ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION SURGERY

Aspects of graft choice, graft fixation and bone mineral loss

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To Mariann, Viktor, Mikael and Olle

ABSTRACT

The aim of this thesis was to measure bone mineral changes in the calcanei, hips and lumbar spine of patients reconstructed with bone-patellar tendon-bone (BPTB) or hamstring tendon (HT) autografts following anterior cruciate ligament (ACL) injury. Furthermore, the aim was to compare the clinical results after ACL revision reconstruction with either reharvested ipsilateral or contralateral BPTB autografts A third aim was to compare bone tunnel widening after ACL reconstruction using either bioabsorbable or metal interference screws. In Study I, bone mineral areal mass (BMA) was measured in the calcanei using the dual-energy photon absorptiometry (DPA) technique in 92 male patients scheduled for ACL reconstruction using BPTB autografts. The patients had a significantly lower BMA on the injured side compared with the uninjured side, before the reconstruction and two years after the reconstruction. A high level of activity correlated with the BMA on both the injured and the uninjured side two years after the reconstruction. In Study V, BMA was prospectively measured using the dual-energy X-ray absorptiometry (DEXA) technique in 67 patients scheduled for ACL reconstruction with HT autografts. After five years both female and male patients had lost more BMA in the calcanei and the hips compared with the age-dependent decrease in reference populations made up of normal healthy individuals. The BMA loss was not correlated with activity level, knee function scores or the health-related quality-of-life score EQ-5D. In Study III, 77 patients, scheduled for ACL reconstruction using HT autografts were randomised to poly-L-lactide acid (PLLA) or metallic screw fixation of the grafts. After eight years, the bone-tunnel widening was significantly larger on the femoral side but not on the tibial side in the PLLA group compared with the metal group. There were no differences in the clinical evaluation parameters between the two groups after eight years. In Study II, 24 patients underwent surgery using reharvested or primary harvested patellar tendon grafts in ACL revision reconstruction and they were assessed after two years in terms of their subjective and objective outcome, activity level and MRI findings relating to the patellar tendons. The patients who were given primary harvested, contralateral BPTB grafts had a significantly better outcome in the Lysholm knee score than the patients who were given reharvested BPTB grafts. Magnetic resonance imaging (MRI) findings were unable to detect any differences in the length, width, thickness or size of the residual gaps in the reharvested tendons compared with the primary harvested tendons. In Study IV, patients from the reharvested group returned for histological, radiographic and clinical evaluation three and ten years after the ACL revision reconstruction. Histological evaluation revealed that, after three years, the tendons showed signs of "ligamentisation" with an increased number of cells, capillaries and glycosaminoglycan content.

Keywords: Anterior cruciate ligament, Reconstruction, Revision, PLLA, Bone mineral areal mass, DEXA
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SAMMANFATTNING PÅ SVENSKA

Syftet med avhandlingen var att mäta förändringar av benmineralhalt i hälbenen, i höfterna och i ländryggen hos patienter som opererats med knäskålsenegraft (BPTB) eller hamstringssenegraft (HT) efter en främre korsbandsskada. Ytterligare ett syfte vara att jämföra det kliniska resultatet efter revision av en främre korsbandsskada med antingen samma sidas återskördade knäskålssena eller knäskålssena från det friska, icke opererade knät. Ett tredje syfte var att jämföra det kliniska resultatet och benkanalernas storlek i två randomiserade grupper opererade med resorberbara eller metallskruvar åtta år efter främre korsbandsrekonstruktion. I delarbete I mättes benmineralhalt, "bone mineral areal mass" (BMA) i båda hälar hos 92 manliga patienter före och efter främre korsbandsrekonstruktion med BPTB graft. Patienterna hade signifikant lägre BMA i hälen på den skadade sidan jämfört med den oskadade sidan före rekonstruktion och två år efter rekonstruktion. En hög aktivitetsnivå korrelerade med högre BMA i både det opererade och i det icke opererade benet två år efter rekonstruktionen. I delarbete V följdes 67 patienter av båda könen med "dual-energy X-ray absorptiometry" (DEXA) mätning av benmineralhalt i hälbenen, i höfterna och i ländryggen under fem år med upprepade mätningar. Fem år efter rekonstruktion med HT graft hade båda könen i studien förlorat mer BMA i båda hälbenen och i båda höfterna jämfört med den förväntade åldersbetingade minskningen hos en normalpopulation. BMA förlusten korrelerade inte med aktivitetsnivån, knäfunktionen eller livskvalitéscore. Delarbete III var en 8-årsuppföljning av en randomiserad studie av patienter opererade med antingen resorberbara poly-L-lactide acid (PLLA) skruvar eller metallskruvar och HT graft. Benkanalerna var efter åtta år signifikant större på den femorala sidan men inte på den tibiala sidan och fler benkanaler var helt beninläkta i metall gruppen jämfört med PLLA gruppen. Skillnaden i kanalernas storlek korrelerade inte med det kliniska utfallet av rekonstruktionen. I delarbete II revisionsoperades 24 patienter uppdelade i två grupper med knäskålsena. I den ena gruppen med 12 patienter där knäskålssenan skördades för andra gången blev två-årsutfallet sämre jämfört med hos 12 patienter i den andra gruppen där operationen utfördes med andra sidans friska knäskålssena. Lysholms knäscore visade signifikant sämre resultat samtidigt som aktivitetsnivå, knästabilitet och andra knätest visade lika bra resultat i de båda grupperna. En magnetkamera undersökning efter två år visade att senans längd, bredd och tjocklek samt storleken på defekten i senan inte visade någon skillnad beroende på om senan hade skördats en gång eller två gånger. En fördjupad radiologisk och histologisk undersökning av fyra patienter från den återskördade gruppen gjordes i delarbete IV. Efter både tre och 10 år förekom ett dåligt kliniskt resultat men vid knäartroskopi och ljusmikroskopisk undersökning av den återskördade senan sågs en tendens till transformation från sena till ligament, s.k. ligamentisering med hög förekomst av celler, kapillärer och glycosaminoglykaner.

LIST OF PAPERS

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

I. **Bone mineral assessments in the calcaneus after anterior cruciate ligament injury. An investigation of 92 male patients before and two years after reconstruction or revision surgery.**
Kartus J, Stener S, Nilsén R, Nilsson U, Eriksson BI, Karlsson J
Scand J Med Sci Sports. 1998;8(6):449-455

II. **Ipsi- or contralateral patellar tendon graft in anterior cruciate ligament revision surgery. A comparison of two methods.**
Kartus J, Stener S, Lindahl S, Eriksson BI, Karlsson J
Am J Sports Med. 1998;26(4):499-504

III. **A long-term, prospective, randomized study comparing biodegradable and metal interference screws in anterior cruciate ligament reconstruction surgery: radiographic results and clinical outcome.**
Stener S, Ejerhed L, Sernert N, Laxdal G, Rostgård-Christensen L, Kartus J
Am J Sports Med. 2010;38(8):1598-1605

IV. **The reharvested patellar tendon has the potential for ligamentization when used for ACL revision surgery.**
Stener S, Ejerhed L, Sernert N, Movin T, Papadogiannakis N, Kartus J
Knee Surg Sports Traumatol Arthrosc 2012;20(6):1168-1174

V. **Anterior cruciate ligament reconstruction reduces bone mineral areal mass.**
Stener S, Kartus J, Ejerhed L
Arthroscopy; accepted for publication DOI: 10.1016/j.arthro.2013.08.13

Additional relevant papers by the author.

Is bracing after anterior cruciate ligament reconstruction necessary? A 2-year follow-up of 78 consecutive patients rehabilitated with or without a brace

Kartus J, Stener S, Köhler K, Sernert N, Eriksson B.I, Karlsson J
Knee Surg Sports Traumatol Arthrosc 1997;5(3):157-161

Factors affecting donor-site morbidity after anterior cruciate ligament reconstruction using bone-patellar tendon-bone autografts

Kartus J, Stener S, Lindahl S, Engström B, Eriksson B. I, Karlsson J
Knee Surg Sports Traumatol Arthrosc 1997;5(4):222-228

CONTENTS

ABBREVIATIONS

ACL	Anterior Cruciate Ligament
BMA	Bone Mineral Areal mass
BMD	Bone Mineral Density
BMU	Basic Multicellular Unit or Bone Metabolic Unit
BPTB	Bone- Patellar Tendon-Bone
CI	Confidence Interval
CRP	C-reactive protein, a marker of early inflammatory reaction
CV	Coefficient of Variation
DPA	Dual-energy Photon Absorptiometry
EQ-5D	Euroqol, Quality of life-5 Dimensions
HT	Hamstring Tendon
HRQoL	Health-Related Quality of Life
IKDC	International Knee Documentation Committee
KOOS	Knee injury and Osteoarthritis Outcome Score
M-A	Meta-Analysis
MRI	Magnetic Resonance Imaging
PBM	Peak Bone Mass
PCL	Posterior Cruciate Ligament
QCT	Quantitative Computed Tomography
RCT	Randomised Controlled Trial
SD	Standard Deviation

BRIEF DEFINITIONS

BMA	The bone mineral content divided by the area of the image of a bone projected in two dimensions, which is the type of bone density that is produced by dual- and single-energy X-ray absorptiometry. BMA is measured in grams/mm². BMD is used in some literature instead of BMA and is presented in grams/mm².
DEXA	Dual-energy X-ray absorptiometry machine, used for the diagnosis of osteoporosis.
Osteoporosis	Defined by the Working Group of the World Health Organisation as a bone density T-score at or below 2.5 standard deviations (SDs) below normal peak values for young adults.
Osteopenia	A term coined by the Working Group of the World Health Organisation to refer to T-scores between 1.0 and 2.5 SD.
keV	The unit 1000 electron volts which is the energy produced by the isotopes or X-ray cathode in the DPA and DEXA machines.
Bq	Bequerel. SI-derived unit of radioactivity. One Bq is defined as the activity of radioactive material in which one nucleus decays per second. Units in s-1.
Sv	Sievert. SI-derived unit of ionising radiation dose often expressed in mSv. The unit is joule/kilograms. A measure of the health effect of radiation on biological tissue by radiation. One Sievert carries a 5.5% chance of developing cancer during a person's remaining lifetime.
Trabecular bone	Bone structured with thin trabeculae and large holes, web-like appearance. Four to five times more surface area than cortical bone.
Cortical bone	The compact bone surrounding the medullary canal of long bones usually where tendons and ligaments connect with the bones.
Modelling of bone	Formation of new bone without prior bone resorption in the BMU during growth.
Remodelling of bone	Resorption by osteoclasts and formation by osteoblasts in the BMU during adulthood.
CRP response	CRP binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. CRP thus participates in the clearance of necrotic and apoptotic cells.

Closed kinetic chain exercises	Physical exercises performed where the hand or foot is fixed to the ground or base of a machine. Usually involve more than one muscle group (agonists and antagonists). Generally induce compressive forces on joints and are therefore considered safer and more functional.
Open kinetic chain exercises	Physical exercises performed where the hand or foot is free to move. Usually involve only one muscle group. Generally induce shear forces on joints but can selectively target certain muscles which is advantageous in later stages of rehabilitation.
Plyometric exercises	Exercises based around having muscles exert maximum force in as short time as possible. The training focuses on learning to move from deceleration in the eccentric mode to acceleration in the concentric mode, thereby increasing both power and speed. This often includes jumping and landing in explosive manner.
T-score	The difference in the number of standard deviations between the mean bone mineral density value of the individual and the mean value of a group of young healthy adults of the same sex.
Z-score	The difference in the number of standard deviations between the mean bone mineral density value of the individual and a group of people of the same sex and age.
Coefficient of variance	Standard deviation divided by the mean value. A parameter expressed in % to describe the precision of a measuring device.
Concentric contraction	A muscle contraction in which the muscles shorten while generating force.
Eccentric contraction	A muscle contraction in which the muscles elongate while under tension due to an opposing force greater than that the muscle generates.
High-power field	The area visible in microscopy under the maximum magnification power of the objective being used. Often, this represents a 400x magnification level when referenced in scientific papers.

01 INTRODUCTION

1.1 BACKGROUND

Since anterior cruciate ligament (ACL) ruptures are among the most common sport and recreational injuries, there is an ongoing debate among surgeons, physiotherapists, researchers and trainers regarding the optimal treatment, rehabilitation and preventive efforts. With a yearly incidence of 80/100,000 in Sweden, ~5,800 patients suffer an ACL injury every year, approximately 3,000 of whom are treated surgically, (www.aclregister.nu). Indications for surgical treatment are repeated symptoms of knee instability and the failure of conservative treatment. More than 95% of the patients undergoing surgery last year in Sweden underwent arthroscopic reconstruction using hamstring autografts. Graft fixations with cortical buttons on the femoral side and bioabsorbable interference screws on the tibial side are the most common fixation methods. Since different surgical techniques and fixation methods render good, reliable results, it has become increasingly important to take account of surgical morbidity when decisions are taken before surgery. Postoperative extension deficit, hamstring weakness, anterior knee pain and the loss of skin sensation are some of the most common problems related to surgery.[87,88,55] In addition to the symptoms documented at follow-up, there are also other side-effects related to the surgical trauma. Bone-tunnel widening as seen on plain radiographs or computed tomography (CT) after surgery can produce problems at revision surgery and is discussed in Study III in this thesis. Another side-effect is a reduction in bone mineral areal mass (BMA), which is discussed in Studies I and V. Study II is a controlled two-year follow-up study comparing two different patellar-tendon grafts in ACL revision surgery and, in Study IV, patients from this study are re-evaluated after three and ten years.

One of the aspects discussed in this thesis is bone mineral changes after ACL reconstruction surgery. In the decision between conservative and surgical treatment of an ACL injury, a return to the pre-injury level of activity should be one of the main goals. With a successful rehabilitation and return to sports or recreational physical activity, bone mineral loss can hopefully be prevented. A reduction in BMA and a deterioration in bone structure increase the risk of fractures (Figure 1). In Scandinavia, it is estimated that 50% of women and 30% of men aged 50 and more will sustain a fracture during their remaining lifetime.[140,139,83] The highest incidences of osteoporosis-related fractures have been described in Northern Europe and Scandinavia. The lifetime risk of a hip fracture is 16-18% in Caucasian women and 5-6% in Caucasian men.[84,82] Borgström et al. report a rise in the annual cost of fragility fractures in Sweden from 15,183 MSEK in 2013 to 26,301 MSEK in 2050.[21] A sedentary lifestyle impairs and physical activities enhance BMA and therefore bone strength.[66,18] Factors acting directly on bone cells in adults, such as calcium, vitamin D, hormones and genes, together with other non-mechanical factors, only contribute 3% to as much as 10% of bone strength.[57] On the other hand, mechanical effects from muscles pulling on bones during movement determine more than 40% of bone strength.[57] Physical activity in early childhood and maintaing this activity in older age is therefore probably the most effective way to slow the physiological age-related reduction in BMA, thereby reducing osteoporosis and fracture incidence.[66] In Figure 1, an osteoporotic trabecular bone is compared with normal bone.

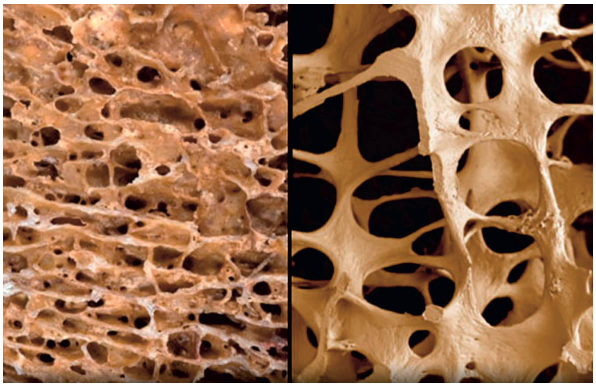


FIGURE 1 *A trabecular bone with a normal (left) and a deteriorated structure and reduced BMA (right). (With kind permission from Demetech AB)*

In Study I, BMA assessments were made in the calcaneus of 92 male patients before (30 patients) and after (62 patients) ACL reconstruction. Kannus et al.[85] among others, had previously found that knee ligament injuries, fractures to the lower extremities and even meniscal injuries led to permanent bone mineral loss that appeared to remain six years after the injury.[147,8,119] Lower extremity BMA is easily measured in the calcanei and this is therefore a useful tool for monitoring temporal changes in patients after injury.[36,37] In Study V, 67 patients were examined after ACL reconstruction using the dual-energy X-ray absorptiometry (DEXA) method. Apart from BMA assessments, health-related quality of life (HrQOL) was evaluated with the EQ-5D questionnaire and the activity level was assessed using the Tegner activity score. The patients were examined during a period of five years and they were participants in an osteoporosis project at the NU Hospital Group.

