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Long-Term Effects of ACL Transection on the Gastrocnemius Muscle in Rats

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Abstract

We investigated long-term changes in the morphological and contractile properties of the gastrocnemius muscle in a rat Anterior Cruciate Ligament (ACL) tear model. The experimental animals were 8-week-old male Wistar rats in which the right ACL was transected. The gastrocnemius muscle in the affected limb and the same muscle in the contralateral limb were harvested 4 and 48 weeks after ACL transection. We created serial frozen sections of the tissues and performed immunohistochemical staining. In results, at 4 weeks, the muscle wet weight-to-body weight ratio and the Cross-Sectional Area (CSA) of type I and IIb muscle fiber in the deep portion of the muscle were both significantly smaller on the transection-affected side than on the contralateral side ($p < 0.05$, respectively). In addition, at 48 weeks, the CSA of type I muscle fibers in the deep portion was significantly smaller on the affected side than on the contralateral side ($p < 0.05$). We surmise that the changes were due mainly to modulation of muscle activity as a result of physical joint instability and functional failure of proprioception, which both occurred as a result of the ACL transection. We showed that these morphological changes may persist over the long term, particularly in the case of type I fibers. Results of this study suggest that long-term rehabilitation for not only the thigh but also the leg is necessary when ACL injuries are encountered clinically.

Keywords: ACL; Gastrocnemius muscle; Muscle fiber; Rehabilitation; Sports injury

Introduction

An Anterior Cruciate Ligament (ACL) tear is a traumatic injury that frequently occurs during sporting activities. The ACL restrains the stability of the knee joint, and a torn ACL results in excessive anterior laxity of the tibia relative to the femur and rotational instability of the lower limb, which pose significant obstacles to sports performance. Thus, ACL reconstruction is usually performed for athletes with such an injury [1-3]. When ACL injury occurs in non-athletes or elderly individuals who do not participate in sporting activities, conservative treatment focusing on improving muscular strength is generally performed [4-6].

Muscular strength training and detailed clinical evaluation at regular intervals are extremely important during the rehabilitation period, whether after surgery or in conservative treatment. The muscles most commonly targeted for this purpose are the quadriceps at the front of the thigh and hamstrings at the back. Both of these particular muscles cross the knee joint [7], and some reports have indicated that the knee joint instability caused by an ACL tear results in changes to the muscle morphology and contractile properties [8]. Of the periarticular knee muscles, the gastrocnemius is the one that most greatly affects lower limb function. This muscle has the function of plantar flexion at the ankle and flexion at the knee joint. Gastrocnemius muscle has two heads (medial and lateral), and, like the quadriceps and hamstrings, it crosses the knee joint. Therefore, we believe that an ACL tear has some effect on the gastrocnemius

[8]. However, basic myophysiological research focusing on the gastrocnemius muscle after ACL injury appears to be lacking.

On the other hand, clinically, the observation period after ACL reconstruction usually lasts a few years [9,10]. However, when the injury is managed conservatively, the knee joint instability can persist indefinitely, so we believe that extended follow-up at intervals of a few decades is important. There have been a few previous studies on this matter [5,11,12], but the effects of an ACL tear throughout the patient's lifetime remain largely unknown.

The purpose of this study was to investigate the morphological and contractile changes (wet weight, cross sectional area and fiber type composition) of gastrocnemius muscle in rats at 4 weeks and 48 weeks after ACL transection. We hypothesized that an ACL transection results in changes in the morphological and contractile properties of the gastrocnemius muscle and that these changes persist over a very long time.

Methods

Animals and Grouping

The study was performed in accordance with the St. Marianna University School of Medicine Animal Experiment Guidelines and was approved by the St. Marianna University School of Medicine Laboratory Animal Experiment Committee (Approval number: 1502019).

Twelve 8-week-old male Wistar rats were used for the study. All were kept in a breeding room under a 12-hour/12-hour light/dark cycle at a constant room temperature (24 ± 1˚C) and humidity (55 ± 10%) and were allowed free access to food and water.

All were subjected to two surgical procedures: transection of the right ACL and a sham operation on the left hind limb. Gastrocnemius muscles were assigned as ACL-transected (A) (n=12) and contralateral Control (C) (n=12) groups, respectively.

