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RESEARCH ARTICLE

Beyond weakness: Exploring intramuscular fat and quadriceps atrophy in ACLR recovery

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Abstract

Muscle weakness following anterior cruciate ligament reconstruction (ACLR) increases the risk of posttraumatic osteoarthritis (OA). However, focusing solely on muscle weakness overlooks other aspects like muscle composition, which could hinder strength recovery. Intramuscular fat is a non-contractile element linked to joint degeneration in idiopathic OA, but its role post-ACLR has not been thoroughly investigated. To bridge this gap, we aimed to characterize quadriceps volume and intramuscular fat in participants with ACLR (male/female = 15/9, age = 22.8 ± 3.6 years, body mass index [BMI] = 23.2 ± 1.9, time since surgery = 3.3 ± 0.9 years) and in controls (male/female = 14/10, age = 22.0 ± 3.1 years, BMI = 23.3 ± 2.6) while also exploring the associations between intramuscular fat and muscle volume with isometric strength. Linear mixed effects models assessed (I) muscle volume, (II) intramuscular fat, and (III) strength between limbs (ACLR vs. contralateral vs. control). Regression analyses were run to determine if intramuscular fat or volume were associated with quadriceps strength. The ACLR limb was 8%−11% smaller than the contralateral limb ($p < 0.05$). No between-limb differences in intramuscular fat were observed (p 0.091−0.997). Muscle volume but not intramuscular fat was associated with strength in the ACLR and control limbs ($p < 0.001$−0.002). We demonstrate that intramuscular fat does not appear to be an additional source of quadriceps dysfunction following ACLR and that muscle size only explains some of the variance in muscle strength.

KEYWORDS
ACL, knee, ligament, muscle, rehabilitation

1 | INTRODUCTION

Following anterior cruciate ligament (ACL) injury, patients can elect to undergo ACL reconstruction (ACLR) to restore the mechanical stability of the joint. Surgical reconstruction is then followed by rigorous and prolonged rehabilitation (>6 months) to return patients to physical activity. Despite this standard of care, patients often encounter impairments such as persistent quadriceps weakness and an elevated risk of developing early onset posttraumatic osteoarthritis (PTOA).[1] One of the predominant theories of PTOA development following ACL injury is that quadriceps weakness diminishes the quadriceps' capacity to absorb shock, altering joint biomechanics and the distribution of load across articular cartilage.[2] Consequently, considerable efforts has been directed toward elucidating the sources of muscle weakness following ACLR. To date, muscle weakness has been primarily attributed to neuromuscular changes and muscle atrophy post-ACLR. However, these factors do not fully explain the experienced

muscle dysfunction long-term following rehabilitation.[3–5] Most studies investigating quadriceps weakness post-ACLR have often focused on whole muscle metrics such as isometric, and isokinetic strength,[6] muscle volume,[7] and cross-sectional area.[8] However, they frequently overlook the quality of muscle tissue such as quantities of non-contractile tissue (e.g., fibrosis[9,10] and intramuscular fat[11]). Since non-contractile elements can directly influence muscle strength and size (e.g., volume and cross-sectional area), examining their presence and functional impact on strength at a chronic time-point post-ACLR may offer additional mechanistic insights into muscle weakness following ACLR.

In the current study, we were particularly motivated to investigate the influence of intramuscular fat as it resides in the extracellular matrix (ECM), which provides structural support for many cellular processes and is essential for adequate force transmission.[12] Importantly, an accumulation of intramuscular fat can restrict force transmission between myofibrils, aponeuroses, and the tendon.[12–15] Following ACL injury and/or ACLR, it has been shown that the ECM expands due to collagen accumulation, which occurs alongside elevated quantities of fibro-adipogenic progenitors.[16–18] This is important as fibro-adipogenic progenitors can disrupt ECM remodeling and promote differentiation into fat and fibrotic tissue,[16–18] which has been associated with quadriceps weakness in other populations[19–21] and found to be a better predictor of knee osteoarthritis than muscle size alone.[11] Following ACLR, others have shown increases in intramuscular fat of the vastus lateralis (VL),[22] but not the rectus femoris or medialis using B-mode ultrasound imaging.[22,23] While important, B-mode ultrasound assesses intramuscular fat via echo intensity, which can be confounded by factors such as subcutaneous fat overlying muscle. In addition, ultrasound most often reveals a single cross section or a two-dimensional panoramic view of muscle. Alternatively, magnetic resonance imaging (MRI) is a reliable imaging technique that enables the separation of fat and water through their unique chemical-shift properties, offering a noninvasive approach to assess fat accumulation more comprehensively in three-dimensions.[24,25] While this imaging modality has been utilized in other populations,[26,27] there has only been a single study to utilize MRI following ACLR. This study was performed on two slices of the rectus femoris and found no differences between limbs 1 and 5 years following surgery.[23] However, this modality has not been applied robustly to measure intramuscular fat across the vasti muscles and in regional compartments following ACLR.

