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Correlations between Achilles tendon material and structural properties and quantitative magnetic resonance imagining in different athletic populations

Andrea Monte [a,b,1,*], Jiri Skypala [a,1], Dominik Vilimek [c], Vladimir Juras [d], Daniel Jandacka [a]

[a] Human Motion Diagnostic Center, Department of Human Movement Studies, University of Ostrava, 70200 Ostrava, Czech Republic
[b] Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[c] Department of Cybernetics and Biomedical Engineering, Faculty of Electrical Engineering and Computer Science, VSB - Technical University of Ostrava, 17. listopadu 15, Ostrava - Poruba 70800, Czech Republic
[d] High Field MR Centre, Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Austria

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ABSTRACT

Achilles tendon stiffness (kAT) and Young's modulus (yAT) are important determinants of tendon function. However, their evaluation requires sophisticated equipment and time-consuming procedures. The goal of this study was twofold: to compare kAT and yAT between populations using the classical approach proposed in the literature (a combination of ultrasound and force data) and the MRI technique to understand the MRI's capability in determining differences in kAT and yAT. Furthermore, we investigated potential correlations between short and long T2* relaxation time, kAT and yAT to determine whether T2* relaxation time may be associated with material or structural properties. Twelve endurance and power athlete, and twelve healthy controls were recruited. AT T2* short and long components were measured using standard gradient echo MRI at rest, while kAT and yAT were evaluated using the classical method (combination of ultrasound and dynamometric measurements). Power athletes had the highest kAT (3064 ± 260, 2714 ± 260 and 2238 ± 189 N/mm for power athletes, endurance athletes and healthy control, respectively) and yAT (2.39 ± 0.28, 1.64 ± 0.22 and 1.97 ± 0.32 GPa for power athletes, endurance athletes and healthy control, respectively) and the lowest T2* short component (0.58 ± 0.07, 0.77 ± 0.06 and 0.74 ± 0.08 ms for power athletes, endurance athletes and healthy control, respectively). Endurance athletes had the highest T2* long component value. No correlations were reported between T2* long component, kAT or yAT in the investigated populations, whereas the T2* short component was negatively correlated with yAT. These results suggest that T2* short component could be used to investigate the differences in AT material properties in different populations.

1. Introduction

In human movement, the viscoelastic tissues (e.g., tendons) serve diverse functions, including metabolic energy conservation, amplification of muscle power output and attenuation of muscle power input (Roberts and Azizi, 2011). Hence, tendon adaptation in response to mechanical loading seems essential to maintain movement efficiency and reduce the risk of damage.

After acute exercise or training, collagen synthesis is elevated (Langberg et al., 2001, 1999; Miller et al., 2005; Moerch et al., 2013) and short- and long-term exposure to increased stress leads to tendon material and morphological changes. In many cases, increased loading causes an increase in stiffness (i.e., resistance to deformation) and Young's modulus, which characterize material properties as a measure of stiffness when tendon dimensions are taken into account (Arampatzis et al., 2007). However, changes in structural (stiffness) and/or material properties (Young's modulus) have been reported during ageing, disuse or disease (Magnusson and Kjaer, 2019). For example, as revised by Magnusson and Kjaer (2019) the tendinopathic process could reduce tendon stiffness and affect collagen organization. Therefore, the investigation of the tendon's structural and material properties is of scientific interest.

* Corresponding author at: University of Ostrava, Department of Human Movement Studies, Czech Republic.
E-mail address: andrea.monte@univr.it (A. Monte).
[1] These authors contributed equally to the manuscript and share the first position.

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Among the viscoelastic tissues of the human body, the Achilles tendon (AT) is considered one of the key evolutionary advantages of human locomotion (Malvankar and Khan, 2011). AT can adapt its structural and material characteristics in response to mechanical loading. Indeed, different groups of participants displayed different AT structural and material properties. For example, Wiesinger et al. (2016) showed that elite athletes showed distinctive sets of the AT's structural, material and morphological properties. In this regard, power athletes (e.g., ski jumpers) showed higher AT stiffness compared to healthy control or water polo players. Chang et al. (2020) showed similar results, observing that basketball players had higher AT stiffness compared to healthy control. Lastly, AT structural and material measurements are a potential diagnostic method for assessing Achilles tendinopathy and AT degeneration (Coombes et al., 2018; Obst et al., 2018). Hence, it is evident the importance of a correct evaluation of AT's structural and material properties.

