

# Ultrasound Echo Intensity of the Rectus Femoris Correlates Muscle Strength in the Post-Operative Rehabilitation of Anterior Cruciate Ligament Reconstruction

Original Research

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## Abstract

**Introduction:** To assess the correlation between corrected rectus femoris echo intensity (C-RF-EI), rectus femoris cross-sectional area (RF-CSA), and quadriceps strength during a 6-month period after anterior cruciate ligament reconstruction (ACL-R).

**Methods:** A retrospective subanalysis of data collected in a randomized controlled clinical trial (IRB 19-008473, NCT ID NCT04302558). Twenty-eight patients aged 13 to 50 years undergoing ACL-R were assessed for C-RF-EI, RF-CSA, and quadriceps strength.

**Results:** The correlation (R) between C-RF-EI and RF-CSA after ACL-R ranged from  $-0.25$  (24 weeks after ACL-R) to  $-0.34$  (4 weeks after ACL-R), which is indicative of a negative linear association. The overall repeated measures correlation was  $-0.31$  ( $P < .001$ ). The correlation between C-RF-EI and isometric knee extensor strength at each time point ranged from  $-0.11$  (4 and 8 weeks after ACL-R) to  $-0.41$  (2 weeks before ACL-R), which suggests a weakly negative linear association. The overall repeated measures correlation was  $-0.21$  ( $P = .01$ ). C-RF-EI, RF-CSA, and knee extensor strength significantly differed with time after ACL-R (all  $P < .001$ ). In comparison with 24 to 28 hours before ACL-R, postoperative C-RF-EI increased, and RF-CSA decreased.

**Conclusions:** These findings suggest that C-RF-EI is a feasible method for evaluating RF-CSA after ACL-R but not an effective method for evaluating progression of quadriceps strength.

**Key Words:** quadriceps muscle; sports medicine; ultrasonography

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## Introduction

Ultrasonography is an increasingly popular imaging method in health care because it is noninvasive, cost-effective, and portable.<sup>1</sup> Ultrasonography allows for real-time dynamic imaging of various structures of the musculoskeletal system, such as bones, muscles, tendons, ligaments, and nerves.<sup>2</sup> Because of these benefits, ultrasonography has been increasingly used in rehabilitation, rheumatology, sports medicine, and orthopedics for diagnosis and monitoring of musculoskeletal conditions.<sup>3</sup>

During assessments of muscle function and quality, ultrasonography is particularly useful because it allows for direct visualization of the muscle and surrounding structures.<sup>4</sup> Clinical research studies frequently use ultrasonography to quantify muscle size and cross-sectional area (CSA), which is correlated to muscle strength.<sup>5,6</sup> Previous studies have also shown that ultrasonography is an accurate method for estimating muscle mass.<sup>7</sup> Moreover, ultrasonography of quadriceps muscle thickness has good intrarater and interrater reliability, is a promising tool to evaluate muscle wasting,<sup>8</sup> and is highly associated with MRI findings.<sup>9</sup> The rectus femoris (RF) muscle—one of the 4 quadriceps muscles located in the anterior compartment of the thigh—is one of the most commonly imaged muscles in studies.<sup>5,6</sup> The primary functions of the RF are knee extension and hip flexion, and the RF is commonly affected in various musculoskeletal disorders, such as anterior cruciate ligament (ACL) injuries and knee osteoarthritis. However, evidence of a correlation between the CSA of the RF (RF-CSA) and strength is limited, and this association has not been thoroughly investigated for patients undergoing ACL reconstruction (ACL-R).<sup>6,10</sup>

One emerging parameter that can be measured with musculoskeletal ultrasonography is echo intensity (EI). EI refers to the degree of brightness of the muscle on ultrasonography images, which is measured via a histogram and is related to the muscle architecture, composition, and contractile properties.<sup>11</sup> The histogram can be read instantly by an ultrasound machine, which permits assessment of muscular adipose tissue, phase angle, fascicle pennation angle, fascicle length, and muscle fiber type.<sup>12</sup> An increase in EI may be indicative of an increase in muscle adipose tissue or intramuscular fibrosis. Therefore, changes in EI may indicate changes in muscle composition and may then be associated with muscle force production abilities.<sup>12</sup> Previous studies have reported that the EI of the RF is associated with muscle strength and function for patients without musculoskeletal impairment<sup>13,14</sup>; however, the association between EI of the RF and knee extensor strength has not been investigated for patients undergoing ACL-R.

