

Anterior cruciate ligament tear induces a sustained loss of muscle fiber force production

Jonathan P Gumucio, PhD^{1,2}, Kristoffer B Sugg, MD, PhD^{1,2,3}, Elizabeth R Sibilsky Enselman MEd¹, Alexis C Konja, BS¹, Logan R Eckhardt¹, Asheesh Bedi, MD¹, Christopher L Mendias, PhD^{1,2,4,*}

Departments of ¹Orthopaedic Surgery, ²Molecular & Integrative Physiology, and ³Surgery, Section of Plastic Surgery, University of Michigan Medical School, Ann Arbor, MI

⁴Hospital for Special Surgery, New York, NY

*To whom correspondence should be addressed:

Christopher L Mendias, PhD
Hospital for Special Surgery
535 E 70th St
Research Institute - S.602
New York, NY 10021
MendiasC@hss.edu
917-260-3783

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Abstract

Introduction: Patients with anterior cruciate ligament (ACL) tears have persistent quadriceps strength deficits that are thought to be due to altered neurophysiological function. Our goal was to determine the changes in muscle fiber contractility independent of the ability of motor neurons to activate fibers.

Methods: We obtained quadriceps biopsies of patients undergoing ACL reconstruction, and additional biopsies one, two, and six months after surgery. Muscles fiber contractility was assessed *in vitro*, along with whole muscle strength testing.

Results: Compared to controls, patients had a 30% reduction in normalized muscle fiber force at the time of surgery. One month later the force deficit was 41%, and at 6 months the deficit was 23%. Whole muscle strength testing demonstrated similar trends.

Discussion: While neurophysiological dysfunction contributes to whole muscle weakness, there is also a reduction in the force generating capacity of individual muscle cells independent of alpha motor neuron activation.

Keywords: anterior cruciate ligament; anterior cruciate ligament tear; muscle atrophy; muscle contractility; rehabilitation; isokinetic strength

Introduction

Anterior cruciate ligament (ACL) tears are a frequent and debilitating injury, with rates in the US estimated to be up to 250,000 per year¹. Patients who tear their ACL have persistent quadriceps weakness of 20-40% or more, even after they have undergone ACL reconstruction (ACL-R) and returned to sport²⁻⁴. Several studies and reviews have suggested loss of proprioception, impaired neuromuscular control, and other neuroplastic changes as the primary causes of quadriceps weakness after ACL-R⁵⁻⁷. However, it is also possible that the whole muscle weakness observed after ACL tear occurs due to intrinsic changes within the muscle fibers themselves, as is observed in chronic rotator cuff disease and muscular dystrophy^{8,9}. To gain a better understanding of the cellular mechanisms of muscle weakness in patients with ACL tears, we measured the force generating capacity of muscle fibers *in vitro* independent of the ability of motor neurons to activate these fibers, and hypothesized that ACL-R patients would reduce muscle fiber force production independent of the ability of motor neurons to activate these fibers.

Author

Methods

Subjects. This study was approved by the University of Michigan Institutional Review Board and conformed to the Declaration of Helsinki. Informed consent was obtained before enrollment. Eighteen to forty year old subjects with a unilateral complete ACL tear were eligible. Patients who were undergoing revision surgery, required a meniscal repair, had a previous injury to either knee, or had a myopathy or rheumatologic disorder were excluded. One week prior to surgery, patients performed strength measurements, and completed the International Knee Documentation (IKDC) survey, a patient reported outcomes assessment used to measure knee function¹⁰. Normative IKDC values have been reported¹⁰. A biopsy of the vastus lateralis muscle was obtained at the time of surgery. Together these events were considered as the first study visit. Patients also completed the IKDC survey, underwent strength testing, and had a muscle biopsy performed at one month, two months, and six months (± 1 week) after surgery.

Surgical Repair and Rehabilitation. Each patient sustained a tear within 12 weeks of surgery, and completed approximately 8 weeks of preoperative rehabilitation prior to surgical reconstruction. ACL reconstructions were performed by a single, fellowship-trained sports medicine surgeon using a hamstring tendon autograft and a single-bundle, anatomic reconstruction to recapitulate native ligament footprints and ligament obliquity. Patients participated in a standardized, accelerated post-operative rehabilitation program¹¹. Approximately six months following repair, patients were discharged to full sport activities based on return to play criteria¹².

Isokinetic Strength Measurements. Bilateral isokinetic strength measurements were performed in a System 3 dynamometer (BioDex, Shirley, NY) as described³. Briefly, isometric strength was measured at 90° of knee flexion, and isokinetic extension strength was measured at

60°/sec over an arc of 90° to 0° of knee flexion. The highest force from five repetitions was used, and force data of the involved side was normalized to the uninjured side at each visit.