Magnetic resonance imaging (MRI) and ultrasound (US) are commonly used imaging modalities to investigate structural changes in tendons. Although the former can be considered an important diagnostic tool, it is not possible to quantify AT stiffness and Young's modulus (e.g., material properties). Indeed, assessing these parameters needs combining ultrasound measurement with other equipment (e.g., dynamometric and electromyographic data), making this approach unsuitable for a large audience. Recent advances in MRI analysis could avoid this methodological problem. Quantitative MRI, based on ultra-short time to echo (UTE) imaging with T2* mapping of the tendon is a reproducible method (Agergaard et al., 2021), which has been proposed to correlate with collagen structure and water content in tendons (Juras et al., 2013). For example, Pownder et al., (2021) showed that the quantitative MRI technique of the UTE provided a means to non-invasively evaluate the healing process of a mechanically damaged tendon. The method is based on UTE recordings with varying short echo times (TE), which can be used to calculate the T2* by plotting the signal intensity against the TE (Juras et al., 2015, 2013). In this regard, longer T2* relaxation time in tendons has been suggested to represent a greater amount of free water protons between collagen fibres that could reflect increased water content and/or disorganized collagen alignment (Juras et al., 2013, 2012). Furthermore, T2* relaxation times can be computed using a mono- or a bi-exponential function. In the former case, the result represents the weighted mean of fast and slow T2* relaxation times (Juras et al., 2013), while in the latter case, it can be possible to analyze the short and long component of the T2* relaxation time which reflect increased content of bound or unbound "free" water molecules, respectively. However, it is unknown if T2* mapping can be a suitable non-invasive methodology to evaluate subtle differences in tendons over healthy populations (e.g., between endurance and power athletes or controls). Furthermore, it is not known if the T2* and its components (short and long components) are more related to structural (stiffness) or material (Young's modulus) tendon properties.

Hence, the study used quantitative MRI and ultrasound techniques to compare AT material and structural properties among endurance, power athletes and healthy control populations and to determine the relationships between T2* relaxation time, Young's modulus and stiffness. To accomplish this, our protocol relied on a standard T2* gradient echo sequence (according to Jandacka et al., 2020), which better suited the 1.5 T MRI scanner's capabilities. As previously reported in the introduction, we hypothesize significant differences between populations. For example, higher values of tendon stiffness and Young's modulus are expected in power athletes compared to the other populations. Furthermore, we hypothesize that the T2* relaxation time was correlated to the material (Young's modulus) rather than to the structural properties of the AT (stiffness). Moreover, since the T2* short component is related to the bound water molecules restricted in motion due to the high level of collagen organization, we hypothesized significant negative correlation between T2* short component and AT material properties.

2. Methods

2.1. Participants

Thirty-six participants aged 18–30 participated in this study. We recruited 12 power athletes, 12 endurance athletes and 12 untrained healthy control (see Table 1). The sample size calculation was performed using G*Power. Input parameters for the one-way ANOVA were: effect size = 0.57, Alfa level = 0.05, Estimated Power = 0.8, and groups = 3. The effect size was calculated based on the differences between populations in AT stiffness and Young's modulus collected during pilot tests.

Participant exclusion criteria were related to AT or triceps surae health problems. Participants with AT pain, a history of AT rupture or surgery, pain in calf muscles, and neurological and progressive severe illnesses were not recruited for this project. A healthy control participant was defined as a person who may be recreationally active but is not training for or participating in a competitive sport. Endurance and power athletes were the people who participated in competitions and training activities finalized to increase performance levels. Male and female power athletes were basketball, volleyball and floorball players. Endurance athletes were runners competing at the national level, while control participants were moderately active (see Table 1). The running volume per week of the runners in the group was 68.8 ± 33.2 km. The competitive specialization of the runners was 1500 m–10 km. The best personal times for 5 km and 10 km ranged from 17:25 to 25:00 min and from 36:47 to 58:15 min for female, respectively, while ranged from 14:32 to 16:30 min and from 30:26 to 35:00 min for male, respectively.

All participants provided written informed consent before the test. The research project was approved by the University of Ostrava ethics committee (OU-53107/45-2022).

2.2. Protocol

Before the laboratory visit, participants had no physical activity for 24 h. Each participant visited the laboratory on one single occasion. After 20 min rest in the lying position, the AT of the right lower limb was analyzed using a 1.5 T Siemens Magnetom Sempra Scanner (Siemens, Erlanger, Germany). Afterwards, the participant was accompanied in the biomechanical lab. The free AT (from the soleus MTJ to its insertion) material and structural properties were evaluated using a combination of ultrasound, kinetic and kinematic data after warm-up.

2.3. Procedures

2.3.1. MRI

Magnetic resonance (1.5 T Siemens Magnetom Sempre Scanner) data were acquired with the 16-channel head coil modified for AT imaging. Each participant was supine with the right ankle fixed at a 90° angle

Table 1
Participants characteristics and AT parameters and joints angles are reported as mean ± SD.

