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Title: Adolescent exercise-related lower leg pain musculotendinous characteristics

Running Title: Adolescent ERLLP musculotendinous characteristics

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Introduction: Exercise-related lower leg pain (ERLLP) is one of the most common injuries among adolescent runners, however there is limited information available on lower extremity

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musculotendinous characteristics in relationship to injury. Ultrasound imaging has previously been used to evaluate musculotendinous structures among adults with chronic lower limb injuries. Similar measurement approaches may be adopted to assess young runners with ERLLP.

Objective: To compare ultrasound-derived lower extremity musculotendinous thickness, echogenicity, and muscle fiber pennation angles between adolescent runners with and without ERLLP.

Design: Cross-sectional design.

Setting: Hospital-affiliated sports injury prevention center.

Participants: Twenty-eight adolescent runners with (N=14) and without ERLLP (N=14).

Interventions: Runners' patellar and Achilles tendons, and tibialis anterior, medial gastrocnemius, abductor hallicus, and flexor digitorum brevis muscles were assessed with ultrasound imaging using standardized procedures.

Main Outcome Measures: Separate repeated measures multivariate analyses of covariance (covariate: sex) were used to compare groups and limbs for mass-normalized musculotendinous thickness, musculotendinous echogenicity, and extrinsic ankle muscle fiber pennation angles.

Results: The adolescent ERLLP group had reduced average muscle size for all structures except the tibialis anterior compared to the uninjured group (mean difference [MD] range: -0.12- -0.49 mm/kg; p-range: 0.002-0.05), and reduced average medial gastrocnemius pennation angles on their case limb compared to their contralateral limb and the uninjured group (MD range: -3.7 - -6.4°; $p<0.001$). The ERLLP group additionally had reduced average patellar and Achilles tendon size (MD range: -0.14 - -0.15 mm/kg; p-range:0.02-0.03), and lower Achilles tendon echogenicity compared to uninjured counterparts (MD: -18; $p=0.02$).

Conclusions: Adolescent runners with ERLLP exhibited morphological musculotendinous changes that may occur either as a result of or as a contributing factor to pain and persistent dysfunction. Our findings highlight key targets for rehabilitation for young, injured runners, particularly intrinsic foot muscle strengthening.

Keywords: musculoskeletal ultrasound; tibial stress fracture; shin splints; medial tibial stress syndrome; strength

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Introduction

Exercise-related lower limb injuries are among the most prevalent adolescent running-related injuries,[1–3] with 34-82% of young runners reporting a history of "shin splints" and tibial stress overuse injuries.[4,5] One of the primary risk factors for incurring lower extremity running-related injuries is injury history,[5] and, as such, between 49% and 75% of exercise-related lower limb injuries are repeat injuries.[6] Exercise-related lower leg pain (ERLLP) broadly encompasses a variety of repetitive stress injuries localized to the region spanning between the tibial plateau and the malleoli.[7,8] There are multiple factors that have been cited as potential contributors to ERLLP injury, such as reduced lower limb muscle strength, reduced posterior chain flexibility, and greater navicular drop.[5,7,9,10] However, there is currently no consensus on direct mechanism of injury or tissue-level risk factors for injury. This gap in the existing literature highlights the need for continued research into contributory factors associated with injury and recovery.

ERLLP symptomology typically includes perceived generalized pain, tenderness, and weakness across multiple static and dynamic structures throughout the lower extremity,[7,9] including but not limited to the tibialis anterior, gastrocnemius-soleus complex, and the flexor digitorum muscles.[11–13] One of the primary hypotheses regarding the development of ERLLP is that runners have lower extremity laxity and poor shock absorption from dynamic stabilizing structures due to reduced lower limb tissue strength, lending to excessive and inefficient lower extremity loading during activity.[14] In support of this theory, adult runners with ERLLP have been found to have reduced extrinsic ankle muscle size and strength compared to uninjured counterparts.[13,15] However, there is little available information about adolescent runners' muscle quality and morphology, especially in the context of muscle measures in relationship to pain and injury development. Given that these injuries frequently occur among adolescent runners and can

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lead to repeated pain and injuries throughout the lifespan,[5] understanding contributing factors in earlier stages of life may help to guide rehabilitative efforts and interrupt cyclical injury and pain patterns.

