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Karolinska Institutet, Stockholm, Sweden

ACUTE ACHILLES TENDON RUPTURE

PREDICTORS AND INTERVENTION TO PROMOTE OUTCOME

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**Karolinska
Institutet**

Stockholm 2015

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Published by Karolinska Institutet.

Printed by AJ E-print AB

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ISBN 978-91-7549-936-9



Department of Molecular Medicine and Surgery, Section of Orthopaedics

Acute Achilles Tendon Rupture

Predictors and Intervention to Promote Outcome

THESIS FOR DOCTORAL DEGREE (Ph.D.)

at Karolinska Institutet publicly defended at Thoraxaulan, Karolinska University Hospital, Solna

Friday May 29th, 2015, at 09.00 am

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To my family

ABSTRACT

Background: Orthopaedic trauma and surgery is still associated with major complications related to immobilization, which results in reduced circulation, thromboembolic events, impaired healing and functional deficits. An acute Achilles tendon rupture (ATR) is associated with a high risk of deep venous thrombosis (DVT) and varied extent of impaired physical ability. The knowledge of underlying factors leading to hampered functional outcome one year after surgery of ATR is still limited. Since pharmacological DVT-prophylaxis has low or no effect during lower leg immobilization it is speculated whether adjuvant mechanical treatment with intermittent pneumatic compression (IPC) applied during lower limb immobilization can reduce the incidence of DVT.

Aims: The purpose of this thesis was to assess predictors of outcome after acute ATR and to investigate if an intervention using IPC could reduce the risk of immobilization-induced complications, i.e. to reduce DVT-incidence and to enhance the healing response.

Results and Discussion: In a prospective cohort of ATR patients using combined patient reported- and functional outcome measures predictors of outcome were investigated. This thesis established that three independent factors predict patient outcome at one year post-operatively. Thus, it was demonstrated that postoperative DVT during leg immobilization, aging and male gender are independent predictive factors of patient outcome. Moreover, more than half of the patients exhibited significant functional deficits at one year post-operatively. These results imply that specific interventions are warranted to prevent DVT.

In a prospective randomized study, intervention with IPC under plaster cast was compared to treatment-as-usual with plaster cast only. DVT incidence was assessed using compression duplex ultrasound (CDU), by two ultrasonographers blinded to the treatment. The study ended prematurely since an interim analysis demonstrated a high, non-significant incidence of DVT in both groups, IPC (75%) and controls (50%), and a malfunctioning of the IPC device under plaster cast. These findings suggest that other means of applying IPC during immobilization should be evaluated.

The above conclusions resulted in a prospective randomized trial comparing adjuvant IPC applied under an orthosis versus plaster cast only. CDU analysis demonstrated significantly reduced incidence of DVT at 2 weeks post-operatively, 21% in the IPC-group compared to 37% in the control group. Patients aged ≥ 40 years exhibited an almost fivefold increased odds of DVT. Moreover, patients that received no IPC treatment exhibited an almost threefold increased odds for DVT, independently of age. Furthermore, using microdialysis technique, adjuvant IPC treatment was shown to increase the metabolic activity at 2 weeks post-operative ATR. The demonstration that adjuvant IPC effectively reduced DVT incidence, and also is capable of enhancing the metabolic response suggests that IPC treatment may not only be a viable means of prophylaxis against DVT in an outpatient setting, but possibly also a method of promoting healing.

Conclusions: This thesis established that poor outcome is common after ATR and that three specific, independent risk factors can predict a negative outcome after ATR. One of these risk factors, i.e. DVT, can be prevented by IPC used under an orthosis during lower limb immobilization. The results suggest that all patients with lower leg immobilization should be screened for risk factors of DVT and that IPC may be an effective, non-pharmacological outpatient approach to reduce the risk of DVT, maybe also for enhancement of healing.

Keywords: Achilles tendon rupture, Epidemiologic factors, DVT, Intermittent pneumatic compression, Outcome assessment, Functional evaluation, Tendon healing, Microdialysis

ISBN 978-91-7549-396-9

LIST OF SCIENTIFIC PAPERS

- I. **Aging, gender and deep venous thrombosis are predictors of functional outcome after acute Achilles tendon rupture.**
E.Domeij-Arverud, P. Anundsson, E Hardell, G. Barreng, G. Edman, P.W. Ackermann.
Manuscript
- II. **Can foot compression under a plaster cast prevent deep-vein thrombosis during lower limb immobilisation?**
E.Domeij-Arverud, A. Latifi, F.Labruto, G. Nilsson, P.W. Ackermann.
Bone Joint J. 2013 Sep;95-B(9):1227-31.
- III. **Intermittent pneumatic compression reduces the risk of DVT during post-operative lower limb immobilisation: A randomized controlled trial of acute Achilles tendon ruptures**
E.Domeij-Arverud, A. Latifi, F.Labruto, G. Nilsson, G. Edman, P.W. Ackermann.
Accepted Bone Joint J 2015;97-B:??-??
- IV. **Metabolic activity in early tendon repair can be enhanced by intermittent pneumatic compression.**
K.Greve*, E. Domeij-Arverud*, F.Labruto, G. Edman, D. Bring, G. Nilsson, P.W. Ackermann
Scand J Med Sci Sports 2012 Aug; 22(4):e55-63

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ABBREVIATIONS

ACOS	Achilles Combined Outcome Score
ADL	Activities of Daily Life
ATR	Achilles Tendon Rupture
ATRS	Achilles tendon Total Rupture Score
BMI	Body Mass Index
CDS	Color Duplex Sonography
CDU	Compression Duplex Ultrasound
CI	Confidence Interval
DVT	Deep Venous Thrombosis
EQ-5DTM	EuroQol, a generic health-related quality of life score
FAOS	Foot and Ankle Outcome Score
IPC	Intermittent Pneumatic Compression
LSI	Limb Symmetry Index
OR	Odds Ratio
PAS	Physical Activity Scale
Qol	Quality of Life
RCT	Randomized Controlled Trial
ROM	Range of Motion
RR	Relative Risk or Risk Ratio
SD	Standard Deviation
VAS	Visual Analogue Scale
VTE	Venous Thromboembolism

DEFINITIONS

Concentric muscle contraction	When a muscle shortens while producing a force
Eccentric muscle contraction	When a muscle lengthens while producing a force
Heel-rise	The exercise in which the subject performs a plantar flexion when standing and back down again
Incidence	The number of new cases of a condition/injury that develop during a specific time period
LSI	The ratio of the involved limb score and the uninvolved limb score expressed in percent (involved/uninvolved x 100 = LSI)
Non-parametric statistics	A statistical method where the data is not required to fit a normal distribution
Odds ratio	Describes the strength of association between two binary data values (effect size)
Parametric statistics	A statistical method that relies on assumptions of a normal distribution
Power	<ol style="list-style-type: none">1. In statistics: the probability that a test will not commit a type II error (Power = 1 – probability of type II error)2. The rate at which work is performed. The product of force and velocity (W).
Predictor	The independent variable used to explain or to predict the outcome variable
Relative risk	The ratio of the probability of the event to occur in the exposed group versus the non-exposed group
Risk factor	A variable associated with an increased risk of injury or disease
Work	The product of a constant force and the distance the object is moved in the direction of the force (J)

1 INTRODUCTION

1.1 BACKGROUND

Modern orthopaedic surgery care aims at restoring musculoskeletal function of the patient as soon as possible without inflicting complications related to operation or to post-operative immobilization. The most common post-operative complication is venous thromboembolism resulting in deep vein thrombosis (DVT) or pulmonary embolism (PE) (1, 2). Secondary complications include oedema formation, wound infections and delayed healing, often leading to prolonged rehabilitation time and inferior functional outcome (3).

Following acute rupture of the thickest and strongest tendon of the human body, the Achilles tendon, the way to recovery is associated with a high risk of DVT and varying degrees of disability (4-8), despite a middle-aged, relatively healthy patient group (9). However, a more recent epidemiological study has shown an increasing mean age of patients with acute Achilles tendon rupture (ATR) (10), which implies an elevated risk of metabolic and other chronic diseases (11, 12). The knowledge of underlying factors causing impairment and the extent of impairment is still limited.

How can we predict the outcome following an acute Achilles tendon rupture?

Early mobilization post-operatively has been a milestone in reducing the risk for DVT as well as to enhance healing (13). However, an acute ATR, no matter if surgically treated or not, still demands stabilization in an orthosis or plaster cast to protect the healing tissue. The detrimental effects of immobilization on tissue repair, as well as an increased risk of DVT, have been observed in experimental studies as well as human observations (13-16).

How do we counteract the effects of immobilization in order to minimize post-operative complications and to expedite recovery?

1.2 FUNCTIONAL ANATOMY

The Achilles tendon is known as the strongest tendon in the human body and played a key role in the evolution of human running (17). It is biologically important for fast movement because it is needed in order to lift of the heel in the final stages of the stride (18). Though, it is also one of the longest tendons in the human body, it is prone to injury since it has to resist a load of up to twelve times body weight during running and jumping (19, 20). It is the conjoint tendon of the gastrocnemius and soleus muscles (triceps surae) (21). The plantaris tendon lies in close proximity to the Achilles tendon but is absent in approximately 10% of the population (20). The contribution of the soleus muscle and gastrocnemius muscle to the Achilles tendon varies. The gastrocnemius muscle is the most superficial muscle of the dorsal aspect of the leg. It has two heads of origin, the medial that arises from the medial condyle of the femur and the lateral arising from the lateral condyle. The soleus muscle originates below the knee joint at the posterior aspect of the proximal fibula, and at the middle third of the medial border of the tibia. The Achilles tendon is formed by the aponeuroses from the two muscles and as the tendon extends

distally it becomes progressively rounder in shape until it flattens again approximately four centimeters from the insertion site at the superior calcaneal tuberosity. The tendon fibers rotate 90° during its descent, which contributes to the ability of elongation and elastic recoiling of the tendon (22, 23) (Figure 1). The gastrocnemius muscle acts on the knee joint by flexion of the knee and at the ankle joint by plantar flexion of the ankle and supination of the foot. The soleus only acts over the ankle joint where it is the main plantar flexor but also contributes to supination of the foot. The soleus consists mainly of the slow twitch, type I muscle fibers and is important for maintaining posture (24) while the gastrocnemius muscle contains a larger proportion of fast twitch, type II muscle fibers, which are important for explosive and rapid movement such as running (25, 26).

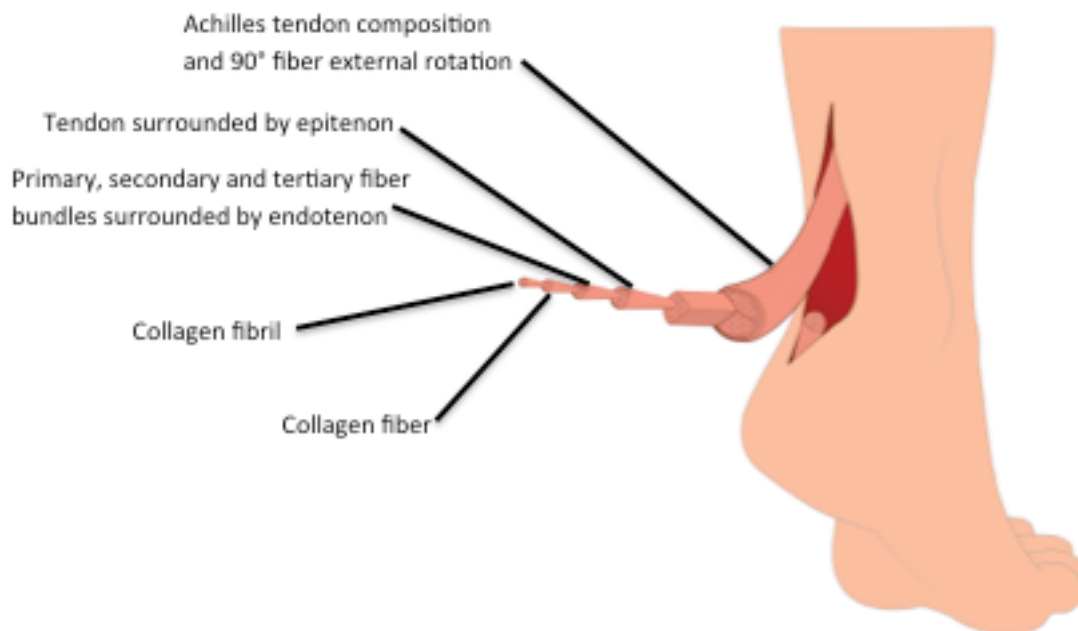


Figure 1. Structure of the Achilles tendon demonstrating the 90° rotation of the fascicles, published with courtesy by Dr Mike Carmont and DJO.

Tendon structure

Type I collagen dominates the Achilles tendon (30%), which explains its considerable strength. Type III collagen represents 1-1,5% and elastin accounts for 2% but is important for the shock-absorbing capacity of the tendon. These three components are embedded in the extracellular matrix consisting of about 67% water. The collagen fibers are organized in primary, secondary and tertiary bundles (27, 28) containing nerve, blood, and lymphatic vessels, forming fascicles (29). The fascicles are surrounded by endotenon and grouped together forming the tendon proper. The tendon is folded by epitenon, which in turn is enveloped by the paratenon, a layer of thin areolar tissue. To reduce friction during motion the epitenon and the paratenon are

separated by a thin fluid layer. The paratenon confluent with the fascia cruris at a distance of 17-58 mm proximal to the postero-superior calcaneal tubercle (30).

Innervation

The triceps surae muscles are innervated by the tibial nerve (21) but the tendon proper is predominantly devoid of nerve fibers. The innervation derives from superficial overlying and deep tissue nerve trunks of the sural nerve in the vicinity of the tendon. The majority of the nerves are situated near the musculo-tendinous junction, the paratenon and also the bone-tendinous junction. This neuroanatomy reflects that normal tendon homeostasis is regulated from the tendon surroundings.

Moreover, accumulating data show that the nervous system, in addition to afferent functions, via efferent pathways through specific neuronal mediators plays an active role in regulating pain, inflammation and tendon homeostasis. For nerve supply during healing, please see 1.9.

Circulation

The blood supply of the Achilles tendon is provided by three sources, (1) the musculo-tendonous junction, (2) vessels in the surrounding connective tissue, and (3) the osteotendinous junction (31). The posterior tibial artery supplies the majority of the Achilles tendon, the proximal and distal part and the peritendinous tissue. The mid-section is supplied by the peroneal arteries via the paratenon and has scant vascularity (29, 32), which may explain its vulnerability to rupture.

1.3 EPIDEMIOLOGY

Current attempts of quantifying the incidence of acute Achilles tendon ruptures are based on small sample populations, with the consequent risk of selection bias. Based on epidemiological studies available the incidence is rising, at least in the developed world. The annual incidence peaks at 55 per 100 000 person years (10, 33-38). The increase in sporting activity, especially in the middle-aged population aged 30-49, as well as a rise in the duration and intensity of training (10, 20, 33, 37) is most likely the explanation. Some studies report a bimodal distribution corresponding to younger athletes and older non-athletic individuals, the later aged 50-59 years (33, 35).

The increased risk of a contralateral rupture in patients who have previously suffered an Achilles tendon rupture is almost 200-fold (39). There is a gender difference in rupture incidence with the odds ratio for being male varies between 3:1 and 18:1, in general approximately 10:1 (33, 40, 41).