Bone mineral loss after ACL reconstruction is also found in the distal femur and proximal tibia.[129,176,14] One consequence is bone-tunnel widening that can be documented with plain radiographs, magnetic resonance imaging (MRI) or computed tomography

(CT).[13,27,33,50,61,76,77,96,165] Previous reports have revealed that bioabsorbable interference screws used for fixating HT autografts in the bone tunnels could induce more bone-tunnel widening than metal interference screws.[100] On the other hand, two previous studies have reported that bioabsorbable screws did not induce more osteolysis or tunnel widening compared with metal interference screws when used for BPTB autografts.[109,112] In Study III, 77 patients were evaluated eight years after ACL reconstruction surgery with hamstring tendon autografts fixated with poly-L-lactide acid (PLLA) or metal interference screws on both the femoral and the tibial side. Two randomised groups were compared in terms of postoperative C-reactive protein (CRP) analysis, postoperative standard radiographs, functional scores, single-legged hop tests and Tegner activity level.

Osteolysis and bone-tunnel widening are a major problem when primary ACL reconstructions fail. ACL revision surgery is hazardous if the knee region is compromised, with large bone defects. In this situation, there is often a need for bone transplantation in order to fill the defects before revision surgery can be accomplished. In a few studies, bone-tunnel widening was associated with increased knee laxity although the association between laxity and tunnel widening does not imply causality.[77,113,164] No study so far has established that tunnel widening causes laxity or that laxity causes tunnel widening. One alternative in revision surgery was to use a BPTB graft from the injured or the uninjured knee. The idea was that the bone blocks would be an advantage if there were bone deficits on both sides of the knee. Some surgeons who are accustomed to ACL revision surgery use the ipsilateral BPTB as a graft, despite the possibility that the tendon could be of compromised quality. Three of the studies[34,125,122] addressing this problem reported good results using ipsilateral BPTB grafts, despite the fact that the donor-site tendon in some histological studies revealed scar tissue with disorganised collagen fibres and pathological MRI.[153,152,89] In Study II, 24 patients with a second ACL injury to the same knee underwent surgery in two groups where the origin of the grafts was different. They were given reharvested ipsilateral or contralateral BPTB grafts and were re-examined after two years in terms of the clinical outcome. In Study IV, two male patients and two female patients from Study II were further examined with second-look arthroscopy and histological analysis of biopsy specimens from the intra-articular graft and they were also examined radiographically with MRI 10 years after ACL revision surgery.

1.2 BONE BIOLOGY

Bone is mainly composed of osteocytes embedded in a matrix of type I collagen. Mineralised crystals of calcium hydroxyapatite fill the space between the collagen protein chains. The density of crystals determines the stiffness and/or flexibility of the bone. Bone modelling is the formation of new bone without prior resorption mainly present during childhood. In bone remodelling, bone resorption by osteoclasts is followed by bone formation, where osteoblasts synthesise collagen and the calcified matrix. This takes place in the basic multicellular unit (BMU) on bone surfaces (Figure 2). Approximately 900 BMU sites are initiated every day in trabecular bone. In cortical bone, about 180

BMU sites are initiated every day throughout life in the human body.[41] Annually, 25% of trabecular bone and 3% of cortical bone are replaced and renewed. After peak bone mass has been reached at the age of 20 to 30 years, there is a small negative balance, with more resorption and less formation in every remodelling cycle event, resulting in a small yet measurable bone loss, especially in the inner juxtamedullary area of the long bones and in trabecular bones.[142] One purpose of remodelling among adults is to remove spontaneous microcracks and replace the damaged bone with new.

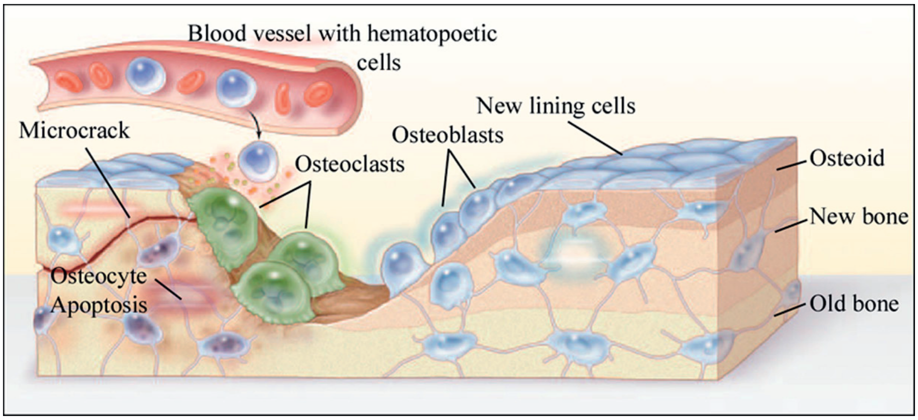


FIGURE 2 *The remodeling cycle on a trabecula, basic multicellular unit (BMU). (Reproduced with kind permission from Seeman et al., ©Massachusetts Medical Society).*

At least three other major stimuli, including the loss of mechanical loading, low blood calcium and alterations in hormones and cytokines, can activate bone remodelling.[111] After only three days of immobilisation or after the propagation of a microcrack, a bone remodeling event begins at one BMU. The involved osteocytes lack oxygen and undergo apoptosis (cell death).[3,11] Unloading also has an effect on articular cartilage and growth-plate chondrocytes with a two- to three-fold increase in chondrocyte apoptosis.[11] An unknown signal from the osteocyte recruits osteoclasts from bone-marrow hematopoetic cells and the bone resorption begins. After approximately three weeks of resorption, the developed lacunae are invaded by osteoblasts from the bone-marrow mesenchymal stemcells (MSCs). Bone formation concludes the remodeling cycle event and takes approximately three months in human bone.[111] Direct cell-to-cell interaction between osteoblasts and osteoclasts and important cell membrane proteins, cytokines and prostaglandins determine the magnitude of newly formed bone, according to Wolff's law and the mechanostat theory (see next chapter).[58,111,20,92] Finally, the osteoblasts either transform into a bone lining cell or an osteocyte embedded in the osteoid. Some osteoblasts undergo apoptosis after the production of the matrix collagen and hydroxyapatite.

Estrogen deficiency, corticosteroid therapy, trauma, immobilisation and advancing age increase the resorption phase and the consequence is the reduction of bone mineral.[142]

1.3 BONE HEALTH AND PHYSICAL EXERCISE

Transduction is the conversion of mechanical energy into chemical signals and is one of the functions accomplished by osteocytes. Three different mechanisms are believed to be responsible for the mechanical effect on bones; the movement of ions surrounding the cells, mechanical strain on the cell membrane and cytoskeleton and fluid shear stress in the canaliculi surrounding the cell extensions of the osteocytes.[19] All three act together so that the bending and flexion of long bones induce shear stress on the osteocytes through the interstitial fluid and their dendritic processes. This flow activates the cells to produce signaling molecules that control bone remodelling. Cyclic bending on long bones and cyclic compression on trabecular bones is needed for bone formation to be induced.[136,159] This is the basic principle behind dynamic exercises. Non-cyclic (continuous) compression, which is the principle behind isometric, static training, is not enough and could even have the opposite effect, with bone loss as a result.[19,98]

The mechanostat model that Harold Frost and co-workers promoted is an engineering model that describes bone growth and bone loss and is a refinement of Wolff's law.[58] According to this model, bone growth and bone loss are stimulated by the local mechanical deformation of bone generated by the muscles connected to the bone. The deformation is expressed by the unity strain and represents the amount of shortening divided by the original length. Strain is therefore a unit-less measurement (ratio) of change in length (Δ length) and is expressed by the Greek letter epsilon, (ε).[149] In physiological conditions, the long bones never exceed more than 3,000 microstrains (με). When strain increases above a threshold range of approximately 1,500 με, bone modelling slowly increases bone density and strength in cortical bones to reduce subsequent strain by increasing cortical thickness and cross-sectional area.[56] In contrast, the strain threshold level for the mechanically controlled remodelling in BMUs (which is more common in trabecular bones) is only 100-300με. This level is needed to repress remodelling events, thereby preserving bone tissue. Consequently, strain levels under this threshold increase bone remodelling events in BMUs and bone will be lost during physical inactivity or deprivation of gravity, as seen in spaceflights. (Figure 3)

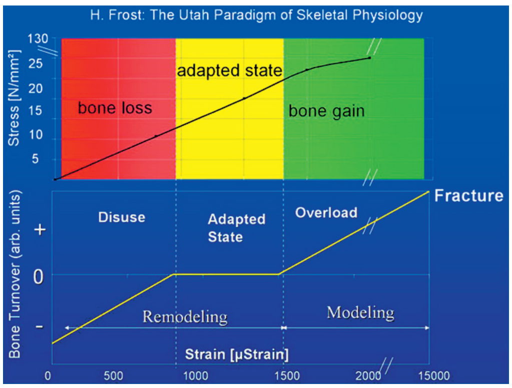


FIGURE 3 *The adaptation of bone to strain (see text) (source: www.en.wikipedia.org)*

The question of how much strain has to be generated for bone osteogenic effect depends on the inter-relationship between strain magnitude (peak impact level), strain rate (number of impacts) and strain frequency (impacts per time unit). High strain magnitude, high strain rates, but also high-frequency, low-magnitude signals used in whole-body vibration, have the best osteogenic effect.[135,69] Recent studies have proposed that bone cells become desensitised to prolonged mechanical stimulation and further loading cycles do not yield an appreciable increase in bone formation. When rest periods, longer than eight hours, are inserted, substantially fewer load cycles are required to precipitate a significant bone formation response.[130,65] So, it is also important to take rest periods into consideration when designing the best bone-protective training. As mentioned above, thresholds differ between cortical and trabecular bones and also at different parts of long bones.[97,72] Cortical bones need four to five times body weight impact for bone formation while trabecular bone responds with bone formation of only 1-1.5 x body weight impact.[162,161] In general, multiple studies show that exercises requiring high forces or generating high impacts have the greatest osteogenic potential.[66,97,142,124] Jumping exercises, (plyometric exercises) induce high ground-reaction forces and weightlifting induces high joint-reaction forces. These two training methods are therefore superior for retaining bone density and should be performed at least three times a week for osteogenic result. On the other hand, unloaded exercises like swimming and cycling have a weak positive influence on bone density, while walking and running have limited positive effects. Eccentric training is more effective than concentric training because of the 15-20% higher forces generated by the muscles during lengthening. One consequence of the mechanostat model is that there is a substantial variance in BMA among different athletes in sports, as illustrated in Figure 4. (BMA values from the home page of The American Society for Bone and Mineral Research, www.asbmr.org).

Soccer player	**Runner**	**Canoeist**	**Weightlifter**
Spine 7% higher	Spine no difference	Spine no difference	Spine 12% higher
Hip 20% higher	Hip 10% higher	Hip no difference	Hip 6% higher
Arm 14% higher	Arm no difference	Arm 10% higher	Arm 20% higher
Leg 16% higher	Leg 10% higher	Leg no difference	Leg 11% higher

FIGURE 4 *The figure shows how the bone mineral density in different sports competitors compares with the bone density in controls.*

Accelerated rehabilitation after ACL reconstruction entails full weight bearing, unlimited range of motion (ROM), strengthening exercises with full extension of the knee and an early return to athletic activity.[144,145] This concept has reduced some of the problems seen after ACL surgery, such as knee stiffness and muscle weakness, and might also have positive effects on the bone integrity after surgery. In the discussion section of this thesis, bone density reduction will be discussed in relation to modern rehabilitation after ACL injury.

1.4 TENDONS AND LIGAMENTS

Davi's law is the analogue of Wolff's law in soft tissues. Tendons and ligaments can adapt and change their structure according to external forces and changes in demand. A microgravity study by Reeves et al. on volunteers subjected to 90 days of bed rest indicated a more than 50% reduction in gastrocnemius tendon stiffness.[128] Ligaments and tendons exhibit different histological appearances. Intra-articular ligaments like the ACL contain more cells with more DNA, more collagen III and more glycosaminoglycans (GAGs) than tendons and are therefore more metabolically active than tendons.[6,110] Table 1.

	Hamstring tendon	Patellar tendon	ACL
DNA	+	+	+++
Collagen I	≈95%	≈95%	≈88 %
Collagen III	<5%	<5%	≈9-12%
GAG	+	+	+++
Cells	+	+	+++

TABLE 1 *Collagen content expressed as a percentage of total collagen. GAG= glycosaminoglycans, cells=fibroblasts, tenocytes.*

The biochemical differences between intra-articular ligaments like the ACL and tendons reflect the fact that they differ in their structure and biomechanical function. Ligaments contain 60% water with viscoelastic properties and can change their length and stiffness to compensate for changes in mechanical loading.[54,16] Fibrocytes in intra-articular ligaments are more heterogeneous, with different sizes and shapes, and contain larger nuclei with more DNA content. On the other hand, fibrocytes in tendons are easily recognised in a light microscope, with thin, spindle-shaped nuclei between bundles of collagen (Figure 5). In clinical terms, the normal ACL is capable of microscopic adjustments to internal stresses over time. Laxity and kinematics can therefore change, depending on the different stress to which the joint is exposed.

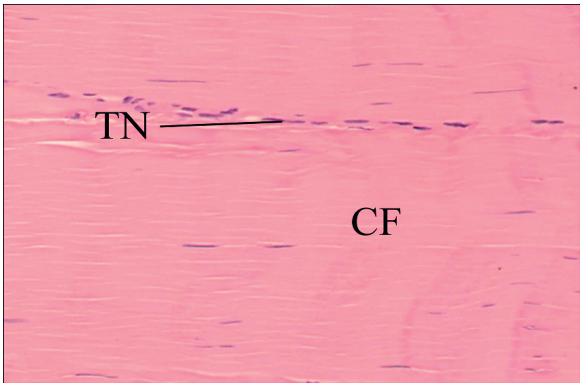


FIGURE 5 *A biopsy section of a normal patellar tendon, TN = tenocyte nucleus, CF = collagen fibres.(Reprinted with permission from Springer Science and Business Media)*

1.5 TENDON-BONE INTERFACE

In ACL surgery, there are two main sites where healing of the graft is accomplished by two slightly different biological processes. Transformation of the tendon to a ligamentous structure is called "ligamentisation", [7,141,110,32] and it is described in detail in Study IV and in the discussion section. In the bone tunnels and at the tunnel entrance, a second biological process is mandatory for successful surgery. There are no sites at which a tendon or ligament enters a bone. Instead, all the tendons and ligaments in the body connect to the bone through the surfaces of bones. This attachment site of a tendon or a ligament to bone is called the "enthesis" or "enthesis organ" because of its complexity.[17] There are two histologically different entheses based on the way the collagen fibres are attached to bone. Direct insertion, also called fibrocartilaginous enthesis or endochondral ossification is composed of four zones; tendon/ligament, un-calcified cartilage, calcified cartilage and bone and it is seen in particular with intra-articular ligaments like the ACL. Indirect insertion, also called fibrous insertion, is more common where tendons or extra-articular ligaments, like the medial collateral ligament (MCL), are attached to bone. In this case, the tendon or ligament collagen molecules pass into the periosteum and into the bone cortex with fibres called Sharpey's fibres.

