General discussion

The anterior cruciate ligament (ACL) is an important stabilizing ligament of the knee and commonly injured in pivoting sports. When surgical intervention is needed to restore knee stability, autogenous hamstring tendons are the graft of choice for ACL reconstructive purposes. The surgical harvesting of one or two hamstring tendons is required and subsequently used to reconstruct the ruptured ligament. In the light of potential donor site morbidity and functional deficits, both patients and orthopaedic surgeons voice concerns about the harvest of healthy and functional tendon tissue. However, in 1992 Cross et al. were the first to describe the potential of hamstring tendons to regenerate following harvesting procedures[21]. If regeneration takes place and regenerated tendons resemble the native ones, the post-harvest morbidity might be limited.
Therefore, the general aim of this thesis was to improve the outcome following harvest of the hamstring tendons through a better understanding of tendon regeneration. This might contribute to the identification of new (therapeutic) targets and ultimately result in an improved outcome after ACL reconstruction procedures entailing the hamstring tendons.

THE REMARKABLE CAPACITY OF HAMSTRING TENDONS TO REGENERATE

Despite the efforts to use synthetic materials or allografts, autografts remain the first choice for ACL reconstruction purposes[53, 56]. Today, the hamstring tendons are the most commonly used autograft to reconstruct the torn ACL[34, 56]. More specifically it requires the surgical resection of either the semitendinosus tendon only, or both the semitendinosus and gracilis tendon to prepare the graft. Another popular and widely used tendon autograft is the bone-patellar tendon-bone (BPTB) graft entailing the central third of the patellar tendon[34, 56]. Although the quadriceps tendon and iliotibial band are less frequently used as autografts, these are suitable alternatives too. Regardless the choice of autograft, surgical resection of healthy and functional tissue is needed. Both patients and surgeons raise concerns about the potential donor site morbidity and lack of this tendon for functional deficits.

What are the regeneration rates for hamstring tendons?

In 1992, Cross et al. were the first authors describing the remarkable regeneration capacity of the hamstring tendons after being entirely resected[21]. In our systematic review we showed that regeneration of the semitendinosus and gracilis tendons occurs in 70% within the first year following harvesting procedures (**Chapter 2**). Studies reporting about hamstring tendon regeneration used different imaging techniques, such as computed tomography (CT), magnetic resonance (MR) imaging and ultrasound.

In addition, various definitions were used to assess (in)complete regeneration of the hamstring tendons, regeneration was assessed dichotomously and evaluated only after a single follow-up period. This does not reflect a dynamic and continuous process such as regeneration of the hamstring tendons. Therefore, we described regeneration rates both one and two years after harvest using MR imaging (**Chapter 3**). In line with previous findings, we found that the semitendinosus tendons regenerated in 65.7% of the cases and that the gracilis tendons regenerated in 82.9% of the cases. Interestingly, regeneration rates in the second year after harvest were found to be lower compared to the regeneration rates one year after surgical resection. This might be explained by the observation that the initial structure is predominately fibrous with only a few collagen fibers[27]. Over time the regenerated tendon starts the remodeling process and becomes similar to the native tendon with longitudinally oriented collagen fibers that appear to be of appropriate orientation and dimension[33, 67, 80]. In line with these findings, biomechanical properties of regenerated tendons improve over time[54]. Therefore, regenerated structures might be most prone to rupture within the first period following harvest and result in a decline in the regeneration rates over time. Patients with a rupture of the regenerating structure often experience a sudden, persistent and sharp pain at the posterior thigh[63].

Hamstring tendons are not the only tendons that are frequently harvested for reconstructive purposes. Another often-used graft for ACL reconstruction procedures is the BPTB autograft, involving the harvest of the middle-third of the patellar tendon. Previous studies reported similar regeneration rates for the patellar tendon as aforementioned for the hamstring tendons[7, 88]. Another tendon that is harvested for ligamentous reconstruction tendon interposition of the carpometacarpal joint is the flexor carpi radialis tendon. Reported regeneration rates for this tendon reach up to 79% 4 years after surgery[5].

Regenerated hamstring tendons are thicker and longer compared to the native tendons

Tendons are important for the transmission of skeletal muscle forces to bone. Appropriate regeneration of the tendon is therefore important to withstand mechanical loads, resulting in an increase of cross-sectional areas (CSA)[38, 48]. In line with these findings, regenerated semitendinosus and gracilis tendons show a doubling of the CSA of compared to the CSA of the native tendons (**Chapter 3**). A similar increase of the CSA is observed after tendon resection for the BPTB autografts[4, 6, 7, 20, 45]. There are several feasible hypotheses that explain the increased CSA of regenerated tendons. First of all, the organization and composition of the extracellular matrix (ECM) in regenerated tendons might be inferior to the original tendon, causing diminished biomechanical properties. Therefore, more tissue is required to withstand the same mechanical forces as before. Secondly, the tendon is exposed to more mechanical stress per unit and therefore increases its CSA. A

third feasible explanation is that the increase in CSA serves as a protective mechanism to strengthen vulnerable tendons, as seen in Achilles tendinopathy and after Achilles tendon rupture[46, 74].

In line with the first hypothesis, it has been observed that in the first months after resection the regenerated structure is predominately fibrous with only a few collagen fibers[27]. Over time the regenerated tendon starts the remodeling process and becomes similar to the native tendon with longitudinally orientated collagen fibers[33, 67, 80]. Along with this improved organization of the ECM, the biomechanical properties of the newly formed structure ameliorate with the passage of time[54]. However, the ultimate load, stiffness and the modulus of the regenerated structure do not become identical compared to the native tendons. These inferior biomechanical properties may be partially due to the decreased cross-sectional diameter of the collagen fibers in the regenerated tendons[33]. This all fits within the first hypothesis and explains our observation that regenerated tendons have increased CSA compared to the native tendons.