1.4 ETIOLOGY

The etiology of Achilles tendon rupture is multifactorial and sometimes divided into intrinsic factors and extrinsic factors. Proposed intrinsic factors include tendon vascularity, aging, gender, body weight and body height, gastrocnemius-soleus dysfunction, pes cavus and lateral-

ankle instability (42). Hypoxic degenerative changes have been reported in ruptured tendons (43, 44). Aging reduces the collagen fiber diameter (45), which can make the tendon more vulnerable. This, in combination with increased body weight and an intense activity can explain the high rupture incidence in the middle-aged group.

Among the extrinsic factors sporting activity is the most well documented one. Between 60-75% of acute ATRs are related to sporting activities (33, 46). Mechanical wear and overuse because of repeated microtrauma may cause tendon weakening and inhibit normal tendon regeneration (43). The use of certain drugs has been implicated in the development of Achilles tendon ruptures. Fluoroquinolone antibiotics have been shown in laboratory studies to reduce proteoglycan synthesis and increase matrix-degrading proteolytic activity (47), but the role in humans is unclear. The negative effects of corticosteroids have also been described (48). One study showed that the risk of tendon rupture was increased after local steroid injections (OR 2.2), and after oral corticosteroids increased to a minor extent (OR 1.4) (43).

1.5 DIAGNOSIS

The acute Achilles tendon rupture presents as a sudden pain in the conjoint tendon of the triceps surae muscle, often without any previous symptoms. Frequently, patients describe that it felt like someone or something struck them from behind and an audibly snap is associated. The most common mechanism is when patient pushes off the weight-bearing forefoot as the knee is extended, which is seen in jumping and racket sports (49). At examination, adjacent to the incident, there is a palpable gap at the rupture site. The ability to plantar-flex the ankle is often absent or very weak. However, due to the plantaris muscle and posterior tibial muscle, some patients may have an almost preserved function. A partial Achilles tendon rupture is more difficult to diagnose and may be overlooked. The diagnosis is clinical and several different clinical tests are described. The calf-squeeze test, also known as Thompson's or Simmond's test, has the highest sensitivity and specificity (0.96 and 0.93 respectively) (50). The patient lies in a prone position while the examiner squeezes the affected calf muscle. If the tendon is ruptured, the normal plantar flexion is absent or minimal – the test is said to be positive (51).

1.6 OUTCOME

Several studies show functional deficiencies after an acute Achilles tendon rupture, both in the short and long term (5, 7, 52). The knowledge about factors responsible for hampered function is sparse and has mainly focused on comparing different treatment protocols.

1.6.1 Surgical vs non-surgical treatment

There is no consensus about the optimal care after an acute Achilles tendon rupture. Several randomized controlled trials and meta-analysis have mainly focused on comparing surgical and non-surgical treatment (8, 53-56). The increased risk of re-rupture after non-surgical treatment and the possibility of early mobilization after tendon suture have been the strongest arguments for surgery. However, the risk of surgery-related complications such as superficial or deep

infection, scar problems and suralis nerve injury, is not negligible. The risk of DVT has been shown to be equally high, no matter if surgically treated or not (9). Regarding the functional outcome, none of the treatment strategies has been shown to be superior the other (57).



Figure 2. Surgical repair of an acute Achilles tendon rupture.

1.6.2 Predictors

Since no treatment protocol has been proven to exceed the other, it is of interest to investigate other variables that may affect outcome. The factors may be intrinsic, such as age, gender, and body mass index (BMI), or extrinsic, such as physical activity level and rehabilitation program.

1.6.2.1 Age

Increasing age has earlier been suggested to be a strong predictor of decreased functional outcome (57). The reduction in tendon elasticity and the ability to resist to loads has correlated to increasing age (58). Age-related metabolic interactions in tendon collagen production may also be an important factor (11, 59). Increasing age is also associated with an elevated risk of complications, which naturally affects outcome (3).

1.6.2.2 Gender

Since there is a male dominance among ATR patients, the knowledge of gender differences is sparse and contradictory. Leppilahti et al. showed that muscle strength was reduced in females (34), whereas a more recent study by Bostick et al. found that strength in females was higher (60).

1.6.2.3 Body Mass index (BMI)

Treatment of Achilles tendon injuries appears to have inferior outcome among individuals with increased adiposity (61). An increased BMI is also associated with more symptoms (57). Since over-weight is a preventable and adjustable factor it is interesting to investigate to which extent BMI affects outcome.

1.6.3 Mobilization vs immobilization

Both in human studies and in experimental studies early mobilization after Achilles tendon rupture has been shown to enhance functional outcome (62-64). It has been demonstrated that early mobilization protocols have decreased the risk of re-rupture, a benefit both for surgically- and non-surgically treated patients (65, 66). To assess the effect of immediate weight-bearing after an acute Achilles tendon rupture Costa et al. performed two independent randomized studies on surgically treated patients with immediate weight-bearing (n=48) and cast treated patients without weight-bearing (n=48). Surgically treated patients with immediate weight-bearing showed improved functional outcome, without a higher rate of complications. However, this positive effect could not be reproduced in the non-surgically treated group. An immediate weight-bearing protocol was concluded to be safe and recommendable after an acute Achilles tendon rupture. Another study compared early weight-bearing and ankle mobilization after ATR suture with a less active rehabilitation protocol (65). The rehabilitation time was shorter and patient satisfaction higher, but no significant differences were found regarding muscle strength and muscle hypotrophy.

The problem of long-term functional deficits following immobilization is however not negligible, especially since it appears to become permanent (67, 68). Many patients report difficulties with running and jumping and a fear of re-rupture preventing them from return to sports (67, 69). Immobilization causes hypotrophy of the calf muscle, reduced calf muscle endurance and strength explaining persistent loss of function (68, 70, 71). Yet, there are patients with full recovery after ATR. The reason for this variation is still largely unknown.

1.7 DEEP VENOUS THROMBOSIS (DVT)

1.7.1 Mechanism

The pathophysiology of venous thromboembolism (VTE) was first presented by the German pathologist Rudolf Virchow (1821-1902) in the 1850s (72). He described the process of clot formation in the veins in the famous “Virchow’s triad”, which includes impaired blood flow, increased coagulability of the blood and an injury in the endothelial wall acting as a trigger factor for clot formation. A blood clot may form in the deep venous system (DVT) or the superficial venous system, also called the muscular veins. The mechanism of thrombus propagation and subsequent embolization from the veins to the pulmonary arteries, pulmonary embolism (PE), is a serious and potentially life-threatening condition (73).

1.7.2 Incidence

DVT is a known and feared complication following trauma and surgery requiring prolonged immobilization of the lower limb (1, 16, 74). However, the majority of postoperative DVTs are asymptomatic and therefore the true incidence is unknown. Symptomatic DVTs can also be missed since normal postoperative findings, such as limb swelling and local pain, can mimic a DVT (1, 75). Most studies on DVT incidence following below-knee trauma and surgery are retrospective and Level II-IV studies (76-79) showing much lower DVT risks compared to

over-knee surgery/-trauma. But patients suffering from an acute Achilles tendon rupture may be at increased risk of DVT, presumably due to immobilization in an equinus position (3). This position reduces the venous blood flow of the lower limb (80).

Meta-analysis and Cochrane database reviews shows that without prophylaxis the VTE rate ranges from 4.3% to 36% after lower limb immobilization (81, 82). Following plaster cast treatment of acute Achilles tendon ruptures the VTE incidence verified with color duplex sonography (CDS) was 36%, irrespective of surgery or conservative treatment (9).

Patient-related risk factors for VTE have been well-documented and especially the following should be considered (83): age over 40 years, obesity, reduced weight-bearing status, immobilization, physical inactivity, past history of VTE, smoking, oral contraceptives and hormone replacement therapy, air travel and rheumatoid arthritis.

1.7.3 Diagnosis

Phlebography has for long been considered the "gold standard" for verifying a DVT. The technique first described by Rabinov and Paulin (1972) is valid and reliable (84, 85), even though the reproducibility has been questioned (86). The method is, however, invasive and demanding for the patient, and contrast media reactions makes it sometimes inappropriate. Technical advances and clinical experience have increased the advantage of colour duplex sonography (CDS), which is a valid, non-invasive, less expensive and more convenient investigation for the patient (87-89).

1.7.3.1 Symptomatic vs asymptomatic DVT

The majority of postoperative DVTs and PEs are asymptomatic (90). Lapidus and co-workers verified, from a register of 668 patients treated for an acute Achilles tendon rupture, 47 (7%) symptomatic DVTs within six weeks from injury (91). Using CDS-screening to detect post-operative DVT, the incidence is as high as 34-36% (9, 92), the majority being asymptomatic. Yet, DVT symptoms are difficult distinguished from normal postoperative findings, and even without disturbing postoperative findings the clinical diagnosis of DVT is unreliable (75).

In the study by Lapidus et al. (91), only 15% of the patients with a PE had a concomitant symptomatic DVT. Thus, the majority of PEs were caused by asymptomatic DVTs. This reflects the relevance of using asymptomatic DVT as an endpoint in clinical trials.

The clinical significance of distal DVTs vs proximal DVTs, has also been debated (93). Between 17 - 28% of distal DVTs propagate to proximal DVTs (94, 95) but no clinical or radiological finding can predict the outcome of a single distal DVT. Since a DVT is necessary for the development of PE as well as post-thrombotic syndrome (PTS), it is reasonable to believe that even distal DVTs should be prevented and treated. In the absence of strong evidence to support anticoagulation over imaging surveillance with selective anticoagulation, either method of managing calf DVT are considered current standards (96).

1.7.4 DVT prevention

Pharmacological thromboprophylaxis in orthopaedic surgery has been thoroughly studied. Following major orthopaedic surgery standardized thromboprophylaxis regimens with low-molecular-weight heparin (LMWH) are usually established since a significant risk reduction is seen and the risk of bleeding low (91, 97). In patients immobilized after so-called minor lower limb injuries, including surgery, there is no consensus whether thromboprophylaxis is needed since varying incidences are reported (81). Additionally, the DVT-preventive effect of LMWH after Achilles tendon surgery has been reported absent or low (92, 98), while some other studies have found beneficial effects (99, 100).

Mechanical prophylaxis with graduate compression stockings (GCS) and intermittent pneumatic compression (IPC) devices administered in the hospital has been shown to reduce the risk of thrombosis in High Risk patients. Since a systematic review did not find any significant differences between IPC and compression stockings (101), the NICE guidelines recommend both modalities (102). Systematic reviews have shown that IPC after total hip arthroplasty (THA) and total knee arthroplasty (TKA) can reduce the DVT incidence significantly (103, 104). However, there is a lack of randomized controlled trials assessing the DVT-preventive effect of mechanical compression therapy post-operatively in outpatients, especially when it comes to lower-limb immobilization.

1.8 INTERMITTENT PNEUMATIC COMPRESSION (IPC)

1.8.1 Mechanism of action

Pneumatic compression for improving circulation has actually been known in the medical literature since the 1800th century, when physicians used compressive therapy against such varied diseases as cholera and tromboangiitis. By supplying external cyclic compressions and relaxations to upper or lower extremities, IPC mimics the muscle pump. The compression pressure is chosen dependent on the type of cuff; foot compression requires 130 mm Hg, while calf and thigh compression requires 40-50 mm Hg, to reach the same clinical effects (55). IPC exerts its effect through mechanical, chemical and molecular mechanisms (105). Mechanical compression increases venous flow with >200%, which decreases the peripheral resistance, whereby arterial blood flow is enhanced, and thereby also the nutritive supply to the tissue. Mechanical compression also affects the interstitial circulation, which reduces oedema and thus diffusion distance for metabolites. The stretch deformation of tissues and cells caused by the venous flow and mechanical compression produces shear stress on the endothelial cells, which increases the production of anti-thrombotic, pro-fibrinolytic, vasodilatory and repair promoting substances (Figure 3). These effects are followed by structural tissue changes and alterations in cellular gene expression (105).

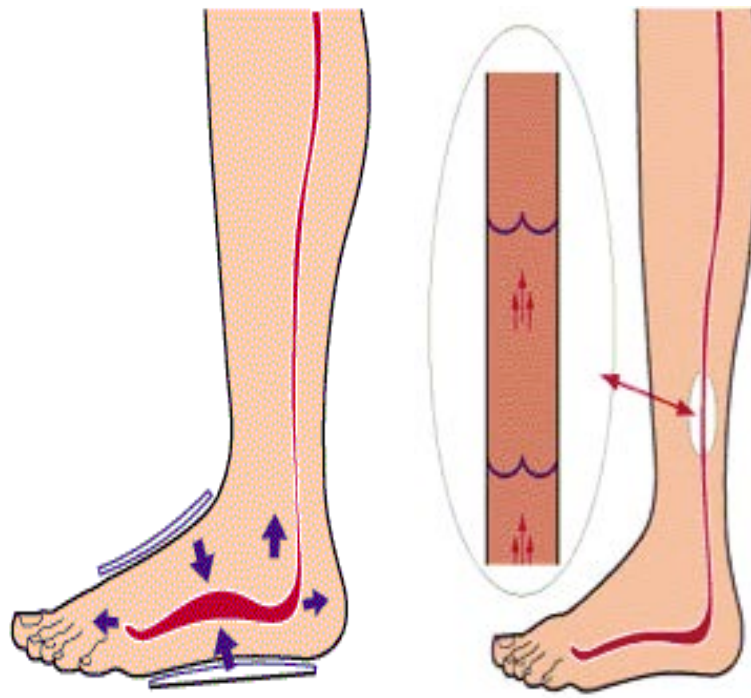


Figure 3. Mechanisms of action of IPC applied to the lower limb: (1) increased venous circulation (2) increased arterial circulation (3) increased interstitial circulation with return of fluid to the veins. (4) Increased venous flow produces shear stress on the endothelial cells, which (5) causes the production and release of ant-thrombotic and proliferative substances.

Several different IPC devices with different settings, such as variations in inflation rate, compression frequency and mode of compression (uniform/graded/sequential), maximum pressure, pressure duration exists, but there are little evidence that these differences affect the DVT-preventive efficiency. However, compression therapy with foot-cuff is less haemodynamically effective than in other segments of the lower limb when it comes to peak speed flow in the femoral vein (106).

1.8.2 DVT prophylaxis

IPC has been shown to cause release of nitric oxide (NO), which has a vasodilating effect, and tissue factor pathway inhibitor that acts antithrombotically. IPC also cause release of tissue plasminogen activator that is pro-fibrinolytic, and has been found to up regulate mRNA for factors involved in angiogenesis and vasodilation (105). Today, IPC is clinically used to prevent venous thromboembolism post-surgery (107). IPC has been clearly proven to have an effect comparable to low molecular weight heparin (LMWH), but without any real side-effects and hereby offers an alternative to heparin when there is increased risk for bleeding complications (108). Studies also suggest that IPC in combination with LMWH can even further reduce the risk of DVT (109) The risk of poor patient compliance has sometimes been brought up as a drawback, and Eisele et al. showed that fewer than six hours of IPC usage per day lead to more DVTs than usage exceeding six hours per day (110). As the devices are technically improved the conditions for adequate usage are enhanced. The America College of

Chest Physicians (ACCP) has stated that further study of the efficacy of portable compression is needed (111).

Whether mechanical compression in an outpatient setting, can reduce the incidence of DVT during leg immobilization after ATR, is yet unknown.

1.8.3 Promotion of healing

The potential role of IPC in enhancing healing post-surgery has been investigated to a minor extent, mainly demonstrating that IPC can reduce postoperative oedema (112-115). IPC has proven positive effects on wound and fracture healing in experimental studies (115-118), although the mechanisms are still unexplored. However, IPC has been demonstrated to enhance neuro-vascular ingrowth in a tendon repair model such as to increase the expression of sensory neuropeptides by up to 100% (112). In the same model, IPC was able during immobilization to improve maximum force by 65%, energy by 168%, organized collagen diameter by 50%, and collagen III-VI occurrence by 150% compared with immobilization only (116). Whether IPC can counteract the negative effects of immobilization in patients still needs to be further investigated.