	Power athletes (6F, 6M)	Endurance athletes (7F, 5M)	Control (6F, 6M)
Age (year)	23.92 ± 2.67	23.30 ± 3.26	23.32 ± 3.52
Height (m)	1.77 ± 0.09	1.74 ± 0.09	1.74 ± 0.11
Body mass (kg)	75.65 ± 12.94	64.10 ± 8.12*	65.35 ± 10.87*
BMI (kg/m2)	22.85 ± 3.99	21.23 ± 1.76	21.51 ± 2.59
Experience (year)	13.33 ± 3.45	9.55 ± 4.52	/
Session (nr per week)	5.25 ± 1.14	7.09 ± 2.77	/
AT CSA (cm^2)	0.83 ± 0.25	0.80 ± 0.24	0.75 ± 0.17*^
AT length (cm)	4.83 ± 0.93	4.69 ± 1.11	4.78 ± 0.95
F_{max} (N)	1438 ± 210	1171 ± 142*	1112 ± 107*
Knee angle at F_{max} (°)	88 ± 1	88 ± 1	89 ± 1
Ankle angle at F_{max} (°)	89 ± 1	89 ± 1	89 ± 1

*Significant difference compared to power athletes: $P < 0.05$.
^Significant difference compared to endurance athletes: $P < 0.05$.

inside the proper coil. The protocol consisted of morphological part using the axial Turbo Spin Echo (TSE) T2-weighted, sagittal Spin Echo (SE) T1-weighted, coronal TSE T1, and axial and sagittal fat saturated TSE PD weighted and quantitative using the two identical gradient echo T2* sequences with a total of ten echo times: TE = 3.78, 7.28, 10.77, 14.28, 17.15, 21.28, 23.52, 28.28, 29.89, and 35.28 ms. Other parameters were set as follows: repetition time = 485 ms, field of view = 205 × 256 mm, resolution 0.6 × 0.6 × 3 mm, flip angle 60°, bandwidth 415, slices 11, resulting in a total acquisition time of 6:40 min. The acquisition time of whole protocol is approximately 20 min. The sequence parameters of the whole MR examination protocol have been reported by Jandacka et al., (2020).

2.3.2. *Ultrasound protocol*

The participants performed 5 min of running at the self-selected speed and ten squat-jump as a warm-up.

The participants were then seated in a custom-made chair with the hip, knee, and ankle at 90°. The lower-limb joints were blocked to this position by steel crossbars to prevent lower limb motion (see Fig. 1), creating a set-up able to test the plantar flexor muscles in an "isometric" (i.e., fixed-end contraction) condition. Indeed, the participants were fixed at the knee level, allowing the plantar flexor to push as hard as possible without heel detachment.

A total of seven reflective markers were attached to the skin. Three markers were attached to the AT insertion, 1.5 and 3 cm above the insertion, while the fourth marker was placed on the ultrasound probe (Tecchio et al., 2022). These markers were used to reconstruct the AT line of action, considering the curvature (Tecchio et al., 2022). Finally, the remaining three markers were placed on the lateral malleolus, foot toe and knee. Marker positions were acquired using a motion capture system (Qualysis, 9x Oqus 700+, 1x Oqus 510, 240 Hz, Gothenburg, Sweden).

The participants performed three maximum voluntary isometric contractions (from resting to maximum contraction intensity) of the plantar flexors. The force generated by the muscles was collected using a force plate (Kistler, 9287CAQ02, 1200 Hz, Winterthur, Switzerland). Electromyography activity of the soleus and gastrocnemius lateralis muscles was recorded to monitor muscle activation. AT length was