Surgical Procedures and Muscle Harvest and Preparation

To simulate an ACL tear, each rat's right hind limb ACL was transected. The rats were placed under deep general anesthesia induced with isoflurane (Forane®, Abbott Japan, Tokyo). The skin covering the anteromedial region of the right hind limb knee joint was incised longitudinally, and the patella was inverted laterally, exposing the joint. With the knee in deep flexion and use of a scalpel, ACL transection was performed under direct vision. Anterior instability of the tibia and rotational instability of the femur were confirmed manually, and the joint capsule and skin were then sutured. A sham operation performed on the left hind limb. With the rats still under deep anesthesia, the left hind knee joint was exposed as described above, and the joint capsule and skin were sutured without ACL transection having been performed.

The right and left gastrocnemius muscles were harvested from six rats at 4 weeks and from the remaining six rats at 48 weeks. Muscle wet weight was measured at the time of harvest and recorded as the muscle wet weight-to-body weight ratio. The tissues were then rapidly frozen in liquid nitrogen and stored at −80˚C.

Immunohistochemical Analysis

Specimens obtained at both 4 weeks and 48 weeks were subjected to immunohistochemical analysis. The belly of the medial head of the frozen gastrocnemius muscle was cut serially into 12-μm thick horizontal sections with a cryostat (CM 1900, Leica, Wetzlar, Germany).

Immunostaining was performed as follows: The frozen sections were warmed at 36˚C for 30 minutes, washed in Phosphate Buffered Saline (PBS), and then covered and soaked in blocking buffer (Blocking One Histo, Nacalai Tesque, Kyoto, Japan). The specimens were left to stand at room temperature for 5 minutes, the blocking buffer was removed, and the specimens were washed twice for 2 minutes with PBS. The following primary antibodies were added to a blocking buffer that was diluted 20-fold with PBS + 0.1% Tween 20 (PBST), and the tissue sections were reacted for 1 day at 4˚C with anti-type I myosin heavy chain (MyHC) isoform (MyHCI, 1:100, BA-F8; Developmental Studies Hybridoma Bank [DSHB], Iowa City, Iowa, USA); anti-type IIa MyHC isoform (MyHCIIa, 1:100, SC-71, DSHB); anti-type IIb MyHC isoform (MyHCIIb, 1:100, BF-F3, DSHB); and anti-laminin (Z0097; diluted 1: 500; Dako Cytomation, Glostrup, Denmark).

The sections were then washed with PBS and soaked in the following secondary antibodies: Alexa Flour 488-labeled goat anti-mouse IgG2b (1:1000, Invitrogen, Carlsbad, California, USA); Alexa Flour 568-labeled goat anti-mouse IgM (1:1000, Invitrogen); Alexa Fluor 350-labeled goat anti-mouse IgG1 (1:200, Invitrogen); and Alexa Flour 488 F (ab') 2 fragment of goat anti-rabbit IgG (H+L) (1:2000, Invitrogen). The sections were reacted for 1 hour in a dark room at room temperature, then washed in PBS, and covered with glass cover slips and cryopreserved.

Histochemical analysis of the stained specimens was performed under an optical microscope (KEYENCE Japan, Osaka, Japan). Superficial and deep portions of the gastrocnemius were extracted randomly from one of two visual fields, as described by Armstrong and Phelps [13]. Using Image J (Ver.1.51, Wayne Rasband, National Institutes of Health, USA) for analysis, we counted 300 to 400 muscle fibers per visual field, then classified the muscle fibers by type (Type I, IIa, IIx, or IIb) (Figure 1) in the superficial and deep portions and recorded the percentage of each type. The muscle fiber Cross-Sectional Area (CSA) was also measured for each muscle fiber type [8,14].