The musculotendinous junctions (MTJ) of regenerated hamstring tendons appear to be found more proximal compared to the MTJ of native tendons, resulting in an increase of the semitendinosus and gracilis tendon length (**Chapter 3**). The average length of this proximal shift ranges from 3.1 to 7.3 cm[17, 62]. On the contrary, it is interesting to note that the patellar tendon is significantly shortened between 0.4 and 1.8 cm after harvest of the middle third of the tendon[7, 12].The opposite post-harvest remodeling of the patellar tendon and the hamstring tendon length might be explained by the different anatomic situations after surgical intervention. The hamstring tendons are harvested from insertion to the MTJ, leaving a free anatomic space between the fascial planes of the medial thigh. However, a BPTB graft requires a longitudinal incision directly over the patellar tendon, involving harvest of the middle third and leaving two thirds of the native tendon in situ. In addition, some surgeons close the harvest gap. This surgical approach might result in formation of exaggerated pathologic fibrous hyperplasia causing a shortening of the patellar tendon and a subsequent tendon shortening[69].

Recently, Laako et al. described a surgical technique in which the distal head of the harvested semitendinosus and/or gracilis muscle is drawn towards its anatomical location and attached to the semimembranosus muscle[50]. This technique could be of value in patients with increased lengths of the semitendinosus and gracilis tendons, since increased tendon lengths might cause symptoms such as posterior thigh pain. Alternatively, one could choose to only harvest 75% of the tendons, leaving the other 25% *in situ*. Another, relatively rare, symptom caused by the altered thickness and length of regenerated tendons is a snapping syndrome. Gali et al. proposed a method of percutaneous lengthening of regenerated tendons alleviating symptoms and resulting in minimal morbidity[30].

The work in this thesis emphasizes that tendons regenerate and that regenerated hamstring tendons are thicker and longer than the native ones, altering the anatomy and biomechanics. Therefore, patients may experience symptoms that are related to this new situation. Orthopaedic surgeons should be aware of the potential of the semitendinosus and gracilis tendons to regenerate, their altered morphological properties and the potential accompanying clinical symptoms. If needed, surgical interventions should be considered.

Should hamstring tendons be reconsidered as graft of choice for ACL reconstruction purposes?

Patients often express their concerns regarding potential muscle strength deficits following tendon harvesting procedures. Previous studies measuring the peak torque of knee flexion reported a full recovery of hamstring strength[19, 55, 87]. This recovery might be attributed to a functional restoration of muscle-tendon-bone complex of the hamstring tendons. Alternatively, it might also be caused by compensatory hypertrophy of the remaining knee flexors, such as the biceps femoris and semimembranosus[26, 42]. Since the semitendinosus and gracilis muscles insert at the pes anserinus and are therefore more important for higher angles of knee flexion (beyond 75 degrees)[65], strength deficits might be found in deep knee flexion. In line with this hypothesis, three studies reported strength deficits ranging from 20 to 30% at knee flexion angles beyond 75 degrees[17, 64, 82]. However, the clinical relevance of this limited strength deficit is debatable (**Chapter 4**).

Orthopaedic surgeons worldwide have not reached consensus yet regarding the selection of the best graft to reconstruct ruptured ACLs. Based on several studies, hamstring tendons and bone-patellar tendon-bone are the most frequently used autograft[1, 34, 56]. However, BPTB autografts result in significantly more donor site morbidity and lower patient reported outcome measurements and hence hamstring tendons are the graft of choice for ACL reconstructions[1, 34, 56, 57]. However, the semitendinosus and gracilis tendons are primarily internal rotators of the tibia, withstanding excessive external rotation and protecting the (reconstructed) ACL[3, 68, 75]. Patients without hamstring tendon regeneration have impaired internal tibial rotary strength[2, 3] and might therefore be at increased risk for ACL re-ruptures[3]. Preoperative identification of patients that are likely to lack regenerative capacity of the hamstring tendons might alter the graft choice. Since there is currently no literature available that supports this hypothesis, future studies should investigate if regeneration indeed decreases the risk of ACL re-rupture.

Another interesting aspect of regenerated hamstring tendons is their potential to be re-harvested. Although it has been reported that the semitendinosus and gracilis tendons have been reharvested for ACL[89] and medial patella-femoral ligament (MPFL)[80] reconstructions, the re-use of these tendons is still very questionable for several reasons. First of all, tendon regeneration does not occur in every patient and therefore harvesting

is not always possible. Given the morphological features of the remodeled tendons and the importance of graft thickness and graft length, newly formed tendons could be considered as potential interesting candidates for reharvesting procedures. However, it is important to address that the strength and stiffness of the newly formed tendons is expected to be inferior to the native tendons. In addition, histological studies revealed that the remodeled tendons show areas of scar tissue that could be expected to alter graft strength[25, 27, 67].

Taken together regenerated tendons resemble the native tendons, but are not identical in terms of morphology, histology and biomechanical features. It would be helpful to identify key role players of tendon regeneration and remodeling because it contributes to the identification of targets to direct and improve these processes. Ultimately, this knowledge might be used to improve treatments for tendon-related diseases.