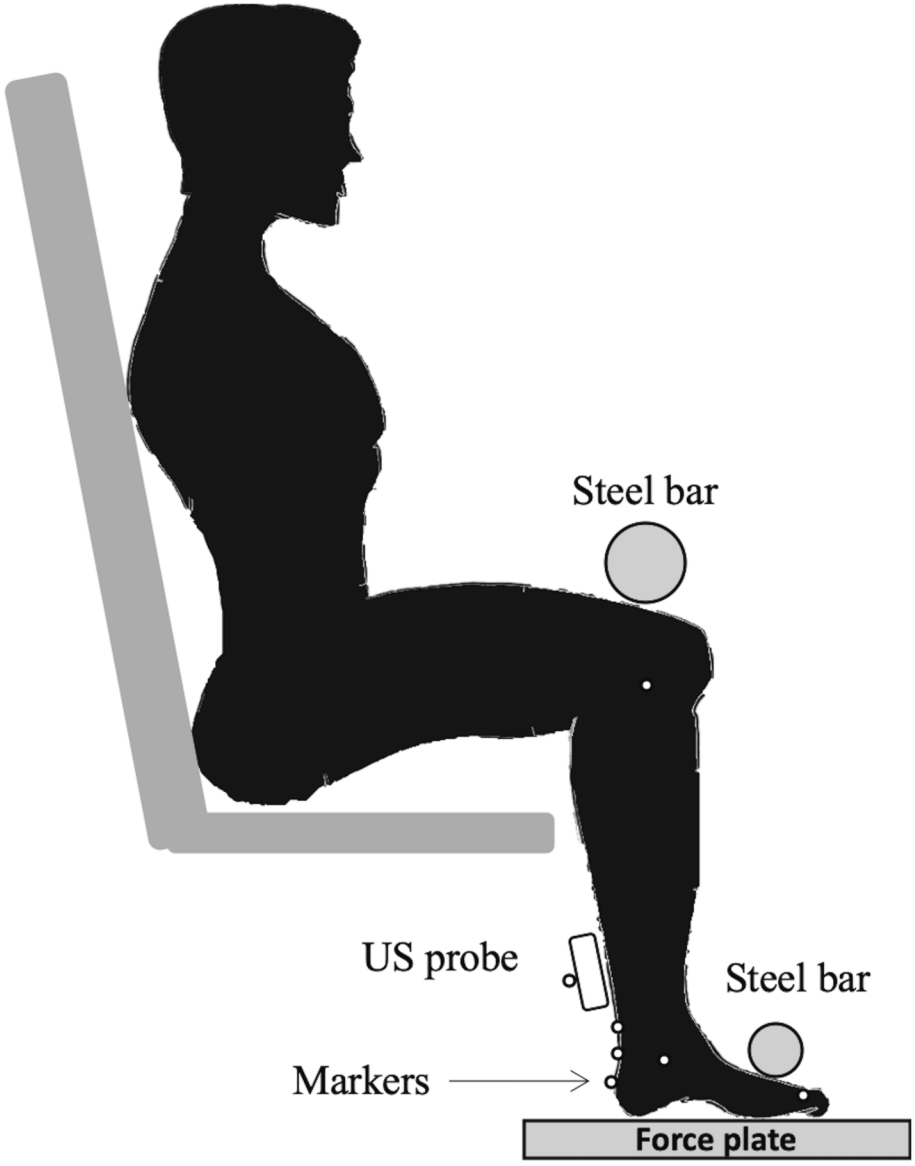

Fig. 1. Experimental design for the collection of force data and ultrasound data. Participants were seated in a custom designed chair with their right foot placed on a force plate. Subjects were seated with hips and knees in 90° of flexion and the foot in a neutral position (0° dorsiflexion). The ultrasound transducer was positioned on the AT MTJ. Iron cross-bars firmly blocked the participants' position.

recorded using B-mode ultrasound imaging (Mindray Z5, Shenzhen, China) with a 4.5 mm linear array probe (sample frequency: 37 Hz) positioned along the tendon to visualize the soleus muscle–tendon junction. All instrumentations (motion capture, force plate and ultrasound) were synchronized.

2.4. Data analysis

2.4.1. MRI

The T2* short and long components were calculated for the entire free tendon (from the soleus MTJ to the calcaneus insertion). For these measurements, the regions of interest (ROIs) at the AT were manually drawn from sagittal images using a custom-made MATLAB script (MathWorks v. R2020a, Natick, MA, USA) (see Fig. 2b). AT T2* relaxation time values were calculated from the mean intensity of ROI in respective echo times using a bi-exponential fitting procedure in a custom-made Interactive Data Language (IDL v.6.3, Boulder, CO, USA). These analyses were performed in accordance with previous research (Juras et al., 2013, 2012). The T2* short and long components were obtained at the end of these procedures by the following equation:

$$S_m = A_0 \cdot exp\left(-\frac{TE}{A_1}\right) + A_2$$

Where A_0 represents the signal intensity at the TE < 1 (or M_0, equilibrium magnetization and local receiver coil gain), A_1 corresponds to the T2*m, and A_2 is the baseline (mostly the noise). Bi-exponential fitting was performed using the equation below:

$$S_b = B_0 \cdot exp\left(-\frac{TE}{B_1}\right) + B_2 \cdot \exp\left(-\frac{TE}{B_3}\right) + B_4$$

Where the short component of bi-exponential fitting (T2*s) is represented by B_1, the long component of bi-exponential fitting (T2*l) by B_3. The B4 is the offset given primarily by noise. The B_0 and B_2 are the component ratios.

2.4.2. Ultrasound protocol

AT force was calculated as the ratio of the plantar flexor moment (product of ground reaction force and external moment arm, where the latter was calculated as the distance between the lateral malleolus and the force vector) and the AT moment arm (computed taking into account the AT curvature as the perpendicular distance from the ankle centre of rotation and the AT line of action; (Tecchio et al., 2022)).

For each participant, the contraction with the greatest AT force (Fmax) was used for further analysis.