In addition to expanding assessments to adolescent runners, it is important to consider additional muscle groups throughout the lower extremity that may contribute to the development and persistence of symptoms associated with ERLLP. Specifically, intrinsic foot and ankle muscles serve to stabilize and attenuate forces throughout the foot during weight acceptance during gait, yet there is minimal research exploring the role of these muscles in ERLLP.[16,17] Intrinsic foot muscle volume has been shown to be reduced in other chronic lower limb pathologies, such as chronic ankle instability[18] and plantar fasciitis.[19] Exploring muscle volume and quality throughout the lower limb may help to better understand the extent of lower extremity muscle change in response to injury. Furthermore, expanded assessments to other muscle groups may provide potential directions for clinical rehabilitation, given that the most current consensus statements on treatments for ERLLP solely recommend posterior chain muscle group stretching and strengthening.[10,20] While addressing extrinsic ankle muscle flexibility and strength is important, clinical efforts to address these deficits alone may not target all injury-related lower limb muscle deficits, especially given the perpetually high rates of injury and injury recurrence.

Ultrasound imaging is a non-invasive, reliable and valid manner to assess musculotendinous tissue characteristics, including tissue thickness, echogenicity, and fiber alignment.[19,21–23] This clinical measurement approach has been primarily used to examine adults with lower extremity pathologies.[18,19] More recently, ultrasound imaging has been used to explore musculotendinous characteristics among uninjured adolescent runners, supporting the

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use of this assessment technique in younger athletic populations.[24] Therefore, the purpose of this study was to compare ultrasound-derived lower extremity musculotendinous thickness and echogenicity, and extrinsic ankle muscle pennation angles between adolescent runners with and without ERLLP. The specific lower extremity structures of interest were the patellar and Achilles tendons, along with the tibialis anterior, medial gastrocnemius, abductor hallicus, and flexor digitorum brevis muscles. We hypothesized that the adolescent runners with ERLLP would have decreased musculotendinous size, higher muscle echogenicity, lower tendon echogenicity, and reduced extrinsic muscle pennation angles across all structures compared to uninjured counterparts, indicative of reduced musculotendinous strength and tissue quality.

Methods

Participants

We conducted a cross-sectional study of adolescent runners (ages 11 to 17 years) with and without ERLLP. Adolescent runners in the ERLLP group were recruited from a hospital-affiliated Injured Runners' Clinic, and were included for study participation if they had pain during running in the antero-medial or -lateral aspect of the lower leg for at least the past four weeks, which was confirmed using a clinical history and physical assessment conducted by a sports medicine physician.[5,25] Conditions included under the ERLLP categorization included medial tibial stress syndrome, grade 1 stress reactions, and/or non-specific lower leg pain (i.e., "shin splints"). Patients were excluded if they had a medical diagnosis of compartment syndrome or grade 2 or higher stress fractures, primary complaint of pain over the Achilles tendon or popliteal fossa due to potential neurovascular involvement, or were currently unable to run due to functional limitations or antalgia secondary to leg pain.[7,8] Uninjured adolescent participants were recruited from a community afterschool running program, and were included if they were

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not currently experiencing any pain during running, and did not report any lower extremity injuries within 3 months of the study.[24,26] Participants in both groups were excluded if they had any other lower extremity injuries, and/or a history of lower extremity surgery. All participants' legal guardians provided informed consent, and adolescent participants provided informed assent. This study was approved by the Hospital's Institutional Review Board (IRB P00039834).

An *a priori* sample size estimation reflected the need for 14 participants per group to achieve a minimally detectable difference in ultrasound-derived musculotendinous size (0.59 mm) with 80% power and alpha set to 0.05.[27] Therefore, 28 adolescent runners were recruited for participation (14 exercise-related lower leg pain; 14 uninjured). In the event that participants with ERLLP had bilateral symptoms, the subjectively "worse" limb was labeled as the "case limb" with the other limb labeled as the "contralateral limb," and uninjured participants' limbs were matched accordingly. All participants completed questionnaires to obtain pertinent demographic, running, and injury information (Table 1).

Procedures

All participants first completed an intake questionnaire form to determine their height, mass, use of assistive devices, and pain intensity on the 100-mm Visual Analog Scale. Following questionnaires, standardized ultrasound imaging assessments were conducted for all participants by a single researcher with athletic training credentials and a PhD in sports medicine with over 6 years of musculoskeletal ultrasound imaging experience (A.F.D.L.). The assessor used a portable 10-MHz linear ultrasound imaging transducer plugged into a smartphone with associated secure software (Butterfly iQ+, Butterfly Network, Inc., Guilford, CT, USA) to obtain bilateral B-mode images of all musculotendinous structures. The software was standardized to the musculoskeletal

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soft tissue setting, with 1-6cm scanning depth, 7-10 MHz frequency, 40-50% gain, 0.01 thermal index for soft tissues, and a range of 0.07-0.3 for the mechanical index.

