

ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION USING SEMITENDINOSUS AND GRACILIS AUTOGRAFTS

Evaluation of the clinical outcome, radiographic findings, histology and biochemistry

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Anterior cruciate ligament reconstruction using semitendinosus and gracilis autografts

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Cover illustration: (Figure 1b) Image of regenerated ST and G tendons. This is a 25-year-old (at surgery) male patient who underwent ACL reconstruction using an ST/G autograft. Due to pain and snapping from the regenerated tendons, they were reharvested two years and 11 months after the initial harvest. The patient then healed uneventfully without further symptoms.

Graphic design: Annika Samuelsson Enderlein, A little company AB

To my family

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ABSTRACT

The present thesis focuses on different aspects of anterior cruciate ligament (ACL) reconstruction using hamstring tendon (HT) autografts. In Study I, two groups of patients were compared. One group underwent surgery in the sub-acute setting, a median of three months after injury (30 patients), and one group underwent delayed surgery a median of 30 months after the injury (31 patients). At clinical evaluation two years post-operatively, the patients in the sub-acute group had a significantly better clinical outcome in terms of the Tegner activity level and Lysholm knee scoring scale. In Study II, 19 patients underwent examination a minimum of six years after ACL reconstruction using HT autografts. MRI of the operated and the contralateral non-operated knee was performed to investigate the cross-sectional area and insertion site of the regenerated tendons. Furthermore, the patients underwent muscle strength measurements using a Biodex dynamometer. The semitendinosus tendon regenerated in 17 of 19 (89%) of the patients and the gracilis in 18/19 (95%). The tendons regained an almost normal point of insertion at the pes anserinus and a cross-sectional area similar to that of the non-operated contralateral side. There was a significant strength deficit in deep knee flexion but not in internal rotation. In Study III, 18 patients underwent bilateral biopsies under ultrasonographic guidance to investigate whether the regenerated tendon-like tissue seen on MRI in Study II is histologically real tendon tissue and/or

scar tissue. The biopsies revealed tendon tissue similar to the normal tendon, but, in some of the regenerated tendons, “scar tissue formations” were seen. In Study IV, synovial fluid was aspirated from both knees in 11 patients to evaluate inflammatory components and disturbed cartilage metabolism in the long term (eight years) after unilateral ACL injury and reconstruction. The patients underwent bilateral weight-bearing radiographs and bilateral MRI to evaluate degenerative changes and meniscal and cartilage damage. There were no significant differences between injured and non-injured knees in terms of cartilage markers and inflammatory cytokines, but there were significantly more degenerative changes on radiographs and MRI in the reconstructed knees.

Keywords: Anterior cruciate ligament, hamstring tendon regeneration, biopsies, cartilage markers, inflammatory cytokines, MRI, strength measurements

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SAMMANFATTNING PÅ SVENSKA

Syftet med avhandlingen var att undersöka olika faktorer som är av betydelse för patienter som genomgått främre korsbandsoperation med egna hamstringsenor. Delstudie I fokuserar på tidpunkten för operation efter en korsbandsskada. Två grupper av patienter jämfördes. En grupp opererades subakut, median 3 månader efter skada (30 patienter) och en grupp median 30 månader efter skada (31 patienter). Vid klinisk utvärdering 2 år efter operation hade patienterna i den subakut opererade gruppen signifikant bättre utfall vad det gäller Tegnors aktivitets nivå och Lysholms knä score. I delstudie II genomgick 19 patienter som opererats minimum 6 år tidigare för främre korsbandsskada med semitendinosus- och gracilissena MR undersökning och styrkemätning i en Biodex maskin. Både det opererade och icke opererade knät undersöktes. MR visade att semitendinosussen återbildats hos 17 av 19 (89%) och gracilis hos 18/19 (95%). Senorna återfick en nära nog normal infästning på skenbenet och en tvärsnittsarea liknande de normala senorna på den icke opererade sidan. Patienterna hade en signifikant kraftnedsättning i djup knäböjning men inte i inätrotation. I delstudie III, togs ultraljudsledda biopsier från återbildad sena och jämfördes med normal semitendinosussen för att undersöka om den återbildade "senlika" vävnaden som sågs på MR undersökningarna i studie II histologiskt är senvävnad eller ärrvävnad. Biopsierna visade att det är senvävnad med en histologisk bild som liknar normal semitendinosussen men i tre av de

återbildade senorna sågs fokala områden med mer ärrlik fiberstruktur. I delstudie IV togs ledvätska från båda knäna hos 11 unilateralt korsbandsrekonstruerade patienter för att undersöka om nivåerna av inflammatoriska cytokiner samt biomarkörer för broskomsättning var förhöjda i det korsbandsopererade knät. Åtta år efter korsbandsrekonstruktion var cytokiner och broskmarkörer ej signifikant förhöjda jämfört med patienternas icke skadade knä, men det fanns signifikant mer degenerativa förändringar på röntgen samt mer brosk och meniskförändringar på MR i det opererade knät.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. A comparison of the clinical outcome after anterior cruciate ligament reconstruction using a hamstring tendon autograft with special emphasis on the timing of the reconstruction.**
Åhlen M, Liden M
Knee Surg Sports Traumatol Arthrosc. Mar 2011;19(3):488-494

- II. Bilateral magnetic resonance imaging and functional assessment of the semitendinosus and gracilis tendons a minimum of 6 years after ipsilateral harvest for anterior cruciate ligament reconstruction.**
Åhlen M, Liden M, Bovaller A, Sernert N, Kartus J
Am J Sports Med. Aug 2012;40(8):1735-1741

- III. Histological evaluation of regenerated semitendinosus tendon a minimum of 6 years after harvest for anterior cruciate ligament reconstruction.**
Åhlén M, Lidén M, Movin T, Papadogiannakis N, Rostgård-Christensen L, Kartus J
Orthopaedic Journal of Sports Medicine 2014 2: DOI: 10.1177/2325967114550274

- IV. Inflammatory cytokines and biomarkers of cartilage metabolism eight years after anterior cruciate ligament reconstruction from operated and contralateral knees.**
Åhlén M, Roshani L, Lidén M, Struglics A, Rostgård Christensen L, Kartus J
Am J Sports Med. Mar 2015 epub ahead of print

ABBREVIATIONS

ACL	Anterior Cruciate Ligament
ARGS	ARGS-Aggrecan (generated by aggrecanase cleavage in the TEGE↓ARGS site of aggrecan)
BPTB	Bone Patellar Tendon Bone
COMP	Cartilage Oligomeric Matrix Protein
dGEMRIC	Delayed Gadolinium Enhanced MRI of Cartilage
ECM	Extra Cellular Matrix
GAGs	GlycosAminoGlycans
G	Gracilis
HT	Hamstring Tendon
HPF	High Power Field
IL-1β	Inter Leukin 1 beta
IL-6	Inter Leukin 6
KOOS	Knee Osteoarthritis Outcome Score
MRI	Magnetic Resonance Imaging
OA	OsteoArthritis
PCL	Posterior Cruciate Ligament
PTOA	Post Traumatic Osteo Arthritis
ROM	Range Of Motion
sGAG	Sulphated GlycosAminoGlycan
ST	SemiTendinosus
ST/G	SemiTendinosus/Gracilis
TNF- α	Tumour Necrosis Factor Alpha

01

INTRODUCTION

The present thesis focuses on different aspects for a patient who sustains an anterior cruciate ligament (ACL) injury, such as the timing of reconstruction, the

regeneration capacity of the tendon used for reconstructive surgery, graft morbidity and the metabolism of the knee joint cartilage in the long term.

1.1 TIMING OF ACL RECONSTRUCTION

The treatment routine after an ACL injury in Sweden is rehabilitation of the injured knee for at least three to six months and only after finishing the rehabilitation programme do the patient and the physician make a decision on whether or not to reconstruct the ACL. If the patient is satisfied with the function of the knee and is able to adapt his/her activity and lifestyle, he/she may not need surgery, but, if the knee is still unstable and the patient's ambition is to return to high-level sport, including pivoting/cutting manoeuvres, the preferred treatment is reconstruction of the ACL. The ACL injury is frequently combined with concomitant meniscal and cartilage damage. The treatment protocol is different if there is a bucket handle meniscal injury. The repair of the meniscus is then urgent and the ACL should be reconstructed simultaneously to protect the meniscal repair. In non-surgically treated ACL-injured knees, the incidence of meniscal injuries increases with time [89]. An ACL injury predisposes the knee to post-traumatic osteoarthritis (PTOA) [33,75,95,128,163]. If the ACL injury is

combined with a meniscal injury, the incidence of PTOA is increased [115,116].

There is no consensus today about the optimal timing for ACL reconstruction. In 2013, the mean delay from injury to reconstruction was more than one year in Sweden (www.aclregister.nu).

Historically, patients often underwent ACL reconstruction within the first week after injury and this sometimes resulted in arthrofibrosis and accordingly a stiff joint. In 1991, Shelbourne [141] suggested that delaying surgery would minimise the risk of arthrofibrosis, which was supported by several subsequent reports from the beginning of the 1990s [54,139,159]. On the other hand, there are advantages to early reconstruction, with a shorter period of abnormal knee kinematics and instability and therefore less risk of meniscal and cartilage damage due to recurrent pivoting trauma while waiting for surgery [46,69,103]. In the 1980s, arthroscopic techniques were developed [31]. Arthroscopic techniques led to reduced post-surgical morbidity, shorter

sick leave and less time away from leisure activities. The modern standards are that the patient should have undergone rehabilitation, regained full range of motion (ROM) and show no signs of arthrofibrosis or quadriceps hypotrophy before reconstruction [16]. In a meta-analysis from 2013 [81], the conclusion was that “if a modern surgical technique and an accelerated rehabilitation protocol are used, there is no increased risk of knee stiffness when ACL reconstruction is performed as early as one week after injury”. In a review by Smith et al. [143], there was no significant difference between early and delayed reconstruction in terms of the Lysholm knee scoring scale or Tegner activity level. In their study, early ACL reconstruction was undertaken three weeks after the injury and delayed reconstruction a minimum of six weeks after the injury. Likewise, in a randomised study from 2010 [126], there were no significant differences in terms of the Lysholm knee scoring scale and Tegner activity level between early reconstruction within two weeks and delayed reconstruction undertaken four to six weeks after injury. However, with the current practice in Sweden, where the patient should first undergo rehabilitation for three to six months, these time intervals are not applicable. Using the bone-patellar tendon-bone (BPTB) autograft, Karlsson et al. [69] reported a higher Tegner activity level in a group reconstructed within three months compared with a group reconstructed between 12 and 24 months. These intervals are more realistic in relation to the current conditions in Sweden.

In a recent comprehensive review of the timing for ACL reconstruction by Andernord et al. [6], the given inter-

vals for the included articles for early reconstruction ranged from two days to seven months and the “delayed or late” reconstruction from three weeks to 24 years. This highlights the difficulty involved in comparing studies, as there is no accepted consensus on “early” vs “delayed” reconstruction. The reported advantages of early reconstruction in some studies are therefore regarded as delayed in other studies [16]. Other difficulties when comparing studies include differences in outcome measurements, time to and method of evaluation, surgical techniques, autografts vs allografts, gender and patients’ age [16,81]. In Sweden, there is a prospective, randomised, high-quality study comparing early ACL reconstruction with rehabilitation with optional delayed reconstruction and, at the two-year follow-up, the authors report that “rehabilitation with early ACL reconstruction was not superior to rehabilitation with optional delayed reconstruction” [47], while, at the five-year follow-up, there was no difference between the groups in terms of the KOOS, Tegner activity level, meniscal injuries and OA findings [48].

Taken as a whole, the initial approach after an ACL injury should be structured rehabilitation, followed by an individual decision on whether or not to reconstruct the ACL. Some 3,500 ACL reconstructions are performed annually in Sweden (www.aclregister.nu), but there is still no consensus in terms of the optimal timing for reconstruction after the injury.

1.2 SEMITENDINOSUS AND GRACILIS AUTOGRAFTS

Several potential autografts for ACL reconstruction have been proposed; the bone-patellar tendon-bone (BPTB), hamstring tendon (HT) (semitendinosus (ST) alone or semitendinosus and gracilis (ST/G)), fascia lata and quadriceps tendon. The most common autograft in Sweden today is the HT autograft. The BPTB autograft was the dominant autograft for almost three decades. However, due to donor-site problems, such as difficulties when kneeling and anterior knee pain [34,72], there has been a shift towards HT autografts, starting in the mid-1990s. There are several randomised studies and systematic reviews that compare clinical outcomes between BPTB and HT (ST, ST/G) autografts [1,17,42,87,91,104,133,134]. Some report less laxity in the knee joint when using the BPTB autograft and, in the Cochrane Review from 2011 [104], the conclusion was that there might be less anterior-posterior laxity after using the

BPTB. At least in the short term, more donor-site morbidity appears to be related to the BPTB autograft [34,36,42]. In the long term, the difference between the BPTB and HT grafts in terms of anterior knee pain and kneeling pain for the BPTB graft appears to diminish [134].

The ST and G tendons are able to regenerate after harvesting the whole tendon from the musculotendinous junction to the insertion. Previous studies are contradictory in terms of the function, regeneration capacity, insertion point and cross-sectional area of the tendons after harvest. With increasing numbers of ACL reconstructions, the number of revision procedures after failed primary reconstructions will increase as well. If total regeneration of the tendons really occurs, this might be a potential source of future graft material in conjunction with ACL revision surgery.

1.3 REGENERATION RATE AND MORPHOLOGY

There is no agreement in the literature with regard to whether the regenerated ST and G tendon tissue should be termed regenerated or neo-tendon. In the present thesis, the term “regenerated tendon” will be used. In 1992, Cross et al. [29] were the first to report that the ST and G tendons were able to regenerate, as seen using magnetic resonance imaging (MRI). Several subsequent studies have verified this using MRI [28,38,39,59,99,127,142,153], computed tomography [108,110] and ultrasonography [122]. The reported regeneration frequency differs from 46% [153] to 93%

[108]. Moreover, the description of the insertion point of the regenerated tendons differs. Some studies [39,108] report an almost normal insertion location, while others report a more proximal [142,160] or medial insertion [29,122]. One explanation for this could be that only the ST tendon is harvested and the G tendon is left in place in some studies [39,108]. This cannot be the only explanation, since Choi et al. [28] harvested both tendons and reported a slightly more distal insertion on the pes anserinus. For this reason, additional factors, such as different time intervals between the harvesting proce-

ture and the radiographic examination, must be considered. When it comes to the area of the regenerated tendons, the time aspect also plays a role. There are reports that the regenerated tendons are hypertrophied at first [160], but that their cross-sectional area then gradually decreases [122]. Other factors contributing to the inconsistency in the reported regeneration rate and insertion location could be differences in the harvesting procedure, the way of defining the inser-

tion point, differences in the size of the study population and the female/male ratio. Evidently, there are studies with long follow-ups from harvest to tendon assessment, where a few patients do not display a regenerated tendon at all and the cause of this is not known. Co-morbidities or smoking might influence the regeneration rate and overly aggressive post-operative rehabilitation could potentially damage a weak tendon structure in the first months after harvest [109].

