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Achilles tendon structure is associated with regular running volume and biomechanics

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ABSTRACT

Achilles tendinopathy was reported to have the highest incidence proportion of all running-related injuries. The purpose of this study was to analyse the association between the Achilles tendon structure and running activity status. 350 healthy participants (runners and inactive controls, 30–50 years) participated in this research. Each participant completed questionnaires: socioeconomic, psychological, physical activity habits, running status and history and VISA-A. Magnetic resonance imaging, anthropological, running biomechanics and 14 days of physical activity monitoring assessments were performed. There was a higher odd of being in the upper quartile of the Achilles tendon T2* relaxation time with higher maximal knee extension moment independent of age and sex. Compared with runners who ran 21–40 km per week, non-runners and those who ran more than 40 km per week had increased odds of having longest the Achilles tendon T2* relaxation time. Regular running of 21 to 40 km per week is related to the Achilles tendon T2* relaxation time indicating possibly better water content and collagen orientation in these runners with compare to inactive non-runners or highly active individuals. In addition, Achilles tendon T2* relaxation time as indirect indicator of the Achilles tendon structure was positively related to the maximal knee extension moment during running.

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Introduction

The Achilles tendon (AT) is considered to represent one of the key evolutionary advantages of humans for locomotion. It has been hypothesized that the unique energy storage and release functions of the AT has enabled humans to engage in rapid locomotion (Malvankar & Khan, 2011). Its interaction with the contractile muscles allows for metabolic energy conservation, power amplification and power attenuation during running (Monte et al., 2020). The contribution of tendon elastic strain energy to the positive work generated by the triceps surae muscle tendon unit is more than 50% during running (Lai et al., 2014; Monte et al., 2020). Nevertheless, the chronic overloading of the AT is a major running-related injury with a 5 to 20% incidence depending on the population studied (Kakouris et al., 2021; Lagas et al., 2020; Lopes et al., 2012). In recent years, Achilles tendinopathy was reported to be the pathology with the highest incidence proportion of all running-related injuries (Kakouris et al., 2021). Therefore, a better understanding of the tendon response to physical (in)activity of the middle-aged population of runners may help both suggest ways to enhance performance and create strategies to promote and protect health in the general population.

A measure that has been used as a marker to assess AT injury, including tendinopathy, is magnetic resonance T2*

relaxation time (T2*RT) which reflects changes in water content and collagen orientation (Juras et al., 2013). Longer T2*RTs have been shown to correlate highly with scores of a subjective measure of AT injury in clinical populations (Juras et al., 2013; Qiao et al., 2017). In addition, it has been shown that AT cross-sectional area and T2*RT is increased in participants with AT tendinopathy, a significant running-related injury (Gärdin et al., 2016; Lopes et al., 2012; Romero-Morales et al., 2019).

Recent evidence has shown that long-term running exercise increases AT cross-sectional area in highly trained runners compared to inactive controls (Devaprakash et al., 2020; Hjerrild et al., 2019). The aforementioned authors concluded that a greater cross-sectional area may reduce the risk of tendon damage from mechanical stress due to an increase in tendon stiffness and a subsequent decrease in tendon stress at a given strain (Hjerrild et al., 2019). Despite this suggestion, it was later shown that there was a longer T2*RT indicating tendinopathy in trained middle-distance runners when compared to healthy inactive controls (Devaprakash et al., 2020). In addition, runners also showed greater cross-sectional area, suggesting a similarity to a condition that is also reported in pathological tendons (Devaprakash et al., 2020).

The properties of the tendon determine its function and injury risk (Arya & Kulig, 2010; Devaprakash et al., 2020). Several previous case control studies have provided evidence

that the AT may be adapted in response to the process of ageing and physical activity levels (Devaprakash et al., 2020; Kongsgaard et al., 2005; Stenroth et al., 2012). For example, acute exercise in humans have been shown to be followed by an increase in both the synthesis and degradation of collagen (Magnusson et al., 2010). In addition, high running distance volume has been shown to be associated with altered biochemical AT properties T2*RT (Devaprakash et al., 2020). However, studies focusing on relationship between running distance and AT properties have only been carried out in two groups (highly active runners and controls) (Devaprakash et al., 2020; Grosse et al., 2015) rather than investigating association between different running distances on AT properties. Therefore, it is unclear whether an increased AT T2*RT may be a sign of running overload and may depend on the structure-specific cumulative load per running session (running biomechanics and technique, the way in which the AT is loaded) and/or the structure-specific capacity when entering a running session (physical (in)activity characteristics, running distance per week) (Bertelsen et al., 2017).

Presently, however, there has been little discussion about the association of running biomechanics and indicators of the AT structural properties such as T2*RT. This question is becoming increasingly important if we consider that the early stages of tendinopathy, although often asymptomatic, usually alters biochemical but not morphological tendon properties (Samiric et al., 2009). The research to date has tended to focus on association of running biomechanics and AT loading. Moreover, more research on AT tendinopathy comes from cross-sectional studies focusing on the differences in running biomechanics between runners with AT tendinopathy and healthy controls, rather than from studies investigating the association between running biomechanics and structural properties of AT or development of AT injury (Almonroeder et al., 2013; Donoghue et al., 2008). It has been shown that non-rearfoot footfall patterns during running induces greater AT forces during the stance phase compared to rearfoot running (Almonroeder et al., 2013). A systematic literature review of studies concentrating on the differences between runners with AT tendinopathy and healthy controls (Munteanu & Barton, 2011) suggested that those with Achilles tendinopathy increased ankle eversion, altered ankle joint dorsiflexion movement and reduced knee flexion during gait. Those with AT tendinopathy also displayed altered knee kinetics (Donoghue et al., 2008; Munteanu & Barton, 2011). A load-induced non-rupture tendinopathy in humans has been associated with localized biochemical and structural changes of the tendon tissue (Pingel et al., 2014).