To compensate for variations in ultrasound equipment and mixed results previously reported by others, Young et al developed an equation to account for the potential influence of subcutaneous fat (Equation 1).<sup>15</sup> The resulting corrected EI (C-EI) values were strongly associated with those obtained with MRI.<sup>15</sup> The primary aim of this study was to determine the correlation between C-EI of the RF and isometric knee extensor strength, as well as to further investigate the association between muscle quality and CSA. This study hypothesized that after a 6-month postoperative ACL-R rehabilitation period, corrected rectus femoris echo intensity (C-RF-EI) will be correlated with RF-CSA and/or isometric knee extensor strength.

## Scientific Methods

### *Participants*

This study was approved by the Mayo Clinic institutional review board (IRB #19-008473, IRB# 23-003312). All study participants, or their legal guardian, provided informed written consent before study enrollment. This study performed a retrospective subanalysis of data collected in a recently completed randomized controlled clinical trial titled "Low-Intensity Blood Flow Restriction Training to Improve Post-Operative Outcomes for ACL Reconstruction" (IRB 19-008473, NCT ID NCT04302558). Briefly, patients aged 13 to 50 years who had an ACL tear requiring reconstruction were enrolled in the trial from December 18, 2020, through May 4, 2022. Patients with a personal or family history of deep-vein thrombosis, a history of multiligamentous injury to the knee, or previous ACL-R were excluded from the study. All patients were treated at a hospital-based orthopedic and sports medicine clinic in the US Southwest. This study conforms to all STROBE guidelines and reports the required information accordingly (see Supplementary Checklist).

### *Protocol*

The ultrasonography images used for this study were obtained as part of the protocol of the randomized controlled trial. The images were deidentified and stored in a secured electronic folder that was accessible only to study investigators. These images were acquired longitudinally at 7 different time points before and after each participant underwent ACL-R. These timepoints were selected due to surgeon follow-up timeframes with subjects as part of their standard of care.

RF imaging was performed with a Fujifilm Sonosite (Bothell, WA, US) ultrasound machine with a curvilinear probe with a frequency between 2-5 megahertz (MHz) and a depth up to 30 cm optimized for musculoskeletal imaging. To collect these images, the subject was placed supine for at least 5 minutes which has been demonstrated to stabilize EI measurements.<sup>16</sup> An adequate amount of ultrasonic gel was placed on the participant's thigh, and the ultrasound transducer was then moved around the area to obtain images of the musculature and soft tissue. The physical therapist performing the imaging was trained and deemed competent for this technique by a radiologist to ensure that proper

measurements were collected. The anatomic landmarks used during ultrasonography of the quadriceps are depicted in Figure 1. The image used for the RF was taken at 50% of the length of the thigh, as measured from the anterior superior iliac spine (ASIS) to the top of the patella. A representative image of the RF is shown in Figure 2.



**Image Reliability:** All image reviews were completed by a blinded physiatrist with subspecialty certification in musculoskeletal ultrasonography. Previous research from our group demonstrated good to excellent interrater reliability for C-RF-EI measurements (intraclass correlation coefficient [ICC] = 0.89, 95% CI: 0.82-0.94) and RF-CSA measurements (ICC = 0.92, 95% CI: 0.87-0.96) between physicians and physical therapists.<sup>16</sup> The polygon function in ImageJ software (v1.53e, National Institutes of Health) was used to outline the border of the RF in each image individually. Care was taken to ensure that fascia along the borders of the muscle were not included in the analysis. The measure function was then used to determine the CSA (in cm<sup>2</sup>) of all muscles individually, and the histogram function was used to assess the raw EI values of the RF. The histogram function calculates and displays a

histogram of the distribution of gray values in the image, with the x-axis displaying possible gray values and the y-axis representing the number of pixels found for each gray value. EI was only used for the rectus femoris because it was the only muscle completely within the frame. The reviewer measured subcutaneous fat with the straight-line function as the distance between the skin-muscle interface and the superior border of the RF's aponeurosis at 3 locations (left, center, and right) in centimeters (cm). The mean of the 3 subcutaneous thickness values from each image was calculated and recorded. The values from each image were input into an encrypted REDCap database hosted by an institutional network to ensure security of the measurements. The C-RF-EI was calculated with these values and Equation 1 (below).