Given the associations between intramuscular fat, quadriceps weakness, and idiopathic OA,[11,16,18,28] it remains plausible that intramuscular fat may interfere with quadriceps strength recovery following ACLR. As such, our primary objective was to use MRI to characterize muscle size and intramuscular fat in the vasti muscles (both globally and regionally) in those with a chronic history of ACLR and in controls. We also explored if intramuscular fat and fat-cleared muscle volume were related to isometric strength in the ACLR and control limbs. We hypothesized that an increase in intramuscular fat (e.g., fat fraction), and smaller fat-cleared muscle volume would correlate with weaker muscles.

2 | METHODS

2.1 | Experimental design

Twenty-four individuals who had undergone primary unilateral ACLR were recruited from the Department of Orthopaedic Surgery and the general student population at the University of Michigan. ACLR individuals were eligible for this study if they: (1) were between 14 and 45 years of age, (2) had no prior knee injury or surgery other than current ACL, (3) were between 1.5 and 5 years post-ACLR, (4) had received a bone patellar tendon bone autograft, and (5) had a body mass index (BMI) under 30 kg/m^2. ACLR participants were excluded if they had multiple ACLR's unilaterally or bilaterally. To characterize control profiles, a convenience cohort of 24 individuals was also recruited that had no history of lower extremity injury or surgery and had a BMI below 30 kg/m^2. A BMI below 30 kg/m^2 was chosen as it aligns with the clinical definition of obesity.[29] Obesity is correlated with increases in adiposity, which in turn has been linked to elevated levels of intramuscular fat.[30] Demographic information for included participants can be found in Table 1. Note, this study sample consisted of the same subjects that participated in our previous study that examined quadriceps fascicle mechanics post-ACLR.[31]

All participants meeting these criteria underwent two experimental sessions over 2 days. The first experimental session consisted of patient reported outcomes and quadriceps strength testing, while

TABLE 1 Data are reported as mean ± standard deviation.

	ACLR (n = 24)	Control (n = 24)	p Value
Females/males	15/9	14/10	-
Age (years)	22.8 ± 3.6	22.0 ± 3.1	0.406
Height (m)	1.70 ± 0.1	1.70 ± 0.1	0.856
Mass (kg)	68.7 ± 8.9	69.9 ± 13.6	0.732
Body mass index	23.2 ± 1.9	23.3 ± 2.6	0.801
Time after surgery (year)	3.3 ± 0.9	N/A	-
Tegner activity scale	Pre: 8.4 ± 1.1	Pre: 6.8 ± 1.3	Pre: <0.001*
	Post: 6.8 ± 1.6	Post: 6.7 ± 1.2	Post: 0.815
KOOS symptoms	84.7 ± 10.6	95.7 ± 5.4	<0.001*
KOOS pain	95.4 ± 5.3	98.7 ± 2.3	0.007*
KOOS activities of daily living	99.0 ± 1.4	99.8 ± 0.9	0.039*
KOOS sports and recreation	89.0 ± 8.5	98.3 ± 4.3	<0.001*
KOOS quality of life	83.1 ± 14.6	99.2 ± 2.1	<0.001*
IKDC	88.9 ± 8.8	98.7 ± 3.0	<0.001*

Abbreviations: ACLR, anterior cruciate ligament reconstruction; IKDC, International Knee Documentation Committee; KOOS, knee injury and osteoarthritis outcome score.

*Denotes statistical significance at the level of $p < 0.05$.

the second experimental session consisted of MRI to measure quadriceps intramuscular fat. When possible, testing sessions were arranged to take place on the same day. Otherwise, the time between experimental testing sessions was minimized (less than 2 weeks) to manage the potential of any physiological variances in muscle tissue that could change with time. All participants provided informed consent, and all protocols were approved by the University of Michigan Institutional Review Board (IRBMED: HUM00169174).

2.2 | Patient reported outcomes

Participants completed a series of questionnaires to evaluate self-reported function including the Tegner Physical Activity,[32] International Knee Documentation Committee (IKDC),[33] and knee injury and osteoarthritis outcome score (KOOS).[34]

2.3 | Isometric strength

Isometric knee extensor strength was assessed as previously described using an isokinetic dynamometer (CSMi Humac Norms Stoughton).[35] The knee joint was positioned at 60° of flexion[36] and participants completed a standardized warm-up protocol consisting of two practice repetitions at each of the following intensities: 25%, 50%, and 75% of perceived maximal effort. Following these warm-up contractions, participants completed one practice trial at 100% of their perceived maximal effort. The maximal voluntary knee extensor torque during this final practice trial was used to set a visual torque target for the subsequent MVC assessments. For test trials, participants performed three MVC's where they were instructed to kick out as hard and fast as they could and were provided strong verbal encouragement. Participants were given 2 min of rest between each trial. Peak knee extension torque (N × m) was extracted from each MVC trial. The largest of the three peaks was used in the subsequent analyses.

