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Alterations in whole muscle quality and physiological cross-sectional area measured with quantitative MRI following ACL injury

Meredith K. Owen[1], Lauren Richardson[2], Darren L. Johnson[3], Moriel H. Vandsburger[4], Thorsten Feiweier[5], Katherine L. Thompson[6], Christopher S. Fry[7], Peter A. Hardy[8,9] and Brian Noehren[1*]

Abstract

Background Emerging evidence suggests that there are morphological and physiological changes to the vastus lateralis after an anterior cruciate ligament (ACL) tear. However, it is unclear whether these alterations are limited to just the vastus lateralis or are more representative of widespread changes across the thigh musculature and/or if these changes precede reconstruction. The purpose of this study was to determine T1ρ relaxation time, a measure of extracellular matrix organization in muscle, and physiological cross-sectional area (PCSA) for muscles of the quadriceps and hamstrings of the ACL-deficient and contralateral limbs soon after ACL injury.

Methods T1ρ and diffusion tensor magnetic resonance imaging were performed on both limbs of 10 participants after primary ACL tear (< 10 weeks). T1ρ relaxation time and PCSA were calculated for all muscles of the quadriceps and hamstrings. Shapiro-Wilks tests were performed to assess normality. Outcomes were compared between limbs for each muscle of interest with paired t-tests or Wilcoxon signed-rank tests with the alpha level set to 0.05.

Results T1ρ relaxation times were significantly longer for the vastus lateralis (7.0%), rectus femoris (15.4%), and vastus intermedius (9.4%) of ACL-deficient limb; whereas, relaxation times were similar between limbs for all hamstring muscles. PCSA was smaller for the vastus lateralis (-19.6%), vastus intermedius (-20.9%), vastus medialis (-26.0%), and semitendinosus (-15.0%) of the ACL-deficient limb compared to the contralateral limb.

Conclusions These results provide evidence that morphological and physiological alterations occur within multiple muscles of quadriceps but not the hamstrings prior to ACL reconstruction. Establishing these differences between the quadriceps and hamstrings suggests there is a differential response within the thigh musculature to an ACL injury, providing a framework for more targeted interventions.

Keywords Quadriceps, Hamstrings, T1ρ imaging, Diffusion tensor imaging, Anterior cruciate ligament

*Correspondence:
Brian Noehren
b.noehren@uky.edu

Full list of author information is available at the end of the article

Background

Muscle strength loss following an anterior cruciate ligament (ACL) injury is ubiquitous and prolonged. Understanding the factors that result in protracted muscle weakness is critical in order to improve patient outcomes, facilitate return to sport, and mitigate the risk of post-traumatic osteoarthritis [1–4]. Based on evidence from a recent, comprehensive meta-analysis, knee extensor strength deficits are still decidedly prevalent (~10%) at 1 year post ACL reconstruction and then do not improve meaningfully long term; whereas, knee flexor strength deficits at 1 year are smaller (5–7%) and do normalize by 5 years post ACL reconstruction [5]. The difference in strength deficits suggest the quadriceps might be differentially affected following ACL injury and reconstruction. Emerging research suggests underlying changes to muscle morphology and physiology may be contributing to protracted quadriceps strength loss after ACL injury [6, 7].

Morphologically, decreases in muscle size, and physiologically, skeletal muscle fibrosis, impact overall strength following ACL injury [6, 8, 9]. To date though, these insights have been limited to the vastus lateralis muscle of the quadriceps muscle group, and only after reconstruction surgery. These limitations make it impossible to determine if observed changes are occurring in just the vastus lateralis after surgery or if these alterations are more representative of a larger phenomenon occurring in all muscles of the thigh prior to reconstruction. Therefore, a more detailed assessment of muscle quality for both the quadriceps and hamstrings muscles after injury but before reconstruction surgery is warranted.

