

Biochemical and Structural Alterations in Skeletal Muscle Following ACL Injury: A Narrative Review

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ABSTRACT

Background: Anterior cruciate ligament (ACL) injuries are some of the most common knee injuries that occur in the US, accounting for around 200,000 documented cases per year. Varying levels of severity can determine whether surgery is required or if physical therapy will suffice. One of the most common complications for patients is that there is significant atrophy of the impacted limb. Yet, there has not been definitive proof explaining this mechanism. **Objective:** The primary goal for this review was to examine some of the biochemical differences that tend to occur within and surrounding an ACL injury and the mechanisms involved in skeletal muscle atrophy and regenerative capabilities. **Outcome:** Multiple studies have found a connection between time spent inactive from the injury and the percentage of retained muscle after exercising again. Among decreases in muscle mass and muscle volume changes, analyses have also revealed alterations in alpha-2 macroglobulin, myostatin, heat shock protein-72, mechano GF-C24E, synovial fluid, and histochemical alterations in collagen and cartilaginous states which all seem to be primary factors in regulating effectiveness and speed of recovery from ACL injury. **Conclusion:** the influx of various cytokines as a response to the initial injury in relation to inflammation change the chemical and physical environment of the knee, making recovery significantly more difficult and time-consuming. Timing of injury, surgery, and re-initiation of movement after surgery are very important factors that can minimize overall damage and reduce recovery time.

Key words: Anterior Cruciate Ligament, Collagen, Satellite Cells, Heat Shock Protein, Synovial Fluid, Musculoskeletal

INTRODUCTION

Many novice, amateur, and professional athletes have likely experienced an injury in some capacity and have rebounded to return to play without any confounding issues. Those who have experienced an ACL injury; however, have unfortunately experienced that recovering from an injury of this degree is no easy task. Joseph et al. (2013) conducted a comprehensive analysis of ACL injuries and found that 97.6% of those who incurred an ACL injury returned to play in some capacity, while the remaining 2.4% did not return to play. This figure represents both athletes who underwent surgery and those who were not recommended to have surgery. There have been various findings of methods that can be used to rehabilitate a person from an ACL injury, some using more conventional methods such as neuromuscular stimulation and the incorporation of water activities (Hauger et al., 2018; Hsieh & Yang, 2018), and others using treatments such as α 2-macroglobulin upregulation and myostatin inhibition (Wang et al., 2014; Wurtzel et al., 2017). It is important to note that not all subjects discussed within these articles

incurred an ACL reconstruction. Several of the participants did not have an injury severe enough to require surgery. The lasting effects that can be present after an ACL injury have been consistent among patients and often include early onset of arthritis of the knee, muscular atrophy in the affected limb, and fibrogenic cell accumulation. By the time that an individual usually notices some of these symptoms, it is often too late to take action, or it will take a significantly longer amount of time to reduce these symptoms. The timing between when a patient has the surgery and/or the injury incurs is very important to the rehabilitation process in that starting rehabilitation too early or too late will impact movement and pain in the knee (Evans, Shaginaw, & Bartolozzi, 2014).

BIOCHEMICAL DIFFERENCES

While there are numerous changes on the surface of the knee after an injury, there are even greater changes that occur within the joint itself and the surrounding muscle. Further examining some of these biological markers, regenerative as well as structural capabilities are compromised including

satellite cell abundance, dystrophin content, and myostatin regulation, among others.

Satellite Cells

Satellite cells are a very important component to muscular regeneration in response to an injury or stress placed on a muscle group. The primary function of these stem cells in muscle fibers is to proliferate and fuse to the existing myofibril nuclei to support greater protein synthesis (Tiidus et al., 2012). Any issue in the regulatory factors regarding these cells can lead to the inability to regenerate muscle fibers. In a recent study by Fry et al. (2017), comparison between the injured and non-injured legs of individuals with an ACL injury revealed a significant decrease in satellite cell abundance of the injured leg. An explanation given for the reduced satellite cell abundance is due to the integrity of the extracellular matrix (ECM) regarding the transcriptional and translational ability to activate the parent satellite cells. Specifically, Tcf4+ fibroblast accumulation inhibits satellite cells from functioning properly, leading to a decrease in myogenesis. A major component of satellite cell proliferation is the movement of chemicals signaling their activation. As is with most ACL surgeries, the injured leg is immobilized and restricted from movement for varying periods of time. An issue with this is that there is a decreased amount of blood flow through that knee resulting in slower activation of the quiescent satellite cells. Furthermore, the excess immobilized time that is spent post-surgery allowing structures to be degraded, is also time that decreases the satellite cell pool to be activated upon return to activity.