Participants remained seated for at least five minutes prior to all ultrasound assessments to ensure they were in a rested state. The researcher then used established ultrasound imaging protocol scanning techniques with standardized patient positioning that have demonstrated excellent validity and intra-rater reliability to ensure consistency across assessments.[19,21–23] Patients were first placed in a supine position on a treatment table to obtain bilateral short- and long-axis images of the patellar tendon, tibialis anterior, abductor hallicus, and flexor digitorum brevis at the mid-belly of each structure (Figure).[23,24,28,29] Patients then were positioned in prone on the treatment table with their ankle in a neutral position to obtain bilateral short- and long-axis images of the medial gastrocnemius and Achilles tendon structures (Figure, Supplementary Figure 1).[23,24,28,29]

Data Processing

All musculotendinous ultrasound images were processed using ImageJ software (v1.52k, National Institutes of Health, Bethesda, MA, USA). Image files were only labeled with participant study ID numbers, and as such the assessor measuring the images was blinded to participant group to remove bias. Musculotendinous thickness measures were obtained from long-axis ultrasound images by measuring a vertical line spanning between the superior and inferior borders of each structure. Thickness measures were normalized to participant mass (kg)[24,30,31] to ensure an equivocal comparison across groups. Echogenicity measures were obtained by a computerized histogram analysis (0-255 scale) for the cross-sectional area of each structure. Lower echogenicity values (darker hues) are favorable for muscles, as this indicates hypertrophic muscle fascicle characteristics and little fat or scar tissue content within the

muscle.[32] Higher echogenicity values (brighter hues) are favorable for tendons, as this indicates more organized collagen fiber orientation and increased tissue strength.[33] The final measures obtained from ultrasound images were pennation angles for the medial gastrocnemius and tibialis anterior muscles, and calculated as the arc between the line along the muscle fibers and deep aponeuroses.[34]

Statistical Analyses

Independent samples t-tests were used to compare continuous demographic outcomes between ERLLP and uninjured adolescent groups, and Chi-square tests were used to compare categorical variables. Preliminary Pearson's correlations were used to assess the presence of multicollinearity ($r \geq 0.60$) across all ultrasound-derived outcomes (musculotendinous thickness, echogenicity, and muscle pennation angles). Multicollinearity existed across several outcomes (muscle echogenicity r-range: 0.62-0.78; muscle thickness r-range: 0.37-0.64; tendon thickness r: 0.69; Supplementary Figure 2). Therefore, separate 2x2 multivariate repeated measures analyses of covariance (MANCOVA) were used to compare groups (exercise-related lower leg pain, uninjured) and limbs (case/matched case limb, contralateral/matched contralateral limb) for a) all mass-normalized muscle thickness measures, b) all muscle echogenicity measures, c) all muscle pennation angles, d) all mass-normalized tendon thickness measures, and e) all tendon echogenicity measures. Participant sex was the covariate included in each model. Tukey's post-hoc analyses were conducted for significant findings, with alpha set *a priori* to 0.05. Cohen's d effect sizes were used to assess the magnitude of differences across comparisons, and interpreted as ≤0.2 negligible, 0.21-0.49 small, 0.50-0.79 moderate, and ≥0.80 large.[35] All analyses were conducted in R (RStudio, v1.2.1335).

Results

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On average, adolescent athletes with ERLLP generally had been experiencing pain for over nine months (mean ± standard deviation [Mean SD]: 9±5), with moderate pain (Mean SD: 51mm ± 15mm) as determined from the 100-mm Visual Analog Scale.[36] There was no significant differences in athlete age, body mass, height, running experience, nor running training volume in the ERLLP group as compared to the uninjured adolescent group (Table 1); however, there were more female participants in the ERLLP group. Ultrasound-derived myotendinous measurements were normalized to body mass to account for the potential influence of anthropometric characteristics on primary outcomes. Four of the ERLLP patients used a walking boot in the past as a part of their treatment for the injury (ranging 2-6 weeks); however, the use of the walking boot was at least 30 weeks prior to study participation (range: 32 to 44 weeks; Table 1), with subsequent return to normal weightbearing and running, thus likely minimizing the effect of boot immobilization treatment on musculotendinous measures at the time of evaluation. However, between-limb comparisons were used to assess the potential atrophying effects of using these assistive devices.

Ultrasound-Derived Muscle Measures

There was a significant group main effect for ultrasound-derived muscle thickness measures (p=0.01); post-hoc analyses demonstrated that the ERLLP group had significantly reduced mass-normalized thickness measures for the medial gastrocnemius (mean thickness difference [MD]: -0.49mm/kg; p=0.01), abductor hallicus (MD: -0.21mm/kg; p=0.002), and flexor digitorum brevis muscles (MD: -0.12mm/kg; p=0.05) as compared to the uninjured group (Table 2). All significant between-group differences were associated with moderate to large effect sizes (d-range: 0.51-0.86; Table 2). There was no significant difference found in tibialis anterior thickness measurements between groups (MD: -0.15mm/kg, p=.37). There were no

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significant differences between groups (p=0.53), limbs (p=0.70), nor a group by limb interaction (p=0.83) for any muscle echogenicity measures (Table 2).