Quantitative magnetic resonance imaging (MRI) techniques, such as T1ρ mapping and diffusion tensor imaging (DTI), provide the unique opportunity to noninvasively (1) evaluate skeletal muscle extracellular matrix (ECM) organization and (2) characterize muscle atrophy via physiological cross-sectional area across all muscles of the thigh. Recently, a significant association between T1ρ relaxation times and collagen abundance and organization within the extracellular matrix following an ACL injury was validated within the vastus lateralis muscle [10], lending utility to employ this technique across all muscles of the thigh. In other diseases tissues, such as cartilage and liver, longer T1ρ relaxation times are suggested to be a result of increased extracellular water [11, 12], indicative of alterations in ECM abundance and organization. After ACL injury, the ECM becomes dysregulated, which results in changes in the accessible free water [13] and thus longer T1ρ relaxation times [10]. Additionally, diffusion tensor imaging can be leveraged to characterize important parameters of muscle force production through the ability to derive fiber tract length and pennation angle to calculate physiological cross-sectional area (PCSA) [14]. PCSA, which is the cross-sectional area of the muscle perpendicular to the orientation of the muscle fibers, is often considered a more representative metric for the force generating capacity of a muscle than anatomical cross-sectional area [15], as it takes into account the angle at which the fibers are acting.

Investigating the underlying morphologic and physiologic differences between the quadriceps and hamstrings after ACL injury can provide a framework for more precise treatments targeting muscle dysfunction. Quantitative MRI provides the opportunity to evaluate these differences in all muscles of the quadriceps and hamstrings. Thus, the purpose of this study was to characterize T1ρ relaxation time and PCSA for all muscles of the thigh of the ACL-deficient and contralateral limbs of individuals with recent ACL tear. We hypothesized that the quadriceps muscles of the ACL-deficient limb would have longer T1ρ relaxation times and smaller PCSA compared to the contralateral limb, whereas there would be no between limb differences for the hamstring muscles.

Methods

Participants

This study is a retrospective cohort study, level of evidence 3, including an analysis of a subset of participants enrolled in a larger clinical trial (NCT03364647) [16] and was conducted in accordance with the ethical standards laid forth in the 1964 *Declaration of Helsinki*. This study was approved by the University of Kentucky Institutional Board Review, and all participants completed informed consent or parental consent and participant assent prior to completing any study related activities. Inclusion criteria consisted of a primary ACL tear within 10 weeks of enrollment, age between 14 and 40 years, and a minimum activity level of 5/10 on the Tegner Activity Scale [16]. Exclusion criteria included previous ACL injury or reconstruction or a complete knee dislocation at the time of initial knee injury. ACL tears were diagnosed by a single orthopaedic surgeon, based on clinical evaluation and clinical MRI, prior to study enrollment. All participants were scheduled to receive a surgical repair of their torn ACL after completing study activities.

Imaging

Magnetic resonance imaging of both limbs was performed on a 3T MAGNETOM Prisma scanner (Siemens Healthineers AG, Erlangen, Germany). All MR imaging was performed prior to randomization for the clinical trial, and prior to surgical reconstruction. Participants had not completed any pre-operative rehabilitation or strengthening programs prior to MR imaging. Participants were positioned supine and feet-first. The ACL-deficient limb was centered on the table and a flexible, 18-element, phased array body coil was secured around

the thigh. Following standard localizer scanning, participants underwent T1ρ imaging, diffusion tensor imaging (DTI), and turbo spin echo imaging for muscle volume calculations. This same process was then repeated for the contralateral limb.