Human studies of ACL autograft incorporation into bone are difficult to design but animal studies have described the biological background.[118,173,62,75,63,44] After fixation of the graft in the bone tunnel, the healing begins with an inflammatory reaction involving fibroblasts, neutrophils and macrophages. These cells are recruited to the tunnels and peak just 24-48 hours after implantation.[40] Macrophages release interleukin 1, interleukin 6, tumor necrosis alpha and prostaglandin E2. High levels of these cytokines activate osteoclasts which leads to bone resorption and excessive bone loss in the same manner as in inflammatory joint diseases. Counteracting this effect, pluripotent mesenchymal stem cells (MSCs) from the nearby bone marrow and synovial membrane produce new trabecular bone in the vicinity of the tendon.[114,131,167,105]

In a study by Wen et al., ACL reconstructions were performed on rabbits and the bone tissue graft complex was examined with microscopic examination, quantitative computed tomography (pQCT), microCT and biomechanical testing.[167] The bone mineral density (BMD) was reduced by 13% and 22% on the tibial and femoral sides respectively after six weeks. Although the BMD partially reversed at 12 weeks, it was still significantly lower than at time zero, confirming that there was significant bone loss on both sides after surgery. On the femoral side but not on the tibial side, there was significant bone-tunnel widening after six weeks. The biomechanical testing revealed that, before 12 weeks, the grafted tendon was pulled out of the tunnel together with a bony attachment.[167] After 12 weeks, the graft ruptured in the tendinous part. At 12 weeks, the strength of the construct was one tenth of the native ACL-to-bone attachment, which is 400N.[167] The mechanical strength of the tendon-to-bone tunnel attachment correlates with the amount of osseous ingrowth and mineralisation of newly formed bone.[105,9] The bone integrity of the tunnels is therefore probably the most important factor for the mechanical strength and stiffness of the ACL graft. Since healing of the graft in the bone tunnel depends on bone ingrowth into the interface, excessive osteoclastic activity may contribute to bone resorption, bone-tunnel widening and impaired graft incorporation, with an increased risk of graft rupture.

Animal studies have also revealed that there are differences between the femoral and the tibial insertion site. The distal femoral metaphysis, where the tendon should be incorporated, is mainly composed of cancellous bone.[64] A histological study revealed that the graft heals with a fibrocartilaginous insertion, especially at the tunnel entrance, and has twice the pull-out strength compared with the tibial insertion.[167] The tibial bone structure is mostly composed of bone marrow, with a structurally weaker microarchitecture, and the insertion is mainly fibrous, with Sharpey's fibres. The pull-out strength is higher at the tunnel entrance and the graft and tunnel length therefore matters less than the bone integrity of the tendon-bone interface (TBI).[174,105] Human studies are scarce, but there are a few studies with histological analyses of the tendon-bone construct when patients return for ACL revision surgery.[49,75,126] In the study by Ishibashi et al., the healing process spanned a longer time period but resembled the process described in animal studies.[75] Petersen et al. found in their study that hamstring grafts healed mostly with indirect, fibrous insertion, while BPTB grafts healed with direct, fibrocartilaginous insertion with all four zones, especially if interference screws had been used.[126]

1.6 BIOABSORBABLE IMPLANTS

Bioabsorbable implants were introduced in the early 1970s in maxillo-facial and oral surgery.[60,38] In orthopaedic surgery, Rokkanen et al.[132] and Böstman et al.[22] started to use bioabsorbable screws, rods and even plates for fracture surgery of the ankle. The first randomised controlled study with one-year follow-up results showed promising results with polyglycolic acid (PGA) implants in displaced malleolar fractures, but two years later eight percent of the patients suffered a foreign-body reaction in the soft tissues.[22] Radiographic examination revealed signs of osteolysis and bone loss and these implants are therefore no longer used in fracture surgery.

Most implants used in ligament surgery in the knee are composed of poly-alpha-hydroxy acids in crystalline form which should have enough strength to withstand stress during rehabilitation before they are degraded and absorbed.[166] Poly-L-lactide acid (PLLA) was the most common bioabsorbable product used in ACL surgery during the 1990s. With water uptake, the chains undergo hydrolytic scission and the material is degraded into shorter chains. The chain fragments are phagocytosed by macrophages and polymorphonuclear leucocytes and the rest products enter the Krebs cycle and are excreted as CO2 and H2O. During phagocytosis, osteoclasts are recruited and cytokines are released, which is mandatory for the degradation of the implant. Bone resorption is therefore inevitable during the removal of the bioabsorbable material. Osteoclasts release hydrogen ions (H+), which dissolve the bone matrix molecules during bone resorption. The degradation of PLLA is accompanied by a reduction in pH, which further potentiates the degradation of hydroxyapatite and ground substance of bone. Because of the problems seen with PLLA, most implants used today are composite products with a calcium salt added to the hydroxy-acid chain. Calcium salts can neutralise the acidic effect of PLLA and PGA substance.

02 AIMS OF THE STUDIES

The overall aim of the thesis was to assess bone mineral changes and bone-tunnel widening after ACL reconstruction surgery. Moreover, the aim was to compare the results after ACL revision surgery with BPTB autografts using reharvested or primary harvested, contralateral patellar tendons.

Study I

To assess the bone mineral areal mass (BMA) in the calcanei of male patients with unilateral ACL injuries before and after reconstruction or revision surgery and to assess whether the BMA ratio or the BMA of the injured and non-injured leg correlated with activity level, functional performance or the time period between the injury and the reconstruction.

Study II

To assess the clinical outcome after ACL revision reconstruction using either ipsilateral reharvested patellar tendon or primary harvest of the contralateral patellar tendon and to compare the findings with the results of primary ACL reconstructions in a matched group of patients. A further aim was to compare the length, thickness, width and size of the residual donor-site gap of the two different patellar tendon grafts two years after reconstruction.

Study III

To evaluate the long-term (eight years) radiographic results relating to bone-tunnel widening and clinical outcome after ACL reconstructions using either bioabsorbable poly-L-lactide acid (PLLA) or metal interference screws. The aim was also to compare the C-reactive protein (CRP) reactions between these two implants during the first postoperative period.

Study IV

To evaluate the clinical, radiographic and histological results of four of the patients from Study II postoperatively, two, three and ten years after ACL revision reconstruction using ipsilateral reharvested patellar tendon and to assess whether the reharvested patellar tendon has the potential for “ligamentisation”.

Study V

To assess BMA changes in both calcanei, both hips and the lumbar spine in female and male patients for five years after ACL reconstruction using HT autografts. Furthermore, the aim was to evaluate the clinical outcome, the Tegner avtivity level and the health-related quality of life using the EQ-5D questionnaire.

03 PATIENTS AND STUDY FLOW CHARTS

3.1 STUDY I

Ninety-two males participated in the study and were evaluated in terms of bone mineral areal mass (BMA) in the calcaneus. They had sustained a unilateral ACL rupture and underwent surgery with a bone-patellar tendon-bone (BPTB) autograft from the ipsilateral knee. In Group A, 30 patients, with a median (range) age of 26 (15-41) years were studied just prior to an ACL reconstruction a median (range) of 11 (2-192) months after the injury (Figure 6). In Group B, 49 patients aged 29 (18-49) years were assessed 24 (23-29) months after a primary ACL reconstruction and 48 (26-192) months after the injury. In Group C, 13 patients aged 27 (21-39) years were analysed 24 (20-35) months after ACL revision surgery. The revised patients had had their first ACL reconstruction 87 (50-167) months before.

STUDY I

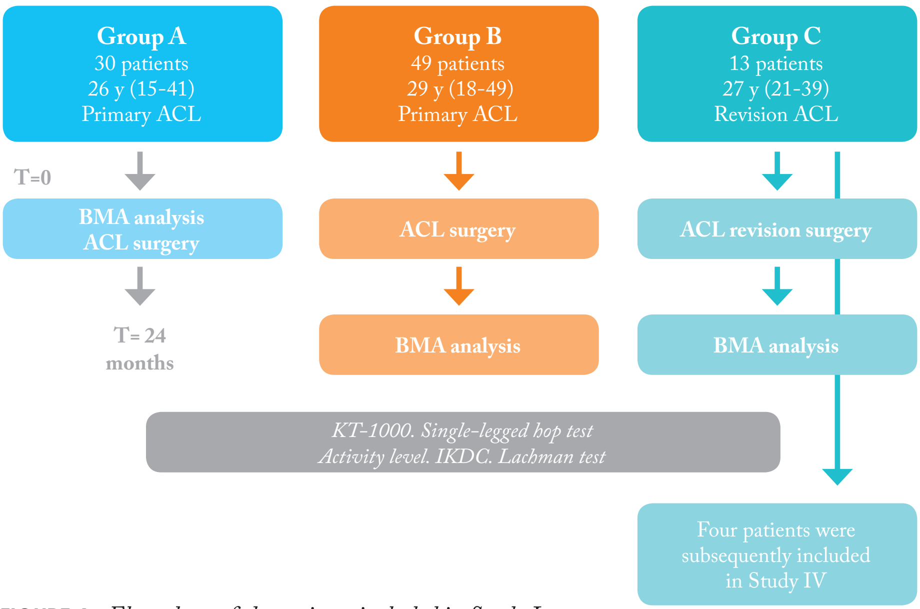


FIGURE 6 *Flow chart of the patients included in Study I.*
Note: T = 0 is the time at which the patients underwent their ACL reconstruction.

3.2 STUDY II

Twenty-four consecutive patients underwent ACL revision reconstructions with either ipsilateral reharvested BPTB graft (Group A, N=12), or a BPTB graft from the contralateral, non-injured leg (Group B, N=12). For comparison 12 patients, age- and gender-matched, underwent an ACL primary reconstruction with an ipsilateral BPTB graft (Group C), (Figure 7).

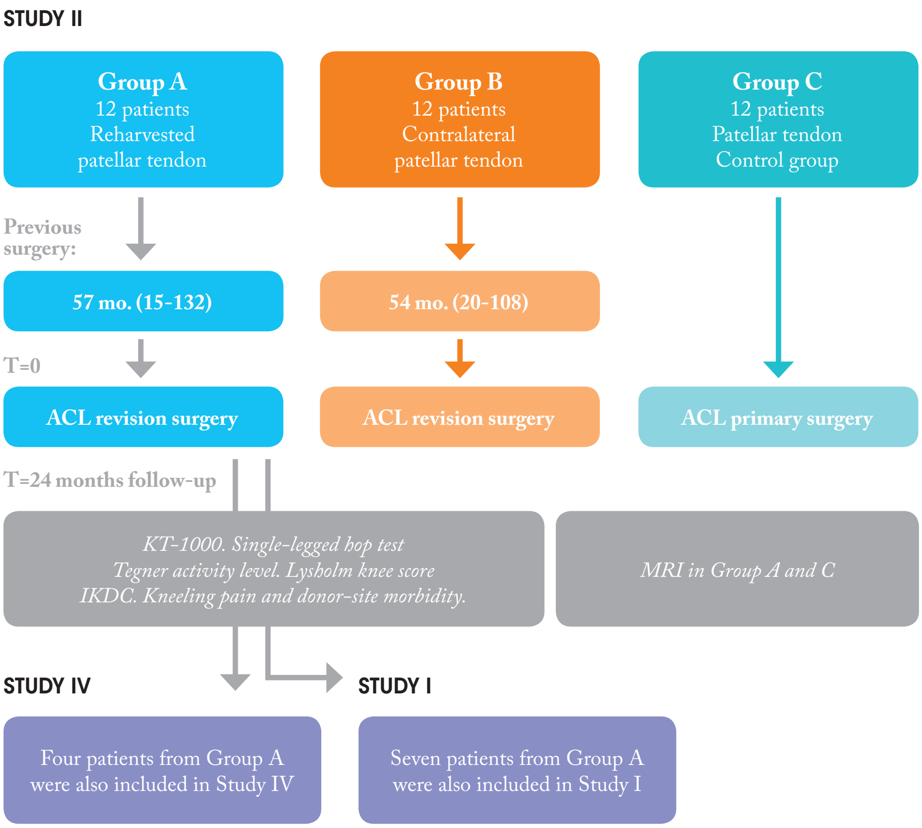


FIGURE 7 *Flow chart of the patients included in Study II. Four and seven of the patients in Group A also participated in Studies IV and I respectively.*

In Group A, all the previous reconstructions were performed with open surgery and medial- (6 patients) or central- (6 patients) third BPTB autograft, 57 (15-132) months previously. The cause of failure was malpositioning of the grafts in 10 patients and a new trauma in two patients. In Group B, all the previous reconstructions were performed with open surgery and medial-third BPTB autograft, 54 (20-108) months previously. The cause of failure was malpositioning of the graft in eight patients and a new trauma in four patients. In Group C the median (range) time since injury was 18 (3-90) months. The median (range) age of the patients at revision surgery was 27 (23-33) years in Group A and 27 (24-33) years in Group B. In Group C, the median (range) age of the patients at the time of primary reconstructions was 27 (19-32) years.

3.3 STUDY III

Seventy-seven consecutive patients, 20 female and 57 male patients, with a symptomatic ACL rupture, were prospectively randomised for ACL reconstruction using an ipsilateral hamstring tendon (HT) autograft and either bioabsorbable poly-L-lactide acid (PLLA), (PLLA group) or metallic (metal group) interference screw fixation (Figure 8). The inclusion criteria were a unilateral ACL injury verified clinically by a positive Lachman test and a positive pivot-shift test or through a previous diagnostic arthroscopy. The exclusion criteria were associated posterior cruciate ligament (PCL), collateral ligament or contralateral ligament knee injury. Further exclusion criteria were a previous ACL injury or radiographically verified osteoarthritis.

STUDY III

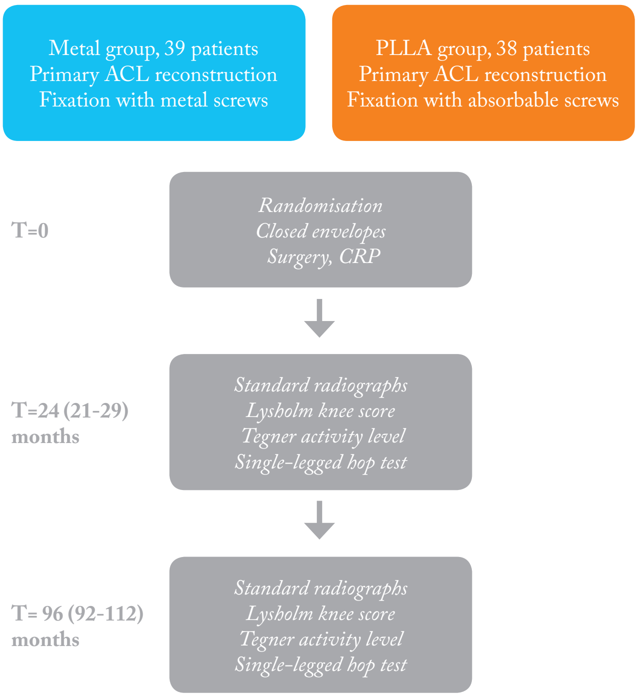


FIGURE 8 *Flow chart of the patients included in Study III.*

3.4 STUDY IV

Two male patients, aged 28 and 26 years at the time of revision, and two female patients, aged 29 and 26 years at the revision, were included in this study. The patients underwent a primary reconstruction using the medial third, (N=2) or the central third, (N=2) 60, 45, 96, and 120 months respectively before the revision procedure (Figure 9). At the time of revision surgery, three/four patients revealed concomitant meniscal injuries requiring partial resection and debridement. All four patients underwent ACL revision surgery with an ipsilateral reharvested BPTB graft 71 (45-120) months after the primary reconstruction.

STUDY IV

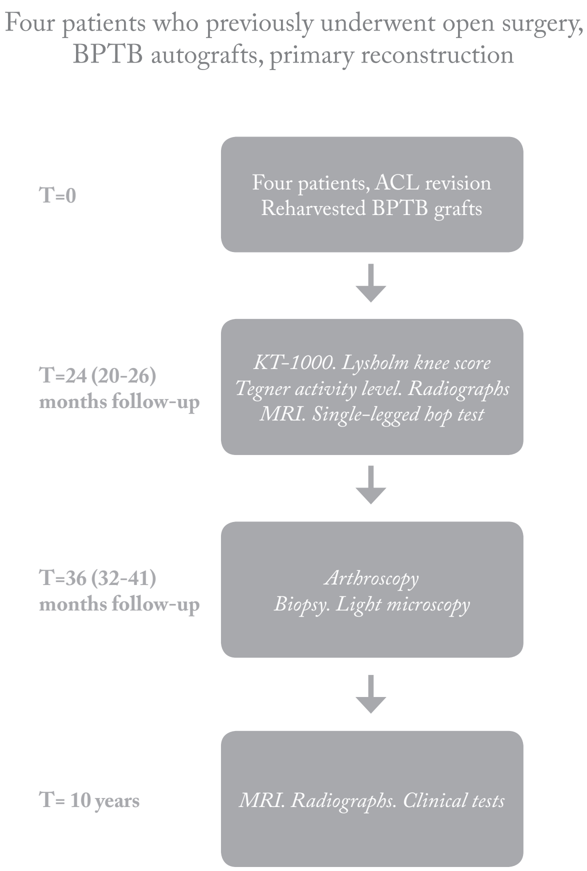


FIGURE 9 *Flow chart of the patients included in Study IV.*

3.5 STUDY V

Sixty-seven patients were enrolled in the study starting in November 2004. Twenty-six females, median (range) age of 31 (17-55) years and 41 males, median (range) age of 29 (17-64) years were prospectively followed after ACL reconstruction with assessments of bone mineral areal mass (BMA). Forty-eight patients, 21 females and 27 males completed the study attending all follow-ups after a median (range) of six (5-10), 18 (17-21), 37 (36-43) and 60 (46-94) months. The patients had sustained a unilateral ACL injury a median (range) of 9.5 (2-245) months before the reconstruction. Patients with a contralateral knee injury or osteoarthritis were excluded from the study (Figure 10).