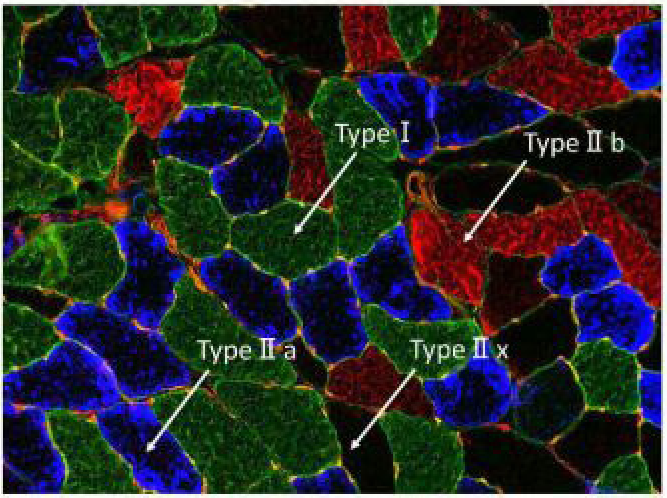

Figure 1: Classification of muscle fiber types.

Muscle fiber type (Type I, IIa, IIx, and IIb) was classified by a standard immunohistochemical staining with anti-myosin heavy chain monoclonal antibodies.

Statistical Analysis

Data are expressed as mean ± SEM values. Differences in measured variables were subjected to two-way (treatment × time) Analysis Of Variance (ANOVA), followed by Tukey's HSD test. Especially, it was used particularly to evaluate the effects of treatment in relation to time. All statistical analyses were performed with SPSS Statistics ver. 21.0J (IBM Japan, Tokyo, Japan), and p values < 0.05 were considered significant.

Results

Wet Weight-To-Body Weight Ratio

Transection of the ACL was found to have a significant main effect of treatment on the gastrocnemius muscle, and there was an interaction between treatment and time. As shown in Figure 2, 4 weeks after the operative procedures, the gastrocnemius wet weight-to-body weight ratio was significantly smaller on the transection-affected side (A group) than on the contralateral side (C group) ($p < 0.05$). At 48 weeks, however, the ratio did not differ significantly between A and C groups.

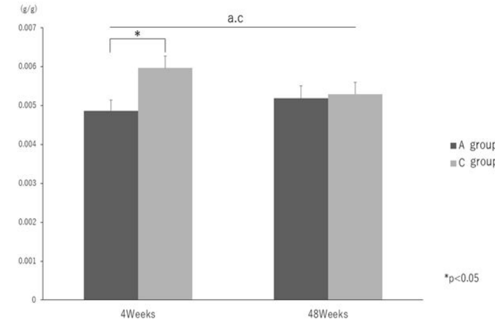

Figure 2: Muscle wet weight to body weight ratio of the gastrocnemius muscle.

4 Weeks: 4 weeks after ACL transection; 48 Weeks: 48 weeks after ACL transection. Right and left limbs of rats were assigned as ACL-transected (A group) and contralateral control (C group), respectively. Values are means ± SEM. n=6 in each group. a: a significant main effect of treatment. c: a significant interaction (treatment x time). * p<0.05.

Muscle Fiber Type Classification

Percentages of the type I, IIa, IIx, and IIb muscle fibers in the superficial portion and deep portion of the harvested gastrocnemius muscles are shown in Figures 3 and 4, respectively. In the superficial portion, there was significant main effect of treatment in type IIx muscle fiber. In the deep portion, however, no significant differences were observed.

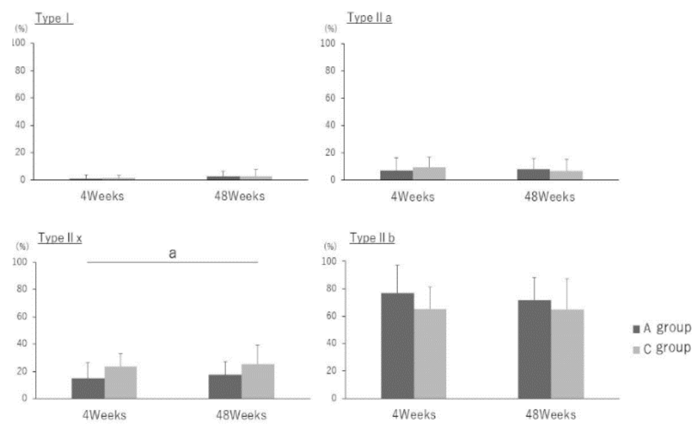

Figure 3: Relative distribution of muscle fiber type in superficial portion of the gastrocnemius muscle.