T1ρ imaging

Recent work validated T1ρ imaging to assess extracellular matrix dysregulation in skeletal muscle [10, 17], with longer T1ρ relaxation times associated with greater degrees of ECM dysregulation. T1ρ imaging was completed using a research sequence as previously reported [10, 18]. Briefly, images were obtained using a spin lock amplitude of 300 Hz and ten spin lock hold times (0/10/20/30/40/50/60/70/80/90 ms) using a four shot, segmented, gradient echo acquisition (repetition time of 5.8 ms, echo time of 2.5 ms, receiver bandwidth of 560 Hz/pixel, 2 excitations) and a shot repetition time of 1000 ms. The field of view was set to 192×192 mm^2, with a matrix size of 96×96 yielding a spatial resolution of 2×2 mm^2. A single axial slice of 10 mm thickness was obtained at the center of the vastus lateralis, determined by measuring the distance from the greater trochanter to lateral femoral condyle from the scout scan. Spin lock preparation included a B1 and B0 compensated spin lock preparation pulse followed by a chemical shift selective saturation pulse. Centric phase encoding was used to provide exclusive T1ρ weighting. For analysis, each pixel of the image was fit to a mono exponential decay curve of signal vs. spin-lock time using custom code (MATLAB, Natick, MA), which has been previously described [10]. T1ρ values were calculated pixel by pixel and averaged over each muscle of interest, including the vastus group (vastus lateralis, vastus medialis, vastus intermedius), rectus femoris, semitendinosus, semimembranosus, and biceps femoris long head. Muscle boundaries were segmented manually using region-of-interest tools within MATLAB by trained raters.

Imaging for PCSA calculation

PCSA was calculated using the equation $PCSA = (Vol/FL)*cos(\theta_{PA})$ from Kan et al. [19], where Vol is the total muscle volume, FL is the fiber length calculated from tractography, and θ_{PA} is the pennation angle calculated from tractography. Diffusion tensor imaging for tractography was conducted following previously published methods [20]. Summarized, a research sequence combining a stimulated echo diffusion preparation with an echoplanar readout module (STEAM) was used to acquire 21 axial slices (slice thickness = 6 mm) with no interslice gap. DTI sequence parameters included 24 diffusion-encoding directions at a high diffusion weighting (b-) value of 500 s/mm^2, repetition time (TR)/echo time (TE) of 7600/40.0 ms, three excitations, a mixing time of 173.0 milliseconds, gradient separation and duration of 185.0 ms and 5.4 ms, respectively, and. A nominal diffusion weighting b-value of 45 s/mm^2 was used. A GRAPPA factor of two was used to reduce image filtering from signal decay and also to reduce image distortion from susceptibility-induced inhomogeneities of the magnetic field. Signal to noise ratio reduction due to the applied parallel imaging was partially compensated by the accompanying shorter echo time. The field of view (FOV) was set to 192×192 mm^2 with an acquisition matrix of 96×96, producing image voxels with a dimension of $2 \times 2 \times 6$ mm^3. Fat suppression was achieved by applying an adiabatic SPAIR suppression pulse prior to beginning the DTI acquisition and alternating the polarity of the slice select gradients during excitation and store/restore RF-pulses so that fat excited in the first pulse was not fully refocused by subsequent pulses in the STEAM acquisition. Eddy-current induced distortions in the diffusion weighted images were corrected by registering all diffusion weighted images to the b = 45 s/mm^2 images during data post-processing. Diffusion tensor and fractional anisotropy calculations and fiber tracking were performed using custom code (MATLAB, Natick, MA). Fiber length and pennation angle were determined from the initial fiber tracking [20, 21]. Fiber tracking was performed within each muscle of interest, excluding the rectus femoris due to its bipennate characteristics, by defining muscle boundaries using region-of-interest tools. All other quadricep muscles were assessed. A representative visual of the muscle fiber tracking for the biceps femoris long head is shown in an additional file (see Additional file 1).

To determine muscle volume, two consecutive stacks of 33 slices (thickness = 6 mm) with a single overlapping slice were captured using T1-weighted turbo spin echo (TSE) imaging. Sequence parameters included TR = 4400 ms, TE = 10 ms, and bandwith = 195 Hz/pixel. The FOV was 192×192 mm^2 with a matrix size of 256×256 resulting in voxel dimensions of $0.75 \times 0.75 \times 6$ mm^3. The image stacks were concatenated and muscle cross-sectional area for each muscle of interest was defined for each slice for the total length of the muscle (MIPAV, NIH, Bethesda, MD). Total muscle volume was determined as the sum of each slice volume (cross-sectional area x slice thickness).