Therefore, the purpose of this study was to assess the association between the AT structure (T2*RT) and selected biomechanical variables (e.g., footfall pattern and ankle and knee kinematics and kinetics) that have been identified in previous literature to be related to AT loading or chronic running injuries of the AT. We hypothesized that as T2*RT increased, the measured biomechanical variables (e.g., increased knee valgus moment, decreased knee flexion angle, altered footfall pattern) would change to suggest the risk for an AT injury. A second purpose of this study was to determine the association between AT structure and running activity status. We

hypothesized that runners with the highest running distance per week will have an increased probability for increased AT T2*RT levels compared to runners that run a lesser distance. In addition, those biomechanical variables which may be related to AT T2*RTs may also impact the association between AT T2*RT and running distance. For example, runners with the highest running distance per week will have an increased probability for increased AT T2*RTs as compared to runners that run a lesser distance only if they have a running technique that is associated with overuse of the AT.

Methods

This study was part of a larger study, Healthy Ageing in the Industrial Environment (4HAIE) which investigated the influence of air pollution on the incidence of sports-related injuries, physical activity-related injuries, physical (in)activity, health, and quality of life across the lifespan (Jandacka et al., 2020). This 4HAIE study was a prospective cohort study of Czech adults aged 18–65. The general methodology and protocol are described elsewhere (Cipryan et al., 2020; Elavsky et al., 2021; Jandacka et al., 2020).

Participants

Participants were recruited through a professional social science and marketing research company (Focus, Brno, Czech Republic). The original sample ($N = 1314$) was recruited using quota sampling based on the location, age, sex and activity status (active runners and inactive). Participants who did not report diabetes were included. As the highest incidence of AT injury has been reported between 30 and 50 years of age, for this study we selected 350 participants who had valid data in this age range (Lagas et al., 2020; Lantto et al., 2014). The basic characteristics of the research sample according to running distance are presented in Table 1. Ethical approval was obtained from the Ethics and Research Committee of the University of Ostrava, and all participants signed an informed consent form prior to data collection.

Participant inclusion and exclusion criteria were evaluated using an online screening survey (Elavsky et al., 2021). To be included in this study, participants had to be between 30 and 50 years of age upon enrolment. Active runners were required to spend at least 150 minutes per week in moderate-intensity physical activity or 75 minutes in high-intensity physical activity including running (World Health Organization, 2020). They had to run regularly for 6 weeks or longer, at least 10 km per week. Inactive controls had to be otherwise capable of physical activity including running but they did not meet public health recommendations for physical activity performing less than 150 minutes of moderate/vigorous physical activity per week (World Health Organization, 2020). Participants had to own a smartphone with Android or iOS operating system. Participants were excluded if they were smokers and/or reported experiencing acute problems that prohibited physical activity in the last six weeks. Additional exclusion criteria involved contraindications to magnetic resonance imaging or Dual Energy X-ray Absorptiometry (pregnancy, radiological examination in the last 7 days using iodine/barium contrast

Table 1. Baseline characteristics and measurements of the participants according to running distance.

	Running Distance (km/week)			
	0–5 N = 69 Mean ± SD	6–20 N = 114 Mean ± SD	21–40 N = 115 Mean ± SD	≥ 40 N = 52 Mean ± SD
Age (years)	40.9 _a ± 6.1	40.0 _a ± 5.2	39.8 _a ± 5.5	39.7 _a ± 6.1
Sex (female/male)	54%/46%	45%/55%	30%/70%	33%/67%
Mass (kg)	78.8 _a ± 17.4	77.0 _a ± 12.6	77.1 _a ± 12.1	70.4 _b ± 11.6
Height (m)	1.75 _a ± 0.10	1.76 _a ± 0.08	1.78 _a ± 0.09	1.76 _a ± 0.10
Running Velocity (m/s)	2.6 _a ± 0.03	2.9 _b ± 0.4	3.0 _b ± 0.4	3.3 _c ± 0.5
Stride Length (m)	2.0 _a ± 0.03	2.2 _b ± 0.3	2.3 _{b,c} ± 0.3	2.4 _c ± 0.3
Strike Index (%)	12.3 _a ± 14.4	12.7 _a ± 15.1	14.7 _a ± 17.5	18.4 _a ± 19.1
Cadence (steps/minute)	157.4 _a ± 10.4	159.8 _{a,b} ± 9.5	159.0 _a ± 8.4	163.5 _b ± 9.8
Running Distance (km/week)	0.4 _a ± 1.3	14.8 _b ± 8.0	28.2 _c ± 8.0	54.0 _d ± 20.0
Physical activity (step mean count per day wake-hours)	12336 _a ± 3167	14398 _b ± 4350	16744 _c ± 5381	20000 _d ± 5913
VO2 max mL/(kg·min)	34.0 _a ± 6.6	43.5 _b ± 7.3	47.0 _c ± 8.2	52.7 _d ± 7.3
VISA-A Score (%)	83 _a ± 15	95 _b ± 7	95 _b ± 7	92 _b ± 12