4 Weeks: 4 weeks after ACL transection; 48 Weeks: 48 weeks after ACL transection. Right and left limbs of rats were assigned as ACL-transected (A group) and contralateral control (C group), respectively. Unit: %. Values are means ± SEM. n=6 in each group. a: a significant main effect of treatment.

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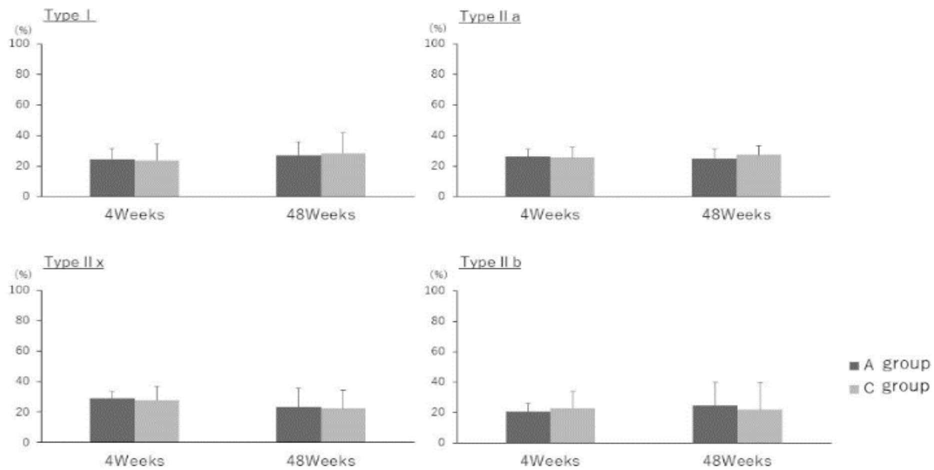

Figure 4: Relative distribution of muscle fiber type in deep portion of the gastrocnemius muscle.

4 Weeks: 4 weeks after ACL transection; 48 Weeks: 48 weeks after ACL transection. Right and left limbs of rats were assigned as ACL-transected (A group) and contralateral control (C group), respectively. Unit: %. Values are means ± SEM. n=6 in each group.

Muscle Fiber CSA

CSAs of the type I, IIa, IIx, and IIb muscle fibers in the superficial portion and deep portion of the harvested gastrocnemius muscles are shown in Figures 5 and 6, respectively. In the superficial portion, CSAs of the 4 types of muscle fiber did not differ significantly between A and C groups at 4 weeks or at 48 weeks. In the deep portion, however, significant main effects of both treatment and time in type I and interaction between treatment and time in type IIb were observed. Four weeks after ACL transection, CSA of A group was significantly smaller than that of C group in type I and type IIb ($p < 0.05$, respectively). At 48 weeks, in addition, the CSA of type I differed significantly between limbs, with that of the transection-affected side (A group) being smaller than that of the contralateral side (C group) ($p < 0.05$).

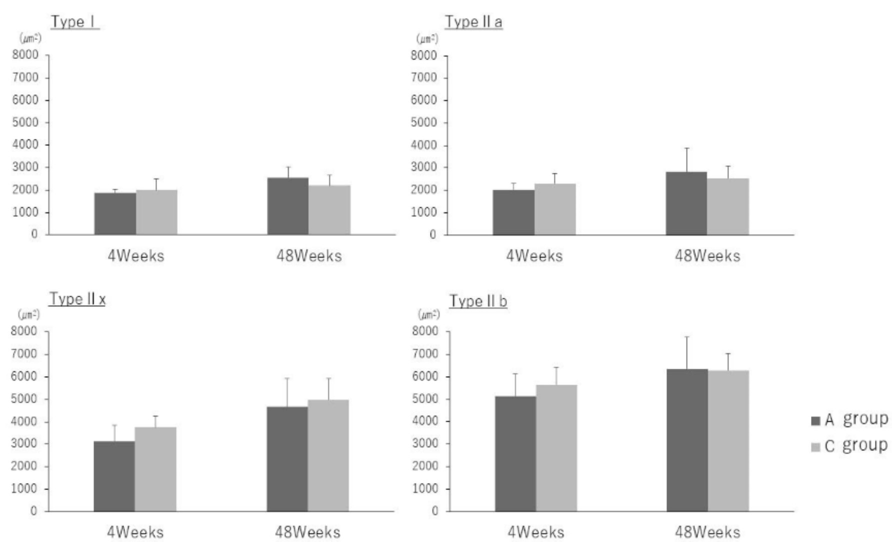

Figure 5. Fiber Cross-Sectional Area (CSA) in each muscle fiber type in superficial portion of the gastrocnemius muscle.

