT is correlated with calf muscle size and the height and age of the individual (Koivunen-Niemelä & Parkkola 1995). It has also been demonstrated that the thickness of the AT is load-dependent (Rosager et al. 2002, Kallinen & Suominen 1994), and that continuous loading of the AT results in exercise-induced tendon hypertrophy (Woo et al. 1980, Birch et al. 1999).

The tensile strength of a healthy tendon in humans increases until 30 years of age and gradually weakens thereafter, being about 40% lower by the age of 70 years (Barfred 1973, Thermann et al. 1995a). This is caused by alterations in collagen crosslinking (Ippolito et al. 1993).

### 1.1.3 Histology of the normal AT

Tendon tissue is composed of cellular and extracellular materials. The number of cells in a tendon is limited, and fibroblasts are encountered most often. These fibroblasts produce collagens and other proteins (Karpakka 1991), the collagens being secreted into the extracellular matrix as procollagens and prepared by enzymatic cleavage for collagen matrix binding. The residual amino acids resulting from the cleavage of type I collagen are called N-terminal (PINP) and C-terminal (PICP) polypeptides (Kielty et al. 1993) and are measurable in vitro and in secretory substances. The rate of collagen metabolism is relatively slow, the turnover time for tendon collagen ranging from 50 to 100 days (Curwin & Stanish 1984). A balance between synthesis and breakdown prevails in normal tendon (Josza & Kannus 1997), but net synthesis can speed up during growth and after injury, while immobilization will slow down synthesis and accelerate breakdown, at the same time causing alterations at the molecular level to reduce tendon strength (Tipton et al. 1986, Karpakka 1991).

The extracellular matrix of the AT is mainly formed of type I collagen, as is the case with all the tendons in the human body, but small amounts of type II collagen are found at the osteotendinous junction and types III, IV and V in the endotenon and vascular wall (Josza & Kannus 1997). The dry weight collagen content of the Achilles tendon is about 70% (Josza et al. 1989b). There are other minor extracellular proteins such as elastin, proteoglycans, glycosaminoglycans and wide variety of other small molecules between the collagen fibres (Karpakka 1991). The function of the extracellular matrix is to support and regulate the cellular elements and the structure of the tendon. The collagen molecules form microfibrils, fibrils
and finally collagen fibres by intermolecular crosslinking, which is important for tissue stiffness and strength, i.e. the more crosslinks there are, the stiffer the tissue (Zernicke & Loitz 1992).

Type I collagen has a micromolecular construction in which two \( \alpha_1 \) chains and one \( \alpha_2 \) chain form a left-handed triple helix. Each \( \alpha \) chain has a repeated glycine – X – Y aminoacid sequence in which X and Y are often (in 25% of cases) represented by proline and 4-hydroxyproline. Some Y positions are occupied by hydroxylysyl amino acids, and the collagen triple helix can form intermolecular crosslinks at these sites (Kivirikko & Myllylä 1982). The mature type I collagen crosslinks in tendon are 3-hydroxylysylpyridinoline and 3-lysylpyridinoline (Eyre et al. 1984). The number of crosslinks is important for the structure of the tendon, and even slight changes in their number, which can happen in a variety of diseases and during ageing, will weaken and alter its mechanical properties (Last & Reiser 1984).

1.2 Achilles tendon rupture

1.2.1 Epidemiology of AT rupture

The incidence of AT rupture has increased dramatically during the last three decades. AT injury was reported before the 1950’s, but no study of its incidence is available for the period 1900–1950. The highest incidence in the age group 40–50 years in Malmö (Sweden) between 1950 and 1973 was 8.5 / 10^5 / year (Nillius et al. 1976), while the average reported incidence in the Salo region of Finland between 1980 and 1991 was 2 / 10^5/ year (Rantanen et al. 1993). In Scotland the annual incidence increased from 4.7 /105 in 1981 to 6/10^5 in 1994 (Maffulli et al. 1999). In the area concerned here, that of Oulu, Finland, the average incidence between the years 1979 and 1986 was 2 / 10^5 / year and that between 1987 and 1995 12 / 10^5 / year, with a peak incidence of 18 / 10^5 / year in 1994 (Leppilahti et al. 1996a).

Most AT ruptures occur in men, the ratio ranging from 2:1 (Carden et al. 1987) to 19:1 (Zollinger et al. 1983). The peak incidence is reached at 30–40 years of age, earlier than that for other spontaneous tendon ruptures (Jozsa et al. 1989a). AT injury is most often unilateral, and a slight left leg predominance has been reported (Hattrup & Johnson 1985, Jozsa et al. 1989a).
1.2.2 Aetiology of AT rupture

AT injuries occur most often (75%) in sports requiring jumping and rapid acceleration (Josza et al. 1989a, Leitner et al. 1991). Although there are considerable national differences in the frequencies of particular sports, ball games constitute up to 60% in many series (Nillius et al. 1976, Josza et al. 1989a, Järvinen 1992, Cetti et al. 1993, Möller et al. 2001). Approximately 10% of cases involve professional athletes, 80% recreational athletes and 10% non-athletes (Nistor 1981, Leppilahti et al. 1998, Möller et al. 2001).

Most AT ruptures occur spontaneously, without any direct trauma to the tendon. These indirect mechanisms may be sudden, unexpected dorsiflexion of the ankle, violent dorsiflexion of a plantarflexed foot, and pushing off with a weight-bearing forefoot while extending the knee joint (Arner & Lindholm 1959). Direct trauma mechanisms include accidental forceful contact on the activated tendon, and open tendon lacerations with broken glass, knives or axe cuts and rare cases of bone fracture lacerations from within.

The two most popular pathogenesis theories are the degenerative theory and the mechanical theory. The degenerative theory is based on studies showing histological signs of repetitive microtrauma and hypovascularity in the AT (Kannus & Josza 1991, Carr & Norris 1989), these two being regarded as predisposing factors for chronic degeneration, which will weaken the AT.

Special aetiological cases include the use of anabolic hormones in competitive sports (Michna & Hartmann 1989, Laseter & Russell 1991) or courses of fluoroquinolone antibiotics (McGarvey et al. 1990, Jagose et al. 1996). There are also clinical case reports of AT ruptures related to systemic corticosteroids (Smaill 1961, Melmed 1965, Baruah 1984) or local corticosteroid injections (Kleinman & Gross 1983), but no reliable studies of the risk of rupture associated with corticosteroid use have been published.

Most younger patients have never had any symptoms in the AT region before the rupture. In Scotland, for example, only 5% of the 176 AT rupture patients studied had had previous symptoms (Maffulli 1999). Other studies have reported figures from 4% to 32% of their patients being symptomatic (manifested by pain, tenderness or stiffness in the Achilles tendon region) prior to rupture (Lea & Smith 1972, Bradley & Tibone 1990, Böhm et al. 1990). Preceding AT symptoms are more common in older patient groups, sometimes involving up to 40% of cases (Nestorson et al. 2000).

1.2.3 Diagnosis of AT rupture

The majority of AT ruptures are typical with respect to their clinical symptoms and findings and also in terms of their causative mechanism (Maffulli 1998). The clinical diagnosis should be easy to make. The patients usually report a sudden pain in their calf with a simultaneous snapping sound from behind, followed by loss of push-off strength. Occasionally the pain may be slight or even absent. Painless ruptures have sometimes been reported in as many as one-third of the patients (Christensen 1954).

AT rupture typically occurs 2 to 6 cm above the heel bone (Schönbauer 1964, Fox et al. 1975). Almost always there is a palpable gap in the tendon (Figure 2), but this can be obscured by local oedema and swelling. About 20% of all AT rupture diagnoses are missed since the long toe flexor muscles can imitate plantar flexion of the ankle, or else there is an uninjured plantaris longus tendon medial to the AT (Simmonds 1957, Inglis et al. 1976, Carden et al. 1987). The most common clinical diagnostic test is Thompson’s test, which involves squeezing the calf in order to achieve plantar flexion (Thompson & Doherty 1962). In the sphygmomanometer test a cuff applied around the calf muscle with the knee flexed to 90° is inflated to approximately 100 mm Hg with the ankle plantar flexed. Passive dorsiflexion of the ankle will cause no change in the mercury in the case of a ruptured AT, but a healthy tendon will cause a rise of about 40 mm Hg depending on the control value as tested on the patient’s healthy calf (Copeland 1990).

The diagnosis can be confirmed with ultrasonography (US) or magnetic resonance imaging (MRI). The US examination is cheap, rapid and widely available, but investigator-dependent, whereas MRI has many diagnostic advantages, including superior soft-tissue contrast, non-invasiveness, direct three-dimensional imaging and lack of ionizing radiation (Panageas et al. 1990, Mink et al. 1991), but it is quite expensive and time-consuming. US and MRI are also valuable tools for the differential diagnosis of AT rupture, with respect to partial AT rupture, calf muscle strain and rupture, rupture of the flexor hallucis longus tendon, rupture of the plantaris tendon, posterior tibial tendon injury, peroneal tendon injuries, posterior tibial

Fig. 2. On the left picture palpable gap is clear when thumb is carried down on the Achilles tendon.

1.3 Surgical treatment

1.3.1 Surgical techniques

Two types of surgical technique, with subgroups, are used by surgeons today. The major distinction is between open and percutaneous surgery. Open surgery is then divided into three subgroups: conventional open surgery, open surgery with several types of augmentation (one central gastrocnemius fascia flap, two gastrocnemius fascia flaps, plantaris longus tendon augmentation, flexor hallucis longus tendon, flexor digitorum longus and peroneus brevis tendon) and open surgery with multiple stab wounds. At least 41 open surgery options have been reported in the literature (Wong et al. 2002). We have found only one randomized trial comparing open surgical techniques. A recent study in Turkey compared a simple end-to-end suture (Krackow) to suture with plantaris longus tendon augmentation in 30 patients and found no difference between the groups (Aktas et al. 2007). Some retrospective trials have compared open surgery techniques, but they, similarly, have not been able to show any difference between the groups (Jessig & Hansen 1975, Rantanen et al. 1993, Nyyssönen et al. 2003). Different augmentations have been used in non-comparative studies, however (White & Kraynick 1957, Quickley & Scheller 1980, Mann et al. 1991, Wapner et al. 1993), and a mechanical experiment has shown the augmented technique to provide extra strength at the suture (Gebauer et al. 2007).
Percutaneous surgery constitutes a category of its own, with a variety of
technical devices available. Good results have been reported (Ma & Griffith 1977,
2001), but no randomized comparison between the techniques has been found.
Open surgery has been compared with percutaneous techniques in several studies,
and the latter has been reported to give a better cosmetic result (Bradley & Tibone
1990), cause lower rates of wound complications (Wong et al. 2002), cause more
sural nerve injuries (Wong et al. 2002) and give an overall lower complication rate
(Lim et al. 2001, Khan et al. 2005). The evidence for all these findings is weak,
however (Khan et al. 2005).
Casting for 4 to 9 weeks without weight bearing was the standard postopera-
tive treatment regardless of the surgical technique until the 1980’s, but more detai-
led and faster rehabilitation protocols have been developed since then as know-
ledge of tendon healing has accumulated (Hurme et al. 1990, Maxwell & Enwem-
eka 1992, Rantanen et al. 1999). In some controlled randomized series early func-
tional postoperative rehabilitation has been found to result in faster recovery than
the conventional postoperative cast immobilization (Cetti et al. 1994, Mortensen et
al. 1999, Maffulli et al. 2003, Costa et al. 2003), and a functional postoperative
protocol has also been observed to improve patient satisfaction without any inc-
crease in the complication rate (Suchak et al. 2006).