1.9 TENDON HEALING

1.9.1 Blood supply

The major blood supply to the tendon originates from the paratenon, the musculo-tendinous junction and the tendon-bone insertion. The most common site of Achilles tendon rupture and tendinopathy is the mid-substance, approximately 3-6 cm from the bone insertion. Since this area is sparsely vascularized, hypoxia has been suggested as an important factor of tendinosis and tendon degeneration. Additionally, histopathological studies have demonstrated hypoxic changes like increased lactate levels in tendinosis (119). The increased neoangiogenesis demonstrated in tendinopathy may be explained as a response to hypoxia and thus represent a more advanced state of tendinosis. Eccentric training, established as a treatment method of tendinosis, has been shown to reduce neovascularization (120, 121).

Whether IPC via increase in blood flow during leg immobilization in ATR-patients can improve healing conditions is still unclear.

1.9.2 Nerve supply

The tendon proper is normally aneuronal. Nerve fibers originate from surrounding soft tissues, skin and muscles and are found at the musculo-tendinous junction (122). However, some fibers pass the musculo-tendinous junction within the septa of the endotenon. Additionally, branches from the more profoundly innervated paratenon stretches via the epitenon and penetrate the tendon through the endotenon (Figure 4).

Recent findings demonstrate that peripheral nerve fibers exhibit efferent effect via specific neuronal mediators, which play an active role in regulating pain, inflammation, tendon

homeostasis and repair. Thus, after injury and during tendon repair there is extensive nerve ingrowth into the tendon proper, followed by a time-dependent release of sensory, autonomic and glutamatergic mediators, which amplify and fine-tune inflammation and regulate tendon regeneration.

Under the pathological condition tendinopathy, ingrowth of sensory nerve fibers into the tendon proper has been observed in several studies. This neoinnervation is sometimes found co-localized with blood vessel, while at other times without vascular co-existence (123, 124) (Figure 4).

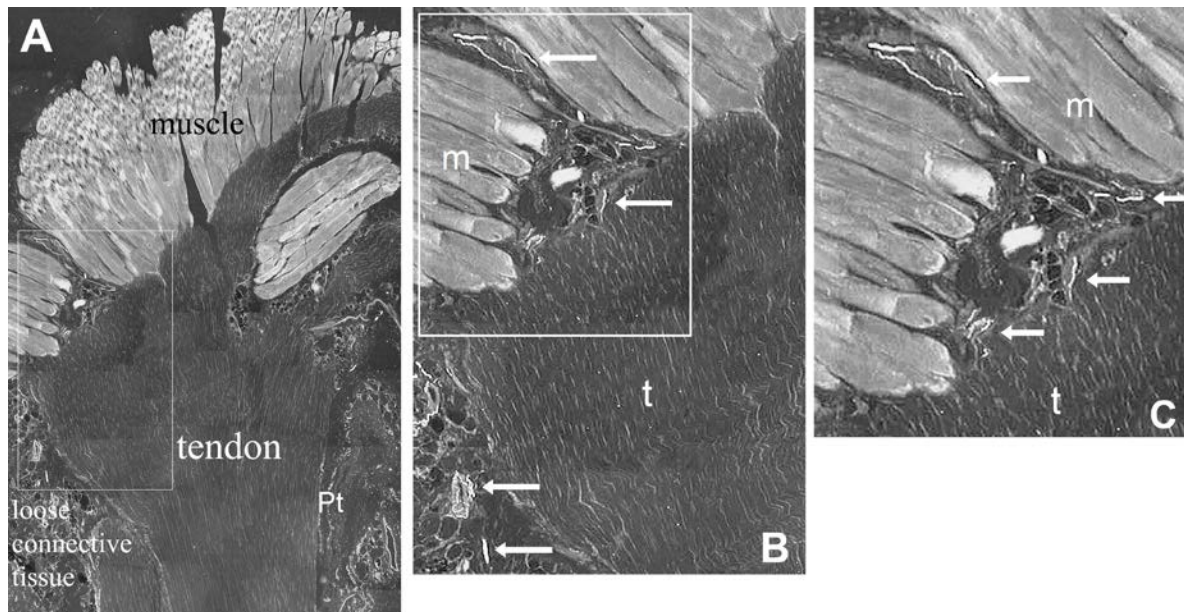


Figure 4A-C. Micrographs of longitudinal sections through the Achilles tendon at increasing magnifications. Arrows denote varicosities and nerve terminals. The immunoreactivity is seen in the paratenon and surrounding loose connective tissue while the tendon proper is almost devoid of nerve fibers. Reproduced with permission from John Wiley and Sons.

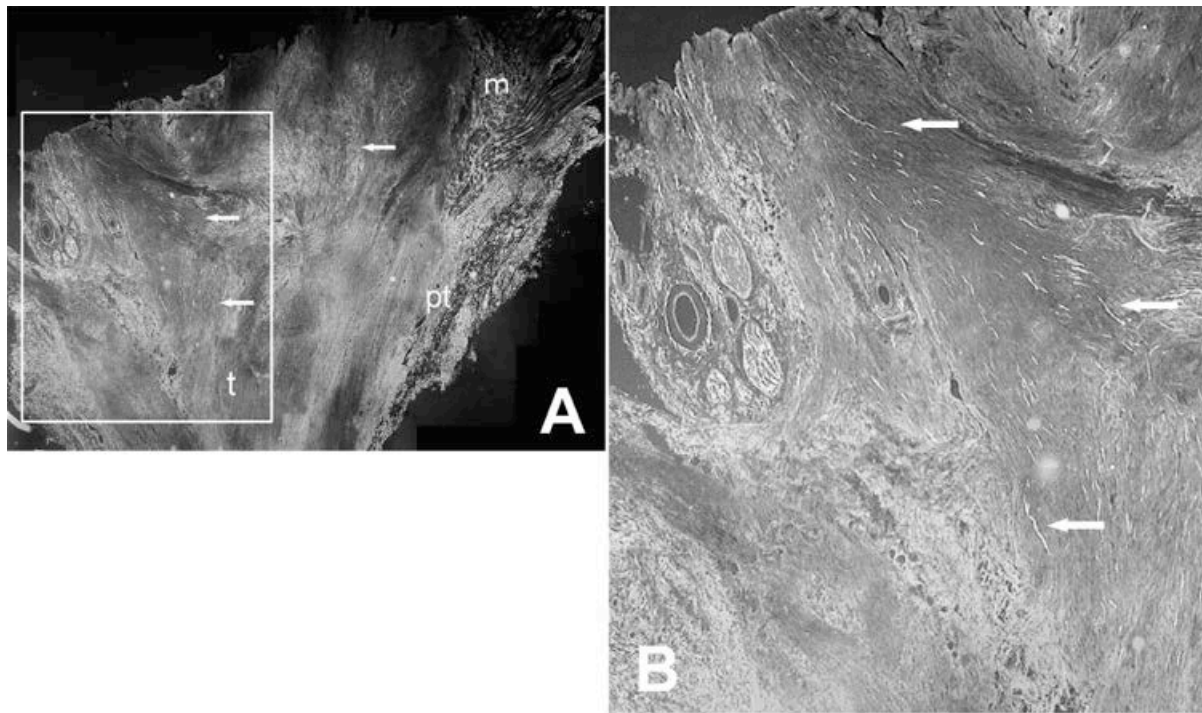


Figure 5A-B. Overview micrographs of longitudinal sections through the Achilles tendon at two weeks post rupture. Incubation with antisera to a nerve growth marker, GAP-43. Micrographs depict the proximal half of the Achilles tendon at increasing magnification in figures (A-B). Arrows denote varicosities and nerve terminals. The GAP-positive fibers, indicating new nerve fiber ingrowth, are abundantly observed in the healing proper tendon tissue. Reproduced with permission from John Wiley and Sons.

1.9.3 Tendon metabolism

Research has revealed that tendons are vascularized mainly in the surrounding sheaths, i.e. paratenon, epitenon and endotenon. Fibroblasts are constantly remodelling the tissue and are increased in number during healing (125). Three kinds of metabolism are used by the fibroblasts; Krebs cycle, anaerobic glycolysis and the pentose phosphate shunt (44). All three mechanisms are active in the growing tendon. In the adult tendon, anaerobic glycolysis remains on the same level of activity while the other metabolic pathways decrease their activity (44). It can be noted that the pentose phosphate shunt not only supplies the tendons with energy but also substrates for nucleic acid synthesis and therefore could be particularly important at times of intense collagen production (44). The metabolic rate in the tendon is relatively low compared to that of the muscle tissue, and even more restricted during immobilization (14, 116, 126, 127). A low metabolic activity combined with a sparse blood circulation may explain why tendon healing is a protracted process.

1.9.4 Phases of healing

The healing process aims at achieving tissue integrity, homeostasis and load-bearing capability. A complex repair mechanism involving blood-derived cells (leukocytes, platelets, monocytes and lymphocytes), tissue-derived cells (macrophages, fibroblasts, endothelial cells, mast cells

and stromal cells) and mediators (vascular-, cellular- and neuronal-) starts at the wound site and continues over five different sequences: 1) induction 2) production 3) orchestration 4) conduction and 5) modification (Figure 5). The healing events are influenced by multiple factors such as injury site, age, sex, genetics, nutrition- and health status of the patient, and neurovascular status, including the supply of mediators (128). Disturbances may occur at any phase of the process and a good understanding of the repair mechanism is crucial to optimize the treatment outcome.



Figure 5. Tendon repair (1) Induction - Blood-derived cells (2) Production – Tissue-derived cells (3) Orchestration – Neuro-vascular ingrowth (4) Conduction – Tissue matrix (5) Modification – Mechanical stimulation

1. Induction of healing

After injury, the wound site is infiltrated with blood-derived cells, which stop bleeding, clean up tissue debris and direct further traffic by release of inflammatory mediators, e.g. cytokines, nitric oxide and growth factors. Clot formation is followed by the migration of polymorphnuclear leukocytes into the healing site, which is stimulated by SP and CGRP (129, 130) This is the initiation of the inflammatory healing phase. Experimental studies have suggested that pharmacological thromboprophylaxis, if given continuously, impair tendon healing (131).

2. Production of callus

The inflammatory mediators released by the blood clot attract tissue derived cells, which during the following three days are triggered to transform e.g. fibroblasts into myofibroblasts and subsequently activate production of tendon callus (132). In that way granulation tissue, i.e. extracellular matrix and collagen type III, is formed from the tissue derived cells, which normally reside in the extrinsic peritendinous tissues and the intrinsic tissue of the epitenon and endotenon. During the first week, collagen synthesis commences and reaches its maximum by week four – the reparative, collagen-forming phase. The fibroblasts respond to mechanical loading by increased production of collagen (133). The mechanical tension across the repair

site is important for the quality of tendon healing since it speeds the realignment of collagen fibers, increases tensile strength and minimizes deformation (134).

The tissue- and blood derived cells that infiltrate the wound area moreover release a cascade of mediators (growth factors, cytokines, bone morphogenetic proteins, and neuropeptides). Supplements of these factors have in numerous experimental studies demonstrated promising results for optimization of the repair process.

In addition to growth factors, neuromediators released by ingrowing nerve fibers during tendon repair have essential effects on the healing process (123, 124, 135-137). Nerve sprouting and growth within the tendon proper is followed by a time dependent expression of neuromediators during the tendon healing process (136). During inflammatory and early proliferative healing mainly sensory neuropeptides (eg. Substance P) are released (136). Subsequently, after the healing process is finished sprouting nerve fibers within the tendon proper retract to the surrounding structures, ie. the paratenon and the surrounding loose connective tissue. Presumably, nerve retraction is also essential for healing progression.

3. Orchestration of callus formation

During initiation of matrix production the healing tendon, which normally is practically devoid of nerves and vessels, is successively infiltrated by new nerves and vessels providing essential neuro-vascular mediators that can orchestrate the repair process (132, 135-137) (Figure 4).

New nerve ingrowth within the tendon proper, which normally is aneuronal, is followed by a time dependent expression of neuropeptides during the tendon healing process (135-137). During the inflammatory and early proliferative phase, i.e. 2-6 weeks after injury, there is a conspicuous increased occurrence of sensory neuropeptides, substance P (SP) and calcitonin gene related peptide (CGRP) in the healing tendon tissue. SP and CGRP are located partly in free sprouting nerve endings among fibroblasts in the healing tendinous tissue and partly perivascular to newly formed blood vessels. These localizations reflect a stimulatory role of sensory neuropeptides on cell proliferation, as demonstrated in cultured fibroblasts (138) and endothelial cells (139). Thus, SP is known to enhance angiogenesis (139). A stimulatory effect on tendon healing has been strengthened by studies demonstrating that supplementation of SP clearly promoted tendon repair partly by increasing tensile strength more than 100% compared with controls (140-142). Accordingly, selective denervation leading to reduced amount of SP results in impaired tendon healing (143).

However, for healing to progress, the ingrown nerves and vessels have to retract, a process that is promoted by adequate mechanical stimuli. In case of tendinopathy an increased number of vessels and sensory nerves with elevated SP-levels have been observed within the proper tendon, indicating an unaccomplished healing process. Thus, signals that regulate nerve and blood vessel retraction are critical in the understanding of preventing tendinopathy. Factors that may regulate nerve retraction and are released at mechanical stimulus during tendon healing

include IL-6 family members (123, 144, 145), neurotrophic factors (14), glutamate (146) and their receptors (15, 147, 148).

4. Conduction of ingrowth

A prerequisite for healing to commence is an existing and functioning tissue matrix into which cells, vessels and nerves can grow in and where production of new granulation tissue can occur. If a tissue defect exists the repair process will be prolonged or will not be able to take place at all. Hence, in conservative as well as in surgical treatment of patients with tendon injuries it is an important principle to bring the disrupted tendon parts close together for regulation of fibril fusion and new tissue ingrowth.

5. Modification of healing callus

Mobilization leading to mechanical tendon loading is the most well-known extrinsic factor adapted to regulate tendon protein synthesis and degradation (133). One exercise bout in human tendons activates an initial increase in both the synthesis and degradation of collagen. The initial loss of collagen after loading is thus over time ensued by a net gain in collagen. Increasing mechanical loading activates myofibroblasts and fibroblasts to increase the production of collagen type I to increase the callus size, and enhance the capacity to withstand high mechanical load. With loading of the tendon the orientation of fibroblasts and collagen changes to the longitudinal axis of the tendon by 4 weeks after injury. By 4 weeks, the mechanical strength of the repairing tendon increases, as there is consolidation and remodelling of the maturing granulation tissue under tension, and the collagen synthesis under load changes from type III to type I. Various factors influence the rate and quality of tendon healing. The most important is the mechanical tension across the repair which speeds realignment of collagen fibers, increases tensile strength and minimizes deformation at the repair site (134). Early mobilization accelerates the nerve plasticity, i.e. nerve regeneration, expression of neuromediators and their receptors and nerve retraction (15, 149) (Figure 4).

1.9.5 Mechanical loading vs immobilization

Convincing data demonstrate that mechanical loading and tension over ligament and tendon tissue enhance tendon healing (133, 150). In human models, mechanical load shows an increase in collagen synthesis and tendon size (151). In an experimental model early motion and physical activity accelerate the nerve ingrowth and thereby possibly promote the repair process (149).

However, the impact of immobilization seems to be even more pronounced than the effects of mechanical stimuli. Following Achilles tendon rupture in a rat model plaster cast immobilization of the hind-limb reduced the tensile strength by 80% at two weeks post-rupture compared to a freely-mobilized group (116). Lack of mechanical stimulation is equally detrimental in humans (43, 152).