4 Weeks: 4 weeks after ACL transection; 48 Weeks: 48 weeks after ACL transection. Right and left limbs of rats were assigned as ACL-transected (A group) and contralateral control (C group), respectively. Unit: μm². Values are means ± SEM. n=6 in each group.

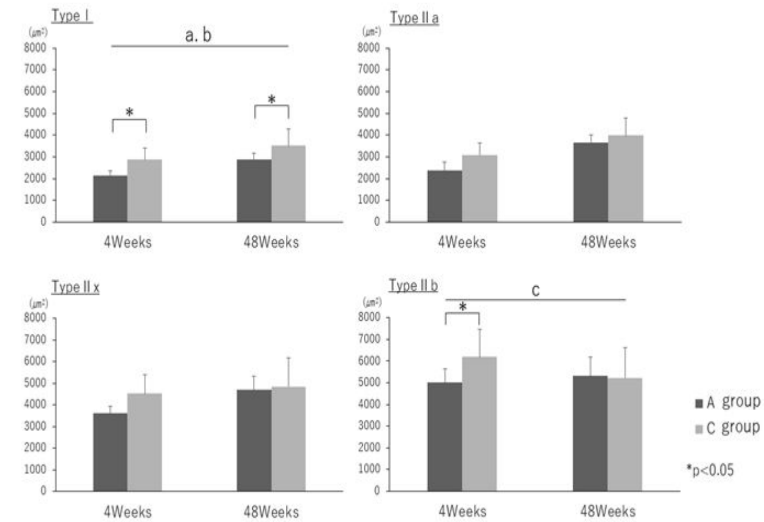

Figure 6: Fiber Cross-Sectional Area (CSA) in each muscle fiber type in deep portion of the gastrocnemius muscle.

4 Weeks: 4 weeks after ACL transection; 48 Weeks: 48 weeks after ACL transection. Right and left limbs of rats were assigned as ACL-transected (A group) and contralateral control (C group), respectively. Unit: µm^2. Values are means ± SEM. n=6 in each group. a: a significant main effect of treatment. b: a significant main effect of time. c: a significant interaction (treatment x time). * p<0.05.

Discussion

Most reported studies of the periarticular knee muscles after ACL injury have been clinical studies of muscle strength subsequent to ACL reconstruction [3,15,16]; very few have entailed basic physiological research. Ohno et al., however, used a rat ACL injury model for investigation of long-term changes in the morphology and contractile properties of the quadriceps (rectus femoris) and hamstring (semimembranosus) muscles [8]. They found a decrease in the CSA of the rectus femoris muscle fibers on the side of ACL transection and conversion of fast-twitch to slow-twitch fibers in the contralateral rectus femoris muscle. These changes also occurred, though less dramatically, in the semimembranosus muscles, and major discrepancies in physiological changes were seen, due to anatomical differences within the same periarticular knee muscle.

To the best of our knowledge, the study we describe herein is the first detailed analysis of changes that occur in the lower limb muscles rather than in the thigh after ACL transection. In clinically, it is well understood that severe atrophy of the triceps surae muscle group included the gastrocnemius muscle occurs during cast immobilization after lower limb trauma. The atrophy is commonly encountered in clinical settings, and early recovery is frequently challenging [17]. Thus, we investigated both short- and long-term changes (at 4 weeks and 48 weeks, respectively), in the morphology and contractile properties of the gastrocnemius muscle after ACL transection.

