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Title: Adolescent marathon training: prospective evaluation of musculotendinous changes during a 6-month endurance running program

Running Head: Musculotendinous changes over adolescent marathon training

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Abstract

Objectives: Assess changes in lower extremity musculotendinous thickness, tissue echogenicity, and muscle pennation angles among adolescent runners enrolled in a 6-month distance running program.

Methods: We conducted prospective evaluations of adolescent runners' lower extremity musculotendinous changes at 3 timepoints (baseline, 3-months, 6-months) throughout a progressive marathon training program. Two experienced researchers used an established protocol to obtain short- and long-axis ultrasound images of the medial gastrocnemius, tibialis anterior, flexor digitorum brevis, abductor hallicus, and Achilles and patellar tendons. ImageJ software was used to calculate musculotendinous thickness and echogenicity for all structures, and fiber pennation angles for the ankle extrinsic muscles. Repeated measures within-subject analyses of variance were conducted to assess the effect of endurance training on ultrasound-derived measures.

Results: We assessed 11 runners (40.7% of eligible runners; 6F, 5M; age: 16±1 years; running experience: 3±2 years) who remained injury-free and completed all ultrasound evaluation timepoints. Medial gastrocnemius muscle ($F_{2,20}$=3.48, p=0.05), tibialis anterior muscle ($F_{2,20}$=7.36, p=0.004), and Achilles tendon ($F_{2,20}$=3.58, p=0.05) thickness significantly increased over time. Echogenicity measures significantly decreased in all muscles (p-range: <0.001-0.004), and increased for the patellar tendon (p<0.001) during training. Muscle fiber pennation angles significantly increased for ankle extrinsic muscles (p<0.001).

Conclusions: Adolescent runners' extrinsic foot and ankle muscles increased in volume and decreased in echogenicity, attributed to favorable distance training adaptations across the 6-month timeframe. We noted tendon thickening without concomitantly increased echogenicity, signaling intrasubstance tendon remodeling in response to escalating mileage.

Keywords: lower extremity; intrinsic foot muscles; ultrasound imaging; running; hypertrophy

Introduction

Running is one of the most popular forms of physical activity among adolescent students in the United States.[1,2] High school cross-country participation has increased by about 11% in the last 10 years, attracting almost half a million youth runners in 2019.[3] Development of functional movement skills in early life has been associated with extensive health benefits, including increased musculoskeletal strength and endurance in the lower extremities.[4] However, specific lower extremity musculotendinous tissue-level changes in response to endurance running have not yet been explored in this younger population.

To date, the majority of research describing musculotendinous response to endurance training has been conducted in cohorts of adult runners, finding that the repetitive loading exposure inherent to running leads to lower extremity tissue remodeling.[5,6] Some amount of remodeling may be favorable as these changes signal increasing musculotendinous resilience, strength, and architectural adaptations. Particularly, running increases lower extremity muscle volume and alters muscle fiber orientation along the aponeuroses (pennation angle; an established predictor of muscle force generation)[7] over time among healthy competitive adult runners.[8–11] Healthy adult runners also have increased Achilles tendon cross-sectional size compared to non-runners.[12] However, for tendinous tissues, extended periods of lower extremity loading associated with long distance running competitions leads to tendinous intrasubstance microdamage, excessive tendon thickening, increased cellularity, and collagen fiber disorganization, even among runners without pain or injury.[13–16] While these studies form an important foundation for expected positive and negative tissue-level responses to running in skeletally mature adults, adolescents may respond differently to repetitive loading during endurance training, feasibly due to factors ranging from rapid skeletal growth velocity,

development of neuromuscular patterning resulting in age-specific differences in gait patterns, age-related differences in tendon mechanical (modulus and stiffness) and growth (collagen turnover rate and cross-link patterns).[17] In light of these factors, additional investigations are needed to further elucidate the lower extremity musculoskeletal changes specific to adolescent runners participating in endurance running training programs.

Ultrasound imaging is a portable, non-invasive, and reliable means to assess musculotendinous size, architecture, and tissue quality parameters as surrogate markers of tissue response to training.[18–20] Among adult runners, ultrasound has been extensively incorporated into clinical assessments of the Achilles and patellar tendons,[9,14–16] and foot and ankle extrinsic and intrinsic muscle structures.[12,18,21] Ultrasound measurement protocols for these structures have been extensively validated in the literature, supporting the use of this imaging modality for longitudinal measurements of training-induced changes over time. However, tissue changes in response to distance training by adolescent runners has yet to be examined.

The purpose of this study was to prospectively evaluate lower extremity musculotendinous thickness and tissue quality, and muscle pennation angle changes over 6-months' time among adolescent runners enrolled in a progressive, structured marathon training program. We hypothesized that all musculotendinous structures would increase in thickness and size, and that muscle pennation angles would increase over time. We also hypothesized that muscles would show decreased echogenicity, and tendons would show increased echogenicity in response to training over time, suggesting improved tissue quality due to repetitive loading adaptations while marathon training.