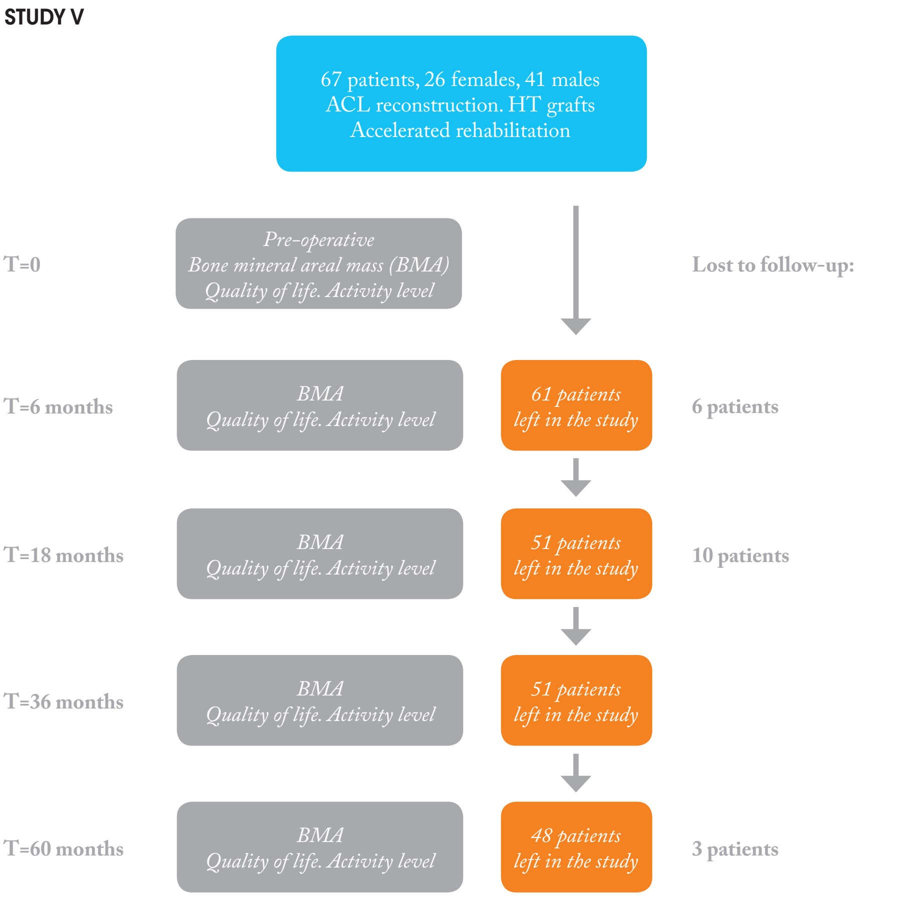


FIGURE 10 *Flow chart of the patients included in Study V.*
Note: Nineteen patients, shown to the right, were lost to follow-up. Quality of life was measured using the EQ-5D questionnaire. Activity level was measured using the Tegner activity score.

04 METHODS

4.1 SURGICAL TECHNIQUE

Studies I, II and IV

BPTB autografts and metal interference screw fixation were used. The central third of the patellar tendon was harvested from the ipsilateral knee (Study I) or both ipsi- and contralateral knee (Study II) through a 7-to 8-cm-long single vertical incision. At completion of the surgery, the tendon defect was left open and the paratenon was carefully sutured. At both ends of the tendon an 8-9 mm diameter bone block was prepared. All the patients in Study I were operated on by the same experienced surgeon and, in Study II, three experienced surgeons performed all the operations. The bone tunnels were created with stepped drills (Smith and Nephew, Andover, Massachusetts). The femoral tunnel was drilled through the tibial tunnel with the knee in 90° of flexion. The grafts were fixed at both ends with metal interference screws.

Studies III and V

Hamstring tendon autografts were used. Through a 3-4 cm skin incision over the pes anserinus, the semitendinosus and gracilis tendons were harvested. The tendons were folded to create a four-stranded graft with a diameter of 7-8 mm. The femoral tunnel was drilled through a medial portal with the knee in maximum flexion. In Study III, the graft was fixated with a 7-8 mm bioabsorbable PLLA or metal interference screw on the femoral side and with an 8-9 mm bioabsorbable PLLA or metal interference screw on the tibial side with the knee in full extension. In Study V, only metal screws were used.

In both groups, meniscal injuries were addressed with either a small debridement or a partial resection, depending on the magnitude of the injury. No meniscal sutures were performed.

4.2 CLINICAL EXAMINATION

The Lachman test

Studies I, II and IV.

With the patient's knee held between full extension and 15 degrees of flexion, the femur is stabilised with one hand, while firm pressure is applied to the posterior aspect of the proximal tibia in an attempt to translate it anteriorly. A positive test indicating disruption of the anterior cruciate ligament is one in which there is proprioceptive and/or visual anterior translation of the tibia in relation to the femur with a characteristic mushy or soft end point. This is in contrast to a definite hard end point elicited when the ACL is intact[157] (Figure 11).

FIGURE 11
The Lachman test.
© Lars Ejerhed

The instrumented Lachman test; KT-1000 arthrometer test

Studies I-IV

The KT-1000 arthrometer (MedMetric, San Diego, California) was used to evaluate the total sagittal stability of the knee.[39] The examination was standardised with the patient supine on the table and the examined leg strapped in 30° of flexion. After calibrating the instrument to zero before the test, the median value of three measurements for each knee was registered using a force of 89 Newtons. In Study III, the force was changed to 134 Newtons. The reproducibility has been found to be good in several studies if the same experienced examiner perform the test and if the side-to-side difference between knees is presented.[172,151,143] In Studies I and IV, the total anterior/posterior side-to-side difference was presented at the follow-up assessments and, in Study II, both the anterior and the total anterior/posterior side-to-side difference were presented. In Study III, a manual maximum force (MMT) was applied and the anterior side-to-side difference was presented. In all four studies, only one experienced examiner performed the tests (Figure 12).

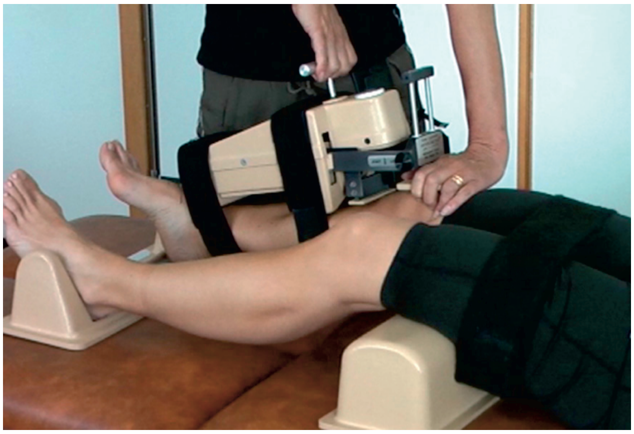


FIGURE 12
The KT-1000 arthrometer.
© Sven Stener

Lysholm knee score

Studies II-IV

The Lysholm knee score was first proposed and tested for the evaluation of knee instability (giving-way) symptoms. Since 1985, when it was presented, the revised version has also been used for patients with meniscal and chondral injuries.[155,106,25,107] The subscores are limp (5 points), support (5 points), locking (15 points), instability (25 points), pain (25 points), swelling (10 points), stair-climbing (10 points) and squatting (5 points). The maximum score is 100 points and the test was self-administered by the patients in Studies II, III and IV, according to Höher et al.[73]

Tegner activity score

Studies I-V

The Tegner activity score was used to evaluate activity level.[155] The score is graded numerically according to work and sports activity and spans from level 10 (competitive sport at national or international level) to level zero (sick leave because of knee problems). In most articles discussing knee surgery, the patients' return to work and sports is often documented as the Tegner activity level and it is therefore not regarded as a score.

Single-legged-hop test

Studies I-IV

The test is performed with the patient jumping three times on each leg as far as possible with his/her hands behind his/her back (Figure 13). The best distance for the injured leg compared with the non-injured leg is registered and the quotient is calculated in per cent. [156]

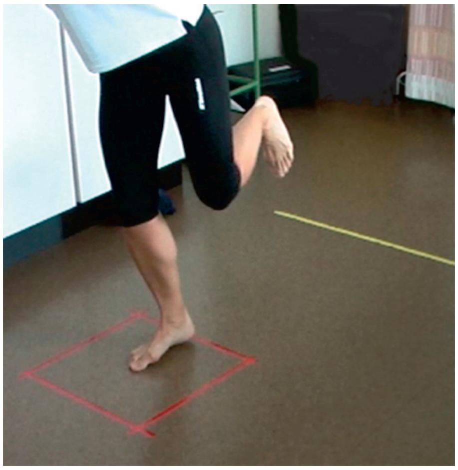


FIGURE 13 *The Single-legged-hop test.* *© Sven Stener*

IKDC International Knee Documentation Committee evaluation form

Studies I-II

The IKDC evaluation form, originating in 1987, is used to document the treatment of knee ligament injuries.[68] Apart from a documentation section, in which the patient and the injury are documented, it includes a qualification section and an evaluation section. For evaluation, there are four major problem areas (subjective assessment, symptoms, range of motion and ligament examination). They are supplemented by four additional areas that are only documented but not included in the evaluation (compartment findings in the medial and lateral part of the knee, donor-site pathology, radiographic findings and functional tests). In this thesis these four additional areas were not evaluated. Each parameter is classified as normal, nearly normal, abnormal or severely abnormal. For evaluation, the parameters of the four major problem areas are classified for the group qualification. The poorest qualification within the group renders the group qualification. The poorest group qualification gives the final evaluation. If the knee is "abnormal" in any of the problem areas, it is evaluated as an abnormal knee.

4.3 BMA MEASUREMENTS

Dual-energy photon absorptiometry (DPA)

Study I

The bone mineral assessments were performed using a gamma camera (Picker SX-300), as described by Jonsson et al.[79] This method uses two energies from two different isotopes, a 125 I source (mean photon energy, 28 keV) of activity 3.0 GBq. and a 99 Tc source (mean photon energy, 140 keV) of activity 150 MBq. The foot is placed in a bath of water (Figure 14) and both calcanei are measured in addition to a transmission measurement of the water bath alone. The bone mineral density is expressed as the bone mineral areal mass (BMA) with the quantity unit g/cm^2. The mean short-term precision expressed as a coefficient of variation (CV) in vivo, has been reported to be 2.1% and the long-term reproducibility in vitro on an aluminum phantom has been reported to be 1.8%.[80] During measurements of both calcanei the patients were exposed to an effective dose of 0.02 mSv. For comparison, the effective dose during mammography and quantitative computed tomography (QCT) is 0.2 to 0.5 mSv.

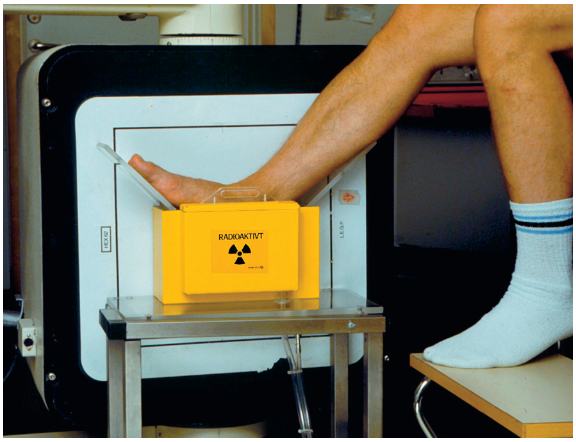


FIGURE 14 *The dual-energy photon absorptiometry machine (With kind permission from Elsevier)*

Dual-energy X-ray absorptiometry (DEXA).

Study V

BMA measurements were made with the DEXA machine (Lunar Prodigy Advance, GE Medical Systems, Waukesha, Wisconsin, USA), (Figure 15) for the hip and lumbar spine. The machine uses one X-ray fan beam with a constant 76 kV of energy. The radiation dose for the patients equals 0.037mSv for both the hip and the spine measurements. When measuring the BMA of the hip and the spine, the specific values of the patient are given, along with the T-score comparing BMA with a 25-year-old healthy female. For comparison, the Z-score, which is the BMA in relation to the same gender and age, is also presented. BMA measurements with DEXA have been found to predict future fracture risk.[108] The area examined in the hip includes the trochanter and the neck and together they are presented as "total hip" (Figure 16). In the appendix section, a monitor screen is shown as presented during DEXA measurements.

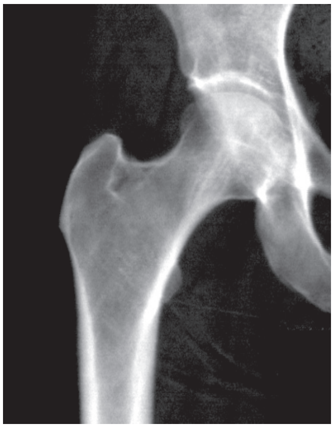


FIGURE 15 *The dual-energy X-ray absorptiometry (DEXA) machine*

FIGURE 16 *The total hip measurement is the addition of BMA from the neck and the trochanter (with kind permission from GE Healthcare)*

For measurements of the calcaneus, a dual-energy X-ray and laser (DXL) Calscan machine (DemeTech Co, Miami, Florida, USA) was used (Figure 17). This device uses fan-beam X-rays of 35 and 68 kV. At the same time the heel thickness was measured with laser technology. A calibration was performed before every patient assessment with a liquid phantom and the coefficient of variation (CV) precision for the machine has been reported to be 0.2%.[95] The short-term CV has been reported to be 1-2%.[95] The laser is used to measure the soft tissue and extract this from the calculation of the bone mineral area. Bone mineral measurements with the Calscan have been shown to predict future fracture risk.[26] The yellow area across the body of the calcaneus represents the part of the bone that is measured (Figure 18). Apart from general information about the patient and BMA values, trend curves with age-related bone reduction are shown (Figure 19).

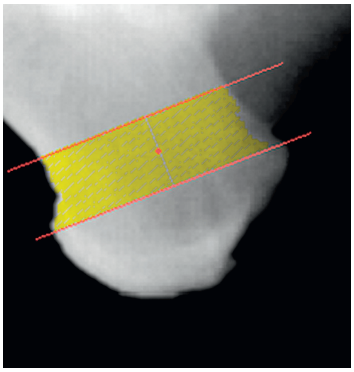


FIGURE 17
The Calscan machine

FIGURE 18
The yellow area across the body of the calcaneus represents the part of the bone that is measured (with kind permission from Medetech AB)

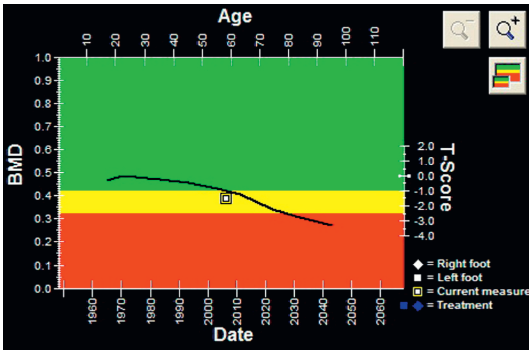


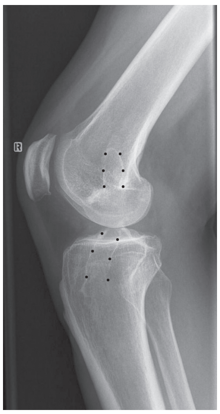
FIGURE 19 *A trend curve on a patient as given by the DXL machine showing the progression of BMA compared with the reference values according to Kullenberg et al.[95] (with kind permission from Medetech AB)*

4.4 RADIOGRAPHIC ASSESSMENTS

Standard radiographs

Studies III and IV

In Study III, the radiographic measurements were made after a mean (range) of 99 (84-120) months in the PLLA group and after a mean (range) of 96 (78-108) months in the metal group. They included standardised weight-bearing radiographs with the knee in 30° of flexion. The radiographs were digital and the tunnels were measured by an experienced radiologist. Only the lateral view was used for tunnel width measurements. The tunnels were easily determined in both the tibia and the femur by using the sclerotic margins as landmarks. Six points, two at each end and two in the centre of the tunnel, were established. The distances between these points were determined and the tunnel width was then calculated as the sum of the three distances divided by 3 (Figure 20a, 20b). The inter-rater and intra-rater test-retest performed before the study at our institution were considered good, with an interclass correlation coefficient value of between 0.84 and 0.97 for the tibial measurements and between 0.88 and 0.93 for the femoral measurements.