MODULATORS FOR THE INFLAMMATORY RESPONSE

Tendon repair is a complex and multistage process, which is initiated after tissue damage and ultimately results in function restoration. Although numerous cells are involved in the process of tendon repair, macrophages are thought to be key role players in this process[35, 49].Various *in vitro* studies showed that the different macrophage phenotypes produce distinct factors, having different effects on tendon repair. Considering the specific effects of the specific macrophage phenotypes, it is not surprising that macrophage subsets are differently recruited during the process of tendon repair[58, 73]. More specifically, it has been shown that M1-like macrophage are recruited first and stimulate tenocytes to produce catabolic enzymes, such as matrix metalloproteinases[73]. In addition, M1-like macrophages typically produce factors such as IL-1β, IL-6, and TNF- α[84]. In **Chapter 5**, we describe that pro-inflammatory cytokines negatively influence the production of structural and fibril-associated ECM components. Then, M2-like macrophages are recruited resulting in higher ECM densities and increased collagen I expression presenting an orientation along the longitudinal axis of the tendon[58]. This macroscopically resembles a normal and healthy tendon. Together, these findings emphasize the pivotal role of macrophages in the tendon repair process. However, it is important to note that both pro-inflammatory and anti-inflammatory macrophages/cytokines are highly required for optimal tissue healing: inhibition of pro-inflammatory factors and stimulation of anti-inflammatory factors will not necessarily result in proper tissue healing. Tissue healing strongly depends on the finely regulated balance between pro- and anti-inflammatory factors. The question remains whether aging and smoking that are known to negatively influence regeneration chances (**Chapter 4**) also affect macrophages and/or their phenotypes.

Aging

Aging is associated with decreased chances for regeneration of the hamstring tendons (**Chapter 4).** In general, it is known that aging negatively affects the function of the immune system[61].

Currently, it is unclear whether the generation of macrophages from monocytes is impaired with age[39, 66, 85]. Regarding macrophage polarization, it has been reported that more M2-like macrophages are found in a mouse-model of age-related macular degeneration[47]. These ocular macrophages had decreased levels of TNF-α and IL-12, whereas the anti-inflammatory cytokine IL-10 was upregulated. In addition, an increase in M2-like macrophages was observed with aging in spleen, lymph nodes and bone marrow[43].

Aging influences the cytokine secretion patterns by macrophages. Compared to cytokine production in young mice, peritoneal macrophages from old mice secrete less pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF- α, and more anti-inflammatory cytokines such as IL-10[9, 14, 72]. Microarray analysis further investigated the molecular basis for this decrease and revealed signal transduction genes were specifically reduced in macrophages from old mice[15]. First of all, decreased expression of toll-like receptor (TLR) 4 has been suggested as a reason for the observed age-related alterations[72]. However, other studies indicated that TLR4 remains unchanged with age[9]. Another explanation might be alterations in the JAK-STAT signaling pathway. As shown in **Chapter 6**, macrophage phenotypes have a characteristic STAT phosphorylation pattern. Previous studies showed that STAT1 phosphorylation levels are decreased in macrophages from old mice, compared to young mice[23]. Since STAT1 phosphorylation is mainly found in M1-like macrophages, decreased phosphorylation levels might therefore contribute to reduced production of pro-inflammatory cytokines.

Taken together, aging impacts on macrophage function and potentially disturbs the highly regulated balance between pro- and anti-inflammatory factors, which is required for optimal tendon healing. A failure to repair the ECM of tendons might therefore be caused by an impaired macrophage function.

Smoking

Smoking has been shown to have deleterious consequences for several orthopaedic conditions, such as higher rates of hip fracture, nonunion of fractures and osteomyelitis. In addition, smoking is also associated with impaired regeneration of the hamstring tendons (**Chapter 4**).

M1-like macrophages play a central role in defending the human body against invading pathogens and foreign material. Cigarette smoking reduces the phagocytic ability of macrophages[40] and decreases levels of molecules that are needed for intracellular killing, such as nitric oxide (NO) and reactive oxygen species (ROS)[90]. This finding suggests that

cigarette smoke induces macrophage polarization towards a M2-like phenotype. This hypothesis is further strengthened by the activation of STAT3 pathways in macrophages after exposure to cigarette smoke[32]. Activation of this pathway is seen in M2-like macrophages (**Chapter 6**) and needed for the production of anti-inflammatory cytokines. In addition, low levels of cytokines that are typically produced by M1-like macrophages are found in response to cigarette smoke[16]. Therefore, STAT3 is considered to be a pivotal signaling molecule for macrophage polarization towards an M2-like phenotype.
Similar to the effect of aging, the exposure to cigarette smoke influences the tightly regulated equilibrium between pro- and anti-inflammatory factors and herewith potentially affects the repair capacity of the ECM.

Genetics

The aforementioned factors only partially explain the interindividual variation in tendon repair. Today, there is mounting evidence suggesting that genetics has a pivotal role in the healing tendency of tendons[70, 76].
Tendons are subject to mechanical loads that reach up to ten times an individual's body mass. Therefore, the ECM of tendons continuously needs to undergo remodeling in order to withstand these loads and maintain homeostasis. It has been described that tendons are able to respond to these mechanical loads by initiating several matrix remodeling pathways[52, 86]. Previous studies showed that inflammatory gene expression profiles of tenocytes are modulated in response to mechanical loading triggering tenocyte apoptosis and ECM degradation. Therefore, it is reasonable to hypothesize that polymorphisms within genes encoding for inflammatory proteins modulate risk of tendinopathy.
Previous studies showed that genes encoding several interleukin proteins including interleukin-1β (*IL1B* rs16944 C>T), interleukin-6 (*IL6* rs1800795 G>C) and interleukin-6 receptor (*IL6R* rs2228145 A>C) modulate risk of Achilles tendinopathy in cohorts from South Africa[77], Australia[77] and the United Kingdom[10]. All these polymorphisms are located at the promotor site, affecting the produced amounts of the respective interleukines[12, 28, 31, 51].
It has been shown that *IL1B* rs16944 is independently associated with an increased risk of acute Achilles tendon ruptures[10]. For chronic Achilles tendinopathy, no independent associations were noted[77]. However, *IL1B* and *IL6* variants were associated with increased risk of Achilles tendinopathy in inferred gene-gene interactions models[77]. Although it has been well described that IL-1β and IL-6 are responsible in the activation of many downstream signaling cascades, it remained unclear how these polymorphisms contribute to tendinopathy[70, 76]. The work in this thesis indicated that polymorphisms within genes encoding IL-6 and IL-1β modulate the production of structural and fibril-associated ECM components (**chapter 5**) and herewith potentially contribute to an impaired capacity to repair the ECM.