$$\text{Corrected EI} = \text{Raw EI} + (\text{mean subcutaneous fat in cm} \times 40.5278) \text{ (Equation 1)}^{15}$$

Isometric muscular strength of the quadriceps was evaluated by using a handheld dynamometer (HHD) (Lafayette Instrument Company, Lafayette, IN, US). Participants were seated at the short edge of an examination table with their knees in 90° of flexion. A strap was secured over the proximal quadriceps and around the examination table to prevent excess elevation of the hips and movement of the lower extremities. The measuring physical therapist held the HHD against the shin of the affected leg, and the participants were instructed to extend their leg out as hard as possible until the HHD beeped twice, which indicated a complete measurement of strength threshold. This test was repeated 3 times, and the mean value (in Newtons, N) of the replicates was recorded. Muscle strength testing was conducted at 5 of the 7 instances of imaging to protect the integrity of the reconstructed ACL. Only the instances with both strength testing and imaging were used in this analysis.

### Statistical Analysis

Continuous variables were summarized as mean (standard deviation [SD]), and categorical variables were summarized as frequency (%). Associations between variables were estimated with the Pearson correlation coefficient (R) and repeated measures correlation coefficient (R) by using the R package rmcrr (R Foundation for Statistical Computing). A linear mixed-effects model of the primary outcomes was fit, with sex, time, and baseline measurement as fixed effects, and summarized these data as  $\beta$  and 95% CI. A random intercept for each participant to account for individual differences among participants was included. P values <.05 were considered statistically significant. Echo intensity values are reported as grayscale values (GV) on a 0-255 scale. R statistical software, v4.1.2, was used for analyses.

## Results

### Demographics and Clinical Characteristics

Table 1 summarizes the baseline demographics and clinical characteristics of study participants. A total of 28 participants were included in the current subanalysis. The mean (SD) age of the participants was 23.9 (9.4) years, and 46% of participants were male.

**Table 1.** Baseline Demographics and Clinical Characteristics (N=28)<sup>a</sup>

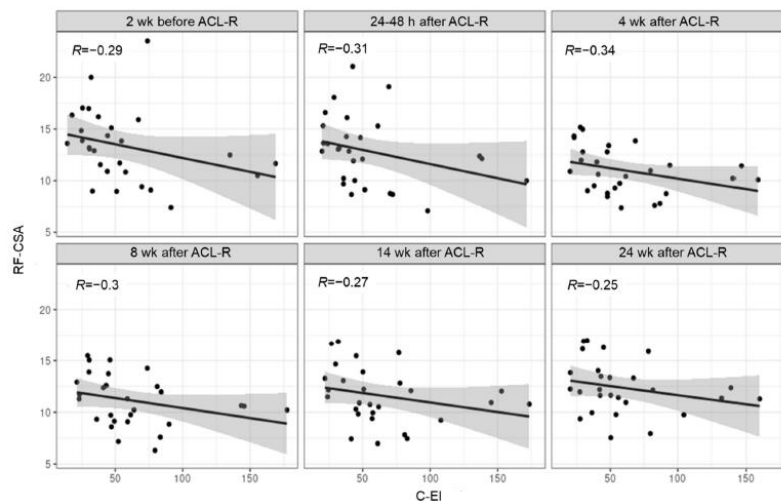
| Characteristic                      | Value       |
|-------------------------------------|-------------|
| Age, y                              | 23.9 (9.4)  |
| Sex                                 |             |
| Female                              | 15 (54)     |
| Male                                | 13 (46)     |
| Time from injury to ACL-R, d        | 40.3 (17.9) |
| Affected leg                        |             |
| Left                                | 12 (43)     |
| Right                               | 16 (57)     |
| Activity level                      |             |
| Collegiate                          |             |
| NCAA                                | 1 (4)       |
| Junior college                      | 5 (18)      |
| High School                         | 10 (36)     |
| Recreational                        | 9 (32)      |
| Professional                        | 1 (4)       |
| None                                | 2 (7)       |
| Sport type                          |             |
| Contact                             | 18 (64)     |
| Noncontact                          | 8 (29)      |
| None                                | 2 (7)       |
| Graft type                          |             |
| Allograft                           | 1 (4)       |
| Bone-patellar tendon-bone autograft | 10 (36)     |
| Hamstring tendon autograft          | 17 (61)     |

Abbreviations: ACL-R, anterior cruciate ligament reconstruction; NCAA, National Collegiate Athletic Association.