2.4 | Intramuscular fat and muscle volume

MRI data of the bilateral upper thighs were acquired with a 3-Tesla MRI Philips Ingenia scanner using a 16-element anterior torso coil and 12-element receiver coil located within the table coil. Three stacks spanning the full length of the quadriceps muscle were acquired with a 30 mm overlap covering a field of view of 420 × 284 × 140 mm^3. Stack-specific shimming was applied independently to each stack to reduce B0 field inhomogeneity. An axial Dixon Quant sequence was performed with the following key acquisition parameters: 3D gradient echo; number of echoes = 6 (TE1 = 1.2 ms, delta TE = 0.9 ms); TR = 7.283 ms; flip angle = 3°; acquired matrix size = 264 × 178 mm; reconstructed matrix size = 288 × 288 mm; acquired voxel size = 1.6 × 1.6 × 2.2 mm^3; reconstructed voxel size = 1.3 × 1.3 × 1.1 mm^3; parallel imaging factor SENSE = 2; number of slices/stack = 148.

Postprocessing was conducted via custom written MATLAB code. Five slices on the proximal and distal ends of each stack were removed to improve signal inhomogeneity that occurs at the ends of each stack. In the regions of overlap, slices closest to the center of the stack were retained. Stacks were then stitched together to generate fat only, water only, in phase, out of phase, and fat fraction images. To reduce the number of slices and maintain the signal-to-noise ratio from the acquisition, signals were averaged over every four slices. The VL, vastus intermedius (VI), vastus medialis (VM), and the femur were manually segmented on a limited number of slices on the water-only image and a semi-automatic method using a combination of diffeomorphic registrations was used to propagate these segmentations to the remaining slices to result in a full thigh segmentation (Figure 1A,B).[37,38] Propagations were manually refined in ITK snap.[39] A single voxel border from all whole muscle segmentations was eroded and all whole muscles were divided into subregions of interests (ROIs). Proximal, central, and distal sub-ROIs were created by splitting each muscle into three equal lengths in the proximo-distal direction (Figure 1C). To further quantify the extent of local damage, segmentations of each muscle were partitioned into two depth levels. Deep and superficial sub-ROIs were discriminated using the distance from the femur gravity center as previously described (Figure 1D).[40]

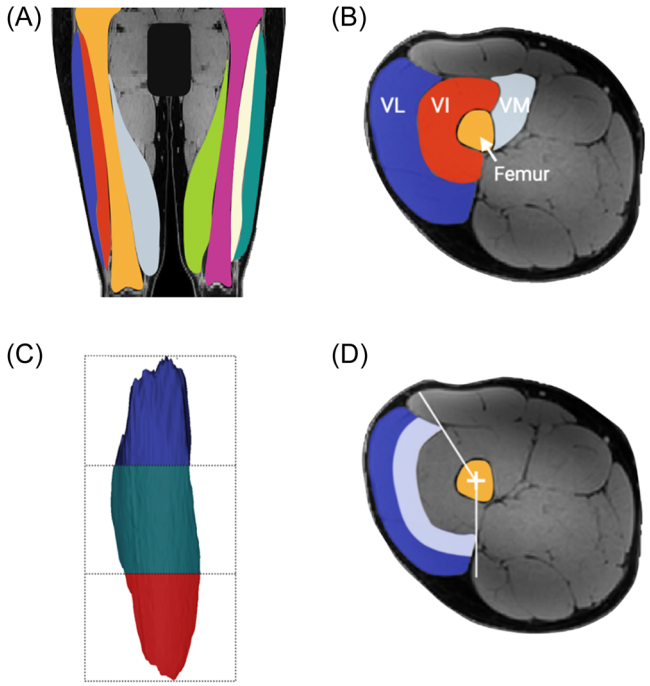

FIGURE 1 Representative muscle segmentations. (A) and (B) represent whole muscle regions of interest (ROI) in the frontal (A) and axial (B) planes. (C) represents an example of the proximal (top), central (middle), and distal (bottom) sub-ROI's for the VL where the muscle was split into three equal lengths in proximo-distal direction. (D) represents an example of the superficial and deep muscle sub-ROIs for the VL, where the muscle was partitioned to two different depths using the distance from the femur gravity center. VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

Absolute muscle volumes were extracted from each whole ROI. Intramuscular fat was measured as the median fat fraction from all whole and sub-ROIs. The distribution of intramuscular fat was also evaluated descriptively as a function of muscle length from the distal to proximal axis as previously described.[41] Briefly, slice numbers were expressed as a percentage of muscle length where the most distal slice was set to 0% and the most proximal slice was set to 100%.