AT length was calculated as the distance between the position of the soleus MTJ (manually tracked using Tracker, Physlets. org) and AT insertion, taking into account AT curvature (calculating the vector sum between each of the markers; Tecchio et al., 2022),

By combining AT force (F) and tendon elongation (L) values, a force–length relationship was obtained for each participant (Maganaris and Paul, 2002). This relationship was fitted with a 2nd-order polynomial fit (the determination coefficient (R^2) of this relationship was: 0.94 ± 0.03) (Maganaris and Paul, 2002). Tendon stress and strain were calculated by dividing tendon force and deformation by the AT cross-sectional area (CSA) and the resting length of the free AT, respectively (Maganaris and Paul, 2002). AT CSA was calculated based on the MRI images (transversal plane) collected at the level of malleoli, while AT resting length was obtained measuring the distance between the origin and the insertion of the free tendon by means of the MRI image on the longitudinal plane. Tendon stiffness (ΔF/ΔL) and Young's modulus (Δstress/Δstrain) were calculated in the final 20 % of the force–displacement and stress–strain curves, respectively, assuming a linear relationship between force and elongation in this region (Maganaris and Paul, 2000; Monte and Zignoli, 2021).

2.5. Statistics

Data were checked for normality distribution using the Komogorov-Smirnov test. Between-group differences in all the investigated parameters were tested with a one-way ANOVA. A Bonferroni corrected post hoc test was performed in case of significant effect. Correlations between variables were assessed using Pearson's correlation coefficients. All the statistics were performed using R (v.3), and the significance level was set to alfa = 0.05.

3. Results

No significant differences in age, body height and AT length were observed between groups. On the other hand, significant differences were reported in body mass, AT CSA and maximal force (Fmax). In particular, power athletes showed higher values of body mass, AT CSA and Fmax compared to all the other groups. In contrast, no significant differences were observed between healthy controls and endurance athletes (for more details, see Table 1). To note, no significant differences were reported in ankle and knee joint angles between populations. Furthermore, the displacements of ankle and knee joints could be considered negligible (<2°).

T2* short and long components showed a significant main effect of group ($P < 0.001$) (see Fig. 3). T2* long component was significantly lower in healthy control, compared to the other populations, while no significant difference was reported between power and endurance athletes. Power athletes showed the lowest T2* short component values, while no significant differences were observed between healthy controls and endurance athletes.

Significant differences between groups were observed for AT stiffness and Young's modulus ($P < 0.001$). Healthy control and endurance athletes showed the lowest AT stiffness and Young's modulus values, respectively (see Fig. 3).

T2* short component was significantly correlated with the Young's modulus in all the investigated populations (control: $R^2 = 0.38$, $P = 0.032$; endurance: $R^2 = 0.35$, $P = 0.039$; power: $R^2 = 0.54$, $P = 0.007$; see Fig. 4 for further details). However, T2* long component was not correlated with the AT material properties (i.e., Young's modulus). In addition, T2* short and long components were not correlated with AT

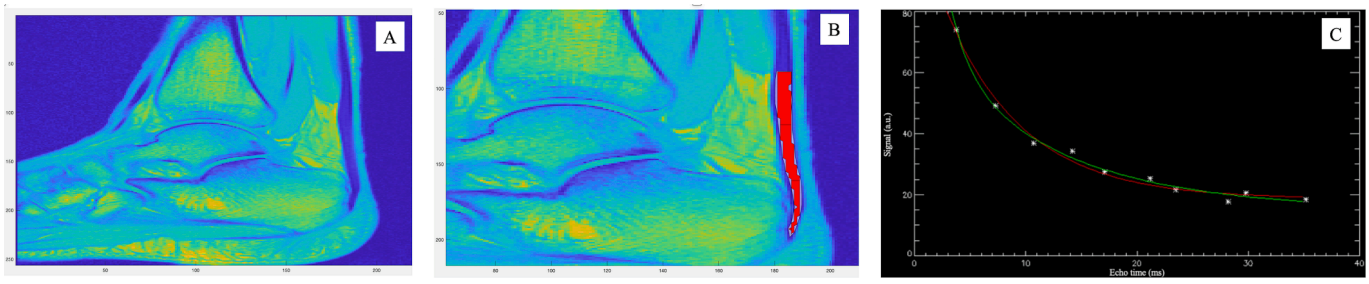

Fig. 2. Overview of methodology: (A) Magnetic resonance image, (B) Achilles tendon ROI (red region) and T2*-weighted image, (C) T2* relaxation time of the free Achilles tendon fitted by a bi-exponential function.