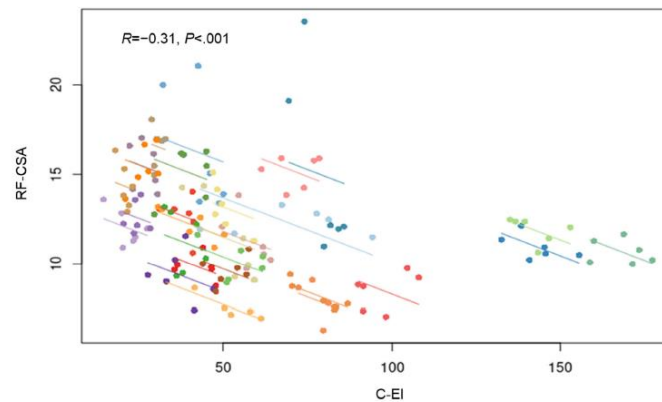
<sup>a</sup> Categorical data are summarized as No. (%) of participants, and continuous data are summarized as mean (SD).

#### Correlation Between C-RF-EI and RF-CSA

The correlation (R) between C-RF-EI and RF-CSA ranged from  $-0.34$  at 4 weeks after ACL-R to  $-0.25$  at 24 weeks after ACL-R (Figure 3), which suggests that C-RF-EI and RF-CSA have a negative linear association. The overall repeated measures correlation (R) was  $-0.31$  ( $P < .001$ ) (Figure 4).



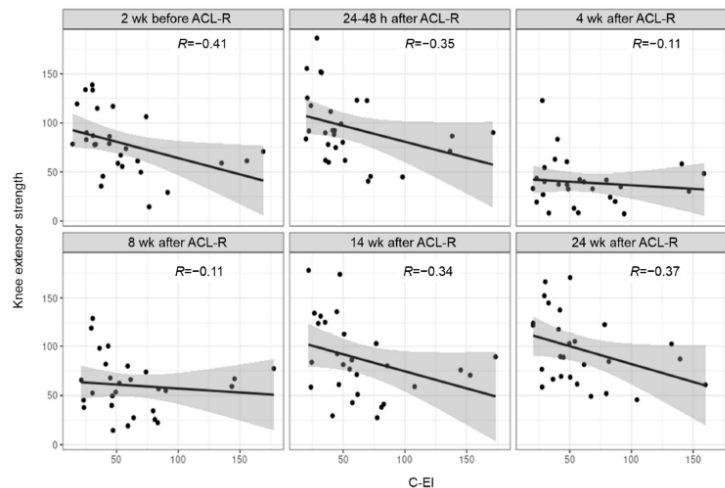
**Figure 3.** Correlation between corrected rectus femoris echo intensity (C-RF-EI, in GV) and rectus femoris cross-sectional area (RF-CSA, in  $\text{cm}^2$ ) at individual time points after ACL reconstruction. Each panel displays Pearson correlation coefficients (R) demonstrating negative linear associations at 4, 8, 14, and 24 weeks post-operatively, with strongest correlation ( $R = -0.34$ ) observed at 4 weeks post-ACL-R.



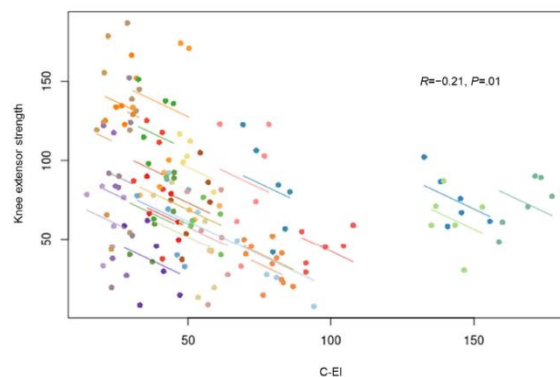
**Figure 4.** Repeated measures correlation between C-RF-EI (GV) and RF-CSA (cm<sup>2</sup>) across all time points ( $R=-0.31$ ,  $P<.001$ ). Each colored line represents an individual participant's trajectory over time, demonstrating consistent negative associations between echo intensity and cross-sectional area throughout the 24-week rehabilitation period.