1.4 GRAFT MORBIDITY AND STRENGTH DEFICIT

Tendons attach muscles to bone and the primary function of a tendon is to transmit the force created in a muscle. The proximal tendon-to-bone attachment is called the muscle origin and the distal one is called insertion. The ST and G tendons normally insert at the pes anserinus. If the ST or G tendons fail to regenerate completely or if they have a more proximal insertion, the biomechanics of the knee will change. The ST/G muscle tendon complex is important, particularly in terms of deep flexion and internal rotation of the knee, and there are concerns that the ST and G autografts may be less suitable for athletes dependent on strength in deep knee flexion, such

as gymnasts, ballet dancers, orienteers, climbers and wrestlers. In addition, it is possible that weakness in internal rotation results in a reduced ability to protect from pivoting and external rotation of the knee, during cutting manoeuvres, for example [15,170,171]. Consequently, the use of HT autografts in young female athletes has been questioned [15]. If the regenerated tendon has a more proximal insertion site, the lever arm would be shorter and a proximal shift of the musculotendinous junction would result in shortened muscle bellies and, as a result, the ability of the muscle to flex the knee would be limited (Figure 2b and 2d).

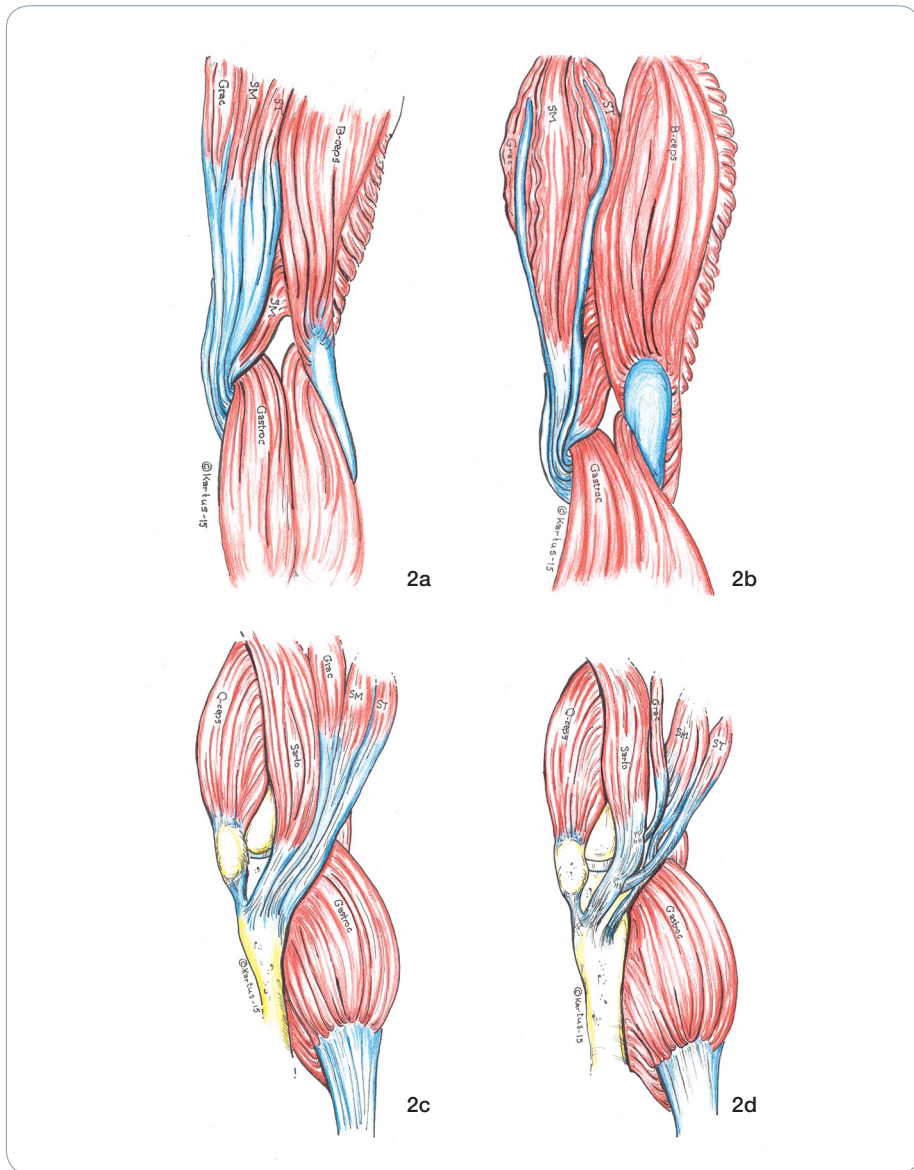


Figure 2a Illustration of normal muscles and tendons from the backside of the thigh.

Figure 2b Hypothetical illustration of regenerated tendons where the musculo-tendinous junction has shifted proximally, resulting in shortened muscle bellies and compensatory hypertrophy of the biceps femoris and semimembranosus muscles.

Figure 2c Normal insertion on the tibia of the ST and G tendons.

Figure 2d Hypothetical illustration with a more proximal insertion of the regenerated ST and G tendons.

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An insertion site located at the medial tibia fascia or popliteal fascia would reduce the ability to generate force in internal rotation. There have been studies reporting that patients regain strength in knee flexion [142,168]. However, they studied the peak torque which is generated at low knee flexion angles (15-30°) and, at those angles, the biceps femoris

muscle is the primary knee flexor [119]. The peak torque is therefore a less sensitive measure of ST and G strength. The ST/G complex is most important in deep knee flexion angles, e.g. more than 75° [119]. Tashiro et al. [154] also suggested that the tendons play a greater role in flexion when the hip is extended (Figure 3).

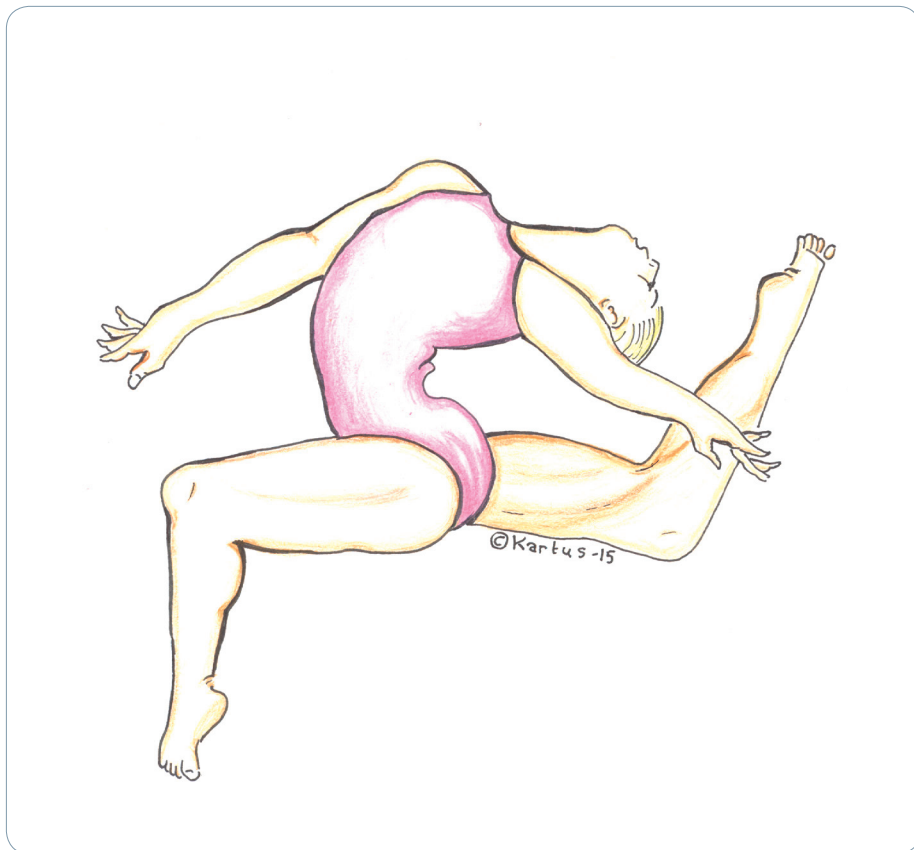


Figure 3 *Illustration of a gymnast performing a jump where deep knee flexion is executed with the hip in extension.*
© C. Kartus

Several researchers report remaining weakness in deep flexion [28,111,154] and internal rotation more than two years after harvest [12,136,161]. In addition, further studies have shown that the semimembranosus and biceps femoris undergo compensatory hypertrophy after harvesting the ST/G or ST alone [37,65,98,160]. There are reports of a greater strength deficit for internal rotation [136] and deep knee flexion [154] after harvesting both ST and G tendons compared with only harvesting the ST tendon. In a randomised, prospective study, Tashiro et al. [154] reported a significant weakness of up to 30% in muscle strength at knee flexion angles greater than 70°. They compared single- and double-tendon harvest and found that the weakness could be minimised if

the gracilis tendon was preserved. Snow [144] reported that G and ST muscles showed hypotrophy nine to eleven years after ACL reconstruction with ipsilateral ST/G autografts, while Nishino et al. [113] studied the relationship between knee flexion torque and the morphology of the ST muscle after ACL reconstruction and found that deficits in deep knee flexion were associated with hypotrophy and shortening of the ST muscle. Likewise, Choi et al. [28] and Williams et al. [160] found that the muscle bellies of the ST/G remained shortened after regeneration of the tendons. To summarise, there are several reports of remaining shortening and hypotrophy of the ST and G muscles, resulting in weakness in deep knee flexion, even if the tendons have regenerated (Figure 2a-d).

1.5 HOW DOES THE TENDON REGENERATE?

The functional cells in tendons are tenoblasts and tenocytes. These cells are elongated fibroblasts and fibrocytes between collagen fibres [67]. The cells produce the extracellular matrix (ECM) which consists mainly of collagen type 1. There is also a small amount of elastin and ground substance. The ground substance consists of proteoglycans, glucosaminoglycans (GAGs), glycoproteins and other small molecules. Proteoglycans are proteins with GAGs and together they form a negatively charged molecule that attracts water (hydrophils). The number of GAGs

is considerably smaller in tendons than in cartilage [67]. The smallest unit of the tendon is a collagen fibril, which consists of interconnected collagen strands. A group of collagen fibrils forms a collagen fibre. The collagen fibre is surrounded by endotenon. Fibre bundles are formed by a group of collagen fibres which are bound together in a sheath of endotenon. A group of fibre bundles forms a fascicle. A group of fascicles forms the tendon (Figure 4). The fascicles are bound together by an inferior sheath of endotenon and the exterior sheath is called epitenon [67].

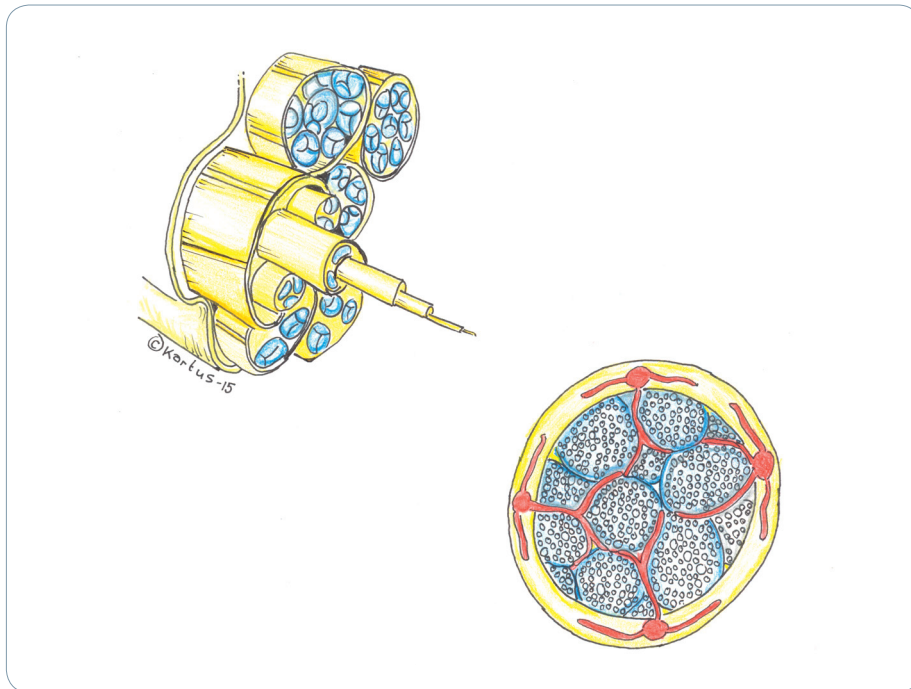


Figure 4 *Illustration of tendon structure.* © C. Kartus

The way the tendon regenerates is not fully understood. First, it was proposed that the tendon regenerates from proximal to distal [127] and Leis et al. [88] termed it “the lizard tail phenomenon”. The present opinion is that the tendon matures uniformly along the harvest site. Carofino et al. [26], when conducting a comprehensive review, suggested that the regeneration process proceeds through several stages and that it takes approximately one and a half years to yield a regenerated tendon that is similar to a normal tendon in structure and composition. Their statement is partly based on a prospective study with serial ultrasonographies on the same patients by Papandrea et al. [122]. They registered an initial haematoma followed by an oedema with gradual solidification along

the entire harvest site. Collagen fibres were detected after six months and, at 18 months, there was a structure with the morphology of a normal tendon at the harvest site. Eriksson et al. [38] proposed that the haematoma that initially forms at the harvest defect acts as a scaffold for fibrocyte migration and the subsequent tendon regeneration. Hadjicostas et al. [53] suggested that the ST tendon has better potential for remodelling than the patellar tendon due to its significantly higher density of collagen fibrils and fibroblasts. Okahashi et al. [117] proposed that the surgical method of “stripping” when harvesting the tendons plays an important role in the regenerative process, as synovial cells possess the ability to differentiate when subjected to mechanical stress.

Taken as a whole, a haematoma initially forms along the harvest defect and the tendon maturation appears to occur uniformly. Synovial cells differentiate into fibrocytes and there is gradual collagen deposition. After approximately one and a half years, there is a structure with the morphology of a normal tendon at the harvest site.

Histological samples are obtained using biopsies from the tendons. Eriksson et al. [38] were the first to obtain biopsies from regenerated ST tendons. They obtained

biopsies in five patients and reported that the regenerated tendons displayed the features of a normal tendon but with focal small scar-like areas. There are other published histological studies of regenerated ST tendon [43,117,169], confirming that it is essentially real tendon tissue that regenerates and not just scar tissue. However, one of the longest reported time periods between harvest and the biopsy procedure is still less than 2.5 years [38] and it is possible that the post-operative maturation process is not completed even at that time [38].

1.6 POST-TRAUMATIC OSTEOARTHRITIS (PTOA)

Studies consistently demonstrate that ACL injury is a significant risk factor for the subsequent development of PTOA, regardless of whether the injury is treated non-surgically or surgically reconstructed [33,94,95,115]. Likewise, there is an increased risk of PTOA after an intra-articular fracture, even if the fracture is anatomically reduced [8]. An ACL injury combined with meniscal tears and/or articular cartilage injury entails a greater risk of developing PTOA [115]. When patients with an ACL injury develop symptomatic PTOA, they are on average 15 to 20 years younger than patients who develop primary osteoarthritis (OA) [131]. At the time of injury, there is a

large increase in inflammatory cytokines and cartilage degradation products in the synovial fluid. The post-injury duration of these elevated levels has not been fully established. One study reported elevated levels up to three years after the injury [100]. The mechanisms of PTOA have not been elucidated. One theory is that inflammatory cells present acutely in high concentrations remain in the joint on a chronic long-term basis and cause ongoing joint degradation, independently of whether or not adequate joint stability is restored. On the other hand, the question of why certain patients do not develop OA after an ACL injury remains unanswered.