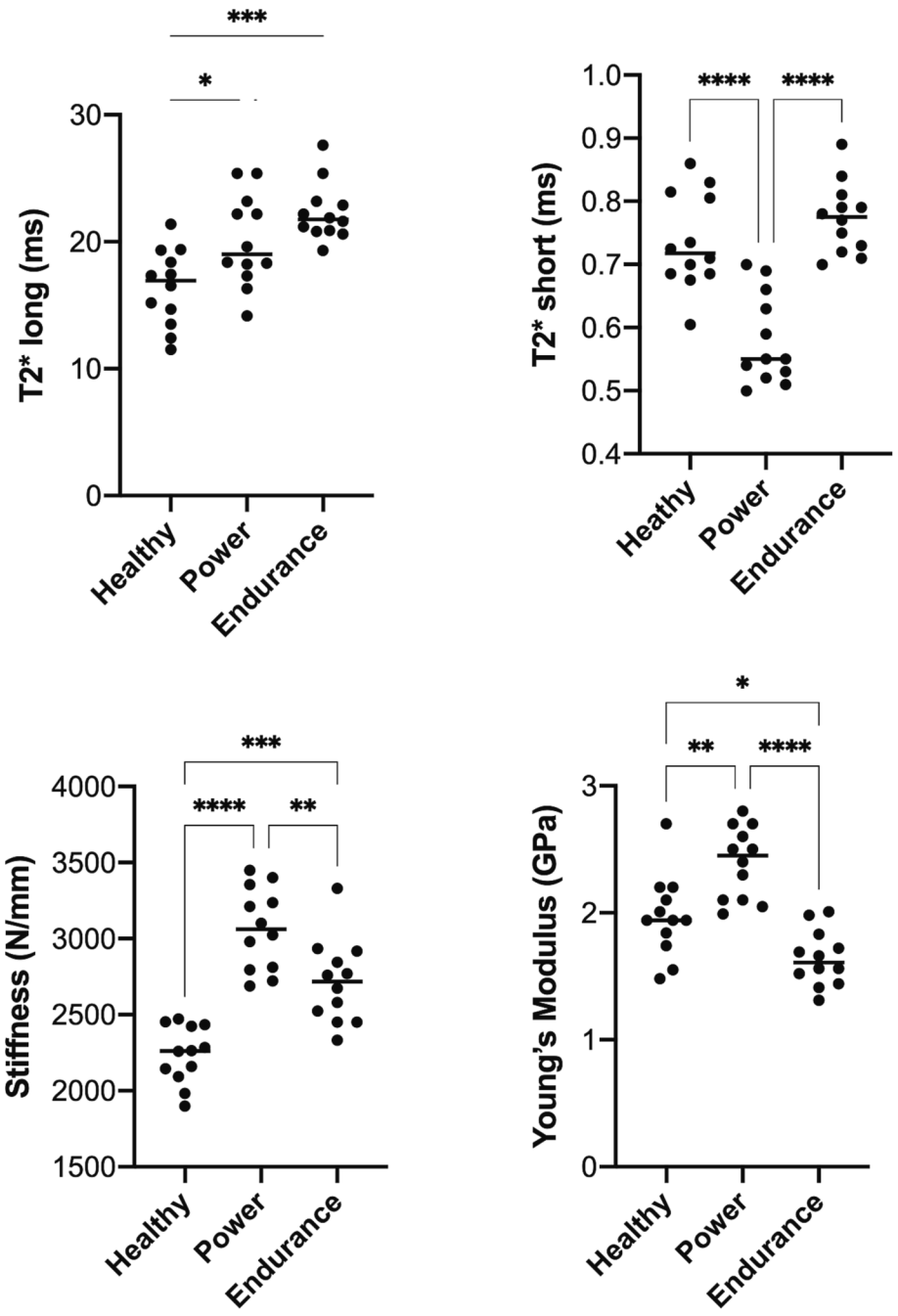

Fig. 3. T2* short and long components, Young's modulus and AT stiffness for all the investigated populations. Dots refer to individual values. Mean values and standard deviation are also reported.

stiffness in all the investigated populations.

4. Discussion

This study used quantitative MRI and ultrasound techniques to compare AT material and structural properties among endurance, power athletes and healthy control populations. In addition, we further tested the relationships between T2* relaxation time, Young's modulus and stiffness. To note, our data showed that the T2* long component was longer in trained populations, which is believed to be indicative (at least in part) of collagen disorganization (Gärdin et al., 2016; Juras et al., 2013). On the other hand, T2* short component was comparable between healthy controls and endurance athletes, whereas it was lower in power athletes. These results suggest that power athletes had superior collagen organization.

This is the first study that used a bi-exponential function to investigate the differences between populations and the possible correlations between T2* short/long component and the AT properties in trained and untrained populations.