It is interesting to note that additional associations were found between polymorphisms within genes encoding for *IL1B* and *IL6*, and other soft musculoskeletal injuries. In line with the findings for acute rupture of the Achilles tendon, the *IL1B* rs16944 promotor polymorphism independently increases the risk of ACL rupture[71]. In addition, inferred allele combinations of *IL1B*, *IL6* and *IL6R* were found to be associated with risk of ACL ruptures, both two independent South African cohorts[71] and a Swedish cohort (**chapter 5**). The risk of developing carpal tunnel syndrome has also shown to be modulated by polymorphisms within IL6R[11]. Collectively, these results underline the potential implication of interleukin signaling pathways in the underlying mechanism that predisposes for both acute and chronic soft musculoskeletal injuries. The investigated polymorphisms were found to be associated with increased risk for ACL rupture in a Swedish cohort (**Chapter 5**), and other cohorts[71]. In addition, these polymorphisms were previously associated with increased risk of chronic Achilles tendinopathy[70, 76, 77]. Therefore, it might be suggested that these polymorphisms negatively affect the odds for hamstring tendon regeneration.

Personalized medicine is a central dogma in current clinical practice, proposing the tailor made clinical assessment of an individual patient based on their extrinsic and intrinsic risk factors[18]. Tendon healing is based on a poorly understood complex interaction between a variety of extrinsic and intrinsic risk factors. Clinicians should be aware of this when considering any clinical application of genetic testing and prevent the use of terms such as diagnostic, prognostic or preventive. Instead, clinicians should rather consider injury susceptibility through the identification of both known intrinsic and extrinsic risk factors (Figure 1). Individuals who are at increased risk to develop a soft musculoskeletal injury should then be referred to a sports-trained physiotherapist to be managed personally by appropriate prehabilitation exercises to reduce risk.

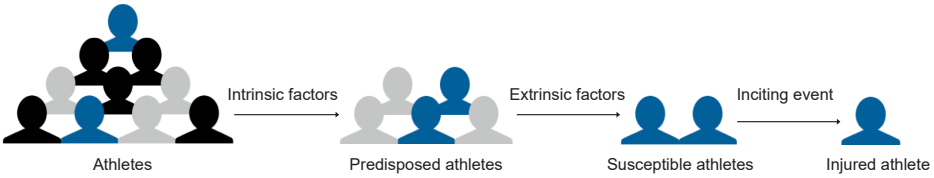

Figure 1: Schematic overview of the complex relationship between intrinsic and extrinsic factors, as well as the role of the inciting event. Future multifactorial models could be of great value to distinguish low-risk (black), predisposed (grey) and susceptible (blue) athletes.

FUTURE PERSPECTIVES

Remaining uncertainties about tendon regeneration

The work in the current thesis shows that the semitendinosus and gracilis tendons regenerate in 70% of patients within the first two years following tendon harvest for reconstructive purposes. It appears that these tendons regenerate in a proximal to distal fashion, and that regenerated tendons are longer and have increased cross-sectional areas compared to the native tendons. However, the exact mechanism that underpins hamstring tendon regeneration remains unclear. In the field of Developmental Biology and Cancer, lineage tracing is currently the golden standard to determine a cell's origin. In this technique, a single cell is labeled in such a way that the mark is transmitted to the cell's progeny. The advantage of this approach is that it can be performed without any prior knowledge of what genes or markers should be expressed. However, a disadvantage this technique requires to physically stop the development process to see how cells look. Therefore, more recent developments have enabled *in vivo* barcode generation, targeting a locus for rearrangement or mutagenesis such that a different set of outcomes is generated in different cells[44]. The barcodes are generated over a limited amount of time resulting in a deep and precise lineage tracing. A great advantage of this strategy is the ability to continuously record a cell's development. Another more indirect method to identify the origin of the cells residing in the newly formed tendon tissue is to compare its methylome with the methylomes of cells derived from the surrounding tissues, such as fat, muscle and tendon sheets. A methylome is the methylation of cytosines, contributing to the epigenetic layer and defining the transcriptional and regulatory potential of genomic DNA[41].

Mid- and long-term follow-up of patients with harvested hamstring tendons is necessary to further evaluate the clinical implications of tendon regeneration. The semitendinosus and gracilis tendons withstand excessive external tibial rotation protecting the ACL. An impaired repair capacity of the hamstring tendons following harvesting procedures might therefore impact ACL reconstruction survival rates. Although there is currently no literature available on this topic, hamstring tendon regeneration is potentially of importance for the clinical outcome after ACL reconstruction procedures. Additionally, complete hamstring tendon regeneration theoretically re-establishes a functional muscle-tendon-bone complex. This hypothesis is supported by the current literature, suggesting that tendon regeneration would result in no or limited loss of muscle strength. However, it remains unclear whether this is caused by high-quality regeneration or compensatory hypertrophy of other (posterior) thigh muscles. In this light, it would be highly valuable to evaluate the volumes of all thigh muscles following harvesting procedures. Next to this, the quality of the regenerated tendons might be indirectly measured by the radiologic appearance of the hamstring muscles. Previously tendon-related disorders of the rotator

cuff have been described to cause a myriad of changes in the cuff musculature on MR imaging, such as fatty infiltration, atrophy and fibrosis. In line with these observations, one might hypothesize that impaired regeneration of the hamstring tendons causes similar deviations of the semitendinosus and gracilis muscles.