Statistical analysis

Data sets included in the analysis were selected after screening MR images to ensure all muscles of interest were within the field of view and had clearly discernable borders. Included data sets were selected prior to image processing and analysis. Primary outcomes for the study included T1ρ relaxation time and PCSA for each muscle. Values for component parts of PCSA (muscle volume, pennation angle, and fiber length) are included in the

Table 1 Mean T1ρ relaxation time (in milliseconds) for each isolated muscle

Muscle Group	ACL-D	Contralateral	*p*	Effect Size [CI][a]	% Diff
VL	30.0 (3.2)	27.9 (1.3)	**0.02**	0.90 [0.14, 1.62]	7.0 (7.7)
RF	30.8 (2.9)	26.4 (2.8)	**0.01**	0.99 [0.21, 1.74]	15.4 (15.2)
VI	29.9 (2.9)	27.1 (1.7)	**< 0.01**[#]	-0.81[#]	9.4 (6.9)
VM	29.7 (4.4)	26.9 (2.8)	0.06	0.66 [-0.04, 1.34]	9.4 (15.3)
BF	29.1 (1.7)	29.0 (1.5)	0.81	0.08 [-0.55, 0.70]	0.5 (7.0)
ST	28.0 (2.3)	28.6 (1.9)	0.61	-0.17 [-0.79, 0.46]	-2.2 (12.9)
SM	29.3 (3.3)	29.1 (1.9)	0.64[#]	0.16[#]	0.4 (13.3)

VL = vastus lateralis; RF = rectus femoris; VI = vastus intermedius; VM = vastus medialis; BF = bicep femoris; ST = semitendinosus; SM = semimembranosus; CI = confidence interval

Reported as Mean (Standard Deviation)

Bolded p-values denote statistical significance

[a]Cohen's d; [#]nonparametric analysis (effect size as r)

Table 2 PCSA (in cm^2) for each isolated muscle. Data for rectus femoris is excluded as fiber tracking was not performed for this muscle

Muscle Group	ACL-D	Contralateral	*p*	Effect Size [CI][a]	% Diff
VL	47.8 (10.1)	57.9 (11.3)	**< 0.01**	-1.7 [-2.67 -0.69]	-19.6 (11.6)
RF	-	-	-	-	-
VI	43.0 (9.8)	52.9 (11.3)	**< 0.01**	-1.17 [-1.96 -0.33]	-20.9 (14.2)
VM	57.8 (16.5)	74.4 (16.5)	**< 0.01**	-1.7 [-2.67 -0.69]	-26.0 (16.3)
BF	29.1 (7.4)	26.1 (5.2)	0.11	0.57 [-0.16 1.23]	9.7 (18.7)
ST	29.6 (10.8)	34.8 (12.6)	**0.03**	-0.81 [-1.52 -0.07]	-15.0 (17.8)
SM	39.4 (9.5)	40.0 (7.0)	0.74	-0.11 [-0.73 0.52]	-2.7 (17.1)

VL = vastus lateralis; RF = rectus femoris; VI = vastus intermedius; VM = vastus medialis; BF = bicep femoris; ST = semitendinosus; SM = semimembranosus; CI = confidence interval

Reported as Mean (Standard Deviation)

Bolded p-values denote statistical significance

[a]Cohen's d

supplemental data for reference. Normality of between limb difference was assessed using Shapiro-Wilk tests. T1ρ relaxation time and PCSA were compared between limbs for each muscle of interest were analyzed separately using paired t-tests if normally distributed or Wilcoxon signed rank tests if not. As an exploratory analysis, we compared potential relationships between T1ρ relaxation time and PCSA using linear regressions and report the hypothesis test for Pearson's correlation. Significance was determined for p-values less than 0.05.