Materials and Methods

Participants

We conducted a prospective cohort study of high-school aged runners involved in a structured 6-month afterschool marathon training program (November 2021-May 2022). Athletes were eligible for participation if they were 14-18 years of age, free from lower extremity injuries within 3-months of the start of the program, and if they did not have any history of lower extremity surgery. All participants provided informed assent, and parents or guardians provided consent prior to study participation. This study protocol was approved by the researchers' affiliated hospital institutional review board (IRB-P00039725). RedCap questionnaires were used to assess key demographic information from all participants (age, sex, height, weight), and to assess running experience, baseline weekly running mileage, and shoewear at the start of the season.

Instrumentation

Portable ultrasound imaging probes (163 x 56 x 35mm; 10 MHz linear transducer) and the associated HIPAA-compliant software (Butterfly iQ+, Butterfly Network, Inc., Guilford, CT, USA) were used to obtain B-mode musculotendinous ultrasound images. The ultrasound probes were plugged into smartphone devices with the Butterfly iQ+ encrypted software to obtain still ultrasound images, and stored in a secure hospital-affiliated research account. The researchers conducted ultrasound assessments using the musculoskeletal soft tissue setting during scanning to standardize imaging procedures and ultrasound settings. Ultrasound setting parameters were standardized to 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.

Study Procedures

Participants were assessed using B-mode ultrasound imaging prior to the start of the training season, 3-months into the training program, and 6-months into the training program for a

total of three scans. Ultrasound imaging procedures were always conducted on Saturdays just prior to the participants' scheduled long weekend runs. Participants remained seated for at least 5 minutes upon arrival to the training site prior to assessment to ensure they were in a rested state. Two researchers (one Sports Medicine physician [fellowship-trained in advanced musculoskeletal ultrasound], one athletic trainer with a PhD in sports medicine) each with focused training in ultrasound and over 6 years of ultrasound imaging experience conducted all study imaging procedures. The researchers scanned key lower extremity musculotendinous structures using standard ultrasound imaging protocol techniques[14,22–24] that have excellent validity and inter- and intra-rater reliability (Table 1).[20,24,25] Standardized protocols included specifications regarding the location of structure imaging capture and measurement, and which component of the structure was captured (e.g., transducer placement at the mid-substance of the patellar tendon). Additionally, patient positioning was standardized to ensure consistency in resting tensile forces on the structures during assessment. More detailed descriptions and images of patient have been published elsewhere.[14,22–24] Standardized short- and long-axis images were obtained on participants' dominant limb for the patellar and Achilles tendons, and the medial gastrocnemius, tibialis anterior, abductor hallicus, and flexor digitorum brevis muscles at verified anatomic soft tissue and bony landmarks; each image was cross-checked in both short-axis and long-axis images to ensure consistent placement and reproducibility (Figures 1a-f). Following imaging procedures, the participants completed their scheduled marathon training "long runs" as determined by their marathon training program goals and as advised by running program coaching staff under adult volunteer supervision.

Data Processing

Ultrasound images were processed using ImageJ software (v1.52k, National Institutes of Health, Bethesda, MA, USA). Thickness measures were obtained from long-axis ultrasound images by drawing a vertical line from superior to inferior borders of the target structures. Pennation angles were calculated for the medial gastrocnemius and tibialis anterior muscles as defined by the angle generated at the intersection between the line along the muscle fascicles and the muscles' deep aponeuroses.[10,11,26] Echogenicity measures as a proxy of tissue quality were obtained from short-axis images by tracing the borders of the target structures, thereby highlighting the cross-sectional area. Echogenicity measures were determined using a computerized histogram analysis on a 0 (black) to 255 (white) scale. In muscles, lower echogenicity values indicates better muscle quality (muscle hypertrophy; minimal infiltration of fatty or scar tissue),[27] while, conversely, in tendons, lower echogenicity indicates poorer tissue quality (collagen fiber disorganization, higher fluid content within the tendon).[28]

Statistical Analyses

Descriptive analyses were used to assess participant demographics, running experience, and accumulated mileage throughout the training program. All ultrasound data were normally distributed as determined from Shapiro-Wilk tests (p-range: 0.09-0.81), supporting parametric assessments. Separate within-subject repeated measures analyses of variance (ANOVAs) were used to assess changes in musculotendinous thickness measures, echogenicity, and muscle pennation angles over time. Post-hoc pairwise comparisons were conducted in the event of statistically significant ANOVA models. Alpha was set *a priori* to 0.05, and all analyses were conducted in R (RStudio, v1.2.1335).