1.9.6 Metabolites

Adequate supply of glucose and oxygen are essential for fibroblast metabolism during ATR repair. Therefore study of the energy substrates needed for the repair process is of particular interest.

1.9.6.1 Glucose metabolism

The energy substrates glucose is processed in the glycolytic pathway into pyruvate. In an anaerobic environment pyruvate is converted to lactate, pyruvate and lactate (153, 154). The blood supply is rather poor throughout the tendon and therefore tissue glucose in the Achilles tendon is not necessarily as high as arterial blood glucose. Langberg et al. have previously measured interstitial glucose in healthy ATs to about 4.0 mmol/l during rest while arterial blood glucose was 5.2 mmol/l (123). It has not previously been assessed what interstitial glucose concentrations are in healing AT and whether that glucose concentration is sufficient for an effective healing process.

1.9.6.2 Pyruvate metabolism

Aerobic metabolism is normally down regulated in the adult compared to the growing AT, and pyruvate concentration is therefore expected to be low in adult healthy tendons (43). In muscle tissue pyruvate concentration has been measured to about 13 $\mu\text{mol/l}$ but few studies have investigated pyruvate in other tissues (155). Anaerobic metabolism, on the other hand, remains at the same level in the adult, as in the growing tendon, with a slight decrease in tendons of elderly (43).

1.9.6.3 Lactate metabolism

Lactate has previously been found to be increased in tendinosis (2.15mmol/l) compared to healthy tendons (1.14 mmol/l), indicating anaerobic conditions (156). Further, Langberg et al. found lactate concentrations in the peritendinous space to be increased during exercise compared to resting conditions, indicating that anaerobic metabolism is frequently used to supply the AT with energy (157). This increase in lactate during exercise occurred despite an increase in blood flow during the exercise. With the energy needs of the repair process it would be plausible that both pyruvate and lactate concentrations increase during healing of ATR but that has not yet been thoroughly investigated.

1.9.6.4 Glutamate metabolism

Both free glutamate and glutamate NMDAR1 receptors have been found in ATs (156). Elevated concentrations of free glutamate were detected in ATs with tendinosis (78-250 $\mu\text{mol/l}$) compared with healthy ATs (16-34 $\mu\text{mol/l}$), a difference associated with pain at first. However, later studies from the same group have shown no such correlation (158). More recent experimental studies have shown increased glutamate concentrations in healing ATs (112) together with nerve and vessel sprouting. Increased free glutamate concentrations may result

from nerve sprouting and hence be a part of the healing process. Whether this holds true also in humans has not yet been verified.

1.9.6.5 Glycerol metabolism

Glycerol is the end product of cell membrane degradation, which is constituted mainly of phospholipids. Glycerol has been investigated with MD in brain tissue, where it has been found to indicate tissue damage secondary to ischemia. However, glycerol has also been suggested to indicate lipolysis in skeletal muscle (159) and could therefore as well be considered as an energy substrate. Since insertion of the microdialysis probe causes some trauma glycerol measurements may be considered less reliable. Gutierrez et al. (160), found initially elevated glycerol concentrations after introduction of catheters in skeletal muscle, while Langberg et al. found glycerol concentrations in peritendinous space of healthy ATs to be stable (150-200 $\mu\text{mol/l}$) during rest, exercise, and recovery (157). Glycerol concentrations during the healing process after ATR in humans have not previously been investigated.

2 AIMS OF THE THESIS

This thesis was designed to first investigate predictors of patient outcome after an acute Achilles tendon rupture (ATR), and secondly to reduce complications and promote outcome by targeted interventions.

The specific aims were:

1. To explore predictors of outcome in patients at one year post ATR (study I).
2. To assess the effect of adjuvant foot-IPC under plaster cast on the incidence of deep venous thrombosis (DVT) during post-operative leg immobilization (study II).
3. To determine the effect of adjuvant calf-IPC under an orthosis on the incidence of DVT during post-operative leg immobilization (study III).
4. To examine whether adjuvant IPC can enhance for tendon healing essential metabolites during post-operative leg immobilization (study IV).

3 SUBJECTS

The study was approved by the Regional Ethical Review Committee in Stockholm and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients provided written informed consent prior to participation in the study.

The inclusion criteria included acute unilateral ATR in patients aged 18-75 years. The exclusion criteria were: inability to give informed consent, current anticoagulation treatment (including high dose acetylsalicylic acid), planned follow-up at other hospital, inability to follow instructions, known kidney failure, heart failure with pitting oedema, thrombophlebitis, thromboembolic event during the previous three months, other surgery during the previous month, known malignancy, haemophilia and pregnancy.

Study I

Patients were included from a cohort of 150 patients with acute Achilles tendon rupture included in a prospective, randomized controlled trial conducted at Karolinska University Hospital, Stockholm, Sweden (study III). A total number of 111 patients were assessed at the one-year follow-up (Figure 13).

Study II

Between February and December 2010, 87 patients with suspected acute ATR at Karolinska University Hospital, Stockholm, Sweden, were assessed for eligibility. Of the eligible patients, 40 were excluded and 21 declined to participate. The remaining patients were randomized between adjuvant IPC (n = 14) and plaster cast (n = 12) (Figure 14). According to the power calculation the plan was to include 100 patients. However, frequent malfunctioning of the IPC device beneath the cast resulted in patients requiring a second plaster cast, which correlated with an increased incidence of DVT. Therefore this study was ended prematurely.

Study III

Between March 2011 and June 2013, 389 patients with ATR were screened for eligibility at the Karolinska University Hospital and Stockholm Söder Hospital. 150 patients (126 men and 24 women) at a mean age of 40 years (range 18-71) were included, and operated at the Karolinska University Hospital, Stockholm (Figure 15). Patients were randomized and allocated to either IPC and orthosis immobilization (n = 74) or plaster cast (n = 74).

Study IV

Patients with acute ATR were between August 2010 and February 2012 recruited at the Karolinska University Hospital, Stockholm, Sweden. Postoperatively, the first 10 patients were randomized in blocks of five into the two treatment groups, while the additional 10 patients in the control group were recruited at a later time point. The IPC-group (n = 5)

received foot-IPC under plaster cast and the control group (n = 15) standard plaster cast treatment.

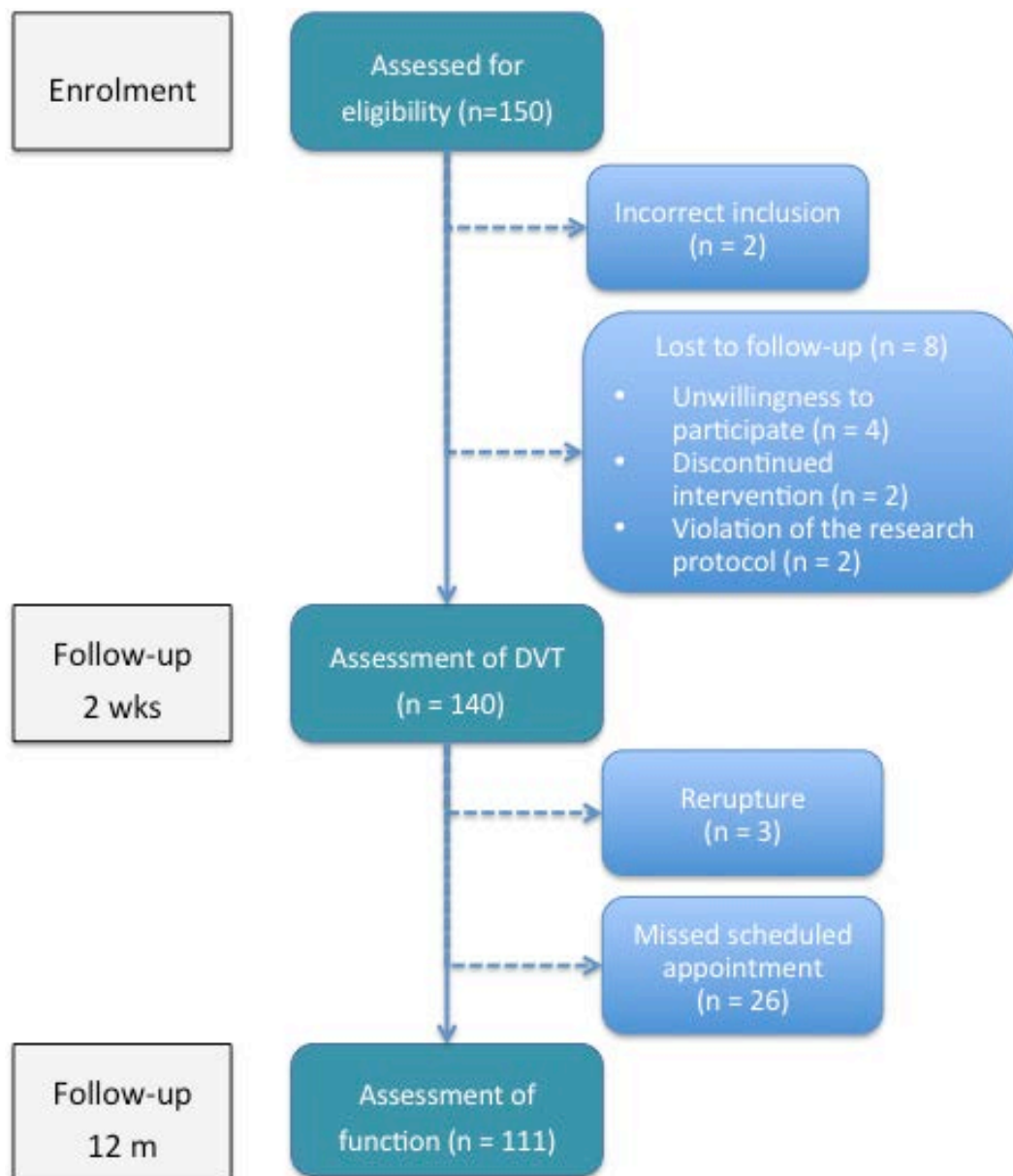


Figure 13. Flow chart for outcome follow-up at 12 months (Study I).

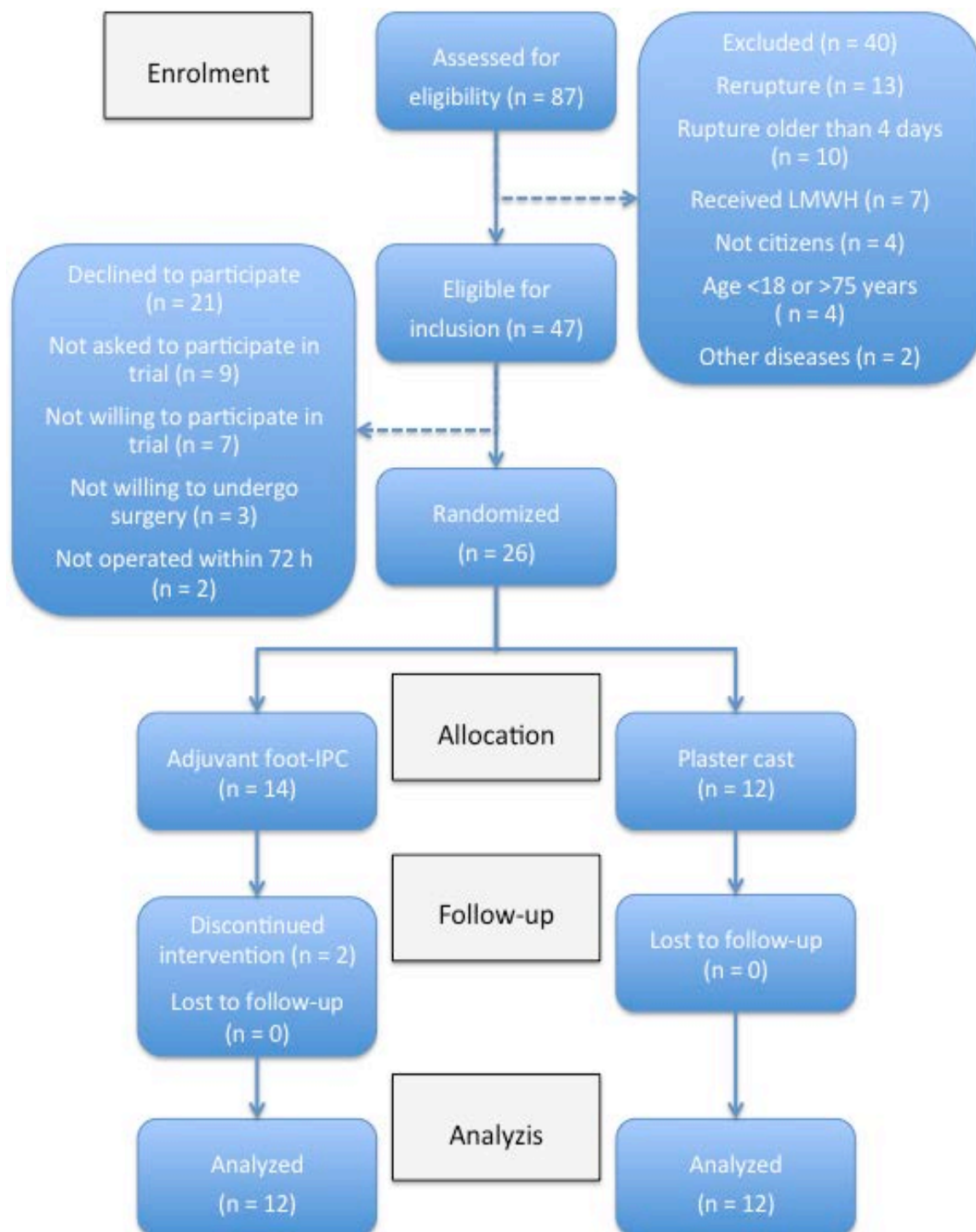


Figure 14. Intermittent pneumatic compression (IPC) with foot-cuff under cast; CONSORT flow diagram (Study II).