2.5 | Statistical analyses

Statistical analyses were conducted using R Statistical Software (4.2.2) with a significance level set at $p \leq 0.05$. To characterize the sample, participant demographics and patient reported outcomes between the ACLR and control groups were compared using independent *t*-tests (Table 1). Between-limb variability in the control subjects were assessed using paired *t*-tests. No significant differences were found between control limbs for all strength, volume, and intramuscular fat outcomes ($p = 0.205–0.969$). As such, both control limbs were included in subsequent linear mixed effect model analyses to create a 3-limb comparison (ACLR, $n = 24$; contralateral, $n = 24$; and control, $n = 48$). An outlier analysis was also performed and outliers that were below the first quartile-1.5 × interquartile range (IQR) or above the third quartile + 1.5 × IQR were identified and removed.

The following variables were analyzed using linear mixed effects models (lmer function from the lmerTest, lme4, and emmeans packages in R)[42–44]: (I) Peak torque, (II) global and individual muscle volume and intramuscular fat, (III) individual vasti intramuscular fat by region (proximal, central, distal, superficial, and deep). To understand if intramuscular fat was distributed differently within limbs, we also ran linear mixed effects models for individual vasti muscles and investigated the interaction of region and limb. A random intercept was included for each subject. Sex and BMI were included as control variables because they have the potential to confound the relationship between fat, muscle size, and strength.[45,46] In case of a significant main effect, post hoc pair wise comparisons with Tukey adjustments were performed. In the case of a significant pair wise comparison, effect sizes were computed as the estimate of the mean difference between limbs divided by the pooled standard deviation of included limbs. Separate regression analyses were run to determine intramuscular fat or volume were associated with quadriceps strength, controlling for sex and BMI. For the ACLR limb, associations also included time since surgery.

3 | RESULTS

3.1 | Patient reported outcomes

KOOS subscale and IKDC results are summarized in Table 1. As expected, the ACLR cohort has significantly worse outcomes on all KOOS subscales and IKDC knee-specific outcomes than the control cohort ($p < 0.001–0.039$).

3.2 | Muscle strength volume, and intramuscular fat

No differences between limbs were identified for peak torque ($p = 0.115–0.311$; Table 2). Significant differences were found for muscle volume between limbs ($p < 0.001$; Figure 2A). Post hoc analyses revealed the vasti muscles in the ACLR limb were on average 142 cm^3 (or 9%) smaller than the contralateral limb (95% CI = 97–186 cm^3), with an effect size of $d = 0.54$. No significant differences in intramuscular fat were found between limbs ($p > 0.05$; Figure 2B, Table 3). These findings were consistent for what was found for individual vasti muscles. Significant differences in muscle volume were found between limbs for VI, VL, and VM ($p < 0.001$; Table 3). Post hoc analysis revealed that the VI in the ACLR limb was 50 cm^3 (or 9%) than the VI in the contralateral limb (95% CI = 28–72 cm^3; $d = 0.49$). Similarly, the VL in the ACLR limb was 49 cm^3 (or 8%) smaller (95% CI = 26–72 cm^3; $d = 0.45$) and the VM was 43 cm^3 (or 11%) smaller compared to the contralateral limb (95% CI = 27–59 cm^3; $d = 0.55$).

3.3 | Regional comparison and variation of intramuscular fat

Proximal, central, and distal intramuscular fat content is summarized in Table 4, while superficial and deep intramuscular fat content is summarized in Table 5. No significant differences in intramuscular fat were observed between limbs for any region ($p = 0.091–0.997$).

When exploring if the amount of intramuscular fat varied by region within a limb (region by limb interaction) no significant interactions were identified ($p > 0.05$), indicating that both groups and limbs had similar variations of fat throughout the muscle. Regional distributions of intramuscular fat for each vasti muscle are represented in Figure 3 and Supporting Information S1: Figure 4.

TABLE 2 Isometric quadriceps strength by limb.

	ACLR	Contralateral	Control	*F*	*p* Value
Peak torque (N × m)	203.8 ± 45.8	213.8 ± 36.3	217.7 ± 77.3	1.20	0.311

Note: Data are reported as mean ± standard deviation. *F* statistic and *p* value reported is result of the main effect of limb from the linear mixed effects model. At the level of $p < 0.05$, no between-limb differences were detected.