*Correlation Between C-RF-EI and Isometric Knee Extensor Strength*

The correlation ( $R$ ) between C-RF-EI and isometric knee extensor strength ranged from  $-0.41$  at 2 weeks before ACL-R to  $-0.11$  at 4 and 8 weeks after ACL-R (Figure 5), which suggests that these 2 variables have a weak negative linear association. The overall repeated measures correlation ( $R$ ) was  $-0.21$  ( $P=.01$ ) (Figure 6).



**Figure 5.** Correlation between C-RF-EI (GV) and isometric knee extensor strength (N) at individual time points. Pearson correlation coefficients range from  $R=-0.41$  (2 weeks pre-ACL-R) to  $R=-0.11$  (4 and 8 weeks post-ACL-R), indicating weak to moderate negative associations that vary throughout the rehabilitation timeline.



**Figure 6.** Repeated measures correlation between C-RF-EI (GV) and knee extensor strength (N) across all measurement time points ( $R=-0.21$ ,  $P=.01$ ). Individual participant trajectories (colored lines) demonstrate the overall weak negative association between muscle echo intensity and strength during ACL-R rehabilitation.

*Linear Mixed-Effects Models*

Table 2 summarizes the fitted linear mixed-effects models for C-RF-EI, RF-CSA, and knee extensor strength. Male sex was a significant predictor of larger RF-CSA ( $P=.001$ ) but was not a significant predictor of C-RF-EI ( $P=.80$ ) or knee extensor strength ( $P=.60$ ). Associations for time before/after ACL-R significantly differed for RF-CSA, C-RF-EI, and knee extensor strength (all  $P<.001$ ). C-RF-EI increased from 24 to 48 hours before ACL-R for all time points after ACL-R. In contrast, RF-CSA decreased with time after ACL-R. Knee extensor strength decreased at 4 weeks and 8 weeks after ACL-R but did not significantly differ from 24 to 48 hours before ACL-R for later time points.

**Table 2.** Linear Mixed-Effects Model Results for C-EI, RF-CSA, and Knee Extensor Strength

| CHARACTERISTIC                                    | C-EI (GV)            |          | RF-CSA (GV)            |          | KNEE EXTENSOR STRENGTH    |          |
|---|----------------------|----------|------------------------|----------|---------------------------|----------|
|   | $\beta$ (95% CI)     | <i>P</i> | $\beta$ (95% CI)       | <i>P</i> | $\beta$ (95% CI)          | <i>P</i> |
| <b>SEX<br/>MALE VS FEMALE</b>                     | 0.8<br>(-5.9 to 7.5) | .80      | 1.8<br>(0.7 to 2.8)    | .001     | 4.5<br>(-11.0 to 19.0)    | .60      |
| <b>ACL-R TIME POINT</b>                           |                      | <.001    |                        | <.001    |                           | <.001    |
| <b>24-48 H BEFORE</b>                             | Reference            |          | Reference              |          | Reference                 |          |
| <b>4 WK AFTER</b>                                 | 6.4<br>(3.4 to 9.4)  |          | -1.9<br>(-2.4 to -1.3) |          | -56.0<br>(-66.0 to -47.0) |          |
| <b>8 WK AFTER</b>                                 | 8.6<br>(5.6 to 12.0) |          | -1.7<br>(-2.3 to -1.1) |          | -36.0<br>(-45.0 to -26.0) |          |
| <b>14 WK AFTER</b>                                | 9.6<br>(6.7 to 13.0) |          | -1.2<br>(-1.8 to -0.7) |          | -8.7<br>(-18.0 to 0.8)    |          |
| <b>24 WK AFTER</b>                                | 5.6<br>(2.5 to 8.6)  |          | -0.6<br>(-1.2 to 0.0)  |          | -0.4<br>(-10.0 to 9.2)    |          |
| <b>C-EI (2 WK BEFORE ACL-R)</b>                   | 0.9<br>(0.9 to 1.0)  | <.001    |                        |          |                           |          |
| <b>RF-CSA (2 WK BEFORE ACL-R)</b>                 |                      |          | 0.4<br>(0.2 to 0.6)    | <.001    |                           |          |
| <b>KNEE EXTENSOR STRENGTH (2 WK BEFORE ACL-R)</b> |                      |          |                        |          | 0.7<br>(0.5 to 0.9)       | <.001    |

