REVIEW

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Lower-Limb Connective Tissue Morphologic Characteristics in Runners. How Do They Relate with Running Biomechanics and Tendon Pathology? A Systematic Review

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SUMMARY

Although the role of connective tissue in running injuries and biomechanics has been widely investigated, systematic reviews on this issue were rarely reported.
The aim of this study is to systematically review the current literature regarding the morphological characteristics (*i.e.*, cross-sectional area and thickness) of the main connective tissue of the lower limb in runners and its relationship with running biomechanics and tendon pathology.
The main keywords used were: Achilles and patellar tendons, plantar fascia, ultrasound, thickness, cross-sectional area and running. Observational design English-written studies published between January 2000 and September 2020 were included.
After exclusion criteria were applied, 34 studies remained. 16 studies analysed connective tissue related to sample characteristics where mainly the differences between runners and controls were studied along other factors such as gender, weight and nationality. Regarding running biomechanics, 10 studies assessed connective tissue on running biomechanics with focusing on foot strike pattern and footwear characteristics. Regarding tendon pathology, 8 studies analysed the connective tissue assessing, mainly, whether the pathological tendons are thicker.
Runners show higher tendons, in terms of thickness and CSA, than control subjects and athletes from other disciplines whose tendons are subjected to lighter loads. Adaptation to load leads to better performance, what seems to explain these morphological differences.
Study registration. None.

KEY WORDS

Connective tissue; morphology; performance; review; running; tendinopathy.

INTRODUCTION

Tendons are connective tissue structures composed mainly by collagen (65-80%) and elastin embedded in a proteoglycan-water matrix (1). The most typical analysed morphological characteristics of the connective tissue are the cross sectional area (CSA) and thickness (2, 3). To assess these morphological characteristics, the main imaging techniques used are ultrasounds and nuclear magnetic resonance, which have proven to be useful and reliable for such use (4, 5).

Connective tissue, specifically tendons, plays an essential role in the locomotor system function. Activities such as walking, running or jumping need a correct tendon functioning as these structures are responsible for loading transmission that allows physiological movement and joint stabilization (6). In relation with its function, tendons are classified as load transmitters or movement transmitter, being the main lower limb tendons (*i.e.*, Achilles tendon [AT] and patellar tendon [PT]) load transmitters (6). Although the plantar fascia (PF) is not properly considered a tendon, some studies refer to the PF such as an extension of the AT due to the common insertion at the calcaneus bone aiding in the function of this tendon (7).

Given the elongation and shortening of these lower limb structures (*i.e.*, AT, PT and PF), they made a huge contribution to the energy storage and release during movements such as running (8). It is suggested that during running the lower limb behaves like a spring that continually compresses and decompresses allowing the movement (9). The main responsible for such compressions and decompressions are the muscle-tendon units through the stretch-shortening cycle (10). Considering that, the characteristics and condition of the aforementioned connective tissues might play a key role in both athletic performance and injury management contexts.

It has been proposed that the AT plays an essential role in running economy (11). Fletcher *et al.* (11) proposed that the AT stiffness is one of the main mechanism behind an enhanced running economy. Tendon stiffness allows the muscle acts at the appropriate length and velocity, being essential for running economy. Tendons optimize the relation between muscle's force, length, and velocity by minimising length change during muscle contraction (11). During running, the AT accommodates much of that muscle-tendon unit length change (12). Seemingly, the AT mechanical properties are optimal to accommodate the mentioned length change, and any change in those properties might result in an increase in running energy cost (11). Supporting this importance of AT in running economy, several studies show the relationship between longer AT length and better running economy (13, 14). For its part, the PT has also been shown to be an influential element in running economy. Thus, a lower PT stiffness is related to a better running economy (11). Depending on the circumstances, a higher stiffness tendon or a lower stiffness tendon can be more beneficial in running economy (11). So, when power is more important, a lower stiffness tendon helps the muscle to shorten at the velocity associated with peak-power output (15). This fact could explain why lower PT stiffness correlates with a better running economy given that it would contribute more effectively to power generation (11).

It is known that during running the main lower limb tendons are exposed to repetitive loadings. In response to these repetitive loads, tendons need to be adapted. These adaptations occur both at the morphological level (*i.e.*, higher CSA and thickness) (16, 17) and in the percentage of the type of collagen fibers (18). Thus, these adaptations would seek for a tendon better prepared for the demanding activity and therefore with a better performance such as with a better velocity (19). However, when the tendon is unable to adapt to these loads, the result is an overloaded tendon that can lead to the development of tendinopathy (20). Thus, for running, when there is not a good adjustment of the load in relation to volume and frequency, the excess load might lead to tendon injury (20). Although some studies show higher CSA and thickness in Achilles tendinopathy (21), these morphological changes are present depending on the phase of tendinopathy in which it is found (20). The most typical injuries associated to running are Achilles tendinopathy and plantar fasciitis (22). A previous study found that shorter races, where runners use more a forefoot strike pattern which demands more the AT, increase the risk of suffering an AT tendinopathy (23). In relation with the PT, patellar tendinopathy has been frequently reported among amateur runners reporting a weekly mileage between 20 and 50 km (24). However, this pathology did not show an important prevalence between marathon runners (24), so that running experience with a correct tissue adaptation could result in a protector factor for patellar tendinopathy (22). Finally, master runners seem to be more affected by plantar fasciitis (24). One of the main functions of the PF during running is to absorb the elastic tension (22). With aging, this capacity might decrease, which could explain why master runners show a higher prevalence of plantar fasciitis (22).

Actually, both the relation between the tendon injuries and running and the influence of the tendon on running biomechanics have been widely investigated (25-28).

Despite the aforementioned information, there still exist gaps that need to be bridged in regards with connective tissue morphologic characteristics and running. Furthermore, how these characteristics are related to running biomechanics and tendon pathology is not well understood. In this way, to the best of the authors' knowledge, a systematic review that analyses the literature carried out on the morphological characteristics of connective tissue in runners and how these characteristics are related to running biomechanics and tendon pathology variables has not been done yet.

Therefore, the main purpose of the present systematic review was to analyse the current literature regarding the morphological characteristics (*i.e.*, CSA and thickness) of the main connective tissue of the lower limb in runners and its relationship with running biomechanics and tendon pathology variables.

METHODS

A systematic review was completed following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (29). Findings were also reported accordingly.

Eligibility criteria

The following inclusion criteria were considered: 1) studies published in English; 2) only human studies. The following exclusion criteria were taken into account: 1) Clinical trials, 2) case reports, 3) case series, 4) systematic reviews and 5) editor letters.

All manuscripts related to lower limb connective tissue morphology (*i.e.*, AT, PT and PF thickness and CSA) and running were included.