Note: VO2 max, Maximum rate of oxygen consumption; VISA-A, index of the clinical severity of Achilles tendinopathy (Robinson, 2001); Subscripts – Values in the same row and sub-table not sharing the same subscript are significantly different at $p < 0.05$ in the two-sided test of equality for column means. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction.

agents, pacemaker, radioactive body, surgical staples, insulin pump, cochlear implant, other metal implants, and foreign bodies such as shrapnel, etc.).

Experimental set-up

Running at their self-selected speed overground, participant's lower extremity kinematics were recorded using a 10-camera high-speed motion capture system (Oqus, Qualisys, Inc., Gothenburg, Sweden). Three force plates built into a 17 m long runway (Kistler, Kistler Instruments AG, Winterthur, Switzerland) were used to collect ground reaction force (GRF) data. Kinematics and ground reaction force data were sampled at a frequency of 240 Hz and 2160 Hz respectively. Overground running speed was controlled using two photocells (OPZZ, EGMedical s.r.o., Brno, Czech Republic) located at intervals of 3 m along the runway. Magnetic resonance data were acquired at a 1.5 T Magnetom Semptra scanner (Siemens, Erlangen, Germany) by a radiologic technologist (D.V., 5 years of experience) with the 16-channel head coil modified for imaging the AT. The protocol consisted of both morphological and T2* mapping using a multi-echo multi-slice sequence (Jandacka et al. (2020)).

Protocol

As part of the 4HAIE study, participants completed baseline assessments in the laboratory. On the first day of the lab visit, participants completed questionnaires on physical activity, running history survey of physical activity habits (KOHL et al., 1988), running status and history questionnaire (Wiegand et al., 2019) and the VISA-A survey as an index of the clinical severity of AT tendinopathy (Robinson, 2001). A graded exercise test in order to determine the maximum rate of oxygen consumption was also conducted on the first day (Cipryan et al., 2020). On the second laboratory visit, magnetic resonance imaging was performed followed by anthropological assessments. Finally, functional lower extremity tests and biomechanical testing were performed by each participant. These two-day baseline measurements were followed by 14 days of

physical activity monitoring using the Fitbit Charge 3 monitor (Fitbit, San Francisco, California, USA), which sampled physical activity during 10 weekdays and two weekends. Fitbit data were downloaded from the Fitbit cloud using the public Application Programming Interface access (or API).

For the biomechanics protocol, thirty-two retroreflective markers and four marker clusters were attached bilaterally to the pelvis, thigh, shank and foot (Jandacka et al., 2020; Malus et al., 2021). Markers were attached on laboratory neutral running shoes (Brooks Launch 5, Brooks Sport Inc., Seattle, WA, USA). Before running, each participant's self-selected running speed was established. For active runners, we used the question: What is your usual speed when you go running for 45 minutes? Non-runners were asked to set the running speed at a pace that will allow them to run as far as possible if they were unable to imagine their running pace for 45 minutes. The participant then ran for two minutes, and the self-selected speed was measured in the last 30 s with photocells (Jandacka et al., 2020). Overground running measurement consisted of eight successful trials on a 17 m long runway at the participant's self-selected speed. The running trial data was considered successful if a participant landed on the force plate with the whole right foot and speed was within $\pm 5\%$ of their self-selected speed.

Data analysis

The kinematic and kinetic data were processed using QTM (Track Manager; Qualisys, Sweden, Göteborg) and Visual3D software (C-Motion, Germantown, Kentucky, KY, USA). Ground-reaction force and marker kinematic data were filtered using a fourth-order Butterworth low-pass filter with cut-off frequencies of 50 and 12 Hz respectively. The proximal and distal ends of the coordinate systems of the lower extremity segments were determined from a calibration trial (Hamill et al., 2014). Foot, ankle and knee three-dimensional angles were calculated using an X-y-z Cardan rotation sequence. Three-dimensional (3D) foot, ankle and knee angles were determined throughout the entire stance phase of gait cycle. Sagittal foot, ankle and knee angles at initial contact with force plate were determined.

In addition, maximum ankle dorsiflexion, knee flexion and ankle eversion during stance phase were also determined. Running speed, stride length and running steps per minute were also calculated (Stanhope et al., 1990). The 3D net internal ankle and knee joint moments were calculated using a Newton–Euler inverse dynamics technique (Selbie et al., 2014). Ankle joint power was calculated as the product of the 3D ankle joint moment and the ankle angular velocity. Positive power indicated energy generation through a concentric action while negative power suggested an eccentric action. All net joint moments and ankle powers were normalized to body mass. The maximum ankle plantar flexion moment, knee extension moment and ankle power generation were determined from the sagittal plane ankle moment signal, sagittal plane knee moment and ankle power, respectively, as the global minimum of the stance phase. Strike index was estimated as initial centre of pressure location and reported as a relative foot length from the posterior calcaneus (Cavanagh & LaFortune, 1980). We calculated the mean and standard deviations for all biomechanical discrete variables from the eight running trials.