Improved understanding of tendon- and ligament related injuries

ACL injuries are often associated with knee instability, take up to 9 months to rehabilitate and lead to gonarthrosis in 40% of the patients 10 years after injury[59]. In addition, treatment of ACL injuries is costly and only 50% of the athletes successfully return to preinjury levels[29, 59]. Therefore, the current focus of musculoskeletal research is the identification of factors associated with increased susceptibility to ACL injuries. Independent associated factors with ACL injury include anatomical variations, neuromuscular control, sex, female sex hormone concentrations, genetic polymorphisms and previous injuries[78, 79]. To date, studies that evaluated risk of ACL injury using combinations of risk factors by developing multivariable models are limited[36, 83]. These models exclusively focus on the anatomic features and do not provide a full understanding of ACL injury risk. However, it is probable that tendon- and ligament related injuries depend on multiple risk factors. Therefore, it is important to focus on creating comprehensive and clinically applicable risk models identifying individuals with increased susceptibility to both tendon- and ligament-related injury. Besides, the models should provide more direction for preventive programs, as well as appropriate counseling for those who are at increased risk.

The healing process of tendons involves an inflammatory phase. Macrophages are considered to be pivotal in the onset and perpetuation of tendon diseases[22, 60, 81]. Some studies indicate that macrophage depletion improved morphological and biomechanical properties in injured Achilles tendons or following ACL reconstruction procedures[24, 37]. On the other hand, other studies reported that aspecific inhibition of macrophages is detrimental to ECM formation and tensile strength[8, 13]. In line with these findings, aspecific targeting of inflammation by common anti-inflammatory drugs including diclofenac, celecoxib and naproxen seem to have deleterious effects on tendon healing. These findings emphasize the functional role of macrophages in the tendon-healing process but suggest the need for further clarification on the interplay between macrophages and tenocytes. *In vitro* studies are limited but suggest that different macrophages have different effects on tenocyte behavior[58, 73, 81]. Given these different effects of the different macrophage subsets, it might be interesting to specifically target macrophages in order to improve the tendon remodeling process. This can either be done by using specific antibodies to specifically target a monocyte or macrophage subset, or by modulating specific intracellular signaling proteins, such as STAT proteins.

REFERENCES

1. Ahlden M, Samuelsson K, Sernert N, Forssblad M, Karlsson J, Kartus J. The Swedish National Anterior Cruciate Ligament Register: a report on baseline variables and outcomes of surgery for almost 18,000 patients. *Am J Sports Med.* 2012;40(10):2230-2235.
2. Ahlen M, Liden M, Bovaller A, Sernert N, Kartus J. Bilateral magnetic resonance imaging and functional assessment of the semitendinosus and gracilis tendons a minimum of 6 years after ipsilateral harvest for anterior cruciate ligament reconstruction. *Am J Sports Med.* 2012;40(8):1735-1741.
3. Armour T, Forwell L, Litchfield R, Kirkley A, Amendola N, Fowler PJ. Isokinetic evaluation of internal/external tibial rotation strength after the use of hamstring tendons for anterior cruciate ligament reconstruction. *Am J Sports Med.* 2004;32(7):1639-1643.
4. Atkinson TS, Atkinson PJ, Mendenhall HV, Haut RC. Patellar tendon and infrapatellar fat pad healing after harvest of an ACL graft. *J Surg Res.* 1998;79(1):25-30.
5. Beall DP, Ritchie ER, Campbell SE, et al. Magnetic resonance imaging appearance of the flexor carpi radialis tendon after harvest in ligamentous reconstruction tendon interposition arthroplasty. *Skeletal Radiol.* 2006;35(3):144-148.
6. Berg EE. Intrinsic healing of a patellar tendon donor site defect after anterior cruciate ligament reconstruction. *Clin Orthop Relat Res.* 1992(278):160-163.
7. Bernicker JP, Haddad JL, Lintner DM, DiLiberti TC, Bocell JR. Patellar tendon defect during the first year after anterior cruciate ligament reconstruction: appearance on serial magnetic resonance imaging. *Arthroscopy.* 1998;14(8):804-809.
8. Blomgran P, Blomgran R, Ernerudh J, Aspenberg P. A possible link between loading, inflammation and healing: Immune cell populations during tendon healing in the rat. *Sci Rep.* 2016;6:29824.
9. Boehmer ED, Goral J, Faunce DE, Kovacs EJ. Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. *J Leukoc Biol.* 2004;75(2):342-349.
10. Brown KL, Seale KB, El Khoury LY, et al. Polymorphisms within the COL5A1 gene and regulators of the extracellular matrix modify the risk of Achilles tendon pathology in a British case-control study. *J Sports Sci.* 2017;35(15):1475-1483.
11. Burger MC, de Wet H, Collins M. Interleukin and growth factor gene variants and risk of carpal tunnel syndrome. *Gene.* 2015;564(1):67-72.
12. Burks RT, Haut RC, Lancaster RL. Biomechanical and histological observations of the dog patellar tendon after removal of its central one-third. *Am J Sports Med.* 1990;18(2):146-153.
13. Chamberlain CS, Leiferman EM, Frisch KE, et al. The influence of macrophage depletion on ligament healing. *Connect Tissue Res.* 2011;52(3):203-211.
14. Chelvarajan RL, Collins SM, Van Willigen JM, Bondada S. The unresponsiveness of aged mice to polysaccharide antigens is a result of a defect in macrophage function. *J Leukoc Biol.* 2005;77(4):503-512.
15. Chelvarajan RL, Liu Y, Popa D, et al. Molecular basis of age-associated cytokine dysregulation in LPS-stimulated macrophages. *J Leukoc Biol.* 2006;79(6):1314-1327.