Information sources

A systematic search was conducted in the electronic databases Pubmed, Web of science, SPORTDiscus and Scopus for relevant studies from 1 January 2000 to 30 September 2020. Keywords were collected through experts' opinion, a systematic literature review, and controlled vocabulary (*e.g.*, Medical Subject Headings: MeSH). Boolean search syntax using the operators "AND" and "OR" was applied. Following an example of a PubMed search is shown: (Achilles OR gastroc* OR triceps surae OR patella* OR quadriceps OR rotulien OR plantar fascia) AND (Tendon* OR Tendin*) AND (Ultrasonograph* or Sonograph* or Ultrasound or US or MSUS OR cross-sectional area OR thickness) AND (Run* or Sprint* or Jog* or Interval or Long Distance or Marathon OR running OR runner). Filters: Publication date from 2000/01/01; Humans; English.

After an initial search, accounts were created in the mentioned databases. In this way, the search was updated until the initiation of manuscript preparation on September 30, 2020. Following the formal systematic searches, additional hand-searches were conducted. In addition, the reference lists of included studies and previous reviews and meta-analyses were examined to detect studies potentially eligible for inclusion.

Study selection

During the selection of studies, a filter was initially made by title, in which those articles that were not considered relevant were excluded. After this, the same procedure was carried out after evaluating the abstract. Finally, the full text of the remaining studies was assessed excluding those that did not meet the review criteria.

Methodological quality in individual studies

Selected studies were evaluated for methodological quality using the modified version of the Quality Index developed by Downs and Black (30). A good test-retest (r = 0.88) and inter-rater (r = 0.75) of the original scale was reported. Furthermore, a good reliability and high internal consistency (Kuder-Richardson Formula 20 (KR-20) = 0.89) were shown. The modified version of the Downs and Black Quality Index is scored from 1 to 14 where higher scores indicate higher-quality studies. Two independent reviewers (ARP-FGP) performed this quality assessment, and, in the event of a disagreement, a third reviewer (LERS) analysed the quality and made the final decision. Agreement between reviewers was assessed using a Kappa correlation for methodological quality. The agreement rate between reviewers was k = 0.91.

RESULTS

Study selection

The study selection process is shown in a flow diagram (**figure 1**). A total of 978 studies were found after the systematic search. 275 from Pubmed, 456 from Web of Science, 187 from Scopus and 60 from SPORDiscus. Additionally, one study was identified from a different resource. After all the selection criteria mentioned above, 34 studies were included. Regarding the methodological quality, results derived from Modified Downs and Black scale are shown in **table I**. Scores for this scale ranged from 8 to 13 out of 14.

The results of the selected studies are shown separately according to next different aspects: sample characteristics (**table II**), running biomechanics variables (**table III**) and tendon pathology (**table IV**).

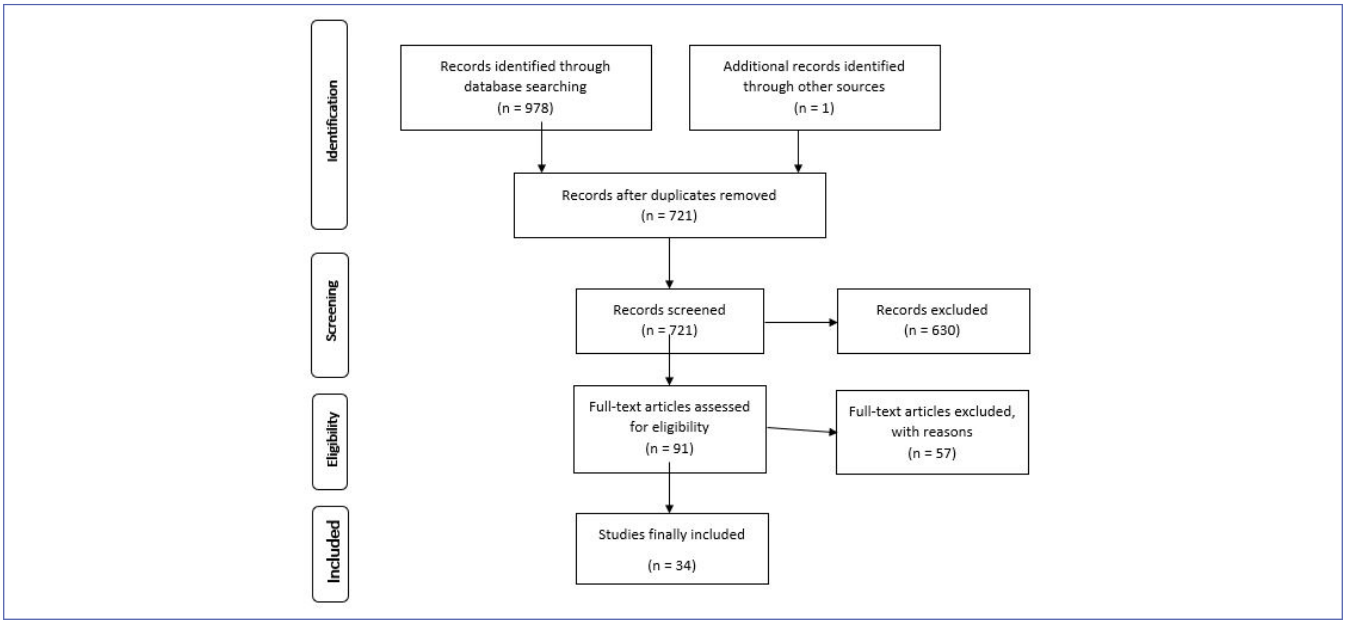

Figure 1. PRISMA flow diagram.

Table I. Modified Downs and Black scale.

Study	Item 1	Item 2	Item 3	Item 6	Item 7	Item 10	Item 12	Item 15	Item 16	Item 18	Item 20	Item 22	Item 23	Item 25	Total (Out of 14)
Chen *et al.* (2019) (50)	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**11**
Dar *et al.* (2019) (38)	1	1	1	1	1	1	0	1	1	1	1	0	0	1	**11**
Devaprakash *et al.* (2020) (31)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Farris *et al.* (2012) (43)	1	1	1	1	1	1	0	0	1	1	1	1	0	0	**10**
Freund *et al.* (2012) (51)	1	1	1	1	1	1	1	1	1	1	1	1	1	0	**13**
Hagan *et al.* (2018) (52)	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**11**
Hall *et al.* (2015) (58)	1	1	1	1	1	1	0	0	1	1	1	1	0	0	**10**
Hirschmuller *et al.* (2012) (53)	1	1	1	1	1	1	0	1	1	1	1	1	0	1	**12**
Histen *et al.* (2016) (44)	1	1	1	1	1	1	0	1	1	1	1	0	0	0	**10**
Hullfish *et al.* (2018) (32)	1	1	1	1	1	1	0	1	1	1	1	1	0	1	**12**
Kernozek *et al.* (2018) (45)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Kongsgaard *et al.* (2005) (37)	1	1	1	1	1	1	0	0	1	1	1	0	0	1	**10**
Kubo *et al.* (2010) (17)	1	1	0	1	1	1	0	0	1	1	1	0	0	1	**9**
Kubo *et al.* (2011) (2)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Kubo *et al.* (2015a) (39)	1	1	0	1	1	1	0	0	1	1	1	0	0	1	**9**
Kubo *et al.* (2015b) (46)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Kubo *et al.* (2015c) (16)	1	1	0	1	1	1	0	0	1	1	1	0	0	1	**9**
Kubo *et al.* (2017) (3)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Kudron *et al.* (2020) (40)	0	1	1	1	1	1	1	0	1	1	1	0	0	1	**10**
Kunimasa *et al.* (2014) (41)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Lieberthal *et al.* (2019) (54)	1	1	1	1	1	1	0	0	1	1	1	1	0	0	**10**

