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Original research

Achilles tendon morphology assessed using image based spatial frequency analysis is altered among healthy elite adolescent athletes compared to recreationally active controls

Michael Cassel[a,*], Lucie Risch[a,1], Frank Mayer[a], Hannes Kaplick[a], Aaron Engel[b], Kornelia Kulig[c], Greg Bashford[b]

[a] University Outpatient Clinic, Dept. Sports Medicine, University of Potsdam, Germany
[b] Department of Biological Systems Engineering, University of Nebraska-Lincoln, USA
[c] Division of Biokinesiology and Physical Therapy, University of Southern California, USA

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ABSTRACT

Objectives: Although expected, tendon adaptations in adolescent elite athletes have been underreported. Morphologically, adaptations may occur by an increase in collagen fiber density and/or organization. These characteristics can be captured using spatial frequency parameters extracted from ultrasound images. This study aims to compare Achilles tendon (AT) morphology among sports-specific cohorts of elite adolescent athletes and to compare these findings to recreationally active controls by use of spatial frequency analysis.
Design: Cross-sectional observational study.
Method: In total, 334 healthy adolescent athletes from four sport categories (ball, combat, endurance, explosive strength) and 35 healthy controls were included. Longitudinal ultrasound scans were performed at the AT insertion and midportion. Intra-tendinous-morphology was quantified by performing spatial frequency analysis assessing eight parameters at standardized ROIs. Increased values in five parameters suggest a higher structural organization, and in two parameters higher fiber density. One parameter represents a quotient combining both organization and fiber density.
Results: Among athletes, only ball sport athletes exhibited an increase in one summative parameter at pre-insertion site compared to athletes from other sport categories. When compared to athletes, controls had significantly higher values of four parameters at pre-insertion and three parameters at midportion site reflecting differences in both, fiber organization and density.
Conclusions: Intra-tendinous-morphology was similar in all groups of adolescent athletes. Higher values found in non-athletes might suggest higher AT fiber density and organization. It is yet unclear whether the lesser structural organization in young athletes represents initial AT pathology, or a physiological adaptive response at the fiber cross-linking level.

Practical implications

- Spatial frequency analysis enables differentiation of Achilles tendon morphology between adolescent athletes and recreationally active controls.
- Lower parameters in healthy adolescent athletes indicate an increased disorganization of intra-tendinous fiber alignment compared to controls.
- Results suggest that Achilles tendons fail to respond to higher loading by an increase in fiber density.
- It has to be clarified, whether the lower fiber density and organization among adolescent athletes represent a physiological adaptive response (increase on fiber cross-linking level) or an initial pathological alteration.

1. Introduction

Physiological tendon thickness adaptation in response to loading is debatable.[1–3] On one hand, Achilles tendon (AT) thickness among 12 year old adolescent athletes has been found to be at adult athletes' level.[4] On the other hand, adolescent athletes from ball

* Corresponding author.
E-mail address: mcassel@uni-potsdam.de (M. Cassel).
[1] Contributed equally to this work.

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Table 1
Anthropometrics and training data of 4 sports categories (B = Ball sports; C = Combat sports; E = Endurance sports; S = Explosive strength sports), athletes and controls [mean ± SD].