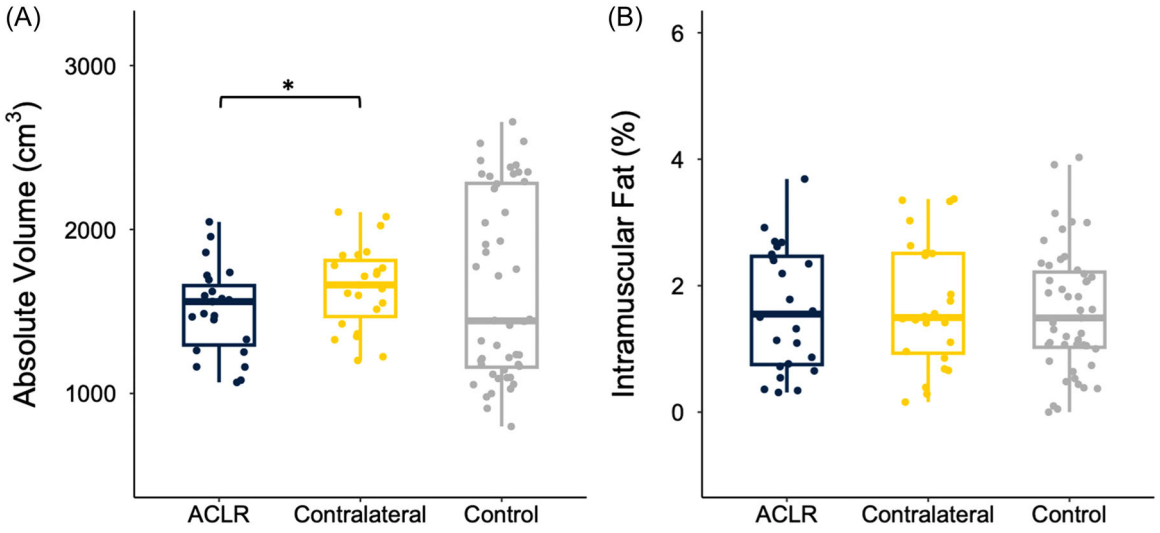

FIGURE 2 Muscle volume and intramuscular fat. Significant differences in absolute volume (A) were observed between limbs. Post hoc analyses revealed that the ACLR limb was, on average, 142 cm^3 (or 9%) smaller than the contralateral limb with * denoting statistical between-limb differences at the level of $p < 0.001$. No differences in intramuscular fat were observed (B). ACLR, anterior cruciate ligament reconstruction.

TABLE 3 Individual volume and intramuscular fat for the VL, VI, and VM.

	Muscle	ACLR	Contralateral	Control	*F*	*p* Value
Absolute volume (cm^3/kg)	VL	**592 ± 112**	**641 ± 104**	666 ± 226	13.45	<0.001*
	VI	**500 ± 101**	**550 ± 104**	517 ± 176	15.34	<0.001*
	VM	**418 ± 71**	**469 ± 83**	460 ± 174	20.58	<0.001*
Intramuscular fat (%)	VL	1.64 ± 1.26	1.90 ± 1.12	1.75 ± 1.24	0.29	0.752
	VI	1.60 ± 1.00	1.59 ± 1.03	1.57 ± 0.99	0.00	0.997
	VM	1.69 ± 0.87	1.60 ± 0.98	1.53 ± 0.85	0.26	0.774

Note: Data are reported as mean ± standard deviation. *F* statistics and *p* values reported are results of the limb main effect from the linear mixed effects model, with * denoting a statistically significant main effect at the level of $p < 0.05$. Bold text indicates that post hoc analyses revealed significant between-limb differences ($p < 0.05$), where the ACLR limb had a smaller volume relative to the contralateral limb. No statistically significant differences in intramuscular fat were identified between limbs ($p > 0.05$).

Abbreviations: ACLR, anterior cruciate ligament reconstruction; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

3.4 | Associations between intramuscular fat and muscle volume with strength in ACLR and control limbs

In the ACLR limb, covariates explained 30.0% (adjusted R^2) of variance in peak torque ($F[3, 19] = 4.19$, $p = 0.020$) where, males had increased peak torque (sex: $t = 3.03$, $p = 0.007$; BMI: $t = -0.687$, $p = 0.509$; time since surgery: $t = 1.56$, $p = 0.136$). Muscle volume ($\Delta F[1, 18] = 6.42$, $\Delta p = 0.020$) but not intramuscular fat ($\Delta F[1, 18] = 1.29$, $\Delta p = 0.270$), helped to explain additional 15.8% of variance in the model ($F[4, 18] = 5.64$, $p = 0.004$, adjusted $R^2 = 45.8\%$). In the control limb, covariates explained 45.1% (adjusted R^2) of variance in peak torque ($F[2, 44] = 19.88$, $p < 0.001$), where males had increased peak torque (sex: $t = 5.90$, $p < 0.001$; BMI: $t = 1.33$, $p = 0.191$). Similarly to the ACLR limb, muscle volume ($\Delta F[1, 43] = 21.49$, $\Delta p < 0.001$) but not intramuscular fat ($\Delta F[1, 43] = 3.62$, $\Delta p = 0.063$), helped to explain additional 17.4% of variance in the model ($F[3, 43] = 26.59$, $p < 0.001$, adjusted $R^2 = 62.5\%$).