1.3.2 Non-surgical treatments

Various brace and cast options have also been used to treat AT ruptures. The
non-surgical option has been preferred for elderly patients with skin problems and
chronic diseases affecting wound healing (Maffulli 1999). The traditional
treatment regimen has most often consisted of a below-knee plaster boot with the
ankle held in the plantar flexed position for 8 weeks and thereafter a heel rise for 4
weeks, allowing walking and calf muscle exercises (Lea & Smith 1972, Stein &
Lukens 1976, Movin et al. 2005). Some authors have even advocated 12 weeks of
casting with simultaneous reduction of the plantar flexed angle to neutral (Blake &
Ferguson 1991). As knowledge on tendon healing has accumulated the use of more
active non-surgical options has been advocated (Hurme et al. 1990, Maxwell &
followed by controlled early mobilization in a splint was introduced in the 1990’s,
and the authors reported more rapid recovery of ankle motion and return to normal
activities than with the traditional 8 weeks of cast treatment (Saleh et al. 1992). A
randomized 50-patient comparison of functional bracing (CAM walker) for 8 weeks with cast treatment pointed to less re-ruptures in the CAM walker group, but no difference in patient satisfaction, muscle strength or ankle mobility (Petersen et al. 2002). Non-operative treatments have been compared in five randomized studies to operative treatments (Nistor 1981, Cetti et al. 1993, Thermann et al. 1995b, Möller et al. 2001, Metz et al. 2008). Operative treatments have resulted in 2%, 4% and 5% re-rupture rate and respectively non-operative treatments in 8%, 15% and 21% re-rupture rate. If a re-rupture can be avoided the results are comparable (Möller et al. 2001). Non-operative treatments have gained in popularity over the last five years, especially since modern walkers are adjustable for ankle motion limitations and are more comfortable to use.

1.3.3 Complications of surgical treatment

Surgery has been reported to cause re-rupture rates of 0–10% (Khan et al. 2005), regardless of the treatment option employed (Jessing & Hansen 1975, Rantanen et al. 1993, Nyyssönen & Luthje 2000, Maes et al. 2006). In a study from Norway the re-rupture incidence after surgical treatment was equal between groups with postoperative immobilization by means of either a cast or a brace (Borchgrevink & Crøntvedt et al. 2005). Deep wound and soft tissue infections are to be feared as complications, as the AT has limited soft tissue protection. The rates reported for superficial infections are between 3 and 14% and those for deep infections 1–5% (Khan et al. 2005). There are known risk factors which increase the rate of wound complications, most notably smoking and steroid use (Bruggeman et al. 2004). Percutaneous techniques have been reported to cause sural nerve injuries (Wong et al. 2002, Maes et al. 2006). Surgically treated patients also have the same risks as non-surgically treated ones in the postoperative period, since all modern protocols involve some method of immobilization. Deep venous thrombosis is less common, but skin irritation and muscle atrophy are to a great extent problems that affect every patient.

1.3.4 Complications of non-surgical treatment

The major complication after non-surgical treatment is re-rupture, the incidence of which has also been shown to be higher than after surgery in randomized series (Nistor 1981, Cetti et al. 1993, Möller et al. 2001). Re-rupture rates have varied from 8% to 21%, and a meta-analysis suggests that there is a three to four-fold risk
of re-rupture in non-surgical relative to surgically treated patients (Khan et al. 2004). Less numerous complications are venous thrombosis and pathological tendon elongation. Problems that recur very regularly are skin irritation and muscle atrophy after prolonged immobilization. The results following non-surgical treatment are usually good if re-rupture can be avoided (Möller et al. 2001).

1.3.5 Treatment of complications

If the guidelines on how to treat a fresh AT rupture vary, even less evidence-based data are available on how to treat typical complications. When non-surgical primary treatment fails and re-rupture occurs it is feasible to continue with open operative treatment. Re-ruptures after a percutaneous first attempt can be turned into open surgery with an end-to-end or augmented technique, and a failed non-augmented surgical repair can be re-operated on with an augmented technique of any kind. The results of surgery after re-rupture of an AT are not well documented, perhaps because the material is scarce. There are case or technique-based reports, but comparisons of different techniques for handling re-ruptures is missing.

Deep infections are dangerous complications, because the soft tissue coverage over the AT is very limited and there may be no repairable AT tissue left after thorough removal of the infected tissue, making reconstruction difficult and rendering the results unpredictable. A loss of tendon tissue can be dealt with either by means of a free tendon transplant or with turn-over flaps of the gastrocnemius fascia or a frozen cadaver transplant. Skin coverage can be achieved with free vascularized flaps, local vascularized flaps or local non-vascularized flaps (Ronel et al. 2004). A meshed cutaneous transplant can be used in the case of a very limited loss of skin coverage. Antibiotic therapy should be given according to the culture findings, the most commonly encountered bacteria being the normal skin bacteria Staphylococcus aureus and Streptococcus epidermidis.

Deep venous thrombosis should be treated according to healthcare guidelines. A first-time thrombosis is not an indication for life-long warfarin.

Damage to the nerves, which is sometimes seen with percutaneous techniques, is difficult to treat. If obvious total damage to a certain nerve is seen immediately after surgery, a re-operation attempt should be made to free the nerve and repair the AT rupture by open surgery.

One reported means of dealing with a troublesome excess AT lengthening involves a shortening Z-plasty (Mafulli & Ajis 2008). The operative method is
simple, although there is no hard evidence supporting the need for such a pro-
cedure.

1.4 Tendon histology changes upon AT rupture

1.4.1 Collagen changes

Tendon is normally formed mainly (98%) of type I collagen (Josza & Kannus 1997). This is synthesized by fibroblasts and its metabolism is well regulated by the surrounding extracellular matrix components. The collagen turnover period in a tendon is 50–100 days. Any damage to a tendon will dramatically alter its composition, however, so that four normal phases of healing after injury have been distinguished: inflammation, proliferation, remodelling and maturation (Gelbermann et al. 1999). At strains below 8% the injury may be microscopic, but at strains above 8% it will be macroscopic, implying total rupture of the tendon. The healing process after a macroscopic rupture is quite clearly defined and follows the given four phases, whereas microscopic tendon damage is more complex and it has been looked on as preceding total rupture (Josza et al. 1989a).

Type I collagen, the major substance making up a normal tendon, is partially replaced by type III collagen in injury areas (Leadbetter 1992, Liu et al. 1995), the latter appearing in wounds after 48 hours and reaching its peak during the first week (Haukipuro et al. 1990). Type III collagen is weaker in resisting tensile forces and its fibre bundle formation is less firmly oriented, since its crosslinking differs from that of type I collagen. After two weeks type III collagen synthesis will decrease and it will slowly be replaced with type I collagen, which will gradually increase the tensile strength of the tendon and restore its mechanical properties (Leadbetter 1992). In good biochemical surroundings healing will result in similar tendon tissue to that which preceded the injury.

Microscopic rupture, in other words repeated microtrauma, is still a partly unknown mechanism and is more difficult to treat. Ruptured tendons have been shown to exhibit significant changes in collagen structure relative to the time before trauma (Kannus & Jozsa 1991, Järvinen et al. 2004). The theory based on such findings is known as the degenerative theory. Repeated microtrauma in the area of maximum loading in a tendon will prolong the healing process and prevent it from proceeding to the next phase. This will result in excess type III collagen and in mucoid and hypoxic tissue changes similar to those seen in degenerative tendon diseases. These changes will naturally weaken the tendon and it will finally
break if the tensile forces exceed its altered capacity. Biopsies of healthy Achilles tendons show markedly less degenerative changes than those of ruptured Achilles tendons (Maffulli et al. 2000).

1.4.2 Tenascin-C

The tenascins form a group of small glycoproteins to be found in the extracellular matrix. There are several types that differ in molecular size and tissue specificity (Chiquet-Ehrismann & Tucker 2004), and their function in the extracellular matrix is not well known, but it has been suggested that they may control cell-to-cell and cell-to-ECM binding (Sage & Bornstein 1992). Tenascins are highly elastic and can be stretched three to four-fold relative to their resting length without damage (Oberhauser et al. 1998). Their molecular construction is well developed for multiple adhesion to surrounding cells and other ECM proteins. With these features it is understandable that they are often to be found in locations where heavy mechanical stresses are present (Settles et al. 1996). Tenascin-C is encountered in tendon, cartilage, bone and skeletal muscle, around tumours and in wounds (Mackie et al. 1988, Riley et al. 1996, Erickson 1997, Kaarteenaho-Wiik et al. 2002, Chiquet-Ehrismann & Tucker 2004). It has three forms, with molecular weights ranging from 50 to 200 kDaltons. The amount of tenascin-C in normal musculoskeletal tissue is very limited, but it has been shown to be expressed more abundantly in tissue pathologies where extracellular matrix activity is increased (Erickson 1997). Thus elevated expression of tenascin-C has been found in the musculotendinous and osteotendinous junctions of the musculoskeletal system after loading periods (Fluck et al. 2000, Järvinen et al. 1999) and in stressed fibroblasts in vitro (Chiquet-Ehrismann et al. 1994). Its exact function in musculoskeletal tissue is unclear, but it is evidently also present in degenerative diseases such as osteoarthritis (Salter 1993) and in supraspinatus tendons (Riley et al. 1996) and supraspinatus bursae (Hyvönen et al. 2003). Whether its expression in degenerative tissues is a part of the repair process or part of the degeneration itself is not known, but tenascin-C gene variation has recently been linked with an increased risk of Achilles tendon injury (Mokone et al. 2005). Interestingly, when mice tenascin genes are blocked, the other ECM proteins replace the tenascin function in tissues (Forsberg et al. 1996). Despite all the data available, the actual function of tenascin remains unknown.
3 Purpose of the research

The specific aims of this research were as follows:

A prospective, randomized study to compare the results of the open augmented and end-to-end surgical repair techniques for the treatment of fresh complete AT ruptures. The null hypothesis was that augmented repair entails no advantage over simple end-to-end repair.

The incidence of complete AT ruptures has increased, but we are not aware of any reports on the incidence of re-ruptures and deep infections following its treatment. The outcome after successful treatment of an acute AT rupture is good, but that after complications is presumably much worse. We therefore determined the incidences of primary ruptures and deep infections and re-ruptures at Oulu University Hospital over a 20-year period and examined the late results after treatment for complications.

We measured by biochemical means the amounts of type I and type III collagen, their synthesis and crosslinked telopeptides at the rupture site and compared the results with those for two other sites in the same tendon for samples collected from six healthy cadaver controls. Our hypothesis was that there collagen type III will be elevated and the crosslinking of collagen fibrils reduced at the rupture site.

We examined the expression of tenascin-C and type I and III collagen in the ruptured human AT by comparing expression at the rupture site with that at two other sites within the same tendon. The hypothesis was that there would be elevated expression of tenascin-C and type III collagen at the rupture site.
4 Materials and methods

The research was performed at the Department of Surgery, Oulu University Hospital, Oulu, Finland, in co-operation with the Department of Physical Medicine and Rehabilitation (I, II), the Department of Diagnostic Radiology (I, II), the Department of Clinical Chemistry (III, IV) and the Department of Clinical Pathology (IV).

4.1 Materials

*Paper I:* Eighty-three patients with a closed total ATR were treated at Oulu University Hospital between October 1998 to January 2001. Twenty-three of these were excluded from the present study on the grounds of age over 65 (six patients), rupture over 7 days old (four patients), local corticosteroid injections around the AT within six months of the rupture (one patient), open ATR (one patient), skin problems over the AT (one patient), living abroad (two patients), occurrence while the main author was out of office (six patients) and refusal to participate (two patients). Thus there were 60 patients eligible for randomization, 49 of whom (82%) had sustained the rupture during a sports-related activity, most frequently ball games (70%).

*Paper II:* Out of a total of 409 patients with a complete closed Achilles tendon rupture were treated at Oulu University Hospital between 1979 and 2000, 23 had a re-rupture. This group included twenty-one men and two women, with a mean age of forty years. These patients represented all social classes and a variety of occupations. Nineteen of the twenty-three patients (83%) had sustained the primary rupture during participation in sports activities, most commonly badminton (seven patients) and volleyball (five patients). Nine patients had sustained a deep infection after surgery. This group included seven men and two women with a mean age of fifty-three years. Only two of these patients had sustained the primary Achilles tendon injury during participation in sports, the other seven having suffered accidents of various kinds during normal activities of daily life. In six cases the infection had occurred after the primary operation, and in three it had occurred after a second operation had been performed to treat a re-rupture.

Altogether twelve patients with a re-rupture and seven with a deep infection were available for a follow-up evaluation at a mean of 4.1 years after the primary Achilles tendon rupture. Of the remaining ten patients, one had died, one (who had a well-healed re-rupture) was unable to attend because of work obligations, three
had a follow-up visit scheduled within less than six months, and five (one of whom was living abroad) did not reply to our questionnaire.