Results

There were 11 participants (40.7% of eligible runners) who completed ultrasound evaluations at all three timepoints (Table 2). All runners wore neutral shoe models throughout the duration of the training program, removing the influence of shoe wear on lower extremity muscle size.[21,29] All athletes followed the same weekly running mileage and cross-training activity plan as a part of the structured training program (Supplementary Table). The program focused on a gradual increase in total weekly mileage (1 long run per week, 2 mid-distance runs, 1 cross-training day, and 2 rest days) with increasing long-distance runs up to a maximum of 20 miles before initiating a brief taper 4-weeks prior to the event day. This training program emulates a highly popularized marathon training regimen adopted by adult first-time marathoners and running coaches across the United States.[30] Runners accumulated an average of 124±9 miles at 3-months with the longest training run logged at 12 miles, with a recommended average weekly mileage of 12 miles (maximum: 19 miles; Supplementary Table). At the 6-month timepoint, runners had an average of 460±31 total accumulated miles with the longest training run logged at 20 miles, and a recommended average weekly mileage of 19 miles (maximum: 27 miles; Supplementary Table).

Muscle Ultrasound Measures

The omnibus ANOVA models were statistically significant for the medial gastrocnemius and tibialis anterior muscle thickness measures; however, there were no statistically significant changes in intrinsic foot muscle thickness measures over time (Table 3). Medial gastrocnemius thickness significantly increased from baseline to 6-months ($p=0.02$; Table 3; Figure 2a), and that tibialis anterior measures increased from baseline to 6-months ($p=0.01$), and 3-months to 6-months ($p=0.01$; Table 3; Figure 2b).

Echogenicity measures significantly changed over time across all muscles (Table 3). All measured muscles developed significantly decreased echogenicity across longitudinal study timepoints (p-range: <0.001-0.02; Table 3; Figures 2c-d). The foot intrinsic muscles including the flexor digitorum brevis, and abductor hallicus all showed significantly decreased echogenicity at every consecutive time interval (Table 3). Similar trends were observed for the ankle extrinsic muscles including medial gastrocnemius, and the tibialis anterior muscle (Table 3; Figures 2c-d). The tibialis anterior muscle showed significantly decreased echogenicity at 6-months compared to 3-months and baseline (Table 3; Figure 2d). Although tibialis anterior muscle echogenicity was lower at 3-months compared to baseline, the difference in that time interval was not statistically significant (Table 3; Figure 2d).

Pennation angles significantly increased for the medial gastrocnemius and tibialis anterior muscles across all study timepoints (p-range: <0.001, 0.04; Table 3; Figures 2e-f).

Tendon Ultrasound Measures

Achilles tendon thickness measures alone were found to significantly change over time (Table 3). Post-hoc analyses reflected that Achilles tendon thickness significantly increased from baseline to 6-months (p=0.05; Table 3; Figure 3a), whereas the Achilles tendon did not statistically change in echogenicity despite tendon thickening across accumulated mileage (Table 3). Conversely, echogenicity decreased within the patellar tendon across all study timepoints (p-range: 0.003-0.05; Table 3; Figure 3b), without a concomitant change in patellar tendon thickness measures (Table 3).

Discussion

We identified key musculotendinous adaptations induced by a 6-month marathon training program among adolescent runners. Long-distance running resulted in positive effects on

extrinsic ankle muscles including increased size, tissue quality, and muscle architecture, suggesting muscle hypertrophy and strengthening effects induced by endurance training. While intrinsic foot muscle size did not significantly change, tissue quality improved over time as a favorable adaptation to training.

Although the runners remained asymptomatic across the 6-month long endurance training season, we identified tendon thickening and a gradual reduction in tendon echogenicity as total cumulative running volume and total mileage increased over time. While some extent of tendon thickening is expected as a physiological adaptation to repetitive loading, increased tendon thickness without a concomitant increase in echogenicity as a surrogate measure of tissue quality has previously been associated with the development of tendinopathic changes and future injury.[14,31,32] While our findings suggest favorable muscular effects of running training for adolescents, changes identified in the tendons suggest susceptibility to some adverse tissue changes with chronic loading during long-distance training. As such, these athletes may benefit from a brief period of off-loading and/or non-impact cross-training in the several weeks following long-distance running programs to mitigate tendon microdamage and allow for tendon remodeling with some rest.

Our muscle morphology findings are consistent with previous studies among adult runner populations.[8,33,34] Running requires extrinsic foot and ankle muscle co-contraction for foot positioning control at initial contact and force-generating power for forward propulsion,[35] thus contributing to increased muscle strength secondary to repeated muscular action at increasing volume and duration associated with long-distance training.[34] Increased ankle plantarflexor muscle thickness measures correlate strongly with weekly running mileage in adult runners as a direct training effect on muscle quality.[8] In our adolescent population, we identified steady

increases in medial gastrocnemius muscle thickness measures by approximately 12.6% at 6-months. The tibialis anterior muscle is highly active in ankle dorsiflexion, and undergoes its greatest eccentric workload to control plantarflexion at the time of initial contact through to loading in midstance during the running gait cycle. This high muscle activity and use during endurance running led to a notable increase in muscle thickness by approximately 17% at 6-months. Collectively, these findings highlight novel in-vivo evidence that, among adolescent runners, endurance running training enhances foot and ankle extrinsic muscle size.