Category	N (m/f)	Age* [years]	Height* [cm]	Weight* [kg]	BMI* [kg/m²]	Training* [h/week]	Training* years	Thickness insertion	Thickness* midportion
B	89 (67/22)	13.4 ± 1.7#	165 ± 13	54 ± 15	19.3 ± 2.7	8.1 ± 4.6#	5.5 ± 2.3#	3.7 ± 0.6#	5.4 ± 0.7
C	91 (68/23)	14.0 ± 1.7#	163 ± 11#	55 ± 16	20.3 ± 3.7	10.1 ± 5.5#	5.2 ± 2.9#	3.8 ± 0.6	5.5 ± 0.8
E	50 (28/22)	14.2 ± 1.9#	167 ± 12	58 ± 13	20.4 ± 2.7	11.8 ± 5.8	4.5 ± 2.1#	3.6 ± 0.7	5.2 ± 0.7
S	104 (51/53)	12.9 ± 2.2#	159 ± 16	48 ± 16	18.4 ± 3.3#	9.3 ± 5.8#	3.9 ± 2.4#	3.6 ± 0.7	5.2 ± 0.7
Athletes	334 (214/120)	13.5 ± 2.0#	163 ±14#	53 ± 15#	19.5 ± 3.3#	9.5 ± 5.6#	4.7 ± 2.6#	3.7 ± 0.6#	5.3 ± 0.8
Controls	35 (17/18)	14.5 ± 2.2#	169 ± 9#	58 ± 10	20.4 ± 2.6#	2.6 ± 2.4#	2.5 ± 3.1#	3.5 ± 0.4	5.0 ± 0.9
p-value		<0.01	0.01	0.02	0.06	<0.01	<0.01	0.10	0.03

Data not normally distributed.
* Significantly different between sport categories: anthropometric data (ANOVA; p < 0.006), training data (p < 0.003) and midportion thickness (p = 0.015).

sports and cycling have significantly higher AT thickness compared to recreationally active controls. Longitudinal, three-year, follow-up of this cohort during maturation has not shown an increase in AT thickness, neither in athletes of different sport categories nor in controls, although a significant increase in body height and weight leading to higher tendon loading has been reported.[5] Consequently, it is speculated that during maturation tendon adaptation to higher loading occurs by physiological intra-tendinous changes suggestive of increase in collagen fiber density and/or organization.[5–7]

Spatial frequency analysis using eight parameters developed by Bashford et al.[8] can be used to evaluate intra-tendinous-morphology, collagen fiber alignment and organization.[8,9] The method has shown to be sensitive in capturing loading induced changes of intra-tendinous-morphology. Immediately following tendon loading, intra-tendinous-morphology exhibited increased values in spatial frequency parameters related to tendon structure, reflecting higher fiber density and organization.[9] Furthermore, the method has been able to discriminate between healthy and tendinopathic tendons.[10] Comparison of volleyball athletes with and without patellar tendinopathy has revealed significant lower values in the parameter "peak spatial frequency radius" in symptomatic compared to asymptomatic participants.[11] Investigations regarding reliability have revealed high intersession reliability in image acquisition of healthy and tendinopathic ATs among adults.[8,10] In adolescents, intra-tendinous-morphology has not yet been evaluated. The use of sonographic spatial frequency analysis in healthy adolescent athletes and recreationally active controls might provide insight into tendon structural properties and their adaptive capacity.

This study aims to compare AT morphology among healthy adolescent elite athletes (1) from different sports, and (2) to recreationally active controls, using spatial frequency parameters. It is hypothesized that adolescent athletes from sports with high and repetitive loading of the lower extremity (i.e. ball sport) present higher AT density and organization than athletes from other sports as well as recreationally active controls indicated by changes in spatial frequency parameter values.

2. Methods

In this retrospective cross-sectional observational study of 402 participants (<18 years of age), AT ultrasound images obtained from 334 healthy adolescent elite athletes (m/f: 214/120) and 35 healthy recreationally active control participants (m/f: 17/18) were analyzed. Adolescent athletes from 17 different sport disciplines were recruited by a sports medicine physician during a pre-participation examination or annual health evaluation for elite schools of sports at a university outpatient clinic. Control participants were recruited from a local secondary school. Participants having clinical and/or imaging-based signs of Achilles tendinopathy (intra-tendinous alterations in ultrasound: hypo/hyperechogenicities, pathological intratendinous vascularization higher than grade I according to Öhberg-score) were excluded (31 participants) from this analysis.[12–14] Likewise, adolescents with history apophysitis calcanei (1 subject) and rheumatic disease (1 subject) were not included. Anthropometric and training related data are presented in Table 1. Parents and participants of the athletic and control population gave their written informed assent and consent before study inclusion. This investigation was approved by the local ethics committee.