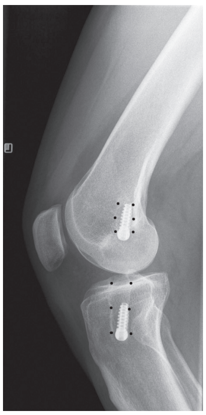


FIGURE 20a *Bone tunnels in a knee with PLLA screws (reprinted with permission from Sage Publications)*

FIGURE 20b *Bone tunnels in a knee with metal screws (reprinted with permission from Sage Publications)*

In Study IV, standard weight-bearing radiographs were taken with the knee in 30 degrees of flexion. Degenerative changes were graded according to the Ahlbäck[4] and Fairbank classifications. In the Fairbank classification, originally proposed for the documentation of radiological changes after meniscectomy, flattening (F), ridging (R) and narrowing (N) are documented in both the medial and the lateral knee compartments[51] (Figure 21). In the Ahlbäck classification, grade I is narrowing of < 50 % in either compartment. Grade II is total cartilage loss in either compartment. Grade III is bone attrition of < 5 mm. Grade IV is bone attrition of > 5 mm. Grade V is translation of the tibia.

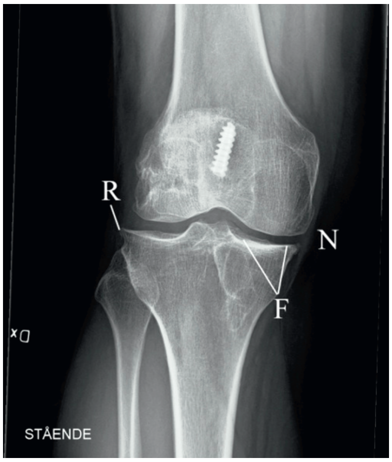


FIGURE 21 *Weight-bearing radiograph. According to the Fairbank classification, N is narrowing of the medial compartment, F is flattening of the tibial surface and R is ridging of the lateral and medial femoral condyle. © Sven Stener*

Magnetic resonance imaging (MRI).

Studies II and IV

In Study II, MRI examination was performed with a Siemens Magnetom 1.0-Tesla magnet (Siemens, Erlangen, Germany), using a flexible knee coil method. The ratios of the length, width and thickness of the patellar tendon in the injured and non-injured leg were evaluated by an experienced skeletal radiologist. A three-dimensional reconstruction program was used for axial reconstructions from which a mean average value for the width and thickness of the patellar tendon was calculated, using three different levels through the upper-, mid- and lower-third of the patellar tendon. The midpoint of the patellar tendon on the operated knee was then evaluated for the gap size in the axial dimension. The length of the patellar tendon from the apex of the patella to the insertion at the tibial tuberosity was calculated (Figure 22a). In Figure 22b, a calculation of width, thickness and residual donor-site gap is shown.

In Study IV, the patients were re-evaluated with MRI after two and ten years. The MRI appearances of the grafts were documented by an experienced skeletal radiologist.

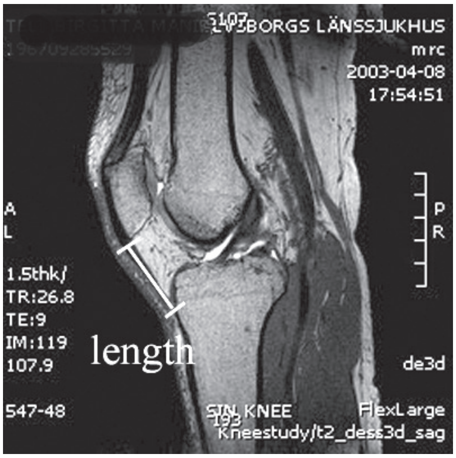


FIGURE 22a *Magnetic resonance image scan of the knee with measurement of tendon length after harvesting for ACL revision surgery. © Sven Stener*

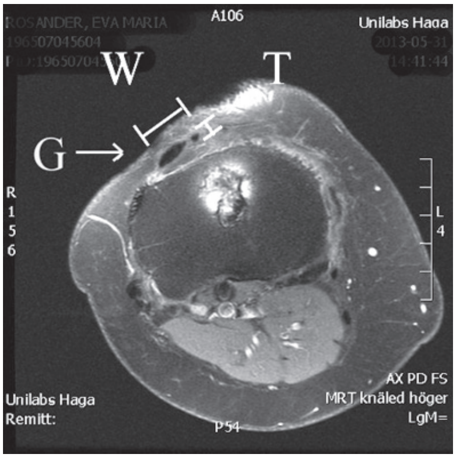


FIGURE 22b *Magnetic resonance image scan of the knee with measurement of the width (W), thickness (T) and residual donor-site gap (G) of the patellar tendon after harvest. © Sven Stener*

4.5 REHABILITATION

In Study I, the patients started closed-chain exercises during the first postoperative week. In Group C, 7/13 patients and, in Group B, 24/49 patients used a knee brace (Genu Syncro Quick-lock S 2300), locked in full extension when they walked and slept (Figure 23). During exercises, the brace was open for unrestricted range of motion training during the first four weeks. The patients in Group A did not use a brace.

In Studies II and IV, all the patients used a knee brace for the first four weeks (Genu Syncro Quick-lock S 2300). Early weight-bearing with crutches and unrestricted range of motion training was encouraged. Closed-chain exercises were started at three weeks. In Studies III and V, no brace was used.[24,90,171] In all studies, open-chain exercises were allowed after six weeks and light jogging was allowed after two to three months. Depending on the patient's fitness, a return to contact sport was permitted after six to 12 months.

FIGURE 23 *The knee brace used in Studies I, II and IV Genu Syncro S 2300 (Mediband, Stockholm, Sweden)*

4.6 LABORATORY EXAMINATIONS

In Study III, blood samples for C-reactive protein (CRP) were taken preoperatively, on day one, after one to two weeks and after four to six weeks. The samples were analysed using an immunotubidimetric method, (Hitachi 917 machine, Tokyo, Japan). The machine has a lower reference value of < 6 mg/L.

4.7 HISTOLOGICAL EXAMINATIONS

In Study IV, biopsies were obtained from the reconstructed ACL during the second-look arthroscopy using a biopsy forceps. For comparison, a biopsy from another patient's normal patellar tendon obtained at primary ACL reconstruction was used. The specimens were approximately 2mm^3 in size and fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 4-5 nm. The sections were stained with hematoxylin and eosin (HE) to evaluate fibre structure, cellularity and vascularity. The Alcian Blue (pH 2.5)/Periodic Acid-Schiff (AB/PAS) method was used to detect elevated levels of glycosaminoglycans (GAGs). An experienced pathologist and an orthopaedic surgeon with a special interest in tendon pathology evaluated the specimens (Figures 24 and 25). Both examiners were blinded to the location in the graft where the specimen was obtained. The grading was based on a semi-quantitative (non-parametric) score in terms of cellularity, vascularity, fibre structure and the presence of glycosaminoglycans (GAGs) [89] (Table 2). The fibre structure, vascularity and GAG content were graded after examining the whole section while the number of cells was estimated in a high-power field representative of the section.

	Grade 0	Grade1	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	200-299 cells	>300 cells
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	Marked increase in alcianophilia

TABLE 2 *The semi-quantitative score, Kartus et al.[89]*

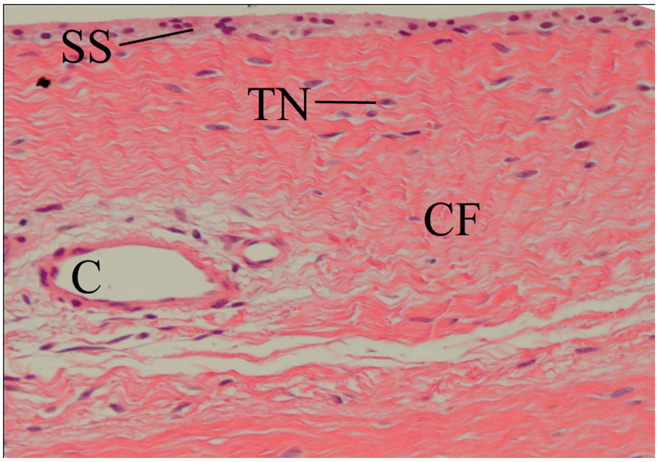


FIGURE 24 *A biopsy section showing the ligamentisation of a reharvested patellar tendon used as a graft for ACL revision surgery. SS = synovial sheet, TN = tenocyte nuclei, C = capillary, CF = collagen fibre in a wavelike appearance (approximate original magnification x200) (reprinted with kind permission from Springer Science and Business Media)*

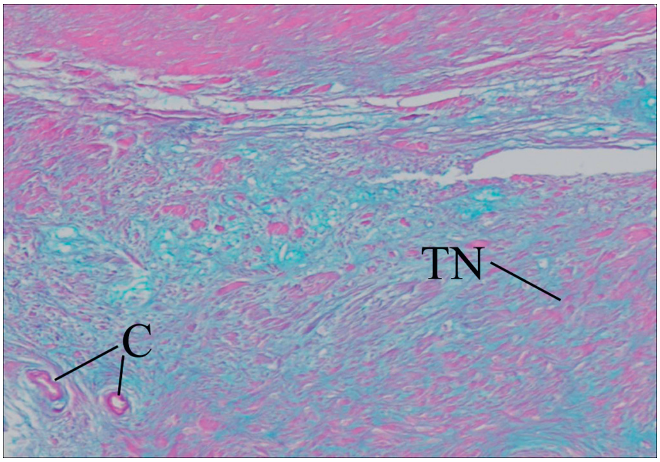


FIGURE 25 *A biopsy section, intensively stained, with a markedly increased occurrence of glycosaminoglycans. TN = tenocyte nuclei, C = capillary (approximate original magnification x200). (reprinted with kind permission from Springer Science and Business Media).*

4.8 STATISTICS

In all studies, median (range) values are presented unless the mean (SD) values are indicated. In all studies, a p-value of < 0.05 was considered statistically significant.

Study I

Wilcoxon's signed-rank, non-parametric, two-tailed test was used for the intra-individual comparison of the BMA of the injured and uninjured side. The chi-square test was used for the longitudinal comparison of stability and IKDC scores within treatment Groups B and C. Spearman's rank correlation coefficient was used to test the correlation between the variables within the treatment groups. The Mann-Whitney U test was used for the comparison of the BMA of the patients rehabilitated with or without a brace. Median (range) values are presented, apart from the total BMA for the injured and non-injured leg, where the mean (SD) is presented.

Study II

The Mann-Whitney non-parametric, two-tailed test and the chi-square test were used for comparisons of the two treatment groups. Group A and Group B were included in the primary analysis and all statistical calculations were based on this cohort. Group C was included as a reference group.

Study III

In terms of both continuous and non-continuous variables, the Mann-Whitney U test was used for comparisons between the study groups. The chi-square test was used to compare the dichotomous variables between the two groups. The appearance of the tunnels was regarded as the primary variable. We expected a difference of more than 2 mm in the appearance of the tunnels between the study groups in the medium and long term and estimated that the standard deviation within each group would be approximately 2 mm. The level of 2 mm difference between the study groups was chosen arbitrarily, but it was based on knowledge of the visibility of the tunnels in bone after the insertion of biodegradable fixation devices in the short and long term. With a sample size of 30 patients in each group, the power of the study was estimated at 90%.

Study IV

Median (range) values are presented.

Study V

Median (range) values are presented for the KOOS and the Tegner activity score. Mean values and standard deviations (SD) were used for the BMA and EQ-5D results. The paired t-test was used to compare the pre-operative and post-operative BMA values and to compare the BMA on the injured and uninjured side. BMA reduction during the five-year follow-up is also shown as the percentage decrease from the pre-operative values. The Wilcoxon's signed-rank test was used to compare the Tegner activity score and EQ-5D index changes over time. Spearman's rank correlation coefficient (rho) was used to test correlations between variables. The power of the study was estimated at $> 80\%$ with 25 patients and was based on standard deviations and BMA reductions found in patients after ACL reconstruction in former studies.

05 RESULTS

5.1 STUDY I

The pre-operative values in Group A in terms of Tegner activity level, KT-1000 laxity, manual Lachman test, IKDC classification and single-legged-hop test are shown in Table 3. In both Group B and Group C, the Tegner activity level, the manual Lachman test and the IKDC classification revealed a significant improvement as compared with the pre-operative values (Tables 4 and 5).

In Group A and Group B, the BMA in the calcaneus was significantly lower on the injured side as compared with the uninjured side before the reconstruction (p=0.001), Group A, and two years after reconstruction (p<0.001), Group B. In Group C, the BMA in the calcaneus was significantly lower on the injured side as compared with the uninjured side two years after ACL revision surgery (p=0.01). The pre-operative BMA ratio in the calcaneus (injured side/non-injured side in per cent) was 96(88-105)% in Group A. The post-operative BMA ratio at the two-year follow-up was 96(86-118)% in Group B and 95(83-111)% in Group C. In Group B, the BMA on the injured and non-injured side correlated with the Tegner activity level (rho=0.5).

Variable	Pre-operative
Tegner activity level	4 (2-8)
KT-1000 total side-to-side diff. (mm)	4 (-2.5-20)
Lachman test +2, +3	29/30
IKDC normal	0
IKDC nearly normal	0
IKDC abnormal	8/30 (27%)
IKDC severely abnormal	22/30 (73%)
Single-legged-hop test inj/non-inj (%)	81 (0-110)

TABLE 3 *Pre-operative values in Group A.*

Variable	Pre-operative	2-year follow-up
Tegner activity level	4 (2-9)	7 (3-9)
KT-1000 total side-to-side diff. (mm)	Not performed	2 (-5.5-10)
Lachman test +2, +3	47/49	2/49
IKDC normal	0	13/49 (27%)
IKDC nearly normal	0	24/49 (49%)
IKDC abnormal	13/49	11/49 (22%)
IKDC severely abnormal	36/49	1/49 (2%)
Single-legged-hop test inj/non-inj (%)	Not performed	94 (50-126)

TABLE 4 *Group B; results after a median of 24 (23–29) months.*
Note: significance: Tegner activity level, p<0.001, Lachman test, p<0.001, IKDC, p<0.001.

Variable	Pre-operative	2-year follow-up
Tegner activity level	3 (2-7)	6 (2-7)
KT-1000 total side-to-side diff. (mm)	Not performed	3 (-4-8.5)
Lachman test +2, +3	13/13 (100%)	3/13 (23%)
IKDC normal	0	1/13 (8%)
IKDC nearly normal	0	6/13 (46%)
IKDC abnormal	0	5/13 (38%)
IKDC severely abnormal	13/13	1/13 (8%)
Single-legged hop-test inj/non-inj (%)	not performed	88 (0-104)

TABLE 5 *Group C; results after revision, median 24 (20–45) months.*
Note: significance: Tegner activity level, p<0.001, Lachman test, p<0.001, IKDC, p<0.01.

5.2 STUDY II

Follow-up examinations showed that there were no significant differences in terms of KT-1000 arthrometer laxity total side-to-side measurements between the groups, but the Lysholm knee score was significantly higher for patients with contralateral, primary harvested tendon grafts than for patients with ipsilateral, reharvested tendon grafts. No significant differences were found between Groups A and B regarding the Tegner activity level or the single-legged-hop test (Table 6). The IKDC classification is shown in Table 7.

Magnetic resonance imaging screening revealed no difference between primary harvest and reharvest of the patellar tendon in terms of length, width, thickness and residual donor-site gap.