While we identified morphological changes for extrinsic foot and ankle muscles, these adaptations were not seen in the foot intrinsic muscles in this group of adolescent runners. The smaller foot intrinsic muscles serve to stabilize and attenuate forces through the intricate multiplanar motion of the midfoot during loading, and as such do not require the same power-generating propulsive and force demands of the larger foot and ankle extrinsic muscles.[36] Furthermore, these muscles have more slow-twitch muscle fibers than larger muscle structures due to their role in motor control,[37] thus supporting our null findings. Previous studies have identified significant changes in intrinsic foot muscle size among adult runners training in minimalist footwear;[21,29] however, all athletes in our study wore neutral shoes. Our findings suggest that there are minimal expected changes in intrinsic foot muscle size over time attributed to running training alone for younger distance runners. Given the role of the intrinsic foot muscles in functional foot stability and motion control, future research should explore the effects of adding dedicated foot core strengthening or footwear modifications into adolescent running programs on intrinsic foot muscle morphological change.[38]

Although we did not identify significant changes in intrinsic foot muscle size, we noted significantly decreased echogenicity measures for these muscles and for the ankle extrinsic

muscles, suggesting globally improved muscle quality in the lower extremity. We additionally found increased muscle fiber pennation angle for the medial gastrocnemius and tibialis anterior muscles. Fiber orientation and echogenicity have both been linked to muscle force generating capacity.[33,39] Increased pennation angle is associated with increased muscle tone,[39,40] and lower echogenicity signifies larger hyperechoic muscle fibers relative to the surrounding hyperechoic perimysium connective tissue, along with a lower presence of fatty and fibrous tissue, suggesting tissue optimization and remodeling from repetitive contraction during the gait cycle.[41] Despite the global benefits identified across our assessments, not all participants responded equivocally across the training program as seen in the individual patient responses for ultrasound-derived muscle measures. We believe that some individualized responses could be, in part, attributed to factors external to the training program, such as other physical activity. However, overall, we identified that long-distance running training largely induces multiple muscular benefits for young runners.

While we expected some extent of tendinous thickening as a result of running training, it was unexpected that echogenicity measures would stay the same or decrease across the training program for the Achilles and patellar tendons, respectively. There have been mixed findings on tendinous adaptations and their association with future development of lower extremity tendinopathies and pain among adult runners.[14,42,43] Achilles tendon thickening correlates with higher weekly training mileage among competitive adult runners and change across training cycles,[9,42] which has been attributed to increased collagen amalgamation and tissue resiliency.[44] Achilles tendon thickness has additionally been associated with improved distance running performance due to increased capacity to absorb and transmit ground reaction forces from absorption into forward propulsion.[8] Some extent of Achilles thickening may therefore be a

favorable training effect in younger runners. This notion is substantiated as all participants were fairly novice at the start of the program (average of 3 years of recreational or school-level running experience), and as all athletes remained healthy throughout the training program.

Our findings are consistent with available evidence that supports the idea that the development of muscle strength during a training process is not necessarily accompanied by concurrent modulation of tendon quality and stiffness, resulting in increased tendon stress and strain due to dysynchrony in musculotendinous development.[45] The differences between muscle and tendon changes in the time course of adaptation and in the mechanical stimuli that trigger tissue adaptation result in this dissociation of the muscular and tendinous development. It is likely that this is due, in part, to high levels of circulating sex hormones during adolescence. These hormonal changes may augment the imbalance in development of muscle strength and tendon mechanical properties, increasing tendon strain and overload changes as demonstrated in our study population.[45]

While patellar tendon thickness did not significantly change, we noted decreased echogenicity which suggests the presence of tissue microdamage. However, hypoechoic patellar tendon adaptations have not been consistently found to be associated with future tendinopathy among runners.[14,43] In fact, previous research has identified a minimal extent of fluid infiltration into patellar tendons among asymptomatic adult athletes.[6] Therefore, echogenicity changes may be an expected response to long-distance running and repetitive loading more broadly. Adolescent athletes are undergoing a phase of peak skeletal height growth velocity and development; as such, a higher presence of tendon microdamage may signal tissue remodeling in response to imposed training demands in a musculotendinous unit under developmentally-related high tensile strain. Clinicians and coaches should be cautiously aware of these tissue quality

changes inherent to long-distance running, and may consider incorporating strategic recovery opportunities for off-loading and non-impact cross-training to mitigate extensive tendon microdamage. There may also be a role for tendon injury preventive strategies which may help increase tendon tissue quality in order to modulate mechanical properties of the tendon to accommodate for the increased force-generating capacity of muscle in adolescent long distance runners during training.