Study	Item 1	Item 2	Item 3	Item 6	Item 7	Item 10	Item 12	Item 15	Item 16	Item 18	Item 20	Item 22	Item 23	Item 25	Total (Out of 14)
Magnusson *et al.* (2003) (33)	1	1	1	1	0	1	0	0	1	1	1	0	0	0	**8**
Monte *et al.* (2020) (19)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Neves *et al.* (2014) (47)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Ooi *et al.* (2015) (55)	1	1	1	1	1	1	0	1	1	1	1	0	0	0	**10**
Rosager *et al.* (2002) (34)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Salinero *et al.* (2020) (35)	1	1	0	1	1	1	0	0	1	1	1	0	0	0	**8**
Shaikh *et al.* (2012) (56)	1	0	1	1	1	1	0	1	1	1	1	0	0	0	**9**
Shiotani *et al.* (2020) (42)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Sponbeck *et al.* (2017) (48)	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**11**
Tillander *et al.* (2019) (57)	1	0	1	1	1	1	0	0	1	1	1	1	0	1	**10**
Ueno *et al.* (2018) (14)	1	1	1	1	1	1	0	0	1	1	1	0	0	0	**9**
Wiesinger *et al.* (2016) (36)	1	1	1	1	1	1	0	0	1	1	1	0	0	1	**10**
Zhang *et al.* (2018) (49)	1	1	1	1	1	1	0	1	1	1	1	0	0	0	**10**

0 = no / unable to determine; 1 = yes. Item 1: clear aim/hypothesis; Item 2: outcome measures clearly described; Item 3: patient characteristics clearly described; Item 6: main findings clearly described; Item 7: measures of random variability provided; Item 10: actual probability values reported; Item 12: participants prepared to participate representative of entire population; Item 15: Blinding of outcome measures; Item 16: analysis completed was planned; Item 18: appropriate statistics; Item 20: valid and reliable outcome measures; Item 22: participants recruited over same period; Item 23: Randomised; Item 25: adjustment made for confounding variables.

Table II. Sample characteristics and morphological characteristics of the connective tissue.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Dar *et al.* (2019)	**Amateur road LDR** n = 26 (15M, 11F) 42.2 ± 9.1 years 168.6 ± 8.2 cm 62.4 ± 8.0 Kg BMI: 21.9 ±1.9 Kg/m^2 Km/wk: 37.5 ± 20.6 **Amateur trail runners** n = 17 (9M, 8F) 41.0 ± 9.5 years 171.2 ± 8.4 cm 67.1 ± 12.1 Kg BMI: 22.7 ±2.8 Kg/m^2 Km/wk: 38.2 ± 16.3	Unilateral crossover Laboratory conditions	- **AT-Thickness** **- AT-CSA** - AT UTC	- Proprioceptive ability (AMEDA) - Dynamic postural balance (Y balance Test) - Triple hop distance test - Hip abduction isometric test	- LDR AT-Thickness (cm) 0.6 ± 0.1 - Trail AT-Thickness (cm): 0.5 ± 0.1 - LDR AT-CSA (cm^2) 0.5 (0.4-0.6) - Trail AT-CSA (cm^2) 0.5 (0.4-0.6) - No significant differences for thickness and CSA