1.7 CARTILAGE BREAKDOWN AND BIOMARKERS OF CARTILAGE METABOLISM

Cartilage consists of cells, the chondrocytes and extracellular matrix (ECM). About 75% of the ECM is water. The chondrocytes synthesise and degrade proteins in the ECM. Collagen II and

aggrecan are two major components of the ECM [55]. Aggrecan is heavily substituted with sGAG. The cartilage has to sustain compression and shearing forces. sGAG is considered to play an important

role in cartilage homeostasis, as it is negatively charged and attracts water into cartilage [149]. In between the collagen network, there are proteins with a high affinity to collagen, which stabilises the network. One of these is cartilage oligomeric matrix protein (COMP).

Cartilage breakdown in OA is a complex process that involves many cell types, signalling pathways and changes in the extracellular matrix. The degradation of cartilage matrix molecules can be estimated by measuring released components in the synovial fluid. Heinegård [56] summarised the existing knowledge of cartilage breakdown based on *in-vitro* studies where catabolic cytokines, mostly IL-1, have been used. Heinegård [56] describes the sequence of cartilage

degradation as follows: in the first step, the aggrecan molecules with the GAG chains are released from the aggrecan domains. This is followed by the fragmentation of COMP and fibromodulin (Figure 5a and b). Finally, the collagen fibres become degraded (Figure 5c).

An acute injury to the ACL causes markedly increased concentrations of cartilage fragments in joint fluid [93] and it has been suggested that COMP and ARGS-aggrecan can be used as biomarkers of knee injuries and OA [84,97,146,147]. Measuring the total amount of sGAG, which is a substitute measurement of aggrecan, can contribute to a better understanding of the degradation process in synovial fluid [77,150].

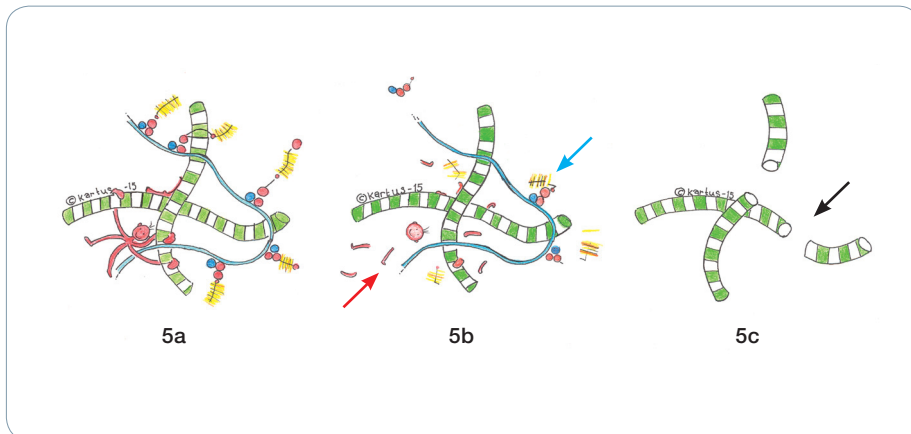


Figure 5a-c Schematic illustrations of cartilage degradation. © C. Kartus, with inspiration from original illustrations by Heinegård. Figure 5a. First aggrecan (with sGAG) is cleaved (blue arrow), followed by COMP (red arrow) and fibromodulin, Figure 5b. Finally, the collagen (black arrow) is destroyed, Figure 5c.

1.8 INFLAMMATORY CYTOKINES

Cytokines are proteins secreted by inflammatory leukocytes and, in the joint, by synoviocytes and chondrocytes, which act as intercellular mediators. In rheumatoid arthritis, interleukin (IL)-1 β , IL-6 and tumour necrosis factor (TNF)- α act as major inflammatory mediators [11,101] and it has been suggested that the same cytokines mediate the cartilage loss in PTOA [49]. These cytokines are elevated in knees with ACL injuries [58,100,150]. It has been suggested that inhibiting interleukin-1 and TNF- α might prevent PTOA [85]. The same authors suggest that the initial trauma causes an initial inflammatory response in the joint and this inflammatory response, mediated ultimately through IL-1 and TNF- α , then leads to a state of cartilage breakdown.

Kraus et al. [78] found elevated levels of pro-inflammatory cytokines up to one month after ACL injury and found that the administration of IL-1 receptor antagonist reduced knee pain and improved function. Andersson et al. [7] suggested a three-phase tissue damage process after an intra-articular fracture, where chondrocyte death leads to an acute inflammatory response, where IL-1- β is elevated and, finally, chronic inflammation and remodelling/degeneration. A recent review [118] investigating the role of cytokines in PTOA concluded that the early inhibition of the intra-articular inflammatory response may improve the clinical outcome.

1.9 STANDARD RADIOGRAPHIC EXAMINATION

The diagnosis of OA is a combination of radiographic findings and symptoms. On radiographs, evidence of cartilage loss, osteophyte formation and subchondral bone remodelling indicates the development of OA. When the OA appears on radiographs, the cartilage loss is often severe. If biomarkers of cartilage degradation can be identified, it is possible to improve the treatment for OA and, if inflammatory cytokines play a role, there is an opportunity for targeted treatment. It is therefore important to investigate the progression of post-traumatic cartilage breakdown in the long term. At the same time, it is important to remember that radiographic findings and patient symptoms seldom correlate.

There are different classification systems for OA. The Ahlbäck, IKDC, Kjellgren

& Laurence and the Fairbank system are frequently used classification systems. Studies using both the Fairbank and Ahlbäck systems report more OA findings for the Fairbank system [2,92]. The Ahlbäck system has been shown to have poor inter- and intra-observer agreement [50].

If targeted treatment for cartilage breakdown is to have an effect, there has to be cartilage left to save. The advantages when it comes to detecting early changes on radiographs are that specific treatment, such as rehabilitation programmes and the adjustment of activity, could begin at an earlier stage. However, the difficulty is identifying patients in whom small radiographic findings will lead to symptomatic OA. A specific biomarker for OA would therefore be of great interest.

02

AIMS

To investigate whether the timing of ACL reconstruction affects the clinical outcome and whether the number of cartilage and meniscal injuries differ if the patient has waited longer for reconstruction

To assess the regeneration rate, insertion site and area of the semitendinosus and gracilis tendons as seen on MRI after harvest compared with the normal tendons in the same patient

To assess the muscle strength in deep knee flexion and internal rotation of the reconstructed knee after semitendinosus and gracilis harvest

To evaluate whether the regenerated semitendinosus tendon has a histology similar to that of the normal semitendinosus tendon in the long term

To evaluate whether inflammatory cytokines and biomarkers of cartilage metabolism are elevated in an ACL-injured knee eight years after reconstruction and to assess whether more degenerative changes can be seen on radiographs in the ACL-reconstructed knee compared with the non-reconstructed knee

03

PATIENTS & METHODS

Table 1. Patients			
	Total number	Age Women/men	Allocation of patients
Study I	61	Group A 26 (9) 15/15 Group B 27 (6) 12/19 Mean (\pm SD) age at surgery	19 patients were also included in Studies II, III and IV
Study II	19	23 (17-40) 9/10 Median age at surgery (range)	All 19 patients were also included in Study I
Study III	18	23 (17-40) 8/10 Median age at surgery (range)	All 18 patients were also included in Studies I and II
Study IV	11	35 (26-47) 6/5 Mean age at fluid aspiration (range)	All 11 patients were also included in Studies I, II and III

STUDY I: Sixty-one patients, with a unilateral primary ACL rupture and a maximum of +1 medial or lateral laxity, underwent reconstructive surgery using ST or ST/G autografts. The exclusion criteria were contralateral ACL injury or reconstruction, any PCL injury or reconstruction and previous or acute fractures. Thirty patients (Group A) underwent surgery within five months (median 3, range 2-5) after the injury and 31 patients (Group B) underwent surgery more than 24 months (median 30, range 24-48) after the injury. The follow-up examination was performed after a median of 25 months (18-43) after the reconstruction. The study

design was retrospective, but the data were collected prospectively and entered into a local database from where patients fulfilling the inclusion and exclusion criteria were extracted to form the study group. The patient demographics are presented in Table 1 in Paper I page 489.

STUDY II Nineteen patients, who had undergone unilateral ACL reconstruction a median of 8.5 years (6-11) earlier using ST/G autografts from the ipsilateral leg, were included in the study (Table 1, Paper II, page 1736). The patients were a subgroup from Study I. From these patients, those that were reconstructed

at least six years earlier and agreed to undergo bilateral MRI examination were selected. The patients underwent clinical examinations, subjective assessments and functional testing commonly used for the evaluation of ACL-reconstructed patients, as well as bilateral MRI of their knees. Their muscle strength in internal rotation and knee flexion was tested using a Biodex Dynamometer (Biodex Multi-Joint System 4 Pro, New York, USA).

STUDY III: Eighteen patients, who underwent ACL reconstruction using ipsilateral ST/G tendon autografts, were included in the study. The patients were a subgroup from Study II who agreed to undergo biopsy. Percutaneous specimens were obtained from the regenerated tendon and the contralateral non-harvested normal ST tendon under ultrasonographic guidance a median of 8.4 years (100.5 months, range 77 to 129) after the harvesting procedure. Specimens from the non-harvested side served as controls. In all, 36 biopsies were obtained. One patient had undergone ACL reconstruction on the contralateral side, while in one patient the regenerated ST tendon and in four patients the non-harvested

tendon specimen contained insufficient amounts of tissue for evaluation. This left 16 specimens from regenerated tendons and 13 specimens from the contralateral side. Twelve patients (24 specimens) were therefore available for paired specimen comparison.

STUDY IV: Eleven patients who underwent ACL reconstruction eight years (6-11) years before synovial fluid sampling were included in the study. The patients in Study IV were a sub-group from Study II. The initial aim was to include all 19 patients from Study II, but, based on the power analysis and due to patient-related discomfort during aspiration, the study was terminated after recruiting 11 patients. Meniscal and cartilage injuries had previously been registered at the index operation (Table 1, Paper IV). The mean age was 35 years (26-47) at the time of synovial fluid sampling. At fluid aspiration, the patients underwent bilateral weight-bearing radiographs and bilateral MRI. Synovial fluid from the reconstructed and the contralateral knee was obtained under ultrasound guidance by an experienced radiologist.

3.1 SURGICAL TECHNIQUE

At the index operation, the ST or ST/G autograft was harvested through a 3-cm oblique incision over the pes anserinus. The sartorius fascia was incised parallel to the fibres of the fascia just above the thicker and more distally inserted ST tendon. After the vinculae had been cut under visual control, the full length of the tendons was harvested with a semi-

blunt, semi-circular open tendon stripper (Acufex, Microsurgical Inc., Mansfield, MA, USA). The femoral bone tunnels were prepared using a standard transtibial or medial portal approach. Metal screws were used on the femoral and tibial sides. No harvest-site drain was used and only the skin was sutured.



6a



6b

Figure 6a *An image showing how the vinculae are exposed and cut under visual control. © J. Kartus*
Figure 6b *An image showing how the tendons are harvested with a tendon stripper. © J. Kartus*

3.2 REHABILITATION

All the patients were rehabilitated according to the same accelerated protocol, permitting immediate full weight-bearing and full ROM [18,140]. No rehabilitation brace was used [20,62,71,164]. Closed-chain exercises were started immediately post-operatively [165]. Terminal extension with an external load other than the weight

of the operated leg was not permitted during the first six post-operative weeks. Running was permitted after three months and contact sports after six months at the earliest, provided that the patient had regained full functional stability in terms of strength, co-ordination and balance as compared with the contralateral leg.

3.3 MAGNETIC RESONANCE IMAGING

In Study II, an experienced musculoskeletal radiologist evaluated all the MRI examinations. A Siemens (Erlangen, Germany) Avanto 1.5 Tesla scanner with an 8 CH High Resolution Knee Coil was used. Both knees were examined in slight flexion. Images were acquired using a three-dimensional sequence (T2, FISP 3D), followed by a three-dimensional reconstruction program to obtain axial reconstructions. From these, values for the cross-sectional areas and diameters (the largest diameter and one perpendicular to it) of the ST and G tendons were obtained and calculated 4.0 cm above the centre of the medial joint compartment of the knee (Figure 7a and 8a). The insertion site of the tendons was defined as the level in relation to the centre of the medial joint compartment where the two tendons merge (Figure 7e and 8e) and, in cases where only one tendon was visible, the level was measured where the tendon

attached to the pes anserinus. The tendons were followed in the axial dimension using a slice thickness of 0.45 mm with 1 mm distances between the slices to their point of insertion (Figures 7b, c, d and Figure 8b, c, d). The evaluations, as well as the three-dimensional reconstructions, were made using an AGFA Impax DS 3000 workstation with computerised distance and area measurements.

In Study IV, bilateral MRIs of the knees using the same 1.5 T machine were performed to assess the presence of ACL, PCL and meniscal and cartilage injuries, as well as graft integrity. In the comparison with the contralateral knee, no meniscal or cartilage injury was graded as 0, meniscal rupture or thinning as 1, cartilage injury or thinning as 1 and both meniscal and cartilage injury/thinning as 2.

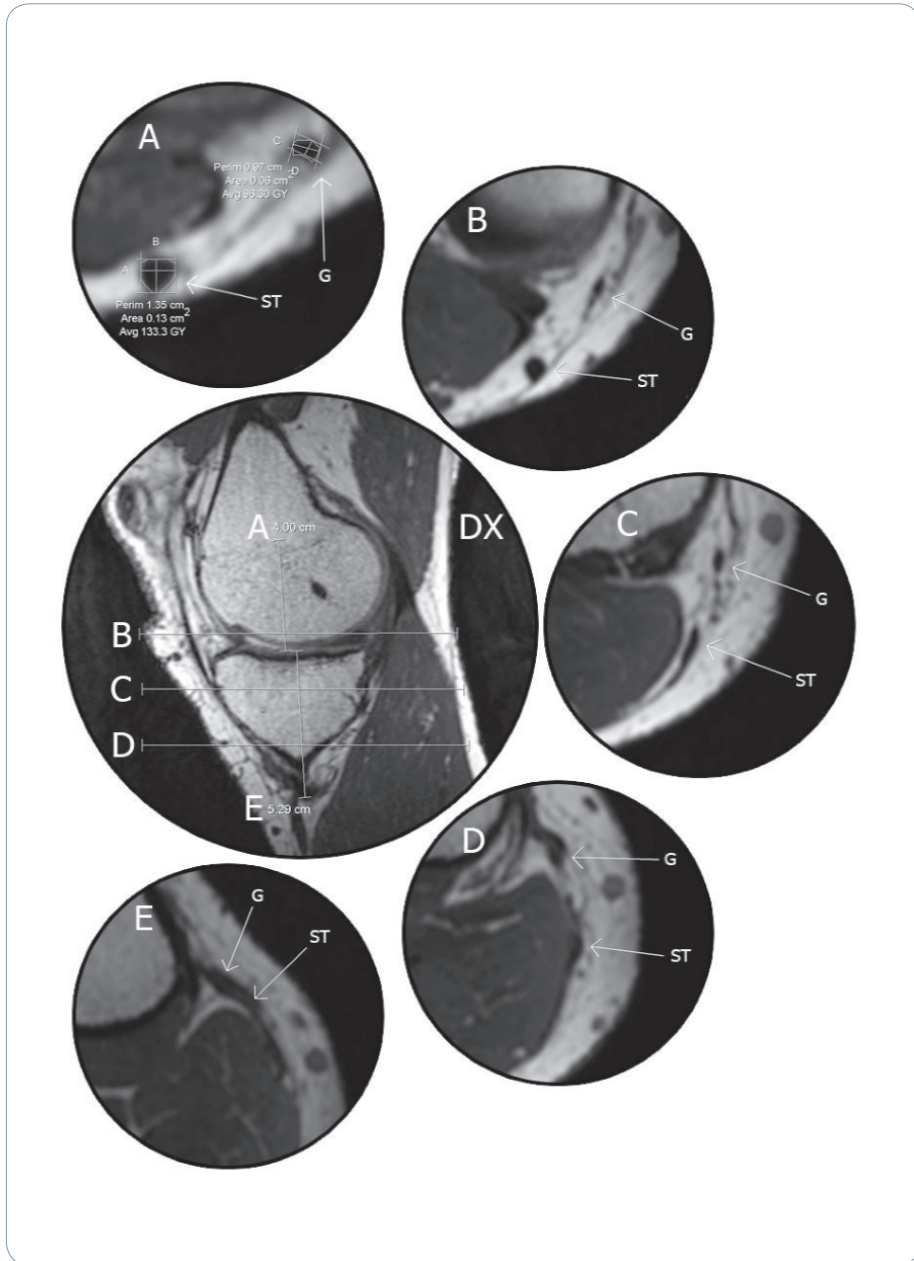


Figure 7 Axial magnetic resonance imaging of the operated right side demonstrating how the measurements were made. The cross-sectional area was calculated 4 cm above the centre of the joint line (A) and the tendons were followed until they merged on the pes anserinus (B–E). DX, right; G, gracilis; ST, semitendinosus. Reprinted with kind permission from SAGE publications.