Our study results should be interpreted within the context of certain limitations. Although we assessed within-participant changes, thereby allowing participants to serve as their own controls, we nonetheless had a relatively small sample size. We were not able to obtain long-term follow-up regarding runner wellness and injury, thus it is not clear if musculotendinous changes relate to future pain development. There was some variability in total accumulated mileage, though runners followed the same general training plan. Similarly, athletes enrolled in the training plan did not complete the prescribed training runs on necessarily the same days during the week apart from the Saturday long run. As such, runs conducted more recently to the ultrasound scan dates may have partially influenced the musculotendinous measures. Finally, we did not capture additional physical activity outside of the context of the prescribed running training program, and acknowledge that other factors may have influenced musculotendinous changes.

Conclusions

We found that adolescents enrolled in a 6-month long-distance running training program in preparation for a marathon underwent significant musculotendinous changes over time with increases in ankle extrinsic muscle size and pennation angles suggesting increased force-generating capacity, and globally improved lower extremity muscle quality. However, we

additionally noted Achilles tendon thickening and patellar tendon hypoechoic changes suggestive of potential microdamage. These findings may be related to non-uniform musculotendinous development in response to adolescent hormonal effects on muscle and tendon, as well as dyssynchronous timing of adaptations in response to running training related loading. As such, future investigations into the effect of incorporating cross-training and other off-loading activities in training programs for adolescent distance runners in order to preserve tendon health and allow for tissue recovery over time should be considered.

Conflicts of Interest: Dr. Meehan receives royalties from 1) ABC-Clio publishing for the sale of his books, *Kids, Sports, and Concussion: A guide for coaches and parents,* and *Concussions;* 2) Springer International for the book *Head and Neck Injuries in Young Athlete* and 3) Wolters Kluwer for working as an author for *UpToDate*. His research is funded, in part, by philanthropic support from the National Hockey League Alumni Association through the Corey C. Griffin Pro-Am Tournament and a grant from the National Football League. Dr. DeJong Lempke has a pending grant from VALD Performance for a separate project. The authors have no other pertinent conflicts of interest to disclose.

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Table 1. Ultrasound imaging methods for assessing myotendinous lower extremity structures.

Myotendinous Structure	Patient Positioning	Ultrasound Probe Orientation
Patellar Tendon[14]	Supine, knees extended to 0°, ankles flexed to 90°	6mm from the attachment at interior patellar pole
Achilles Tendon[14]	Prone, feet hanging over edge of table, ankles passively flexed to 90°	20 mm from distal attachment at calcaneus
Medial Gastrocnemius[23]	Prone, feet hanging over edge of table, ankles passively flexed to 90°	Mid-belly at the proximal 1/3 of posterior shank (medial head), superior to the myotendinous junction
Tibialis Anterior[23]	Supine, knees extended to 0°, ankles flexed to 90°	Mid-belly at the proximal 1/3 of the anterior shank, superior to the myotendinous junction
Abductor Hallicus[22,24]	Supine knees extended to 0°, ankles flexed to 90°	Mid-belly just anterior to the medial foot, and inferior to the navicular tubercle
Flexor Digitorum Brevis[22,24]	Supine, knees extended to 0°, ankles flexed to 90°	Mid-belly at 50% of total foot length

Table 2. Participant demographics, baseline running experience, and training mileage.

Measure	**Group Outcomes (Mean ± SD)**
Sex	6 Females, 5 Males
Age (Years)	16 ± 1
Height (cm)	169.7 ± 8.9
Mass (kg)	59.6 ± 9.8
Running Experience (years)	3.1 ± 2.3
Weekly Running Distance (km)	25.7 ± 6.4
Runs Per Week	4 ± 1
Running Pace (min/km)	5:43 ± 0:45
Accumulated Weekly Mileage	3-Months: 124±9 miles 6-Months: 460±31 miles

Table 3. Ultrasound measures over time.