Ultrasound examinations were performed with the same portable high-resolution commercial ultrasound machine (Viamo SSA-640A Toshiba, Tokyo, Japan) using a wideband linear transducer (PLT-704SY) with standardized transducer settings (center frequency 11 MHz, gain = 93, DR = 50, penetration depth = 3 cm, one transmit focus at 0.5 cm).[4,15] The examinations were performed by three examiners, trained in musculoskeletal ultrasound imaging. Participants lay in the prone position on an examination table with the ankle being passively placed and held by the examiners thigh in neutral position (0° of the ankle). Both left and right ATs were scanned longitudinally in B-mode with the transducer being placed strictly parallel and orthogonal to the fiber direction. AT thickness was assessed at the proximal calcaneal insertion and in tendon midportion (thickest distance). All scans were stored as JPEG files for subsequent analysis of intra-tendinous-morphology analysis.

All ultrasound images were imported into MATLAB (Mathworks, USA R2016a) to select a polygonal region of interest (ROI) for spatial frequency analysis (performed by one examiner) described by Bashford et al.[8] The ROI was selected in a standardized manner by measuring 1 cm longitudinally proximal to the most proximal visible point of the calcaneus (pre-insertion), and 1 cm longitudinally in the tendon midportion. Subsequently, the locations of all 2 mm × 2 mm sub-ROI's ("kernels") that fit within the vertices of the main ROI were identified. For each kernel, a two-dimensional Fast Fourier Transformation (FFT) based procedure was performed extracting 8 spatial frequency parameters. The eight resulting spatial frequency parameters named "Peak Spatial Frequency Radius" ("PSFR"), "P6 width", "Q6 Factor", "Mmax", "Mmax Percent", "Axis

Table 2
Results of reliability testing among spatial frequency parameters (Intraclass correlation coefficient [ICC], standard error of measurement [SEM], test-retest variability [TRV]).

Location	Test	PSFR	P6 Width	Q6 Factor	Mmax	Mmax%	Axis Ratio	Ellipse Rot	Sum
Pre-insertion	ICC	0.52	0.28	0.47	0.79	0.83	−0.31	0.64	0.91
	SEM	0.21	0.03	0.27	673	0.66	0.07	0.55	3617
	SEM %	11.2	4.0	10.3	8.6	8.8	4.2	0.6	3.4
	TRV ± SD	14.8 ± 11.8	6.0 ± 4.4	12.8 ± 10.4	10.4 ± 8.3	10.6 ± 8.6	5.5 ± 2.6	0.9 ± 0.6	4.2 ± 2.2
Midportion	ICC	0.9	0.36	0.89	0.83	0.84	0.79	0.71	0.95
	SEM	0.13	0.01	0.19	492	0.54	0.05	0.76	3090
	SEM %	6.9	1.3	7.3	5.8	6.0	3.1	0.8	3.3
	TRV ± SD	8.0 ± 4.7	3.5 ± 4.7	8.3 ± 5.8	10.0 ± 6.1	8.3 ± 8.2	3.0 ± 2.8	0.9 ± 0.8	3.9 ± 2.5

SEM% = (SEM/mean M1&M2) × 100.

Ratio", "Ellipse Rotation", and "Sum" were then saved in an excel sheet for statistical analysis.[8] Higher values of parameters "PSFR", "P6 width", and "Q6 Factor" primarily indicate a more "pure" (parallel) alignment of tendon fibers. "Axis Ratio" is a measure of fiber alignment anisotropy. "Ellipse Rotation" indicates the angle of acoustic beam with the tendon. Higher values of "Mmax" and "Sum" represent the density of the most prominent fiber spacing and the overall tissue density, respectively. "Mmax Percent," a comparison of the strength of dominant and randomly-aligned fibers, contains both aspects (fiber organization and density) as it is a ratio of "Mmax" to the total intensity of pixels in the 2-D FFT.[8,9,11]