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Devaprakash *et al.* (2020)	**Trained elite LDR** n = 16 (10 M, 6 F) 25.2 ± 5.0 years 175.5 ± 7.3 cm 64.4 ± 8.4 kg BMI: 20.9 ± 1.8 Kg/m^2 **Healthy controls** n = 16 (11 M, 5 F) 30.3 ± 4.9 years 172.4 ± 10.5 cm 71 ± 16.8 kg BMI: 23.8 ± 4.5 Kg/m^2	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT lenght - AT strain - AT stress - AT elongation - AT stiffness and Young's modulus - AT force - AT torque **Measured by RNM		- AT-CSA significantly larger in LDR
Hulfish *et al.* (2018)	**Competitive runners** n = 22(12 M, 10 F) 19 ± 1.5 years BMI: 20.3 ± 1.6 Kg/m^2 **Untrained subjects** n = 12(5 M, 7 F) 25 ± 2 years BMI: 23.8 ± 2.4 Kg/m^2	Unilateral crossover Laboratory conditions	***- AT-Thickness*** - AT structure - Collagen organization - Echogenicity	- VISA-A - Foot strike pattern - Neovascularity	- AT-Thickness 48% greater in competitive runners
Kongsgaard *et al.* (2005)	**Elite LDR** n = 8 M 28.6 ± 2.2 years 182.4 ± 2.7 cm 69.5 ± 2.9 kg **Elite volley players** n = 8 M 25.3 ± 0.8 years 193.8 ± 2.8 cm 89.5 ± 2.6 kg **Elite Kayak players** n = 9 M 20.5 ± 1.1 years 181.9 ± 1.3 cm 79.9 ± 1.9 kg **AT rupture patients** n = 6 M 31.0 ± 0.7 years 181.5 ± 2.8 cm 86.0 ± 6.3 kg	Unilateral crossover Laboratory conditions	***- AT-CSA (maximal, narrowest and Nrm to mass)*** - AT moment arm AT peak tendon force - AT length - Maximal AT force - Peak AT stress	- Leg length - MVIC plantar flexion - Triceps Surae CSA	- LDR AT-CSA (mm^{2}) Maximal (106 ± 8.6), Narrowest (55.1 ± 2.1) - LDR larger Nrm AT - CSA than kayak and rupture subjects - LDR narrowest Nrm AT-CSA higher than kayak and rupture subjects - The narrowest AT-CSA correlated to maximal AT force
Kubo *et al.* (2010)	**LDR** n = 12 M 20.3 ± 1.1 year 171.0 ± 4.7 cm 57.3 ± 3.5 Kg **Untrained subjects** n = 21 M 21.2 ± 1.8 years 172.2 ± 5.6 cm 64.5 ± 5.9 Kg	Unilateral crossover Laboratory conditions	***- AT-Thickness*** (Abs and Nrm to mass) ***- PT-Thickness*** (Abs and Nrm to mass) - AT and PT elongation - AT and PT stiffness	- Muscle thickness (knee extensors and plantar flexors) - MVIC (knee extensors and plantar flexors) - Resting twitch properties - Neural activation level	- LDR thickness (mm): PT: 3.24 ±0.37, AT: 4.65 ± 0.57 - Untrained thickness (mm): PT: 3.36 ± 0.36, AT 4.67 ± 0.56 - Nrm AT and PT thickness greater in LDR No differences in Abs both tendon thickness.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Kubo *et al.* (2011)	**100-m sprinters** n = 15 M 20.8 ± 1.0 years 174.5 ± 4.3 cm 67.6 ± 5.3 Kg **Untrained subjects** n = 15 M 20.7 ± 1. 8 years 173.1 ± 4.2 cm 70.2 ± 7.7 Kg	Unilateral crossover Laboratory conditions	***- AT-Thickness*** ***- PT-Thickness*** - AT and PT elongation - AT and PT stiffness	- Muscle thickness (knee extensors and plantar flexors) - MVIC (knee extensors and plantar flexors) - Running performance	- Runners thickness (mm): PT: 3.15 ± 0.47, AT: 4.28 ±0.54 - Untrained thickness (mm): PT: 3.27 ± 0.50, AT: 4.34 ± 0.63 -No differences in tendon thickness -No significant correlations between best 100-m record and tendon thickness
Kubo *et al.* (2015a)	**Faster LDR** n = 32 M 20.4 ± 1.2 years 171.1 ± 4.5 cm 56.7 ± 3.5 Kg Experience 7.7 ± 1.9 years **Slower LDR** n = 32 M 20.3 ± 1.0 years 170.5 ± 5.6 cm 58.3 ± 4.8 Kg Experience: 6.8 ± 2.9 years	Unilateral crossover Laboratory conditions	***- AT-Thickness*** ***- PT-Thickness*** - AT and PT elongation - AT and PT stiffness - At and PT strain	- Muscle thickness (knee extensors and plantar flexors) - MVIC (knee extensors and plantar flexors) - Running performance	- Faster Thickness (mm): PT: 4.9 ± 0.5, AT: 3.2 ± 0.4 - Slower Thickness (mm): PT: 5.1 ± 0.6, AT: 3.1 ± 0.4 -No differences in tendon thickness -No significant correlations between the best 5000m record and tendon thickness
Kubo *et al.* (2015c)	**LDR** n = 20 M 20.4 ± 1.0 years 171.2 ± 4.8 cm 57.4 ± 4.6 Kg **Untrained subjects** n = 24 M 22.2 ± 3.6 years 172.3 ± 5.5 cm 66.4 ± 8.1 Kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** (Abs and Nrm to mass) - AT stiffness - AT elongation	- Muscle thickness plantar flexors - Passive and Active muscle stiffness - MVIC plantar flexors - EMG plantar flexors	- LDR AT-CSA (mm^2). Abs: 74.4 ± 10.7, Nrm: 4.99 ± 0.72 - Untrained AT-CSA (mm^2). Abs: 73.5 ± 11.6, Nrm: 4.48 ± 0.63 - Nrm AT-CSA higher in LDR
Kubo *et al.* (2017)	**Sprinters** n = 14 M 20.7 ± 1.1 years 173.2 ± 5.5 cm 65.4 ± 4.3 Kg **Untrained subjects** n = 24 M 22.2 ± 3.6 years 172.3 ± 5.5 cm 66.4 ± 8.1 Kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT stiffness - AT elongation	- Muscle thickness plantar flexors - Muscle fascicle length - Pennation angle - Active muscle stiffness - MVIC plantar flexors - Twitch properties	- Sprinters AT-CSA (mm^2): 74.0 ± 12.0 - Untrained AT-CSA (mm^2): 73.5 ± 11.6 - AT-CSA no differences

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Kudron *et al.* (2020)	**Pro CCR** n = 27 (11 M, 16 F) 19.5 ± 1.4 years 67.2 ± 3.4 in 127.6 ± 18.1 lb BMI: 19.8 ±1.5 kg/m² Miles/wk: 64.1 ± 21.6	Unilateral crossover Laboratory conditions	**- AT-Thickness** **AT-CSA** - AT structure	- Dominant *vs* Non dominant side - Demographic characteristics	- Non dominant AT-CSA (cm²): 0.71 ± 0.17 - Dominant AT-CSA (cm²): 0.74 ± 0.19 - Non dominant AT-Thickness (cm): 0.56 ± 0.1 - Dominant AT- thickness (cm): 0.53 ± 0.09 - Positive correlation height and weight with AT-CSA - Positive correlation Miles/wk with AT-CSA and thickness - Males larger AT-CSA - Underweight subjects smaller AT-CSA
Kunimasa *et al.* (2014)	**Kenyan LDR** n = 22 M 21.9 ± 4.5 years 1.74 ± 0.06 m 57.2 ± 4.8 Kg BMI 18.9 ± 1.5 Kg/m² **Japanese LDR** n = 22 M 20.2 ± 2.2 years 1.73 ± 0.05 m 56.9 ± 4.6 Kg BMI 19.0 ± 0.9 Kg/m²	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT moment arm	- Running performance - Thigh length - Leg length - Forefoot length - Gastrocnemius-AT length -Soleus-AT length	- Kenyan AT-CSA (mm²): 60.5 ± 9.3 - Japanese AT-CSA (mm²): 53.6 ± 9.8 - AT-CSA higher in Kenyan
Magnusson *et al.* (2003)	**Runners** n = 6 M 36 ± 7 years 1.84 ± 0.05 m 70.9 ± 4.4 Kg **Control no runners** n = 6 M 34 ± 3 years 1.81 ± 0.02 m 81.2 ± 8.7 Kg	Unilateral crossover Laboratory conditions	***- AT-CSA:*** 7 sites from 10 mm to 70 mm to calcaneus		- Runners greater AT-CSA at 10, 20, 30 and 40 mm sites
Rosager *et al.* (2002)	**LDR** n = 5 M 34 ± 6 years 1.82 ± 0.09 m 72.1 ± 4.6 kg **Control no runners** n = 5 M 33 ± 8 years 1.80 ± 0.03 m 82.2 ± 4.2 kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** - AT length - AT strain - AT stress - AT displacement - AT stiffness and Young's modulus - AT moment arm	- EMG flexor and extensor ankle muscles - Angular ankle joint motion - Maximal dorsiflexion moment	- AT-CSA larger in LDR