Effect of ACL Transection for Gastrocnemius Muscle

The greatest ACL transection-induced change in the gastrocnemius muscle was observed at 4 weeks. At this time point, muscle wet weight was significantly lower on the transection side than on the contralateral side, and the CSAs of all types of muscle fiber in the deep portion of the gastrocnemius muscle of the transection-affected limb tended to be smaller than CSAs of the same types of fiber of the gastrocnemius muscle of the contralateral limb. The volume of gastrocnemius muscle of the transection-affected limb was clearly lower than that of the contralateral limb.

One of the reasons for the difference in gastrocnemius muscle volume is the instability during internal rotation of the lower limb that results from ACL transection [7]. Generally, skeletal muscle tends to atrophy more during relaxed states than during extended states [18]. In our rat model, excessive internal rotation of the lower limb was followed by relaxation of the medial head of the gastrocnemius muscle, and it is supposed that muscle atrophy occurred in this state. We also suspect that the ACL transection itself would typically decrease the animal's activity. Thus, muscle atrophy due to both a relaxed state and disuse probably occurred.

Furthermore, the transaction would have had one or more neurological effects, such as proprioception failure [19,20]. The ACL mechanoreceptor functions as a tension sensor and acts to protect the knee joint by modulating the tone of the periarticular knee muscles via the γ-muscle spindle system. The factors are the

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original muscle tone and these reflexes [21]. Interruption of the afferent reflex pathway in this nervous system causes gastrocnemius muscle atrophy, and the muscle activity may thereby decrease [22,23].

These changes have been commonly observed in the deep portion of the gastrocnemius muscle. According to Armstrong and Phelps, rat skeletal muscle consists of a high percentage of slow-twitch muscle fibers in the deep portion, close to the skeleton, and a high percentage of fast-twitch muscle fibers in the superficial portion [13]. Accordingly, it appears that the effects of ACL transection are greatly reflected in the slow-twitch muscle fibers, which function as postural muscles.

Generally, of the various types of skeletal muscle fiber, type I fibers are slow-twitch fibers, type IIb are fast-twitch fibers, and types IIa and IIx are intermediate fibers with characteristics somewhere between type I and type IIb fibers [14,24,25]. Fibers can convert from one type to another, with various possible factors responsible for such conversion [26].

However, despite the fact that some changes were observed only in our rats' type IIx muscle fibers, no major change in fiber type, such as conversion to either slow-twitch or fast-twitch fibers, or in contractile properties was observed at either 4 or 48 weeks. This means that there were virtually no qualitative ACL transection-related changes (in contraction characteristics, such as contraction speed and resistance to fatigue) in the lower limb gastrocnemius muscle, despite interrupted proprioception and joint instability.

Long-Term Changes in Gastrocnemius Muscle

We evaluated the long-term effects of the ACL injury, i.e. when almost 1 year had passed after transection. As noted above, very few animal experiments have entailed long-term observation of the effect of ligament injury on periarticular knee muscles. Typically, Wistar rats live 48 to 72 weeks [27]**,** and 48 weeks of age in rats corresponds to ≥ 60 years of age in humans. Naturally, a simple cross-species comparison is not possible, but we guess that the effects of an ACL tear may persist for the remainder of a patient's life. We attempted to identify overall trends in gastrocnemius response over the long-term and found that the wet weight-to-body weight ratio of gastrocnemius muscle obtained from the ACL-transected limb at 4 weeks was significantly lower than that of the gastrocnemius muscle obtained from the contralateral limb at 4 weeks but that the ratios did not differ significantly at 48 weeks. In addition, the significant between-limb CSA-based differences in the deep portion type IIb fibers observed at 4 weeks were not observed at 48 weeks.

The reason for these time-related changes is unknown. With the overall increase in gastrocnemius wet weight-to-body weight ratio and fiber CSAs on the transection-affected hind limb, the difference between muscles on the two hind limbs decreased. Konishi, et al. reported that quadriceps muscle strength decreased on the side contralateral to a unilateral ACL injury in 13 patients (mean follow-up period of 9.3 months after injury) [28]. This might have been due to decreased muscle contraction power on the contralateral side as a result of γ-loop neurophysiological modulation due to the ACL tear. Results of our study suggest that an ACL tear affects the contralateral gastrocnemius muscle as well as the ipsilateral gastrocnemius muscle. The body might also constantly attempt to equalize lower limb balance following knee instability or neurological proprioception failure resulting from the ACL injury. Basic other researches are needed in the future.