**Paper III:** Tissue samples from 10 consecutive patients (9 men, 1 woman, average age 38 years, range 30–48) with a closed total Achilles tendon rupture were taken during surgery at Oulu University Hospital. Exclusion criteria were a previous Achilles tendon injury, age over 60 years, use of corticosteroids and a rupture more than 48 h before the operation. The study was approved by the Research Ethics Committee of Oulu University Hospital, and voluntary informed consent was obtained in writing from all the patients. Presumably healthy Achilles tendon cadaver samples from corresponding sites were obtained from 5 men and one woman (average age 43, range 13–56) within 72 hours post mortem. Permission for the use of the cadaver samples was obtained from the National Board of Medicolegal Affairs, Helsinki, Finland.

**Paper IV:** The material for this paper consisted of tissue samples from the same 10 consecutive patients as for Paper III above.

### 4.2 Methods

#### 4.2.1 Treatments

**Paper I**

Sixty patients with acute complete Achilles tendon rupture (ATR) were randomized preoperatively to receive end-to-end suturation by the Krackow locking loop technique either without augmentation (simple repair (SR) group n=32) or with one central down-turned gastrocnemius fascia flap (augmented repair (AR) group n=28) (Silfverskiöld 1941) (Figure 3). The first available operation time within 48 hours of randomization was scheduled. All the patients were operated on by the main author (AP) under spinal anaesthesia in a prone position using a tourniquet. In both groups a posteromedial skin incision was made, curving to the middle line in the more proximal part of the calf. The fascia and paratenon were divided on the same line. Irregular tendon ends were cleaned and repaired by the Krackow technique (Mandelbaum et al. 1995) with two separate 0-gauge absorbable PDS (polydioxanone) sutures (Ethicon, Johnson & Johnson Inc. Somerville, New Jersey) and smaller 2–0 gauge apposition sutures made with Vicryl (polylactin, Ethicon). No augmentation was used in the SR group, whereas in the AR group a 10mm wide
central gastrocnemius aponeurosis flap was turned down over the suture line and stitched to the distal AT with 2–0 gauge Vicryl. Titanium marker clips were placed on both sides of the ruptured tendon ends after suturing. The fascia was carefully re-sutured with Vicryl, and the skin was closed with Ethilon (nylon) sutures (Ethicon®). At the end of the operation a below-knee rigid plaster splint was applied, with the ankle in a neutral position in all cases.

![Diagram](image)

**Fig. 3.** The Krackow suture (used in simple repair group) in Achilles tendon rupture on the left side and the Krackow suture and augmentation with one down turned gastrocnemius fascia flap (used in augmented repair group) on the right side.

All the patients were observed overnight at the hospital and then received a custom-made below-knee dorsal brace of Soft Cast® on the first postoperative day which allowed active free plantar flexion of the ankle while dorsiflexion was restricted to neutral. The brace and skin sutures were removed at three weeks. Twenty kg weight bearing was allowed for three weeks, half weight bearing between the third and sixth weeks and full weight bearing after six weeks. The patients in both groups were advised to perform postoperative exercises according to a standard rehabilitation programme. None of the patients received professional physiotherapy. Jogging was commenced at 12 weeks, and swimming and cycling exercises were recommended. Running at full speed, ball games and all other types of sports were allowed after 6 months.
Primary treatment. Twenty-eight of the twenty-nine patients had initially been treated operatively, and one had been treated non-operatively with a cast. Our standard protocol for operative treatment was tendon suturing with absorbable size-0 non-braided sutures. The repair was augmented with one turn-down flap of the gastrocnemius aponeurosis (the Silfverskiöld technique) in fourteen cases, with two flaps (the Lindholm technique) in five cases, and with the plantaris tendon (the Lynn technique) in one case. For twenty of the twenty-eight patients who had had surgical treatment the planned postoperative rehabilitation protocol consisted of immobilization in a below-the-knee cast for six weeks, with the ankle in plantar flexion for three weeks and then in a neutral position for three weeks. Weight-bearing was allowed gradually after the first three weeks, with full weight-bearing achieved at six weeks. The other eight patients who had had surgical treatment were enrolled in a clinical trial in which a customized below-the-knee brace was used postoperatively. This device allowed active free plantar flexion of the ankle but restricted dorsiflexion to neutral. Weight-bearing was limited to one-half of body weight until six weeks, at which time active ankle exercises with full weight-bearing and strengthening exercises were allowed. All of the patients who were treated surgically were instructed to begin jogging and controlled sports activities at three months. Jumping sports and professional activities were begun at six months. The one patient who had been treated non-operatively wore a below-the-knee cast with the ankle in plantar flexion for three weeks and then a second cast with the ankle in a neutral position for an additional three weeks.

Treatment of re-ruptures. The twenty-three re-ruptures occurred at a mean of seventy-nine days (range, two to 209 days) after the primary operation. Nineteen were treated operatively and four non-operatively with six weeks of immobilization in a below-the-knee cast. The repair of the re-rupture was augmented with one turn-down flap of the gastrocnemius fascia in nine cases, with two turn-down flaps in two cases, and with the plantaris tendon in three cases. In one case the re-rupture was reconstructed with exogenous material (Leeds-Keio; Howmedica, Rutherford, New Jersey). The operative technique was not accurately described in the records for two of the patients. (Table 1)

Two patients had a second re-rupture. The first had had two previous surgical repairs that had been performed with the end-to-end technique, and the third repair was performed with one turn-down flap for augmentation. The other patient had
had two previous surgical repairs with plantaris tendon augmentation and the third repair was augmented with two turn-down flaps. (Table 1)

Treatment of infections. There were a total of nine deep infections. Six occurred after the primary repair and three after the repair of a re-rupture. Wound revision was performed in all cases, and necrotic Achilles tendon tissue was totally removed from five patients during the surgical treatment. The results of bacterial cultures, recorded for six of the nine patients, were positive for Staphylococcus aureus (four patients), Staphylococcus epidermidis (one patient) and Propionibacterium acnes and diphtheroid species (one patient). (Table 2)

Six patients were treated with repeat débridement and primary split-thickness skin-grafting. Four of these patients lost the Achilles tendon entirely during the course of treatment, while in the other two cases the infection resolved before all the tendon tissue had been removed. Two patients were managed with a microvascular radial forearm flap and a tensor fasciae latae graft after débridement. One of these microvascular reconstructions was successful, but the other failed because of thrombosis. The latter patient ultimately had total loss of the tendon and was eventually treated with split-thickness skin grafting. One patient needed a local two-tail full-thickness transposition flap to cover the exposed tendon after débridement. (Table 2)
Table 1. Data on the patients in the re-rupture group.

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1 GFA = gastrocnemius fascia augmentation with one turn down flap (Silfverskiöld).
1 GFA = gastrocnemius fascia augmentation with two turn down flap (Lindholm).
Lynn = plantaris longus tendon augmentation.
End-end = end to end suture.
Table 2. Data on the patients in the deep infection group.

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<td>Yes</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>–</td>
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</tr>
<tr>
<td>26</td>
<td>End-end</td>
<td>–</td>
<td>56</td>
<td>Yes</td>
<td>Yes</td>
<td>–</td>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>27</td>
<td>1 GFA</td>
<td>–</td>
<td>36</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
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<tr>
<td>28</td>
<td>2 GFA</td>
<td>–</td>
<td>32</td>
<td>–</td>
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<tr>
<td>29</td>
<td>2 GFA</td>
<td>–</td>
<td>45</td>
<td>–</td>
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<td>6</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>1</td>
</tr>
</tbody>
</table>

1 GFA = gastrocnemius fascia augmentation with one turn down flap (Silfverskiöld).
1 GFA = gastrocnemius fascia augmentation with two turn down flap (Lindholm).
Lynn = plantaris longus tendon augmentation.
End-end = end to end suture.
*Cases 3, 5, and 6 are included in both the rupture group and the deep infection group.
Papers III and IV

All ten patients with a complete closed Achilles tendon rupture were operated on using the one turn-down flap augmentation technique as described by Silfverskiöld.

4.2.2 Evaluation methods

Achilles tendon rupture score (Papers I and II)

Paper I: All available patients were examined clinically at 3, 6, 12 and 52 weeks and the clinical outcome was assessed at the 12 and 52-week check-ups by the clinical scoring method described by Leppilahti (Leppilahti et al. 1998), which included subjective factors such as pain, stiffness, muscle weakness, footwear restrictions and subjective outcome and objective factors such as the range of active ankle motion and isokinetic calf muscle strength. The patients were asked to fill in a non-validated subjective symptoms questionnaire sheet. The assessors, two physiotherapists and A.Pajala, of the objective clinical outcome were not blinded to the treatment groups.

Paper II: Twenty-three re-ruptures (prevalence 5.6%) and nine deep infections (prevalence 2.2%) occurred in twenty-nine patients. We reviewed the records of these patients retrospectively to determine the overall incidence of ruptures, re-ruptures and deep infections and to record the known risk factors for these major complications. The final clinical outcome for twelve patients with a re-rupture and seven with a deep infection at a mean of 4.1 years after the initial treatment was assessed in terms of the Achilles tendon rupture score.

Strength measurements (Papers I and II)

Isokinetic and isometric muscle function parameters were assessed for the two groups at 3 months and 12 months using a Lido Multi-Joint II dynamometer linked to a computer (Loredan Biomedical Inc, Davis, CA). One physiotherapist performed all the isokinetic tests. All the patients were informed of the procedure, and a ten-minute warm-up period of ergometer cycling was included prior to the test. The testing position was supine, and the patient was fixed to the testing apparatus with straps round the foot and the pelvis, with the knee supported in extension. The extent of ankle motion was from 40 degrees of plantar flexion to 20 degrees of dor-
siflexion. Before testing, the patient performed some submaximal and maximal repetitions of the ankle flexion and extension movements at the isokinetic test velocity. The isokinetic dorsiflexion and plantar flexion strengths were measured, first at a speed of 60°/sec, then at 120°/sec, and finally at 180°/sec after two minutes of rest. Five maximal voluntary muscular torque contractions were required. After the isokinetic tests, the maximal isometric plantar flexion strength was measured with the ankle in the neutral position.

For evaluation of muscle strength, the peak torques (PT, unit: Newton meters) of plantar flexion and dorsiflexion of the ankles were analysed. The isokinetic strength scale for scoring the peak torque of the ankle during plantar flexion and dorsiflexion at three test velocities was used to analyse the strength results (Leppilähti et al. 1996b).

Peak work (unit: joule, Paper I) was defined as the sum of the total area under all the torque versus angular displacement (time) plots in the best repetition of the test. The peak work-displacement curves for both legs were divided into five parts, making it possible to calculate the PW deficits at intervals of 10 degrees over the range of ankle motion.

**Radiographic measurements (Paper I)**

Standardized radiographs for the measurement of previously placed radiographic markers were taken on the first day postoperatively and at 3, 6, 12 and 52 weeks. The ankle was fixed in the plantigrade position in the brace, the distance between the x-ray source and the film plate was fixed at 100 centimetres and the radiograph was focused at the midpoint of the AT. A magnification of 1.1 was taken into account. The AT elongation curves for both groups were analysed and correlated with the clinical data.

**Biochemical and histopathological characterization (Papers III and IV)**

Tissue samples (III, IV) were taken from the rupture site (RUPT), from the lower end of the flap (CONT1) and from the top corner of the flap (CONT2) (Figure 4).
Fig. 4. Locations of tissue samples taken for Papers III and IV. A is the rupture site, B is control 1 and C is control 2. The distances from the heel bone insertion are A 4cm, B 8cm and C 16cm. As seen in the picture, tendon-like tissue is still available at site C.

Tissue sample preparation (Paper III). The Achilles tendon samples from the rupture patients (weight 13 to 335 mg) and cadavers (weight 61 to 273 mg) were cut into small pieces, suspended into PBS, pH 7.2 (20 mg/ml), homogenized by sonication (4 times for 15 seconds in an ice bath), incubated in ice for 30 minutes and centrifuged (8 000 g, 30 min) to separate the soluble tissue from the insoluble pellet. The supernatants were collected for type I and III procollagen propeptide analyses. The insoluble pellet was lyophilized for further processing (see below).

Stabilization of collagen crosslinks and degradation of insoluble pellets (Paper III). The pellets were suspended in PBS, pH 7.2 (20 mg/ml), and reduced with NaBH₄ (1 mg of NaBH₄ to 40 mg of tissue, reaction time 2 h with magnetic stirring) to stabilize any immature, divalent collagen crosslinks (Knott et al. 1997). The samples were washed several times with distilled water, centrifuged (8000 g, 30 min), decanted and finally lyophilized.