For the medial head of the gastrocnemius muscle, there was a significant group by limb interaction found for pennation angles (p<0.001). Post-hoc analyses reflected that the ERLLP case limb had significantly decreased muscle pennation angles compared to the uninjured group (MD: -6.4°; p<0.001) and compared to their contralateral limb (MD: -3.7°; p<0.001; Table 2), with large effect sizes (d-range: 1.28-2.03; Table 2). There was not a significant difference between groups or limbs for the tibialis anterior muscle pennation angles (Table 2).

Ultrasound-Derived Tendon Measures

There was a significant group main effect for ultrasound-derived tendon thickness measures (p=0.02); post-hoc analyses showed that the ERLLP group had significantly reduced mass-normalized Achilles tendon thickness (MD: -0.15mm/kg; p=0.03) and patellar tendon thickness measures compared to the uninjured group (MD: -0.14mm/kg; p=0.02; Table 3). These significant differences were accompanied by moderate effect sizes (d-range: 0.61-0.66). Similarly, there was a significant group main effect for tendon echogenicity measures (p=0.05). The ERLLP group had significantly reduced Achilles tendon echogenicity values compared to uninjured counterparts (MD: -18; p=0.02; Table 3), with a moderate effect size (d=0.70).

Discussion

Ultrasound assessments of musculotendinous structures among adolescent runners helped to identify morphological features across foot intrinsic muscles and ankle extrinsic myotendinous units that were associated with ERLLP, as well as more proximal patellar tendon changes. Our findings suggest that reduced plantarflexor musculotendinous tissue quality and thickness, as well as reduced intrinsic foot muscle size are associated with ERLLP among adolescents, and

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may contribute to chronic dysfunction and persistent symptomatology. Furthermore, our findings support that there are additional injury ramifications throughout the kinetic chain, which we postulate are associated with altered movement patterns due to lower extremity pain. This is one of the first studies to our knowledge that has reported lower extremity musculotendinous features among injured adolescent runners. These findings may serve to guide future clinical interventions to optimize approach to management, rehabilitation, and injury mitigation practices in the course of care for young runners with ERLLP.

During running gait, the central 'core' of the foot, comprised of both active and passive subsystems, is requisite to normal foot function.[37] The plantar foot intrinsic muscles serve a key role as local stabilizers in the foot core system, contributing to active motor control and attenuation of forces through the complex multiplanar motion of the midfoot during gait.[16,17] Through modulating and synergistic activity of the foot intrinsic muscles, the foot core provides stability at initial contact and propulsion, but during midstance becomes a dynamic adapter. The intrinsic foot muscles help to attenuate load, store elastic energy by way of the deformation of the arch, and release that energy during the propulsive phase of gait. Furthermore, intrinsic foot muscles are strongly recruited at higher running speeds, thereby providing force-dependent alterations in stiffness to facilitate effective ground reaction force transmission and offloading strain on the foot passive subsystem.[37–39] When the foot intrinsic muscles are not functioning optimally, the foot loses its capacity to absorb force and loses its dynamic stability, resulting in detrimental alterations in foot function, posture, and motion.[40,41] These changes may result in injurious tissue strain within the foot, and secondarily, problems in more proximal structures up the kinetic chain.

In a previous study among adult runners with ERLLP, injured runners had increased

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motion about the great toe and the ankle accompanied with decreased ankle strength compared to uninjured counterparts, supporting the theory that poor support from dynamic stabilizing muscles contributes to decreased force attenuation and subsequent pain patterns.[15] Our findings build upon this previous work as we identified reduced muscle size of both the abductor hallicus and flexor digitorum brevis intrinsic foot muscles among adolescents with ERLLP compared to uninjured runners. Cumulatively, these findings suggest a key link between reduced foot intrinsic muscle size, impaired lower extremity motor function, and chronic lower limb injuries.[15]

Decreased medial longitudinal arch integrity attributed to poor intrinsic foot muscle volume and strength has previously been associated with more rapid pronation of the foot during functional activities among runners with other chronic lower extremity injuries, such as patellofemoral pain and ankle sprains.[42,43] Previous researchers have proposed that impaired foot strength and inability to provide a stable base of support to adjust to the imposed demands during high-intensity sporting activities may alter the moments and forces acting on the joints, increasing the risk of overuse injuries.[44] Our findings align with this postulation given that we identified decreased foot muscle thickness among patients with ERLLP. Additionally, the relationship between foot morphology and the influence on more proximal joint loading were further substantiated with the concomitant finding of altered morphological characteristics at the patellar tendon.