Results

10 participants (9 female) had MR images meeting the selection criteria, screened from a cohort of 35 participants. Included participants were 20.9 ± 4.8 years of age, 1.66 ± 0.06 m in height, 65.3 ± 10.2 kg in mass, and were imaged 17.8 ± 10.5 days from the initial injury. All variables were normally distributed except for between limb difference in T1ρ relaxation time for the vastus intermedius and semimembranosus. Effect sizes are listed (Cohen's d if normally distributed or r if not) and percent difference is reported as ACL-deficient limb in reference to the contralateral limb (i.e. a positive percent difference indicates a higher value for the ACL-deficient limb).

T1ρ relaxation time was significantly longer for the vastus, rectus femoris, and vastus intermedius of the ACL-deficient limb (Table 1). There were no significant differences for the vastus medialis or for any muscles of the hamstrings.

Table 2 lists the means, standard deviations, p-values, effect sizes, and percent differences for PCSA of each muscle. PCSA was significantly smaller for the vastus lateralis, vastus intermedius, vastus medialis, and semitendinosus of the ACL-deficient limb. There were no significant differences for the biceps femoris or the semimembranosus. Values for component parts used in PCSA calculation (muscle volume, fiber length, and pennation angle) and graphs displaying all raw data points are included as additional files (see Additional file 2 and Additional file 3).

We also sought to explore potential relationships between PCSA and T1rho relaxation time and observed a negative relationship in the VM ($R^2 = 0.54$ and $p = 0.02$) and VI ($R^2 = 0.49$; $p = 0.02$) but not the VL ($R^2 = 0.28$; $p = 0.12$).

Discussion

Using T1ρ and diffusion tensor imaging, we show that the quadricep muscles experience greater morphologic and physiologic changes following an anterior cruciate ligament tear than the muscles of the hamstrings. Comparing the ACL-injured limb to the contralateral limb within participants soon after initial injury, we found elevated T1ρ relaxation times and decreased PCSA in all muscles of the quadriceps. Similar differences were not present for the muscles of the hamstrings indicating the muscles of the quadriceps respond uniquely to the initial ACL-injury.

Three out of the four quadricep muscles had statistically significantly longer T1ρ relaxation times in the ACL-deficient limb, whereas T1ρ relaxation time was similar between limbs for all hamstring muscles. While there is there is limited prior work in healthy thigh muscles, the relaxation times for the contralateral limbs in this study were within the range of those of the calf muscles of young, healthy individuals determined using a mono-exponential fitting model [22]. Longer relaxation times in the quadriceps of the ACL-injured limb indicate greater amount of collagen and unfolding in these muscles [10], and because the ECM plays a critical role in muscle maintenance and force transmission [23], the quadricep muscles may not be able to recover from muscle strength loss as readily as the hamstring muscles. Previously, we have shown, through histology, immunohistochemistry and imaging, that extracellular matrix dysregulation occurs in the vastus lateralis after ACL injury [6, 10, 20]. The results of this work expand upon these previous studies and provide evidence of greater increase in T1ρ relaxation time for the remaining muscles of the quadriceps.

We extend prior work to show that not only is PSCA of the vastus lateralis smaller but that PCSA for the other heads of the quadriceps is also smaller [20]. While all quadriceps evaluated had significantly smaller PCSA for the ACL-deficient limb, the same was true for only one of the three hamstring muscles assessed. Further, the biceps femoris actually had larger PCSA of the ACL-deficient limb; perhaps because the biceps femoris is a biarticular muscle that may be activated more as a compensatory mechanism after ACL injury. Values for PCSA determined in this study were larger than previously reported for human thigh muscles [24]; however, this previous work was done on cadavers with an average age above 80 years. Consequently, we expect larger values for this study cohort as it is composed of young, active individuals. Assessment of PCSA may be more valuable than standard cross-sectional area or volume measures as it is a stronger predictor of absolute muscle strength [15].