The reduced samples were dissolved in 0.2 M NH₄HCO₃, pH 7.8 (20 mg/ml). The tissue pieces were heat-denatured at 65°C for 30 minutes, cooled to 37°C and digested with TPCK-treated trypsin (Worthington Biochemicals, Lakewood, NJ)
After incubation for 6 h at 37°C, the heat denaturation and trypsin digestion were repeated and the samples were incubated overnight. The samples were treated similarly for a third time on the next day and then incubated for 6 h at 37°C. After final heat denaturation, they were centrifuged (8000 g, 30 min) and the supernatants analysed for hydroxyproline, ICTP, IIINTP and PIIINP.

Measurement of type I and III collagen antigens and total collagen content (Paper III). Procollagen synthesis in the soluble tissue extracts was assessed by radioimmunoassays (RIA) for the aminoterminal (PINP) and carboxyterminal (PICP) propeptides of type I collagen and for the aminoterminal propeptide (PII-INP) of type III collagen (Orion Diagnostica, Oulunsalo, Finland) (Melkko et al. 1990, Melkko et al. 1996, Risteli et al. 1988). The results are expressed per wet weight of the original tissue sample.

Type I collagen in the insoluble tissue digests was analysed with an assay for the trivalent pyridinoline cross-linked carboxyterminal telopeptide structure, ICTP, and with an in-house SP 4 assay based on a synthetic peptide sequence (SAGFD-FSFLPQPPQEKY, Neosystem Laboratories, Strasbourg, France) (Bode et al. 2000a, Sassi et al. 2000, 2001). The trivally pyridinoline-crosslinked aminoterminal telopeptide of type III collagen, IIINTP, was also analysed by means of an in-house RIA as described earlier (Bode et al. 1999, Kauppila et al. 1999, Bode et al. 2000a,b), and PIIINP was also analysed in the digests. To determine the total collagen content, hydroxyproline was measured with a novel colorimetric microtitre plate assay (Brown et al. 2001), on the assumption that this accounts for 12.4% (w/w) of the total collagens. These results were expressed per dry weight of the insoluble tissue residues.

Reverse phase HPLC (Paper III). The insoluble tissue digests of the RUPT site of one individual with a ruptured tendon and one cadaver used for C8 reverse phase HPLC (228TP1010, Vydac, Hesperia, CA, U.S.A) with 0.4% ammonium acetate (pH 7.4) and 75% acetonitrile. Amounts of sample equivalent to 100 µg of ICTP were loaded and their pyridinoline crosslink fluorescence (ex 320 nm, em 405 nm) was monitored. ICTP and IIINTP were measured from the fractions collected.

Tissue sample preparation for Paper IV. All the tissue samples were fixed in 10% buffered formalin, embedded in paraaffin and cut to a thickness of 5 μm. Immunohistochemical staining was carried out by the avidin-biotin-immunoperoxidase technique, and the tissue sample slides were counterstained with haematoxylin-eosin. A monoclonal mouse antibody reacting to two major isoforms of human tenascin-C was employed to visualize tenascin. Polyclonal antibodies were raised
in rabbits, cross-absorbed with other connective tissue antigens and purified by immunoabsorption on the relevant antigens coupled to Sepharose 4B. These were used to characterize the type I and III procollagens and collagens deposited in the tissue samples. Since anti-PINP detects the aminoterminal propeptide of type I procollagen, positive staining indicates newly synthesized type I collagen still having the aminoterminal propeptide attached. This form is also called type I pN collagen. Anti–PICP detects the carboxyterminal propeptide and is also used as a marker of type I collagen synthesis. Anti–PIIINP detects the aminoterminal propeptide of type III collagen, which can be found in free form in the extracellular matrix or as type III pN collagen on the surfaces of type III collagen fibrils. It is used as a marker of type III collagen synthesis. Anti–IIIINTP detects the type III collagen which is bound to collagen fibrils with normal intermolecular crosslinking.

The amounts of tenascin-C and of type I and III procollagens and collagens were determined by analysing the immunoreactivity of the tissue sections, the evaluation being performed on a semiquantitative scale from 0 to 3, corresponding to the abundance of labelled tissue in the samples (0: reactivity absent; 1: under 33%; 2: 33–66%; 3: over 66%). The reactivity was evaluated independently by two pathologists (Figure 12).

4.2.3 Statistical methods (I, II, III, IV)

Paper I: The summary statistics are expressed as means and standard deviations (SD) unless other stated. The mixed model approach was used to analyse continuous variables measured repeatedly using a combined covariance pattern and random coefficient model. P values are reported as follows: p between groups ($P_g$), indicates a level of difference between the groups, p-measure*group ($P_{m\times g}$), indicates group measurement interaction and ($P_m$) indicates a change between measurement points, the word measurement was substituted for time when presenting the AT elongation results. The Mann-Whitney U test or Student’s t test was used to assess the distribution of continuous variables between the groups (the former if the t-test assumptions were not met), and the paired t-test was used to compare the 3 and 12-month strength measurements. Fisher’s exact test was used for categorial variables. Spearman’s correlation coefficient ($\rho$) was calculated to present simple correlations between two continuous variables. Two-tailed significance levels are reported. Readers should treat the p values with caution, since several comparisons
are made and no p value correction coefficient method is used. The analyses were performed with SPSS (version 15.0; SPSS, Chicago, Illinois).

Paper II: The summary statistics for continuous variables were expressed as means and standard deviations. The Mann-Whitney U test was used to calculate differences between continuous variables, and Fisher's exact test for differences between frequencies. Two-tailed p values were reported, and the level of significance was set at $p < 0.05$. The analyses were performed with SPSS (version 10.0; SPSS, Chicago, Illinois).

Paper III: Wilcoxon’s signed-rank test and the Mann-Whitney test were used to assess the statistical significances of the differences. The data were expressed as medians and ranges (min-max). Linear regression analysis was used for the correlation analysis. The analyses were performed with SPSS software (SPSS, Chicago, Illinois).

Paper IV: The non-parametric rank test was used to assess the statistical significances of the differences. The data are expressed as frequencies of rankings on the semiquantitative scale. Statistical significances of differences in ranking between the three sites studied were calculated using the Friedman test. P values in the Sign test used for comparing the rupture and control sites were calculated if Friedman’s test gave $p < 0.05$). The statistical analyses were performed using SPSS software (SPSS, Chicago, Illinois).

4.2.4 Ethics (I, II, III, IV)

All four papers were approved by the local Research Ethics Committee. Permission for the use of cadavers for Paper III was obtained from the National Board of Medicolegal Affairs, Helsinki, Finland.
5 Results

5.1 Augmented vs. non-augmented surgical repair of acute total AT rupture. A randomized prospective study (I)

The average surgery time was 77 (SD 12.1) minutes in the AR-group and 52 (SD 8.0) minutes in the SR-group (p<0.001), and the length of the incision was 18 (SD 1.3) cm and 11 (SD 1.4) cm, respectively (p<0.001), with circulatory arrest times of 64 (SD 9.8) minutes and 44 (SD 8.5) minutes (p=0.01).

There were six re-ruptures, three in each group, and two deep infections, both in the AR group. These re-ruptures and deep infections were regarded in the analysis as early treatment failures. There is one patient in the simple repair group and three in the augmented repair group for whom we could not perform strength tests with Lido-device as they refused to any given appointment and this affects the group sizes in Leppilahti score and isokinetic strength score. Also one patient in the simple repair group was totally lost after he moved abroad and this reduces group size in all outcome variables of that group.

The primary outcome measure, Leppilahti’s outcome score, was classified as excellent at the 12-month check-up in nineteen cases (63%) in the SR-group and as good in eight (27%), with three early failures (3 re-ruptures; 10%). The corresponding scores in the AR-group were excellent in fourteen cases (56%) and good in six (24%), with five early failures (3 re-ruptures, 2 deep infections; 20%). (0.68) (Table 3)

Twenty-two of the patients in the SR group (71%) were very satisfied at 12 months, and six (19%) were satisfied with minor reservations, whereas twenty patients in the AR-group (71%) were very satisfied and three (11%) were satisfied with minor reservations. The early failures three (10%) in SR-group and five (18%) in AR-group. (p=0.55) (Table 3)

Twenty-eight Achilles tendons in the SR group (90%) were painless at 12 months, whereas twenty-two tendons (79%) were painless and one (3%) mildly painful in the AR-group. The early failures three (10%) in SR group and five (18%) in AR-group. (p=0.35) (Table 3)

Twenty-three patients (74%) in the SR group and thirteen (46%) in the AR group reported no stiffness at the 12-month check-up, while five patients (16%) in the SR group and ten (36%) in the AR-group reported occasional mild stiffness. The early failures three (10%) in SR-group and five(18%) in AR-group. (p=0.10) (Table 3)
Twenty patients in the SR group (65%) had no subjective calf muscle weakness at the 12-month check-up, while eight (26%) had mild weakness, whereas twenty in the AR group (71%) had no subjective calf muscle weakness, two (7%) had mild weakness and one (4%) had moderate weakness. The early failures three (10%) in SR-group and five (18%) in AR-group. (p=0.14) (Table 3)

Twenty-eight (90%) patients in the SR group had no footwear restrictions at 12 months, while twenty-two in the AR group (79%) had no footwear restrictions and one (4%) had mild restrictions but tolerated most shoes. The early failures three (10%) in SR-group and five (18%) in AR-group. (p=0.35) (Table 3)

Twenty-six patients (84%) in the SR group had a normal range of ankle motion at the 12-month check-up and two (6%) had mild limitation, whereas nineteen patients in the AR-group (68%) had a normal range of motion and four (14%) had mild limitation. The early failures three (10%) in SR-group and five (18%) in AR-group. (p=0.40) (Table 3)
Table 3. Results at the 12-month follow-up evaluation in study groups.

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Simple repair group</th>
<th>Augmented repair group</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leppilahti’s score *</td>
<td>19</td>
<td>14</td>
<td>0.68</td>
</tr>
<tr>
<td>Excellent</td>
<td>19</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Subjective result **</td>
<td>22</td>
<td>20</td>
<td>0.55</td>
</tr>
<tr>
<td>Very satisfied</td>
<td>22</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Satisfied, with minor reservations</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Satisfied, with major reservations</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dissatisfied</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Pain **</td>
<td>28</td>
<td>22</td>
<td>0.35</td>
</tr>
<tr>
<td>None</td>
<td>28</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Mild, no limitations on recreational activities</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Moderate, limitations on recreational activities, but not on daily activities</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Severe, limitations on recreational and daily activities</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Stiffness **</td>
<td>23</td>
<td>13</td>
<td>0.10</td>
</tr>
<tr>
<td>None</td>
<td>23</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Mild, occasional, no limitations on recreational activities</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Moderate, limitations on recreational activities, but not on daily activities</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Severe, limitations on recreational and daily activities</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Calf muscle weakness (subjective) **</td>
<td>20</td>
<td>20</td>
<td>0.14</td>
</tr>
<tr>
<td>None</td>
<td>20</td>
<td>20</td>
<td></td>
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<tr>
<td>Mild, no limitations on recreational activities</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Moderate, limitations on recreational activities, but not on daily activities</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>Severe, limitations on recreational and daily activities</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Footwear restrictions **</td>
<td>28</td>
<td>22</td>
<td>0.35</td>
</tr>
<tr>
<td>None</td>
<td>28</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Mild, most shoes tolerated</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Moderate, unable to tolerate fashionable shoes, modified shoes tolerated</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Active ROM difference between ankles **</td>
<td>26</td>
<td>19</td>
<td>0.40</td>
</tr>
<tr>
<td>Normal (≤ 5°)</td>
<td>26</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Mild (6°–10°)</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Moderate (11°–15°)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Severe (≥16°)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Isokinetic muscle strength (score) *</td>
<td>11</td>
<td>9</td>
<td>0.43</td>
</tr>
<tr>
<td>Excellent</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>14</td>
<td>7</td>
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<td>Fair</td>
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<tr>
<td>Poor</td>
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<td></td>
</tr>
<tr>
<td>Early failure</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

* Patients available for outcome variable evaluation in simple repair group 30 and in augmented repair group 25
** Patients available for outcome variable evaluation in simple repair group 31 and in augmented repair group 28
Isokinetic and isometric calf muscle strength

The isokinetic strength outcome score at the 12-month check-up was excellent for eleven patients in the SR-group (36%), good for fourteen (47%) and fair for two (7%), whereas it was excellent for nine patients in the AR-group (36%), good for seven patients (28%), fair for three patients (12%) and poor for one patient (4%). The early failures one patient (4%). There were early failures three (10%) in SR-group and five (20%) in AR-group. (p=0.43) (Table 3)

The mean relative peak torque deficits for plantar flexion in the injured limb at velocities of 60, 120 and 180°/sec did not differ significantly between the groups at the 3-month and 12-month follow-up examinations. The figures at 12 months were 7%, 7% and 2%, respectively, for the SR group and 7%, 5% and 2% for the AR-group (Table 4). The differences between the 3-month and 12-month results were significant at velocities of 60°/sec and 120°/sec in both groups (Table 4).