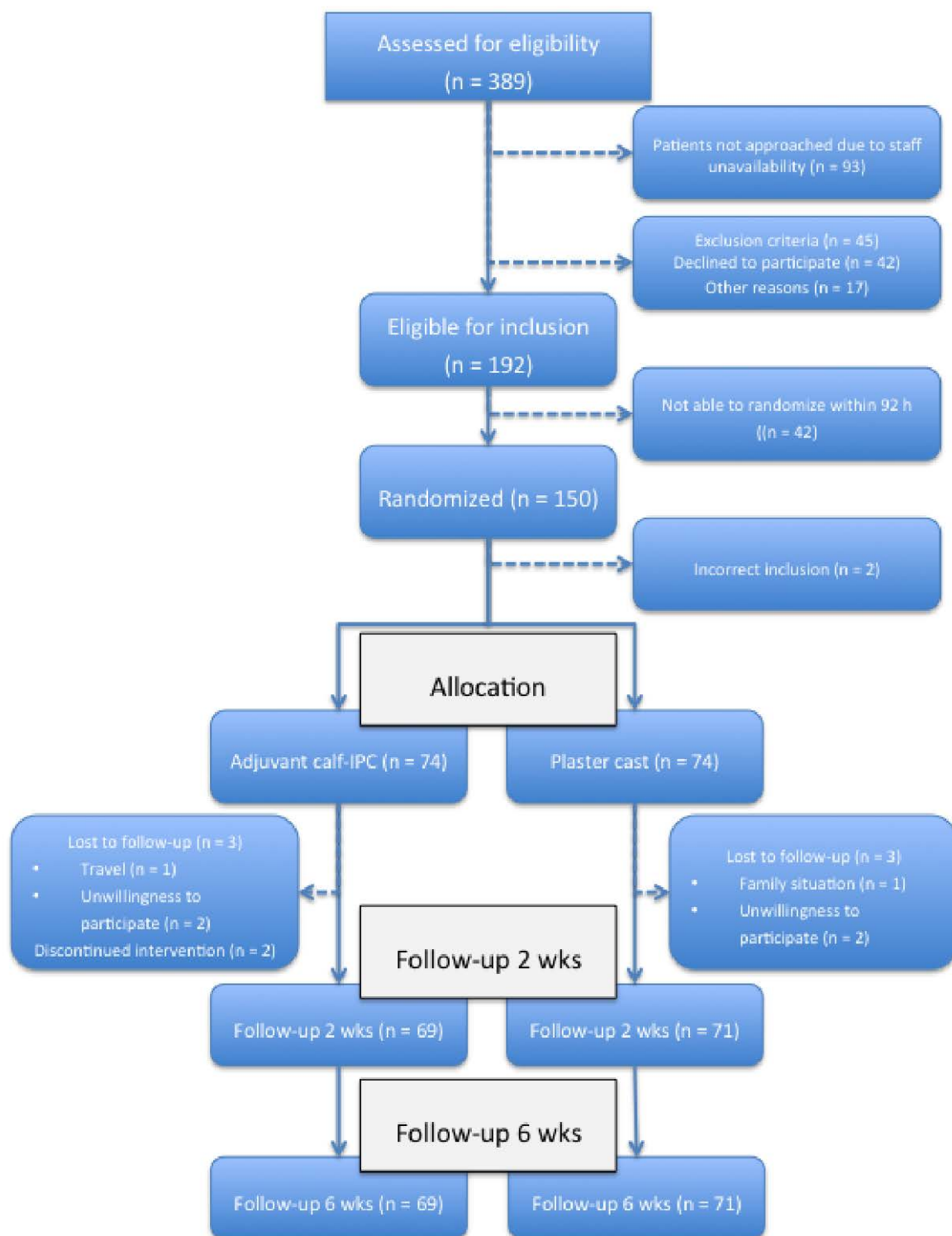


Figure 15. Intermittent pneumatic compression (IPC) with calf-cuff under Aircast Walker® orthosis for DVT prevention; CONSORT flow diagram (Study III)

4 METHODS

4.1 SURGERY

All patients included in paper I-IV were treated with open surgical repair of the ruptured Achilles tendon. Surgery was performed within 96 hours from the injury under local or spinal anesthesia. With the patient in the prone position, a posteromedial skin incision was made over the rupture site. The fascia cruris and paratenon was incised centrally over the rupture site. An end-to-end suture was placed using a modified Kessler suture technique (161) with two 1-0 polydioxanone (PDS) sutures, with closure of the paratenon and fascia cruris using absorbable sutures (Vicryl 3-0) and skin closure using nylon sutures (Ethilon3-0). All sutures were supplied by Ethicon, Sommerville, New Jersey. The operation technique was standardized and performed by experienced orthopaedic surgeons familiar with the method.

4.2 RANDOMIZATION

After Achilles tendon rupture surgery, consecutive patients were randomized to treatment with either mechanical prophylaxis with a) foot-IPC under plaster cast (Study II and IV) or calf-IPC under orthosis (Study I and III) or b) standard treatment with plaster cast. Patients were enrolled and assigned to the interventions either by a third party nurse or by a research nurse. Randomization was performed with use of computer-generated block size of four, through an independent software specialist, and consecutively numbered, sealed, opaque envelopes opened after surgery and prior to treatment.

4.3 IMMOBILIZATION

4.3.1 Cast

To standardize the cast application, patients in the control group received a below-knee plaster cast with the ankle in 30 degrees equinus position in the outpatient clinic shortly after the completion of surgery. Patients were non-weight-bearing with crutches during the first two weeks.

4.3.2 Cast and foot-IPC

Patients in the foot-IPC group (n = 14) had the IPC system applied to the foot under the cast. The Covidien A-V impulse system (Ortofix Vascular Novamedix, Andover, UK) uses special foot cuffs for inclusion under a plaster cast, and as the device cycles the distal chamber inflates to 130 mmHg over a 0.4-second period, with 20-second intervals (Figure 6). The patients were instructed to apply the IPC when they were either sitting, or lying in bed sleeping, for at least of six hours daily. The IPC treatment was registered by the patient and by the device and was discontinued two weeks post-operatively.



Figure 6. Intermittent pneumatic compression (IPC) with foot-cuff applied under a below-knee cast in equinus position.

4.3.3 Orthosis and calf-IPC

The patients in the intermittent pneumatic compression group received two weeks of six hours daily bilateral calf-IPC (Venaflo® Elite, DJO LLC, Vista, CA, USA) applied under an orthosis (Aircast® XP Walker™, DJO LLC, Vista, California) (Figure 7). As the IPC-device cycles, the distal chamber inflates to 73 mm Hg over a 0.5-second period. During the last 0.2 second of this period, the proximal chamber inflates to 63 mmHg and then settles at 45 mm Hg. After six seconds of inflation the cuff deflates, and the cycle is repeated every minute. The patients were instructed to apply the intermittent pneumatic compression therapy daily during the time they were sedentary, i.e. sitting or lying in bed sleeping, 6 hours at a minimum. Patient compliance was registered by the patient and by the device. Intermittent pneumatic compression treatment was discontinued two weeks post-operatively.

4.3.4 Orthosis

At two weeks post-operatively, patients with previous cast immobilization received an orthosis and were allowed to weight-bear as tolerated until six weeks post-operatively when immobilization was ended. Hence, the immobilization was identical in both the IPC group and the control group during the last four weeks. Patients were allowed to remove the orthosis during nighttime and one hour daily.



Figure 7. Photograph showing bilateral calf cuffs applied to the operated limb and the contra lateral limb, both connected to an intermittent pneumatic compression device. An orthosis (Aircast® XP Walker™, DJO, Vista, California) is applied to the operated limb.

4.4 DVT ASSESSMENT

At 2 and 6 weeks post-operatively, all patients were screened for DVT in the operated leg by unilateral colour duplex sonography (CDS). Either of two experienced ultrasonographers, blinded to the treatment regimens, performed all the CDSs using a Philips CX 50 ultrasound machine (Philips Medical Systems, Andover, MA, USA) (Figure 8). The standard procedure included evaluation of all deep proximal and distal veins, including muscle veins, as well as vena saphena magna. The criteria for DVT diagnosis and the diagnostic procedure have been described earlier (87). Proximal DVT was defined as a thrombosis that involved the popliteal vein or any more proximal veins, with or without involvement of the calf veins. Briefly, DVT was diagnosed based on abnormal wall compressibility of the blood vessel with a transversal ultrasound compression test. Prior to this, an assessment of blood flow in the veins by color Doppler flow was performed, which is especially important in the calf to identify the multiple vein segments.

The results denoting a verified DVT were re-evaluated after completion of the study. This second assessment was made in a blinded fashion, without any knowledge of the primary outcome. There was no difference in the post hoc examination. Patients who were diagnosed as having a DVT received LMWH, according to the protocol of the Department of Haematology of our hospital.



Figure 8. Screening for deep venous thrombosis with colour duplex sonography (CDS).

4.5 PATIENT REPORTED OUTCOME AND PHYSICAL ACTIVITY

As evidence-based medicine has become accepted, the need for valid and reliable outcome measures is obvious. Injury-specific outcome measures increase the quality of research and facilitate clinical work for physicians since assessment of progress of treatment and rehabilitation is possible. Combining several outcome measurers is recommended since various aspects are covered by different tests.

The first injury-specific questionnaire for acute ATR was developed by Nilsson-Helander and co-workers – the Achilles tendon Total Rupture Score (ATRS) (162). The questionnaire consists of 10 items that evaluates aspects of symptoms and physical activity, where the score for each item ranges from 1-10 on a Likert scale, with a maximal score of 100; a lower score indicates more symptoms and greater limitation of physical activity. The test has been shown to have a high reliability (test-retest reliability ICC=0.98), validity and responsiveness (an effect size of 0.87-2.21) for evaluation of Achilles tendon rupture treatment. The test has been cross-culturally adapted to English, and has been shown to have an equally good reliability, validity and responsiveness (163). A review article of patient-reported outcome measurers concluded that ATRS was the only valid outcome measure for Achilles tendon rupture patients (164).

The Physical Activity Scale (PAS) was constructed for self-assessment of middle-aged' and former athletes' present intensity/frequency of physical activity (165). The score ranges from 1 to 6 where 6 equals regular intense training several times a week. The scale was based on a population aged over 70 years, which should be borne in mind.

The Foot and Ankle Outcome Score (FAOS) is a region-specific questionnaire that assesses patients' functionality and foot- and ankle-related quality of life (166). This test has five different subscales and has got a high reliability and validity in patients with ankle ligament injuries, but has not been evaluated for Achilles tendon ruptures.

The EuroQol Group's questionnaire (EQ-5D) (167, 168) is a standardized instrument to measure health status. It is based on a health profile consisting of five dimensions with three levels and provides a single index value. An EQ-5D score of 0 is considered to be the worst imaginable health state and a score of 1.00 the best imaginable health state. The instrument is used worldwide in clinical and economic evaluation of health care.

In this thesis, patients' symptoms and physical activity levels were assessed using four reliable and valid scores; the Achilles tendon Total Rupture Score (ATRS) (162), Physical Activity scale (PAS) (8), Foot and Ankle Outcome Score (FAOS) (166) and EuroQol Group's questionnaire (EQ-5D) (167).

4.6 FUNCTIONAL ASSESSMENT

Following an Achilles tendon rupture patients often demonstrate deficits in the range of motion (ROM) in the joint, strength, endurance and jumping ability (10-12). To evaluate outcome after treatment it is therefore important to have objective measurements that is both reliable and valid.

By comparing the injured side with the contralateral intact side it is possible to establish the limb symmetry index (LSI) expressed as a percentage ($\text{injured/intact} \times 100 = \text{LSI}$). The LSI is commonly used to evaluate functional outcomes. For return to sports the LSI for strength and functionality should reach 85-90%.

Calf circumference is used in clinical settings to estimate muscle hypotrophy (Figure 9A). However, its weak correlation to calf muscle endurance and strength makes it less useful (4, 169). Muscle strength improvements can be measured with isokinetic dynamometry with a high reliability but is only moderately correlated to functional performance (8, 52, 62).

The muscular endurance is another measurement of muscle function, often measured with the heel-rise test. Evaluation of the calf muscle endurance by repetitive plantar flexion of the ankle upon standing until fatigued, is a commonly used test with high reliability (ICC 0.78-0.84) (169, 170). By also evaluating maximum heel-rise height and total work performed differences between injured and intact sides can be more easily detected (70).

One year after injury all patients returned for assessment of functional outcome. The functional evaluation consisted of muscular endurance test and was performed as previously described in the literature (70, 171). The tests have been shown to be reliable and valid (169, 170) and have frequently been used in evaluating outcome after Achilles tendon rupture (7, 57, 60, 70, 172). All evaluations were performed by either of two independent physical therapists. The patients' height and weight were measured and documented. The examiner demonstrated the test for the subjects, and all of them were given standardized instructions.

The test was always performed in the same order, and the uninjured side was always tested first. Verbal encouragement was used and athletic footwear was standardized. Prior to testing, the patients warmed up on a stationary bicycle for 5 min followed by one set of 10 two-legged heel-rises. MuscleLab® (Ergotest Technology, Oslo, Norway), a data collection unit with different sensors, was used for the evaluations.

The heel-rise test for endurance was performed on one leg at a time with the participant standing on a box with an incline of 10 degrees (Figure 9B). The participants were allowed to place the fingertips, at shoulder height, against the wall, for balance. A metronome was used to keep a heel-rise frequency of 30 per minute. The participant was instructed to go as high as possible on each heel-rise and then lower the heel to the starting position, and to perform as many heel-rises as possible. The test was terminated when the patient stopped, could not maintain the frequency, or did not perform a proper heel-rise. The numbers of heel-rises, the time and height of each heel-rise, the total work (the body weight x total distance) in joules and the power (work/time) was used for data analysis. The limb symmetry index (LSI) was defined as the ratio between the injured limb and the uninjured limb, expressed as a percentage ($\text{injured/uninjured} \times 100 = \text{LSI}$) and was used for comparison between the limbs.



Figure 9A-B. (A) Calf circumference measurement and (B) Heel-rise test for muscular endurance.

4.7 MICRODIALYSIS

4.7.1 Principle

Tissue microdialysis (MD) is a technique that allows for determination of interstitial concentration of molecules in various substances in-vivo (173, 174). The method is useful when investigating human metabolism in critical care medicine after multitrauma, sepsis or brain trauma, and pre- and post-surgery, as well as in studies of drug distribution, metabolism and pharmacodynamics.

A thin dialysis probe with a small semi-permeable membrane on its tip is introduced into the tissue of interest, and a fluid resembling the interstitial fluid (perfusate) is being pumped through the probe. (Figure 10a, b). Substances in the interstitial space will diffuse across the membrane in relation to the relative difference in concentration in the tissue and in the perfusate. The fluid, now called dialysate, is collected in vials for immediate, or later, analysis. The concentration of the dialysate reflects the concentration in the investigated tissue. This makes it possible to detect changes in metabolite concentration in the interstitium.



Figure 10A-B. The principles of the microdialysis (A) and the CMA 71 microdialysis catheter (B), published with courtesy by MDialysis AB.

4.7.1.1 Considerations

Although carefully introduced, catheter insertion causes a tissue trauma that affects the first samples. Therefore, the catheter must be calibrated in situ since diffusion over the membrane is also dependent on several factors including the type of membrane, perfusion rate, temperature, tortuosity and tissue type for reliable results (159). The time necessary to reach tissue equilibration before starting the analysis has been estimated to half an hour, since increased levels of thromboxane B2, glucose and lactate return to baseline within this time range (175).

4.7.1.2 Recovery

Concentrations of measured metabolites are influenced by changes in local blood flow (176, 177). Since simultaneously measure the local blood flow is difficult to achieve in the clinical setting, a different method, measurement of recovery, has been developed. Recovery is inversely proportional to blood flow (178) and hence gives an approximation of how much metabolite

concentrations are affected by local blood flow changes. Recovery is assessed by adding a known concentration of an endogenous, freely diffusible substance to the perfusate, and subsequent measuring the concentration of this substance in the dialysate (153, 173). If the substances are metabolized in the tissue of study, the added substance is radiolabelled to distinguish between endogenous and added substance (179). Ethanol has been standard for measuring recovery and has been validated against other methods of blood flow measurements (179, 180). Recently, urea has been found equally reliable as ethanol and in addition easier to analyze (176, 177, 181).

4.7.1.3 Sampling

To assess tendon healing, microdialysis followed by metabolite analysis were performed in principle as described by Langberg et al. (157). Microdialysis was conducted at the 2-week postoperative control, when the plaster cast and the IPC device were removed. The subjects were instructed not to eat, smoke, or use snuff for at least 1 h before the appointment. The subjects had the option of receiving analgesia in the form of codeine and paracetamol taken orally, in order not to interfere with the local tissue metabolism.

A microdialysis catheter (CMA 71; MDialysis, Solna, Sweden; 100 kDa molecular cutoff, 0.5 mm outer diameter; 30 mm in length) was introduced, under ultrasound guidance, from the lateral distal aspect of the heel into the peritendinous space 2–5 mm ventral to the Achilles tendon. The lateral approach was chosen in order to avoid the healing scar since a slightly medial incision on the Achilles tendon was chosen. The active part of the membrane was placed as close to the rupture site as possible on the injured side and with approximately the same distance, 4 cm, to the calcaneal bone on the contralateral side.