Abbreviations: ACL-R, anterior cruciate ligament reconstruction; C-EI, corrected echo intensity; RF-CSA, rectus femoris–cross-sectional area.

## Discussion

The purpose of this study was to evaluate ultrasonography measurements before and after ACL-R to determine associations between C-RF-EI and RF-CSA or knee extensor strength. This study hypothesized that C-RF-EI would be correlated with RF-CSA and knee extensor strength during a 6-month rehabilitation period after ACL-R. C-RF-EI and RF-CSA were negatively correlated at each time point after ACL-R, and C-RF-EI had a weak negative correlation with knee extensor strength throughout the rehabilitative process. These findings suggest that C-RF-EI is a feasible indicator of RF-CSA after ACL-R but is not effective for evaluating progression of quadriceps strength. This study also observed that postoperative C-RF-EI, RF-CSA, and knee extensor strength significantly differed from measurements obtained before ACL-R.

A primary goal of ACL-R rehabilitation is to restore normal quadriceps muscle function and hypertrophy. Marked deconditioning of the quadriceps muscle is well documented after ACL-R because of immobilization and unloading of the involved lower extremity.<sup>17</sup> Restoration of normal quadriceps neural activation and muscle fiber size, in addition to attenuation of external loads on the knee, through exercise and neuromuscular electric stimulation is an extensive focus of ACL-R rehabilitation.<sup>18</sup> However, the long-term consequences of quadriceps atrophy may be detrimental to the patient because quadriceps atrophy is associated with poor performance during functional tests for return to a sport, as well as with patient-reported outcomes.<sup>19,20</sup>

The weak correlation ( $R=-0.21$ ) between C-RF-EI and quadriceps strength observed in this study has important clinical implications for ACL-R rehabilitation. While statistically significant, this weak association suggests that echo intensity and muscle strength represent distinct and somewhat independent aspects of muscle function during post-operative recovery.

Evidence suggests that muscle atrophy only partially contributes to quadriceps weakness after ACL-R and that decreased muscle activation and central activation ratios may affect progression years after the procedure.<sup>21,22</sup> Garcia et al longitudinally evaluated EI of the RF after ACL-R (9 weeks after ACL-R and at return to activity).<sup>23</sup> The authors reported that lower EI was associated with greater self-reported function, which was measured by International Knee Documentation Committee (IKDC) scores. That finding is consistent with previous reports that decreased RF-CSA after ACL-R is associated with decreased knee function, lower physical activity levels, and poorer patient-reported outcomes.<sup>23,24</sup> Our study deepens these findings by creating another link between RF-CSA and EI in a population who have undergone ACL-R.

From a clinical perspective, this finding indicates that increases in echo intensity, reflecting greater intramuscular fat infiltration and fibrosis, do not necessarily correspond proportionally to decreases in strength capacity.<sup>25</sup> Several mechanisms may explain this dissociation. First, neuromuscular activation deficits, which are well-documented after ACL-R, may play a more dominant role in strength loss than changes in muscle composition alone.<sup>21,22</sup> Even as muscle quality deteriorates (higher echo intensity), patients may partially compensate through improved neural drive or motor unit recruitment strategies during rehabilitation, thereby maintaining relatively better strength than echo intensity alone would predict.

Second, the temporal pattern of recovery may differ between muscle composition and functional capacity. Our data suggest that compositional changes (reflected in echo intensity) may persist or even worsen in the early post-operative period while strength begins recovering through compensatory mechanisms. This temporal mismatch would naturally result in a weaker correlation between these measures, particularly when assessed across multiple time points.