Variable	Group A	Group B	Group C	Significance
KT 1000 total side-to-side diff. (mm)	3 (-0.5-8.5)	2 (0-5.5)	3 (-7-6.5)	p=0.11
Lysholm knee score	62 (25-89)	84 (55-95)	90 (38-99)	p=0.002
Tegner activity level	5 (1-7)	5 (2-7)	6 (1-9)	p=0.3
Single-legged-hop test inj/non-injured leg (%)	88 (0-118)	88 (62-120)	87 (50-104)	p=0.4

TABLE 6 *Results of ACL revision surgery, after 26 (20–33) months in Group A and 24 (22–30) months in Group B. Results of primary ACL reconstruction after 24 (23–26) months in Group C. Median (range) values are reported.*

Variable	Group A	Group B	Group C
Normal			5
Nearly normal	3	7	5
Abnormal	7	4	2
Severely abnormal	2	1	
Major complication	2		
Re-operation meniscus	1	2	2
Re-operation hardware	1		2

TABLE 7 *IKDC, final evaluation, at a median (range) of 26 (20–33) postoperatively in Group A, at 24 (22–30) months in Group B and at 24 (23–26) months in Group C.*
Note: in Group A, two major complications were registered, one patellar fracture and one patellar tendon rupture.

5.3 STUDY III

At the long-term follow-up, the PLLA group displayed significantly larger bone tunnels on the radiographs than the metal group on the femoral side but not on the tibial side (Table 8). The CI with a 95% confidence limit was 11.4(±0.9) mm and 8.0(±1.2) mm in the PLLA and metal group on the femoral side respectively.

No significant differences were found between the PLLA and metal groups in terms of the KT-1000 knee laxity measurements, Tegner activity level, Lysholm knee score and the single-legged-hop test (Table 9).

On day one postoperatively, the CRP value was a median (range) of 23 (<6-55) mg/L in the PLLA group and a median (range) of 9 (<6-47) mg/L in the metal group ($p<0.001$). At one to two weeks and at four to six weeks, there were no significant differences in the CRP values.

Variable	PLLA group N=33	Metal group N=31	Significance	CI PLLA/metal
Femoral tunnel (mm)	11.4 (3.7)	8.0 (5.1)	p<0.005	±0.9/±1.2
Tibial tunnel (mm)	10.7 (1.7)	10.5 (2.6)	n.s.	±0.4/±0.6
Obliterated femoral tunnel (n)	2/33	9/31		
Obliterated tibial tunnel (n)	0	1/31		

TABLE 8 *Radiographic mean (SD) results of the bone tunnel diameters eight years after ACL reconstruction using HT autografts, n.s. = non-significant. CI = confidence intervals for the mean values with 95% limits. Note: obliterated = completely healed bone tunnel.*

Variable	PLLA group	Metal group	Significance
Follow-up period (months)	99 (84-120)	96 (78-108)	n.s.
Tegner activity level	7 (3-9)	6 (2-9)	n.s.
Lysholm knee score	90 (51-100)	89 (53-100)	n.s.
KT1000 anterior side-to-side diff. (mm)	1 (-2.0-4.0)	1 (-3.0-6.5)	n.s.
Single-legged-hop test inj/non-inj leg (%)	96 (60-132)	96 (0-113)	n.s.

TABLE 9 *Clinical results after eight years, median (range values), n.s. = non-significant.*

5.4 STUDY IV

The clinical results were poor at both two and 10 years postoperatively, with a Lysholm knee score of 43, 85, 25 and 64 respectively for the four patients after two years and 68, 64, 44 and 51 respectively among the four patients after 10 years. At 10 years, three of the patients had degenerative radiographic changes in both the medial and lateral compartments and one of the patients had degenerative changes in the medial compartment (Table 10). After 10 years the reconstructed ACLs were evaluated using MRI by an experienced radiologist and the grafts were classified as normal in one patient, thickened/oedematous in two patients and thin/oedematous in one patient.

At the second-look arthroscopy, three years after the reconstruction, the grafts appeared macroscopically normal in all four patients, with visible blood vessels in two patients. In one patient, the reconstructed ACL had an almost normal histological ligament appearance and in all four patients signs of ligamentisation were present. Two patients had an increase in sulphated glycosaminoglycans. The individual results for the semi-quantitative score are shown in Table 11.

Variable	Patient 1, male 28 y	Patient 2, male 26 y	Patient 3, female 29 y	Patient 4, female 26 y
Meniscal damage at revision	None	Medial	Medial and lateral	Medial
Radiograph medial compartment, 10 years	N, R	Ahlbäck I, N, R	N, R	R
Radiograph lateral compartment, 10 years	Ahlbäck I, Fairbank N, R	Normal	Fairbank F, R	Fairbank F, R
MRI graft appearance, 10 years	Normal	Oedematous, thickened	Oedematous, thin	Oedematous, thickened

TABLE 10 *Results after 10 years. N = narrowing, R = ridging, F = flattening of the articular compartment according to Fairbank's classification.*

Variable	Patient 1, male 28 y	Patient 2, male 26 y	Patient 3, female 29 y	Patient 4, female 26 y
Fibre structure	1	2	1	1
Cellularity	0	2	2	1
Vascularity	0	2	3	0
Glycosaminoglycans	0	3	0	1

TABLE 11 *Individual data on the semi-quantitative tissue score, Grade 0–3.*

5.5 STUDY V

During the study period, the BMA decreased significantly in both calcanei and in both hips among both female and male patients. The reduction in BMA during five years in the calcanei of female and male patients compared with the reference values is shown in Figures 26 and 27. The BMA in the lumbar spine decreased by 1.6% (n.s.) and 1.7% (p=0.021) in the female and male patients respectively after five years. Apart from the male patients, the reduction in the lumbar spine was not significant compared with the pre-operative values, after five years. The Tegner activity levels pre-injury and pre-operatively were a median (range) of 7.5 (1-10) and 2.5 (0-9) respectively (p<0.001). The Tegner activity level and EQ-5D index results revealed significant improvements compared with the preoperative results (Table 12). At two years, the KOOS score had improved significantly in all five subscores compared with the preoperative assessments (Figure 28). The Tegner activity level and the EQ-5D index values did not correlate with the decrease in BMA.

Variable	Pre-operative	6 months	18 months	36 months	60 months
Tegner level females	2 (0-5)	3 (1-10)	4 (2-9)	4 (1-9)	3 (1-9)
Tegner level males	3 (0-9)	4 (0-9)	5 (2-9)	5 (2-9)	4.5 (1-9)
Missing values			7	9	4
EQ-5D index females	0.69 (0.21)	0.84 (0.16)	0.86 (0.11)	0.89 (0.12)	0.87 (0.12)
EQ-5D index males	0.74 (0.24)	0.85 (0.19)	0.85 (0.18)	0.86 (0.20)	0.85 (0.20)
Missing values			6	8	

TABLE 12 *Tegner activity level, median (range) values and EQ-5D mean (SD) results during five years.*

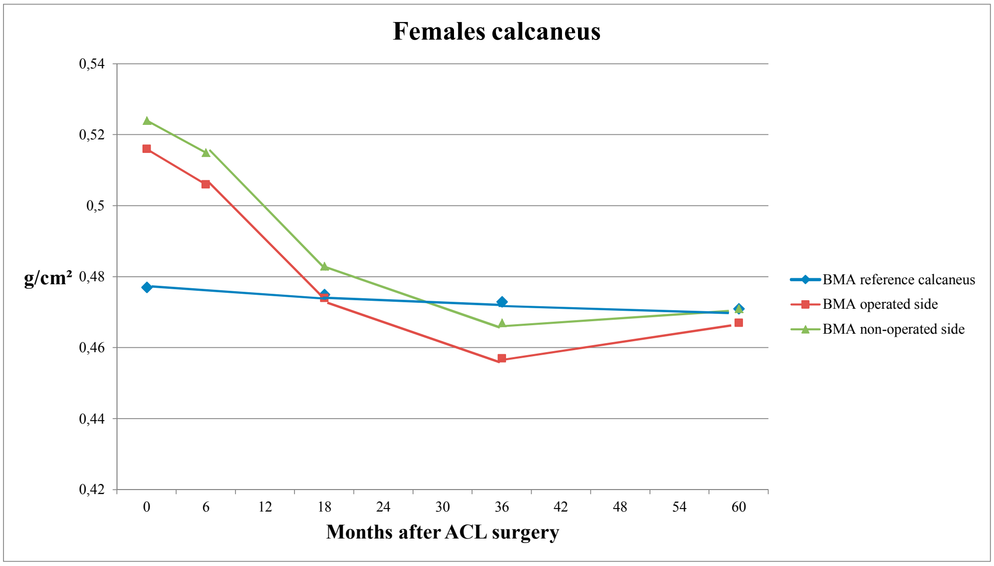


FIGURE 26 *The reduction in BMA of the calcaneus among female patients during five years compared with reference values of the Calscan.*

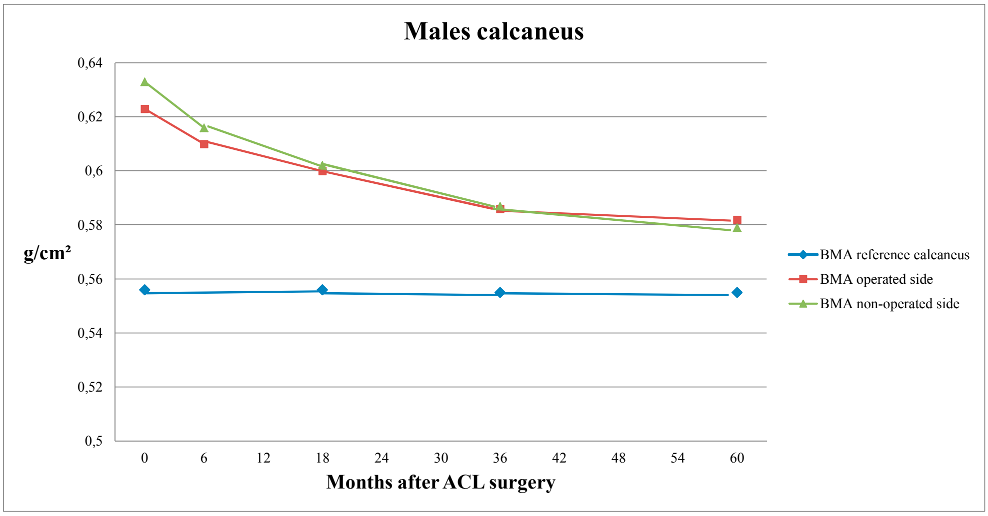
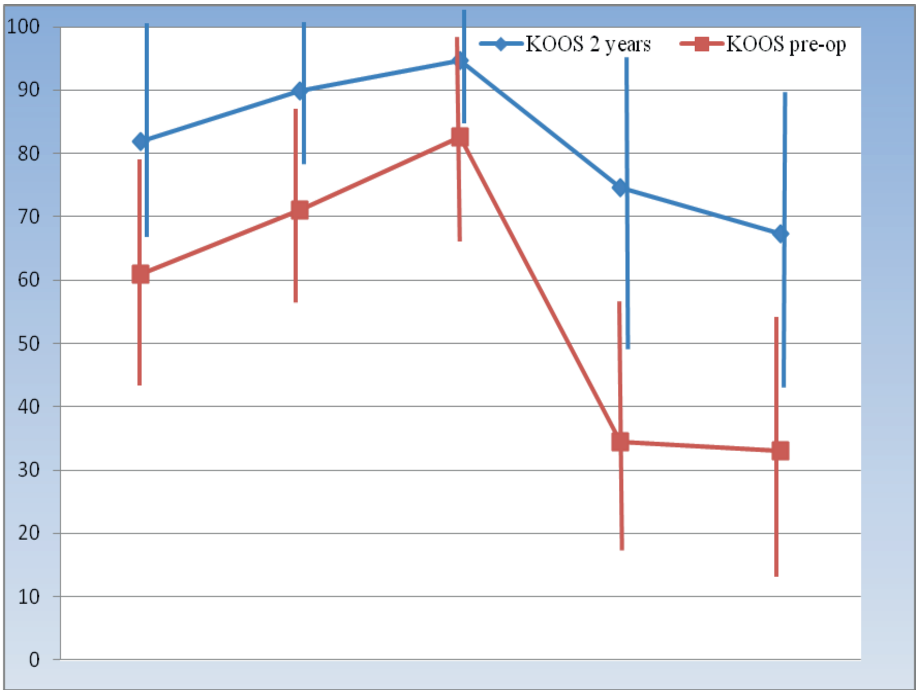


FIGURE 27 *The reduction in BMA of the calcaneus among male patients during five years compared with reference values of the Calscan.*



	Symptoms	Pain	ADL	Sports/Rec	QOL
KOOS Preop	60.9(18.0)	71.1(16.6)	82.6(15.8)	34.4(20.8)	33.0(19.7)
KOOS 2 years	81.9(17.4)	89.9(12.3)	94.7(9.0)	74.6(23.8)	67.4(23.2)

FIGURE 28 *The pre-operative and the two-year results for the KOOS score, mean (SD).*

06 CONCLUSIONS

The BMA of the calcanei on the injured side had decreased compared with the uninjured side both before and two years after ACL reconstruction with BPTB autografts in male patients scheduled for ACL reconstruction.

The reduction in the BMA of the calcanei among the male patients was correlated with the activity level after two years. The reduction was not correlated with the functional performance or the time period between the injury and the reconstruction.

The use of reharvested ipsilateral patellar tendons resulted in a significantly lower Lysholm knee score and poorer IKDC score two years after ACL revision reconstruction compared with using primary harvest, contralateral patellar tendons.

Magnetic resonance imaging revealed that reharvested patellar tendons did not differ from primary harvested tendons in terms of length, thickness, width and the size of the residual donor-site gap two years after reconstruction.

In patients scheduled for ACL reconstruction using HT autografts, significantly more bone-tunnel widening as seen on standard radiographs eight years after surgery was found on the femoral side but not on the tibial side after using PLLA interference screws compared with metal screws. The difference in bone-tunnel widening was not correlated with the clinical outcome of the patients.

There was a significant difference in the CRP response in patients undergoing surgery with PLLA interference screws compared with metal screws on the first postoperative day, but no difference was found after the first week and thereafter.

In an in-depth study of four patients receiving reharvested ipsilateral patellar tendons autografts, inferior clinical results were found after both two and 10 years. Despite this, the patients displayed firm stable knees and the histological and radiographic evaluation revealed a transformation (ligamentisation) from a tendinous to a ligamentous structure of the grafts.

After ACL reconstruction with hamstring autografts, the BMA in the calcanei and in the hips decreased on the operated and the non-operated side more than the expected age-dependent decrease in reference populations of Swedish women and men. The patients increased their Tegner activity level and improved their EQ-5D index during the five-year follow-up period. The decrease in BMA was not correlated with the time from injury to surgery, with the Tegner activity level or with the quality of life EQ-5D index results.

07 DISCUSSION

One important finding in this thesis is that female and male patients lose more BMA in the calcanei and in the hips on the operated and the non-operated side compared with normal reference populations during a five-year period after ACL reconstruction with HT autografts. Moreover, among male patients, the BMA in the calcaneus on the injured side after an ACL injury is significantly reduced compared with the uninjured side before and two years after ACL reconstruction with BPTB autografts.

Another important finding is that bone-tunnel widening is still present eight years after ACL reconstruction with hamstring tendon autografts. The bioabsorbable PLLA screws reveal more bone-tunnel widening after eight years than the metal screws on the femoral side.

Improved clinical results are accomplished when using contralateral instead of reharvested ipsilateral patellar tendons in ACL revision surgery. In a histological and radiographic in-depth study of patients from the reharvested ipsilateral group, the patellar tendon graft appears to transform into ligamentous tissue resembling native intra-articular ligament in spite of inferior clinical results.

7.1 LIGAMENTISATION OF TENDON GRAFTS

In 1986, Amiel et al.[7] reported in an animal study of rabbits how a quadriceps/patellar tendon graft transformed into a tissue similar to a ligament in ACL reconstruction. The process is termed "ligamentisation" and is regarded as the fundamental process in which a transplanted tendon graft is able to function as a new ligament in a patient suffering from an intra-articular ligament injury in the knee. Three important criteria constitute the transformation of a tendon into a functional ligament. First, there is an increase in collagen type III, which is normally more common in ligaments than in tendons. Secondly, the glycosaminoglycan (GAG) content is highly elevated when a tendon is transformed into an intra-articular ligament. The third finding in the ligamentisation of tendon grafts is the transformation between two different kinds of reducible collagen cross-link, as well as a conversion of collagen cross-linkage from reducible to non-reducible. The latter type is four to five times more common in ligaments than in tendons. Without this basic biological process, an ACL reconstruction would probably be unsuccessful.