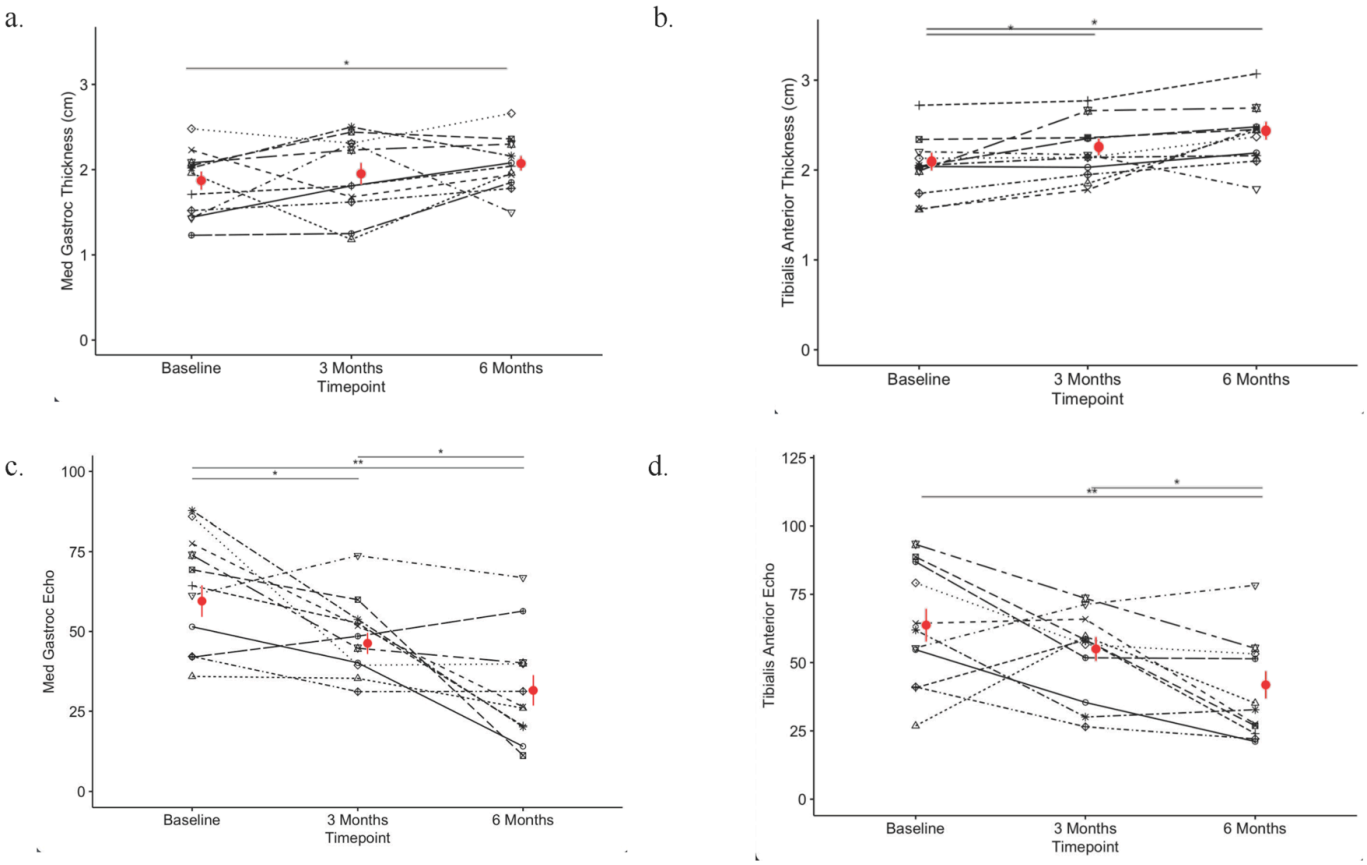
Anatomical Structure	Outcome Measure	Baseline	3-Months	6-Months	ANOVA Model Results ($F_{2,20}$; p-value)
Medial Gastrocnemius	Thickness (cm)	1.83 ± 0.39[b]	1.92 ± 0.47	2.06 ± 0.31[b]	3.48; 0.05*
	Echogenicity	62.87 ± 18.04[a,b]	48.28 ± 12.11[a,c]	32.05 ± 17.43[b,c]	11.60; <0.001*
	Pennation Angle	25.01 ± 2.75[a,b]	26.49 ± 1.97[a,c]	29.74 ± 2.25[b,c]	14.47; <0.001*
Tibialis Anterior	Thickness (cm)	2.03 ± 0.34[a,b]	2.20 ± 0.31[a]	2.38 ± 0.33[b]	7.36; 0.004*
	Echogenicity	63.02 ± 21.95[b]	53.39 ± 16.03[c]	38.88 ± 18.24[b,c]	7.39; 0.004*
	Pennation Angle	11.90 ± 1.29[a,b]	13.28 ± 1.80[a,c]	14.18 ± 2.00[b,c]	12.90; <0.001*
Abductor Hallicus	Thickness (cm)	0.64 ± 0.11	0.60 ± 0.09	0.59 ± 0.11	1.69; 0.21
	Echogenicity	58.32 ± 13.64[a,b]	48.87 ± 15.79[a,c]	32.86 ± 7.14[b,c]	13.47; <0.001*
Flexor Digitorum Brevis	Thickness (cm)	0.75 ± 0.12	0.67 ± 0.28	0.72 ± 0.07	0.74; 0.49
	Echogenicity	71.76 ± 10.91[a,b]	64.10 ± 12.31[a,c]	43.49 ± 17.90[b,c]	12.98; <0.001*
Achilles Tendon	Thickness (cm)	0.61 ± 0.11[b]	0.67 ± 0.12	0.69 ± 0.08[b]	3.58; 0.05*
	Echogenicity	90.82 ± 18.04	83.72 ± 16.25	76.51 ± 18.08	2.24; 0.13
Patellar Tendon	Thickness (cm)	0.45 ± 0.13	0.48 ± 0.11	0.46 ± 0.09	0.42; 0.66
	Echogenicity	114.42 ± 29.58[a,b]	102.79 ± 23.09[a,c]	84.48 ± 18.53[b,c]	10.73; <0.001*