4 | DISCUSSION

The objectives of our study were to use MRI to (I) characterize vasti size and intramuscular fat in those with a history of ACLR and Controls and, (II) determine if intramuscular fat and muscle volume were related to isometric strength in the ACLR and control limbs. The primary results of our study indicated that those with ACLR exhibited smaller vasti volumes compared to contralateral and control limbs, but intramuscular fat content and quadriceps strength did not differ across limbs. The lack of differences in intramuscular fat and quadriceps strength may be attributed to the high level of strength recovery observed in this ACLR cohort, which contrasts with the

TABLE 4 Regional comparisons in proximo-distal direction of intramuscular fat (%) by limb.

Muscle	Region	ACLR	Contralateral	Control	*F*	*p* Value
VL	Proximal	2.24 ± 0.88	2.21 ± 1.05	2.15 ± 1.08	0.32	0.867
	Central	1.42 ± 1.40	1.82 ± 1.16	1.75 ± 1.39		
	Distal	1.58 ± 1.53	1.80 ± 1.29	1.77 ± 1.58		
VI	Proximal	1.64 ± 1.16	1.47 ± 1.16	1.51 ± 0.97	0.59	0.668
	Central	1.10 ± 0.84	1.37 ± 0.95	1.39 ± 0.98		
	Distal	2.39 ± 1.49	2.25 ± 1.41	2.21 ± 1.32		
VM	Proximal	1.57 ± 1.22	1.60 ± 1.03	1.51 ± 0.99	0.86	0.491
	Central	1.41 ± 0.81	1.37 ± 0.89	1.42 ± 0.98		
	Distal	1.97 ± 1.04	1.47 ± 0.71	1.59 ± 0.70		

Note: Data are reported as mean ± standard deviation. *F* statistics and *p* values reported are results of the region by limb interaction from the linear mixed effects model. No significant region by limb interactions were determined ($p > 0.05$).

Abbreviations: ACLR, anterior cruciate ligament reconstruction; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

TABLE 5 Regional comparisons by depth of intramuscular fat (%) by limb.

Muscle	Region	ACLR	Contralateral	Control	*F*	*p* Value
VL	Superficial	1.53 ± 1.26	1.80 ± 1.07	1.73 ± 1.34	0.01	0.989
	Deep	1.80 ± 1.23	2.04 ± 1.15	1.91 ± 1.22		
VI	Superficial	1.67 ± 1.08	1.74 ± 1.07	1.73 ± 1.15	0.18	0.838
	Deep	1.52 ± 0.94	1.41 ± 1.01	1.40 ± 0.85		
VM	Superficial	1.73 ± 0.92	1.66 ± 1.04	1.60 ± 0.87	0.08	0.923
	Deep	1.65 ± 0.85	1.54 ± 0.92	1.42 ± 0.80		

Note: Data are reported as mean ± standard deviation. *F* statistics and *p* values reported are results of the region by limb interaction from the linear mixed effects model. No significant region by limb interactions were determined ($p > 0.05$).

Abbreviations: ACLR, anterior cruciate ligament reconstruction; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

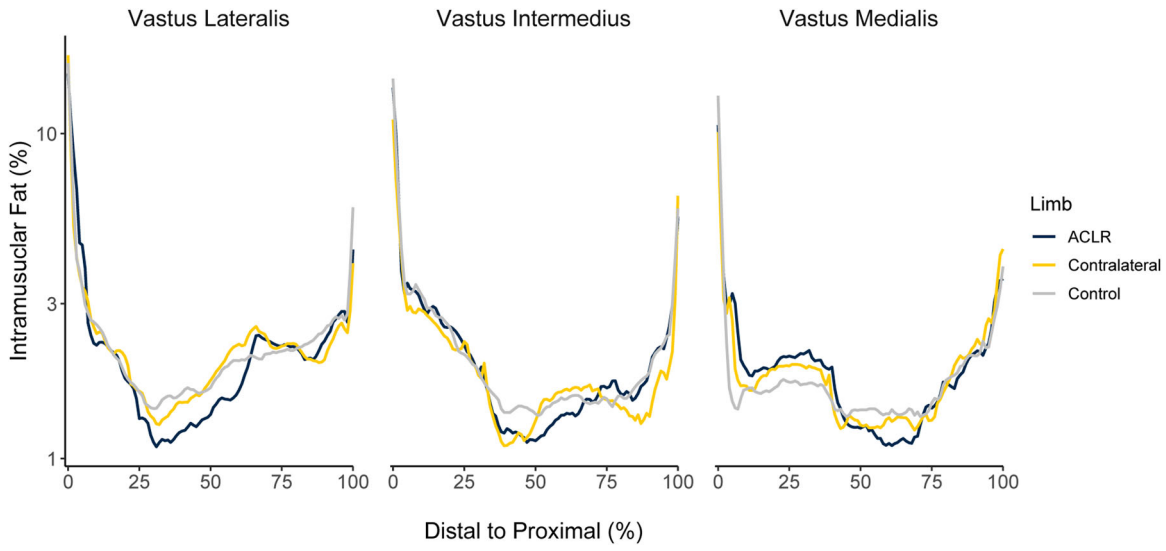

FIGURE 3 Regional distributions of intramuscular fat by limb represented on a logarithmic scale. Left, middle, and right panels demonstrate vastus lateralis, vastus intermedius, and vastus medialis, respectively. ACLR, anterior cruciate ligament reconstruction.