Average daily step count was calculated as an objective measure of overall physical activity level based on the Fitbit data. Specifically, mean step count per day during wake-hours was calculated by summing raw step counts directly measured from the Fitbit monitors for each valid day (i.e., day when data for at least 10 waking hours were available), then calculating the mean across fourteen days (Elavsky et al., 2021). Missing data not available for at least 10 waking hours in a day was 3% of all data.

For the image analysis, the regions of interest (ROIs) of the AT were manually drawn from sagittal images using an in-house written MATLAB script (MathWorks v. R2020a, Natick, MA, USA) by experienced image analysts and checked by an

experienced radiologist. An example is shown in Figure 1. T2* maps were calculated from set of T2* weighted images using bi-exponential pixel-wise fitting. The bi-exponential pixel-wise fitting was performed in a custom-written Interactive Data Language (IDL v.6.3, Boulder, CO, USA). Analysis of the AT was carried out in the three regions (insertion, middle, and musculotendon junction). These analyses were performed in accordance with previous research (Juras et al., 2012, 2013).

Expired air was continuously monitored to analyse oxygen and carbon dioxide gas concentrations during the graded exercise test with a breath-by-breath system (Blue Cherry, Geratherm Medical AG, Germany). The highest average oxygen consumption measured over a 30 s running period was used to estimate body mass normalized maximal oxygen consumption (mL/kg/min) (Cipryan et al., 2020).

Average daily step count was calculated as an objective measure of overall physical activity level based on the Fitbit data. Specifically, mean step count per day during wake-hours was calculated by summing raw step counts directly measured from the Fitbit monitors for each valid day (i.e., day when data for at least 10 waking hours were available), then calculating the mean across fourteen days (Elavsky et al., 2021). Missing data not available for at least 10 waking hours in a day was 3% of all data.

Statistical analysis

Achilles tendon T2*RTs were not normally distributed and none of the transformations (e.g., log, square-root, cubic, square, inverse and others) provided a normal distribution of data according to Wilcoxon and Shapiro-Wilk normality test. Therefore, in order to explore the associations between AT T2*RTs and biomechanical variables, and AT T2*RTs and

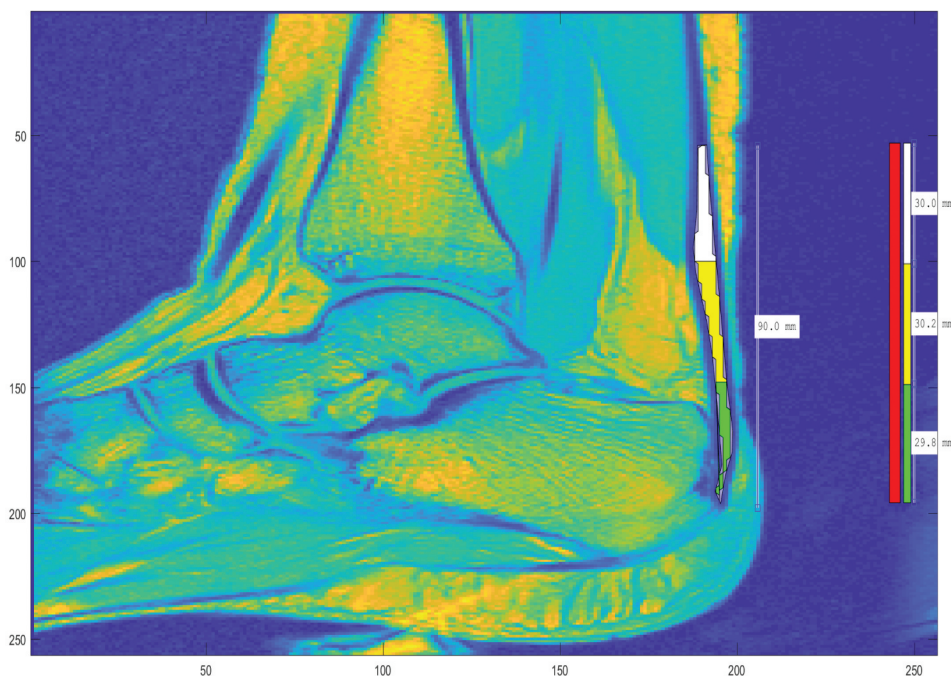


Figure 1. Magnetic resonance image of the Achilles tendon. Region-of-interest placement on T2* image. In green is marked the insertional part of the Achilles tendon which is most often affected by tendinopathy (Juras et al., 2013). This image has been cropped for display purposes.

running distance, we used binary logistic regression and examined whether biomechanical variables or running distance were associated with high values of AT T2*RTs.

There is no standard cut-off for what is considered a "high" AT T2*RT. Therefore, respondents were considered as having high AT relaxation time if they had AT T2*RTs in the upper quartile (more than 1.8 ms). As part of the logistic regression, we also calculated the correlations between the variables that entered the statistical models to understand their inter-relationships. Running distance was used as a categorical variable (≤ 5 km/week; 6–20 km/week; 21–40 km/week; > 40 km/week). Since an increased risk of all running-related injuries when running was reported with distances of more than 64 km per week, and the recommended upper limit of running distance for longevity benefits was suggested to be 48 km per week, we established a reference group of physically active individuals whose training distance was in the range of 21–40 km per week (Lee et al., 2017; Nielsen et al., 2012). All models were controlled for age (continuous) and sex (male; female).