In a recent systematic review of the subject, Claes et al.[32] compared four human biopsy studies of transplanted tendon autografts that discussed the time scale in the ligamentisation process.[1,52,134,138] In contrast to most animal studies, the grafts in these human studies appear to be viable at any time during the course of ligamentisation.[134,52,1] In these human studies, the ACL grafts were histologically viable, with nourishing vessels probably originating from the Hoffa fat pad and the synovium. There were no signs of any significant graft necrosis, except exclusively in the centre of the grafts, and a key aspect in the transformation is the invasion of new cells probably originating from the large pool of multipotent, mesenchymal stem cells in the synovial fluid.[114,137] The synovium probably also plays an important role in nourishing the graft before the ingrowth of new capillaries is completed.

The authors in these four studies propose that ligamentisation comprises three different stages; the "early stage", remodeling stage and maturation stage. Although the time frame differed between these four studies, the authors hypothesised that the remodeling stage of the graft was probably completed in one year.[1,52,134,138] Histologically, a graft in the maturation stage may resemble a normal human ACL, but ultrastructural differences regarding collagen fibril distribution persisted. One interesting conclusion in the studies mentioned above was that the remodelling phase continued for approximately one year, but the authors hypothesised that the maturation phase of tendon grafts probably exceeded at least two years.

It is not known how vulnerable a tendon graft is during this maturation phase, as mechanical studies are not possible in human knees. Alm et al. performed a histological study on 13 patients and found that instability and inferior clinical results correlated with hypocellularity, poor vasculature and disintegration of the grafts.[5] Animal studies have revealed that patellar tendon grafts that are reharvested lose at least 50% of their strength compared with primary harvested tendon grafts.[127,99] In a study by Marumo et al., involving 50 patients surgically reconstructed with either BPTB or HT grafts, the authors reported that the ligamentisation process continued for one year or more and they therefore proposed the use of a low-aggressive rehabilitation programme.[110]

Four of the patients involved in Study II were available for a longer follow-up study in Study IV, with second-look arthroscopy and histological evaluation of the grafts. The aim was to examine the patients with reharvested patellar tendons for a longer time period and to analyse the histological, radiographic and clinical results. As previous studies have shown that the patellar tendon does not regenerate to a normal tendon structure six years after harvest for a BPTB graft, using it a second time for revision surgery might be questionable.

The patients in Study IV with reharvested BPTB grafts regained stable knees during the follow-up and the biopsies performed after three years revealed macroscopic and histological signs of ligamentisation. One interesting finding was that there was a discrepancy in the histological picture in all four patients. This might be due to different stages in the remodelling process between patients or to the possibility that the biopsies were taken from different parts of the grafts. Two of the patients had a marked and a moderate increase respectively in GAG concentration, indicating late stages in the ligamentisation process. GAG is linked to the proteoglycan molecule as a side-chain and, together with water, probably provides the lubrication between tendon collagen fibres. The GAG concentration increases three times in concentration in the conversion from patellar tendon to a functional ACL graft. It is also highly elevated in pathological tendon disorders.[115,6] Another difference in the patient's histological pictures was the amount of new capillaries and cells. Collagen disarrangement, as seen in one of the biopsies, could be caused by mishandling of the biopsies (artifact), but it could also represent signs of a local graft rupture.

A resemblance to a native ACL was only seen in one of the patients otherwise the grafts appeared to be viable and functional. In three/four patients, the grafts appeared to remain in the remodelling stage according to the various histological pictures three years after revision reconstruction. The differences that were found could indicate that the time frame for ligamentisation might be longer than in earlier studies and/or exhibit differences between individuals.

The clinical results in terms of the Lysholm knee score and the Tegner activity level were poor in these four patients. The inferior clinical results were probably due to the degenerative changes in the knee and radiographic signs of incipient osteoarthritis found on the weight-bearing plain radiographs. One of the patients failed to attend MRI after two years and, as a result, only the ten-year assessments were presented in the study.

One limitation, when it comes to histological studies is that biopsies have to be small (2-3 mm^3) and sampled from the surface of the graft. This necessitates further studies of graft ligamentisation, preferably from different parts of the graft, in the future. Comparisons between different histological studies are difficult and necessitate a valid and reliable scoring system. To the best of our knowledge, the scoring system designed by Movin et al. and subsequently used by Kartus et al. is reliable and has been used in tendon pathology studies and in patellar tendon studies as well.[89,115]

Another limitation of Study IV was the limited number of patients. Our purpose was to find at least ten or twelve patients on whom we could perform follow-up studies for ten years, but soon after the study started, the use of hamstring grafts was introduced and only a limited number of patients therefore fulfilled the inclusion criteria.

7.2 GRAFT CHOICE IN ACL REVISION SURGERY

In the modern era of ACL reconstruction through to the 1990s, the patellar tendon graft was the most popular graft choice in primary ACL reconstruction surgery.[59,48,78] Since then, a considerable number of research papers have discussed the difference between BPTB grafts and HT grafts. In systemic reviews and meta-analyses from Spindler et al. and Freedman et al., the two grafts were equivalent, based on objective measurements, and, as a result, none of them can be recommended over the other. PT grafts have the advantage of decreased laxity, increased hamstring strength and a higher percentage of return to high-activity sports compared with HT grafts. HT grafts, on the other hand, usually induce better range of motion, (ROM) and less anterior knee pain.[150,55] Yunes et al. concluded in their meta-analysis (M-A) that patients receiving BPTB grafts had an 18% better return rate to sports and physical activity. Another advantage was that the BPTB grafts rendered significantly less instrumented knee laxity than HT grafts, but the failure rates and number of complications were the same.[175]

When revision surgery is indicated, surgeons have many choices, but the differences in results between grafts have not been thoroughly investigated. In the 1990s, a reharvested patellar tendon constructed to create a BPTB graft was an option among some surgeons.[121,86,34] The main advantage is the absence of surgical trauma to the uninjured knee. Ultrasound studies and computed tomography (CT) have demonstrated that healing of the donor-site patellar tendon could be expected to take place between six months and one year after primary harvest.[31,2] In one MRI study, the donor-site tendon thickness had increased and the remaining residual donor-site gap was still visible two and ten years after reharvesting the central-third of the tendon compared with the healthy contralateral side.[103] Histological and radiographic studies of the donor-site patellar tendon after primary harvest for BPTB reconstruction have also demonstrated insufficient regeneration of the patellar tendon.[12,89,152-154] Histologically, the scar tissue in the tendon does not return to normal tendon structure after six years.[152] This is in line with previous studies that have used MRI or CT to evaluate the donor-site patellar tendon after surgery.[35,133] Nixon et al., on the other hand, propose that the patellar tendon appears to be restituted after two years based on MRI and histological evaluation.[120] Apart from this, there are other unsolved questions regarding the mechanical strength of the patellar tendon after harvesting. In animal studies by LaPrade et al.[99] and Proctor et al.[127] using canine and goat models respectively, the findings revealed that the reharvested, mid-third tendon graft only regained 50-60% of the primary harvested patellar tendon graft strength. The question therefore arises of whether the reharvested tendon graft has the strength needed to withstand the forces imposed on the grafts during daily living and sports activities.

Study II in this thesis was designed to compare the clinical results between reharvest and primary harvest of BPTB grafts in ACL revision surgery. For this purpose, two groups were evaluated after two years regarding MRI of the patellar tendons and clinical outcome. The clinical outcome revealed inferior results in the reharvested group, with a lower Lysholm knee score, poorer IKDC evaluation and more serious complications, including one patellar fracture and one patellar tendon rupture. MRI screening revealed no difference in tendon length, width, thickness and residual donor-site gap between reharvested or primary harvested tendons after two years. Although MRI assessments do not describe the quality of the tissue in the tendon defect we concluded that the tendon has regenerating potential, as there was no difference in tendon size or donor-site gap between the reharvested and the primary harvested groups.

One limitation in the design of that study was that no quality-of-life outcome was used. After this study, we have seen an increasing interest in knee-related scores like the KOOS and health-related quality-of-life scores like the EQ-5D in ACL research. These scores would have been useful for evaluating the clinical relevance of this study. Finding patients for revision surgery is time consuming and the study was therefore performed at two institutions with three surgeons. The relatively small patient groups and the lack of pre-operative data on the patients are therefore a considerable drawback of the study. A further limitation was that, although the groups were comparable in terms of demographics, the patients were not randomised. Furthermore, histological evaluation of the residual donor-site tendon gap with biopsies would have been useful as complement to the MRI study.

In the 1990s, the concept of anatomical positioning of the grafts was not routinely discussed in ACL surgery. Instead, the grafts were most frequently placed vertically with the aim of ensuring an isometric position where the graft was to be placed. Recent studies have revised this opinion and introduced a lower fixation point on the femoral wall. With three different surgeons, it could be questionable whether the grafts were fixated at approximately the same site in all the patients. Plain radiographs postoperatively would have contributed to a more exact comparison between the two groups. Another limitation is the KT-1000 results. At the time of this study, it was customary to use 89 N in laxity testing. Subsequently, 134 N or manual maximum force has been found to determine small differences in laxity between groups and in laxity changes in each patient after surgery. Despite the limitations, it is important to account of the difference between the two groups when dealing with a patient with his or her second ACL injury to the same knee. There are disadvantages when it comes to harvesting the patellar tendon from the uninjured knee, if the patient suffers a contralateral injury later in life. In this case, HT tendons are a potential option, but they can also be a problem if the patients are re-injured and the surgeon has run out of autograft options.

7.3 BIOABSORBABLE SCREWS AND TUNNEL WIDENING

Hamstring grafts are used without bone blocks at each end and therefore heal in the tendon-bone interface inside the tunnels more slowly than BPTB grafts.[131] Histologically, tendon-to-bone healing is different from bone-to-bone healing and results in more bone-tunnel widening primarily during the first six weeks.[177] The first reports that compared BPTB grafts and hamstring grafts revealed that hamstring grafts induce more bone-tunnel widening than BPTB grafts.[33,96,165] Mechanical and biological mechanisms are basically involved in the phenomenon of bone-tunnel widening. Among biological factors, non-specific inflammatory response, cell necrosis due to toxic products in the tunnel, synovial fluid invasion around the graft and heat necrosis as a response to drilling are the most frequently discussed explanations.[160,168] Most researchers agree that the mechanical factors are equally important and accelerated rehabilitation has some negative effects on the bone surrounding the graft. Longitudinal and transverse motions, which are called “bungee effects” and “windshield wiper effects”, increase if the rehabilitation is too "aggressive".[74,67] A less aggressive regimen when rehabilitating patients with HT grafts compared with BPTB grafts could therefore be recommended.

Soon after the introduction of hamstring grafts, bioabsorbable interference screws became increasingly popular. PLLA was the most common product that was used because of the slow degradation of the substance, which allowed the graft to be fully incorporated in the bone tunnels.[166] One early observation made by surgeons was that some of the PLLA implants on the market induced more osteolysis and bone-tunnel widening than metallic implants. In a randomised study of ACL reconstruction with hamstring grafts, Laxdal et al. found more bone-tunnel widening on the femoral and the tibial side using PLLA interference screws compared with metal screws after two years.[100]

Frequent studies presented since 1995 have concluded that the increased bone-tunnel widening caused by bioabsorbable screws does not appear to affect the clinical outcome.[53,15,112,10] These results are in accordance with two recent meta-analyses of randomised controlled trials (RCTs) comparing bioabsorbable interference screws and metal screws. In the first M-A, Shen et al.[146] summarised 10 studies comprising 790 patients. The median follow-up in these analysed studies was 24 months. Hamstring tendons were used in four of the studies and BPTB grafts were used in six studies. Increased bone-tunnel widening was seen in three of the hamstring studies,[100,113,116] but no widening or osteolysis was found in the BPTB studies. This difference was predictable, as there is substantial benefit in terms of the osteogenic effect of the bone blocks in the BPTB grafts. Objective stability, measured with the KT-1000 arthrometer, Lachman test and pivot shift test, revealed no differences between the groups.

In the M-A by Emond et al. in 2011, pooling of the data from the bone tunnel measurements was not possible because of the wide variety in the way the radiographic data were reported.[46] Data were obtained from eight studies and, in four of them, the bioabsorbable screws were made of PLLA. Before the publication of Study III, no RCTs with more than three years of follow-up could be found.

In these two M-As, the only clinical difference that was found was that knee-joint effusions were more common in the bioabsorbable groups compared with the metal groups. Adverse tissue reactions to bioabsorbable implants in general orthopaedics and fracture surgery are common, but there appear to be fewer complications when it comes to intra- and extra-articular synovial reactions, such as knee effusion and pretibial cysts in ACL surgery. ACL surgeons generally use PLLA screws instead of polyglycolic acid (PGA) screws because of the slower degradation time for PLLA. Among the complications in ACL surgery with bioabsorbable screws, the most common are early or late screw breakage, screw migration to the knee with knee effusion as a result and migration to the periosteum with tibial cyst formation.[10,81,28] In Study III, no adverse reactions were seen after eight years, despite some large osteolytic changes in some cases. PLLA is hydrophobic, with a prolonged degradation time, and adverse reactions, such as bone resorption and soft-tissue reactions, can therefore be expected after more than three years.[93,166] The PLLA manufacturers claim that the implants should be slowly replaced by bone during degradation, but this has never been shown in any in vivo study. Drogset et al. performed an MRI study on patients seven years after the implantation of PLLA screws using BPTB autografts. In none of the 16 patients had the PLLA screws been replaced by bone in the tibia.[42] Consequently, long-term studies are needed to evaluate the clinical outcome using bioabsorbable screws.

Former studies by Buelow et al. and Webster et al. had proposed that plain radiographs are comparable to MRI and CT with regard to measurements of bone-tunnel diameters after surgery.[27,165,164] The inter-rater and intra-rater test-retest calculations before Study III began were close to the values presented by Webster et al.[164] In their study of 76 patients with ACL reconstructions and HT grafts, they presented results with substantial measurement errors, especially in the inter-rater test. When widening or enlargement of bone tunnels was defined as a change that exceeded inter-rater measurement error, they found a significant correlation between radiographic widening of the tibial tunnel and anterior knee laxity. Disagreements exist among researchers in this area. Moisala et al. and Järvelä et al. claim that tunnel widening is correlated with knee laxity, but most researchers are of the opposite opinion.[113,77]

One limitation of Study III is the different radiographic techniques that were used. The immediate postoperative and two-year radiographs were taken with analogue films and, after eight years, digital films were introduced. As a result, comparisons of tunnel widening could only be performed between groups after eight years when all films were digital.

One problem that needs to be solved in long-term studies is that patients are lost to follow-up. At two years, nine patients had dropped out and, between two years and eight years, another four patients were lost, leaving a total of 64 patients in the study after eight years (33 in the PLLA group and 31 in the metal group). Large-scale efforts were made at the institution, with letters and phone calls, in order to examine as many patients as possible. The power of the study was calculated as > 90% with 30 patients in each group. Three patients in the PLLA group and one patient in the metal group developed signs of septic arthritis and two of them had positive bacterial cultures. This is an exceptionally high number which we cannot explain. Apart from these misfortunes, three patients were excluded from the laxity calculations because they sustained a re-rupture of the ACL graft before the eight year follow-up assessments.

7.4 BONE MINERAL ASSESSMENTS IN ACL SURGERY

Studies I and V in this thesis examine the bone-mineral reduction in the calcanei after an ACL injury. In Study I, the main finding was that, after an ACL injury and two years after an ACL reconstruction with BPTB grafts among male patients, the bone-mineral areal mass (BMA) in the calcaneus on the injured side was significantly reduced compared with the uninjured side. Only male patients were assessed in order to obtain the most uniform group of patients possible. The reason for involving male patients in the study was that, at the time this study was initiated, female patients with an ACL injury were not as common as male patients at the institution and it would therefore have been difficult to find enough patients during a reasonable time period. In addition, comparisons between groups with both men and women are more difficult, due to different hormone-related mechanisms. Estrogen is known to protect against bone loss and this is primarily mediated through estrogen receptor (ER)α and estrogen receptor (ER)β.[170] [101] The adaptive response of bone to mechanical loading differs between women and men and, in recent studies, the effect of ERα mediated bone formation during mechanical loading in trabecular and cortical bone differs between females and males in animal studies.[170,169]

No association was found between the time period before injury and the BMA assessment just prior to the reconstruction. This finding could support the theory that the BMA reduction on the injured side after ligament injuries to the knee occurs fairly rapidly after the trauma and then stabilises at a lower level.[8,147,85] Despite a fair outcome after surgery and an increase from 4(2-9) to 7(3-9) in the Tegner activity level, there were still significant differences between the operated and the non-operated leg two years after reconstruction.