Third, the weak correlation highlights that muscle strength is a multifactorial outcome dependent not only on muscle quality but also on factors such as central nervous system activation, tendon mechanical properties, joint biomechanics, pain inhibition, and psychological factors such as kinesiophobia. Echo intensity captures only one component, muscle tissue composition, and therefore cannot fully account for the complex physiological determinants of force production.

For clinicians, these findings suggest that C-RF-EI should not be used as a standalone marker for strength recovery. Instead, echo intensity may be most valuable when interpreted alongside direct strength assessments, providing complementary information about the structural and compositional changes occurring within the muscle. An athlete might demonstrate improving strength despite persistent elevations in echo intensity, suggesting successful compensatory adaptations but incomplete muscle quality restoration. Conversely, patients showing improved echo

intensity but minimal strength gains might benefit from interventions specifically targeting neuromuscular activation rather than focusing solely on muscle hypertrophy or composition.

Additionally, the weak correlation between echo intensity and strength emphasizes the importance of multi-modal assessment in ACL-R rehabilitation. Relying exclusively on imaging biomarkers like echo intensity could lead to incomplete clinical decision-making. Comprehensive evaluation should integrate strength testing, functional performance measures, patient-reported outcomes, and imaging parameters to capture the full spectrum of recovery.

Future research should investigate whether specific rehabilitation interventions differentially affect muscle composition versus strength, potentially allowing for more targeted therapeutic approaches. Understanding when and why echo intensity and strength diverge during recovery could identify critical windows for intervention and help optimize rehabilitation protocols for individual patients.

### *Limitations*

All ultrasonography images were obtained by a physical therapist in a clinical setting, which may limit the generalizability of the findings. Indeed, physical therapists have reportedly excellent interrater reliability for ultrasonography image acquisition of the RF but poor reliability when evaluating its CSA.<sup>26</sup> However, this was addressed, in advance but after image acquisition, because a physiatrist who is board-certified in musculoskeletal ultrasonography evaluated the images and verified the accuracy of the CSA and C-RF-EI measurements. In addition, research has shown good reliability in measuring C-RF-EI between physicians and physical therapists.<sup>16</sup>

Another limitation of this study was that the physical therapist who performed the ultrasonography was trained in location and image capture but not in expansion of the field of vision of the ultrasound machine. Thus, some of the muscles may have been outside of the viewing area. However, the RF muscle belly appeared to be collected completely, making this a minimal limitation to the study. The presence of the femur in the view could have significantly altered the measurement of EI. Bone has higher acoustic impedance, which can lead to distortion of ultrasound image and affect EI readings.<sup>25</sup> In addition, panoramic ultrasound was not used in this study. Panoramic ultrasonography has been deemed reliable and valid for assessment of quadriceps CSA but requires a trained operator, which was not available for this study.<sup>27</sup> Additionally, evaluated only C-RF-EI and did not include the vastus lateralis, vastus medialis, and vastus medialis obliquus. The rectus femoris generally shows a strong correlation with torque development at mid-range, but vastus lateralis also plays a significant role.<sup>28</sup> The vastus medialis and vastus intermedius also contribute to knee extension, however to a lesser degree.<sup>28,29</sup>

Lastly, due to the longitudinal aspect of this study, there were no efforts made to control for hydration status. This may have led to differences in ultrasound readings as there is evidence that hydration status can significantly alter both muscle quantity, cross-sectional area and muscle thickness, as well as quality of echo intensity.<sup>11</sup> Future research should attempt to control for hydration status, femur presence, and ensure ultrasound settings are consistent to determine the effects of such variables and their effect over time.

### **Conclusions**

The C-RF-EI and RF-CSA were negatively correlated, and C-RF-EI and knee extensor strength had a weak negative association. Therefore, while C-RF-EI may serve as a feasible adjunct to RF-CSA measurements during ACL-R rehabilitation, its weak correlation with quadriceps strength suggests it should not be used as a standalone measure for evaluating strength progression. The primary value of C-RF-EI may lie in providing complementary information about muscle quality when used in conjunction with CSA measurements, rather than as a replacement for established assessment methods. Clinically, the dissociation between echo intensity and strength underscores the multifactorial nature of muscle function recovery and the importance of comprehensive, multi-modal assessment in guiding rehabilitation decisions.

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