To investigate test-retest reliability of intra-tendinous-morphology assessment, ultrasound images were acquired from AT pre-insertion and midportion of 6 healthy athletes at two measurement time-points with an interval of 2 h by one examiner. Intraclass correlation coefficient (ICC 2.1) and standard error of measurement (SEM, SEM%) as well as test-retest variability (TRV, [%]) were calculated for all spatial frequency parameters. Interpretation of ICC followed recommendations of Landis and Koch[17] being "poor" (<0.0), "slight" (0.0–0.20), "fair" (0.21–0.40), "moderate" (0.41–0.60), "substantial" (0.61–0.80), and "excellent" (0.81–1.00).[16,17] Table 2 summarizes results of reliability analysis, showing only fair results for P6 width and pre-insertional axis ratio and moderate results for pre-insertional PSFR and Q6 Factor. All other parameters showed substantial to excellent reliability at both locations. SEM% varied from 0.6% in "Ellipse Rotation" to 11.2% of "PSFR" at pre-insertion and from 0.8% in "Ellipse Rotation" to 6.9% of "PSFR" in midportion location, TRV ranged from 1 to 15% (Table 2).

Adolescent elite athletes were categorized into 4 groups according to their sports discipline (ball sports (B, n = 89), combat sports (C, n = 85), endurance sports (E, n = 50) and explosive strength sports (S, n = 110); Table 1)[5]. Descriptive statistics for anthropometric, training-related, tendon thickness and spatial frequency analysis data are presented with mean and SD for all athletes, different sport categories and controls. Tendon thickness and spatial frequency analysis data is shown as means of both AT sides. Differences of spatial frequency parameters between sport categories

Table 3
Spatial frequency parameters for AT pre-insertion and midportion of 4 sport categories (B = Ball sports; C = Combat sports; E = Endurance sports; S = Explosive strength sports), from all athletes (n = 334) and controls (n = 35) [mean ± SD].

	Category	PSFR	P6 Width	Q6 Factor	Mmax	Mmax%	Axis Ratio	Ellipse Rotation	Sum
Pre-insertion	B	1.79 ± 0.18	0.76 ± 0.03	2.48 ± 0.25	7656 ± 884	7.38 ± 0.96	1.62 ± 0.07	89.8 ± 1.2	104825 ± 12379#*
	C	1.84 ± 0.20#	0.76 ± 0.04#	2.55 ± 0.31#	7473 ± 983	7.51 ± 0.98#	1.63 ± 0.07	89.6 ± 0.8	100188 ± 9592
	E	1.87 ± 0.21	0.75 ± 0.05#	2.60 ± 0.31	7428 ± 953#	7.56 ± 1.20	1.63 ± 0.08	90.0 ± 0.9#	99610 ± 12191
	S+	1.84 ± 0.20	0.75 ± 0.05#	2.56 ± 0.30	7368 ± 1141	7.54 ± 1.31	1.62 ± 0.07	90.0 ± 1.2	99543 ± 15069
	Athletes+	1.83 ± 0.20	0.75 ± 0.04#	2.54 ± 0.29	7483 ± 1008	7.49 ± 1.12#	1.62 ± 0.07#	89.8 ± 1.1#	101138 ± 12740#
	Controls	1.91 ± 0.17	0.75 ± 0.03	2.64 ± 0.25	7663 ± 1207	7.34 ± 0.98	1.65 ± 0.07	90.2 ± 1.0#	104801 ± 11535#
	p-value	0.04	0.56	0.05	0.33	0.63	0.05	0.09	0.03
Mid-portion	B+	1.87 ± 0.18	0.76 ± 0.03#	2.25 ± 0.22	7452 ± 842#	8.12 ± 1.17#	1.61 ± 0.07#	90.2 ± 1.2#	93358 ± 13200#
	C	1.87 ± 0.16	0.75 ± 0.02	2.24 ± 0.20	7385 ± 894	8.38 ± 1.13	1.63 ± 0.07	90.3 ± 1.0	89273 ± 11601
	E	1.85 ± 0.15	0.75 ± 0.03	2.22 ± 0.19	7464 ± 1078	8.27 ± 1.35	1.62 ± 0.07	90.2 ± 1.0	91438 ± 11859
	S	1.86 ± 0.18	0.75 ± 0.03#	2.23 ± 0.20	7253 ± 889	8.14 ± 1.28#	1.62 ± 0.06	90.1 ± 1.0	90856 ± 13265#
	Athletes+	1.86 ± 0.17	0.75 ± 0.03#	2.24 ± 0.21	7374 ± 909	8.22 ± 1.22#	1.62 ± 0.07#	90.2 ± 1.1	91172 ± 12641#
	Controls+	1.92 ± 0.20	0.75 ± 0.02	2.31 ± 0.28#	7750 ± 1081	8.10 ± 0.90	1.64 ± 0.07	91.1 ± 1.0	96257 ± 11765
	p-value	0.08	0.51	0.11	0.02	0.97	0.08	<0.01	0.01