Aside from the global changes, the CSA of the type I fiber in the deep portion of the gastrocnemius muscle on the affected side was significantly smaller than that on the contralateral side at both 4 weeks and 48 weeks, once again indicative of a somewhat significant persistence of the effects of the ACL injury. It is possible that our observations might be changed by the follow-up period to last longer than 48 weeks. Some of the effects of ACL transection showed to persist over the long term and those appeared most notably in postural muscles [8,29,30].

During this study, we observed rats over 48 months. This is a long period of time, almost equal to their entire lifespan. Some of the physiological disproportion in both lower limbs due to ACL transection persisted, suggesting that, in both legs, the muscle was not fully restored to its former state after 48 weeks. We predict that the course in humans is similar and that good management is clinically indispensable. One of the most common forms of muscle strength training for the gastrocnemius muscle is the "heel raise" exercise [17]. Accordingly, conservative treatment of an ACL tear entails short sessions of high load-bearing exercise during gastrocnemius muscle strength training on the affected side, and we believe it is especially important to reinforce the slow-twitch muscle fibers. We also expect that correction of the disproportionate muscle state in both lower limbs and good equilibrium are qualitatively and quantitatively important for patients to be able to return to sporting activities.

Study Limitations

The study was limited in that we examined only the medial head of the gastrocnemius muscle. In addition, we did not assess strength of the gastrocnemius as it pertains to function of the muscle. Further studies are necessary in the future to address these limitations.

Perspective

This animal study provided evidence that the leg muscles, rather than just the thigh muscles, should be a focus of rehabilitation during conservative treatment of an ACL tear. It is of note that the injury-related changes can be expected to persist over a very long term. We once again confirmed that ACL reconstruction is an important form of treatment for an ACL tear. Overall, we believe that this surgery is significant in that it contributes greatly to restoring stability of the knee joint in addition to recovering volume and contractibility of the periarticular knee muscles.

Conclusions

We investigated the long-term changes in the morphology and contractile properties of the gastrocnemius muscle that result from an ACL tear in rats. The decreasing of wet weight and the CSA of some fiber types of the muscle were observed and those changes persisted over the long-term period. These results suggested that it was necessary to perform the rehabilitation for not only thigh muscles but also leg muscles after ACL injury.