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Salinero *et al.* 2020	**Marathon runners** n = 96 M 42.0 ± 9.6 years 175 ± 6 cm 73.7 ± 8.6 kg **Non-active people** n = 47 39.9 ± 11.6 years 176 ± 7 cm 79.6 ± 16.1 kg	Unilateral crossover Laboratory conditions	***- AT-CSA*** ***- AT-Thickness***	- Ankle dorsal flexion angle - Marathon performance - Running experience	- Runners AT-CSA (mm^2) 60.74 ± 14.41 - Control AT-CSA (mm^2): 53.62 ± 9.90 - AT-CSA significant greater in Runners - Runners AT-thickness (mm) 4.85 ± 0.75 - Control AT-thickness (mm): 4.60 ± 0.66 - AT-Thickness significant greater in Runners - AT-CSA and thickness correlates with body mass only in controls -AT-CSA and thickness correlates with height -AT-thickness correlates with years of running experience.
Shiotani *et al.* (2020)	**Amateur LDR** n = 10 M 22.0 ± 0.7 years 1.68 ± 0.04 m 55.5 ± 4.2 Kg BMI: 19.6 ± 1.2 Kg/m^2 Experience: 11.0 ± 2.2 years Km/wk: 43.7 ± 35.4 **Untrained subjects** n = 10 M 22.5 ± 1.4. year 1.70 ± 0.05 m 58.4 ± 5.6 Kg BMI: 20.3 ± 1.7 Kg/m^2	Unilateral crossover Laboratory conditions **4 measurements during 10Km test: pre, 30’,60´, post*	***- PF-thickness:*** 3 sites: proximal (in the proximity to the calcaneus), middle (level of navicular tuberosity) and distal (proximity to the second metatarsal head) - PF stiffness	- Foot length - Dorsal height - Navicular height - Arch height ratio - Navicular drop - FSP	- PF-thickness higher in runners at proximal site but not significant

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Wiesinger *et al.* (2016)	**Pro ski jumpers** n = 10 M 22.2 ± 2.9 years 176.3 ± 4.5 cm 64.3 ± 3.9 Kg BMI: 20.7 ± 1.0 Kg/m² **Pro LDR** n = 10 M 31.5 ± 4.6 years 180.9 ± 8.2 cm 72.8 ± 7.6 Kg BMI: 22.2 ± 1.7 Kg/m² **elite water polo players** n = 10 M 24.2 ± 3.2 years 182.4 ± 6.5 cm 84.3 ± 10.8 Kg BMI: 25.3 ± 2.8 Kg/m² **sedentary individuals** n = 10 M 31.0 ± 5.1 years 182.9 ± 7.2 cm 83.9 ± 12.3 Kg BMI: 25.0 ± 2.8 Kg/m²	Unilateral crossover Laboratory conditions	***- AT and PT CSA*** - AT and PT length - AT and PT stiffness- AT and PT stress - AT and PT strain -AT and PT Young's module	- MVIC knee flexors and extensors - MVIC ankle flexors and extensors	- LDR CSA (cm²): PT: 1.1 ± 0.1, AT: 0.7 ± 0.1 - PT-CSA (Nrm to mass and abs) higher in LDR than water polo and sedentary - AT-CSA higher in LDR than in water polo - Nrm to mass AT-CSA higher in LDR than sedentary

LDR: Long distance runners; BMI: Body mass Index; Wk: Week; AT: Achilles Tendon; CSA: Cross-sectional area; UTC: Ultrasonographic tissue characterization NMR: Nuclear Magnetic Resonance; Nrm: Normalized; Abs: Absolute; MVIC: Maximal voluntary isometric contraction; PT: Patellar tendon; CCR: Cross country runners; EMG: Electromyography; PF: Plantar fascia; FSP: Foot strike pattern.

Table III. Running biomechanics variables and morphological characteristics of the connective tissue.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Chen *et al.* (2019)	**Amateur LDR RFS** n = 21 25.14 ± 4.,74 years BMI: 22.32 ± 2.31 Kg/m² Km/wk: 44.58 ± 24.53 **Amateur LDR FFS** N =14 26.85 ± 4.50 years BMI: 21.42 ± 1.29 Kg/m² Km/wk: 43.95 ± 25.73	Unilateral crossover Laboratory conditions	**- PF-thickness** - PF shear wave velocity - Hypoechogenicity	- FSP	- RFS PF-thickness (mm): 3.08 ± 0.35 - FFS PF-Thickness (mm): 3.41 ± 0.89 - PF-Thickness no significant differences
Farris *et al.* (2012)	**Amateur LDR** n = 12 M 27 ± 5 years 1.79 ± 0.06 m 78.6 ± 8.4 kg	Repeated Measures Laboratory conditions * Run 30´ at 12 km/h	**- AT-CSA** (pre and post 30´run) - AT stiffness - AT force -AT length and strain: T_1 (1´), T_2 (15´) and T_3 (30´)	- Ankle ROM - Running Kinematics - Hoping Kinematics	Pre running AT-CSA (mm²) 43 ± 8 -Post running AT-CSA (mm²) 41±8 - AT-CSA no significant differences

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Histen *et al.* (2016)	**Traditionally shod LDR** n = 17 (11 M, 6 F) 25.3 ± 6.5 years 165.1 ± 8.9 cm 67.3 ± 11.9 kg Miles/wk: 18.6 ± 6.7 **Minimalist LDR** n = 14 (12 M, 2 F) 30.1 ± 9.1 years 166.6 ± 8.5 cm 73.1± 7.8 kg Miles/wk: 24.1 ± 9.9	Unilateral crossover Laboratory conditions	**- AT CSA** - AT length - Tendon force - Tendon stress - Tendon elongation - Tendon strain - Tendon stiffness - Young's modulus	Traditionally/ Minimalist Shod	- Traditional AT-CSA (mm^2): 67.4 ± 8.5 - Minimalist AT-CSA (mm^2): 76.6 ± 7.7 - Minimalist runners significant greater AT CSA
Kernozek *et al.* (2018)	**RFS Runners** n = 17 F 21.9 ± 1.5 years 1.70 ± 0.03 m 59.2 ± 6.5 Kg **No RFS Runners** n = 18 F 21.6 ± 1.2 years 1.69 ± 0.07 m 61.4 ± 6.9 Kg	Unilateral crossover Laboratory conditions	**- AT-CSA** - AT stress	- RFS / No RFS - Cadence - Step length	- RFS AT-CSA (cm^2): 0.355 ± 0.02 - No RFS AT-CSA (cm^2): 0.359 ± 0.02 - No significant differences
Kubo *et al.* (2015b)	**41 trained LDR** **FFP** n = 12 M 20.4 ± 1.3 years 169.8 ± 5.2 cm 57.1 ± 4.7 Kg Experience: 6.5 ± 1.9 years **MSP** n = 12 M 20.6 ± 1.3 years 170.7 ± 4.9 cm 56.7 ± 4.0 Kg Experience: 8.4 ± 2.0 years **RFS** n = 17 M 20.6 ± 1.0 years 170.9 ± 5.6 cm 58.5 ± 4.2 Kg Experience: 7.3 ± 3.3 years	Unilateral crossover Laboratory conditions	**- AT-CSA** - AT elongation - AT stiffness - AT strain	- FSP - MVIC plantar flexors - Athletic level	- FSP AT-CSA (mm^2): 74.8 ± 10.6 - MSP AT-CSA (mm^2): 75.7 ± 11.7 - RFS AT-CSA (mm^2): 76.2 ± 12.6 - AT-CSA no significant differences