The link between intrinsic foot function and lower extremity injury has been substantiated by prospective assessments that have identified a 2.42-fold running-related injury risk reduction when foot core strengthening was incorporated into injury prevention programs.[45] Moreover, several studies have identified that foot core strengthening programs for patient with chronic lower limb musculoskeletal injuries lead to increased intrinsic foot muscle volume and

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improved running propulsive forces compared to controls.[45,46] Although the present cross-sectional study demonstrates decreased foot plantar intrinsic muscle size among adolescent runners with ERLLP, further prospective studies are needed to understand biomechanical mechanisms of injury. Such assessments would help to determine the effectiveness of foot core training programs for adolescent recreational runners in increasing foot intrinsic muscle size and strength, and ultimately reducing running-related injury risk among adolescent athletes.

In addition to intrinsic foot muscle morphological changes, we identified that patients with ERLLP had impaired medial gastrocnemius and Achilles tendon size and tissue quality compared to controls. The reduction in tendon echogenicity with greater width can suggest tendinopathic disorganization due to chronic remodeling in response to repetitive strain.[21,47] We believe these musculotendinous changes may be attributed, in part, to altered biomechanical patterns during running contributing to patients' pain and dysfunction. The calf muscle complex contracts isometrically during early stance phases of running gait, while the elastic components of the musculotendinous unit elongate, decelerating the downward movement of the body's mass and storing elastic energy. This elastic energy is returned during mid-to-late stance when the gastrocnemius-soleus complex contract concentrically to control the foot and ankle complex, and facilitate vertical and forward propulsion.[48,49] In light of the important role of the triceps surae in force exchange and movement control during gait, any deficits in neuromuscular patterning in the calf complex attributed to poor neuromuscular control or force capacity may lead to undue tissue strain and microinjury.[50,51] Previous researchers have consistently identified that runners with ERLLP have increased rearfoot eversion throughout the stance phases of gait,[52,53] greater peak eversion,[54,55] and greater overall time in the gait cycle spent in rearfoot eversion compared to uninjured runners, thereby putting excessive strain on the posterior foot and ankle

musculature.[52] Taken together, these findings suggest that altered movement patterns may contribute to morphological foot and ankle muscle changes, although future research directly comparing these measures in adolescent populations are warranted.

Our study demonstrated no significant difference between tibialis anterior muscle thickness nor pennation angles for adolescents with ERLLP compared to the uninjured group. These findings were contrary to our study hypotheses, which were based on previous studies that have identified reduced muscle activation[56] and poor muscle strength among adults with exercise-related lower leg pain.[15] Our findings therefore may suggest that musculotendinous adaptations for anterior ankle musculature may be secondary or compensatory sequelae of the more primary pathologic mechanisms of injury involving the posterior chain and foot core function as described above.

Limitations

Given the cross-sectional nature of the study, it is not possible to determine if musculotendinous changes were present due to ERLLP or contributed to ERLLP injury development. Additionally, we were unable to assess the influence of the developmental stage of the athlete due to the study design. Future prospective studies are needed to ascertain these relationships. The study participants in the injured group were primarily female, which aligns with previously reported ERLLP epidemiology; however, we included sex as a covariate in our MANCOVA model to address this concern. Given the exploratory nature of the study, we did not use a multiple comparison correction and thus there is a risk of type 1 error in the findings. Future research in more robust participant sample sizes is warranted. We were unable to assess additional musculotendinous features such as strength or neuromuscular activation, however we acknowledge that such factors may play a key role in ERLLP injury sequalae. Several

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participants used a walking boot during their clinical recovery period which may have influenced musculotendinous adaptations; all participants discontinued use of assistive devices at least 6-months prior to participation, reducing this potential effect. Additionally, there were no significant between-limb thickness differences within the ERLLP group, signifying that atrophy may not have been present. Finally, we did not have access to physical therapy programs for the injured group, however we acknowledge that the inclusion of these programs may have influenced musculotendinous tissue measures.

Conclusion

Adolescent runners with ERLLP presented with ultrasound-derived morphological musculotendinous changes across intrinsic and extrinsic foot and ankle muscles, and the Achilles and patellar tendons. Such changes may be related to biomechanical alterations and microdamage either as a result of or as a contributing factor to pain and persistent dysfunction. Our findings highlight the opportunity to incorporate intrinsic foot muscle strengthening into rehabilitation plans for young runners with ERLLP.