Figure 11. The microdialysis catheter introduced, under ultrasound guidance, into the peritendinous space ventral to the Achilles tendon.

The subject was lying prone on a gurney during the entire microdialysis investigation in order not to alter the position of, or damage, the catheter. A perfusion fluid was pumped (CMA 107; MDialysis AB, Solna, Sweden) through the inner tube of the catheter into the space between the inner tube and the semipermeable catheter membrane, where the exchange between the interstitial fluid and the perfusion took place. The fluid was transmitted from the catheter and was finally collected in vials (Microvial, MDialysis AB, Solna, Sweden). The perfusion speed was set at 1.0 mL/min. The samples were collected every 30 min during 2 h and were instantly analyzed using an ISCUS Clinical Microdialysis Analyzer (MDialysis AB, Solna, Sweden). Because of the lingering effects of the insertion trauma and the possible differences in fluid pump adjustment during the first few minutes, the first of the four vials was not considered reliable and thus analyzed but excluded from the calculations. To investigate the instantaneous effects of IPC, the treatment group had IPC applied bilaterally during the microdialysis examination as well.

4.7.1.4 Analysis

The substances analyzed were the metabolites lactate, glucose, pyruvate, and glycerol, and the neurotransmitter glutamate. The ISCUS Analyzer was calibrated before every analysis using a commercially available set of reagents (Reagent Set B, MDialysis AB, Solna, Sweden). The greatest advantage of this type of analysis is that it can be performed instantly, thus eliminating the risk of confounding factors, e.g., associated with degradation of metabolites during storing and freezing of the samples. No calculations of relative recovery were made because of reported unequal results using different methods of assessing recovery (180). A prior study, however, with similar setup demonstrated a recovery of 48–62% (range) at rest and 70–76% during exercise for glycerol and glucose (157).



Figure 12. ISCUS Clinical Microdialysis Analyzer was used for sample analysis, published with courtesy by MDialysis AB.

5 STATISTICAL METHODS

The descriptive statistics and statistical analyses were conducted with SPSS, version 19.0 (Study II, III and IV) or version 22.0 (Study I) (SPSS Inc., Chicago, Illinois). All variables were summarized with standard descriptive statistics such as frequencies, means and standard deviations. The significance level in all analyses was 5 percent (two-tailed).

Study I

The Limb symmetry index (LSI) was defined as the ration between the involved limb and the uninvolved limb expressed as a percentage ($\text{involved/uninvolved} \times 100 = \text{LSI}$). Provided that the distribution of a variable was not severely skewed differences between groups were analyzed with oneway analysis of variance (ANOVA) or Student's t-test (for two-groups comparisons). If a variable was severely skewed, i.e. the skewness statistics was greater than 1.5, Mann-Whitney U test was applied for the two-groups comparison.

The dichotomized outcome measure, ACOS, was entered into a logistic regression analysis (stepwise forward with an inclusion level of 5 %) as the dependent variable with six independent variables or predictors: gender (man= 1 and woman= 2) age (age \leq 40 was set to 0, and age $>$ 40 to 1) smoker (nonsmoker= 0, and current smoker= 1) BMI (BMI \leq 26.0 was set to 0, and BMI $>$ 26.0 to 1) DVT (no DVT= 0, and a DVT= 1) and, finally, pre-injury level of activity (exercise up to 1-2 hours/week= 0, and above= 1). For age, BMI, and pre-injury level of activity the median was used as an unbiased cut-off. The relationships between the dependent and independent variables were expressed as odds ratios (OR) with 95 percent confidence intervals (C.I.). Since the regression analysis was a stepwise forward, only significant contributions to the prediction were entered in the regression equation.

Study II

Categorical variables, including the differences between the IPC and the control group in the occurrence of DVT, were analyzed with Pearson's chi-squared test, or Fisher's exact test if one cell had an expected cell count < 5 . Differences between the groups in continuous variables, such as calf circumference and ankle oedema, were analyzed with independent Student's t-tests, as none of these variables were skewed. The correlation between changing to a second plaster cast and the incidence of DVT at two weeks post-operatively was expressed as a Φ coefficient.

Study III

Our and other recent studies showed the rate of CDU verified DVT after ATR surgery to be 40 % (34-36). Based on earlier studies of IPC, and our own pilot study, we estimated a 60% risk reduction of the incidence of DVT (37). Thus, fifty-four patients per group would be required to detect a difference of 24% in the DVT rate (two-sided type-I error rate = 5%; power = 80%).

All variables were summarized with standard descriptive statistics such as mean, standard deviation, and frequency using SPSS v19.0 software (SPSS Inc., Chicago, Illinois). Categorical variables, e.g. differences between the IPC- and control group in occurrence of DVT, were analyzed with Pearson's χ^2 -test or Fisher's exact test if one cell had an expected cell count less than 5. Differences in continuous variables, such as age and time to surgery, were scrutinized for severe skewed distributions or outliers. However, no such distributions were found and, thus, these variables were analyzed with Student's t-test. The level of significance was set to less or equal to 0.05 (two-tailed).

Study IV

Differences between the IPC and control group were analyzed with the non-parametric Mann-Whitney U-test, and differences between the injured and intact legs with Wilcoxon matched pairs test. A power analysis ($\alpha = 0.05$, $b = 0.8$) on available data showed that five patients in each group would give significant results. The power was however low, and therefor, 10 extra controls were included. The statistical power allowed detection of only large effect sizes, i.e. ≥ 0.80 . Thus, trends at the 10% level were also commented and denoted 'almost significant' to compensate for the high risk of type II errors. A post hoc power analysis ($\alpha = 0.05$, $b = 0.8$) for the levels of pyruvate, which in the current study only demonstrated a trend toward significance between the IPC and the control group, demonstrated a need for 10 patients in each group.

6 RESULTS AND DISCUSSION

6.1 PREDICTORS OF OUTCOME AFTER ATR

We sought to assess predictors of outcome in ATR patients using a prospective cohort study design. Prospectively a total of 111 patients (84 men, 16 women; mean age 40.3 ± 8.4) with operated acute total ATR were assessed at one year post-operatively using validated outcome measures. A uniform outcome score, Achilles Combined Outcome Score (ACOS), was obtained by combining three validated, independent, outcome measures: Achilles tendon Total Rupture Score, heel-rise height test, and limb symmetry heel-rise height. A multiple logistic regression analysis was performed based on the dichotomized ACOS (dACOS) assigned 1, good outcome or 0, poor outcome. Multiple logistic regression analysis demonstrated that three independent variables correlated significantly and independently with dACOS at one year; age, gender and the occurrence of deep venous thrombosis at two weeks post-operatively. Thus, age ≥ 40 years (1), was the strongest independent predictor of developing a poor outcome (dACOS=0) at one year after ATR (Table 1). Male gender (2) was the second most important factor for obtaining a poor outcome. Notably, exhibiting a DVT (3) during the post-operative immobilization was also an important independent predictor for a poor outcome after ATR (dACOS=0). BMI, PAS and smoking did not significantly predict the outcome after ATR as assessed with dACOS.

Table 1. Relationship between gender, age, deep venous thrombosis, and outcome

<i>Independent variable</i>	<i>B</i>	<i>S.E.</i>	<i>Df</i>	<i>p</i>	<i>OR²</i>	<i>95% C. I.¹ for OR²</i>	
						<i>Lower</i>	<i>Upper</i>
Gender (Man = 1; Woman = 2)	1.43	0.724	1	0.048	4.18	1.01	17.24
Age (40 or less = 0; above 40 = 1)	-1.59	0.462	1	0.001	0.20	0.08	0.51
Deep venous thrombosis after two weeks? (No = 0, Yes = 1)	-1.19	0.491	1	0.016	0.31	0.12	0.80
Constant	-0.36	0.784					

¹ C.I. = Confidence Interval

² OR = Odds Ratio

6.1.1 Age

The strongest predictor of functional outcome and symptoms at one year was age. Patients aged over 40 years demonstrated a limb symmetry index (LSI) of total concentric work of 58% compared to patients aged below 40 that reached 71% ($p < 0.001$). Maximum heel-rise height was 9.73 cm in the older patient group and 11.51 cm in the younger patients ($p < 0.001$). PAS differed significantly between the two age groups before injury ($p = 0.047$), while the difference was not significant after injury ($p = 0.157$). BMI and patient-reported outcome, ATRS and EQ-5D, however, did not differ between the two age groups (Table 2).

Table 2. Outcome of ATR patients, which are younger (aged ≤ 40 years) compared with older patients (aged >40 years) at one year post-operatively.

Variable	Age					
	≤ 40 (n=63)		> 40 (n=48)		p-value	% Difference
	Mean	SD	Mean	SD		
Heel-rise concentric work (LSI)	71%	17.2	58%	22.4	<0.001	22%
Heel-rise height injured side (cm)	11,51	2.2	9,73	2.6	<0.001	18%
Heel-rise height (LSI)	86%	14.3	74%	14.9	<0.001	16%
Heel-rise concentric power (LSI)	87%	19.0	76%	22.6	0.006	14%
PAS postinjury	3.90	1.2	3.56	1.2	0.157	10%
Heel-rise Repetitions (LSI)	84%	18.1	77%	19.9	0.061	9%
PAS preinjury	4.75	0.9	4.43	0.8	0.047	7%
ATRS	77,55	19.0	77,02	18.9	0.885	1%
BMI	26.9	3.5	26.6	2.9	0.570	1%
EQ-5D	0.917	0.11	0.911	0.10	0.772	1%

LSI=Limb symmetry index; PAS=Physical Activity Scale; ATRS=Achilles tendon Total Rupture Score; EQ-5D=EuroQol Group's questionnaire; BMI=Body Mass Index; SD=Standard deviation; n=number of patients.

The negative outcome associated with aging may have its explanation in both intrinsic and extrinsic factors. Intrinsic factors such as reduced tendon stiffness and resistance to loads has been correlated to increasing age (182). Metabolic interactions in tendon collagen production may be more frequent with increasing age, since high glucose levels and diabetes are more common in elderly (11, 12, 59).

6.1.2 DVT

Patients experiencing a DVT postoperatively exhibited a significantly lower maximum heel-rise height of 9.9 cm as compared to patients without a DVT that reached 11.1 cm ($p = 0.018$). LSI of heel-rise height was significantly different between patients without (84%) and patients with a DVT (75%) ($p = 0.008$). LSI of total concentric work did not reach the recommended levels of 85% in the DVT group (61%), nor the non-DVT group (67%), and the difference between the groups was not significant ($p = 0.164$). ATRS was lower in the DVT group (73.2) than in the healthy group (79.2), but the difference did not reach significance ($p = 0.131$). No other differences were found between the groups (Table 3).

Of the three independent factors associated (age, gender, DVT) with the ATR patient outcome at one year post-operatively, the occurrence of a DVT would be the obvious factor to address in an interventional study to promote the outcome after ATR.

Table 3. Outcome of ATR patients with a DVT compared with patients without a DVT at one year post-operatively.

Variable	DVT					
	No (n=77)		Yes (n=34)		p-value	% Difference
	Mean	SD	Mean	SD		
Heel-rise height injured side (cm)	11.14	2.4	9.89	2.7	0.018	13%
Heel-rise height (LSI)	84%	15.3	75%	15.2	0.008	12%
Heel-rise concentric work (LSI)	67%	17.6	61%	25.6	0.164	10%
ATRS	79.16	18.7	73.21	18.9	0.131	8%
PAS postinjury	3.84	1	3.58	1.1	0.32	7%
Heel-rise concentric power (LSI)	82%	19.7	78%	24.1	0.105	5%
Heel-rise repetitions (LSI)	82%	17.7	78%	22.1	0.356	5%
EQ-5D	0.918	0.1	0.906	0.1	0.604	1%
BMI	26.8	3.4	26.6	2.9	0.778	1%
PAS preinjury	4.6	0.9	4.63	0.8	0.877	-1%

LSI=Limb symmetry index; PAS=Physical Activity Scale; ATRS=Achilles tendon Total Rupture Score; EQ-5D=EuroQol Group's questionnaire; BMI=Body Mass Index; SD=Standard deviation; n=number of patients.

6.1.3 Gender

Patients with male gender exhibited a tendency towards lower LSI of concentric power 81% compared to women who reached 91% ($p = 0.099$). Maximum heel-rise height was 10.6 cm among men and 11.5 cm among women ($p = 0.251$). However, since the BMI in men was higher than in women (27 vs 24) ($p < 0.001$) the LSI of total concentric work (65% vs 71%) did not differ significantly ($p = 0.265$). Interestingly, the difference in PAS between men and women (3.67 vs 4.27) was larger post-injury 16% compared to pre-injury 7% (4.56 vs 4.87).

Magnusson et al. reported that women exhibit less tendon hypertrophy and collagen synthesis in response to training (183), compared to males, findings that are supported by other studies (184, 185).

Our results are in contrast to earlier studies that have found better functional outcome in males, based on the ability to perform a single heel-rise (186). However, contradictory results made the same research group exclude females in further studies on outcome predictors (57). Assessment of outcome predictors made by Bostick et al. showed that female gender was a positive predictor of return of calf muscle endurance after ATR (60).

The higher BMI, 27.2, in male patients suggests that overweight may have reduced early mobilization. Our data at one year post-operatively indicated a tendency towards that female patients were more physically active than males. An increased BMI has been associated with a greater degree of symptoms (57), which may hamper early mobilization. Although neither

BMI, nor physical activity, were found to be independent outcome variables after ATR rupture, these parameters may be important underlying factors affecting gender differences.

6.2 INTERVENTION WITH IPC TO PROMOTE OUTCOME

Having established DVT as an important, independent factor for the outcome after ATR further studies aimed at reducing the risk of DVT during the post-operative immobilization after ATR. Since, pharmacological prophylaxis earlier showed low or no DVT-preventive effect further studies focus on mechanical prophylaxis of DVT during leg immobilization.

6.2.1 DVT incidence during IPC under plaster cast immobilization

The study aimed to evaluate a mechanical compression prophylaxis during leg immobilization using foot-IPC under plaster cast. A new foot cuff for under plaster cast application use was marketed by the company (Ortofix Vascular Novamedix, Andover, UK), however without any previous clinical research. In a prospective randomized trial of 26 included, operated ATR patients we hypothesized that adjuvant foot-IPC during post-operative outpatient lower limb immobilization for two weeks could reduce the incidence of DVT.

Screening for DVT with CDS showed very high rates of DVT but no significant differences between the IPC group (9 of 12, 75% DVT) and the plaster cast group (6 of 12, 50% DVT) ($p = 0.18$) (Table 4). Of 15 diagnosed DVTs, 11 were already diagnosed by the two-week examination. Four new DVTs were detected at six weeks. Of the 11 DVTs diagnosed at two weeks, two (13%) located in muscle veins had disappeared by the six-week examination. None of the DVTs detected were proximal.

Table 4. Incidence of deep venous thrombosis (DVT) adjuvant foot-IPC and controls.