Muscle Biopsy. Biopsies were obtained from the vastus lateralis muscle. At the first visit, the biopsy was obtained intraoperatively, 3-5 cm proximal to the joint line using a percutaneous arthroscopic duckbill biter. Subsequent biopsies were obtained in the clinic under local anesthetic using a percutaneous biopsy needle as described¹³, 3 cm proximal to the previous biopsy.

Muscle Fiber Contractility. Biopsies were prepared for muscle fiber contractility as described^{9,14} (see supplementary material for details). Briefly, biopsies were placed in calcium chelating and cryoprotectant solutions, and stored at -80°C. For contractility testing, fibers were isolated from thawed bundles, and secured to a servomotor (Aurora Scientific, Aurora, ON) and force transducer (Aurora Scientific) using 10-0 nylon suture. Fiber length was adjusted to obtain a sarcomere length of 2.7µm. Fiber cross sectional area (CSA) was calculated from width measurements that were fitted to an ellipse. Maximum isometric force (F_o) was elicited by immersing the fiber in a high-calcium activation solution, and specific force (sF_o) was determined by dividing F_o by CSA. Ten to fifteen type II fibers were tested from each biopsy. Historical control data of uninjured subjects was reported previously¹⁴.

Statistical Analyses. Values presented are mean±SD. SigmaPlot 12.0 (Systat, San Jose, CA) was used for statistical analyses. Differences between time points were tested using a repeated measures one-way ANOVA ($\alpha=0.05$) followed by Fisher's LSD post-hoc sorting.

Results

There were 10 subjects, 5 males and 5 females (age 27.4 ± 6.1 years and BMI 24.6 ± 1.9). At the time of surgery, IKDC scores were 44% lower than uninjured controls (Figure 1). One month later, scores decreased by an additional 27%, but by two months had returned to perioperative levels (Figure 1). Six months after surgery, scores were 29% higher than at the start of the study, but were still 27% lower than normative data (Figure 1).

Isometric knee extension strength at the time of surgery was 13% lower in the injured limb than the uninjured limb (Figure 2A). One month later, there was a 68% decrease in relative strength compared to the perioperative value, and by two months relative strength was still reduced by 41% (Figure 2A). At six months, there was a 17% strength deficit compared to the time of surgery, and a 26% deficit compared to the uninjured limb (Figure 2A). Isokinetic strength generally changed in a similar pattern to isometric values (Figure 2B).

We next measured muscle fiber contractility (Figure 3). Perioperative CSA values were close to those from healthy, uninjured muscles, but decreased by 23% one month after surgery (Figure 3A). Thereafter, there were no significant differences in CSA. At the time of surgery, F_0 was 32% lower than uninjured muscles (Figure 3B). F_0 declined by an additional 34% at 1 month, and by two and six months was not different from the perioperative value (Figure 3B). At the time of discharge, F_0 remained 27% lower than controls (Figure 3B). sF_0 followed generally similar trends, displaying a 30% reduction compared to control muscles at the time of surgery, and at the time of discharge remained 22% lower than controls (Figure 3C).

Discussion

Deficiencies in the ability of the nervous system to recruit and activate muscle fibers have been suggested as the primary factors behind the quadriceps muscle weakness in patients with ACL tears⁷. By measuring the contractility of muscle fibers *in vitro*, we decoupled muscle force generation from motor neuron input, and identified a muscle fiber-autonomous deficit in contractile function in patients with ACL tears.

Various mechanisms of neuromuscular deficiency have been proposed to explain weakness following ACL-R. ACL rupture causes remodeling of the central nervous system¹⁵ that affects both reflexive movement and voluntary movement in the injured limb⁷. One result of central nervous system remodeling is arthrogenic muscle inhibition, during which abnormal signals conveyed from mechanoreceptors or nociceptors in the knee joint after ACL tear inhibit the activation of alpha motor neurons, subsequently reducing muscle force production¹⁵. Much of the literature on neuromuscular changes in ACL-R patients has not considered intrinsic changes to the muscle fibers themselves. However, recent studies have pointed to clear evidence of maladaptive muscle responses, with ACL-R patients demonstrating increased circulating levels of the atrophy-inducing signaling molecules myostatin and TGF- β up to 5 weeks after surgical repair³, and decreased quadriceps muscle stem cell density and muscle fiber CSA^{16,17}. This is further supported by animal studies that reported reduced *in vitro* whole muscle force production after ACL tear, with increased expression of atrophy-associated genes such as the E3 ubiquitin ligases atrogin-1, MuRF-1, and MUSA-1 that can target proteins for enzymatic breakdown^{18,19}. In the current study, the lack of appreciable changes in fiber CSA along with substantial changes in F_0 , suggest that there is a marked reduction in the density of myofibrils, which are the force generating organelles in muscle fibers. Although speculative, the results of the current

manuscript and previous studies suggest that it is possible that after ACL tear and surgical reconstruction there is an initial activation of proteolytic processes within fibers that lead to a reduction in force production through loss of myofibrils. Subsequent to this bout of atrophy, during the rehabilitation period there is then likely insufficient activation of muscle stem cells and protein synthesis signaling pathways to fully restore the myofibrils lost due to the tear and surgical repair, resulting in overall persistent muscle weakness in ACL-R patients.