The lack of change in T1ρ and the overall smaller between limb differences in PCSA for the hamstrings could be a sign of a localized response to ACL injury. Hamstrings coactivation is an important limiter of anterior translation of the tibia in the absence of the ACL, which typically provides critical sagittal plane stabilization [25–28]. Perhaps, underlying pathways, such as upregulated myostatin, which has been previously related to increased fibroblast activity, decreased muscle strength, and muscle atrophy [6, 7, 9], lead to targeted strength loss within the quadriceps to further limit excessive anterior tibial translation. Further, while the PCSA of the semitendinosus of the ACL-deficient limb was significantly smaller, it was no different for the semimembranosus and larger for the biceps femoris, and none of these muscles showed the same degree of fibrotic changes, as measured with T1ρ relaxation time, as the quadriceps. We also observed negative relationships between PCSA and T1rho relaxation time in the VM and VI that offer intriguing evidence in support of a dysregulated ECM that contributes to changes in force production and muscle atrophy. While we did not observe a similar relationship in the VL, we have shown across various injury and chronic illness populations a negative relationship between greater muscle fibrosis (collagen content) and quadriceps strength [29–31]. There is growing recognition of the central role the ECM plays in modulating muscle function and adaptation making the development of clinical biomarkers all the more urgent. While only representing 5–10% of total muscle mass, the ECM plays a central role in muscle function, controlling growth, repair, as well as force transmission [23, 32, 33].

Because we performed this investigation soon after injury and prior to any reconstruction surgery, the between limb difference in both T1ρ relaxation time and PCSA are occurring in the quadriceps before any surgical intervention and are likely related to the initial injury. When considering the rehabilitation implications of these pre-surgical difference, perhaps a pre-surgical focus on quadriceps strengthening may be beneficial to ameliorate the difference found. Additionally, the changes in skeletal muscle ECM indicated by T1ρ relaxation require further investigation of potential strategies to intervene before surgery. Other factors may also contribute to isolated changes and strength loss for the quadriceps commonly seen after ACL injury. Arthrogenic muscle inhibition (AMI) and other neurological changes are also hypothesized to be a reason atrophy and strength loss are limited to just the quadriceps. Pre-reconstruction, AMI is believed to be a protective mechanism to prevent quadriceps activity and increased tibial translation resulting from the reduced anterior stabilization [34]. Additionally, reductions in protein synthesis have recently shown to be another physiological mechanism behind muscle atrophy after ACL injury [35]. Further work is needed to understand the complex interaction of these various pathways.

Limitations

There are several limitations that must be considered with the results of this study. The data included in this study were from a subset of individuals that had images meeting all selection criteria (clearly discernable muscle borders, all muscles of interest were fully within the field-of-view), which resulted in a sample size of $n = 10$. Nonetheless, effect sizes were large (> 0.8) for all significant differences for both T1ρ relaxation time and PCSA. This image screening resulted in a female-biased cohort (90% female). Skeletal muscle response to injury and recovery have been hypothesized to be different between males and females [36], so having a female-biased cohort for this study could influence the overall results and interpretation. However, removal of the male subject did not alter any results or conclusions. Calculation of PCSA for the rectus femoris was not completed due to bipennate structure and current limitations with our DTI processing methods, which do not account for bipennate tracking. However, we were able to calculate muscle volume for the rectus femoris and found no differences between limbs (values provided in the Supplemental Digital Content). Perhaps the biarticular nature of the rectus femoris helps better protect from muscle atrophy that affects the other uniarticulate quadricep muscles. Owing to the cross-sectional nature of the study, we only have information regarding the instantaneous state of the muscle at the time of imaging. The contralateral limb was assumed to be representative of pre-injury status. While we are unable to know for sure if the contralateral limb is truly representative of pre-injury status, the contralateral limb provides the best biological control for muscle related measures. Pre-operative strength has been shown to be crucial for improved post-operative knee function, strength, and return-to-sport [37–40], indicating the importance of identifying muscular changes affecting strength early after injury, and thus, justifying the early pre-operative time point selected for this study. The participants in this study were between 5 and 35 days post-injury; however, all comparisons were intra-participant and thus the effect of time since injury is participant specific and systematic across all muscles. Additionally, while time since injury is a consideration for AMI, our data still shows greater between limb difference in both T1ρ and PCSA for the quadriceps and smaller between limb differences for the hamstrings. Future work investigating the effect of time since injury on the measures would be of interests. Additionally, long-term studies are needed to assess how the muscle changes throughout recovery.