Results

Table 1 presents the socio-demographic characteristics and running-related measures of the 350 participants. When comparing runners based on running activity level (i.e., across categories of running distance), significant differences emerged in several socio-demographic, body composition, fitness level, biomechanical, clinical and physical activity parameters. Specifically, there were differences in sex, mass, aerobic fitness, running speed, spatiotemporal running characteristic, index of clinical severity of AT tendinopathy and overall physical activity level. As would be expected, higher weekly running activity (expressed by running distance) was associated with lower mass and higher aerobic fitness. The groups of inactive

and most active individuals had relatively the fewest individuals engaging in moderate or high-intensity sports outside of their regular running compared to runners with 6–20 km/week and 21–40 km/week. With higher weekly running activity, there was a higher ratio of runners with training for a running race, higher self-selected running speed, running stride length and mean daily step count measured by Fitbit (Table 1). Moreover, the inactive group had a lower subjective index of the clinical severity of Achilles tendinopathy compared to all active groups. The running volume of the groups (as categorized based on the running distance) (Wiegand et al., 2019) was consistent with the running volume data derived from the ACSL (Kohl et al., 1988).

The logistic regression analysis compared participants with affiliations to the highest quartile of AT T2*RTs and the rest of the research sample in the lower quartiles (Table 2). There were more men in the group with the longest AT T2*RTs than in the rest of the sample. The two groups did not differ in age, body mass, level of relative aerobic fitness or subjective level of Achilles tendinopathy. However, those in the highest quartile of AT T2*RTs had significantly lower relative fat, were higher and had a greater maximal knee extension moment normalized to body mass during running. Next, we investigated the association between AT T2*RTs and socio-demographic variables. Results from the logistic regression showed that being male was associated with higher likelihood of having AT T2*RTs in the upper quartile (OR = 2.25), with no differences based on age and education.

Table 2. The basic characteristics and measures according to affiliations of participants in the highest quartile of at T2* RT and the rest of the research sample in the lower quartiles.

	First to Third Quartile		Fourth Quartile	
	Mean/N	SD	Mean/N	SD
Age (years)	40.0a	5.7	40.2a	5.4
Highest level of education				
Primary education	0.0%		0.0%	
Secondary education without matriculation	6.5%		8.0%	
Secondary education with matriculation	36.6%		28.7%	
Higher vocational education	3.1%		5.7%	
University and higher education	53.8%		57.5%	
Sex (Female/Male)	44.5%/55.5%		26.4%/73.6%	
Mass (kg)	75.7a	13.4	78.6a	14.0
Height (m)	1.76a	0.09	1.78b	0.08
Relative fat (%)	28.6a	6.3	27.0b	5.9
Maximum rate of oxygen consumption mL/(kg·min)	43.7a	9.6	45.4a	8.8
VISA-A Total Score (%)	93a	11	91a	12
AT T2* relaxation time (ms)	1.44a	0.72	3.43b	0.58
Running velocity (m/s)	2.9a	0.5	3.0a	0.4
Footstrike angle sagittal (deg)	79.5a	9.5	79.4a	9.2
Max ankle dorsiflexion angle (deg)	85.0a	4.3	85.6a	4.5
Max ankle eversion angle (deg)	−15.5a	4.9	−15.5a	4.6
Knee angle at initial contact (deg)	−10.5a	5.0	−10.7a	4.7
Maximal knee angle (deg)	−39.9a	5.4	−40.1a	4.5
Max ankle plantar flex moment (Nm/kg)	−2.4a	0.4	−2.4a	0.4
Max knee extension moment (Nm/kg)	2.5a	0.5	2.7b	0.5
Max ankle power generation (W/kg)	8.1a	2.1	8.1a	2.5

Note: Subscripts, Values in the same row and sub-table not sharing the same subscript are significantly different at $p < .05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test.

Associations between at T2*RTs and biomechanical variables

We further investigated the association between AT T2*RTs and biomechanical variables using logistic regression. This analysis indicated that there was a greater odd of being in the highest quartile of AT T2*RTs with a higher maximal knee extension moment (OR 1.91; 95% CI 1.07 to 3.42; $p = 0.029$) independent of age and sex. The association for maximal ankle dorsiflexion angle with higher odds of longer AT T2*RTs was not significant (OR:1.06; 95% CI 0.996 to 1.12, $p = 0.069$). There was also association between higher likelihood of having AT T2*RTs in the highest quartile with a higher maximal knee extension moment independent of running speed, age and sex (OR:2.3; 95% CI 1.21 to 4.38; $p = 0.011$). When ankle dorsiflexion was added to the same model the odds of having increased relaxation time with higher knee extension moment decreased to an OR of 1.66 (95% CI 0.87 to 3.2; $p = 0.123$). The correlation of the maximum dorsiflexion angle and maximal knee extension moment in this model was negative ($r = -0.445$). Additionally, if we added maximum knee angle, which is related to the maximal knee joint moment ($r = 0.478$) during running, the OR increased to 2.02 (95% CI 0.96 to 4.25; $p = 0.064$). The correlation of the maximum dorsiflexion angle and the maximal normalized knee extension moment in this model decreased ($r = -0.114$).