The mean relative isometric strength deficit in the injured limb in plantar flexion was 29% for the SR group and 34% for the AR-group at the 3-month check-up and 10% for the SR group and 2% for the AR-group at the 12-month check-up (In both groups p<0.001 between 3 and 12 months) (Table 4).

The differences in isokinetic dorsi flexion strength deficits were not significant between the study groups. There was a slight tendency so that dorsi flexion was weaker in the healthy leg than in the injured side (Table 4).

The mean peak work-displacement relationships upon plantar flexion of the ankle in both groups at the 3-month and 12-month check-ups are shown in Figure 5. The deficit in plantar flexion motion was larger in the injured leg in both groups at the 3–month check-up (p< 0.001), but the differences between the injured and non-injured sides had diminished by the 12-month check-up. The differences in the deficit in dorsiflexion of the ankle at the 3 and 12-month check-ups were not significant between the groups or within the groups (Figure 6).
Fig. 5. Mean peak work in plantarflexion measured in 10-degree intervals at the 3-month and 12-month follow-ups (error bars denote SD). Control value is the healthy leg in each group.

Fig. 6. Mean peak work in dorsiflexion measured in 10-degree intervals at the 3-month and 12-month follow-ups (error bars denote SD). Control value is the healthy leg in each group.
Table 4. Mean (SD) strength deficits in percentages between the operated leg and healthy leg at different angle speed velocities (60°/sec, 120°/sec and 180°/sec). P values are calculated for comparisons between the groups and between the 3-month and 12-month results. Negative values in dorsiflexion results indicate that the operated leg showed better strength than the healthy leg.

<table>
<thead>
<tr>
<th>Tendon elongation</th>
<th>At 3 months</th>
<th>At 12 months</th>
<th>P (3 month vs. 12 month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean plantar flexion strength deficit %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 60° / sec angle speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>20.3 (SD 22.7)</td>
<td>6.5 (SD 11.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>18.3 (SD 12.0)</td>
<td>7.4 (SD 9.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>0.37</td>
<td>&gt;0.9</td>
<td></td>
</tr>
<tr>
<td>At 120° / sec angle speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>17.2 (SD 21.2)</td>
<td>5.2 (SD 13.2)</td>
<td>0.026</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>18.1 (SD 12.1)</td>
<td>6.9 (SD 10.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>&gt;0.9</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>At 180° / sec angle speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>4.0 (SD 17.3)</td>
<td>1.7 (SD 12.7)</td>
<td>0.55</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>4.2 (SD 12.3)</td>
<td>1.6 (SD 10.4)</td>
<td>0.49</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>0.74</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Isometric plantar flexion strength deficit%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>34.4 (SD 13.5)</td>
<td>2.1 (SD 26.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>28.9 (SD 13.5)</td>
<td>10.4 (SD 11.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>0.32</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Mean dorsiflexion strength deficit %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 60° / sec angle speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>-8.3 (SD 18.8)</td>
<td>2.3 (SD 15.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>-8.2 (SD 15.6)</td>
<td>-0.4 (SD 12.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>&gt;0.9</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>At 120° / sec angle speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>-4.8 (SD 17.4)</td>
<td>3.2 (SD 12.6)</td>
<td>0.025</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>-9.3 (SD 20.0)</td>
<td>-3.8 (SD 14.2)</td>
<td>0.094</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>0.45</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>At 180° / sec angle speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation group (n=21)</td>
<td>-2.6 (SD 17.5)</td>
<td>-2.3 (SD 13.1)</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Non-augmented group (n=28)</td>
<td>-5.6 (SD 20.5)</td>
<td>-11.3 (SD 16.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>P (between groups)</td>
<td>0.79</td>
<td>0.027</td>
<td></td>
</tr>
</tbody>
</table>

Tendon elongation

The elongation curves increased significantly up to twelve weeks in both groups and decreased between 3 and 12 months ($P_{time}$<0.001, Figure 7). The elongation was more marked in the non-augmentation group at 3 months and 12 months, although the difference between the groups was not significant. The median AT
elongation was 5.0 mm (25th and 75th percentiles 2.5–9.2) in the SR-group and 6.0 mm (3.0–10.5) in the AR-group at 3 weeks, 8.0 mm (4.8–12.0) and 11.0 mm (7.0–14.5) respectively at 6 weeks, and 14.0 mm (7.0–21.0) and 12.0 mm (8.0–20.5) at 3 months. After 3 months the AT shortened in both groups, the median elongation being 10.0 mm (5.0–19.0) in the SR-group and 5.0 mm (2.0–20.0) in the AR group at 12 months.

AT elongation correlated significantly with the isokinetic peak torque deficits at velocities of 120º/sec ($\rho=0.52$, $p=0.013$) and 180º/sec ($\rho=0.64$, $p=0.001$) and with the isometric strength deficits ($\rho=0.475$, $p=0.026$) in the SR-group.

**Complications**

*Re-ruptures.* There were three re-ruptures in each group. The mean time elapsing before re-rupture was 58 (min. 2 and max. 112) days, and all the cases occurred in males. Three patients had obviously suffered a new injury during the recovery period. The first of these had fallen on a slippery bathroom floor two days after the operation and had to forcefully step on the operated foot, the second was repairing his wife’s bicycle at four weeks and he hit the edge of the pavement during a test.
run, so that he had to put his weight on the toe of the operated foot, and the third was walking in a swimming pool at four weeks and placed the toes of his operated foot on the edge when climbing out of the pool and pushed with his full body weight. The fourth re-rupture occurred at 10 weeks, when the patient was cycling up a steep hill, and the fifth and sixth cases occurred at 12 weeks with a minimal trauma. Both of these patients were in the non-augmented group. When a careful history was taken, they recalled having had a slight trauma during the first 3 weeks as well, while the dorsal brace was still on. The subjective clinical results in all the re-rupture cases were good.

Infections. There were two deep infections in the augmentation group and none in the simple repair group. The first such patient was a 36-year-old male who developed a clear drainage at the incision after three weeks and was immediately taken into hospital. The wound was revised and left open. None of the samples yielded a positive bacterial culture. The infection had been brought under control with intravenous antibiotics by 7 days and we were able to close the wound with a two-tailed cutaneous turnover flap. He was able to walk normally at the one-year check-up. The second patient was a 40-year-old male who had slow drainage from the lower edge of the wound. He was taken into hospital at 6 weeks and the wound was revised and left open. The bacterial cultures were positive for both *Streptococcus aureus* and acinetobacteria. The Achilles tendon had already partially healed. The situation was brought under control after 6 days with repeated revisions and intravenous antibiotics, and it was possible to close his wound with a two-tailed cutaneous turnover flap, which showed epidermal necrosis at first, but eventually healed well. He was walking well at the one-year check-up and is satisfied with the result. There were four superficial wound infections in the non-augmented group and one in the augmented group. All these resolved after oral antibiotics and were followed up according to the research protocol. These cases are included in the results section in the normal way.

There was one case of a deep venous thrombosis in the augmented group that was diagnosed at three weeks and treated with warfarin for six months. The patient was followed up according to the protocol and is included in the results.

Return to previous level of activity. All patients in the study, including the early failures, were able to return to their previous level of activity.
5.2 Re-rupture and deep infection following treatment of total AT rupture (II)

Pain. Six Achilles tendons in the re-rupture group were painless, 5 were mildly painful and one moderately painful. Only one tendon in the infection group was painless, while 4 were mildly painful and 2 moderately painful (p=0.3). (Table 5)

Stiffness. Eight patients in the re-rupture group and 2 in the infection group reported no stiffness in the Achilles tendon region. Four in the re-rupture group and 5 in the infected group reported occasional mild stiffness (p=0.17). (Table 5)

Subjective calf muscle weakness. Six patients in the re-rupture group had no subjective calf muscle weakness and 6 had mild weakness. One of those in the infection group had mild subjective calf muscle weakness, 5 had moderate weakness and one had severe weakness (p<0.001). (Table 5)

Footwear restrictions. None of the patients in the re-rupture group had footwear restrictions, whereas 3 of those in the infection group had no footwear restrictions and 4 had mild restrictions but tolerated most shoes (p=0.009). (Table 5)

Range of ankle motion. Eleven patients in the re-rupture group had a normal range of ankle motion and one had mild limitation. Two of those in the infection group had a normal range of motion, 4 had mild limitations and one had moderate limitation (p=0.01). (Table 5)

Subjective outcome. Three patients in the re-rupture group were very satisfied with the outcome, 8 were satisfied with minor reservations and one was satisfied with major reservations, while one patient in the infection group was satisfied with minor reservations and 6 with major reservations (p=0.004) (Table 5). Two patients reported a definite loss of sensation in the foot, one having undergone an unsuccessful reconstruction with a radial forearm flap and tensor fasciae latae graft and the other sustaining damage to a branch of the sural nerve at the time of the repair of the re-rupture.

Isokinetic and isometric muscle strength. In re-rupture group the isometric strength was 14% lower in injured side when compared to healthy leg (p=0.012). Respectfully in infection group the same difference was 42% in favour of healthy side (p=0.07). In isokinetic tests the differences at speeds 60°/ sec, 120°/ sec and 180°/ sec in re-rupture group were 12%, 12% and 7% in favour of healthy leg (p=0.007, p=0.003 and p=0.039) and in infection group at the same speeds 40%, 40% and 26% in favour of healthy leg (p=0.006, p=0.022 and p=0.022) (Table 6 and 7).
Table 5. Clinical outcome scoring according to Leppilahti in re-rupture and deep infection groups.

<table>
<thead>
<tr>
<th>Clinical factor</th>
<th>Renupture N=12</th>
<th>Infection N=7</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pain</strong></td>
<td></td>
<td></td>
<td>p=0.3</td>
</tr>
<tr>
<td>None</td>
<td>6 50%</td>
<td>1 14%</td>
<td></td>
</tr>
<tr>
<td>Mild, no limitations on recreational activities</td>
<td>5 42%</td>
<td>4 57%</td>
<td></td>
</tr>
<tr>
<td>Severe limitations in ADL</td>
<td>1 8%</td>
<td>2 29%</td>
<td></td>
</tr>
<tr>
<td><strong>Stiffness</strong></td>
<td></td>
<td></td>
<td>p=0.17</td>
</tr>
<tr>
<td>None</td>
<td>8 67%</td>
<td>2 29%</td>
<td></td>
</tr>
<tr>
<td>Moderate, limitations on recreational activities</td>
<td>4 33%</td>
<td>5 71%</td>
<td></td>
</tr>
<tr>
<td>Severe limitations in ADL</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Calf muscle weakness</strong></td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>None</td>
<td>6 50.0%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Moderate, limitations on recreational activities</td>
<td>6 50.0%</td>
<td>1 14%</td>
<td></td>
</tr>
<tr>
<td>Severe limitations in ADL</td>
<td>–</td>
<td>1 14%</td>
<td></td>
</tr>
<tr>
<td><strong>Footwear restrictions</strong></td>
<td></td>
<td></td>
<td>p=0.009</td>
</tr>
<tr>
<td>None</td>
<td>12 100%</td>
<td>3 43%</td>
<td></td>
</tr>
<tr>
<td>Moderate, limitations on recreational activities</td>
<td>–</td>
<td>4 57%</td>
<td></td>
</tr>
<tr>
<td><strong>Active ankle-rom difference</strong></td>
<td></td>
<td></td>
<td>p=0.1</td>
</tr>
<tr>
<td>Normal (+6°)</td>
<td>11 92%</td>
<td>2 29%</td>
<td></td>
</tr>
<tr>
<td>Mild (6°–10°)</td>
<td>1 8%</td>
<td>4 57%</td>
<td></td>
</tr>
<tr>
<td>Moderate (11°–15°)</td>
<td>–</td>
<td>1 14%</td>
<td></td>
</tr>
<tr>
<td>Severe (&gt;15°)</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Subjective result</strong></td>
<td></td>
<td></td>
<td>p=0.004</td>
</tr>
<tr>
<td>Very satisfied</td>
<td>3</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Satisfied with minor problems</td>
<td>8 1</td>
<td>1 14%</td>
<td></td>
</tr>
<tr>
<td>Satisfied with major problems</td>
<td>1 6</td>
<td>6 86%</td>
<td></td>
</tr>
<tr>
<td>Dissatisfied</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Isokinetic muscle strength</strong></td>
<td></td>
<td></td>
<td>p=0.04</td>
</tr>
<tr>
<td>Excellent</td>
<td>–</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>2 67%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>7 8%</td>
<td>1 14%</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>3 6</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td><strong>Ankle performance score</strong></td>
<td></td>
<td></td>
<td>p=0.004</td>
</tr>
<tr>
<td>Excellent</td>
<td>1 8%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>7 58%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>4 33%</td>
<td>2 14%</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>–</td>
<td>5 86%</td>
<td></td>
</tr>
</tbody>
</table>

ADL = activities of daily living.