Conclusions

Understanding the morphological and physiological differences between the quadriceps and hamstrings that are present following and ACL injury provides a framework to develop more targeted treatments and rehabilitation programming. Future work should seek to build upon these initial results to evaluate long term effects of ACL-injury as well as the effects of ACL-reconstruction and rehabilitation on T1ρ and PCSA of all muscles of the thigh.

Abbreviations

ACL	Anterior Cruciate Ligament
MRI	Magnetic Resonance Imaging
DTI	Diffusion Tensor Imaging
ECM	Extracellular Matrix
PCSA	Physiological Cross-Sectional Area
TR	Repetition Time
TE	Echo Time
FOV	Field-of-view
TSE	Turbo Spin Echo
Hz	Hertz
VL	Vastus Lateralis
RF	Rectus Femoris
VI	Vastus Intermedius
VM	Vastus Medialis
BF	Biceps Femoris
ST	Semitendinosus
SM	Semimembranosus
AMI	Arthrogenic Muscle Inhibition

Supplementary Information

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Additional file 1

Additional file 2

Additional file 3

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Author contributions

Experiments were performed in the laboratory of Brian Noehren. Brian Noehren and Meredith Owen acquired funding. Brian Noehren and Meredith Owen were involved with conception and design of the experiments. Meredith Owen, Lauren Richardson, Darren Johnson, Katherine Thompson, Christopher Fry, Peter Hardy, and Brian Noehren collected, analyzed, and interpreted the data. Moreiel Vandsburger and Thorsten Feiweier developed and maintained sequences and analysis methods required for MRI. Meredith Owen and Brian Noehren drafted the article and created figures. Meredith Owen, Lauren Richardson, Darren Johnson, Katherine Thompson, Moreiel Vandsburger, Thorsten Feiweier Christopher Fry, Peter Hardy, and Brian Noehren revised and critically edited the manuscript for important intellectual content. All authors approved the final submitted manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the University of Kentucky Institutional Board Review (Approval #: 42791) and all participants completed informed consent or parental consent and participant assent prior to completing any study related activities.

Consent for publication
Not applicable.

Competing interests
Darren Johnson receives hospitality payments from Smith & Nephew. All other authors declare that they have no competing interests.

Author details
[1]Department of Physical Therapy, University of Kentucky, 900 S Limestone, Lexington, KY 40536-0284, USA
[2]Department of Exercise Science, Brigham Young University, Provo, UT, USA
[3]Department of Orthopaedic Surgery and Sports Medicine, University of Kentucky, Lexington, USA
[4]Department of Bioengineering, University of California, Berkeley, Berkeley, CA, USA
[5]Siemens Healthineers AG, Erlangen, Germany
[6]Dr. Bing Zang Department of Statistics, University of Kentucky, Lexington, KY, USA
[7]Departement of Athletic Training & Clinical Nutrition, University of Kentucky, Lexington, KY, USA
[8]Department of Radiology, University of Kentucky, Lexington, KY, USA
[9]Magnetic Resonance Imaging and Spectroscopy Center (MRISC), University of Kentucky, Lexington, KY, USA

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