Outcome	Total (n=24)	Adjuvant IPC (n= 12)	Control group (n=12)	p-value*
DVTs at 2 weeks	11 (46)	6 (50)	5 (42)	0.33
DVTs at 6 weeks	13 (54)	8 (67)	5 (42)	0.08
DVTs at 2+6 weeks	15 (63)	9 (75)	6 (50)	0.18

*chi-squared test

6.2.1.1 Device complications

At two weeks post-operatively seven of 12 patients in the IPC group complained of malfunction of the IPC device, predominantly because it produced pressures that were too low, caused by less back-pressure within the plaster cast. This suggests that the free space within the plaster cast had expanded due to calf muscle atrophy and/or reduced swelling. For this reason, four patients in the IPC group received a second plaster cast, five to ten days after the first cast, but not exceeding two weeks of cast immobilization. This change to a second plaster cast correlated significantly with an increased risk of DVT at two weeks post-operatively ($\phi = 0.71$; $p = 0.019$), whereas malfunctioning of the IPC device showed a tendency to correlate with increased DVT risk ($\phi = 0.51$; $p = 0.079$). Due to these

complications the study was ended prematurely making it underpowered to answer the question regarding a difference in DVT rate. Based on the detected differences in frequency of DVT between the groups, 120 patients would have been required to permit a statistically significant difference to be detected.

These findings suggest that multiple casting during leg immobilization may be associated with increased risk of DVT, possibly due to the fact that a fresh plaster cast allows less movement and thus reduces circulation. A more rigid immobilization is known to increase the risk of DVT (187). This observation requires validation by more extensive clinical studies.

The frequency of DVT found in this study (63%; 15 of 24) is higher than the previously reported 36% in other studies on DVT after surgical treatment of acute ATR (34, 35). The reasons for this discrepancy could be diverse patient populations, treatment dissimilarities and differences in outcome measurements. The characteristics of our population did not differ significantly from those of the two earlier studies. However, half of the patients in this study received a new untested adjuvant foot IPC cuff under the plaster cast, which was vitiated with device complications. When looking at DVT rates in our control group, receiving a plaster cast in 30° of plantar flexion, half the patients experienced a DVT. These numbers are closer to the frequency of DVT, 40%, as earlier detected by CDS (34). It is known that early CDS can diagnose occasional DVTs that may disappear when examined at a later date, which was in fact the case in our study. At two weeks we detected 73% of all the DVTs at both two- and six weeks, whereas in the study by Lapidus et al (1), CDS performed at three weeks diagnosed 65% of all the DVTs in that study. At the second examination time-point at six weeks, the diagnosed values were similar in our study as compared to the study by Lapidus.

The results of this study imply that the plastic foot IPC cuff developed for beneath plaster cast use is not a viable solution for the prevention of DVT in an outpatient setting, owing to a high degree of malfunction. Another possibility to immobilize patients post-operatively would be with the use of an orthosis. This is a more flexible solution, which allows individual and adaptable change of immobilization, as well as allows for early mobilization.

6.2.2 DVT incidence during IPC under orthosis immobilization

In consequence of the results of the earlier study the following first pilot study established a functioning mechanical compression prophylaxis during leg immobilization using calf IPC under a walker orthosis. Thereafter, in a prospective randomized trial of 150 included, operated ATR patients we hypothesized that adjuvant IPC during post-operative outpatient lower limb immobilization could reduce the incidence of DVT.

6.2.2.1 DVT incidence during IPC intervention

Screening with compression duplex ultrasound (CDU) at two weeks post-operatively demonstrated a DVT in 23% (n= 16/69) in the intention-to-treat analysis and 21 % (n=14/67) in the per-protocol analysis of the patients in the IPC group and in 37% (n= 26/71) of the patients in the control group (Table 2). The patients compliant with the IPC-treatment exhibited a significant reduction in the risk of DVT, after correction for age differences

between groups (ITT analysis: OR= 2.11; 95% C. I. = 0.97-4.62; p=0.061 and PP analysis: OR=2.60; 95% C.I. 1.15-5.91; p=0.022) (Table 5). No proximal DVTs and no clinical PEs were diagnosed.

Table 5. Incidence of deep venous thrombosis (DVT).

Outcome (incidence)	Adjuvant IPC		ITT vs. C		PP vs. C
	ITT*	PP†	Control (C)	p-value	p-value ^a
DVTs at two weeks n (%)	16 (23)	14 (21)	26 (37)	0.083	0.042
DVTs at six weeks n (%)	36 (52)	34 (51)	34 (48)	0.612	0.737

IPC: intermittent pneumatic compression; DVT: deep vein thrombosis.

* ITT = Intention To Treat, i.e. all randomised patients.

† PP = Per-Protocol. Two patients were withdrawn due to non-compliance regarding IPC –usage. Both patients had developed a DVT at the two weeks follow-up.

The significantly reduced incidence of DVT of the IPC group indicates that IPC may prevent the development of DVT during immobilization. This study establishes patient-administered adjuvant calf IPC in an outpatient setting as a feasible method of DVT-prevention after ATR surgery.

The reduction in DVT observed at two weeks in the IPC group is probably related to reduced venous stasis produced by the intermittent mechanical calf compression. IPC has thus been shown to increase venous peak flow velocity by > 200 (105, 188). The reduced incidence of DVT may also be related to effects on intrinsic fibrinolysis observed after IPC. Hence, cyclical tissue shear stress induces the production of chemical substances: antithrombotic tissue factor pathway inhibitor and pro-fibrinolytic substance tissue plasminogen activator (188). In contrast, immobilization in equinus position and a non-weight-bearing regimen have been shown to reduce the venous blood flow significantly compared to full weight-bearing in a neutral cast or neutral pneumatic boot (80).

6.2.2.2 DVT incidence after cessation of IPC intervention

After ending the IPC intervention at week 2 and subsequent orthosis immobilization in both groups, CDS-screening at six weeks post-operatively showed a DVT in 52% (n= 36/69) of the patients in the IPC group and in 48% of (n= 34/71) the patients in the control group (OR=0.94; 95 % C.I. 0.49-1.83). One proximal DVT was detected in the control group. The patient had a DVT in vena fibularis at 2 weeks and was treated with LMWH but still had a progression of the DVT. No clinical signs of PE were observed in any of the patients.

Hence, after the end of the IPC intervention at week two, the DVT incidence at six weeks post-operatively was similar in the two groups, around 50%. This suggests that the DVT preventive effect of the IPC therapy does not persist after cessation of treatment when continued immobilization is applied. Therefore, the DVT frequency at six weeks additionally

indicates that the IPC therapy should be applied and scientifically studied during the whole time of post-operative lower limb immobilization.

From the current study, we cannot fully discriminate to what degree the DVT preventive effect is related to calf IPC or to orthosis mobilization, respectively. However, our data at six weeks, showing increased number of DVTs in the treatment group after the IPC intervention was concluded and all patients were treated with orthosis immobilization, suggest that the major DVT preventive effect is related to the IPC therapy.

The correlation analysis of risk factors demonstrated that leg-immobilized patients aged > 39 years exhibited an almost fivefold increased risk of DVT. Other risk factors, such as BMI, smoking, time to and time in surgery, did not significantly affect the risk of DVT in this study. Increased age is therefore a strong risk factor, as is also stated in many guidelines, e.g. by NICE, UK (102, 189). This study therefore suggests that leg-immobilized patients aged > 39 years, could be considered for prophylactic measures against DVT following ATR surgery.

6.2.3 Metabolic healing activity during IPC therapy

Adequate circulation is a healing prerequisite, and IPC has recently been demonstrated to improve soft tissue repair and experimental Achilles tendon repair (112, 115, 116). Essential metabolites glucose, lactate, pyruvate and glycerol and neuronal transmitters, including glutamate, have been identified in the healing tendon and are regarded to be involved in the repair process (137, 190). In the present study, we hypothesized that vital metabolite concentrations would be elevated during early human Achilles tendon repair, and that adjuvant IPC could further stimulate metabolic activity.

Twenty patients were included in this prospective cohort study. The IPC group and the plaster cast group exhibited no demographical differences - except for a mean 80 h usage of adjuvant IPC in the treatment group during the first two weeks postoperatively.

6.2.3.1 Healing vs intact contralateral tendons in the plaster cast group

The healing Achilles tendons exhibited at 2 weeks post-operatively significantly higher levels of glutamate (59.8 ± 13.9 vs 19.9 ± 11.0 mM; $P = 0.003$), lactate (1.2 ± 0.6 vs 0.6 ± 0.4 mM; $P = 0.007$), and pyruvate (80.6 ± 29.1 vs 34.7 ± 25.1 mM; $P = 0.001$) compared to the contralateral intact tendons. No significant differences were found in levels of glucose ($P = 0.807$), glycerol ($P = 0.345$), or in the lactate/pyruvate ratio ($P = 0.307$).

The observed findings suggest that Achilles tendon healing is associated with significantly elevated metabolic activity. Glutamate exhibited the highest (threefold) up-regulation suggesting the most central role in Achilles tendon repair. The elevation of glutamate concentrations in the healing tendons of the control group is in agreement with earlier experimental studies on Achilles tendon repair (147, 190). Glutamate has been suggested to potentiate tissue repair by stimulating cellular proliferation.

6.2.3.2 Healing vs intact contralateral tendons in the IPC group

Metabolite upregulations were observed in the healing Achilles tendons of the IPC-treated group as compared to the intact, contralateral tendons. Thus, significantly higher concentrations of glutamate (84.4 ± 14.7 vs 16.0 ± 9.1 mM; $P = 0.043$), glucose (3.4 ± 0.6 vs 2.5 ± 1.1 mM; $P = 0.043$), lactate (1.8 ± 0.6 vs 0.8 ± 0.3 mM; $P = 0.043$), and pyruvate (112.7 ± 33.0 vs 39.9 ± 19.4 mM; $P = 0.043$) were found.

The data suggest an upregulation of all metabolites except glycerol. Again, glutamate demonstrated the highest upregulation after IPC treatment. IPC treatment produces shear stress upon the endothelial walls of both intact and injured regenerating vessels. Vascular shear stress gives rise to a multitude of reactions, including synthesis of nitric oxide (NO), a potent vasodilator assumed to stimulate angiogenesis, accelerated tissue healing and improved tissue quality (105).

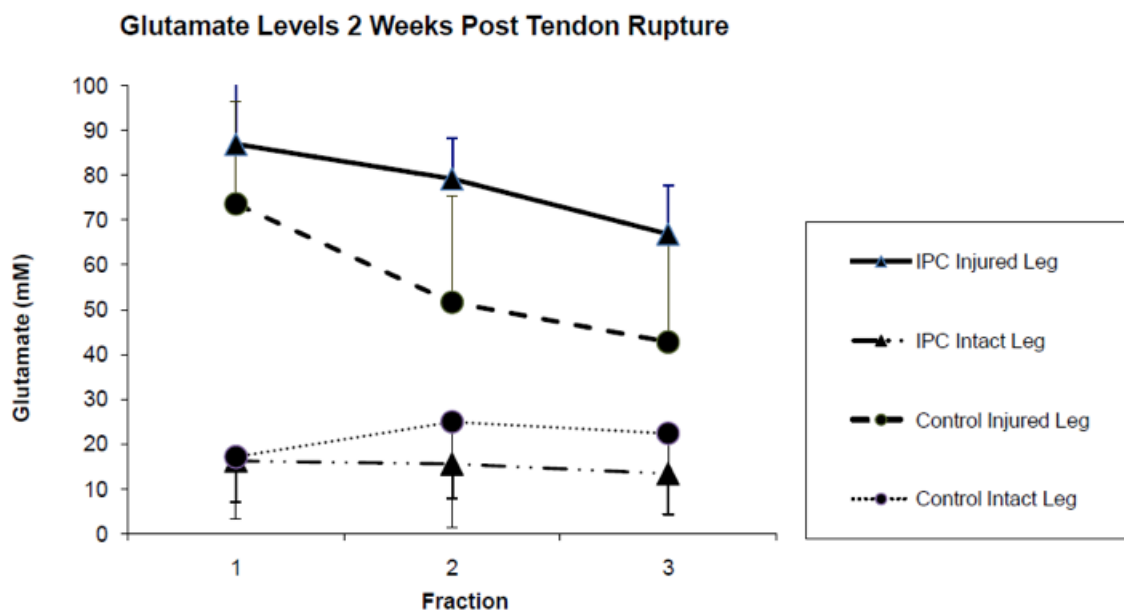


Figure 13: Glutamate concentrations from microdialysis probes placed peritendinously ventral to the ruptured and contralateral intact Achilles tendons in five patients with adjuvant intermittent pneumatic compression and in 15 patients serving as controls. Glutamate was the metabolite exhibiting the highest increase in concentrations both during healing and after IPC treatment. There were significantly higher levels in the healing tendons of the IPC group compared to the healing tendons of the control group.

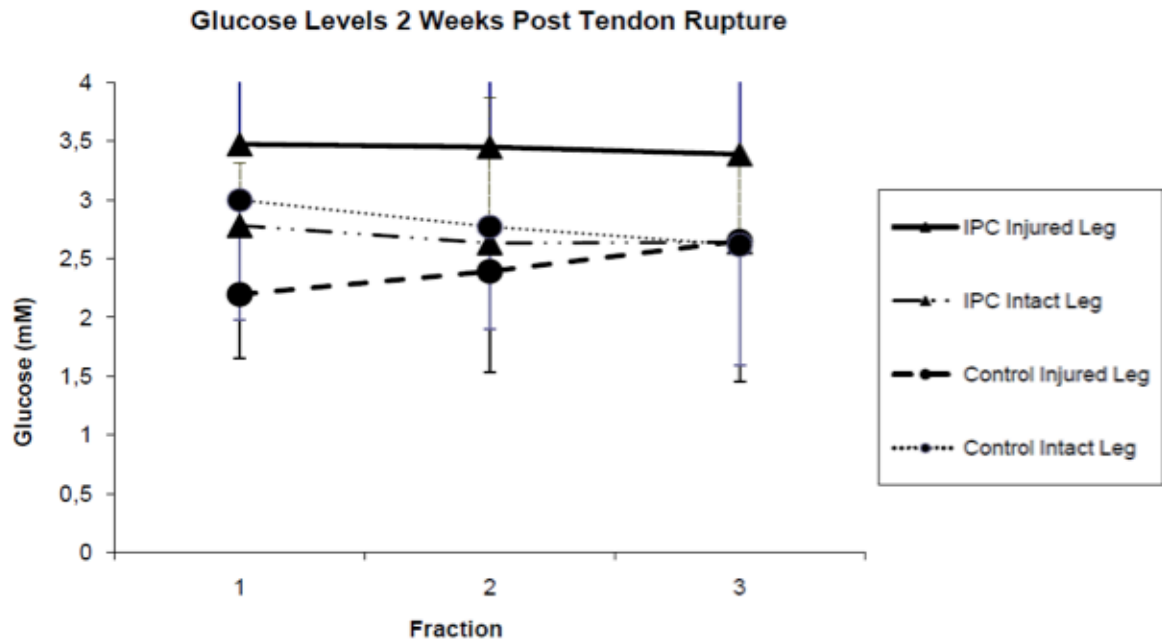


Figure 14: Glucose concentrations from microdialysis probes placed peritendinously ventral to the ruptured and contralateral intact Achilles tendon in five patients with adjuvant intermittent pneumatic compression and in 15 patients serving as controls. The glucose levels displayed a significant increase in the healing tendons after IPC treatment as compared to the healing tendons of the control group.

6.2.3.3 IPC vs plaster cast group: healing tendons

Comparing the healing tendons of the IPC group and the control group the levels of glutamate (84.5 ± 14.7 vs 62.4 ± 16.0 mM; $P=0.035$) and glucose (3.4 ± 0.6 vs 2.6 ± 0.7 mM; $P=0.04$) were significantly higher in the IPC group as compared to the control group (Figure 13 and 14). There were also almost significant trends toward increased levels of pyruvate, lactate and glycerol, but no such trend regarding the lactate/pyruvate ratio ($P=0.76$) (Table 6).

Table 6. Metabolite levels in healing tendons comparing the IPC-group and controls.