Overall result. All the patients in the re-rupture group and one patient in the infection group were able to walk normally. Five patients in the infection group had a mild limp and one needed crutches. All the patients in the re-rupture group were able to participate in at least recreational sports, whereas 6 patients in the infection group had a restricted ability to participate in sports. The ankle performance scores (Leppilahti et al. 1998) were classified as excellent or good in 8
re-rupture cases and as fair in 4, while 2 results in the infection group were scored as fair and 5 as poor (p=0.004). (Table 5)

Table 6. Mean peak torques (Nm) during plantar flexion and dorsiflexion of the ankle at velocities 60°/sec, 120°/sec and 180°/sec and mean isometric strength of plantar flexion in re-rupture group.

<table>
<thead>
<tr>
<th>Test Speed</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Pairwise % difference Mean (SD)</th>
<th>95%CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantar flexion 60°/sec</td>
<td>113.3 (21.7)</td>
<td>128.3 (17.9)</td>
<td>11.6 (12.1)</td>
<td>3.9, 19.3</td>
<td>0.007</td>
</tr>
<tr>
<td>120°/sec</td>
<td>89.3 (16.6)</td>
<td>102.3 (19.9)</td>
<td>12.2 (10.9)</td>
<td>5.3, 19.1</td>
<td>0.003</td>
</tr>
<tr>
<td>180°/sec</td>
<td>69.1 (12.1)</td>
<td>75.2 (14.3)</td>
<td>7.1 (12.5)</td>
<td>-0.8, 15.0</td>
<td>0.039</td>
</tr>
<tr>
<td>Dorsiflexion 60°/sec</td>
<td>30.3 (6.5)</td>
<td>30.8 (6.8)</td>
<td>-0.5 (18.7)</td>
<td>-15.1, 8.7</td>
<td>0.95</td>
</tr>
<tr>
<td>120°/sec</td>
<td>25.8 (5.5)</td>
<td>25.3 (4.0)</td>
<td>-0.5 (18.1)</td>
<td>-13.9, 9.2</td>
<td>0.77</td>
</tr>
<tr>
<td>180°/sec</td>
<td>24.8 (5.5)</td>
<td>24.2 (4.3)</td>
<td>-0.6 (23.7)</td>
<td>-19.3, 10.8</td>
<td>0.74</td>
</tr>
<tr>
<td>Isometric strength (N) Plantar flexion</td>
<td>130.3 (33.8)</td>
<td>154.6 (33.9)</td>
<td>14.3 (18.8)</td>
<td>2.5, 26.4</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Table 7. Mean peak torques (Nm) during plantar flexion and dorsiflexion of the ankle at velocities 60°/sec, 120°/sec and 180°/sec and mean isometric strength of plantar flexion in infection group.

<table>
<thead>
<tr>
<th>Test Speed</th>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Pairwise % difference Mean (SD)</th>
<th>95%CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantar flexion 60°/sec</td>
<td>47.2 (29.3)</td>
<td>78.2 (42.4)</td>
<td>30.5 (11.3)</td>
<td>27.7, 51.4</td>
<td>0.006</td>
</tr>
<tr>
<td>120°/sec</td>
<td>40.4 (19.9)</td>
<td>68.4 (36.5)</td>
<td>28.0 (8.7)</td>
<td>28.6, 50.3</td>
<td>0.022</td>
</tr>
<tr>
<td>180°/sec</td>
<td>40.0 (19.9)</td>
<td>54.8 (26.8)</td>
<td>14.8 (10.7)</td>
<td>12.6, 39.2</td>
<td>0.022</td>
</tr>
<tr>
<td>Dorsiflexion 60°/sec</td>
<td>26.3 (12.2)</td>
<td>21.8 (10.5)</td>
<td>4.5 (8.6)</td>
<td>11.4, 72.9</td>
<td>0.07</td>
</tr>
<tr>
<td>120°/sec</td>
<td>21.8 (10.5)</td>
<td>21.8 (8.6)</td>
<td>-0.0 (23.8)</td>
<td>-2.5, 26.4</td>
<td>0.67</td>
</tr>
<tr>
<td>180°/sec</td>
<td>21.4 (8.6)</td>
<td>21.4 (8.6)</td>
<td>-0.0 (23.8)</td>
<td>-2.5, 26.4</td>
<td>0.67</td>
</tr>
<tr>
<td>Isometric strength (N) Plantar flexion</td>
<td>58.8 (40.7)</td>
<td>109.2 (55.9)</td>
<td>42.1 (24.8)</td>
<td>11.4, 72.9</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Incidence of re-rupture and deep infection

The population for which our university hospital served as the only unit treating Achilles tendon ruptures increased from 94 000 to 120 000 during the period stud-
ied here, and the annual incidence of these injuries increased from 4.2 /105 inhabitants in 1979–1990 to 15.2 in 1991–2000, the peak annual figure of 19.0 being recorded in 1999 (Figure 8). Twenty-three of the 409 patients (5.3%) had an Achilles tendon re-rupture and 9 (2.2%) a deep Achilles tendon infection. The annual incidence of re-ruptures increased from 0.25 /105 in 1979–1990 to 1.0 in 1991–2000, the peak of 3.5 being reached in 1999. The incidence of deep infections increased from 0 in the 1980s to 0.63 in the 1990s, with a peak of 2.6 in 1999. The proportion of re-ruptures was 6.0% in 1979–1990 and 6.6% in 1991–2000.

Fig. 8. Incidence of ruptures, re-ruptures and deep infections of the Achilles tendon in our patient population (graph smoothed by calculating a three-year moving means for each incidence.)

Risk factors

The number of patients over 60 years of age treated for an Achilles tendon rupture increased from 2 between the years 1980 and 1989 to 24 between 1990 and 1999. We reviewed the patient records for other risk factors, such as corticosteroid medication, smoking, symptoms in the tendon before injury, diabetes and a delay in treatment, and found that there were 9 patients in the re-rupture group (39%) and 2 in the deep infection group (22%) who had none of these risk factors. The number
of patients with more than 3 risk factors was 5 in the infection group (56%) but only 4 (17%) in the re-rupture group (Table 8). The patients with a deep infection were significantly older than those with a tendon re-rupture without deep infection and they were more often receiving corticosteroid medication. The rupture mechanism in the patients with deep infection was associated with activities of daily living more often than with recreational sports, and a delay before treatment was more common in this group.

Table 8. Incidence of known risk factors in the deep infection group as compared with the re-rupture group.

<table>
<thead>
<tr>
<th>Risk factor variable</th>
<th>Re-rupture group (N=23)</th>
<th>Deep Infection group (N=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No known risk factors</td>
<td>9 (39)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Age over 60 years</td>
<td>3 (13)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Corticosteroid therapy</td>
<td>5 (22)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (39)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Delay in treatment for more than 7 days</td>
<td>3 (13)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Pain in the tendon before injury</td>
<td>4 (17)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Mean number of risk factors</td>
<td>1.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

5.3 Increased type III collagen content at the human AT rupture site (III)

Type I and III collagen synthesis

The amount of newly synthesized type III procollagen, as measured by PIIINP RIA, showed no differences between the tendon sites (Table 9), while the results of type I procollagen propeptide analyses were contradictory, in that no differences in the PICP results were observed between the sites, but the PINP level was significantly lower at the RUPT site than at the CONT1 and CONT2 sites, which did not differ from each other (Table 9).
Table 9. Median (range min-max) content of type I and III collagen markers and total collagen at the sampling sites.

<table>
<thead>
<tr>
<th>Soluble tissue extract (μg/g of wet tissue weight)</th>
<th>Sample site</th>
<th>Rupture</th>
<th>Control 1</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIIIINP</td>
<td></td>
<td>558 (143–4584)</td>
<td>384 (157–1414)</td>
<td>516 (142–1298)</td>
</tr>
<tr>
<td>PINP</td>
<td></td>
<td>227 (50–543) *</td>
<td>428 (233–1107)</td>
<td>524 (173–1019)</td>
</tr>
<tr>
<td>PICP</td>
<td></td>
<td>516 (137–1103)</td>
<td>463 (183–1581)</td>
<td>417 (225–954)</td>
</tr>
<tr>
<td>Insoluble tissue digests (mg/g of dry tissue weights, except PIIIINP μg/g)</td>
<td>Sample site</td>
<td>Rupture</td>
<td>Cadaver</td>
<td></td>
</tr>
<tr>
<td>IINTP</td>
<td>rupture</td>
<td>15.3 (6.5–30.9)**</td>
<td>3.0 (1.2–8.9)†</td>
<td>1.2 (0.7–8.3)</td>
</tr>
<tr>
<td></td>
<td>cadaver</td>
<td>3.7 (2.1–13.6)*</td>
<td>2.1 (0.7–5.5)</td>
<td>1.2 (0.6–1.9)</td>
</tr>
<tr>
<td>Tryptic PIIINP</td>
<td>rupture</td>
<td>15.4 (3.5–82.1)**</td>
<td>3.6 (1.2–19.2)</td>
<td>1.2 (0.7–10.0)</td>
</tr>
<tr>
<td></td>
<td>cadaver</td>
<td>5.7 (2.2–48.4)</td>
<td>3.9 (1.8–30.1)</td>
<td>1.2 (0.7–9.9)</td>
</tr>
<tr>
<td>ICTP</td>
<td>rupture</td>
<td>2.6 (1.5–3.6)</td>
<td>2.4 (1.4–3.9)</td>
<td>1.2 (0.7–3.2)†</td>
</tr>
<tr>
<td></td>
<td>cadaver</td>
<td>2.4 (1.5–18.3)</td>
<td>2.5 (1.5–20.7)</td>
<td>1.2 (0.7–17.2)●</td>
</tr>
<tr>
<td>Total collagen</td>
<td>rupture</td>
<td>731 (540–770)‡‡</td>
<td>725 (674–798)‡‡</td>
<td>705 (375–768)‡‡</td>
</tr>
<tr>
<td></td>
<td>cadaver</td>
<td>881 (830–923)‡‡</td>
<td>905 (873–981)‡‡</td>
<td>927 (853–959)‡‡</td>
</tr>
</tbody>
</table>

* p<0.05 when compared to CONT1 and CONT2, ** p<0.005 when compared to CONT1 and CONT2.
† p<0.005 when compared to CONT2, ‡ p<0.01 when compared to RUPT and CONT1
● p<0.05 when compared to RUPT and CONT1
‡‡ p<0.005 when compared to cadaver, †† p<0.001 when compared to cadaver

**Total collagen content and type I and III collagen structures in the insoluble matrix**

Collagens accounted for about 70% of the dry weight of the insoluble matrix of the ruptured Achilles tendons, whereas the insoluble matrix in the cadavers contained significantly more collagen, about 90% of dry weight at all sites (Table 9).