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Monte *et al.* (2020)	**LDR** n = 32 M 37.9 ± 13.0 years 70.7 ± 7.8 Kg 1.76 ± 0.06 m Experience: 9.5±8.9 years Workout: 3.7±1.8 days/week Km/wk: 43.7 ± 21.4	Unilateral crossover Laboratory conditions	**- AT-CSA** - AT length	**- Kinematic parameters:** CT, FT, SF, SL, RV, - **Spring mass model parameters:** vertical force, trochanter vertical displacement, leg spring compression, Kleg, Kvert **- Metabolic parameters** pulmonary ventilation; Vo2, HR, respiratory exchange ratio, energy cost of running - **Half marathon pace**	- AT-CSA (cm^2) 0.65 ± 0.15 - AT-CSA positive correlation with Kvert and RV
Neves *et al.* (2014)	**LDR** n = 20 F 20.7 ± 1.8 years 1.65 ± 0.06 m 60.5 ± 7.2 Kg Km/wk: 26.8 ± 12.1	Repeated Measures Laboratory conditions *Pre/Post 10´: 7 mph at 0%, 5.5 mph at + 6% and 5 mph at - 6% grade	**- AT-CSA**	- 3D Kinematics	- Pre-run average AT-CSA: 0.39 cm^2 -Post-run average AT-CSA: 0.36 cm^2 - AT-CSA significant decrease
Sponbeck *et al.* (2017)	**Pro CCR** n = 24 (8 M, 16 F) 19.88 ± 2.12 years 168.92 ± 17.16 cm 61.32 ± 20.15 kg Experience: 6.44 ± 5.54 years Run collegiately: 2.56 ± 1.54 years	Repeated Measures Laboratory conditions *4 measures: Before season -3weeks - 6weeks - End season (3-5 weeks)	**- AT-CSA**	- AT-Pain	- AT CSA significantly increased in the 3 and 6 weeks - AT-CSA no difference between the post and pre-season - Sex and mass were significant covariates
Ueno *et al.* (2017)	**Japan well-trained LDR** n = 30 M 20.2 ± 2.9 years 169 ± 4.7 cm 54.1 ± 3.4 Kg BMI: 18.7 ± 1.1 Kg/m^2	Unilateral crossover Laboratory conditions	**- AT-CSA** (Abs and nrm to mass) - AT length (Abs and Nrm to leg length)	-Energy cost at 14, 16 and 18 km/h - Best 5000 m record	- AT-CSA (mm^2): 111.2 ± 17.0 - No correlation AT-CSA and best 5000-m - No correlation AT-CSA and energy cost

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Zhang *et al.* (2018)	**Amateur neutral LDR** n = 11 (7 M, 4 F) 24.6 ± 6.0 years BMI: 22.7 ± 2.3 kg/m² Km/wk: 23.6 ± 10.5 **Amateur MCS LDR** n = 10 (6 M. 4 F) 27.9 ± 8.5 years BMI: 21.4 ± 2.0 kg/m² Km/wk: 26.4 ± 17.3 **Amateur minimalistic LDR** n = 7 (5 M, 2 F) 28.3 ± 8.4 years BMI: 22.2 ± 2.3 kg/m² Km/wk: 25.4 ± 15.0 **Amateur shoe insole LDR** n = 7 (3 M, 4 F) 25.1 ± 5.2 years BMI: 21.7 ± 1.9 kg/m² Km/wk: 26.2 ± 9.9	Unilateral crossover Laboratory conditions	**- PF-thickness:** (proximal, middle and distal) **- AT-Thickness**	- FPI - Arch height - Shoe properties - CSA and Thickness of foot muscles - Heel pad thickness	- Neutral AT- thickness (mm): 4.2 ± 0.5 - MCS AT-thickness (mm): 4.5 ± 0.4 - Minimalistic AT-thickness (mm): 4.6 ± 0.4 - Insole AT-thickness (mm): 4.2 ± 0.2 - Neutral Proximal PF (mm): 3.2 ± 0.4 - MCS Proximal PF (mm): 3.3 ± 0.2 - Minimalistic Proximal PF (mm): 2.9 ± 0.1 - Insole Proximal PF (mm): 3.0 ± 0.2 - Neutral Middle PF (mm): 1.7 ± 0.2 - MCS Middle PF (mm): 1.9 ± 0.3 - Minimalistic Middle PF (mm): 1.7 ± 0.2 - Insole Middle PF (mm)1.7 ± 0.2 - Neutral Distal PF (mm): 0.9±0.1 - MCS Distal PF (mm): 0.9 ± 0.1 - Minimalistic Distal PF (mm): 0.9 ± 0.1 - Insole Distal PF (mm): 0.8 ± 0.1 - Minimalistic 10% thicker AT compared to neutral shoe and insole - Minimalistic 9% and 12% thinner proximal PF than neutral and MCS respectively

LDR: Long distance runners; FSP: Foot strike pattern; RFS: Rear-foot strike pattern; FFS: Forefoot strike pattern; MSP: Midfoot strike pattern; Wk: Week; PF: Plantar fascia; BMI: Body mass Index; AT: Achilles Tendon; CSA: Cross-sectional area; CCR: Cross country runners; Nrm: Normalized; Abs: Absolute; CT (contact time); FT (flight time); SF (Step frequency); SL (step length); RV (running velocity); Kleg (leg stiffness); Kvert (vertical stiffness); HR (Heart Rate); MCS: Motion control shod; FPI: Foot posture index; MVIC: Maximal voluntary isometric contraction