Data not normally distributed.
+ Data at attachment missing in 1 participant of the group.
* Indicates significant higher Sum of B (ANOVA: p = 0.019) in comparison to C (p = 0.004), E (p = 0.021), S (p = 0.013) and E (p = 0.033) at pre-insertion.

were tested either by one-way ANOVA (post-hoc Tukey-Kramer) or Kruskal-Wallis- ANOVA (post-hoc Wilcoxon test) depending on data distribution in Kolmogorov-Smirnov-test. Comparisons of all athletes versus controls were performed using an unpaired t-test or Mann-Whitney U-test. Regression analysis was performed to and control for the effect of age, sex, anthropometric data (weight and height) and training amount (hours/week and training years) on spatial frequency parameters. Results with $p \leq 0.05$ were considered significant.

3. Results

Anthropometric and training related data as well as midportion thickness were slightly different between athletes of different sport categories (Table 1; $p \leq 0.01$). Controls had slightly higher age, height, weight ($p \leq 0.02$) and presented with lower training amount ($p < 0.01$) as well as midportion thickness ($p = 0.03$) than athletes (Table 1).

At pre-insertion, ball sport athletes presented with higher “Sum” compared to athletes from other sport categories (Table 3; $p = 0.02$). Other parameters were not significantly influenced by type of sport participation ($p > 0.05$). Compared to all athletes, controls showed higher values of “PSFR” ($p = 0.04$), “Q6 Factor” ($p = 0.05$), “Axis ratio” ($p = 0.05$) and “Sum” ($p = 0.02$; Table 3). Regression analysis revealed an influence of female sex on an increased “PSFR” (m: 1.82; f: 1.88; $t = 2.7$, $p < 0.01$) and “Q6 Factor” (m: 2.53; f: 2.60; $t = 2.6$, $p = 0.01$). Higher age was associated with a slightly higher “Mmax” ($t = 4.1$; $p < 0.01$) and “Mmax%” ($t = 4.8$; $p < 0.01$) as well as lower “Ellipse Rotation” ($t = -2.6$; $p = 0.04$). Higher weight led to increased “Mmax%” ($t = 2.1$; $p = 0.04$) and decreased “Axis ratio” ($t = 3.0$; $p < 0.01$), an increased height to higher “Axis ratio” ($t = 2.7$; $p < 0.01$). Higher training amount (h/week) was found to be associated with higher “Mmax%” ($t = 2.9$; $p < 0.01$).

At midportion, no relevant differences were detected between different sport categories (Table 3; $p > 0.05$). In contrast, controls presented higher but statistically insignificant “PSFR” and “Axis ratio” ($p = 0.08$) as well as significantly higher “Mmax” ($p = 0.03$), “Ellipse Rotation” and “Sum” ($p \leq 0.01$) compared to all athletes. Female sex was associated with slightly lower “Mmax%” (m: 8.32; f: 8.0; $t = 2.7$; $p < 0.01$) and higher “Sum” (m: 89928; f: 94816; $t = 3.8$; $p < 0.01$). Higher age led to higher “Mmax” ($t = 4.2$; $p < 0.01$) and “Mmax%” ($t = 4.7$; $p < 0.01$). Higher weight was associated with lower “Axis ratio” ($t = 3.0$; $p < 0.01$) and “Ellipse rotation” ($t = 2.6$; $p = 0.01$), an increased height led to higher “Axis ratio” ($t = 2.7$; $p < 0.01$). An increased training amount (h/week) was associated with slightly lower “Axis Ratio” ($t = 2.5$; $p = 0.02$), “Ellipse Rotation” ($t = 2.7$; $p < 0.01$) and “Sum” ($t = 2.1$; $p = 0.04$).