Both IINTP and tryptic PIIINP were markedly increased at the RUPT site in the individuals with total Achilles tendon rupture relative to either the CONT1 or CONT2 site. The CONT1 site contained more IINTP than did CONT2 in the rupture patients but not in the cadavers, while the RUPT site in the rupture patients contained more IINTP than did that in the cadavers. The tryptic PIIINP levels did not differ significantly between the rupture patients and cadavers, but there was tendency in that direction. When the very high tryptic PIIINP value in the cadaver group (for a 13-year-old male) was excluded, the difference between the rupture patients and the cadavers at the RUPT site was statistically significant (p < 0.05). The type III pN-collagen content as calculated from the molar amounts of IINTP and tryptic PIIINP (less than 0.1%) did not differ between the sites or between the rupture patients and cadavers, however.
The ICTP levels of the rupture patients and cadavers were similar. The CONT2 site contained less ICTP than the RUPT or CONT1 site (Table 9). It was noticeable that the youngest of the cadavers (a 13-year-old male) contained ten-fold more ICTP than did the other samples (Figure 9) and that the ICTP content decreased significantly with age at the CONT1 and CONT2 sites in both the rupture patients and the cadavers, but at the RUPT site only in the cadavers (Figure 10).
Fig. 10. Effect of age of the rupture patients (closed dots) and cadavers (open dots) on the ICTP content and SP 4 / ICTP ratio at the CONT1 and CONT2 sites. The decreases with age were significant in the rupture patients (straight line) and in the cadavers (dotted line) at the CONT1 ($r^2=0.755$, $p<0.005$ and $r^2=0.921$, $p<0.01$) and CONT2 sites ($r^2=0.750$, $p<0.005$ and $r^2=0.947$, $p<0.01$), but only in the cadavers at the RUPT site ($r^2=0.291$, $p=\text{ns}$ and $r^2=0.807$, $p<0.05$). The SP 4 / ICTP ratio correlated positively with age at the CONT1 ($r^2=0.630$, $p<0.01$ and $r^2=0.940$, $p<0.005$) and CONT2 sites ($r^2=0.563$, $p=0.05$ and $r^2=0.982$, $p<0.001$), but only in the cadavers at the RUPT site ($r^2=0.370$, $p=\text{ns}$ and $r^2=0.991$, $p<0.001$).

The SP 4 assay detects all the variants of the crosslinked and uncrosslinked structures containing at least one carboxyterminal telopeptide region of the a1-chain of type I collagen, and thus shows broader immunoreactivity than the ICTP assay. Neither the SP 4 assay results nor the SP 4 / ICTP ratios differed between the sites, but there was a positive correlation between age and the SP 4 / ICTP ratio at the CONT1 and CONT2 sites in both the rupture patients and the cadavers, although only at the RUPT site in the cadavers (Figure 10).
ICTP and IIINTP at the RUPT site in the reverse phase run

By loading equal amounts of ICTP antigen, the two runs could be standardized to observe the differences in the IIINTP antigen. In the case of the rupture patients IIINTP eluted at a position where pyridinoline fluorescence was also observed, although the majority of the pyridinoline eluted in the same position as with ICTP. The cadaver sample had less IIINTP, and the pyridinoline peak was also lower (Figure 11). These data show that IIINTP contains pyridinoline.

Fig. 11. HPLC reverse phase analysis of the insoluble tissue digests from the RUPT site of one patient with a ruptured Achilles tendon (A) and from a site corresponding to the rupture site in a cadaver (B). IIINTP (closed triangles) and ICTP (closed dots) were analysed from the fractions. Pyridinoline fluorescence (straight line) was followed during the run. The elution position of the trivalent pyridinoline-crosslinked IIINTP structure is marked with arrows.
5.4 Tenascin-C and type I and III collagen expression in total AT rupture (IV)

Samples. The final series included nine men and one woman, with an average age of 38 years (range 30–48), who were operated on a mean of 29 (10–43) hours after the injury. One sample taken at the rupture site and one sample used in the type I collagen analysis were spoiled during processing.

Tenascin-C. There was no significant difference between the sites (Table 10).

Type I and III procollagens. The type I carboxyterminal (PICP) and aminoterminal (PINP) procollagens were almost equal in expression at the sites (Table 10), but the expression of type III procollagen (PIIINP) was significantly higher at the rupture site than at control site 2 (Table 10, p=0.016 Sign Test).

Mature type III collagen. The amount of mature type III collagen present was significantly higher at the rupture site than at control sites 1 and 2 (Table 10, P=0.008 Sign Test).
Table 10. The expression of tenasin-C, type I and III procollagens and mature type III collagen at the studied sites. Figures represent frequencies in different scales used (0–3).

<table>
<thead>
<tr>
<th></th>
<th>Rupture site rankings</th>
<th>Control 1 rankings</th>
<th>Control 2 rankings</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency in different scales (0–3)</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td>PIINP</td>
<td>5 4</td>
<td>6 1 3</td>
<td>7 3</td>
<td>0.021*</td>
</tr>
<tr>
<td>PINP</td>
<td>1 2 6</td>
<td>1 8</td>
<td>9</td>
<td>0.33*</td>
</tr>
<tr>
<td>PICP</td>
<td>3 4 2</td>
<td>3 3 4</td>
<td>3 3 4</td>
<td>0.60*</td>
</tr>
<tr>
<td>IIIINTP</td>
<td>1 2 6</td>
<td>3 6 1</td>
<td>7 3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Tenasin</td>
<td>7 1 1</td>
<td>9 1</td>
<td>6 4</td>
<td>0.40*</td>
</tr>
</tbody>
</table>

*Statistical significance in rankings between the three studied sites (Friedman test).
# P-values according to Sign test for comparison of rupture and control 1 (†) and control 2 (‡) sites (calculated if Friedman’s test p<0.05).

Fig. 12. Microscopy photographs (Optical magnification 4x) of immunohistologically stained specimen show expression of tenasin-C level 1 scale (under 33% of surface area coverage) in Achilles tendon in picture A, and tenasin-C level 2 scale (33–66% of surface area coverage) in picture B. Expression of PINP in Achilles tendon level 2 scale in picture C, and level 3 scale (over 66% of surface area coverage) in picture D.
6 Discussion

6.1 Augmented vs. non-augmented surgical repair of acute total AT rupture

Surgery has been suggested by many authors as the standard treatment for a complete Achilles tendon rupture, especially where young, active patients are concerned, as the risk of re-ruptures is lower than after conservative treatment. Surgical procedures naturally entail more wound complications, but the majority of these do not affect the final outcome. The most popular surgical approaches today are suture without augmentation, suture with augmentation and percutaneous techniques. Augmented techniques have been justified in terms of the higher tensile strength attained while non-augmented techniques in terms of the shorter incision and lower incidence of wound problems (Khan et al. 2004).

We found only three studies in the literature which compared the results achieved with augmented and non-augmented suture techniques, two of which had a retrospective research design (Jessing & Hansen 1975, Nyyssönen et al. 2003), while the one prospective study involved quite a small number of patients (Aktas et al. 2007).

In our prospective, randomized study we compared the results of surgical repair of a fresh complete Achilles tendon rupture with an end-to-end Krackow locking loop suture alone and augmented with one central down-turned gastrocnemius fascia flap (Silfverskiöld 1941), but failed to find any benefit of using the augmented repair technique in this population, the results in terms of subjective and objective ankle outcomes, isokinetic strength scores, mean peak work-displacement relationships and tendon elongation being equally good and the number of deep infections (2/28 vs. 0/32), re-ruptures (3/28 vs 3/32) and superficial infections comparable. On the other hand, the statistically shorter operating time (a difference of 25 min) and the smaller incision (a difference of 7cm) argue in favour of end-to-end repair. This result is in line with other examinations of the same clinically relevant question (Jessing & Hansen 1975, Nyyssönen et al. 2003, Aktas et al. 2007).

The primary outcome measure employed, Leppilahti’s outcome score, pointed to excellent or good results in 90% of cases in the simple repair group and 81% in the augmented repair group at the 12-month follow up. Early failures amounted to 10% in the simple repair group and 19% in the augmented repair group. No differences were seen between the two groups with regard to pain, stiffness, subjective
calf muscle weakness, footwear restrictions or range of ankle motion. These results were slightly better than in the author’s previous report, where two postoperative regimens were randomly compared after Achilles tendon suture with the augmented technique, yielding excellent or good outcome scores in 88% of cases in the early motion group, fair in 4% and poor in 8%, whereas the scores in the cast group were excellent or good in 92% of cases and fair in 8% (Kangas et al. 2003). Our results were also better than in a previous series from our hospital in which the postoperative treatment consisted of 6 weeks of below-knee cast immobilization with the ankle in an equinus position for 3 weeks and in a neutral position for 3 weeks, allowing gradual weight bearing after three weeks, for which the ankle scores were excellent or good in 79% of the 101 cases, fair in 17% and poor in 4% a mean of 3 years postoperatively (Leppilahti et al. 1998).

The re-rupture rate was about 10% in both of our two groups, which is higher than expected (Khan et al. 2005). One contributing factor may have been the short period of brace immobilization together with considerable weight bearing. The soft cast brace was removed at three weeks and 20 kg weight bearing was allowed for three weeks, half weight bearing for the next three weeks and full weight bearing after six weeks. Even the augmentation technique could not prevent re-ruptures. Similar results regarding early weight bearing have been reported with a cross-stitch suture (Aoki et al. 1998). Three of our patients had an obvious new injury during the recovery period. Some of the re-rupture mechanisms described in the results section evidently involved serious exceeding of the limits laid down in the rehabilitation programme. The other two cases of re-rupture, both in the non-augmented group, occurred as a result of low-force activity at 12 weeks, and it transpired that these patients had also suffered a slight trauma during the first 3 weeks, while the dorsal brace was still on. It is our opinion that this initial trauma had interfered with the repair and caused a gap between the tendon ends. Recovery of strength in the tendon repair process has been shown in canine model to be slower upon gap formation (Gelbermann et al. 1999), and this may have meant that the remaining suture strength and improperly healed tendon could no longer resist normal forces at 12 weeks, a situation that resembles delayed union in bone healing. In the authors’ previous study the re-rupture rate was 6% when full weight bearing was allowed after three weeks but dorsiflexion was restricted to neutral until six weeks (Kangas et al. 2003), and elsewhere active rehabilitation and mobilization has been shown to improve the functional results and tendon healing (Enwemenka et al. 1988, Mortensen et al. 1999), although we think that dorsiflexion should be controlled by means of a brace for longer than three weeks.
There is no universal consensus that a given suture type and suture thickness is the method of choice for ATR repair. We wanted to use a #0 absorbable monofilament, with minimal tissue response but enough tensile strength for tendon healing, and many authorities in Europe use monofilament or braided absorbable sutures, whereas the tendency in North America is to use mechanically stronger stitches extending above and below the site of rupture. No randomized clinical studies of the suture materials used for ATR repair are available. The material that we used (polydioxanon) has been reported to lose 50% of its strength during the first 4 weeks (data from Ethicon, Johnson & Johnson Inc., Somerville, New Jersey) (O’Brien 1995).

Infections occurring after the surgical repair of a ruptured Achilles tendon are often extremely difficult to manage and the final outcome is poor. We were successful in treating our two cases of deep infection with débridement and two-tail cutaneous transfer flaps. We strongly advocate careful postoperative observation of Achilles tendon ruptures by experienced surgeons, since problems tend to arise unexpectedly and often remain unnoticed at first. Prompt and precise action is needed in the early phase of an infection.

The strengths of this study lie in its prospective, randomized design and the homogeneous groups of patients. There were no known biases, all the patients were operated on by the same surgeon and they were advised to perform postoperative exercises according to a standard rehabilitation programme. Also, thorough evaluations were performed using various clinical outcome tools, an isokinetic strength score, peak work-displacement relationships and tendon elongation measurements. The only possible object of criticism could be that our evaluators were not blinded to the assignment of patients to treatment groups. Also the relative small number of subjects at the follow-ups prevent definite conclusions concerning some outcome variables.

6.2 Re-rupture and deep infection following treatment of total AT rupture

The incidence of total Achilles tendon ruptures is increasing (Leppilahti et al. 1996c), and deep infections are reported to occur after surgery in 1% to 2% of cases, re-ruptures in 2% to 15% and minor complications in 15% to 20% (Khan et al. 2005). There are also more re-ruptures after conservative treatment than after surgical treatment (Khan et al. 2005), but overall clinical outcome can be very similar (Möller et al. 2001). The overall incidence of re-ruptures and deep infec-
tions after the treatment of Achilles tendon ruptures has scarcely ever been reported in the literature, and the outcomes after these complications are mentioned only in case reports.