Alpha-2 Macroglobulin and Myostatin

The maintenance of muscle volume is partly regulated by a transforming growth factor- β family member known as myostatin. Myostatin acts on the cellular level by inhibiting the ubiquitin-proteasome pathways along with blocking the IGF-1 receptors within the cell (Wurtzel et al., 2017). While this process is necessary within muscle to elicit the turnover of myofibrils, excess activity of this protein can lead to atrophy of the muscle. It is a known fact that a lack of, or sudden cessation of exercise, will lead to decreased muscle volume. Those suffering from an ACL injury find themselves in this situation and quickly notice a drastic change in the volume and power of the injured leg. In a study done by Wang et al. (2014), they examined the effects that α 2-macroglobulin (A2M) may have on delaying atrophy as a result of injury. Their findings supported that an increase in endogenous A2M inhibited the amount of myostatin activated within the surrounding muscles of the knee such as the vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) (Wang et al., 2014). Furthermore, analysis showed that the expression of MMP-13, a major degrading factor of collagen type I, II, and III, was downregulated which helped to preserve the collagen content and cartilage proteoglycans.

While there is some A2M present in the body naturally, the levels are not significant enough to inhibit the degradation reactions of cytokines such as myostatin. Exogenous application of A2M can be important for attenuating atrophy

in that analysis of synovial fluid in injured knees revealed downregulated amounts of biological A2M. Furthermore, after supplementation of A2M, there was a decrease in protease and cytokine activity as well as an increase in the amount of identifiable A2M protein in synovial fluid. Thus, the supplementation of A2M as a potential suppressor for cytokines could delay the atrophic effects as a result of the cessation of exercise.

Studies have found that injury to the ACL has been shown to increase the amount of MMPs present which can be detrimental through formation of certain collagenase substances that can impact the newly created tendon-to-bone anchoring (Demirag, Sarisozen, Ozer, Kaplan, & Ozturk, 2005). Collagen, specifically type 1 and type 3, are important structures in the formation of new bonds after an ACL reconstruction. With various MMP molecules upregulated after damage to the knee, it is crucial for the structural integrity of the knee to be able to form those collagen molecules. The significance of A2M in the knee post-injury can mean greater inhibition of the collagenase materials to form stronger bonds within the surgical tendon tunnels. As demonstrated through Demirag et al. (2005), just a single injection of A2M can have profound effects on the strength and stability present in the knee during load-to-failure tests just two weeks following surgery. Further studies would be required to determine effective timing and dosage amounts.

Heat Shock Protein 72

Heat shock protein 72 (HSP72) is a stress-activated protein which responds to stress placed on the body typically through high internal temperatures or environments. The overall function of HSP72 in the cell is to unfold and re-fold biosynthetic proteins in order for myogenesis to occur (Mooren & Volker, 2005). HSP72 activation is crucial in that it staves off muscular degradation for the time being. However, during periods of HSP activity, a specific subclass known as HSP70 is decreased due to muscle inactivity, thereby reducing cross-sectional area (CSA) of the muscle. Heat shock proteins are molecular chaperones in that they facilitate movement of proteogenic RNA sequences from the ECM to the nucleus (Okuyama et al., 2003; Senf, 2013). A primary goal of research in the realm of HSP72 and injury is how its activity can be maintained even after suffering an injury. Sustainability of HSP72 is one method that can potentially reduce muscle degradation and preserve existing muscle tissue. Heat shock protein 72 has been shown time and again to induce myogenesis and, in some cases, delay muscle atrophy (Tiidus et al., 2012). One particular study by Gehrig et al. (2012) found that the upregulation of HSP72 was able to preserve the structural integrity and contractile properties in rat models with Duchenne muscular dystrophy. The suspected mechanism of action is through the sarcoplasmic and endoplasmic reticulum Ca^{2+} -ATPase pathways. While upregulation of HSP72 was not able to eliminate degradation of myofibril structures, it was able to significantly decrease the rate at which these tissues were impacted.