4. Discussion

The present retrospective study investigated AT morphology using spatial frequency analysis parameters on B-mode ultrasound images of asymptomatic, sonographically inconspicuous ATs of elite adolescent athletes from different sports and recreationally active controls. Contrary to the hypothesis, adolescent athletes from sports disciplines with high and repetitive loading of the lower extremity (i.e. ball sport) did not present alterations in spatial frequency parameters compared to athletes from other sport disciplines. Instead, controls had higher values in some parameters at both tendon sites, suggesting higher fiber density and organization in control participants.

Several studies have investigated tendon thickness as a possible adaptive mechanism to increasing loads during growth or increased athletic participation. Data indicate that tendon thickness adaptation has physiological limits.[3,18,19] Among athletes as well as recreationally active adults an upper limit of the physiological AT thickness of 6 mm in midportion has been reported.[3,20] Previous data among 131 adolescent elite athletes and 24 controls suggests that AT thickness reaches adult athletes' level at 12 years of age. Longitudinal follow-up data did not see a further increase in AT thickness between 12 and 15 year old adolescents, neither in athletes nor in controls.[5] Furthermore, a recent investigation has revealed sex-specific differences in tendon thickness with females presenting thinner tendons compared to males, independent of sports participation.[5] Taken together, the literature strongly indicates that thickness itself is not a metric of physiological AT adaptation to exercise and sport-dependent load.

Reference values for physiological AT morphology (collagen fiber organization and density) and their age-related developments determined by use of spatial frequency analysis are not yet available. However, this method has shown to be sensitive to acute changes in tendon fiber organization and density. By use of this method, healthy patellar tendons of 15 nineteen year old males assessed before and during maximal isometric tendon loading have revealed an increase in “PSFR”, “Axis ratio” and “Sum”.[9] These alterations have been explained by the viscoelastic characteristics of tendons during elongation leading to a so called “uncrimping” of fibers in longitudinal direction.[21] Thereby a closer longitudinal fiber orientation with less water content was reflected as stronger signals in “PSFR”, “Axis ratio” and “Sum”.[9] Related to these findings, a recently published study found an adaptive response behavior of the AT with a reduced cross-sectional-area during tendon loading.[22] This supports findings from spatial frequency analysis of patellar tendon intra-tendinous-morphology behavior under loading.[9] Results of the present study show higher values in six out of eight parameters (“PSFR”, “Q6 Factor”, “Mmax”, “Axis Ratio”, “Ellipse Rotation”, “Sum”) in controls compared to athletes. This potentially indicates a more aligned tertiary collagen fiber organization as well as higher fiber tissue density of ATs among controls.

The hypothesis of a physiologic adaptation of AT morphology to sport-specific loading has been rejected. Only one parameter (“Sum”) at pre-insertion could be shown to be higher in ball sport athletes compared to athletes of other categories. The linear relationship of higher age with a slight increase in “Mmax” and “Mmax%” at both sites potentially indicates slight changes during maturation leading to a denser intra-tendinous-morphology over time. Therefore, the slightly higher age of controls versus athletes has to be considered when interpreting these results. However, the fact that controls revealed consistently higher values for “PSFR”, “Q6 Factor”, “Mmax”, “Axis Ratio” and “Sum” in both pre-insertion and midportion location negates the conclusion of a primary age-dependent difference. Lower values representing fiber organization (“PSFR”, “Q6 factor” and “Axis Ratio”) among athletes might potentially also be interpreted as an increase in collagen fiber cross-linking associated with higher loading.[7,23] This would be in line with data of Couppé et al.,[24] showing old compared to young men having lower patellar tendon collagen mass in biopsies, but compensating tendon mechanical properties with higher collagen cross-links concentrations.[24] Results of longitudinal follow-up investigations by use of spatial frequency parameter analyses, among young athletes, might clarify this speculation. Additionally, training studies performing standardized tendon loading protocols examining tissue adaptation using biopsies and diagnostic ultrasound could help inform the results presented here.