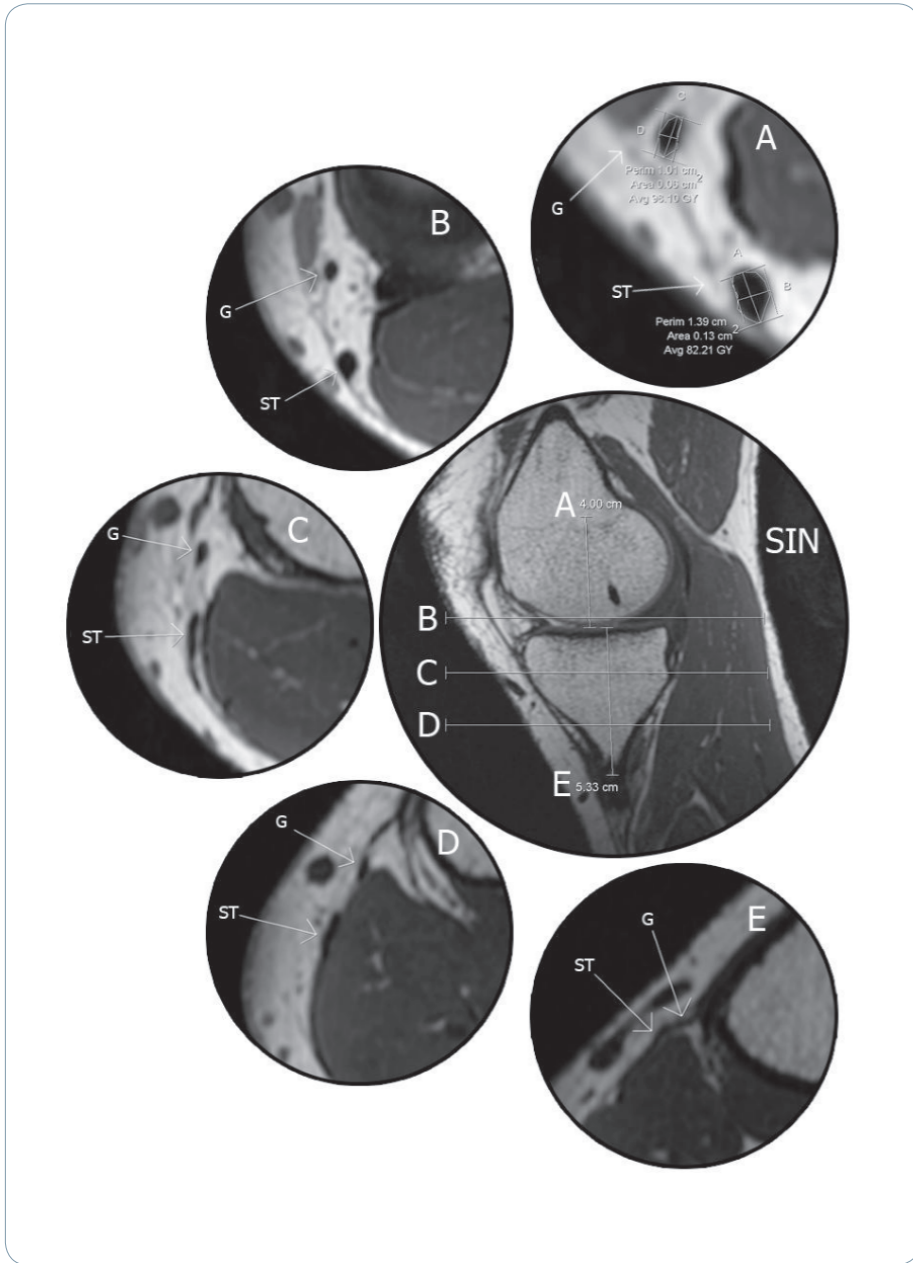


Figure 8 Axial magnetic resonance imaging of the non-operated side of the same patient as in Figure 7, showing that the cross-sectional area of the tendons and the point of insertion on the pes anserinus were similar compared with the operated side. SIN, left; G, gracilis; ST, semitendinosus. Reprinted with kind permission from SAGE publications.

3.4 STANDARD RADIOGRAPHY

Standard weight-bearing radiographic examinations in the antero-posterior and lateral views, with 20° to 30° flexion of the knee, were classified according to the rating system of Fairbank [40]. The Fairbank system dichotomously rates the presence of flattening, narrowing and

ridging of the joint in the medial and lateral compartment respectively. The cumulative number of positive findings from 0 to 6 was calculated for each knee. Grading was assessed by an experienced radiologist.

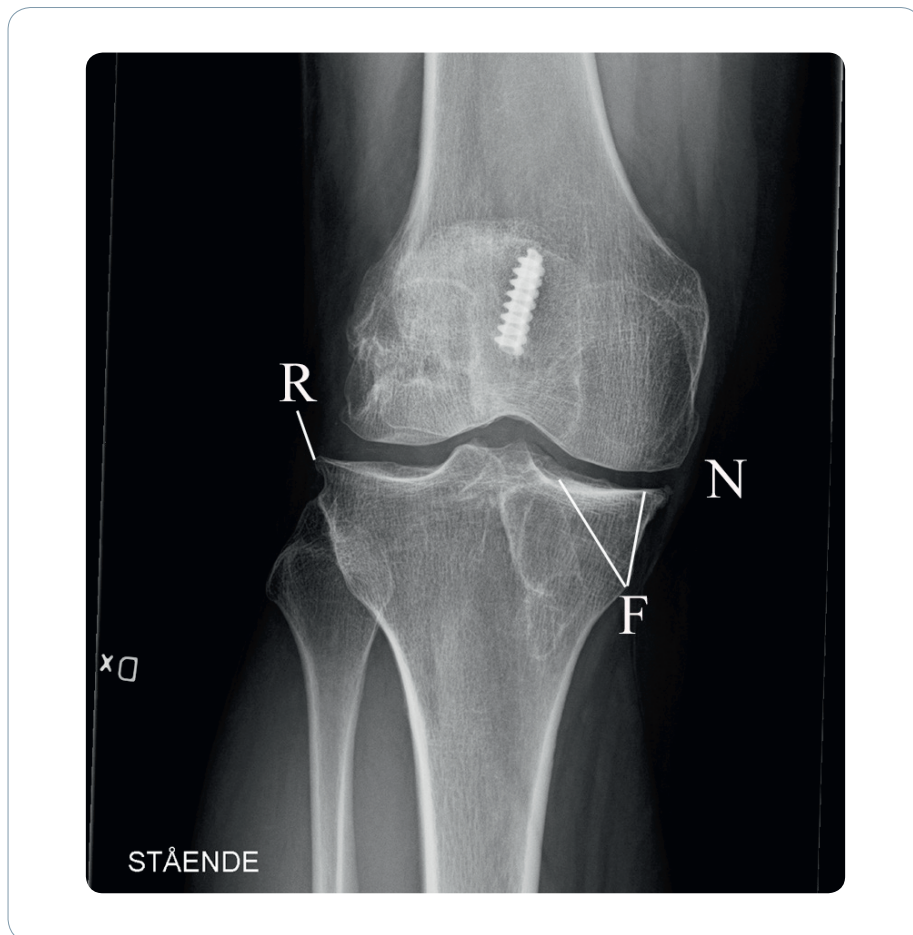


Figure 9 Antero-posterior radiographic view of a weight-bearing knee, illustrating the Fairbank classification. *N* in the picture refers to the narrowing of the medial compartment, *F* refers to the flattening of the tibial surface and *R* refers to the ridging of the lateral and medial femoral condyle. Reprinted with the kind permission of © S. Stener

3.5 OUTERBRIDGE

At index operation, cartilage damage was graded according to Outerbridge. The classification was originally developed for chondromalaciae patella [121]. It consists of four grades: Grade 1, softening and swelling of the cartilage, Grade 2, frag-

mentation and fissuring in an area half an inch or less in diameter, Grade 3, the same as grade 2, but an area more than half an inch in diameter is involved and Grade 4, erosion down to bone.

3.6 BIOPSY PROCEDURE

Specimens were obtained from the ST tendon on the operated and non-operated side of each patient. The biopsy specimens were obtained under ultrasonographic guidance with a free-hand technique using a 1.2 mm Tru-cut Monopty instrument (Bard Inc., Covington, GA, USA). This is a metal handle with a pre-attached disposable biopsy needle. The gun needle moves in two stages when fired. During the first stage, the inner stylet punctures the target and, in the second stage, an outer cannula follows the path of the stylet, covering the sample notch and thus

capturing the sample. Local anaesthesia with adrenaline (5-10 ml) was given subcutaneously. Under ultrasonographic guidance, the ST and G tendons were identified proximally on the thigh and followed to a position approximately 4 cm above the medial joint line with the knee in slight flexion. In this position, the specimens were obtained from the central part of the ST tendon through a small incision. Each specimen was placed separately in a coded tube. The specimens had a depth of 5 mm and a maximum diameter of 1.2 mm.

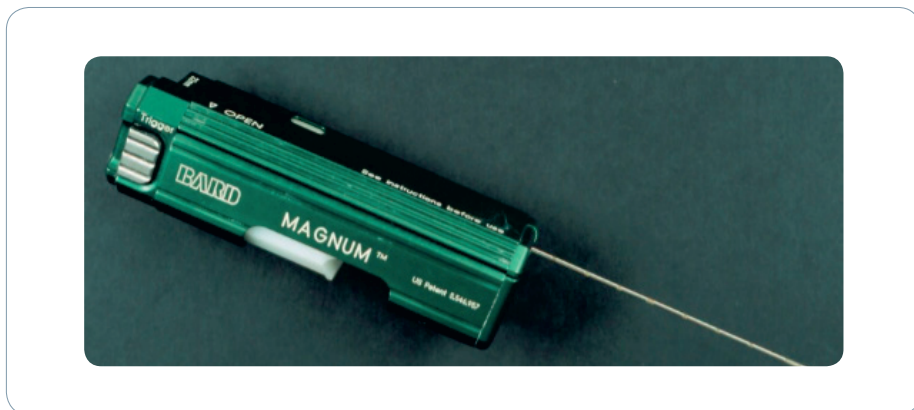


Figure 10 *The biopsy instrument, a 1.2 mm Tru-cut Monopty, in which the needle moves in two steps. ©J. Kartus*

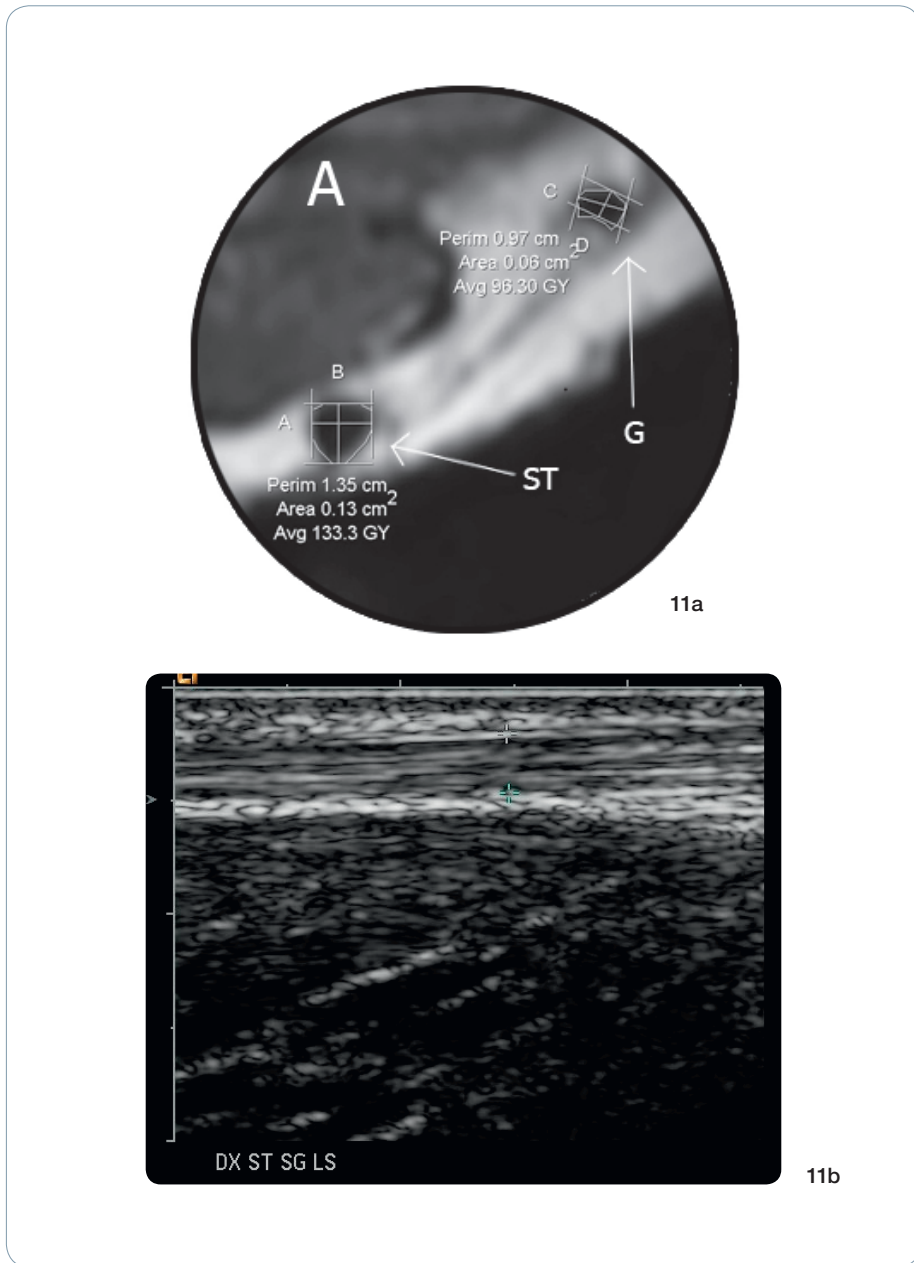


Figure 11a MRI image at the level from which the biopsy was obtained.
Figure 11b Ultrasonographic image of the semitendinosus tendon with markings (+) to highlight the tendon.