The implications of HSP72 expression in ACL injuries could be dramatic by way of preserving muscle tissue

integrity long enough for the patient to recover and begin exercise again. Moresi et al. (2009) demonstrated that overstimulation of HSP72 in regenerating muscle tissue proved to have a significant effect of myofibril modulation. Even after two weeks from being mechanically injured, the HSP72 treated samples displayed much larger CSA; $p=0.0442$ for *ex vivo* samples and $p=0.0188$ for *in vivo* samples of HSP overexpression (Moresi et al., 2009). Repeated tests in humans would be needed to confirm the effectiveness of these methods. Nevertheless, regeneration to that degree in muscle which would normally incur significant atrophy is an example that there are methods of reducing the impact of ACL injuries on recovery and return to play time.

Mechano GF-C24E

Matrix metalloproteinases (MMPs) are a key contributor to a lack of strength and muscular regeneration in muscles and cartilaginous structure surrounding the ACL. The focus of a fair amount of recent research has been on limiting the formation of these MMPs and allow for greater rehabilitation speeds and associated strengths. Mechano GF-C24E (MGF-C24E) is one of the products of IGF-1 splicing and has been shown to exhibit significant response to mechanical stress. Some of the effects that have been reported through use of MGF-C24E have been increased satellite cell production, increased muscle progenitor cells, and enhanced migratory patterns to assist tendon recovery and oxidative stress reduction (Mooren & Volker, 2005; Song et al., 2016). The reduction in oxidative stress in stretching to the new tendon-joint tunnel can result in less overall stress placed on the joint and increased compliance. The same study found through analysis of reactive oxygen species (ROS) that varying doses of MGF-C24E decreased levels by 4.5 to 5.5-fold compared with the control group.

As previously mentioned, upregulation of MGF-C24E can also increase cartilaginous structure in and surrounding the knee. By association, MGF-C24E increases the formation of collagen type I and type III, the fibril-forming collagens, which are the primary components of fibrous cartilage (Mooren & Volker, 2005). For an ACL to recover properly, there needs to be adequate and sturdy structure to the newly formed tendon-joint tunnel. Multiple studies have found that soon after an ACL tear, the joint cavity begins to develop collagenase which breaks down the organized structure of the existing cartilage (Fry et al., 2017; Mooren & Volker, 2005). The application of MGF-C24E can help to attenuate this response which would allow for the proper collagen to develop within the joint.

MUSCULOSKELETAL CHANGES

Muscle Volume Changes

One of the most noticeable changes as a result of ACL injuries is the decline of muscle volume and function in the quadriceps. While this change may not be as noticeable immediately after injury or surgery, there is speculation that the lack of reduction in volume may be a result of edema pres-

ent within and around the knee (Yoh & Infantolino, 2017). Regardless, there is an overall decline in power output shortly after injury. Numerous studies have been conducted to combat this decrease in muscle mass and its associated consequences. One of the primary concerns is the inability to fire the quadricep muscle following surgery, as well as asynchronous contractions of the medial and lateral hamstring muscle (Hauger et al., 2018; Macleod, Snyder-Mackler, & Buchanan, 2014; Mantashloo, Letafatkar, & Moradi, 2019). Several conclusions have attributed this to a protective mechanism of the body to restrict anterior translation of the lower leg. A combination of decreased quadricep muscle volume and ill-timed hamstring contractions leads to the knee giving way suddenly.

The decreased muscle volume that occurs within the quadriceps is a combination of adaptations to muscle fibers and formation of alternative substances within the existing space. One of the primary changes that is observed is the transitioning of muscle fibers from type I to type IIa or IIx, or oxidative to non-oxidative, respectively. These changes are observed differently depending on which muscles are examined, such as the RF, VL, and semimembranosus (SM). Ohno et al. (2017) found that over time there was significant decrease in type I fiber content in RF but an increase in type IIa and IIx content in RF and SM. This can be attributed to the decrease in muscle volume in that type I fibers have a larger CSA due to the amount of myosin heavy chain (MHC) content and the size of the myofibrils. With time, the relative size of the myofibrils decreases from a lack of use and decreased motor neuron stimulation.