We hypothesized that the T2* relaxation time was correlated to the material (Young's modulus) rather than to the structural properties of the AT (stiffness). In this regard, we further hypothesized a significant correlation between T2* short-component and AT material properties. In accordance with our hypotheses, T2* long component was not significantly correlated with AT properties. On the other hand, T2* short component was negatively correlated with the Young's modulus in all the investigated populations. This result suggests that the T2* short component could detect changes in the AT material properties, but not

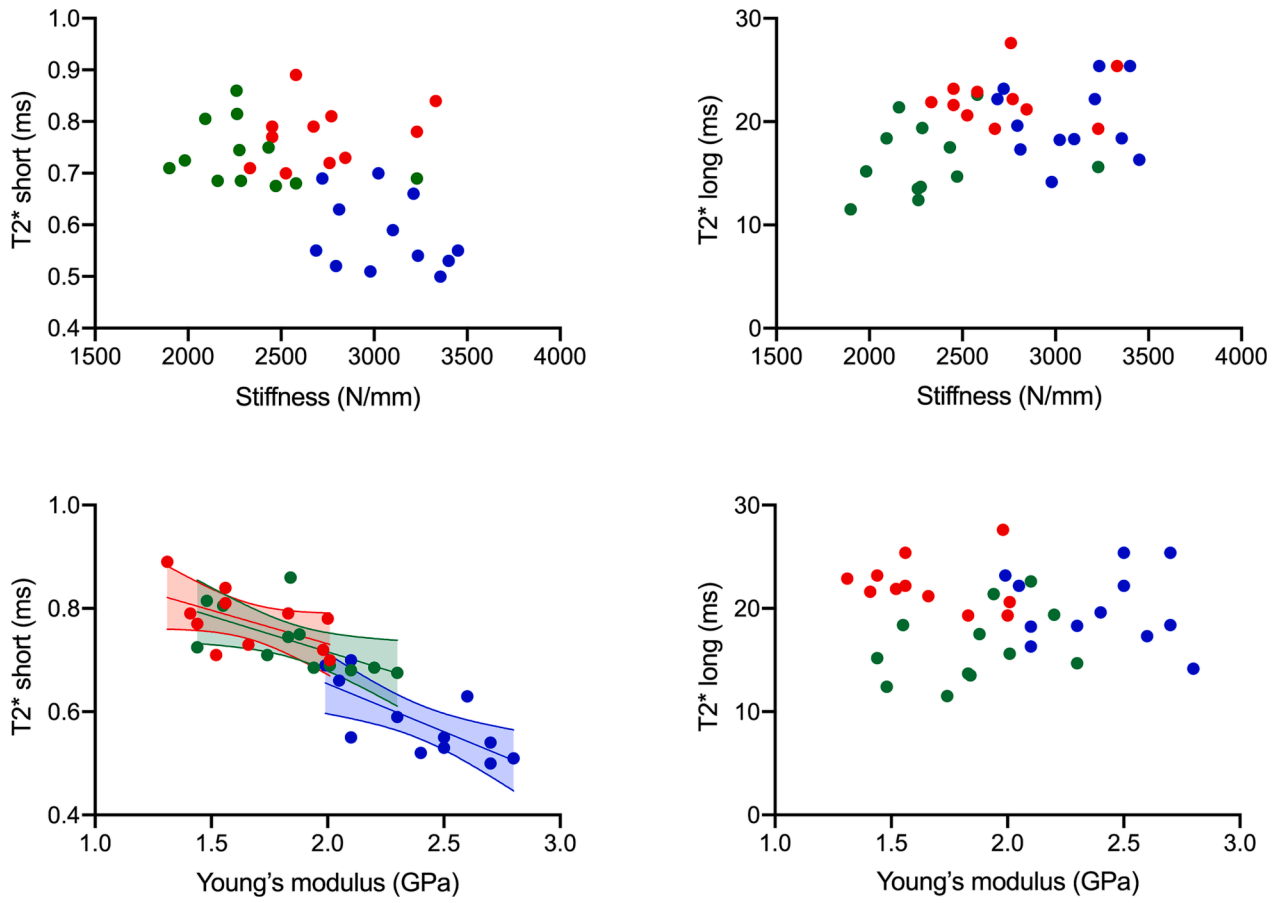

Fig. 4. Upper panels: correlations between AT stiffness and T2* short and long components. Lower panels: correlations between Young's modulus and T2* short and long components. Red, green and blue dots refer to endurance athletes, control and power athletes, respectively. Shaded areas refer to 95 % confidence intervals and were reported only for significant correlations. The statistical results for the correlations between Young's modulus and T2* short were: Endurance athletes: $y = -0.129 \cdot T2^*$ short component + 0.99; $F = 4.19$, $R^2 = 0.35$, $r = 0.59$, $P = 0.039$, 95 % confidence interval = 0.75–1.23 and 4.49–41.12 for the Y and X intercept, respectively. Power athletes: $y = -0.185 \cdot T2^*$ short component + 1.02; $F = 11.57$, $R^2 = 0.54$, $r = 0.73$, $P = 0.007$, 95 % confidence interval = 0.73–1.32 and 4.28–11.49 for the Y and X intercept, respectively. Control: $y = -0.139 \cdot T2^*$ short component + 0.99; $F = 6.17$, $R^2 = 0.38$, $r = 0.62$, $P = 0.032$, 95 % confidence interval = 0.76–1.23 and 4.65–53.29 for the Y and X intercept, respectively.