3.7 EVALUATION OF THE BIOPSIES USING A LIGHT MICROSCOPE

The specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 4-5µm, according to routine procedures. The sections were stained with hematoxylin and eosin, to evaluate fibre structure, cellularity and vascularity, and Alcian Blue (pH 2.5)/Periodic Acid-Schiff (AB/PAS), for the detection of GAG-rich areas. A pathologist and an orthopaedic surgeon, both with a specific interest in and knowledge of tendon pathology, simultaneously examined the tendon specimens using a light microscope (Leica DMRBE, Wet-

zlar, Germany). Both examiners were blinded as to whether the specimens came from regenerated or non-harvested ST tendon. The specimens were evaluated using a semi-quantitative (non-parametric) grading system for the tendon alterations used in multiple previous studies [73,102,105,151]. Grading was based on a four-point scoring system (Table A). Fibre structure, cellularity, vascularity and the level of GAGs were graded after examining the whole section. The number of cells was estimated in a high-power field (HPF) representative of the section.

Table A				
	Grade 0	Grade 1	Grade 2	Grade 3
Fibre structure	Straight, parallel, packed fibres, with slight waviness	Slight separation of fibres, increased waviness	Separation of fibres, deterioration of fibres	Complete loss of fibre structure and hyalinisation
Cellularity	< 100 cells/high-power field (HPF)	100-199 cells/HPF	200-299 cells/HPF	> 300 cells/HPF
Vascularity	Vessels run parallel to the collagen fibre bundles in the septa	Slight increase in vessels, including transverse vessels in the tendon tissue	Moderate increase in vessels within the tendon tissue	Markedly increased vascularity with clusters of vessels
Glycosaminoglycans	No alcianophilia	Slight alcianophilia between the collagen fibres	Moderate increase in alcianophilia	Markedly increased alcianophilia

Table A *The semi-quantitative scoring system.*

3.8 ASPIRATION OF SYNOVIAL FLUID

Synovial fluid from the reconstructed and the contralateral knee was obtained under ultrasound guidance by an experi-

enced radiologist. The synovial fluid did not contain any visible cell aggregates or tissue debris and aliquots of synovial fluid

were therefore collected in small tubes and immediately frozen on dry ice and

stored at -70°C . The obtained synovial fluid volume was between 1 and 5 ml.

3.9 MEASUREMENTS OF CYTOKINES

Concentrations of predetermined synovial fluid cytokines (IL-1 β , IL-6 and TNF α) were analysed using Human Proinflammatory Multiplexed cytokine assays (Meso Scale Discovery, Gaithersburg, USA, K15007C-2), according to the manufacturer's protocol. Briefly, 25 μl /well of duplicates of standards or synovial fluid were added to each well on the 96-well microtitre platform. The platform was incubated at room temperature for two hours on a plate shaker. The unbound materials were washed away three

times with PBST (Phosphate Buffered Saline with Tween), available in the assay kit. Sulfo-Tag conjugated antibodies against analytes were added and incubated for two hours at room temperature on a plate shaker. After being washed three times with PBST, the read buffer was added and quantification of the signals was assessed using a SECTOR Imager 2400 reader (Meso Scale Discovery). The final concentrations were determined through standard curves over a range of 0-2, 500 pg/ml.

3.10 MEASUREMENTS OF GLYCOSAMINOGLYCANS

The amount of sGAG in the synovial fluid was determined with the Alcian blue precipitation method, as described previously by Bjornson and modified by Struglics et al. [19,148]. The absorbance at 600 nm in duplicates of samples and standards (chondroitin sulphate, Sigma,

SAINT LOUIS, MO, USA, C4384) was measured spectrophotometrically in a 96-well plate with a plate reader (Termo lab-systems). The final concentrations were determined through a calibration curve of a series of standards over the range of 3-200 $\mu\text{g}/\text{ml}$.

3.11 MEASUREMENTS OF ARGS-AGGREGAN

The ARGS-neoepitope of aggrecan was measured in synovial fluid using electrochemiluminescence technology [82,84]. Briefly, anti-human aggrecan antibody MAb AHP0022 (Invitrogen, Carlsbad, CA, USA), recognising the G1-G2 binding region of human aggrecan, was used to coat a high-bind MA600 96-well microtitre platform (no. L11XB-1, Meso Scale Discovery, Gaithersburg, MD, USA). Duplicates of 25 μl of deglycosylated synovial fluid

and standards were added to each well [148]. The concentration of the standards ranged from 0.017 to 6 pmol ARGS/ml and the final synovial fluid dilutions were 1:13. A biotin conjugated monoclonal antibody (MAb OA-1) specific to the [124] ARGS-neoepitope, in combination with sulpho-tagged streptavidin (no. R32AD-5, Meso Scale Discovery), were used to detect ARGS-aggrecan. The plates were read in a Sector Imager 6000 (Meso Scale Discovery) within 15

minutes. Sample concentrations of the ARGS-aggregan were calculated from the standard curve using Discovery

Workbench 2006 MSD-3-0-18 software (Meso Scale Discovery).

3.12 MEASUREMENTS OF CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP)

Concentrations of COMP in synovial fluid were determined using a commercially available enzyme-linked immuno-

sorbent assay, according to the manufacturer's instructions (COMP® ELISA, AnaMar Medical AB, Sweden).

3.13 THE LYSHOLM KNEE SCORING SCALE AND TEGNER ACTIVITY LEVEL

The Lysholm knee scoring scale was self-administered according to Höher et al. [61] and the questionnaire did not show the scores for the alternative answers. The questionnaire consists of eight items, where pain and instability each account for 25 of the total score of 100 points [155].

The Tegner activity level is graded between 0-10, where grades 0-4 cover activities of daily living and work and

grades 5-10 indicate whether the patient is able to participate in recreational or competitive sports [155]. The Tegner activity level was assessed by the examiner/physiotherapist during the course of the examination before surgery and at the different follow-ups.

The Lysholm knee scoring scale has been validated and tested for reliability and responsiveness in the long term after ACL injuries of the knee [23].

3.14 KOOS

The KOOS was originally developed for OA. It has been validated for ACL injuries in both the short and long term [129,130]. It is self-administered and consists of five subscales. All questions are graded from 0 to four points, after which a normalised score for each subscale is calculated. The subscales consist of Pain, Other symptoms (Symptoms),

Function in daily living (ADL), Function in sports and recreation (Sports/Rec) and Knee-related quality of life (QoL). There are nine questions for pain, seven for symptoms, 17 regarding ADL, five for sports/rec and four regarding QoL. The maximum score of 100 points indicates no symptoms and zero points indicate extreme/severe symptoms.

3.15 THE LACHMAN TEST

The knee is in approximately 15 degrees of flexion and the femur is stabilised with one hand when performing the Lachman test. Firm pressure is applied to the posterior aspect of the proximal tibia in an attempt to translate it anteriorly. A proprioceptive and/or visual anterior translation of the tibia in relation to the

femur with a characteristic mushy or soft end point is a positive test indicating disruption of the ACL. When the ACL is intact, there is, in contrast, a definitive hard end point. The examiner estimates and grades the test as +1 (<5mm), +2 (5-10mm) or +3 (>10mm), compared with the uninjured contralateral knee [157].

3.16 KT-1000 ARTHROMETER TEST

The total sagittal stability of the knee was evaluated with the KT-1000 arthrometer (MedMetric, San Diego, California). The patients were in a supine position during the examination. Both legs were placed on a thigh support with 30 degrees of knee flexion [60]. A foot-rest and a strap around the thighs kept the legs in a neutral position [45,63]. After calibrating the instrument to zero before the test, the median value of three meas-

urements for each knee was registered. In Study I, a force of 89 Newtons was used both pre-operatively and at follow-up. In Study II, a force of 130 N was used at follow-up. The reproducibility has been found to be good [32,145,166]. However, the reproducibility between two examiners has been found to be fair and using the same examiner in clinical studies has consequently been recommended [137].

3.17 KNEE WALKING ABILITY

The subjective knee walking [72] ability was used to classify kneeling discomfort. The test involves the direct loading of the anterior knee region. The patients were asked to kneel on a hard floor without any protective clothing or other protec-

tion and then asked to walk six steps slowly forward on their knees. They were then asked to grade the test based on the feeling in the operated knee as OK, unpleasant, difficult or impossible to perform.

3.18 RANGE OF MOTION (ROM)

The ROM was measured to the nearest five degrees with a goniometer. The patient first made an active full extension and then an active full flexion. The uninjured leg was always tested first. The measurements were performed in the

supine position using a hand-held goniometer graded in one-degree increments [24]. The examiner always made a visual check to ensure that the measurements appeared reasonable.

3.19 ONE-LEG-HOP TEST

The one-leg-hop test [156] was used to evaluate functional performance. The patient stood on one leg and jumped as far as possible landing on the same foot with his/her hands behind the back [52]. Three attempts were made for each leg and the

best distance was registered for each leg separately. A quotient (%) between the index and uninjured leg was calculated. Before returning to sport activities, a side-to-side symmetry of at least 85% is recommended [13,123].

3.20 MUSCLE STRENGTH MEASUREMENT BIODEX

A physiotherapist who was not involved in the rehabilitation performed all the muscle strength measurements. A Biodex Dynamometer was used for the isokinetic strength testing. The patients warmed up by riding a stationary bicycle for five minutes. The non-operated side was always tested first. During measurements of the internal rotation of the tibia, an ankle brace (DeRoyal Functional Ankle Brace, Tennessee, USA) was used to restrict the inversion and eversion motion

of the talocalcaneal joint, as described by Armour et al. [12]. The isokinetic peak torque measurements for internal rotation were measured at 60 and 180 deg/sec. The patients were in a prone position with their hip in extension when measuring strength in knee flexion, as suggested by Tashiro et al. [154]. To determine the deep knee flexion strength, the torques at 90° of knee flexion were obtained from the torque curves at 60 deg/sec and at 180 deg/sec.



Figure 12 *The strength tests were performed using a Biodex machine (Biodex Multi-joint System 4 Pro, Biodex Medical Systems Inc., Shirley, New York). © N. Sernert*

3.21 STATISTICAL ANALYSIS

Study I

Median (range) values are presented, except for the anterior KT-1000 knee laxity measurements, where mean (range) values are presented. Wilcoxon's signed rank test was used for comparisons of the pre-operative and post-operative data within the groups. The Mann-Whitney U test was used to compare the variables between the groups. The chi-square test or Fisher's exact test was used to compare dichotomous variables. A p-value of < 0.05 was considered statistically significant. The primary variables in the study were the Lysholm knee scoring scale and the Tegner activity level at the two-year follow-up.

Study II

The paired t-test was used to compare the MRI findings and muscle strength measurements between the operated and non-operated leg. The mean (SD) values are reported for the MRI, strength measurements and the anterior KT-1000 knee laxity measurements. All other values are presented as median (range). Wilcoxon's signed rank test was used for comparisons of the pre-operative and post-operative clinical assessments. A p-value of < 0.05 was considered statistically significant. We expected a minimum difference of 10 mm in the insertion point for the regenerated tendons compared with the normal tendons on the contralateral side when planning the study. With a standard deviation of 10 mms, a sample size of 10 patients estimates the power to 80%. In order to improve the power of the study, we included a somewhat larger number of patients.

Study III

Median (range) values are presented. The Wilcoxon signed rank test was used for comparisons between the regenerated and non-operated ST tendon specimens. A value of $p < 0.05$ was considered significant. When planning the study, a difference of one (1) unit in the classification of fibre structure between regenerated and non-harvested tendons was expected. The required sample size would then be 10 paired specimens to reach a power of 80%, if the standard deviation is one unit for the difference between pairs. In order to allow for lost and non-gradable samples, 18 paired specimens were obtained.

Study IV

Mean values with standard deviation are presented for IL-1 β , IL-6, TNF- α , sGAG, ARGS and COMP. Median (range) values are presented for the clinical assessments and the Fairbank score. The paired t-test was used to analyse the differences in biomarkers between the reconstructed and the contralateral knee. The Wilcoxon signed rank test was used to analyse the difference in terms of the Tegner activity level, Lysholm knee scoring scale, Fairbank score and the MRI findings. The Spearman rank correlation test was used to assess the correlations. A p-value of < 0.05 was considered statistically significant. When the study was planned, it was estimated that the difference between the index and the control knee and the standard deviation for the difference in the biomarkers would have the same magnitude. The power was set at 80% and the p-value at < 0.05. The required sample size would then be 10 paired synovial fluid samples.

04

RESULTS

4.1 STUDY I

The patients were examined at a median of 25 months (18-43) after the index operation. The sub-acute treated patients in Group (A) had a significantly better outcome in terms of the Lysholm knee scoring scale ($p=0.01$) and Tegner activity level ($p=0.01$) at follow-up compared with the delayed-treated patients in Group (B), (Table B). Furthermore, there were no significant differences between the groups in terms of the KT-1000 arthrometer test, manual Lachman test, ROM, the ability to knee walk and the one-leg-hop test (Tables 3 and 4, Paper I, page 491). The clinical assessment at follow-up was significantly improved for both Group A and Group B in terms of the KT-1000 arthrometer test, manual Lachman test and the one-leg-hop test. The patients in Group B had a significantly better ROM pre-operatively in terms of both extension and flexion. The ROM of Group A was significantly improved post-operatively and reached the same level at follow-up as in Group B. There were no significant differences between the groups in terms of the pre- and

post-operative knee walking test (Table 4, Paper I, page 491).

There were no significant differences between the groups in the prevalence of meniscal or chondral damage registered at the index operation (Table 5, Paper 1, page 492). Fifty per cent (Group A) and 65 per cent (Group B) of the patients had meniscal injuries (n.s.). In Group A, 3/30 patients and, in Group B, 13/31 patients required resection of their meniscal injury at the index operation ($p<0.01$), (unpublished additional results). Chondral damage classified as Outerbridge [121] II or III was found in 20% of the patients in Group A and 29% in Group B (n.s.). Three patients in Group A and two patients in Group B underwent meniscal sutures at the index operation. Four patients, two in Group A and two in Group B, underwent additional surgery between the index operation and follow-up. All these patients had meniscal injuries; three patients were treated with meniscal sutures and one patient with a partial resection of the meniscus.

Table B			
	Group A (n=30)	Group B (n=31)	Significance
	Lysholm score (points)		
Pre-operatively	71 (34-100)	68 (33-94)	n.s. (p=0.9)
Follow-up	90 (58-100)	81 (38-100)	p=0.01
Significance pre- vs post-operative	p<0.0001	p=0.004	
Missing values at follow-up	0	1	
	Tegner activity level		
Pre-injury	8 (4-10)	8 (5-10)	n.s. (p=0.3)
Pre-operative	4 (0-9)	4 (2-6)	n.s. (p=0.2)
Follow-up	6 (2-9)	5 (0-9)	p=0.01
Significance pre- vs post-operative	p<0.0001	p=0.01	
Missing values at follow-up	0	1	
<p>The evaluations revealed a significant improvement in both groups in terms of the Lysholm score and the Tegner activity level after surgery. Furthermore, the patients in Group A scored significantly higher than those in Group B at follow-up. Median values (range) are presented, significant values in bold (n.s., not significant).</p>			

Table B Pre- and post-operative data in terms of the Lysholm score and the Tegner activity level.