One study by Macleod et al. (Macleod et al., 2014) examined the individual muscle volume and CSA of each quadricep muscle and ratio of quadriceps to hamstring volume in those who were recommended to receive surgery and those who were deemed not necessary to have an ACL reconstruction (copers vs. non-copers). The findings of this study were that the vastus intermedius and vastus medialis did not differ significantly between groups in either volume, CSA, or ratio to the hamstring. However, they did differ significantly between the involved and uninvolved limbs of the body. In the VL of the potential coper group, there was a significant decrease in CSA between the involved and uninvolved limbs (Macleod et al., 2014). The conclusion of this data pointed to the possibility that the level of atrophy may be less directly related to muscle size and more based on the level of neuromuscular function possessed by the individual. This follows the presumption that a higher-level athlete has more precise control over the contraction of their muscles versus a lower level or amateur athlete.

Depending on the type of graft used by the surgeon to reconstruct the ACL can also impact the volumetric changes in the hamstring and quad muscles. For example, using part of the gracilis and semitendinosus of the involved side tends to lead to the largest deficit in volume from pre- to post-surgery (Norte et al., 2018). There are, however, some changes which can occur in the surrounding muscles of the injured limb which can lead to hypertrophied states such as in the gluteal muscles. Post-operative gait can be impacted

due to subtle shifts to limit movement in the injured knee in that the patient may restrict knee flexion by employing more hip movement. This can of course lead to hypertrophy of some muscles (e.g. the gluteal complex and the obturator externus), but atrophy of others (e.g. the gastrocnemius and soleus).

Collagen

There are various forms of collagen that are present in the body, currently 20 are known, of which some are beneficial to the ECM of myocytes while others have degradative effects (Mooren & Volker, 2005). Type I, II, and III are commonly present in healthy cells, comprise 80-90% of all collagen in the body, and contribute to the structural matrix of the cell membranes. Between these three types of collagen, they constitute a majority of the tensile and compliance characteristics of the tissues by having well formed, tightly-knit patterns. Injury to the ACL has been shown to impact the collagen content within the knee and surrounding muscles in that it produces collagenase, in this case through the form of MMP-13 (Hsieh & Yang, 2018). It is also believed that collagen degradation is irreversible and is furthermore replaced by a less structured collagen form which impacts the integrity of the joint structure (Hong Li, Chen Chen, & Shiyi Chen, 2015). One mechanism that is thought to cause the decrease in collagen synthesis is through blocking of the transcription factors specificity protein 1 and specificity protein 3 (Sp1 and Sp3) as well as TGF- β , a key influencer of type II collagen synthesis. Since collagen is such an integral part of the stability of the knee, damage to the content and proliferation of it can have significant effects both acutely and long-term. An issue that can result from decreased collagen synthesis is a decrease in the amount of tissue between the bone surfaces of the knee. This further relates to bone-on-bone contact leading to increased chances for developing post-traumatic osteoarthritis (PTOA).

SYNOVIAL FLUID

In recent years, synovial fluid has been used as a method for determining the degree of injury in patients with ACL injuries and as a way of analyzing the long-term effects of such an injury. By using synovial fluid, researchers can examine various cytokines, proinflammatory, or anti-inflammatory mediators present in the knee at any given time. Some of which have been able to hint at PTOA formations years after the injury. Some of the biomarkers tested for are the interleukin (IL) complex, MMPs, TIMPs, tumor necrosis factor (TNF), and various proteoglycans. While no biomarkers have been able to accurately predict development of PTOA in patients without fail, they have been able to provide a direction in which research should focus its efforts. Given that this method of prognosis is still in its developing stages, there is much progress to be made before it can be used as a reliable predictor. That being said, researchers have identified some of the most likely culprits for causing PTOA and have started to identify the mechanisms through which they influence the environment of the knee.

Starting with the anti-inflammatory markers, chemicals such as tissue inhibitor of metalloproteinase (TIMP) and interleukin-10 (IL-10) have been identified as some of the major chemokines involved in injury rehabilitation. Various studies over the past few years have examined the differences between healthy knees, ACL-injured ones, and ACL combined with other structural injuries. Compared to healthy knees, most knees that have experienced an ACL injury have significantly lower levels of these anti-inflammatory chemokines. The significance with this being that the knee cannot heal itself or respond as quickly as with any other sort of injury, leading to longer periods of inflammation thereby increasing the deleterious effects. The reason for recovery taking longer in knees that have been previously injured is that normal pathways for nutrient distribution have been damaged or altered, making it so that some areas are inaccessible and unable to properly respond to future injuries.