Association between at T2*RTs and running distance

Figure 2 presents the results from the logistic analysis estimating associations between AT T2*RTs and running

distance adjusted for sex and age. These results indicated that, compared with runners who run 21–40 km per week, those who run 0–5 km per week were at higher odds (OR = 2.16; 95% CI 1.06 to 4.40, $p = 0.035$) of having an AT T2*RTs in the highest quartile. Similarly, compared with runners who run 21–40 km per week, those who run more than 40 km per week were at a higher odd (OR = 2.20; 95% CI 1.05 to 4.6, $p = 0.037$) of having AT T2*RTs in the highest quartile. The Wald test was used to test the interaction between AT T2*RTs and running distance. There was no significant evidence ($p = 0.07$) that there were differences in AT T2*RTs according to km/week. We examined whether sex modified this association by using the interaction term between running distance and sex. Sex did not modify the association ($p = 0.11$).

We also did not observe an interaction between the knee extension moment and the association between AT T2*RTs and running distance ($p = 0.54$).

Discussion

The purpose of the present study was to analyse the association between the AT structure and running activity status. We hypothesized that with increased T2*RT: (1) biomechanical variables would change to suggest an increase in the risk for injury; and (2) increased running distance would relate to increased T2*RT.

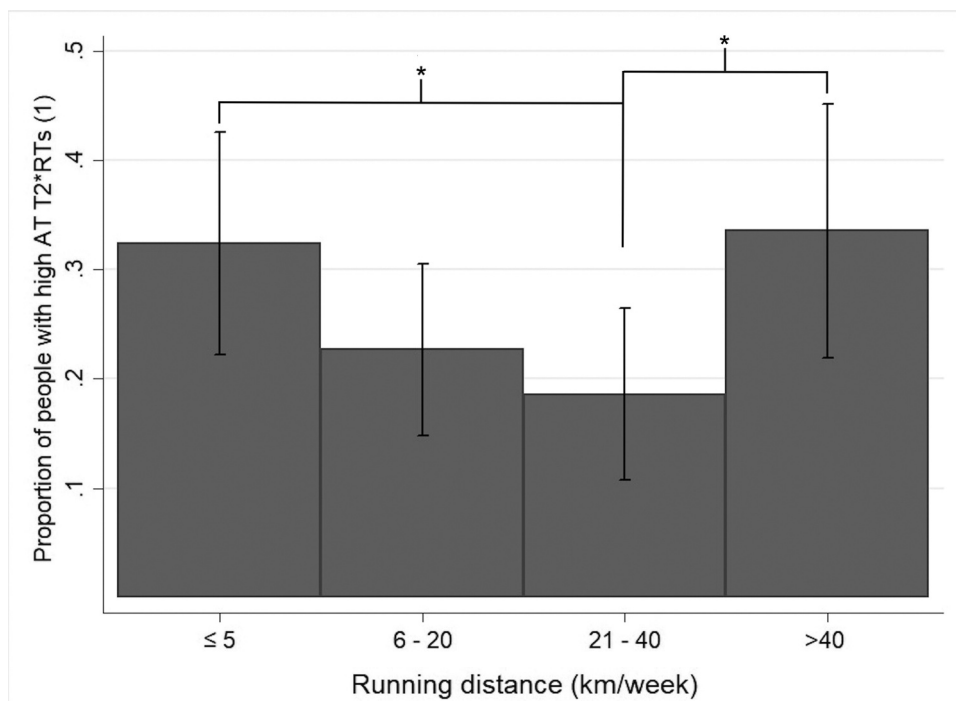


Figure 2. The graph describes proportion of people with Achilles tendon relaxation time (T2* RT) in the upper quartile according to running distance and respective 95% of confidence intervals ($n = 350$). The values for proportion of people with at relaxation time in the upper quartile for each category of running distance were predictions from a generalized linear model including category of running distance (≤ 5 km/week; 6–20 km/week; 21–40 km/week; > 40 km/week), age (continuous) and sex (male; female). *Statistically significant association.

AT T2*RTs and biomechanical variables

A primary purpose of the present study was to assess the association between the AT structure and selected biomechanical variables thought to be related to AT chronic running injuries. We hypothesized that altered running biomechanics were associated with increased AT T2*RTs. The results of our study confirmed this hypothesis. When controlled for possible covariates such as age, sex and running speed, there was a higher odd of being in the upper quartile of AT T2*RTs with higher maximal knee extension moment. A 1 Nm/kg increase in the knee extension moment was associated with a 130% increase in the odds of being in the group with the longest AT T2*RTs. A possible explanation for this might be that, in runners with a disrupted AT structure, there may be a shift of the knee moment during gait from distal to more proximal joint (i.e., knee to hip) due to the reduced capacity to store and release energy by the plantar flexor muscle-tendon units (DeVita & Hortobagyi, 2000). It has been hypothesized in previous studies that the distal to proximal shift of joint moments and powers represent age-related natural changes (DeVita & Hortobagyi, 2000). Some researchers have tested whether the natural age-induced changes in moments during gait from distal to proximal joints could be slowed by regular running. However, research on active senior runners and inactive senior volunteers has shown that physical activity in the form of running 32–63 km per week did not mitigate the age-related distal to proximal shift of joint moments and joint powers in older adults (Krupenevich & Miller, 2021). However, this negative result may have been due to a disturbance in the structure of the AT as a result of an excessively high a running volume. In our middle-aged group of runners, we may have observed a distal to proximal shift not due to ageing, but due to a disrupted AT structure. Therefore, the increased knee moment would not be the cause but rather the consequence of the change in AT structure.