[*]signifies statistically significant differences for the omnibus ANOVA model
[a]signifies statistically significant differences between baseline and 3-months
[b]signifies statistically significant differences between baseline and 6-months
[c]signifies statistically significant differences between 3-months and 6-months

Figure 1. Sample Ultrasound Images.
Caption: Each image depicts an example of a short-axis (or cross-sectional) view and long-axis (or longitudinal) view of the Patellar Tendon, Achilles Tendon, Medial Gastrocnemius Muscle, Tibialis Anterior Muscle, Abductor Hallicus Muscle, and Flexor Digitorum Brevis Muscles. Still frame images are taken in short axis and long axis views relative to the structure of interest. The lead end of the probe is demarcated as the "B" label in the upper left image corner. Yellow lines outline the structures, with labels 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.
Abbreviations: Abbreviations: Patellar Tendon: PT; Achilles Tendon: AT; Medial Gastrocnemius Muscle: MGM; Tibialis Anterior Muscle: TAM; Abductor Hallicus Muscle: AHM; Flexor Digitorum Brevis Muscle: FDBM.

Figure 2. Medial gastrocnemius and tibialis anterior muscle thickness (a-b), echogenicity (c-d), and pennation angle (e-f) changes over time.
Caption: Comparison of muscle thickness, echogenicity, and pennation angles (y-axes) over time from baseline, 3-months, to 6-months (x-axes). Each shape and line connecting the measures over time represents an individual runner. The data were found to be normally distributed on preliminary Shapiro-Wilks analyses, and, as such, red filled circles jittered to the right of the individual measures represent group means with 95% confidence interval bars. Significant differences across timepoints are denotes with a horizontal bar and asterisk ('*' $p \leq 0.05$, '**' $p \leq 0.01$, '***' $p \leq 0.001$)

Figure 3. Achilles tendon thickness (a) and patellar tendon echogenicity (b) changes across the training program.
Caption: Comparison of Achilles tendon thickness, and patellar tendon echogenicity (y-axes) over time from baseline, 3-months, to 6-months (x-axes). Each shape and line connecting the measures over time represents an individual runner. The data were found to be normally distributed on preliminary Shapiro-Wilks analyses, and, as such, red filled circles jittered to the right of the individual measures represent group means with 95% confidence interval bars. Significant differences across timepoints are denotes with a horizontal bar and asterisk ('*' $p \leq 0.05$, '**' $p \leq 0.01$, '***' $p \leq 0.001$)



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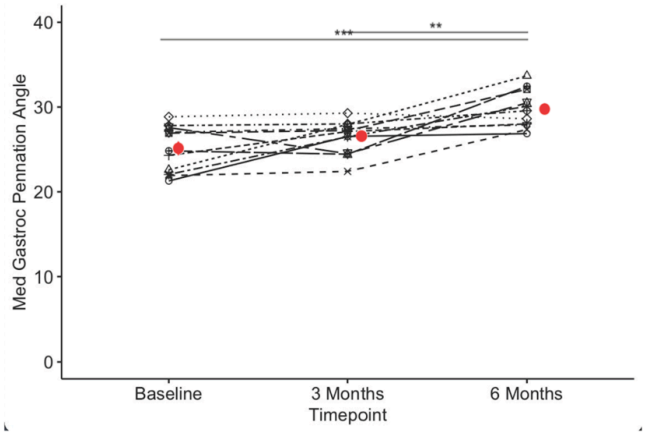

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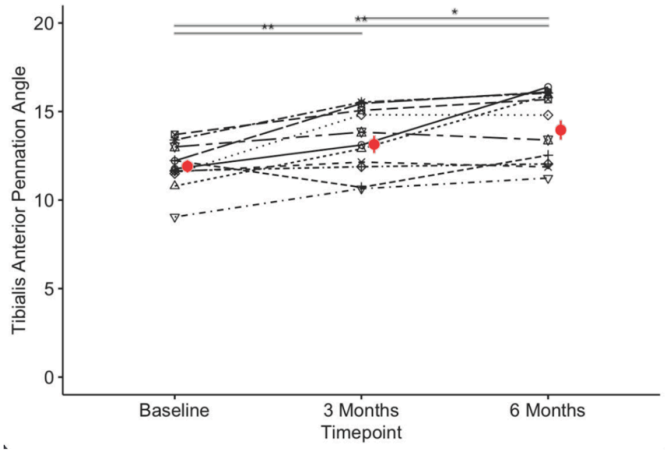