16. Chen H, Cowan MJ, Hasday JD, Vogel SN, Medvedev AE. Tobacco smoking inhibits expression of proinflammatory cytokines and activation of IL-1R-associated kinase, p38, and NF-kappaB in alveolar macrophages stimulated with TLR2 and TLR4 agonists. *J Immunol.* 2007;179(9):6097-6106.

17. Choi JY, Ha JK, Kim YW, Shim JC, Yang SJ, Kim JG. Relationships among tendon regeneration on MRI, flexor strength, and functional performance after anterior cruciate ligament reconstruction with hamstring autograft. *Am J Sports Med.* 2012;40(1):152-162.

18. Collins M. Genetic risk factors for soft-tissue injuries 101: a practical summary to help clinicians understand the role of genetics and 'personalised medicine'. *Br J Sports Med.* 2010;44(13):915-917.

19. Coombs R, Cochrane T. Knee flexor strength following anterior cruciate ligament reconstruction with the semitendinosus and gracilis tendons. *Int J Sports Med.* 2001;22(8):618-622.

20. Coupens SD, Yates CK, Sheldon C, Ward C. Magnetic resonance imaging evaluation of the patellar tendon after use of its central one-third for anterior cruciate ligament reconstruction. *Am J Sports Med.* 1992;20(3):332-335.

21. Cross MJ, Roger G, Kujawa P, Anderson IF. Regeneration of the semitendinosus and gracilis tendons following their transection for repair of the anterior cruciate ligament. *Am J Sports Med.* 1992;20(2):221-223.

22. Dakin SG, Martinez FO, Yapp C, et al. Inflammation activation and resolution in human tendon disease. *Sci Transl Med.* 2015;7(311):311ra173.

23. Davila DR, Edwards CK, 3rd, Arkins S, Simon J, Kelley KW. Interferon-gamma-induced priming for secretion of superoxide anion and tumor necrosis factor-alpha declines in macrophages from aged rats. *Faseb j.* 1990;4(11):2906-2911.

24. de la Durantaye M, Piette AB, van Rooijen N, Frenette J. Macrophage depletion reduces cell proliferation and extracellular matrix accumulation but increases the ultimate tensile strength of injured Achilles tendons. *J Orthop Res.* 2014;32(2):279-285.

25. Eriksson K, Kindblom LG, Hamberg P, Larsson H, Wredmark T. The semitendinosus tendon regenerates after resection: a morphologic and MRI analysis in 6 patients after resection for anterior cruciate ligament reconstruction. *Acta Orthop Scand.* 2001;72(4):379-384.

26. Eriksson K, Larsson H, Wredmark T, Hamberg P. Semitendinosus tendon regeneration after harvesting for ACL reconstruction. A prospective MRI study. *Knee Surg Sports Traumatol Arthrosc.* 1999;7(4):220-225.

27. Ferretti A, Conteduca F, Morelli F, Masi V. Regeneration of the semitendinosus tendon after its use in anterior cruciate ligament reconstruction: a histologic study of three cases. *Am J Sports Med.* 2002;30(2):204-207.

28. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest.* 1998;102(7):1369-1376.

29. Fithian DC, Paxton EW, Stone ML, et al. Prospective trial of a treatment algorithm for the management of the anterior cruciate ligament-injured knee. *Am J Sports Med.* 2005;33(3):335-346.

30. Gali JC, Serafim BLC, Nassar SA, Gali Filho JC, LaPrade RF. Percutaneous Lengthening of a Regenerated Semitendinosus Tendon for Medial Hamstring Snapping. *Arthrosc Tech.* 2019;8(3):e349-e352.