4.2 STUDY II

The patients were examined a median of 8.5 years (6-11) after the index operation. The ST had regenerated in 17/19 (89%) of the patients and the G in 18/19 (95%), which means that, in 16% of the patients, only one tendon had regenerated (Table C). There was no significant difference between the level of the insertion for the tendons on the operated and non-op-

erated side (Figures 7e and 8e). The cross-sectional areas of the regenerated tendons revealed no significant differences compared with the normal tendons on the contralateral side, as measured 4 cm above the joint line (Table C), (Figures 7a and 8a). The patients revealed a significant muscle strength deficit in the operated leg at 90° of knee flexion at angular

velocities of 60 deg/s and 180 deg/s, but they were significantly stronger in terms of internal rotation of the tibia on the operated side when measuring the peak torque at 60 deg/s. At 180 deg/s, there was no significant difference (Table 4,

Paper II, page 1739). At follow-up, the patients had improved in terms of the Lysholm knee scoring scale, Tegner activity level and the one-leg-hop test compared with the pre-operative values (Table 2, Paper II, page 1737).

Table C			
	Operated side	Non-operated side	Significance
Insertion point of the tendons on the pes anserinus below the medial joint line (cm)	3.5 (SD 1.8)	4.2 (SD 0.8)	n.s. (p=0.11)
Cross-sectional area gracilis 4 cm above the medial joint line (cm ²)	0.09 (SD 0.06)	0.08 (SD 0.03)	n.s. (p=0.37)
Missing value	1		
Cross-sectional area semitendinosus 4 cm above the medial joint line (cm ²)	0.15 (SD 0.10)	0.14 (SD 0.03)	n.s. (p=0.74)
Missing value	2		
There was no significant difference in the point of insertion for the tendons on the operated and non-operated sides. The cross-sectional areas of the regenerated tendons revealed no significant differences compared with the normal tendons on the contralateral side, as measured 4 cm above the joint line; n.s. (not significant). The missing values represent those tendons that did not regenerate.			

Table C Insertion points and cross-sectional areas of the regenerated semitendinosus and gracilis tendons compared with the non-operated side.

4.3 STUDY III

The patients had a Tegner activity level of median 6 (range 5-7) and a Lysholm knee scoring scale of median 87 (range 47-100) at the time of the biopsy procedure. Bilateral biopsy specimens were obtained in all patients. The patients experienced no pain or discomfort during or after the

biopsy procedure. The semi-quantitative scoring system revealed no significant differences in the fibre structure, cellularity, vascularity and the amount of GAGs between the regenerated and non-harvested contralateral side (Table D). The fibre structure in both the regenerated

and the non-harvested tendons was classified as median grade 1. However, of the 16 gradable specimens from the regenerated tendon, three was classified as fibre structure grade 3 in focal areas and, in three, increased levels of GAGs were detected. Furthermore, five specimens from the regenerated tendons had > 200 cells/

HPF. In the remaining specimens from the regenerated tendon tissue and in all 13 normal gradable tendon specimens, no areas of grade 3 fibre structure or GAGs could be detected. In terms of cellularity, two of the non-harvested ST tendons had > 200 cells/HPF.

Table D			
	Regenerated ST tendon Median (range)	Normal ST tendon Median (range)	P-value
Fibre structure	1 (0-3)	1 (0-2)	0.20
Cellularity	1 (0-3)	0 (0-2)	0.11
Vascularity	0.5 (0-2)	0 (0-2)	0.36
GAGs	0 (0-1)	0 (0-0)	0.08
Missing value	1	4	
The specimen from one of the regenerated tendons and four specimens from the non-operated side contained an insufficient amount of tissue and one patient had undergone ACL reconstruction on the contralateral side. This left twelve patients for paired specimen comparison.			

Table D *Histological results.*

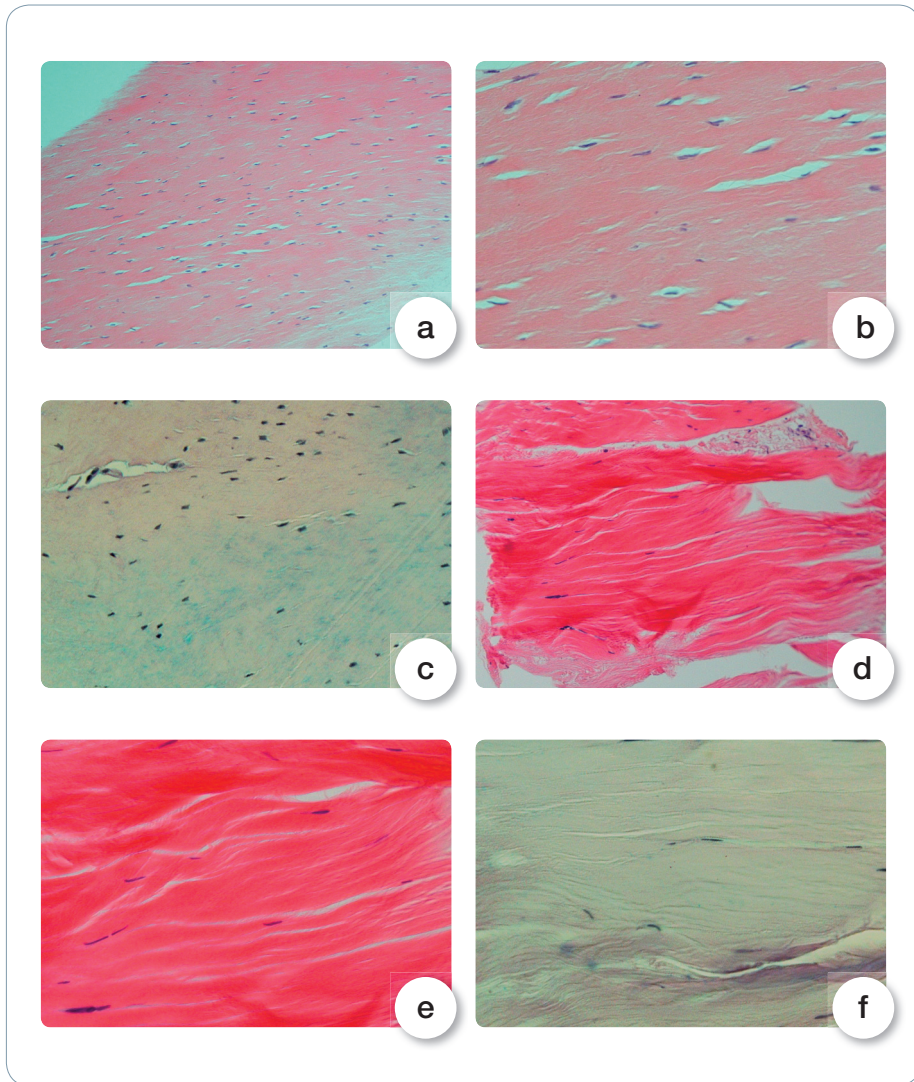


Figure 13 *Light-microscopic views of (a-c) a specimen obtained from tendon-like repair tissue and (d-f) the contralateral specimen from non-harvested semitendinosus (ST) tendon tissue. The specimens were obtained seven years after the harvesting procedure from a male patient who was 24 years old at the time of reconstruction. Both sides show linear, parallel-oriented collagen fibres. The regenerated tissue (a-c) shows slight separation and deterioration of fibres, the number of cell nuclei is increased and there is slight blue-stained alcianophilia between the collagen fibres (c). By comparison, in the contralateral ST tendon (d-f), the fibres are packed; the sparse tendon fibroblasts are thin, oblong and longitudinally oriented in between the fibres and there is no alcianophilia (f). Hematoxylin and eosin staining; original magnification 100 x (a and d), 200 x (b and e), and Alcian blue (pH, 2.5)/periodic acid-Schiff (AB/PAS) staining 200 x (c and f). Reprinted with kind permission from SAGE publications.*

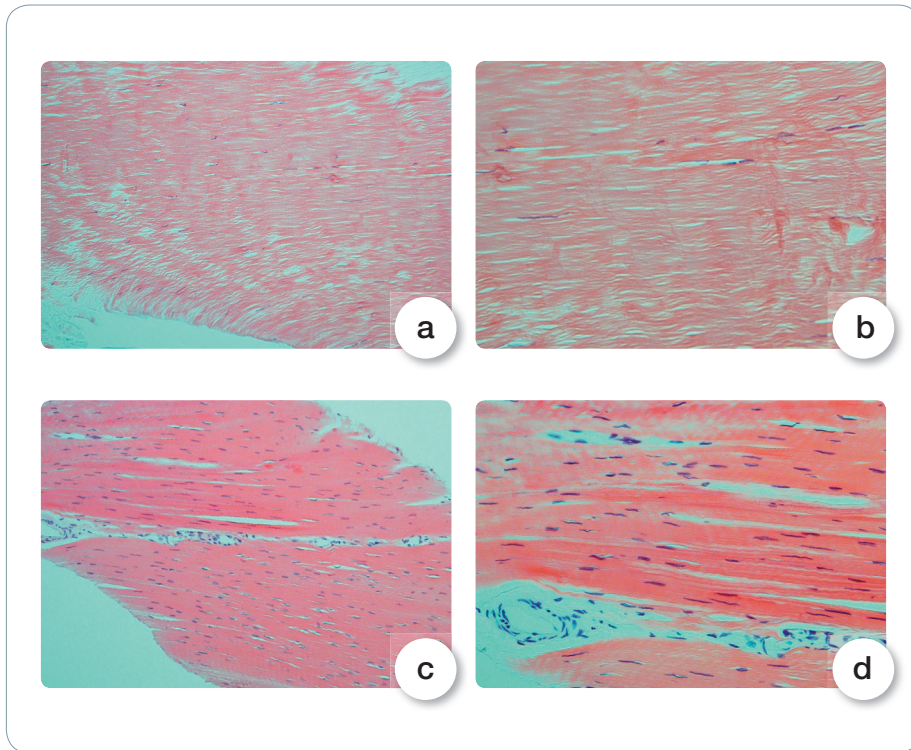


Figure 14 *Light-microscopic views of (a and b) a specimen obtained from tendon-like repair tissue and (c and d) the contralateral specimen from non-harvested semitendinosus (ST) tendon tissue. The specimens were obtained 6.5 years after the harvesting procedure from a female patient who was 18 years old at the time of reconstruction. Parallelism of the collagen fibres can be seen in both the regenerated and the contralateral ST tendon. In the regenerated tendon (a and b), there is slight separation and waviness of the fibres and sparse, thin, slender fibroblast nuclei in between the fibres. The contralateral ST tendon specimen (c and d) shows a vessel running longitudinally within the view and the number of well-oriented fibroblast nuclei is increased. Hematoxylin and eosin staining; original magnification 100 × (a and c) and 200 × (b and d). Reprinted with kind permission from SAGE publications.*

4.4 STUDY IV

There was a significantly higher Fairbank score in the reconstructed knee, indicating more degenerative changes on radiographs, compared with the non-operated knee. All the patients but one had some grading for the Fairbank score on the reconstructed side. Only one patient had a score of one on the non-operated side,

while the rest had no changes. The median (range) for the Fairbank score was 2 (0-6) on the operated side and 0 (0-1) on the non-operated side ($p=0.004$), (Table 1, Paper IV).

MRI revealed an intact graft and intact PCL on the operated side; furthermore,

an intact ACL and PCL on the non-operated side were found in all patients. Significantly more meniscal and cartilage injuries/thinning were seen on the reconstructed side ($p=0.023$), (Table 1, Paper IV).

The mean concentration of TNF- α was higher in the reconstructed knee, while the mean concentration of IL-1 β and IL-6 was higher in the contralateral knee. This finding was not statistically significant (Table E).

The mean concentration of COMP was higher in the reconstructed knee, while the mean concentrations of sGAG and ARGS-aggrecan were higher in the non-operated knee. This finding was not statistically significant (Table E).

At follow-up, the clinical assessment revealed that the Lachman test compared with the contralateral knee was 0 in six patients, +1 in four patients and +2 in one patient. The pivot shift test was normal in all patients. The Lysholm knee scoring scale, KOOS score, one-leg-hop test and the Tegner activity level are reported in Table 3, Paper IV. The median ROM in extension was 0° (range minus 15-10) and 140° (range 125-140) in flexion at follow-up. At follow-up, there was a correlation between the Lachman score and MRI findings ($Rho\ 0.7$, $p=0.037$) but not between the Lachman and Fairbank scores. Four patients reported marked pain in one of their knees after fluid aspiration. All four patients recovered uneventfully. No signs of infection were registered.

Table E			
	Synovial fluid concentrations		
	Mean (SD)		
	Reconstructed knees N=11	Contralateral knees N=11	P-value
IL-1 β (pg/ml)	0.35 (0.3)	0.71 (0.2)	0.5
IL-6 (pg/ml)	0.49 (0.3)	9.11 (21.7)	0.2
TNF- α (pg/ml)	1.62 (0.6)	1.36 (0.7)	0.4
ARGS (pmol/ml)	5.8 (3,4)	7.2 (3.5)	0.2
COMP (U/l)	36.5 (18.4)	29.4 (21.5)	0.4
sGAG (μ g/ml)	33.7 (23.7)	36.1 (22.6)	0.7
There were no significant differences between the operated and contralateral knees in terms of IL- β , IL-6, TNF- α , sGAG, ARGS and COMP.			

Table E Cytokines and cartilage markers in reconstructed and contralateral knees.

05

DISCUSSION

5.1 TIMING OF ACL RECONSTRUCTION

The most important finding in Study I was that patients who underwent sub-acute ACL reconstruction had a better functional outcome in terms of the Lysholm knee scoring scale and Tegner activity level than patients who underwent delayed reconstruction. In this study, the Tegner activity level and Lysholm knee scoring scale were used as primary outcome measurements. Both scores have been validated for this purpose and shown to be reliable in this population [22,23]. A Lysholm knee scoring of 95-100 is considered excellent, 84-94 good, 65-83 fair and less than 65 poor. The Lysholm knee scoring scale was good in Group A and fair in Group B. Both groups reached the activity level of recreational sports [155], but patients in Group A were able to do this at a more advanced level. There are confounding methodological factors related to the activity level. Depending on how the question is asked, patients might report their activity at a certain time or refer to the highest level of activity at any time during a certain time interval [41]. It could also be that the patients undergoing delayed ACL reconstruction did not have the same desire to return to a similar high activity level, compared with patients in the sub-acutely treated group, who were more motivated to do so and accordingly

also returned to a higher activity level. Neither of the two groups returned to the pre-injury Tegner activity level. There are probably many reasons for this and one of them is psychosocial issues [9,79,41]. In addition, the fear of re-injury could be one reason why the patients do not return to their pre-injury activity level [10,80]. In contrast to the findings in Study I, a review by Smith et al. [143] compared the results of early (within three weeks) versus delayed (after six weeks) ACL reconstruction. They found no differences in terms of the Lysholm knee scoring scale and Tegner activity level. However, the time interval between their groups was different from that in Study I and these time intervals are not applicable in Sweden today.