Conversely, lower values in spatial frequency parameters among athletes compared to controls might present the first sign of intra-tendinous-morphological degeneration in direction of tendon pathology since lower values in the analysis were shown in degenerated tendons of symptomatic athletes.[10,25,26] The association of lower “Axis Ratio” and “Sum” at midportion with higher training amount might support this interpretation. It is also in line with data in patellar tendons of asymptomatic volleyball players aged 21 years showing slightly lower PSFR values than recreationally active controls.[11] However, since the study only examined the "PSFR" further comparisons cannot be drawn.[11] Possibly, spatial frequency analysis might have the potential to discriminate for early pathological changes of tendon tissue quality (i.e. early cellular and ground substance changes or intra-tendinous-ruptures) assumed to be the first sign of tendinopathy leading also to pain development.[19,26] A further improvement as well as a better understanding of these imaging modalities is required to identify early intra-tendinous-morphological disorganization in tendon structure helping to understand development of pain in tendinopathy.[26] This would require identifying reference values for tendon-specific spatial frequency parameters among different populations (children, adolescents, adults, elderly, athletes and sedentary) including the parameter transition values between healthy and tendinopathic tissue.

This cross-sectional study aimed to compare AT morphology between adolescent elite athletes of different sports categories. Furthermore, it aimed to compare data of the athletes to a recreationally active control group. Different sample size of sport categories and especially low number of controls has to be considered when interpreting the results. Furthermore, the ultrasound image acquisition and the image analysis have been performed by three different examiners, which could have led to a certain bias. Intra-observer test-retest reliability analysis showed relatively high stability of values for most relevant spatial frequency parameters. Therefore the use of the same ultrasound machine with standardized settings (identical probe and setup guidelines) for all examinations is essential. Nevertheless, the lower reliability found for pre-insertion site has to be taken into account. Additional inter-rater reliability comparisons might be useful for a more comprehensive statement of methodological measurement error.

5. Conclusions

Contrary to the hypothesis, Achilles tendon morphology does not differ between adolescent elite athletes from sports disciplines with different loading demands of the lower extremity. Moreover, recreationally active controls presented higher values of relevant spatial frequency parameters than athletes, suggesting more dense and organized tendon material in controls. This potentially indicates that Achilles tendons do not respond to higher loading by an increase in fiber density. It is yet unclear whether the increased disorganization of intra-tendinous-morphology in young athletes already represents the first sign of tendon pathology,[26] or a physiological adaptive response at the fiber cross-linking level.[7] Longitudinal follow-up investigations are required to confirm the present findings and to identify the predictive value of the intra-tendinous-morphology analysis in development of tendon pathology (tendinosis) and tendon pain (Achilles tendinopathy).