While we did not measure histological or biochemical changes in biopsied tissue, or contractility beyond 6 months after surgery, our study provided insight into the changes within muscle independent of neural input after ACL tear and reconstruction. Studying the pathological changes to muscle fibers is important, as the muscle weakness which results after ACL tear reduces athletic performance, and likely contributes to the development of osteoarthritis^{2,20}. Many ACL rehabilitation programs put substantial focus on neuromuscular re-education, but more emphasis on exercises for muscle hypertrophy may be beneficial. Additionally, studies that evaluate the ability of pharmacological interventions to prevent myofibrillar protein degradation in the acute post-operative period, or increase myofibrillar protein synthesis in the long term, are likely warranted.

Abbreviations

ACL, Anterior cruciate ligament

ACL-R, Anterior cruciate ligament reconstruction

CSA, Cross-sectional area

F_0 , Maximum isometric force

IKDC, International knee documentation committee

sF_0 , Specific force, maximum isometric force normalized to cross-sectional area

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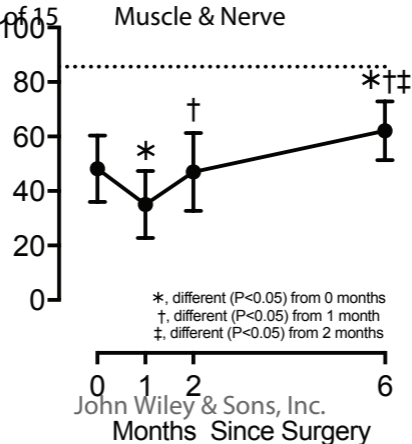
Figure Legends

Figure 1. International Knee Documentation Committee (IKDC) scores. Values are mean \pm SD. Horizontal dashed line indicates the mean normative value previously published¹⁰. Differences tested with a one-way repeated measures ANOVA ($\alpha=0.05$) followed by Fisher's LSD post hoc sorting.

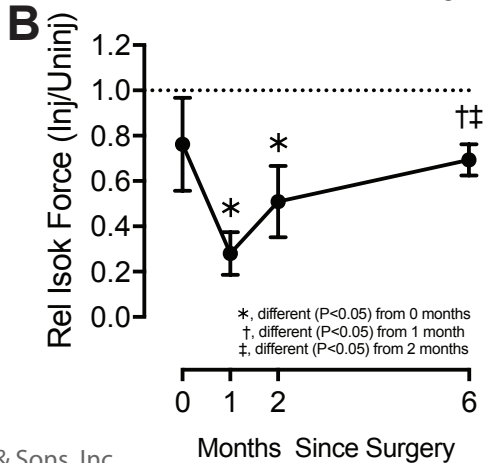
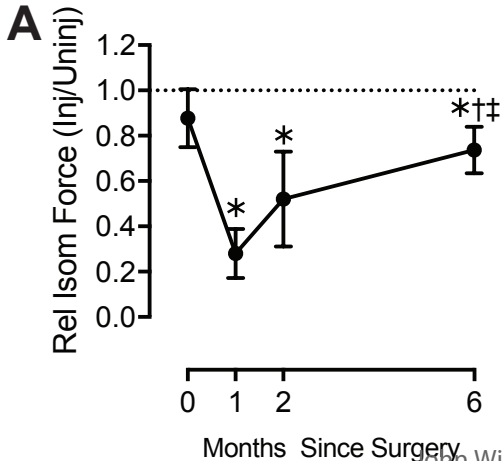
Figure 2. Strength Measurements. (A) Maximum isometric extension strength, and (B) maximum isokinetic knee extension strength. The injured side was normalized to the uninjured side at each time point to generate relative values. Horizontal dashed line indicates bilateral strength symmetry. Values are mean \pm SD. Differences tested with a one-way repeated measures ANOVA ($\alpha=0.05$) followed by Fisher's LSD post hoc sorting.

Figure 3. Permeabilized fiber contractility. (A) Cross-sectional area (CSA), (B) maximum isometric force (F_0), and (C) specific force (sF_0 , F_0 normalized by CSA). Values are mean \pm SD. Horizontal dashed line indicates the mean normative value from uninjured, healthy, similar age subjects (24.5 ± 2.5 years of age) previously published, using the same equipment and techniques as the current study¹⁴. Differences tested with a one-way repeated measures ANOVA ($\alpha=0.05$) followed by Fisher's LSD post hoc sorting.

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