HEALING TENDONS	IPC-group			Control-group					
	<i>n</i>	<i>M</i>	<i>SD</i>	<i>n</i>	<i>M</i>	<i>SD</i>	<i>z</i>	<i>p</i>	% Change
Lactate (mM)	5	1,79	0,56	14	1,17	0,58	1,85	0,064	53
Pyruvate (μM)	5	112,7	33,04	15	80,6	29,10	1,70	0,089	40
Glutamate (mM)	5	84,4	14,71	12	62,4	16,00	2,11	0,035	35
Glucose (mM)	5	3,44	0,62	15	2,62	0,72	2,05	0,040	31
Glycerol (μM)	4	83,3	6,60	15	77,6	46,69	1,90	0,057	7
L/P *1000	5	16,4	4,46	15	20,9	14,95	0,31	0,760	-22

The increased glutamate concentration obtained may be related to increased nerve ingrowth and neurotransmitter release. This observation has earlier been experimentally demonstrated when IPC improved cellular amino acid metabolism in the tendon (116). The upregulated glutamate levels may stimulate proliferation of endothelial cells on blood vessels and of tenocytes to promote angiogenesis and tendon repair (116, 190).

6.2.3.4 IPC vs plaster cast group: intact tendons

Metabolite levels of the intact tendons in the IPC group and the control group showed no significant differences in the metabolite levels. This indicates that the instantaneous effect of IPC on blood flow did not increase the relative recovery of metabolites.

The study demonstrates specific increases in metabolite concentrations during early Achilles tendon repair, which may indicate a central role in cellular proliferation when energy demands are elevated. Elevated levels of metabolites glutamate and glucose imply activated amino acid and carbohydrate metabolic pathways as a result of adjuvant IPC.

The observed increased peritendinous levels of glutamate, lactate and pyruvate in the healing tendons has in a previous study been demonstrated to correlate with corresponding concentrations within the Achilles tendon proper (191). The elevation of glutamate concentrations in the healing tendons of the control group is in agreement with earlier experimental studies on Achilles tendon repair (137, 190). Glutamate has been suggested to potentiate tissue repair by stimulating cellular proliferation.

Adjuvant compression treatment during postoperative plaster immobilization has the ability to additionally elevate the concentration of essential metabolites at the repair site. IPC has been suggested to promote both fracture- and soft tissue repair by enhancing callus formation and biomechanical strength (112, 115, 116). Further studies on metabolic activity should be combined with assessments of tendon repair.

7 LIMITATIONS

7.1 GENERAL CONSIDERATIONS

A clinical randomized controlled trial requires a well-defined and precise study protocol. Conducting the studies, the following limitations have been observed:

- Several exclusion criteria were stated to reduce the risk of selection bias. Additionally, oral contraceptives and earlier thrombotic events (not only the last three months) would further have contributed to reduced selection bias.
- Attempts to differentiate between symptomatic and asymptomatic DVT was made by asking the patients for symptoms when performing CDU. A pre-defined definition of symptomatic DVT and PE would perhaps have made it possible to estimate the proportion of symptomatic VTE events.
- The control group was immobilized in 30° equinus plaster cast and non-weight-bearing during two weeks, which was routinely used at our hospital. Since increasing equinus position and non—weight-bearing is believed to be associated with increased DVT risk, this may not be gold standard at other hospitals and the data less generalizable. However, other hospitals apply longer immobilization periods than 6 weeks, which is used in our hospital, especially in combination with non-operative ATR treatment. Such longer immobilization time may even further increase the risk of DVT.
- After ending the immobilization at six weeks post-operatively, patients were referred to physiotherapists outside the hospital for continued rehabilitation. A standardized rehabilitation protocol, preferably conducted within the hospital, would have reduced the risk of diverse rehabilitation protocols, which may have an effect on outcome.

7.2 SPECIFIC CONSIDERATIONS REGARDING STUDY I-IV

7.2.1.1 *Study I*

There is a potential limitation in Study I due to patients lost to follow-up. Out of 150 patients, 9 patients were excluded and 2 were affected by a re-rupture. Hence, out of 139 patients, 28 (20%) did not come to the scheduled appointment. However, a later follow-up was performed in order to find out whether the characteristics of these absent patients deviated from the others. Our preliminary results indicate that the patients lost to follow-up would not alter the overall conclusions of the study.

7.2.1.2 *Study II*

The aim in study II was to assess the efficacy of IPC with a new foot cuff beneath plaster cast. Due to device complications the study was ended prematurely. The study demonstrated that the new cuff produced for under plaster cast use was not viable in an outpatient setting

during prolonged immobilization. However, we cannot draw any conclusions about the inpatient use of the new cuff during a short time-period. On the whole, we can conclude that new applications, although used within a well-accepted device, always should be tested scientifically before commercialized.

7.2.1.3 Study III

Of the eligible patients a larger part was not included in the study. Recording of eligible, not included patients would have been desirable to reveal if patient characteristics deviated from included patients.

In this study the IPC group had both IPC and orthosis immobilization as different factors of intervention compared with the plaster cast group. However, the results at six weeks post-operatively, when the IPC therapy was ended and patients were immobilized in orthosis, showed equally high DVT incidence in the two groups, around 50%. This suggests that the DVT preventive effect of the IPC therapy does not persist after cessation of treatment when continued immobilization is applied. Neither does weight-bearing in orthosis alone seem to have a DVT-preventive effect.

7.2.1.4 Study IV

One limitation of this study is that only five patients received the IPC treatment, while 15 control patients were treated with plaster cast. Moreover, the relative recovery, i.e. percentage of substance yielded from the microdialysis, as compared to actual tissue concentration was not assessed. This means that the intervention with IPC may have increased the blood flow, which may have caused increased recovery of metabolites, but not for certain elevated their true tissue concentrations. However, our data on the intact limbs that received IPC during 2 hours of microdialysis demonstrated no increase in metabolite levels compared to intact limbs that received no IPC.

8 CONCLUSIONS

The aim of the present thesis was to assess potential predictors of impaired outcome after acute Achilles tendon rupture surgery, and to determine whether interventions, i.e. adjuvant IPC, targeted to the predictors could reduce complications and promote healing and function.

This thesis concludes that postoperative deep venous thrombosis (DVT) during leg immobilization, aging and male gender are independent predictive factors for poor outcome of patients at one-year post Achilles tendon rupture (ATR). These predictors provide the clinician with knowledge that will lead the way towards a more individualized and evidence-based decision when treating ATR patients. Age and gender should be further studied as to understand the underlying molecular causes leading to negative outcome. The results further highlight that risk factor assessment is important in order to address them in a targeted manner, eg. by individualized treatment protocols.

Establishing DVT as a predictor of poor outcome and a very common complication during leg immobilization after ATR warrants that the primary focus to improve outcome after ATR should be to prevent DVT. However, since DVT-preventive effect with pharmacological prophylaxis has no or low effect in ATR patients, an alternative strategy could be the usage of mechanical prophylaxis.

Assessment of DVT incidence after using foot-IPC beneath plaster cast as compared to plaster cast only demonstrated no significant DVT reduction. However, frequent malfunctioning of the new foot cuff device for under plaster cast usage implied that this device is not a viable solution for the prevention of DVT.

A novel approach to combine calf IPC and orthosis immobilization was developed. Adjuvant calf IPC during orthosis immobilization reduced the DVT incidence by almost 50% compared to plaster cast only. This thesis implies that mechanical IPC treatment may be a viable and effective prophylactic treatment against DVT, also in an outpatient setting.

Moreover, IPC can enhance the metabolic response during early tendon healing. Adjuvant IPC produced increased concentrations of, for tendon healing essential metabolites, glutamate and glucose, which may reflect an upregulation of the amino acid/neurotransmitter and carbon hydrate metabolism. Whether adjuvant intermittent pneumatic compression is beneficial for tendon repair should be further investigated.

9 CLINICAL IMPLICATIONS

Trauma, orthopaedic surgery and lower limb immobilization are not without complications. The most common and feared complication is venous thromboembolism resulting in deep vein thrombosis (DVT) and/or pulmonary embolism (PE). Another common complication includes failed or delayed healing, often leading to prolonged rehabilitation time and suboptimal functional outcome.

From the present studies it seems obvious that patients with lower limb immobilization after acute Achilles tendon rupture (ATR), represent a diverse patient population, which is affected by high incidence of DVT, and poor- and variable outcome. Therefore, the increased knowledge acquired about predictors of outcome will give evidence-based guidance to both patients and clinicians in deciding a treatment program.

Patients at age over 40 years and of male gender, which are at risk of poor outcome after ATR, should presumably be prescribed supervised post-operative rehabilitation to optimize enhancement of the healing process. Such rehabilitation protocols as well as the underlying mechanisms leading to poor outcome with aging and male gender should be further scientifically evaluated.

The studies of this thesis clearly demonstrate that interventions to prevent the development of DVT should be the first clinical focus to reduce complications and to promote the outcome after ATR. Pharmacological prophylaxis with LMWH is still widely used although earlier studies have shown that LMWH does not reduce the risk of DVT during leg immobilization after ATR. Moreover, it has been speculated that LMWH may impair tendon healing. Therefore the use of pharmacological prophylaxis during leg immobilization after ATR seems contradictory.

From our studies it is instead apparent that mechanical thromboprophylaxis using IPC in outpatients is an effective method of reducing the risk of DVT during leg immobilization. Our studies moreover demonstrate that patients with an ultrasound verified DVT, although most DVTs are asymptomatic, exhibit an impaired outcome after ATR. Reduced blood circulation may be a common denominator to DVT and poor healing. Therefore it is our belief that increasing the blood circulation with IPC, e.g. reducing the risk of DVT, would also improve patient outcome as indicated by enhanced metabolism owing to IPC.

Regarding the treatment protocol for IPC we recommend based on our studies, using IPC at least six hours daily underneath an orthosis, during the entire leg immobilization period. According to our experience patient education has an essential role to achieve high compliance in an outpatient setting. Moreover, technical advancements such as battery powered IPC devices as well as lighter and more silent models will presumably further facilitate usage.

Our results of IPC usage during leg immobilization may be generalized to non-surgically treated Achilles tendon rupture patients since here the risk of DVT is equally high. Currently,

the proportion of non-surgically treated patients is increasing and treatment protocols are often implying a prolonged plaster casting. The DVT preventive intervention using IPC during lower limb immobilization could possibly also be applied to all lower leg immobilized patients with increased risk of DVT.

Based on the results from our studies and international guidelines, eg. NICE, UK, we recommend that all lower limb immobilized patients undergo stratification of risk for DVT. Our data demonstrating that patients aged 40 years and older exhibited an almost fivefold increased risk of DVT, should be borne in mind when considering a patient's thromboprophylaxis.

The American College of Chest Physicians recommend no pharmacological prophylaxis today to lower limb immobilized patients, based on the low/no efficacy of LMWH. Our studies demonstrate that mechanical DVT-prophylaxis is a viable alternative in selected leg immobilized outpatients. A cost-benefit analysis of IPC therapy is warranted. However, the almost 50% DVT reduction owing to IPC and the lack of other options suggest mechanical compression as a recommendable method of thromboprophylaxis.

In order to prevent complications and improve patient outcome after trauma, orthopaedic surgery and lower limb immobilization IPC may prove to become a widely established adjuvant treatment method. Other parallel approaches should be further explored. Early mobilization is established as an essential part the healing process and further studies on early mobilization protocols, tailor-made for patients at risk, are desirable. Improved orthotic devices, which will allow earlier mobility, loading and improved circulation should be evaluated.

10 ACKNOWLEDGEMENTS

I would like to thank all of you that have contributed and helped me making this thesis possible. Especially I would like to express my deepest gratitude to:

Paul Ackermann, associate professor, my supervisor and friend, for endless support and enthusiasm and by brilliant analyses lifting our research to a higher level. Thank you for always being available for questions and discussions, no matter when, and making this a joyful journey.

Per Renström, professor emeritus and co-supervisor, for encouraging my work and sharing your expertise and wisdom, regarding research and life in general.

Gunnar Nilsson, MD, PhD, co-supervisor, for encouragement and being the critical supervisor, questioning my conclusions and bringing new perspectives.

Daniel Bring, MD, PhD, co-supervisor, for encouragement and valuable advice during this journey that you have already completed.

Professor **Ylva Pernow**, my mentor, for support whenever I needed.

Gunnar Edman, MD, PhD, Neurogenetics unit, co-author, for your expertise in statistics and outstanding skillfulness in analyzing research, and never ending patience.

Luigi Belcastro, research nurse, for valuable assistance in the inclusion, randomization and data collection and being a dear friend.

Eva Hardell och **Gunilla Barreng**, physiotherapists and co-authors, for your valuable work with data collection, your enthusiasm and friendship, and professional rehabilitation of the patients.

Fausto Labruto, MD, PhD och **Ali Latifi**, PhD, for invaluable help and expertise in ultrasonography, making the data collection possible and the work in our research team fruitful and fun.

Professor **Lars Weidenhielm**, for generous support and valuable advice, both in scientific matters and life in general, and providing the opportunity to do this work.

Birgitta Majlöv-Björk, nurse at Stockholm Söder Hospital, for invaluable help identifying eligible patients. Without you this thesis would be far from finished.

Per Anundsson and **Katarina Greve**, co-authors that during their medical education showed dedication and talent to fulfill our research project.

Gustaf Neander, MD, PhD, Head of the Department of Orthopaedics, Danderyd Hospital, for always supporting me and giving me the opportunity to finish this thesis.

My former colleagues and staff at the Department of Orthopaedics, Karolinska University Hospital, for valuable help with the treatment of the study participants. This thesis would not have been possible without your support.

All my colleagues and staff at the Department of Orthopaedics, Danderyd Hospital, for encouragement and daily support in clinical work making my days fun and inspiring.

All the participants in this thesis, for spending time on evaluation and giving me your trust.

All my friends, for all great things far from research.

My parents **Erik and Maj-Lis**, for always being there, encouraging and believing in me, sharing your unconditional love, for invaluable help with our children and thereby making my work possible.

My brother **Daniel** with family, with whom we share wonderful moments in life.

My parents-in-law **Ulla and Lars**, for encouragement, love and family support making the “the puzzle” go together.

My wonderful sons **Gustaf, Oscar and Filip**, the stars in my life. Spending time with you brings me energy and happiness.

But most of all to my best friend and beloved husband **Anders**, for constant support (not only in IT issues), for reminding me of what life is really about, and for endless love in difficult and happy times.

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12 ORIGINAL PAPERS I-IV

- I. **Aging, gender and deep venous thrombosis are predictors of functional outcome after acute Achilles tendon rupture.**
E.Domeij-Arverud, P. Anundsson, E Hardell, G. Barreng, G. Edman, P.W. Ackermann.
Manuscript
- II. **Can foot compression under a plaster cast prevent deep-vein thrombosis during lower limb immobilisation?**
E.Domeij-Arverud, A. Latifi, F.Labruto, G. Nilsson, P.W. Ackermann.
Bone Joint J. 2013 Sep;95-B(9):1227-31.
- III. **Intermittent pneumatic compression reduces the risk of DVT during post-operative lower limb immobilisation: A randomized controlled trial of acute Achilles tendon ruptures**
E.Domeij-Arverud, A. Latifi, F.Labruto, G. Nilsson, G. Edman, P.W. Ackermann.
Accepted Bone Joint J 2015;97-B:??-??
- IV. **Metabolic activity in early tendon repair can be enhanced by intermittent pneumatic compression.**
K.Greve*, E. Domeij-Arverud*, F.Labruto, G. Edman, D. Bring, G. Nilsson, P.W. Ackermann
Scand J Med Sci Sports 2012 Aug; 22(4):e55-63