We conducted the present study in response to a treatment-based suspicion of an increase in the incidence of re-ruptures and deep infections in our hospital. The outcome after successful treatment of a total Achilles tendon rupture is good regardless of the method used, but there are very few conclusive reports on the final outcome after complications have occurred. Ankle flexion forces have been reported to be low in patients who have a re-rupture, and the sequelae after infection are said to be particularly difficult, often requiring plastic surgery interventions. Previous studies have suggested that diabetes, corticosteroid usage, age and previous symptoms in the Achilles tendon area are related to a higher risk of deep infection. A second operation also poses a risk of infection, especially in the Achilles tendon area, where the subcutaneous tissue is very thin. We are not aware of any conclusive report on the final outcome after re-rupture or deep infection. We believe that more profound information on the final outcome after complicated Achilles tendon rupture repair could be very useful for doctors who see these patients in practice and need guidelines for their treatment.

The incidence of total Achilles tendon ruptures in our geographical area increased nearly four-fold between 1979–1990 and 1991–2000, from 4.2 to 15.2 (per 100,000 inhabitants), while that of re-ruptures increased from 0.25 to 1.0 and that of deep infections from 0 to 0.63. The ratio of re-ruptures and deep infections to primary Achilles tendon ruptures did not change substantially over this period. The rate of re-ruptures was in our university hospital-based patient series was 5.6% and the rate of deep infections 2.2%. These figures are reliable and are not affected by any known bias. Similar figures for re-ruptures and deep infections have been reported in a recent meta-analysis (Khan et al. 2005). Increases in the incidence of total Achilles tendon ruptures have been reported in Scotland, Copenhagen, Malmö, and Oulu, and it is evident that the total number of complications is also increasing, and that surgeons must be more aware of these complications. At the time of our highest complication rates, in 1999, we were very aggressively recruiting patients for operative treatment, and some of them were high at risk to receive treatment complications.

The two groups formed in the present study clearly differed in terms of risk factors such as advanced age, diabetes, corticosteroid use, smoking, delayed treatment and previous tendon symptoms, these factors being more numerous in the deep infection group. As the number of known risk factors for surgery in the
Achilles tendon area was markedly higher in the deep infection group, we feel that at least some of the complications could have been predicted and perhaps avoided with better patient selection. Interestingly, the majority of our patients with a simple re-rupture had sustained their original injury during participation in sports activities, whereas the majority of those with deep infections had sustained their initial rupture during normal activities of daily life. It is possible that an Achilles tendon that is liable to rupture during normal daily activities may already be in such a poor condition that normal healing is impaired and the risk of postoperative complications is increased.

Eleven of the twelve patients in the present re-rupture group were subjectively satisfied with the final clinical outcome and mentioned only minor problems, whereas only one out of the seven in the deep infection group was equally satisfied. The results in terms of the clinical outcome score were even worse, with only eight patients in the re-rupture group and none in the deep infection group achieving a good or excellent level of recovery. Likewise, the mean isokinetic plantar flexion strength deficit for three test velocities was 10% in the re-rupture group and 35% in the deep infection group. The results in both groups were nevertheless inferior to those achieved following successful primary surgical treatment. In a previous study of 101 patients with an Achilles tendon rupture without re-rupture or infection who were monitored in our clinic for a mean of three years postoperatively, the mean isokinetic calf-muscle strength deficit was only 7% (Leppilähti et al. 1996c).

A deep infection after surgical repair of an Achilles tendon rupture is a relatively rare but devastating problem, as the skin and soft-tissue defects associated with Achilles tendon loss constitute a major challenge for surgeons. Methods for the reconstruction of soft tissues after failed surgery have been presented in the literature (Maffulli & Ajis 2008), but the results have been variable and there are insufficient data in general to support any of the techniques. In our clinic the infection is first brought under control with débridement and administration of antibiotics. A skin cover is provided with split-thickness skin grafts, local transposition flaps, or free-tissue transfers by means of a microvascular anastomosis. The tendon tissue itself is reconstructed with an autograft or allograft. The present series included two patients who had reconstruction with a lateral radial forearm flap and a tensor fasciae latae graft; the reconstruction being successful in one patient and a failure in the other forever. The cases with deep infection in our randomized series were successfully treated with two-tailed skin and subcutaneous tissue transfers. Their infections were rapidly recognized and prompt action saved us from tendon
tissue reconstructions. It is not possible on the available data to decide which method of reconstruction is best.

6.3 Type I and III collagen expression in total AT rupture

The main purpose of the work reported in Papers III and IV was to examine the patterns of tenascin-C and type I and III collagen expression and collagen cross-linking in the ruptured human Achilles tendon by comparing expression at the rupture site with that at two other sites within the same tendon, and also with that in presumably healthy cadavers in Paper III. The tendon samples from living patients were harvested less than 43 hours after rupture and should at most represent the tendon tissue composition before the trauma (Haukipuro et al. 1990, Fluck et al. 2000). The samples from the cadavers were harvested within 72 hours post mortem.

The level of mature type III collagen was markedly higher at the rupture site than at the two control sites with both methods used. Similar results have been reported in other studies (Kannus & Josza 1991).

The type III collagen content of the insoluble tissue digests of patients with total Achilles tendon rupture was markedly increased at the rupture site. IIINTP represents type III collagen which has been incorporated into the collagen fibrils and stabilized there by intermolecular pyridinoline crosslinks, forming what is known as mature type III collagen. The type III collagen content at the site in the cadavers corresponding to the RUPT site was significantly lower than in the rupture patients. Only one cadaver (a 41-year-old male) exhibited IIINTP levels comparable with those of the rupture patients, but his whole tendon felt much stiffer than the other tendons and could be represent a case of undiagnosed tendinopathy. Type III pN-collagen, immature type III collagen, did not differ in content between the sites, indicating similar processing of type III collagen.

The soluble propeptide antigens represent newly synthesized procollagens or partially processed forms which have not yet been covalently crosslinked into the insoluble matrix, i.e. immature type III collagen. Since the unchanged PIIINP levels in the soluble tissue extracts indicate a slow overall rate of type III procollagen synthesis, the accumulation of a large amount of type III collagen at the rupture site must have taken place over a longish period. This suggests that there a continuous, long-lasting microtraumatic process may have been taking place prior to the total rupture of the Achilles tendon. The reason for this microtrauma is not clear, and the role of mechanical overloading cannot be excluded. Such a gradual accu-
mulation of type III collagen will eventually cause a decrease in the biomechanical strength of the tendon.

The concentration of PINP was lower at the rupture site than at the control sites, but the PICP levels remained unchanged. The lower PINP levels may have been caused by the presence of type I pN-collagen, where part of the PINP is retained on the surface of the newly synthesized collagen molecules in the insoluble matrix. This may lead to thinner type I collagen fibres, as has been found in embryonic skin (Fleishmajer et al. 1983). It is also possible that the PINP antigen may not be as stable as PICP and was degraded during processing.

The ICTP concentration was lowest at the control 2 site in both the rupture patients and the cadavers, probably due to the tendon tissue gradually changing into fascia. Ageing is known to affect collagen crosslinking profiles, and the amount of pyridinoline in the human Achilles tendon, for example, has been reported to increase up to the age of thirty and then to decrease gradually (Moriguchi et al. 1978). The ICTP levels decreased and the ratio of SP4 to ICTP increased with age at both control sites, suggesting a relative increase in the amount of unknown variants of the crosslinked carboxyterminal telopeptide structures that only the SP4 assay is capable of detecting. The 13-year-old male in the cadaver series had ten-fold higher ICTP concentration and a lower SP4/ICTP ratio than did the other cases, suggesting a pyridinoline-like crosslinking pattern. The mature crosslinked telopeptide structure appearing in the Achilles tendon with age could be identical or analogous to the crosslinking structure predominating in the human skin (Sassi et al. 2001). It has been shown that a reduction in pyridinoline crosslink density causes biomechanical weakening of the healing rabbit medial collateral ligament (Frank et al. 1995), and the same authors discussed the possibility that skin-like crosslinking might be the reason for the decrease in the pyridinoline content. The skin-like crosslinked telopeptide can be measured with the SP4 assay, but not with the ICTP assay (Sassi et al. 2001). If the low ICTP content contributes to total Achilles tendon rupture, the change in the collagen fibril organization must be a generalized one.

6.4 Tenascin-C in Achilles tendon rupture

Although elevated expression of tenascin-C has been found in certain regions of degenerated human supraspinatus tendons, our results show no differences between the sites in this respect (Riley et al. 1996). Similarly, higher tenascin-C expression has been reported at the musculo-tendinous junction of a rat Achilles
tendon after increased physical loading (Järvinen et al. 1999). We think that the long-term mechanical loading affecting the rupture site of the human Achilles tendon does not differ from that at other sites in the same tendon. Since any changes in tenascin-C expression should take place after a delay of more than 36 hours (Fluck et al. 2000), the levels obtained in the present ruptured tendons must mainly have originated from the time before the rupture. We suspect that there may be a more universal mechanical loading pathology associated with the degenerative process and rupture of the human Achilles tendon. Abnormal tension in the gastrocnemius apparatus could be one explanation for our findings, and we would agree that tenascin-C has some as yet unknown role in the loading changes taking place in connective tissue (Järvinen et al. 2000).

Since we could not find any correlation between the expression of tenascin-C and type III collagen synthesis or accumulation, we believe that the role of tenascin-C in tendon degeneration is variable. A similar observation has been made regarding the appearance of tenascin-C in supraspinatus bursae, which did not correlate with normal histopathological findings of degeneration (Hyvönen et al. 2003).
7 Conclusions

The augmented repair seems to have no clear advantages over simple end-to-end repair in cases of fresh complete Achilles tendon rupture. The outcome measure tools we used did not show any significant difference between these two groups. Very active postoperative rehabilitation combined with short-term brace immobilization and considerable weight bearing gives excellent or good results with most patients, but there is also an increased risk of early re-ruptures that are unrelated to the surgical technique.

The incidence of Achilles tendon re-ruptures and deep infections has increased. The outcome is satisfactory after a simple re-rupture without infection, but the results after a deep infection are often devastating.

Clear evidence of a slow accumulation of type III collagen at the rupture site of the Achilles tendon was found. This may lead to biomechanically weaker tissue due to the thinner collagen fibres. The unexpectedly low level of ICTP structures in the tendon tissue and the decrease in these with age may exacerbate qualitative changes, which reduce the strength of the matrix before total rupture of the Achilles tendon occurs.

Although tenasin-C is expressed evenly over the whole Achilles tendon, we do not believe that it has any specific value as a predictive or diagnostic marker of tendon degeneration. Our findings support results in which tenasin-C expression has been shown to be elevated after mechanical loading, but its exact function in the extracellular matrix of human tendon tissue still remains unresolved.
8  Future prospects for Achilles tendon rupture research

There is still no definite answer of which method is the best for treatment of an acute Achilles tendon rupture. Large size randomized studies comparing surgical techniques and even surgery and non-surgery are needed for statistically significant results and therefore a multi-centre co-operation would be needed. The incidence of Achilles tendon ruptures, re-ruptures and deep infections has increased, but we still do not know why the tendon tissue is more prone to this injury in some individuals than in others. Future Achilles tendon research can be expected to expand our knowledge of the biochemical alterations, especially with respect to changes in collagen structure. There are already numerous studies available dealing with the matrix metalloproteinases (MMP) and their inhibitors (TIMP), which are said to control the degradation of collagen, and the presence of certain types of MMP’s is known to be correlated with painful tendon syndromes (Jones et al. 2006).

Another area of special interest is the enhancement of tendon healing. Local administration of growth factors or tissue scaffolds, including tendon healing promoting substances, has already been studied in animal models (Anitua et al. 2006).
References


Original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


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1000. Lajunen, Taina (2008) Persistent Chlamydia pneumoniae infection, inflammation and innate immunity
1005. Iinattiniemi, Sari (2009) Fall accidents and exercise among a very old home-dwelling population
1006. Westerlund, Tarja (2009) Thermal, circulatory, and neuromuscular responses to whole-body cryotherapy
1008. Kuusma, Mari (2009) Magnetic resonance imaging of lumbar degenerative bone marrow (Modic) changes. Determinants, natural course and association with low back pain
1010. Löfgren, Johan (2009) Genetic polymorphisms in collectins and Toll-like receptor 4 as factors influencing susceptibility to severe RSV infections and otitis media
Ari Pajala

ACHILLES TENDON RUPTURE

COMPARISON OF TWO SURGICAL TECHNIQUES, EVALUATION OF OUTCOMES AFTER COMPLICATIONS AND BIOCHEMICAL AND HISTOLOGICAL ANALYSES OF COLLAGEN TYPE I AND III AND TENASCIN-C EXPRESSION IN THE ACHILLES TENDON

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