31. Galicia JC, Tai H, Komatsu Y, Shimada Y, Akazawa K, Yoshie H. Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced. *Genes Immun.* 2004;5(6):513-516.
32. Geraghty P, Wyman AE, Garcia-Arcos I, Dabo AJ, Gadhvi S, Foronjy R. STAT3 modulates cigarette smoke-induced inflammation and protease expression. *Front Physiol.* 2013;4:267.
33. Gill SS, Turner MA, Battaglia TC, Leis HT, Balian G, Miller MD. Semitendinosus regrowth: biochemical, ultrastructural, and physiological characterization of the regenerate tendon. *Am J Sports Med.* 2004;32(5):1173-1181.
34. Granan LP, Forssblad M, Lind M, Engebretsen L. The Scandinavian ACL registries 2004-2007: baseline epidemiology. *Acta Orthop.* 2009;80(5):563-567.
35. Guo S, Dipietro LA. Factors affecting wound healing. *J Dent Res.* 2010;89(3):219-229.
36. Hashemi J, Chandrashekar N, Mansouri H, et al. Shallow medial tibial plateau and steep medial and lateral tibial slopes: new risk factors for anterior cruciate ligament injuries. *Am J Sports Med.* 2010;38(1):54-62.
37. Hays PL, Kawamura S, Deng XH, et al. The role of macrophages in early healing of a tendon graft in a bone tunnel. *J Bone Joint Surg Am.* 2008;90(3):565-579.
38. Heinemeier KM, Kjaer M. In vivo investigation of tendon responses to mechanical loading. *J Musculoskelet Neuronal Interact.* 2011;11(2):115-123.
39. Herrero C, Marques L, Lloberas J, Celada A. IFN-gamma-dependent transcription of MHC class II IA is impaired in macrophages from aged mice. *J Clin Invest.* 2001;107(4):485-493.
40. Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M, Reynolds PN. Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol.* 2007;37(6):748-755.
41. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. *Science.* 1975;187(4173):226-232.
42. Irie K, Tomatsu T. Atrophy of semitzendinosus and gracilis and flexor mechanism function after hamstring tendon harvest for anterior cruciate ligament reconstruction. *Orthopedics.* 2002;25(5):491-495.
43. Jackaman C, Radley-Crabb HG, Soffe Z, Shavlakadze T, Grounds MD, Nelson DJ. Targeting macrophages rescues age-related immune deficiencies in C57BL/6J geriatric mice. *Aging Cell.* 2013;12(3):345-357.
44. Kalhor R, Kalhor K, Mejia L, et al. Developmental barcoding of whole mouse via homing CRISPR. *Science.* 2018;361(6405).
45. Kamps BS, Linder LH, DeCamp CE, Haut RC. The influence of immobilization versus exercise on scar formation in the rabbit patellar tendon after excision of the central third. *Am J Sports Med.* 1994;22(6):803-811.
46. Karjalainen PT, Soila K, Aronen HJ, et al. MR imaging of overuse injuries of the Achilles tendon. *AJR Am J Roentgenol.* 2000;175(1):251-260.
47. Kelly J, Ali Khan A, Yin J, Ferguson TA, Apte RS. Senescence regulates macrophage activation and angiogenic fate at sites of tissue injury in mice. *J Clin Invest.* 2007;117(11):3421-3426.
48. Kjaer M, Magnusson P, Krogsgaard M, et al. Extracellular matrix adaptation of tendon and skeletal muscle to exercise. *J Anat.* 2006;208(4):445-450.

49. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. *Expert Rev Mol Med.* 2011;13:e23.

50. Laakso M, Kosola J, Niemi P, et al. Operative treatment for the painful posterior thigh after hamstring autograft harvesting. *Muscles Ligaments Tendons J.* 2017;7(3):570-575.

51. Landvik NE, Hart K, Skaug V, Stangeland LB, Haugen A, Zienolddiny S. A specific interleukin-1B haplotype correlates with high levels of IL1B mRNA in the lung and increased risk of non-small cell lung cancer. *Carcinogenesis.* 2009;30(7):1186-1192.

52. Legerlotz K, Jones GC, Screen HR, Riley GP. Cyclic loading of tendon fascicles using a novel fatigue loading system increases interleukin-6 expression by tenocytes. *Scand J Med Sci Sports.* 2013;23(1):31-37.

53. Legnani C, Ventura A, Terzaghi C, Borgo E, Albisetti W. Anterior cruciate ligament reconstruction with synthetic grafts. A review of literature. *Int Orthop.* 2010;34(4):465-471.

54. Leis HT, Sanders TG, Larsen KM, Lancaster-Weiss KJ, Miller MD. Hamstring regrowth following harvesting for ACL reconstruction: The lizard tail phenomenon. *J Knee Surg.* 2003;16(3):159-164.

55. Lipscomb AB, Johnston RK, Snyder RB, Warburton MJ, Gilbert PP. Evaluation of hamstring strength following use of semitendinosus and gracilis tendons to reconstruct the anterior cruciate ligament. *Am J Sports Med.* 1982;10(6):340-342.

56. Magnussen RA, Granan LP, Dunn WR, et al. Cross-cultural comparison of patients undergoing ACL reconstruction in the United States and Norway. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(1):98-105.

57. Mascarenhas R, Tranovich MJ, Kropf EJ, Fu FH, Harner CD. Bone-patellar tendon-bone autograft versus hamstring autograft anterior cruciate ligament reconstruction in the young athlete: a retrospective matched analysis with 2-10 year follow-up. *Knee Surg Sports Traumatol Arthrosc.* 2012;20(8):1520-1527.

58. Mauro A, Russo V, Di Marcantonio L, et al. M1 and M2 macrophage recruitment during tendon regeneration induced by amniotic epithelial cell allotransplantation in ovine. *Res Vet Sci.* 2016;105:92-102.

59. Meuffels DE. [Increase in surgical treatment of anterior cruciate ligament injury]. *Ned Tijdschr Geneeskd.* 2009;153:A466.

60. Millar NL, Murrell GA, McInnes IB. Inflammatory mechanisms in tendinopathy - towards translation. *Nat Rev Rheumatol.* 2017;13(2):110-122.

61. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. *J Clin Invest.* 2013;123(3):958-965.

62. Nakamae A, Deie M, Adachi N, Nakasa T, Nishimori M, Ochi M. Effects of knee immobilization on morphological changes in the semitendinosus muscle-tendon complex after hamstring harvesting for anterior cruciate ligament reconstruction: evaluation using three-dimensional computed tomography. *J Orthop Sci.* 2012;17(1):39-45.

63. Nakamae A, Ochi M, Deie M, Adachi N. Unsuccessful regeneration of the semitendinosus tendon harvested for anterior cruciate ligament reconstruction: report of two cases. *Orthop Traumatol Surg Res.* 2012;98(8):932-935.

64. Nakamura N, Horibe S, Sasaki S, et al. Evaluation of active knee flexion and hamstring strength after anterior cruciate ligament reconstruction using hamstring tendons. *Arthroscopy.* 2002;18(6):598-602.