Multiple cytokines have also been established as ones that have a degradative nature to other cells. As mentioned above, proinflammatory chemicals have been identified as the IL complex, MMPs, TNF, and various proteoglycans. Many of these cytokines work off each other to degrade structures within the joint capsule and deteriorate the knee. MMPs, as previously stated, break down the ECM of cells and inhibit the regeneration of new ones. One study found that MMP-3, a chemical that inhibits collagen type III production, levels in an ACL injured knee vs. a control knee were upregulated nearly 220% at a period of just a few weeks after injury (Kaplan, Cuellar, Jazrawi, & Strauss, 2017). Another major result of MMP upregulation is the inhibition of the mechanistic target of rapamycin (mTOR) pathway, which is critical in muscle regeneration (Pereira et al., 2014). The combination of the breakdown of ECM and the blocking of mTOR have been identified as two of the major consequences of ACL injuries.

The interleukin complex has several properties that make it part of a large focus in examining SF changes in patients. While certain IL variations, IL-1 α , IL-1 β , IL-6, and IL-8, are known to be degradative in chondrocytes, IL-10 is beneficial to the health of the cartilage (Larsson, Struglics, Lohmander, & Frobell, 2017). Multiple studies have proven that an ACL injury, with or without concomitant knee damage, results in elevated IL levels. The primary significance of this being that the combination of these cytokines results in severe breakdown of chondrocytes and the bone matrix that is in the joint capsule. A secondary result of these upregulated cytokines is that they also inhibit the healing properties of other cells. There are existing quantities of these chemicals in the SF regardless of whether there is an injury; the issue is the concentration at which they are present. One group found that as a result of an ACL injury without concomitant cartilage damage, IL-6 was upregulated by 804% compared to control models (Kaplan et al., 2017).

Interleukin-1 α and IL-1 β by comparison are not as damaging as IL-6 directly; however, they are responsible for collagenase production as well as bone resorption which, over time, creates further degradative issues (Bigoni et al., 2016; Lattermann et al., 2018). Studies have focused on how these levels may change throughout a period of up to 16 years to see if they can be used as predictor chemicals in PTOA.

In longitudinal studies, researchers reported that elevated cytokine levels at the time of injury, declined and returned to normal when measured several years after the injury (Neuman, Dahlberg, Englund, & Struglics, 2017). However, patient reports made it clear that even with normal cytokine levels, the deleterious effects of when they were elevated left an impression that made the remaining structures weaker. As a result, many patients incurred subsequent injuries to the same knee. Explanation for this comes from the degradative natures of the IL complex and MMPs in that recovery of the existing structures may not have been able to fully heal, particularly in the bone tunnel of the ACL reconstruction (Bigoni et al., 2016; Zeng, Xiao, Deng, & Li, 2017). This would imply the significant effect that synovial fluid has on the recovery of the knee where degradation of the bone as a result of the chemokines may play a bigger role than the immediate injury and repair itself. The direction of future studies may deal with the maintenance and mitigation of changes to the synovial fluid in order to preserve the foundational integrity of the joint.

CONCLUSION

Matrix metalloproteinases and interleukins are strong contributors to the structural damage that occurs in the joint following an ACL injury. With minimal blood flow to the tendons and cartilage of the knee, there is a significant issue in the ability to clear the inflammatory cytokines that are present. As mentioned, prolonged cytokine activity can have irreversible effects specifically on the collagen and reconstructed ACL anchor points. Collagen functions not only as an absorbent layer but also as a support substance on bone surface integrity and compliance. Several of these mechanisms are not yet fully understood but the overall impact they have has been observed in numerous studies and will continue to be if these injuries still occur. Alterations to any one of these contributors would have a profound impact and a combination of these can be a career-ending injury for an athlete.

There is no shortage of ACL injuries that occur each year and with that, research being done to improve the recovery speed and quality of those who are impacted is being reexamined constantly. There is not much that can compare to an ACL injury in terms of the ease of incidence and the lasting significance it can have. Chronic differences from muscle atrophy to synovial fluid changes alter the homeostasis of the knee creating a new normal that is extremely difficult to change back. There are methods being developed to limit and shorten the negative results of an ACL injury, such as “prehab” exercises and variances in post-operative therapy practices including timing of knee mobilization and cryotherapy. However, more research is needed to examine the mechanistic changes and how the biochemical alterations of the knee environment can be attenuated.

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