protracted deficits in muscle strength reported in other studies.[6,47] Future studies would benefit from exploring the relationship between intramuscular fat, muscle volume, and strength in a cohort with strength deficits. Nevertheless, these data represent a comprehensive assessment of whole and regional vasti intramuscular fat following ACLR utilizing Dixon MR that helps aid in our understanding of factors contributing to quadriceps dysfunction following ACLR.

In other conditions, such as rotator cuff injuries, there is substantial scientific evidence indicating that excessive fat infiltration and muscle atrophy represent significant obstacles to the healing and recovery process.[48,49] Regarding the knee, heightened levels of intermuscular[50] and intramuscular fat[11] have been linked to those with idiopathic OA. In addition, increased levels of intramuscular fat on the VM have been associated with decreased quadriceps strength ($r = -0.455$) in those with idiopathic OA[51] and a loss of cartilage volume in obese patients.[52] However, given that there are only a few studies to investigate intramuscular and intermuscular fat following knee pathology (e.g., ACLR, idiopathic OA, PTOA), there has not been a general consensus of its prevalence or functional impact on quadriceps strength. Modeling studies have shown that intramuscular fat influences the force-producing and force-transmitting properties of muscle.[12] As such, given the commonly reported strength deficits following ACLR, we hypothesized that poorer quadriceps muscle properties (i.e., atrophy, increased intramuscular fat) would be linked with worse strength in ACLR limbs. In partial contradiction to our hypotheses, we observed that intramuscular fat was not elevated in ACLR limbs and was also not associated with quadriceps strength. However, muscle volume was associated with quadriceps strength in both ACLR and control limbs. This is particularly interesting as there was not any statistical differences in quadriceps strength between limbs, despite that global vasti volume and each individual vasti muscle volume in the ACLR limb was 8%–11% smaller than the contralateral limb. To this point, it is well known that following ACLR many patients experience persistent strength deficits, which often coexists with muscle atrophy.[53] However, it has been shown that muscle size does not fully explain variance in muscle strength. For example, Thomas et al.[53] found that quadriceps cross-sectional area only explained 30% of isometric quadriceps strength deficits in the acute stages (7 months) following ACLR, while Arangio et al.[54] found no correlation between thigh circumference and strength in the ACLR-involved limb 4 years following ACLR. Collectively, and over a range of different measurements of muscle size (e.g., cross sectional area, thigh circumference, three-dimensional muscle volume), the results of Arangio et al.[54] and Thomas et al.,[53] and the findings of the present study indicate that muscle size explains only some of the variance in quadriceps strength and that muscle atrophy can manifest even in the absence of significant strength deficits.

The importance of muscle atrophy coexisting with an absence of statistical strength requires further investigation. It is important to recognize that muscle function has been largely categorized as whole measures of muscle strength (e.g., isometric and isokinetic strength). However, these whole muscle properties do not encompass the intrinsic properties of muscle that give rise to its contractile ability, including its composition (e.g., fat, and fibrotic tissue, fiber types, inflammation), neural activation, or muscle architecture that largely govern force production. While fatty tissue development did not affect our patient cohort, it remains possible that individuals in the current study may have experienced adaptations in other key components of muscle such as a shift to fast type II fibers, changes in muscle architecture, or neural adaptations that enabled effective force production.[17] It should be noted that we previously reported abnormalities in fascicle mechanics during self-selected walking pace in this cohort.[31] Due to the lack of significant findings between-limbs in quadriceps strength, it prompted us to further investigate muscle size and composition in the current study. Together with the addition of muscle volume and intramuscular fat, the data further emphasizes that muscle dysfunction (atrophy, contractile dysfunction) can persist despite the absence of strength differences between contralateral and control limbs. Exploring the relationships between quadriceps muscle size and contractile function and other functional activities beyond isolated strength assessments in standardized lab environments such as walking, running, or more dynamic tasks may help uncover deficits not captured during isometric strength testing and aid in our understanding of the impact of muscle atrophy on additional performance-based outcomes.