Table IV. Tendon pathology and morphological characteristics of the connective tissue.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Freund *et al.* (2019)	**LDR** n = 22 (20 M, 2 F) 49.1 ± 11.5 years 1.74 ± 0.09 m 70.9 ± 11.3 Kg (Trans Europe Foot Race 4487Km).	Repeated measures Laboratory conditions *Run 4487 Km. Each 1000 Km or after abortion of the run	- **AT-thickness**	- SI at AT insertion, mid-portion and at site of lesion - Distance lesion from AT insertion - Number of new lesions - Highest intraosseous SI in any foot bone - Nº of bone bruises /subchondral or osseous lesions - SI of FP was rated	- AT-thickness baseline (mm) 6.8 ± 0.37 - Larger differences on the right side: SI of the AT insertion, the SI of the plantar aponeurosis - Finishers and no finishers showed significant differences at the beginning only in the SI of the PF
Hagan *et al.* (2018)	**Collegiate CCR** n = 22 (13 M, 9 F) 19 ± 1.5years 172 ± 7 cm 60.4 ± 8 Kg **Healthy controls** n = 11 (1M, 10 F) 24 ± 5.1 years 163 ±7.32 cm 59.1 ± 11.6 Kg	Repeated Measures Laboratory conditions *(S1)1 wk before start trainings (S2) 1 wk after the season conclusion (S3) 1 wk before season conclusion	**- AT-Thickness** **- PT-Thickness** - Collagen alignment - Tendon structure	- VISA-A - Neovascularity	- AT TS not confirmed by ultrasound and VISA-A. - CCR lower tendon alignment. - Tendon thickness no changes in the CCR -Tendon collagen alignment improves at S3 compared with S2 CCR underwent remodelling of both the AT and PT without TS
Hall *et al.* (2015)	**Asym runners** n = 39 (20M, 19 F) 39.3 (20–67) years 171.2 (152.4– 190.5) cm 66.0 (52.2–88.2) Kg BMI: 22.2 (18.7–26.4) Kg/m^2	Unilateral crossover Laboratory conditions	**- PF-thickness** - Plantar heel pad thickness and compressibility - PF appearance	- Shoe type preference - Neovascularity	- PF-thicknesses (mm): Right 3.78 (2.4–7.0), Left 3.87 (2.3–6.7). - Neovascularity in 31% PF, and 44% runners. - 35% of heels among 41% of runners showed a thickener PF (> 4 mm). 52% of PF normal texture.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Hirschmuller *et al.* (2012)	**Asym runners** n = 634 (425 M, 209 F) 41 ± 11.2 years 175.8 ± 8.7 cm 71.3 ± 11.1 Kg BMI: 23 ± 2.4 Kg/m² Km/wk: 39 ± 2.4	Repeated Measures Laboratory conditions * 1 year prospective	**- AT-thickness** - Spindle shaped thickening - Echogenicity	- Neovascularity - Training volume - Medical history - AT symptoms	- AT-Thickness (mm): 5.6 ± 1.1 - Echogenicity: 15% Hypo, 2% Hyper. - Neovascularity: 40% - 61 subjects new AT symptoms: 29 mid-portion tendinopathy, 3 insertion tendinopathy, 29 unspecific pain. - AT thickness significantly greater in mid-portion tendinopathy - Neovascularization significant increased risk of developing mid-portion tendinopathy - Neovascularization greatest positive predictive value for mid -portion tendinopathy
Lieberthal *et al.* (2019)	**LDR** n = 37 M 36.0 (32.0-42.0) years 180.0 (174.0-183.5) cm 77.4 (73.8-83.4) Kg	Unilateral crossover Laboratory conditions	**- AT-Thickness** - AT normal / AT abnormal (US findings)	- Standing Lunge Test - Straight Knee dorsal flexion test - Running years - Mileage - Sessions/wk - Nº Marathons or Half Marathons	- 48 Normal AT, 26 abnormal AT. - AT-Thickness (mm): Normal AT 5.4 ± 0.8, Abnormal AT 4.7 ± 0.5 - Abnormal AT significantly more years of running
Ooi *et al.* (2015)	**Marathon runners** n = 21 (13 M, 8F) 37.1 ± 11.3 years 1.75 ± 0.08 m 70.0 ± 11.6 Kg BMI: 22.6 ± 2.4 Kg/m² **Control** n = 20 (12 M, 8 F) 37.5 ± 12.3 years 1.73 ± 0.09 m 72.4 ± 11.8 Kg BMI: 23.9 ± 1.9 Kg/m²	Repeated Measures Laboratory conditions *Pre and post marathon	**- AT-Thickness** **- AT-CSA** - Echogenicity - Elastography	- VISA-A - Neovascularity	- Pre AT-thickness (mm): Runners 0.46 ± 0.05 / Control 0.52 ± 0.07 - Pre AT-CSA (mm²): Runners 0.50 ± 0.09 Control 0.57 ± 0.11 - Pre AT-CSA and AT -Thickness significant higher in runners - Hypoechogenicity significant higher in runners - No significant changes in AT-thickness and CSA post marathon - Marathon induced a significant increase in intratendinous neovascularity - 75% of neovascularity go normal after 4-6 wk.

Study	Subject Description	Study design	Connective tissue morphologic measures	Other outcome measures	Results
Shaikh *et al.* (2012)	**Pro LDR** n = 25 (19 M, 6 F) 34.2 ± 13.0 years 1.74 ± 0.08 m 69.68 ± 9.39 Kg BMI: 22.82 ± 1.71 Kg/m² **Controls** n = 25 (19 M, 6 F) 31.3 ± 15.1 years 1.68 ± 0.08 m 66.16 ± 15.39 Kg BMI: 23.36 ± 4.1 Kg/m²	Unilateral crossover Laboratory conditions	- **AT-Thickness**: 3 sites: CI, MT, MTJ	- Neovascularity - AT symptoms	- LDR MTJ AT -Thickness significant higher - LDR CI and MT AT-Thickness higher but no significant - LDR AT more symptoms - Signs of tendinopathy: 36% LDR AT and 4% control AT
Tillander *et al.* (2019)	**Amateur LDR** N = 21 (15 M, 6 F) 47.5 ± 6.3 years BMI: 23.3 ± 2.0 Kg/m² Km/wk: 28.8 ± 13.5	Unilateral crossover Laboratory conditions	- **AT-Thickness** (Max and 30 mm to calcaneus) - AT structure	- Neovascularity - Tendinosis and bursitis: Yes/No - VISA-A	- Max AT-Thickness (mm): Asym: 5.7 ± 1.0, Sym 6.7 ± 0.8 - 30mm AT-Thickness (mm): Asym 4.5 ± 0.8, Sym 5.9 ± 0.8 - Sym AT were thicker than Asym

LDR: Long distance runners; AT: Achilles Tendon; PT: Patellar tendon; PF: Plantar fascia; SI: Signal intensity; CCR: Cross country runners; Wk: week; TS: Tendinopathy symptoms; BMI: Body mass Index; Asym: Asymptomatic; Sym: Symptomatic; US: Ultrasounds; CSA: Cross-sectional area; CI: Calcaneus insertion; MT: Midtendon; MTJ: Musculotendinous junction.