65. Noyes FR, Sonstegard DA. Biomechanical function of the pes anserinus at the knee and the effect of its transplantation. *J Bone Joint Surg Am.* 1973;55(6):1225-1241.

66. Ogawa T, Kitagawa M, Hirokawa K. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. *Mech Ageing Dev.* 2000;117(1-3):57-68.

67. Okahashi K, Sugimoto K, Iwai M, et al. Regeneration of the hamstring tendons after harvesting for arthroscopic anterior cruciate ligament reconstruction: a histological study in 11 patients. *Knee Surg Sports Traumatol Arthrosc.* 2006;14(6):542-545.

68. Osternig LR, Bates BT, James SL. Patterns of tibial rotary torque in knees of healthy subjects. *Med Sci Sports Exerc.* 1980;12(3):195-199.

69. Paulos LE, Wnorowski DC, Greenwald AE. Infrapatellar contracture syndrome. Diagnosis, treatment, and long-term followup. *Am J Sports Med.* 1994;22(4):440-449.

70. Rahim M, Collins M, September A. Genes and Musculoskeletal Soft-Tissue Injuries. *Med Sport Sci.* 2016;61:68-91.

71. Rahim M, Mannion S, Klug B, et al. Modulators of the extracellular matrix and risk of anterior cruciate ligament ruptures. *J Sci Med Sport.* 2017;20(2):152-158.

72. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. *J Immunol.* 2002;169(9):4697-4701.

73. Schoenenberger AD, Foolen J, Moor P, Silvan U, Snedeker JG. Substrate fiber alignment mediates tendon cell response to inflammatory signaling. *Acta Biomater.* 2018;71:306-317.

74. Schweitzer ME, Karasick D. MR imaging of disorders of the Achilles tendon. *AJR Am J Roentgenol.* 2000;175(3):613-625.

75. Segawa H, Omori G, Koga Y, Kameo T, Iida S, Tanaka M. Rotational muscle strength of the limb after anterior cruciate ligament reconstruction using semitendinosus and gracilis tendon. *Arthroscopy.* 2002;18(2):177-182.

76. September A, Rahim M, Collins M. Towards an Understanding of the Genetics of Tendinopathy. *Adv Exp Med Biol.* 2016;920:109-116.

77. September AV, Nell EM, O'Connell K, et al. A pathway-based approach investigating the genes encoding interleukin-1beta, interleukin-6 and the interleukin-1 receptor antagonist provides new insight into the genetic susceptibility of Achilles tendinopathy. *Br J Sports Med.* 2011;45(13):1040-1047.

78. Smith HC, Vacek P, Johnson RJ, et al. Risk factors for anterior cruciate ligament injury: a review of the literature - part 1: neuromuscular and anatomic risk. *Sports Health.* 2012;4(1):69-78.

79. Smith HC, Vacek P, Johnson RJ, et al. Risk factors for anterior cruciate ligament injury: a review of the literature-part 2: hormonal, genetic, cognitive function, previous injury, and extrinsic risk factors. *Sports Health.* 2012;4(2):155-161.

80. Stevanovic V, Blagojevic Z, Petkovic A, et al. Semitendinosus tendon regeneration after anterior cruciate ligament reconstruction: can we use it twice? *Int Orthop.* 2013;37(12):2475-2481.

81. Stolk M, Klatte-Schulz F, Schmock A, Minkwitz S, Wildemann B, Seifert M. New insights into tenocyte-immune cell interplay in an in vitro model of inflammation. *Sci Rep.* 2017;7(1):9801.

82. Tashiro T, Kurosawa H, Kawakami A, Hikita A, Fukui N. Influence of medial hamstring tendon harvest on knee flexor strength after anterior cruciate ligament reconstruction. A detailed evaluation with comparison of single- and double-tendon harvest. *Am J Sports Med.* 2003;31(4):522-529.

83. Uhorchak JM, Scoville CR, Williams GN, Arciero RA, St Pierre P, Taylor DC. Risk factors associated with noncontact injury of the anterior cruciate ligament: a prospective four-year evaluation of 859 West Point cadets. *Am J Sports Med.* 2003;31(6):831-842.

84. Utomo L, van Osch GJ, Bayon Y, Verhaar JA, Bastiaansen-Jenniskens YM. Guiding synovial inflammation by macrophage phenotype modulation: an in vitro study towards a therapy for osteoarthritis. *Osteoarthritis Cartilage.* 2016;24(9):1629-1638.

85. Wang CQ, Udupa KB, Xiao H, Lipschitz DA. Effect of age on marrow macrophage number and function. *Aging (Milano).* 1995;7(5):379-384.

86. Yang G, Im HJ, Wang JH. Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene.* 2005;363:166-172.

87. Yasuda K, Tsujino J, Ohkoshi Y, Tanabe Y, Kaneda K. Graft site morbidity with autogenous semitendinosus and gracilis tendons. *Am J Sports Med.* 1995;23(6):706-714.

88. Yazdanshenas H, Madadi F, Madadi F, Washington ER, 3rd, Jones K, Shamie AN. Patellar tendon donor-site healing during six and twelve months after Anterior Cruciate Ligament Reconstruction. *J Orthop.* 2015;12(4):179-183.

89. Yoshiya S, Matsui N, Matsumoto A, Kuroda R, Lee S, Kurosaka M. Revision anterior cruciate ligament reconstruction using the regenerated semitendinosus tendon: analysis of ultrastructure of the regenerated tendon. *Arthroscopy.* 2004;20(5):532-535.

90. Yuan F, Fu X, Shi H, Chen G, Dong P, Zhang W. Induction of murine macrophage M2 polarization by cigarette smoke extract via the JAK2/STAT3 pathway. *PLoS One.* 2014;9(9):e107063.