Another possible explanation may be that the increased knee extension moment could be due to the kinematics of the knee and ankle joints. The AT attaches to the triceps surae muscle group, which passes through the ankle and knee joints. Therefore, ankle and knee sagittal coordination may be important for the loading of the AT. The ATs subsequent remodelling, due to the week or over-stimulation of the mechanosensors, may indicate chronic degenerative changes of the microstructure of the AT as a result of the period without overload (Arya & Kulig, 2010; Hein et al., 2014; Passini et al., 2021; Pingel et al., 2014). When we controlled for maximum dorsiflexion of the ankle joint in the logistic regression, the odds ratio of the effect of extensor moment on T2*RTs was reduced. However, when we added maximum knee flexion to the model, again maximum knee extensor moment presented a higher odd of being in the group with the longest T2*RTs. At the same time, the maximum knee moment was positively correlated with knee flexion and negatively correlated with dorsiflexion at the ankle joint. Lower knee flexion during running increases the effective mechanical advantage of the knee and reduces the knee extension muscle forces required to generate the knee extension moment (Wheatley et al., 2021). A restricted range of ankle

dorsiflexion excursion may limit the capacity of the triceps surae to absorb mechanical energy and so result in greater loading rates (Narelle et al., 2010). Therefore, the relationship between the AT structure and the maximum extension knee moment may be associated with the tendon-loading nature of running which has been shown to be related to clinically relevant improvements of tendinopathy (Breda et al., 2021).

In this study, we focused on other biomechanical variables (such as the footfall pattern) that either increase the load on the AT or have been associated in cross-sectional studies with runners with tendinopathy. A non-rearfoot footfall pattern has been shown to have greater AT forces during the stance phase compared to rearfoot running (Almonroeder et al., 2013). In addition, the triceps surae also supinates the foot and the increased frontal plane ankle movement was considered to be one of the possible risk factors for Achilles tendinopathy (Donoghue et al., 2008). However, no other biomechanical variables were associated with changes in AT structure in our study. Based on this cross-sectional study, it is not possible to determine how the biomechanics of the ankle and knee joints should be adjusted to avoid impairment or enhancement of the AT structure. However, this study has shown that there is an association between running biomechanics and AT structure. Further experimental or prospective studies using MRI are needed to understand the effect of specific biomechanics of the ankle and knee joints on AT structure.

AT T2*RT and running distance

We hypothesized that runners with the highest running distance per week would have an increased probability of having higher AT T2*RTs compared to the group of lower distance runners. The findings of the current study support our hypothesis. When controlling for age and sex, the group with the highest running distance had a significantly greater odds of having longer AT T2*RTs of the insertion of the AT than the reference group (21–40 km per week). The present findings seem to be consistent with other research which reported that T2*RTs of the mid-portion of the AT were significantly longer in healthy runners compared to healthy non-runners (Devaprakash et al., 2020; Grosse et al., 2015). The experimental groups of runners in the two aforementioned studies had weekly running distances greater than 30 km and 80 km and may be equivalent to our group that ran more than 40 km per week. Therefore, it is possible to argue that the AT structure is related to training volume (i.e., running distance per week). A longer AT T2*RT may be related to adaptations or pathological conditions of the tendon microstructure and water content induced by long-term mechanical loading (Devaprakash et al., 2020; Grosse et al., 2015).

finding of our study was that, when controlling for age and sex, too much running activity may be related to the odds of being in the upper quartile with longer AT T2*RTs. Regular running with distances between 21 and 40 kilometres per week had been shown to be associated with a reduced odds of being in the highest quartile with longer AT T2*RTs compared to being inactive or running (>40 km per week). MRI quantitatively assesses structural and biochemical properties

of the AT tissues *in vivo* (Fouré, 2016). In previous research, short component T2*RTs showed excellent correlation with structural status as indicated by the histology of cadaver ATs (Filho et al., 2009). In addition, the short component of T2*RTs have been shown to be a marker of Achilles tendinopathy in clinical populations, suggesting that it reflects the changes in water content and collagen orientation (Juras et al., 2013). Moreover, T2*RTs have been shown to be an acute marker to detect Achilles tendinopathy in the early stages (Qiao et al., 2017). Furthermore, our research suggests that there might be an optimal running distance to achieve a healthy AT biochemical structure. In their systematic review, Nielsen et al. (2012) reported that an increased odds of all running injuries occurred when the training distance was more than 64 km per week. Nonetheless, we are not aware of any empirical research to date that suggests an association between inactivity and high activity simultaneously in terms of risk of musculoskeletal injuries. Previous studies (e.g., Magnusson et al., 2010) showed a “supercompensation” phenomenon of the collagen turnover after running. Over the first 24–36 hours, this response results in a net loss of collagen, but is followed by a net synthesis 36–72 hours after exercise (Magnusson et al., 2010). Repeated training with rest periods that are too short, as in the case of a runners with a training volume of more than 40 km per week, can then result in a net degradation of the matrix and lead to overuse injury.