In other neuromuscular diseases, fat has been characterized by fatty streaks or localized deposits that can vary throughout muscle regions.[41] As such, we anticipated that there would be an increase in intramuscular fat that would differ regionally and in its distribution in patients with a history of ACLR. However, we demonstrate through comprehensive measurement techniques (e.g., full thigh segmentation) that there were no global or regional differences, or variability in the distribution of intramuscular fat relative to the contralateral or control limbs on average 3 years following ACLR. Grozier et al.[23] recently reported similar results 1–5 years following ACLR where no differences in intramuscular fat were identified in the rectus femoris. While there are multiple methodological differences between the current study and those of Grozier et al.,[23] (e.g., MR sequence, muscle of interest), collectively, these findings suggest that there might not be excessive fat infiltration after ACLR. To study this phenomenon more rigorously, longitudinal study designs across more diverse subjects (e.g., range of activity levels, BMI's, quadriceps function) are needed to determine if intramuscular fat interferes with muscle recovery earlier or even more chronically (>3–5 years) after ACLR.

There are multiple limitations to consider while interpreting the findings of this study. First, this was a cross-sectional study design that did not capture longitudinal muscle outcomes. As muscle composition is modifiable, understanding the time-course of changes rather than just at a single time point may help better understand the link between muscle strength, volume, and intramuscular fat post-ACLR. It is plausible the interrelationship between these factors differ across the post-operative rehabilitation time course wherein stronger associations are observed earlier in recovery as opposed to later time points, as in the current study. It is also important to recognize that we only explored intramuscular fat and did not explore intermuscular fat, that can

encompass both intramuscular fat within a tissue and irregular fat deposits between muscle groups.[55] Similarly to intramuscular fat, intermuscular fat has been associated with a host of impairments including impaired mobility and muscle dysfunction.[55] Either type of fatty deposit (e.g., intramuscular and intermuscular) can alter mechanical properties of muscle tissue (e.g., stiffness) that disrupts how muscles generate force during movement.[12] Given that intermuscular fat has not been fully characterized or explored following ACLR, future research would benefit from understanding if intermuscular fat is elevated following ACLR. Another compositional component that may interfere with strength recovery in a similar manner to fat is the development of fibrotic tissue. Fibrotic tissue has been less studied following ACLR but there is early evidence to support expansions of the ECM through increases in collagen.[16] As such, this is a compositional component of muscle that was not directly captured by our Dixon MRI sequence but has the potential to influence muscle function. Understanding if there is a replacement of healthy contractile tissue with non-contractile elements like fibrotic tissue development is an important future focus for our field in characterizing muscle tissue quality following ACLR. Interestingly, our ACLR and control limbs had the same quantity of intramuscular fat. However, it remains unknown if intramuscular fat changes over a range of athletic populations. Here, all participants had similar activity levels at the time of testing that could be described as between recreational and competitive sports engagement (Table 1). This may help to explain why we did not see variability in the quantity of intramuscular fat in the ACLR and control limbs as it has been shown that consistent physical activity, endurance, and resistance training can all decrease quadriceps intramuscular fat.[55] However, since the ACLR vasti muscles had atrophy, the percent of fat may change as a function of size. Further research is needed to understand the role of intramuscular and intermuscular fat in muscle function and to understand how much intramuscular and intermuscular fat is normal based on activity level. Given that intramuscular fat has a positive association with age, we limited participants to young adult aged 22 ± 3. As such, it remains plausible that older participants with ACLR may be more susceptible to accumulate intramuscular fat. Understanding the relationship between ACLR, intramuscular fat, and strength in older populations warrants further investigation. Lastly, this was the first study to comprehensively assess intramuscular fat via a Dixon MRI sequence following ACLR. As such, there were not adequate data available to perform a priori power analysis and therefore, we could be underpowered to detect differences between limbs.

In summary, people who achieve adequate quadriceps strength do not differ in intramuscular fat 3 years following ACLR, but do exhibit muscle atrophy of the vasti muscles. Further work should focus on longitudinal investigations of muscle quality (intramuscular fat and fibrotic tissue development) and in more diverse populations (e.g., less active, higher BMI, older) to improve our understanding of sources contributing to muscle weakness following ACLR.

AUTHOR CONTRIBUTIONS

Substantial contributions to research design: McKenzie S. White and Lindsey K. Lepley. *The acquisition*: McKenzie S. White and Thomas L. Chenevert. *Analysis*: McKenzie S. White, Augustin C. Ogier, Thomas L. Chenevert, Elizabeth Zucker, Luke Stoneback, and Constance P. Michel. *Interpretation of data*: McKenzie S. White, Thomas L. Chenevert, Riann M. Palmieri-Smith, and Lindsey K. Lepley. *Drafting the paper or revising it critically*: McKenzie S. White, Riann M. Palmieri-Smith, and Lindsey K. Lepley. *Approval of the submitted and final versions*: McKenzie S. White, Augustin C. Ogier, Thomas L. Chenevert, Elizabeth Zucker, Luke Stoneback, Constance P. Michel, Riann M. Palmieri-Smith, and Lindsey K. Lepley.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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