A positive correlation was found between the Tegner activity level and the BMA of the operated and the non-operated leg in the group of patients assessed two years after surgery. The patients with a higher BMA had a significantly higher activity level, but they did not perform better on the single-legged-hop test. Despite early and accelerated rehabilitation after surgery, there was a significant reduction in BMA on the operated side and it is not clear whether the surgical trauma or the injury itself is responsible for the decrease in BMA. Another finding was that the BMA reduction did not differ between patients wearing a post-operative brace and those that did not.

One limitation was that no KT-1000 arthrometer laxity tests or single-legged-hop tests were performed pre-operatively in the two-year follow-up group and the revision control group. Furthermore, the KT-1000 laxity measurements revealed wide ranges, making statistical evaluation difficult. As mentioned above the 89 N force used in laxity measurements should have been replaced by 134 N or manual maximum force in order to identify small changes in knee laxity.

Study V was initiated after previous studies at our institution had revealed a BMA reduction in the calcaneus after ACL reconstruction using BPTB grafts.[43,91]. Contrary to the findings in Study V, Leppälä et al. and Zerahn et al. did not find a reduction in BMA in the calcaneus after ACL reconstruction, but they did find a significant reduction in the proximal tibia and distal femur.[102,176] Different patient demographics and rehabilitation protocols are two possible explanations for the discrepancy.

In Study V, the patients were assessed with repeated BMA measurements in both calcanei, both hips and the lumbar spine using the dual-energy X-ray absorptiometry (DEXA) method, which is used to confirm a diagnosis of osteoporosis. All 48 patients who completed the study for five years lost BMA in both calcanei and in the hips more than the expected age-dependent loss.[95,104] The surgical outcome was evaluated with the Tegner activity level, the KOOS score and the EQ-5D quality-of-life questionnaire. Despite a successful outcome after surgery, the patients lost 7-10% of BMA in the calcanei, 3-5% in the hips and 1.5-2% in the spine.

There are genetic, ethnic and geographical differences in the reference values of DEXA measurements that make comparisons difficult.[71,104,45] In addition, BMA values cannot be compared between different DEXA machines. For this reason, only the change in BMA during follow-up assessments was evaluated and discussed and not the absolute values. According to reference data on BMA in the hip in different age groups of Caucasian women and men, there should be less than a 0.4% yearly change from 22-49 years of age.[47,158,94] Comparable reference data on BMA in the spine have not been found in the literature, but the BMA should be fairly unchanged from 22 to 45 years of age in the lumbar spine region and in the case of spinal osteoarthritis, an increase in BMA is not uncommon.[94]

Most women and men in Study V were athletes or participating in high level activities and their Tegner activity score dropped considerably during the time before surgery. The BMA in the calcaneus was ~1 SD above the mean reference values among the women and ~1.5-2 SD above the mean reference values among the men. We were unable to conclude that the BMA decrease seen in this study can be transferred to other patients with a lower activity level. One theory behind the dramatic decrease in BMA could be that athletes, with a higher BMA than average, might lose more BMA when sustaining an injury than patients with a lower activity level.

Drilling of the bone tunnels has a direct osteoclastic effect and another contribution to the bone loss could be the catabolic effect of the surgery itself. Scientific progress in bone and osteoporosis research has shown that, in certain situations, bone resorption is uncoupled from bone formation. If there are enough molecular signals for bone resorption, the result will be bone loss. In analogy, for bone formation to take place, a different molecular signal uncoupled from resorption is needed.[19] This is probably the reason why the patients in Study I and V lost so much bone, despite being healthy individuals with above average BMA levels pre-operatively. It is difficult to explain why no recovery of the pre-operative BMA status took place, despite accelerated rehabilitation. Non-mechanical factors such as hormones, dietary calcium, vitamin D metabolites, genes and so on only contribute between three to as much as ten per cent of the postnatal strength of bones. On the other hand, mechanical loading effects on bone modelling and remodelling contribute to more than 40% of bone strength.[57] So, one plausible lesson learnt from Studies I and V is that the rehabilitation protocol might not include enough exercises that augment bone formation.

One limitation was the drop-out of 19 patients since enrolment took place. The power analysis revealed that 25 patients were needed to find a BMA change of 1% during the follow-up period, but, with more patients than 48 attending all the assessments, a subgroup analysis would have been possible. Another limitation was that the time from injury to surgery was a median of 9.5 months and had a range of 3-240 months.

Consequently, some of the patients could have had a problem remembering their Tegner activity level before injury, contributing to a recall bias. Moreover, the long waiting time before surgery could have had an effect on the BMA change found after surgery, even though this is a speculation.

Another limitation was that there could be differences in the rehabilitation protocols used by different physiotherapy centres. Although the protocol embraces certain ground rules, the individual training can diverge depending on different important factors related to the patient. Concomitant injuries, previous surgery, range of motion status, knee stiffness and demands of the patients are some of the factors that physiotherapists have to consider when designing the protocol. In the present study, we gave priority to BMA measurements, which is relatively time consuming and inconvenient for the patients. For this reason, KT-1000 arthrometer laxity testing was only performed at the sixth-month follow-up when all the patients were evaluated in order to find failures. The lack of objective stability tests and ROM measurements is a limitation which could have added more information to the study.

08 FUTURE PERSPECTIVES

8.1 ACL REVISION SURGERY

The true revision rate of ACL injuries in Sweden is not known. Since 2005, more than 700 patients who have undergone ACL reconstruction and are registered in the Swedish ACL Register have undergone revision reconstruction on the same knee. The revision rate therefore accounts for approximately 6%, (www.aclregister.nu). Under the period from 2005 to 2012, the group of patients with the highest revision rate and rate of injury to the contralateral knee is without question female soccer players 15-18 years of age. Of these patients, 27% have undergone surgery either on the same knee, (true revision), or on the opposite knee since 2005. Furthermore, 8% of the re-injured young female soccer players chose to decline a new operation. So, more than 30% of female soccer players 15-18 years of age have run the risk of a new injury during the seven years since the start of the Swedish ACL Register. Efforts should be made to reduce the incidence of ACL injuries and re-injuries, especially in the group of patients with the highest incidences. Caraffa et al., Myklebust et al., and Hewett et al. were some of the first researchers who proposed that a specific proprioceptive or neuromuscular prevention programme could reduce the incidence of ACL injury.[30,117,70] In the large intervention study from 2012, Waldén et al. were able to report a reduction of 64% in ACL injuries among adolescent female soccer players in 230 clubs randomised to an intervention group and a control group.[163] With the currently high re-injury figures, a further improvement in the preventive programmes with individualised training will hopefully entail less need for revision surgery.

HT grafts have been, and will continue to be, one of the main options in revision surgery. When HT grafts were introduced, there were some problems with postoperative knee laxity, probably because of difficulty fixating the tendon securely on the tibial side. Eventually these problems have been solved. The reason for the malfunction of HT grafts in some cases could be the failure of tendon-to-bone healing, especially on the tibial side. Surgeons are now more observant and there is a trend towards using larger interference screws and also using double fixation, with the addition of postfixation buttons, screws with washers and staples on the tibial side. BPTB grafts will still have their place after HT graft failures. One clinical observation is that some patients retain their instability soon after HT reconstruction and, for these patients, the BPTB graft should be the choice for revision surgery, because of the safety with bone-to-bone healing. Non-anatomic positioning of the ACL graft, especially on the femoral side, is probably one of the main reasons for failures. Another reason could be failure of biological healing in the tunnels, which has been discussed in this thesis. Reharvesting the patellar tendon for BPTB grafts is now history. In cases in which HT grafts are not available, it is safer to harvest the contralateral patellar tendon or the quadriceps tendon, despite the inconvenience for the patient.

8.2 BONE MINERAL REDUCTION

There are basically two different ways of retaining bone density in ACL reconstructed patients. Reducing the surgical trauma to bone can be accomplished by drilling shorter bone tunnels. Grafts can easily be shortened by folding the tendons into approximately seven cm long constructs. The bone density surrounding the bone tunnels can be increased by undersize drilling and using dilators to increase the tunnel width to the right diameter.[29] Compaction drilling is probably not more efficient than extraction drilling.[123] Secondly, by putting more emphasis on high-impact training methods, the bone density can be better preserved. A modified training protocol focusing on jumping exercises (plyometric exercises), resistance training with weight vests, weight training and exercises with high impacts on the ground could be included, along with balance and proprioceptive exercises. Plyometric exercises can be noxious for a healing graft and therefore supervision by an experienced physiotherapist is therefore recommended and the exercises should probably be included in the later stages of rehabilitation.

8.3 TENDON-TO-BONE HEALING

The drawback in terms of drilling procedures around the knee is manifested through bone-tunnel widening and could perhaps be an insoluble problem. Despite new composite products with the addition of hydroxyapatite and tricalcium phosphate used for fixation, bone integration around the implants is still problematic. Two of the new composite interference screws on the market were recently compared in an RCT by Bourke et al.[23] In both groups, tunnel widening was apparent after two years and the screws were not replaced by new bone. The ACL revision surgeon has to face bone osteolysis in many revision cases and could therefore be forced to operate in staged procedures, including bone grafting. With the innovative ideas among engineers, there is good reason to believe that there will be new alternatives for the secure fixation of grafts. No matter how strongly and successfully the surgeon manages to secure the graft in the tibial tunnel there will still be patients in whom the laxity remains after surgery. Ultimately, the biological factor is the single most important factor that determines a successful outcome. So, in the future, histological studies are probably even more important. New landmarks have already been achieved when it comes to biological or biophysical methods for enhancing graft fixation strength. Methods that can increase bone formation, reduce bone loss and thereby improve tendon-to-bone healing can be classified into growth factors, chemical and biological agents, cell therapy and biomaterials.[105] The radiographic evaluation of bone effects after ACL surgery has also become more important and new methods, such as high-resolution CT, micro CT and peripheral quantitative CT (pQCT), have been introduced.[167,148]

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11 APPENDIX

11.1 BMA MEASUREMENTS WITH DEXA

DEXA measurements are widely used in osteoporosis research and medical care. The effect of treatment for osteoporosis can be ascertained with repeated measurements of BMA. When patients attend the assessment, a trend curve is calculated by the DEXA machine and the DXL Calscan machine and presented as in Figures 29, 30 and 31 below.

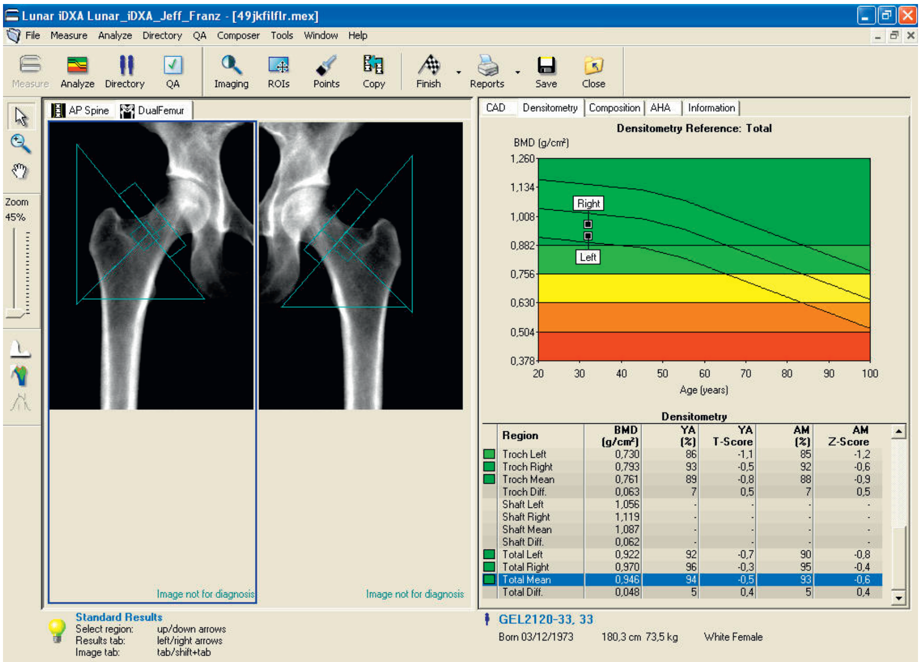

FIGURE 29 *The monitor screen of the DEXA with a total hip measurement (with kind permission from GE Healthcare).*

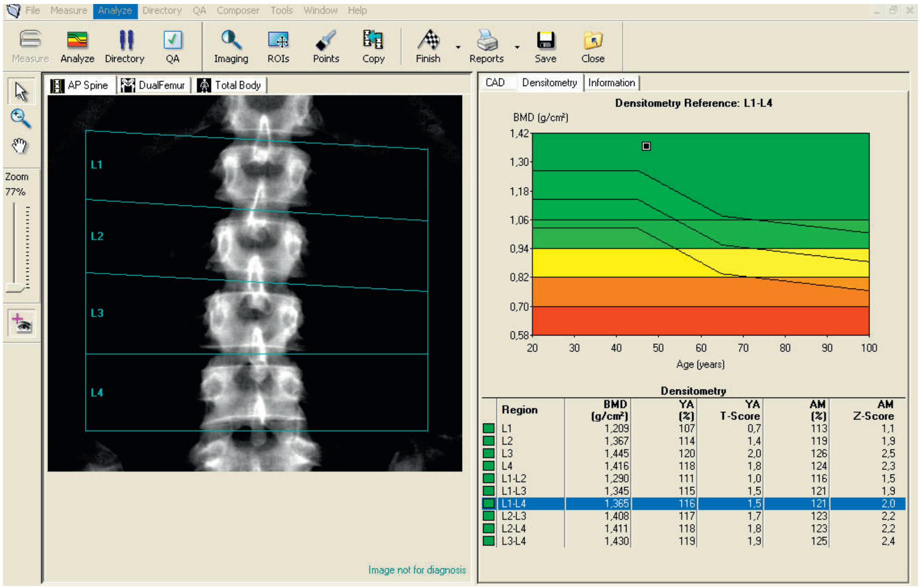

FIGURE 30 *The monitor screen of the DEXA with a lumbar spine measurement (with kind permission from GE Healthcare).*

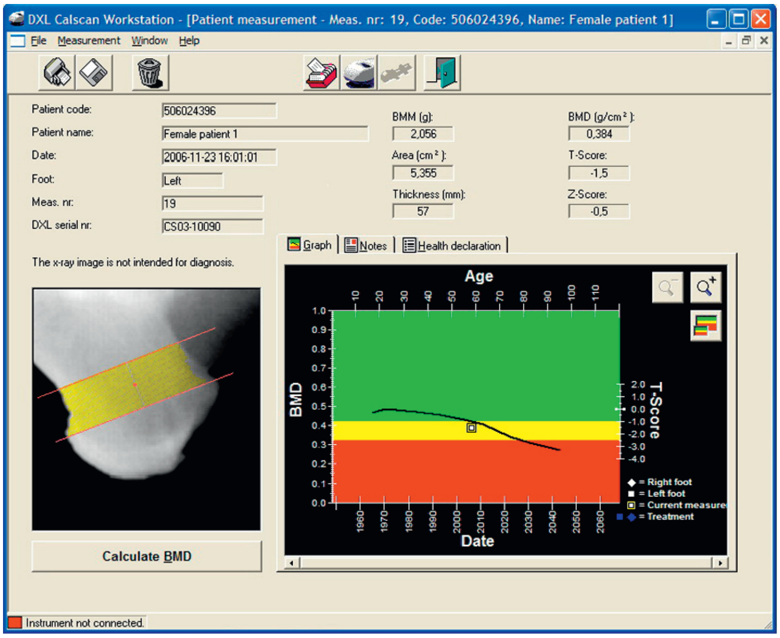

FIGURE 31 *The monitor screen of the DXL Calscan machine with measurement of the calcaneus (with kind permission from Medetech AB).*