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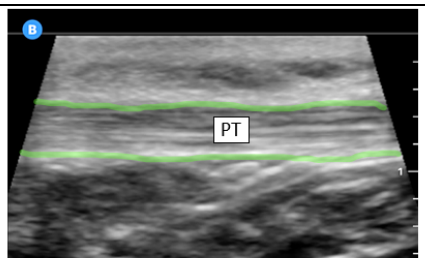
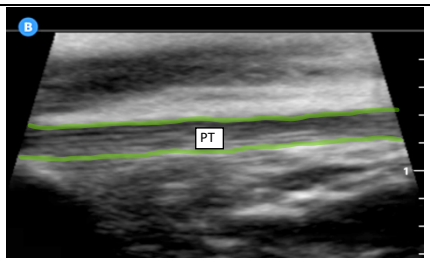
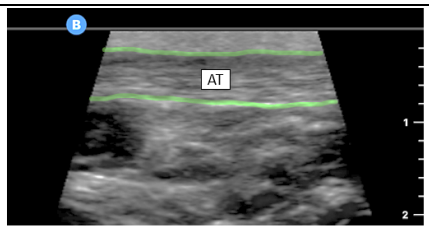
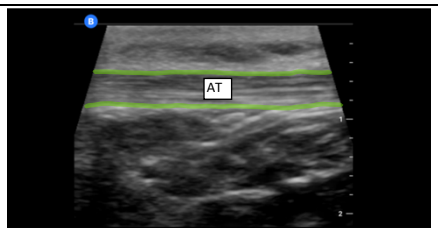
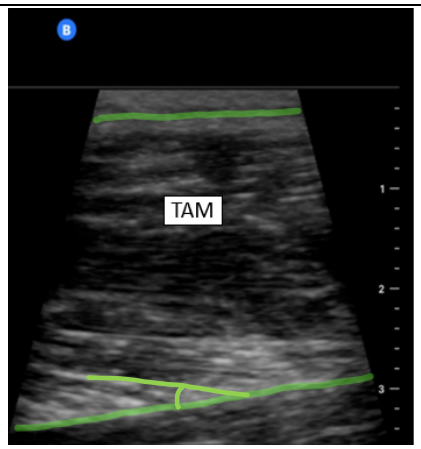
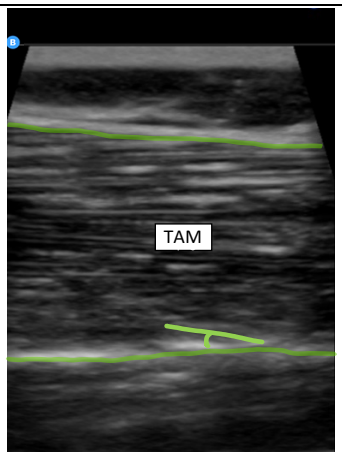
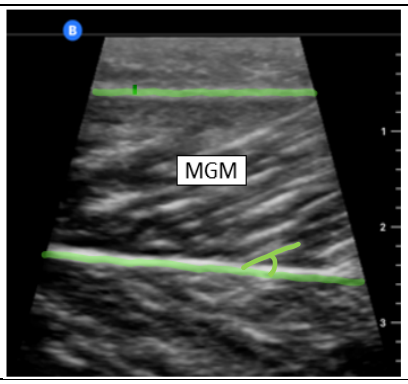
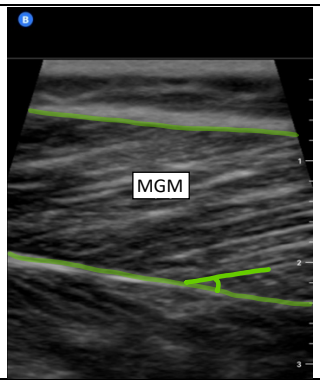
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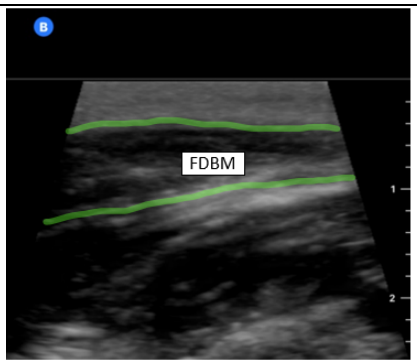
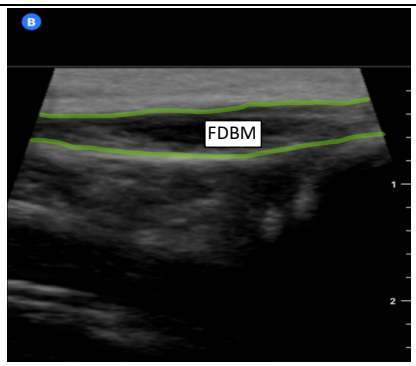
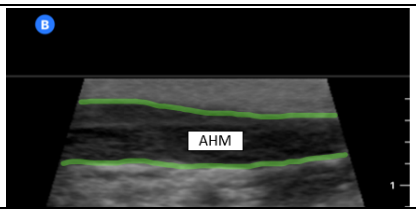
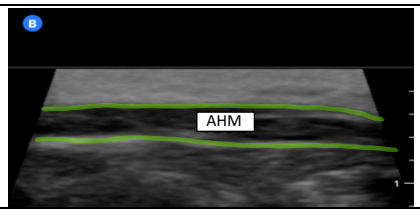
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Figure. Sample long-axis ultrasound images comparing healthy and ERLLP myotendinous structures.