During the last five to 10 years, it has been increasingly underlined that the decision to reconstruct the ACL should be individualised. Frobell et al. [48] conducted a prospective randomised study of early ACL reconstruction versus rehabilitation with optional delayed reconstruction and reported no differences between the groups according to the KOOS, Tegner activity scale, meniscal injury and osteoarthritic findings at the five-year follow-up. In spite of this, a waiting time of one year might be too long if

reconstruction is indicated. This is especially disadvantageous if the patient is not undergoing structured rehabilitation and has not received the information to adjust his/her activity level and lifestyle. For this reason, the risk of sustaining additional injuries to the cartilage and meniscus is most probably substantially increased during the waiting period. In Study I, there were no significant differences between the groups in terms of meniscal injuries and cartilage injuries. However, when comparing meniscal injuries requiring resection, they were significantly more frequent in the delayed group. Karlsson et al. [69] reported more meniscal injuries in the late reconstruction group, a finding that has also been reported by other researchers [14,46,64,66,103]. Several studies have shown that an ACL rupture in combination with meniscal injury is associated with an inferior clinical outcome in the short, mid and long term [74,86,138,167], as well as an increased risk of OA in the long term [75,94,112,115,116]. A recent

study [3] indicated that ACL reconstruction lowered the relative risk of OA changes at 10 years compared with a non-surgical approach. One surprising finding was that the risk of severe OA was higher in the reconstructed group and the authors suggested that this could probably be related to the return to a high activity level. In 2013 [41], Feller stated “Finally, it needs to be recognized that return to sport following ACL reconstruction is associated with a risk of further injury and the development of osteoarthritis”. One of the main indications for ACL reconstruction is the desire to return to a high activity level. However, the high re-rupture frequency among women playing football and also the poor outcome after revision surgery must be taken into consideration. The optimal timing for ACL reconstruction probably depends on different individual factors in each patient, such as the condition of the knee at the time of reconstruction and the patient’s motivation to undergo surgery and rehabilitation.

5.2 THE REGENERATION RATE AND MORPHOLOGY OF SEMITENDINOSUS AND GRACILIS TENDONS

The most important finding in Study II was that the tendons regenerated and regained an almost normal point of insertion and cross-sectional area compared with the non-operated side in the majority of patients. Another important finding was that the patients had a persistent strength deficit in deep knee flexion but not in internal rotation.

In Study II, the ST tendon regenerated in 89% and the G tendon in 95%. In line with the findings in the present study, Nakamae et al. [108] reported a 93%

regeneration rate after 12 months, while Nishino et al. [113] reported a regeneration rate of 91%, 12-43 months after harvesting only the ST tendon. Choi et al. [28], harvesting both tendons, reported 80% for the ST tendon and 76% for the G tendon. Other authors have, however, reported a lower regeneration rate [37,110,153]. Tadokoro et al. [153] found a regeneration rate of 79% for the ST but only 46% for the G tendon after a minimum of two years. Eriksson et al. [37] reported a regeneration rate of 75% after a minimum of six months, while

Nakamura [110] reported a 63% regeneration rate at 37 months after harvesting only the ST tendon. Ferretti et al. [44] conducted a study in which two groups of patients were compared. In the group with “a modified harvesting technique”, 100% of the ST tendon regenerated, while, in the control group, only 50% was found at the joint level.

The regenerated tendons had an almost normal insertion location in Study II. The insertion location of the regenerated tendons differs. Initially, the insertion of the regenerated tendons was reported to be more [142,160] proximal and medial than in the normal knee [29,122]. Simonian et al. [142] used MRI and found a more proximal insertion and an average difference in the insertion site of 26.7 mm for the ST and 47.1 mm for the G, compared with the non-operated side. Cross et al. [29] used MRI and reported that the regenerated tendon inserted on the medial popliteal fascia. Eriksson et al. [38,39] reported that the regenerated tendon inserts at the pes anserinus in an almost anatomical position. One explanation for this could be that only the ST tendon was harvested and the G tendon was left in place in some studies [38,108]. However, in Study II, both tendons were harvested and showed an almost normal insertion location, which is in line with the study by Choi et al. [28] Additional factors, such as different time intervals between the harvesting procedure and the radiographic examination, must be considered. This is supported by Nakame et al. [108] in a study using 3D CT, where they reported that, after one month, no patient had evidence of regeneration, while, after 12 months, all but two patients of 29 had regenerated tendon tissue and the tendons then coursed as expected

from the muscle bellies to their normal insertion site on the proximal tibia. The time interval in the present study is several years longer than in the other studies and we claim that the insertion point might gradually normalise over time. Another explanation for the inconsistency could be the different methods of defining the insertion point.

In Study II, it was decided to define the insertion point as the level at which the tendons conjoin in the pes anserinus below the joint line. This was considered to be the most reproducible way to compare the operated and non-operated sides. The tendon harvest procedure, differences in study population/size, male/female ratios, the use of a drain and the rehabilitation programme are other factors that could be of importance. Choi et al. [28] performed MRI before harvest and a repeat investigation a minimum of two years post-harvest of both the ST and G tendons. They are one of the few authors who noted a more distal insertion of the regenerated tendons than their original site. They used a “delicate reverse L-shaped” incision to preserve the sartorius fascia and protect the regenerating tube when harvesting the tendons. In Study II, the sartorius fascia was incised in a parallel fashion and not sutured. If the sartorius fascia is sutured tight after harvest, the post-harvest haematoma might not reach the normal insertion on the pes anserinus, thus resulting in a more proximal insertion.

There are reports that the regenerated tendons are hypertrophied in the early phase. Williams et al. [160] used MRI and reported that the regenerated tendon remains hypertrophied six months post-operatively. In Study II,

the cross-sectional area was close to the contralateral normal tendons, which is in

line with the studies by Choi et al. and Papandrea et al. [28,122].

5.3 STRENGTH DEFICITS AFTER SEMITENDINOSUS AND GRACILIS TENDON HARVEST

The patients in Study II were significantly weaker in knee flexion at 90 degrees compared with the contralateral side, despite the fact that the tendons had regenerated and regained an almost normal insertion location. This is in line with Murakami et al. [106], who reported a significant strength deficit at 90 and 120 degrees of knee flexion [106]. In addition, several other studies have reported a significant deficit in deep knee flexion [28,99,111,114,154,153]. For both internal rotation [136] and deep knee flexion [154], there are reports of a greater strength deficit after harvesting both the ST and G tendons compared with only the ST tendon. Several researchers have found a persistent shortening and hypotrophy of the ST and G muscles after tendon regeneration [39,113,144,160]. Williams et al. [160] found that the muscle bellies of the ST/G remained shortened after regeneration of the tendons. In line with these findings, Snow et al. [144] reported that, in the long term between nine and eleven years after harvesting the ST and G tendons, there was a reduction in peak cross-sectional muscle area and a compensatory hypertrophy of the long head of the biceps. In addition, Nishino et al. [113] found that deficits in deep knee flexion were associated with hypotrophy and shortening of the ST muscle after ACL reconstruction. This persistent shortening of the ST/G muscles in regenerated tendons was further confirmed by Choi et al. [28], who reported that the musculotendinous junction shifted

proximally by approximately 4 cm for the ST tendon and 3 cm for the G tendon, resulting in significant weakness in deep knee flexion, a minimum of two years after surgery.

A recent report by Nomura et al. [114] showed findings in line with previous reports. Regenerated ST tendon was confirmed in 21 of the 24 patients, but the muscle volume and muscle length of the ST in the operated limb were significantly smaller than those in the normal limb. The percentage of the knee flexion torque of the operated limb compared with that of the normal one was apparently lower at 105° (69.1%) and 90° (68.6%) than at 60° (84.4%). The authors concluded that preserving the morphology of the ST muscle-tendon complex is important and this has also been tried with different harvesting techniques [44,106] [135]. Murakami et al. [106] used an inducer technique, which resulted in regeneration and insertion at the pes anserinus, but there was still a significant torque deficit in deep knee flexion. Sasahara et al. [135] developed a harvesting technique in which they left part of the width of the ST insertion by splitting it. This resulted in significantly less shortening of the ST muscle (mean 8 mm) versus a mean of 36 mm for the whole tendon harvest group and significantly less torque deficit in knee flexion.

The hamstring muscles are considered to be ACL agonists [171]. A weakness

in internal rotation could reduce the protection of the ACL during cutting manoeuvres as a result of less prevention from excessive external rotation [15]. An insertion site located at the medial tibia fascia or popliteal fascia [29,122] would theoretically reduce the ability to generate force in internal rotation. Because of this, Ferretti et al. [44] conducted a study in which they used a modified harvesting technique. They showed that the weakness in internal rotation could be minimised in the group with the modified technique. Segawa et al. [136] reported that male patients regained normal strength in internal rotation after rehabilitation, while in females there was a persistent strength deficit.

In Study II, the regenerated tendons had an almost normal insertion and the patients were significantly stronger in terms of internal rotation at 60 deg/s on the operated side, while, at 180 deg/s, there was no significant difference. One reason for this could be that other muscles are able to undergo hypertrophy and compensate for weakness in internal rotation.

Taken as a whole, we claim that, when the tendons are harvested, a shortening of the muscle bellies occurs. Factors that affect the strength deficit after ST/G tendon harvest are the amount of tendon regeneration, the proximal shift of the musculotendinous junction, muscle hypotrophy, the insertion point of the regenerated tendons and whether other muscles are able to compensate and undergo hypertrophy.

5.4 BIOPSIES OF REGENERATED TENDON

The principal finding in Study III was that the appearance of the regenerated ST tendon was similar to that of the contralateral non-harvested ST tendon when evaluated histologically a median of 8.4 years after harvesting. To our knowledge, this is the first study in which specimens from the regenerated ST tendon are compared with contralateral non-harvested tendon specimens from the same patients.

The way in which the tendons regenerate is not fully understood, but the present opinion is that a gradual solidification takes place along the harvest site. Papandrea et al. [122] performed serial ultrasonography in their patients and they registered an initial haematoma followed by oedema with gradual solidification along the entire harvest site. Otoshi et al. [120] presented additional

support for this theory using an animal model to assess tendon regeneration in Achilles tendons. They describe a similar regeneration and maturation process uniformly along the length of the regenerated tendon and conclude that the haematoma scaffold enhances the migration of fibroblast precursor cells from the surrounding peritendinous tissue and tendon sheath. Histological samples were obtained using biopsies from the tendons. There are previously published studies with biopsies of regenerated ST tendon [38,43,117,169] confirming that it is essentially real tendon tissue that regenerates and not just scar tissue.

Ferretti et al. [43] obtained open biopsies from regenerated tendon in three patients, one after six months and two after two years. In the specimens retrieved two years post-operatively, the central

thicker portion of the specimen was occupied by well-oriented tendon-like fibres, together with uniformly distributed spindle-shaped cells that had the appearance of mature tenocytes. Okahashi [117] collected surgical biopsies of regenerated ST tendons in nine patients. They found that, histologically and immunohistochemically, the regenerated tendon closely resembled normal tendon one year after harvest. However, no regularity in cell nucleus size, shape or distribution was found in the regenerated tendon. Eriksson et al. [38] obtained open biopsies from the regenerated ST tendon 20 months after harvest in five patients. They reported that the regenerated tendons displayed the features of a normal tendon, but there were focal small scar-like areas with more irregularly oriented collagen, increased fibroblastic proliferation and capillary formation compared with normal control tendon. In Study III there was no significant difference in fibre structure and cellularity between the regenerated ST tendon and normal control tendon. The fibre structure in both the regenerated and non-harvested control tendons generally showed slight separation of and increased waviness in the fibres. However, three of the regenerated tendons displayed an appearance that was classified as grade 3 according to the scoring system (Table A) in focal areas in terms of fibre structure. These findings are similar to those reported by Eriksson et al. [38] where they reported small areas with scar formation. Nakamae et al. [109] reported two cases of unsuccessful regeneration of the ST tendon at 12 months using 3D CT imaging. These patients had experienced a sudden sharp pain in the posterior aspect of their thighs when their hamstring muscles were subjected to an aggressive load shortly after surgery.

This raises the question of whether something happened in the early regeneration process to the patients in Study III who were classified as grade 3 in terms of fibre structure. Trauma with a micro-rupture in the weak tendon that did not result in a haematoma as seen after tendon stripping could be the cause of loss of fibre structure. This might also be the reason why some patients do not display tendon regeneration. If several biopsies had been obtained from the entire length of the ST tendon, it is possible that focal scarring would have been found in more of the regenerated ST tendons.

Proteoglycans with GAGs are negatively charged molecules that entrain water. In tendons, they contribute to the elasticity and stabilisation of the collagenous system. Compared with cartilage, the concentration of GAGs is considerably lower in tendons. High levels of GAGs are found in ruptured [68] tendons and tendons subjected to compression [162] forces, as well as in Achilles tendinopathy [105] and patellar tendinosis (“jumper’s knee”) [76]. In the normal patellar tendon, GAGs appear in low concentrations [4,5]. The content of GAGs in the present study was low or non-detectable in most regenerated specimens, which could be regarded as a sign of tendon normalisation.

The four-point scoring system used in the present study was initially developed for evaluating alterations in the patellar tendon, with a score of 0 for all the measured items in the healthy patellar tendon. In the present study, the histological score was slightly higher for the non-harvested ST tendon than for the healthy patellar tendon. However, a different morphological appearance between the patellar tendon and the ST tendon has been de-

scribed by Hadjicostas et al. [53]. They reported increased cellularity, as well as a tendency towards increased vascularity, in the ST tendon in 20 cadavers. This is similar to the findings in the present study.

Since the ST tendon is able to regenerate, the question of whether it can be used in revision surgery has arisen. Re-harvested patellar tendon has been used for revision surgery [73], even though MRI and biopsy studies [70,152] reveal that the patellar tendon does not normalise after harvesting its central third. The results with re-harvested patellar tendon for ACL revision surgery are inferior compared with primary harvested patellar tendon autografts [73,90]. In animal models, the biomechanical strength of regenerated ST [51,88] tendons has been described as

being inferior to that of normal tendons up to one year after harvest but with a trend towards increasing strength over time [88]. Since ST/G tendon regeneration is unpredictable in terms of focal scarring and until studies with long-term biomechanical testing in humans have been performed, it is our opinion that regenerated ST/G tendon cannot be recommended for ACL revision surgery.

To cover the full regeneration process and assess the final histological outcome for the entire tendon, the optimal solution would be a long-term serial study with histological examination of the entire regenerated tissue with comparisons with normal tendon. This would, however, be difficult for ethical reasons.