In non-runners, the mechanisms related to higher odds to have longer AT T2*RTs compared to reference group (21–40 km/week) could be related to recent findings by Passini et al. (2021) who showed that tenocytes detect mechanical forces through the mechanosensitive ion channel. This receptor then senses mechanical shear stress induced by displacement of collagen fibres in the AT. With reduced receptor perception, tendon stiffness was subsequently reduced in rats but also in humans. Conversely, increased mechanical signalling increased tendon stiffness presumably through up-regulation of collagen cross-linking in response to physical activity (Passini et al., 2021). In inactive subjects, there may be reduced stimulation of the mechanosensitive ion channel in AT and we observed altered AT collagen compared to active subjects with a load that is not pathologically high. In addition, it has been shown in a large clinical trial that exercises capable of eliciting tendon strain resulted in clinically relevant improvements at 24-week follow-up (Breda et al., 2021). It has been suggested that during running the AT is subjected to repeated stretch-shortening cycles able to increase collagen turnover, inducing positive adaptation that could be beneficial for AT remodelling (Kjaer et al., 2005). Since, the amount of stretch-shortening cycle is affected by foot strike patterns, it can be argued that the biochemical structure of the AT may be influenced not only by the volume of training but also by the way the run is performed (i.e., the biomechanics of running).

In addition, we suggested that biomechanical variables related to AT T2*RTs also impacted the association between AT T2*RTs and training distance. However, this hypothesis was not confirmed. Thus, it appears that a running technique cannot protect a runner from altering the structure of the AT when performing high training volumes.

Limitations

This study provided both scientifically and practically meaningful findings informed by extensive measurement of a range of running, biomechanical, magnetic resonance imaging, and running distance parameters. However, the findings of this study must be interpreted in the context of the study’s limitations. When we compared the AT T2*RTs of the highest quartile with the rest of the sample, we found that the runners were mostly male, taller, had less fat, and a higher normalized maximum knee extension moment. These results are consistent with the evidence of a higher incidence of AT injury in males compared to females (Lantto et al., 2014). Different groups reported engaging in different sport activities of moderate and high intensity besides running. However, we presume this additional sport activity to have minimal impact on our study results, as the runners in the group with the highest running volume and longest T2* relaxation times also performed the least moderate and high-intensity sport activity. The highest running volume group had the highest proportion of runners training for a race. However, our study did not consider factors such as motivation or other psychosocial variables that may play an important role in AT injuries (Mousavi et al., 2021). Another limitation may be the retrospective description of the training volume via a questionnaire. However, we cross-checked responses across two independent questionnaires and, additionally, included a 14-day objective measure of physical activity, which confirmed the categorization of groups according to their physical activity volume. Another limitation could be that the biomechanics of running is influenced by running speed, and individual groups differed according to running speed. However, we controlled for running speed in the logistic regression. We performed the analysis from only one medial image through the AT cross-section. Thus, we neglected to include fibres from the medial head of the gastrocnemius muscle that are located posteriorly while the fibres from the lateral head are located anteriorly. We also neglected the fibres from the soleus muscle that are located in the medial part of the tendon (Szaro et al., 2009). Furthermore, we excluded the use of other quantitative techniques, such as diffusion imaging or spectroscopy, since the Achilles tendon scan was only one area of interest in our large cohort study. Due to the lengthy examination times associated with the acquisition protocol of the study, we were unable to use these advanced techniques.

Conclusion

We can conclude from the results of this study that there is an association between selected running biomechanics parameters (e.g., maximal net knee extension moment) and the AT T2* relaxation time as indirect indicator of AT structure. In addition, physical inactivity, as defined by the World Health Organization, and excessive physical activity, in the form of regular running, may be related to impairment of the AT T2* relaxation time. Conversely, regular physical activity, that meets the World Health Organization recommendations and regular running of 21 to 40 km per week, may be related to the AT T2* relaxation time indicating a possibly better water content and collagen orientation than in inactive non-runners or highly

active individuals (running distance ≥ 40 km/week). Thus, both running biomechanics and running distance can be thought to be related to the AT T2* relaxation time. Nevertheless, running technique did not appear to protect runners from altering AT T2* relaxation time when performing high training volumes (running distance ≥ 40 km/week).

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Contributorship

DJ, JH, VKJ, JU, SE obtained funding for this project and were responsible for the design of this study. JS, DV conducted data collection. VKJ, VJ, DV, DJ conducted data analysis, DJ, JH, AM, VKJ, VJ interpreted findings. DJ wrote the initial draft of the manuscript, SE, DV and JH wrote critical revision, final manuscript was then revised and approved by all authors.

Ethical approval information

Ethical approval was obtained from the University of Ostrava (Application EK 3/2018) with written informed consent obtained from all participants.

Data availability statement

Data presented in the paper can be found at www.4HAIE.cz

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