	Healthy Participant Sample Images – Long-Axis View	ERLLP Participant Sample Images – Long-Axis View
Patellar Tendon		
Achilles Tendon		
Tibialis Anterior Muscle and Pennation Angle		
Medial Gastrocnemius Muscle and Pennation Angle		

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Flexor Digitorum Brevis Muscle		
Abductor Hallicus Muscle		

Caption: Each image depicts an example of a long-axis (or longitudinal) view of select myotendinous structures for healthy and ERLLP participants in the study. The lead end of the probe is demarcated as the "B" label in the upper left image corner. Outlines of key structures as well as pennation angles for extrinsic ankle muscles are included, and labels are placed in the center of the structure to orient to the ultrasound image. Note that the imaging depth is delineated in centimeters along the scale on the right side of the image. Each image for the healthy and ERLLP participant are depicted in the same scale for ease of comparability.

Abbreviations: ERLLP, exercise-related lower leg pain; Abbreviations: Patellar Tendon: PT; Achilles Tendon: AT; Medial Gastrocnemius Muscle: MGM; Tibialis Anterior Muscle: TAM; Abductor Hallicus Muscle: AHM; Flexor Digitorum Brevis Muscle: FDBM.

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Table 1. Participant demographics.

Outcome Measure	Variable	Exercise-Related Lower Leg Pain Group (N=14) (Mean ± SD)	Uninjured Group (N=14) (Mean ± SD)	p-value
Demographics	Sex (F/M)	11 / 3	6 / 8	0.01*
	Age (years)	15 ± 2	16 ± 1	0.11
	Mass (kg)	60.2 ± 8.3	59.3 ± 9.3	0.79
	Height (cm)	167.4 ± 6.8	170.2 ± 9.4	0.37
	BMI (kg/m^2)	21.6 ± 1.9	20.5 ± 2.7	0.22
Running Experience	Years of Running Experience	4.0 ± 2.8	3.3 ± 2.3	0.48
	Average Weekly Mileage (mi)	16.5 ± 6.8	15.9 ± 4.3	0.78
Injury Details	100-mm VAS Pain Scale (mm)	51 ± 15	-	-
	Duration of Pain (months)	9 ± 5	-	-
	Use of Walking Boot/Assistive Device? (Y/N)	4 Y 10 N	-	-
	Length of Time Since Use of Boot/Assistive Device (wks)	38 ± 5	-	-

Independent samples t-tests comparing group continuous demographic and running experience variables, and Chi-square tests comparing categorical variables. Mean and standard deviation values are presented for continuous outcomes, while counts are presented for categorical variables.

*signifies statistically significant difference between groups at $p \leq 0.05$.

Abbreviations: F, Female; M, Male; kg, Kilograms; cm, Centimeters; mi, Miles; MTSS, Medial Tibial Stress Syndrome; mm, Millimeters; Y, Yes; N, No; wks, Weeks.

Table 2. Between-group Comparisons for Ultrasound-derived Muscle Measures.

Outcome Measure	**Omnibus Repeated Measures MANCOVA Results**	**Muscle**	**Exercise-Related Lower Leg Pain Group (N=14)**			**Uninjured Group (N=14)**			**Post-hoc Between-Participant Group Comparison Results**
			Case Limb *(Mean ± SD)*	Contralateral Limb *(Mean ± SD)*	Limbs Combined *(Mean ± SD)*	Matched Case Limb *(Mean ± SD)*	Matched Contralateral Limb *(Mean ± SD)*	Limbs Combined *(Mean ± SD)*	
Mass-Normalized Thickness (mm/kg)	Participant Group Main Effect: p=0.01* Limb Main Effect: p=0.45 Group*Limb Effect: p=0.58	Medial Gastrocnemius	2.84 ± 0.55	2.84 ± 0.52	2.84 ± 0.53	3.50 ± 0.82	3.16 ± 0.77	3.33 ± 0.80	p=0.01* d=0.72
		Tibialis Anterior	3.48 ± 0.53	3.52 ± 0.53	3.50 ± 0.71	3.70 ± 0.76	3.59 ± 0.68	3.65 ± 0.71	p=0.37 d=0.21
		Abductor Hallicus	0.88 ± 0.24	0.89 ± 0.24	0.88 ± 0.24	1.11 ± 0.25	1.06 ± 0.25	1.09 ± 0.25	p=0.002* d=0.86
		Flexor Digitorum Brevis	1.07 ± 0.23	1.13 ± 0.24	1.10 ± 0.23	1.26 ± 0.24	1.18 ± 0.24	1.22 ± 0.24	p=0.05* d=0.64
Echogenicity (0-255)	Participant Group Main Effect: p=0.53 Limb Main Effect: p=0.70 Group*Limb Effect: p=0.83	Medial Gastrocnemius	53 ± 25	51 ± 22	60 ± 23	58 ± 17	62 ± 17	60 ± 16	p=0.99 d<0.2
		Tibialis Anterior	65 ± 40	64 ± 35	64 ± 36	63 ± 20	62 ± 20	62 ± 20	p=0.80 d=0.07
		Abductor Hallicus	51 ± 19	47 ± 17	55 ± 14	56 ± 14	54 ± 15	49 ± 18	p=0.16 d=0.37
		Flexor Digitorum Brevis	61 ± 26	59 ± 25	66 ± 15	67 ± 15	65 ± 16	60 ± 25	p=0.30 d=0.29
Pennation Angle (°)	Participant Group Main Effect: p=0.15 Limb Main Effect: p=0.01* Group*Limb Effect: p<0.001*	Medial Gastrocnemius	23.1 ± 2.2[a]	26.8 ± 3.5	24.9 ± 3.4	29.5 ± 3.9	26.5 ± 3.9	28.0 ± 4.2	p<0.001* d=0.81
		Tibialis Anterior	11.9 ± 2.9	14.4 ± 1.8	13.1 ± 2.7	13.9 ± 3.5	12.7 ± 2.9	13.3 ± 3.2	p=0.85 d=0.07