5.5 CARTILAGE MARKERS

In Study IV, there were no significant differences in the average mean concentrations of ARGS, COMP and sGAG in the synovial fluids from the reconstructed and the contralateral knee. The diagnosis of OA is defined by a combination of clinical and radiographic findings. When joint space narrowing becomes visible on standard weight-bearing radiographs, cartilage degradation is already advanced. If biomarkers of cartilage degradation can be identified, there is at least a theoretical possibility of improving the treatment options. If there were a treatment that was able to stop cartilage degradation, it would be best to start it before the cartilage is severely damaged. There are, however, patients with radiographic findings indicating degeneration/OA without symptoms. An acute injury to the ACL or menisci of the knee causes

a marked increase in the concentration of ARGS-aggrecan and COMP fragments in the joint fluid and it has therefore been suggested that the levels of ARGS-aggrecan and COMP can be used as biomarkers of knee injuries and OA [84,97,146,147]. Moreover, the synovial fluid sGAG concentration is elevated in the acute phase after a knee injury [150] and it has been suggested that this could be used as an early marker of cartilage disease and breakdown [149]. Since almost 90% of the aggrecan mass is comprised of substituted sGAG chains [77], measuring the total amount of sGAG provides a wider insight into the degradation process of cartilage and the release of aggrecan fragments in synovial fluid [77,150]. It is also important to remember that not all patients develop OA changes after meniscal and ACL injury. There are

many factors, such as weight, genes and co-morbidity, which affect the risk of OA. Andersson et al. [7] suggested three-phase tissue damage after an intra-articular fracture where chondrocyte death leads to an acute inflammatory response, where IL 1beta is elevated and there is then ongoing chronic inflammation, remodelling/degeneration. This raises the question of whether patients have different inflammatory responses leading to ongoing cartilage degradation in some and not in others, as there are many patients with both ACL and meniscal injury that do not develop PTOA. In Study IV, the aim was to examine whether sGAG, ARGS-aggrecan and COMP were elevated several years after ACL reconstruction, indicating ongoing disturbed cartilage metabolism/degradation. For both ARGS and COMP, there was a wide range of concentration values in the synovial fluids from the reconstructed and the contralateral knee and no significant differences were found in the average mean concentrations. This could be due to the non-homogeneous group of patients. However, Larsson et al. [84] found that the synovial fluid ARGS-aggrecan decreases after knee injury and approaches the levels of subjects with healthy knees three to 12 months after injury. The synovial fluid levels found in the subjects in Study IV are similar to the levels found in non-injured reference subjects [82]. Likewise, Dahlberg et al. [30] reported that, in the acute phase, there is an increase in the concentrations of aggrecan and COMP in the joint fluid of the injured knee compared with the unilateral non-injured knee, but, after two to five years, the concentrations were similar in the knees. Moreover, Dahlberg

et al. [30] concluded that the synovial fluid levels of proteoglycan fragments are influenced by the mass of cartilage matrix remaining in the joint, the inflammatory activity in the joint and the metabolic activity of the cartilage cells. It appears that the concentrations of sGAG decrease with time after injury. Elsaid et al. [35] reported that the concentration of sGAG was higher in ACL-injured knees than in contralateral non-injured knees. However, the maximum time between injury and sampling was one year in their study. In Study IV, many years after ACL injury, no significant differences for sGAG were found. In Study IV, the group of patients varied in age, time from reconstruction to fluid sampling and number of meniscal and cartilage injuries at reconstruction and this could be one of the reasons why no differences were found, as studies that have attempted to develop a molecular marker for the detection of the early stages of OA have revealed a considerable range of concentrations within different groups of patients [27,125]. This makes it difficult to develop reference intervals for use in clinical situations. In Study IV, there were significantly more signs of degeneration on the weight-bearing radiographs and significantly more meniscal and cartilage injuries on the MRI of the ACL-reconstructed knees compared with the contralateral knees. Larsson et al. [83] found no differences in the synovial fluid concentrations of ARGS-aggrecan 18 years after meniscectomy compared with non-operated individuals. They stated that higher synovial fluid ARGS-aggrecan concentrations were weakly associated with less progression of radiographic knee OA.

5.6 INFLAMMATORY CYTOKINES

In the early phase after acute ACL injury, the levels of IL-1 β , IL-6, IL-8 and TNF- α have been reported to be significantly higher compared with an age-matched reference group with healthy knees [150]. Kraus et al. [78] found elevated levels of pro-inflammatory cytokines up to one month after ACL injury. If the inflammatory cytokines remain elevated, they could cause an ongoing low-grade inflammation and degradation of the cartilage. In contrast to the findings in Study IV, Marks et al. [100] found that the concentrations of IL-1 β and TNF- α were significantly higher in patients with chronic ACL ruptures than in the contralateral normal knees a mean of three years after the injury. In addition, Higuchi et al. [58] reported similar findings for IL-6 and TNF- α a mean of 27 weeks after ACL rupture. However, in contrast to the results of Marks et al., they reported that the IL-1 β concentration was below the detectable range.

Synovial fluid cytokine levels decrease over time after injury [35]. In Study IV, the time interval between the injury and

synovial fluid sampling was much longer than in previous studies and this might be the reason why no differences were found. Intuitively, the body's initial inflammatory response to injury should disappear with time. It is possible that the inflammation is sustained for longer in some patients, causing an ongoing disturbed cartilage metabolism, but the question of which patients and why remains unanswered. To summarise, the results in Study IV suggest that, by eight years post-injury and surgery, there is no longer an active inflammatory process degrading the joint. It is likely that a combination of biomechanical factors, such as residual instability, initial irreversible chondrocyte damage, acute focal cartilage lesions and meniscal insufficiency, are the mechanism for ongoing degenerative changes. Further research could productively focus on earlier repeated measurements of cytokine profiles and cartilage markers in an attempt to better define and identify a pathological response/concentrations in those patients developing symptomatic PTOA in the long run compared with the ones that do not.

5.7 RADIOGRAPHS

In Study IV, more radiographic degenerative changes were observed in the ACL-reconstructed knees at follow-up. The Fairbank system rates small degenerative changes and it is possible that these changes are not yet symptomatic or will never be. This is also supported by the KOOS score, Lysholm knee scoring scale, Tegner activity level and ROM, which were generally satisfactory. Even if the ACL is reconstructed and the laxity

is improved, the loading pattern of the joint is probably not restored and it is perhaps the change in biomechanics and load on the cartilage after meniscal and ACL injury that causes the degenerative changes and not an ongoing inflammatory process. All the patients in Study IV had intact grafts, but, although the ACL was reconstructed, completely normal biomechanics of the knee were probably never restored [21]. The clinical finding

for the Lachman test supports this, as well as the correlation found between the Lachman test and cartilage and meniscal changes on MRI.

Previous studies have not shown that ACL reconstruction delays the development of PTOA [96]. In contrast to previous reports, a recent systematic review and meta-analysis [3] from 2014 supports the opinion that ACL-reconstructed patients have a reduced risk of developing PTOA at 10 years compared with non-reconstructed ACL injured patients. These authors also suggest that a return to sport after ligament reconstruction might exacerbate the development of PTOA. However, it is important to remember that the surgical technique and positioning of the ACL graft have changed over the years. The arthroscopic technique has evolved over the years and the positioning of the graft has changed from a more vertical to the current concept of anatomical reconstruction. It is important to remember that the

anatomical concept was initiated in the 21st century and the long-term results might be different 10-15 years from now. Taken as a whole, better reconstruction techniques might at least theoretically produce a lower incidence of PTOA after ACL reconstruction.

Some sports involving pivoting and cutting manoeuvres might elevate the risk of PTOA [3]. The patients in Study IV had exposed their contralateral knee to exactly the same activity level over the years. In a study design with bilateral analyses of the knees, like the one we used in Study IV, other risk factors for PTOA, such as weight, genes and co-morbidity, are automatically controlled for.

The Fairbank system rates minor degenerative changes. The advantages when it comes to detecting early changes are that specific treatment, such as rehabilitation programmes and the adjustment of activity, could begin earlier.

06

STRENGTHS & LIMITATIONS

6.1 STUDY I

The strengths of Study I were that the patients in the study groups were comparable in terms of age, gender and pre-injury Tegner activity level, they were reconstructed using the same type of graft and surgical technique and had the same rehabilitation programme. These factors could otherwise

contribute to selection biases. One major limitation is the study design. No power calculation before planning the study and no randomisation of the patients were performed. Further weaknesses are that the study might be under-powered and that no radiographs were obtained.

6.2 STUDY II

The strengths of Study II were the long follow-up period and the fact that bilateral radiographic examinations and muscle strength measurements were performed by independent observers. The limitations

include the cross-sectional and retrospective design without multiple images over time and the fact that no MRIs were obtained from the proximal thigh to measure the muscle volumes.

6.3 STUDY III

The strengths of Study III were its long-term design and the paired biopsies from the patients' regenerated and non-harvested ST tendon, thus enabling the patient to serve as her/his own control. We chose only to include patients in whom both the ST and G tendons were harvested. This means that the biopsy was always obtained from a regenerated tendon. One weakness of Study III is that no intra-observer or inter-observer reliability testing was performed on the score that was used in the study. However, the original score from which the score used in the present study

was developed has been tested for intra-observer reliability with satisfactory agreement for different tendons [105]. Another limitation is that five biopsies contained an insufficient amount of material for analysis and there is a potential risk that no significant differences were found, due to a type-II error. Performing the power analyses on the non-parametric primary variable is also a potential weakness. No biomechanical tests of the regenerated ST tendon have been performed, for obvious ethical reasons, and so the true quality of the regenerated tendon is unknown.

6.4 STUDY IV

The strengths of Study IV were that the findings are consistent with those of prior studies reporting that the high levels of inflammatory markers seen at the time of acute ACL injury and surgery decline over time. Multiple confounding factors for OA are controlled for when using the patient's own uninjured knee as a control. One limitation is the small number of patients, which may result in a cohort that is not representative of the overall patient population and might still involve a type-II error. However, as the patient was his/

her own control, we calculated that the sample size would provide good enough power for the study. Another limitation is that there was a wide age range and time elapsing from injury to surgery. Furthermore, the presence of meniscal and cartilage injury was different among the patients and the system for evaluating cartilage and meniscal injury is rough. It is also important to consider that, if biomarkers circulate in the patients' blood, they might affect the intra-articular concentrations in all joints.

07

CONCLUSIONS

7.1 STUDY I

The patients who underwent sub-acute reconstruction had a significantly better outcome in terms of the Lysholm score and Tegner activity level than the patients who underwent delayed reconstruction. No significant difference was

found between the groups in terms of total meniscal and chondral damage, but there were significantly more partial meniscal resections in the group of patients who underwent delayed reconstruction.

7.2 STUDY II

The ST/G tendons regenerated, as seen on MRI, in a large majority of patients and regained an almost normal insertion point on the pes anserinus a minimum of six years after the harvesting proce-

dure. The regenerated tendons had a cross-sectional area similar to that of the contralateral side. The patients revealed a persistent strength deficit in deep knee flexion but not in internal rotation.

7.3 STUDY III

The ST tendon may regain a histological appearance similar to that of the non-harvested contralateral tendon, a median of

8.4 years after harvesting. However, in some tendons, loss of fibre structure was found.

7.4 STUDY IV

Eight years after ACL reconstruction, there were no significant differences in inflammatory cytokines and biomarkers of cartilage degeneration between the non-operated and the ACL-reconstruct-

ed knee, even though there were more OA changes and meniscal and cartilage damage in the operated knee, as seen on weight-bearing radiographs and MRI.

08

FUTURE PERSPECTIVES

Optimal timing of ACL reconstruction

To be able to compare studies of the timing of ACL reconstruction, there needs to be a general consensus about the definition of time intervals for early, sub-acute and delayed surgery. Evidence-based research should be the foundation of our guidelines. The preferred method for the research question would be a prospective randomised study design (with power calculation), but, as studies have shown an increased incidence of meniscal injuries with time in non-reconstructed ACL-injured knees and meniscal injuries increase the risk of PTOA, there are ethical aspects that have to be considered as well. Register studies could therefore be a better method. To accommodate this research, it would be of great value if the ACL register was able to register all ACL injuries as soon as they are diagnosed and it would also be preferable if the patients for whom a non-surgical approach is chosen were followed and registered in the same way as the reconstructed cases. Register studies of timing could then be performed and data on the amount of meniscal and cartilage injury could be extracted and analysed.

Semitendinosus and gracilis autografts

Graft morbidity with persistent weakness in deep knee flexion after ST and G harvest is probably due to the proximal shift

of the muscle tendinous junction and persistent shortening and hypotrophy of the muscle bellies. The loss of strength in internal rotation is probably caused by an altered insertion of the regenerated tendons. It would be interesting to continue the work of Sasahara et al. [135], who developed a partial harvesting technique in which they left part of the width of the ST insertion by splitting it, resulting in significantly less shortening of the ST muscle and significantly less torque deficit in knee flexion torque.

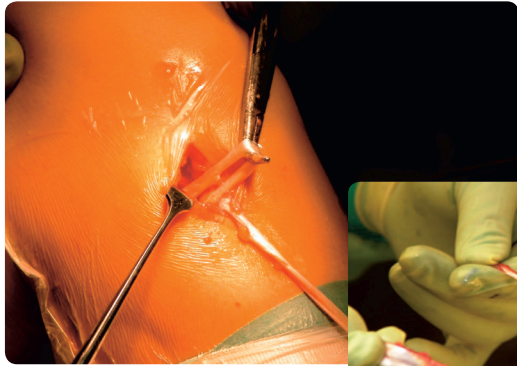
It would also be interesting to further evaluate why there is focal scarring in some regenerated tendons and also why the tendons do not regenerate in some patients. Should the rehabilitation and harvesting technique be modified? Is the use of a drain disadvantageous from a perspective where the post-operative haematoma might be of importance? Could suturing the sartorius fascia stop the post-operative haematoma from reaching the normal insertion site on the pes anserinus?

Taken as a whole, if the ST tendon has enough graft material, it would be beneficial to spare the G tendon, thereby enabling this muscle tendon complex to undergo hypertrophy and compensate for some of the strength loss. Graft choice should be individualised and, especially in athletes dependent on deep knee flexion, the BPTB graft should be considered.

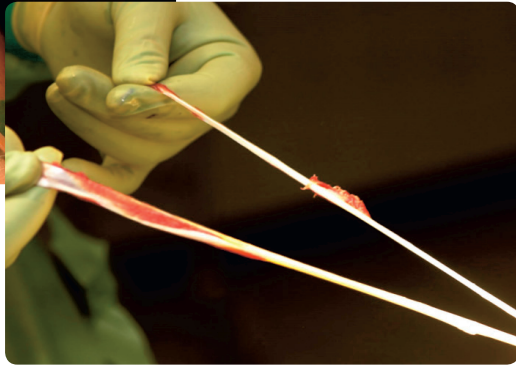
Post-traumatic osteoarthritis

Should the research focus on the ACL- and meniscus-injured patients who do not develop OA? Perhaps there is something unique about these patients. In animal research on mice where the gene for ADAMTS-5 (metalloprotease, enzyme required in proteoglycan degradation) was inhibited, OA did not develop. Future research should probably focus on earlier stages of the inflammatory process and attempt to delineate the time course of the rise and fall of cytokines and biomarkers for cartilage metabolism, with the aim of identifying a possible therapeutic window. If a certain inflammatory response was detected in patients developing PTOA, it might be possible to inhibit this in the same way as in rheumatoid arthritis. Since joint fluid aspiration is associated with risks and serum markers are influenced by

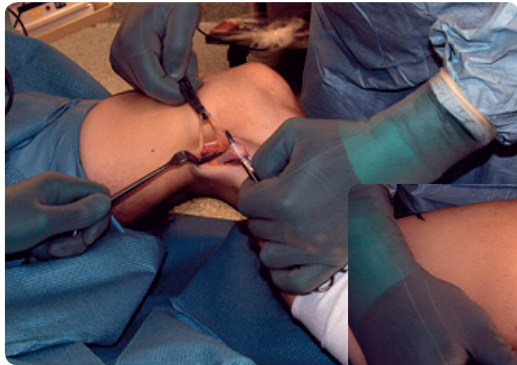
other ongoing biological processes in the body, the research field is complicated and animal studies are of value in distinguishing pathological patterns. Based on animal studies and existing knowledge, a study using bilateral dGEMRIC at injury, together with bilateral joint fluid aspirations which are then repeated over time, could be a useful study design. Most of the PTOA is still probably due to changes in the biomechanics and load on the cartilage after an ACL injury and it could also be that the initial trauma to the cartilage, with bone bruising and chondrocyte death, is the main contributory factor. To summarise, the best thing is to not sustain an ACL injury at all and, as there are studies reporting that the ACL injury incidence can be lowered by using neuromuscular proprioceptive exercise programmes [25,57,107,132,158], the prevention of the injury has to be one of the primary points of focus.



15a



15b



1a



1b

Figure 15a-b *Normal ST and G tendons at harvest during ACL reconstruction (15a and b). For comparison, images 1a and 1b of regenerated ST and G tendons, two years and 11 months after the initial harvest, are shown. The tendons were reharvested due to pain and snapping.*

09

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10

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