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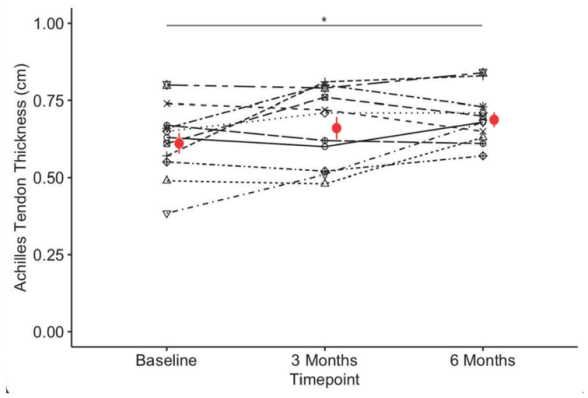

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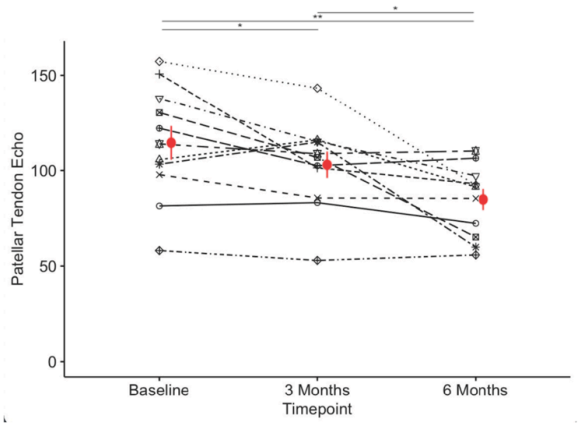

JUM_16105_Fig3a-b_R1.png

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Date: 8/27/2022

Your Name: Danielle Hunt

Manuscript Title: Adolescent marathon training: prospective evaluation of musculotendinous changes during a 6-month endurance running program

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Date: 8/29/2022

Your Name: Kristin E Whitney, MD, MA

Manuscript Title: Adolescent marathon training: prospective evaluation of musculotendinous changes during a 6-month endurance running program

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Your Name: William Meehan

Manuscript Title: Adolescent marathon training: prospective evaluation of musculotendinous changes during a 6-month endurance running program

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Date: 8/25/2022

Your Name: Sarah Willwerth

Manuscript Title: Adolescent marathon training: prospective evaluation of musculotendinous changes during a 6-month endurance running program

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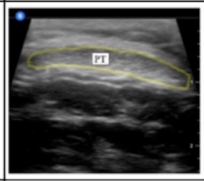
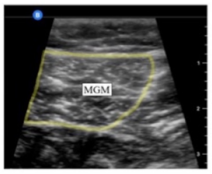
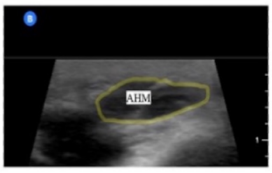
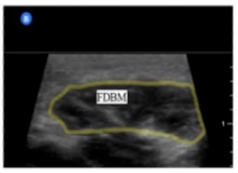
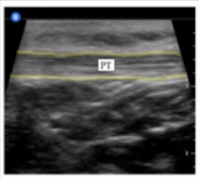
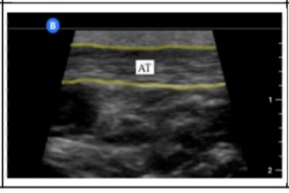
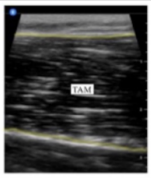
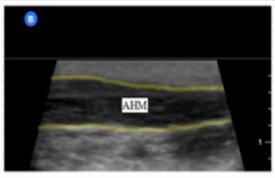
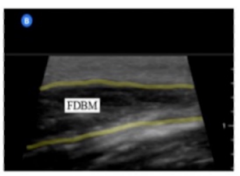
Short-Axis						
Long-Axis						

JUM_16105_JUltrasoundMed_Figure_1.png

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Date: 8/20/2022

Your Name: Alexandra F. DeJong Lempke

Manuscript Title: Adolescent marathon training: prospective evaluation of musculotendinous changes during a 6-month endurance running program

Manuscript Number (if known): Click or tap here to enter text.

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2	Grants or contracts from any entity (if not indicated in item #1 above).	☐ **None**	
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3	Royalties or licenses	☒ **None**	
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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ **None**	
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8	Patents planned, issued or pending	☒ **None**	
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11	Stock or stock options	☒ **None**	
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I continue to work as an Associated Personnel for projects affiliated with Boston Children's Hospital and the Micheli Center for Sports Injury Prevention	

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