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Mean and standard deviation of ultrasound-derived mass-normalized muscle thickness and echogenicity on a 0-255 scale for the medial gastrocnemius, tibialis anterior, abductor hallicus, and flexor digitorum brevis muscles, and pennation angles for the medial gastrocnemius and tibialis anterior muscles. The second column displays the group, lim, and group*limb interaction main effects, while final column displays the results of the post-hoc between-participant group differences identified from the multivariate repeated measures analysis of covariance (covariate: sex), and the Cohen's d effect size for the magnitude of differences identified between groups.

*signifies statistically significant difference between groups at $p \leq 0.05$.

[a]signifies statistically significant difference between limbs at $p \leq 0.05$.

Abbreviations: MANCOVA, multivariate analysis of covariance; SD, Standard Deviation; mm, Millimeters; kg, Kilograms; d, Cohen's d Effect Size.

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Table 3. Between-group Comparisons for Ultrasound-derived Tendon Measures.

Outcome Measure	**Omnibus Repeated Measures MANCOVA Results**	**Tendon**	**Exercise-Related Lower Leg Pain Group (N=14)**			**Uninjured Group (N=14)**			**Post-hoc Between-Participant Group Comparison Results**
			Case Limb *(Mean ± SD)*	Contralateral Limb *(Mean ± SD)*	Limbs Combined *(Mean ± SD)*	Matched Case Limb *(Mean ± SD)*	Matched Contralateral Limb *(Mean ± SD)*	Limbs Combined *(Mean ± SD)*	
Mass-Normalized Thickness (mm/kg)	Participant Group Main Effect: p=0.02* Limb Main Effect: p=0.32 Group*Limb Effect: p=0.66	Achilles Tendon	0.99 ± 0.20	1.03 ± 0.24	1.01 ± 0.22	1.22 ± 0.27	1.10 ± 0.28	1.16 ± 0.27	p=0.03* d=0.61
		Patellar Tendon	0.66 ± 0.19	0.68 ± 0.21	0.67 ± 0.19	0.82 ± 0.24	0.79 ± 0.23	0.81 ± 0.23	p=0.02* d=0.66
Echogenicity (0-255)	Participant Group Main Effect: p=0.05* Limb Main Effect: p=0.90 Group*Limb Effect: p=0.95	Achilles Tendon	89 ± 31	90 ± 29	89 ± 27	109 ± 29	105 ± 19	107 ± 24	p=0.02* d=0.70
		Patellar Tendon	80 ± 24	81 ± 23	80 ± 23	89 ± 15	87 ± 18	88 ± 16	p=0.16 d=0.40

Mean and standard deviation of ultrasound-derived mass-normalized tendon thickness and echogenicity on a 0-255 scale for the Achilles and patellar tendons. The second column displays the group, lim, and group*limb interaction main effects, while final column displays the results of the post-hoc between-participant group differences identified from the multivariate repeated measures analysis of covariance (covariate: sex), and the Cohen's d effect size for the magnitude of differences identified between groups.
*signifies statistically significant difference between groups at $p \leq 0.05$.
Abbreviations: MANCOVA, multivariate analysis of covariance; SD, Standard Deviation; mm, Millimeters; kg, Kilograms; d, Cohen's d Effect Size.

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Title: Adolescent exercise-related lower leg pain musculotendinous characteristics

Running Title: Adolescent ERLLP musculotendinous characteristics

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