References

1. Johnson RJ, Beynnon BD, Nichols CE, Renström P (1992) The treatment of injuries of the anterior cruciate ligament. J Bone Joint Surg Am 74: 140-151.
2. Smith FW, Rosenlund EA, Aune AK, MacLean JA, Hillis SW (2004) Subjective functional assessments and return to competitive sports after anterior cruciate ligament reconstruction. Br J Sports Med 38: 279-284.
3. Urbach D, Nebelung W, Becker R, Awiszus F (2001) Effects of reconstruction of the anterior cruciate ligament on voluntary activation of quadriceps femoris. J Bone Joint Surg Br 83: 1104-1110.
4. Bonamo JJ, Fay C, Firestone T (1990) The conservative treatment of the anterior cruciate deficient knee. Am J Sports Med 18: 618-623.
5. Konrads C, Reppenhagen S, Belder D, Goebel S, Rudert M, et al. (2016) Long-term outcome of anterior cruciate ligament tear without reconstruction: a longitudinal prospective study. Int Orthop 40: 2325-2330.
6. Novak PJ, Bach BR Jr., Hager CA (1996) Clinical and functional outcome of anterior cruciate ligament reconstruction in the recreational athlete over the age of 35. Am J Knee Surg 9**:** 111-116.
7. Kapandji IA (1987) The knee. In: The Physiology of the Joints. Volume Two Lower Limb. (5th Edition). Edinburgh London Melbourne and New York: Churchill Livingstone, pp. 64-146.
8. Ohno M, Fujiya H, Goto K, Kurosaka M, Ogura Y, et al. (2017) Long term changes in muscles around knee joint after ACL resection in rats: comparisons of ACL-resected, contralateral and normal limb. J Sports Sci Med 16: 429-437.
9. Feller JA, Cooper R, Webster KE (2001) Current Australian trends in rehabilitation following anterior cruciate ligament reconstruction. Knee 9: 121-126.
10. Delay BS, Smolinski RJ, Wind WM, Bowmans DS (2001) Current practices and opinions in ACL reconstruction and rehabilitation: results of a survey of the American Orthopaedic Society for Sports Medicine. Am J Knee Surg 14: 85-91.
11. Kostogiannis I, Ageberg E, Neuman P, Dahlberg L, Fridén T, et al. (2007) Activity level and subjective knee function 15 years after anterior cruciate ligament injury: a prospective, longitudinal study of nonreconstucted patients. Am J Sports Med 35: 1135-1143.
12. Neuman P, Englund M, Kostogiannis I, Fridén T, Roos H, et al. (2008) Prevalence of tibiofemoral osteoarthritis 15 years after nonoperative treatment of anterior cruciate ligament injury: a prospective cohort study. Am J Sports Med 36: 1717-1725.
13. Armstrong RB, Phelps RO (1984) Muscle fiber type composition of the rat hindlimb. Am J Anat 171: 259-272.
14. Kishiro S, Fujiya H, Goto K, Ogura Y, Kurosaka M, et al. (2013) Effects of STG tendon harvest and BTB harvest on knee joint muscles: an immunohistochemical evaluation. Journal of St. Marianna University 4: 49-59.
15. Colombet P, Robinson J, Jambou S, Allard M, Bousquet V, et al. (2006) Two-bundle, four-tunnel anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 14: 629-636.
16. Fu FH, Shen W, Starman JS, Okeke N, Irrgang JJ (2008) Primary anatomic double-bundle anterior cruciate ligament reconstruction. Am J Sports Med 36: 1263-1274.
17. Peterson L, Renstörm P (2001) Knee. In: Sports injuries; their prevention and treatments. (3rd Edition). London: Martin Dunitz, pp. 267-330.
18. Booth FW, Kelso JR (1973) Effect of hind-limb immobilization on contractile and histochemical properties of skeletal muscle. Pflügers Arch European Journal of Physiology 342: 231-238.
19. Schultz RA, Miller DC, Kerr CS, Micheli L (1984) Mechanoreceptors in human cruciate ligaments: a histological study. J Bone Joint Surg Am 66-A: 1072-1076.
20. Zimmny ML (1988) Mechanoreceptors in articular tissues. Am J Anat 182: 16-32.
21. Johansson H, Sjolander P, Sojaka P (1991) A sensory role for the cruciate ligament. Clin Orthop Relat Res 268: 161-178.
22. Macefield VG, Gandevia SC, Bigland-Ritchie B, Gorman RB, Burke D (1993) The firing rates of human motoneurones voluntarily activated in the absence of muscle afferent feedback. J Physiol 471: 429-443.
23. Barrack RL, Skinner HB, Buckley SL (1989) Proprioception in the anterior cruciate deficient knee. Am J Sports Med 17: 1-6.
24. Botteinelli R, Schiaffino S, Reggiani C (1991) Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. J Physiol 437: 655-672.
25. Szentesi P, Zaremba R, van Mechelen W, Stienen GJ (2001) ATP utilization for calcium uptake and force production in different types of human skeletal muscle fibers. J Physiol. 531: 393-403.
26. Pette D (2002) The adaptive potential of skeletal muscle fibers. Can J Appl Physiol 27**:** 423-448.
27. Sengupta P (2013) The laboratory rat: relating its age with human's. Int J Prev Med 4**:** 624-630.
28. Konishi Y, Fukubayashi T, Takeshita D (2002) Possible mechanism of quadriceps femoris weakness in patients with ruptured anterior cruciate ligament. Med Sci Sports Exerc 34: 1414-1418.
29. Gutmann E, Schiaffano S, Hanzlikova V (1971) Mechanism of compensatory hypertrophy in skeletal muscle of the rat. Exp Neurol 31: 451-464.
30. Yellin H (1974) Changes in fiber types of the hypertrophy in denervated hemi- diaphragm. Exp Neurol 42: 412-428.