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References

[1]. Hansen P, Aagaard P, Kjaer M et al. Effect of habitual running on human Achilles tendon load-deformation properties and cross-sectional area. *J Appl Physiol* 2003; 95:2375–2380.
[2]. Kongsgaard M, Aagaard P, Kjaer M et al. Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. *J Appl Physiol* 2005; 99:1965–1971.
[3]. van Hauten C, Granrath M, Ostapczuk MS et al. Training adaptation of the achilles tendon in competitive athletes sonographic standard values for the achilles tendon. *Z Orthop Unfall* 2011; 149:699–704.
[4]. Cassel M, Carlsohn A, Fröhlich K et al. Tendon adaptation to sport-specific loading in adolescent athletes. *Int J Sports Med* 2015; 37:159–164.
[5]. Cassel M, Intziegianni K, Risch L et al. Physiological tendon thickness adaptation in adolescent elite athletes: a longitudinal study. *Front Physiol* 2017; 8: 1–8.
[6]. Kjaer M, Langberg H, Heinemeier K et al. From mechanical loading to collagen synthesis, structural changes and function in human tendon. *Scand J Med Sci Sports* 2009; 19:500–510.
[7]. Heinemeier KM, Kjaer M. In vivo investigation of tendon responses to mechanical loading. *J Musculoskelet Neuronal Interact* 2011; 11:115–123.
[8]. Bashford GR, Tomsen N, Arya S et al. Tendinopathy discrimination by use of spatial frequency parameters in ultrasound B-mode images. *IEEE Trans Med Imaging* 2008; 27:608–615.
[9]. Pearson SJ, Engel AJ, Bashford GR. Changes in tendon spatial frequency parameters with loading. *J Biomech* 2017; 57:136–140.
[10]. Kulig K, Chang YJ, Winiarski S et al. Ultrasound-based tendon micromorphology predicts mechanical characteristics of degenerated tendons. *Ultrasound Med Biol* 2016; 42:664–673.
[11]. Kulig K, Landel R, Chang YJ et al. Patellar tendon morphology in volleyball athletes with and without patellar tendinopathy. *Scand J Med Sci Sports* 2013; 23:e81–8.
[12]. Ohberg L, Alfredson H. Ultrasound guided sclerosis of neovessels in painful chronic Achilles tendinosis: pilot study of a new treatment. *Br J Sports Med* 2002; 36:173–175, discussion 176–7.
[13]. Boesen AP, Boesen MI, Torp-Pedersen S et al. Associations between abnormal ultrasound color Doppler measures and tendon pain symptoms in badminton players during a season: a prospective cohort study. *Am J Sports Med* 2012; 40:548–555.
[14]. Risch L, Cassel M, Mayer F. Acute effect of running exercise on physiological Achilles tendon blood flow. *Scand J Med Sci Sports* 2017:16–19.
[15]. Cassel M, Baur H, Hirschmüller A et al. Prevalence of Achilles and patellar tendinopathy and their association to intratendinous changes in adolescent athletes. *Scand J Med Sci Sports* 2015; 25:e310–e318.
[16]. Risch L, Wochatz M, Messerschmidt J et al. Reliability of evaluating achilles tendon vascularization assessed with doppler ultrasound advanced dynamic flow. *J Ultrasound Med* 2017:5–7.
[17]. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33:159–174.
[18]. Hirschmüller A, Frey V, Deibert P et al. Achilles tendon power Doppler sonography in 953 long distance runners – a cross sectional study. *Ultraschall Med* 2010; 31:387–393.
[19]. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med* 2009; 43:409–416.
[20]. Schmidt WA, Schmidt H, Schicke B et al. Standard reference values for musculoskeletal ultrasonography. *Ann Rheum Dis* 2004; 63:988–994.
[21]. Depalle B, Qin Z, Shefelbine SJ et al. Influence of cross-link structure, density and mechanical properties in the mesoscale deformation mechanisms of collagen fibrils. *J Mech Behav Biomed Mater* 2015; 52:1–13.
[22]. Intziegianni K, Cassel M, Hain G et al. Gender differences of achilles tendon cross-sectional area during loading. *Sport Med Int Open* 2017; 1: E135–E140.
[23]. Heinemeier KM, Olesen JL, Haddad F et al. Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. *J Physiol* 2007; 582:1303–1316.
[24]. Couppé C, Hansen P, Kongsgaard M et al. Mechanical properties and collagen cross-linking of the patellar tendon in old and young men. *J Appl Physiol* 2009; 107:880–886.
[25]. Arya S, Kulig K. Tendinopathy alters mechanical and material properties of the Achilles tendon. *J Appl Physiol* 2010; 108:670–675.
[26]. Cook JL, Rio E, Purdam CR et al. Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? *Br J Sports Med* 2016; 50:1187–1191.