in the structural ones. Malmgaard-Clausen et al. (Malmgaard-Clausen et al., 2021), reported a negative correlation between the T2* relaxation time obtained using the mono-exponential function and Young's modulus, reinforcing our data and those reported in the bovine tendon (Diaz et al., 2012). Taken together, these results suggest that the T2* short component could have a functional implication for evaluating the changes in AT material properties. Indeed, Kijowski et al. (2017) showed that patients with patellar tendinopathy had significantly higher T2* long component compared to healthy volunteers, reinforcing the idea that the bi-exponential function can detect changes in the composition and microstructure of tendons.

Noticeably, the variation in the T2* long component among studies is much greater than that of the short component, which can explain the lower clinical specificity and sensitivity of the T2* long component. Accordingly, Breda et al. (2020) showed that quantitative multi-compartment T2*analysis in heterogeneous tissues (e.g., patellar tendon) could be facilitated using a bi-exponential fitting, which in turn, can differentiate between tissue compartments with comparable water pool, leading to the identification of tissue-specific T2* biomarkers with high repeatability.

In agreement with previous studies (Devaprakash et al., 2020; Wiesinger et al., 2016), significant differences were reported between the populations in AT stiffness and Young's modulus, suggesting different structural and material characteristics among the population, as expected. Furthermore, the strong negative correlations reported between T2* short component and Young's modulus suggest that the short component could provide important insight into the AT material properties. The short component has been associated with the water molecule restricted in motion due to the presence of collagen (Juras et al., 2015, 2013, 2012). Therefore, it is possible to speculate that it represents a better representation of the collagen organization and quality than the long component. Our data support this speculation. Indeed, higher values of Young's modulus are associated with superior collagen characteristics. Therefore, the negative correlations between Young's modulus and T2* short component suggest that the subjects with the lower values of T2* short component (e.g., lower values of water molecule inside the tendon) are those with the superior AT material characteristics. On the contrary, the lack of significant correlation between the T2* long component and the AT structural or material properties suggests that the T2* long component should not be considered a valid proxy for the free AT characteristics.

4.1. Limitations

Our study focused exclusively on the AT and included healthy volunteers. Future studies could involve patients with Achilles tendinopathy and expand these results to the patellar tendon.

To note, our study did not investigate the mechanisms responsible for differences in T2* relaxation time between populations. Additional studies correlating T2* relaxation time with collagen, water,

proteoglycan, and lipid content using biochemical assays are needed to investigate the factors responsible for changes in the T2* relaxation time. Indeed, the correlations reported in this study cannot reveal causality between an altered T2* signal and the presence of collagen disorganization or structural alterations. Furthermore, MRI analysis was performed at rest, while the evaluation of AT material and structural properties during maximum in-vivo contraction. These different procedures imply that we are comparing data coming from two different parts of the AT stress–strain (force–elongation) curve. However, with the current MRI apparatus is not possible to solve this methodological limitation due to the impossibility in performing maximum voluntary contraction inside the MRI coil. Moreover, in our study, we focused on one slice of the AT, which may limit the generalizability of our findings. Furthermore, we did not calculate the percentage of short and long T2* within the tendon, which is a potential area for future research. Additionally, inclusion of a clinical assessment could enhance our results to a broader clinical interpretation. In the current project, we did not report repeatability data. In previous projects, we conducted some repeatability analysis on other data, where we utilized the same procedure. However, we do not have this information for the current project. Finally, even if the precision of the fitting curves suggested that a bi-component analysis was a valid and reliable tool to investigate T2* relaxation time, we adopted an MRI scan with a magnetic field of only 1.5 Tesla and therefore we weren't able to use UTE imaging. Further studies with higher magnetic intensity (e.g., 3 Tesla) could provide further insights into the reported correlations.

5. Conclusions

This study provides novel evidence about the role of T2* short and long components in determining the differences between healthy controls and athletes. Last but not least, we showed that the T2* long component do not reflect differences in the material or structural properties of the Achilles tendon. On the other hand, T2* short component was correlated with Young's Modulus of AT, suggesting that T2* short component can be described as reflecting, in general, a similar characteristic of AT material properties.

CRediT authorship contribution statement

Andrea Monte: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.. **Jiri Skypala:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dominik Vilimek:** Writing – review & editing, Validation, Supervision, Software, Data curation. **Vladimir Juras:** Writing – review & editing, Validation, Supervision, Data curation. **Daniel Jandacka:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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