Sample characteristics

Regarding sample characteristics, 16 studies were included. In relation with morphologic characteristics of the AT, 10 studies analysed the differences between runners and healthy untrained subjects (2, 3, 16, 17, 31-36). Regarding AT, Wiesinger *et al.* (36) and Kongsgaard *et al.* (37) studied the differences of the AT morphologic characteristics between runners and other sport athletes. Differences in AT morphologic characteristics were also analysed in relation to other aspects: road runners and trail runners (38), running performance (39), leg dominant side (40) and nationality (Kenyan *versus* Japanese runners) (41). Finally, other subject characteristics such as sex, body mass, height, body mass index and running experience (35, 40) were also studied in relation to the AT morphologic characteristics.

The PT morphologic characteristics were analysed by four studies (2, 17, 36, 39). Kubo *et al.* (2, 17) and Wiesinger *et al.* (36) studied the differences between runners and healthy untrained subjects. Kubo *et al.* (39) analysed the PT differences in relation the running performance. In relation with other sports, PT-CSA was analysed between long-distance runners, water-polo players and ski jumpers (36).

Finally, regarding the PF, the morphological characteristics between runners and untrained subjects were analysed only by Shiotani *et al.* (42).

Running biomechanics variables

In relation to biomechanics variables, 10 studies were included. The morphologic characteristics of the AT were analysed by 9 studies (14, 19, 43-49). The AT morphologic characteristics were assessed in relation to foot strike pattern (45, 46) and the influence of the footwear (*i.e.*, traditional *versus* minimalist shod) (44, 49). Farris *et al.* (43) and Neves *et al.* (47) studied the AT differences pre and post 10-min and 30-min running bouts, respectively. Other aspects that have been studied in relation with AT morphologic characteristics are: lower limb stiffness (19), cross country season (48), foot characteristics (49), running energy cost and running performance (14).

Finally, two studies assessed the relation of the PF-Thickness with running variables. Chen *et al.* (50) analysed the relation between PF-Thickness and foot strike pattern, while Zhang *et al.* (49) assessed the relation between footwear characteristics and PF-Thickness.

In terms of running biomechanics variables any study assessed the morphologic characteristics of the PT.

Tendon Pathology

In relation with tendon pathology, 8 studies were included. The AT morphologic characteristics and tendon pathology variables were analysed by 7 studies (51-57).

Morphologic characteristics of the PT (52) and PF (58) were studied in relation with tendon pathology variables.

DISCUSSION AND IMPLICATIONS

The purpose of this systematic review was to critically analyse the literature that has considered the morphologic characteristics of the lower limb connective tissue (*i.e.*, thickness and CSA) and its influence on running biomechanics and tendon pathology variables in runners. After the systematic analysis described above, thirty-four studies were included. In order to assess the methodological quality of these studies, the modified Downs and Black scale (30) was used in which all the studies reported, at least, 8 points out of a total of 14. In order to obtain a better thematic and visual understanding, the results were shown in relation to 3 aspects: sample characteristics, running biomechanics variables and tendon pathology.

Sample characteristics

In relation with sample characteristics, the main variable that correlates with the morphology of the connective tissue is the running condition. In this way, runners showed higher tendons in terms of CSA and thickness than untrained people or other sports people. Other variables that positively correlate with the morphologic characteristics of the connective tissue are the ethnic, weight, height, sex and running performance.

For the AT characteristics, a large majority of the studies showed that runners had greater AT-CSA and AT-thickness compared to untrained subjects (16, 17, 31-36). Although some studies did not found differences (2, 3), in both studies, Kubo *et al.* studied sprinters (2, 3), while the rest of the studies assessed long distance-runners (16, 17, 31-36). It seems that running speciality might determine whether there exist differences, in terms of AT morphologic characteristics, between runners and untrained subjects. Thereby, long-distance runners apparently show higher CSA and thickness in such tendon. The greater weekly mileage run by these runners, and therefore a repeated load for a longer time, might justify why these differences occur in long-distance runners and not in sprinters.

In relation with the PT, Kubo *et al.* (17) and Wiesinger *et al.* (36) showed higher thickness and CSA, respectively, in long-distance runners compared to untrained subjects showing no differences for sprinters (2). Therefore, as already mentioned, running speciality seems to be determinant when analysing differences of the tendon morphologic characteristics between runners and untrained subjects.

It is worth mentioning that both thickness and CSA values were normalized to the body mass in some studies (16, 17, 36). In fact, in two of these studies (16, 17), the differences between runners and controls only happened for values normalized to body mass and not for absolute values. This finding highlights the importance of normalizing these values (*i.e.*, thickness and CSA) in relation to the body mass when these characteristics are correlated with other variables, especially when the sample characteristics are not very homogenous.

From all the studies considered, only one assessed the PF morphologic characteristics between runners and untrained people (42). Greater thickness was found among runners, but the results were no significant. The lack of consistency of the results and the reduced number of studies suggest that further research is needed to deepen the understanding in relation to the aforementioned structure.

The current literature shows that the thickness and CSA of the AT and PT were higher in long distance runners than untrained people suggesting that the chronic exposure of these tendons to repetitive impacts has resulted in significant tissue adaptation. When comparing with other sports, it seems that this chronic exposure to repetitive loading may explain why kayakers (37) and water-polo players (36), with less lower-limb tendon load, showed smaller AT-CSA and PT-CSA than long distance runners. These differences do not occur in sports with a higher AT and PT load such as volleyball (37) or ski-jump (36).

As mentioned above, there are other variables that are less studied (*i.e.*, ethnics, weight, height, sex and running experience), which seem to show a positive correlation with the morphology of the connective tissue in runners. Kenyan runners had a higher AT-CSA than their Japanese counterparts (41). AT-CSA showed a positive correlation with height (35, 40), weight (40) and sex (*i.e.*, males larger values) (40), while AT-Thickness correlates positively with height (35). Again, the repetitive loading over tendon appears as an influencing factor, since both running experience and kilometres run per week positively correlate with AT-CSA (40) and AT-Thickness (35, 40).

Considering the results, the exposure of the tendons to repetitive loading appears to be the main influencing factor on tendon characteristics. But not exclusively the load, but also the time that load has been repeated seems to be influential. The tendons of the sprinters bear high loads, but such loads are not as repeated in time as in the case of long-distance runners. Thus, the chronicity exposure to repetitive loading seems to be key when analysing differences of the tendon morphologic characteristics between runners and untrained people. To the best of the authors' knowledge, these differences remain uncertain and not well understood by professional and amateur runners. It would be worth

analysing whether tendon morphological characteristics is dependent upon training level within the same sport.
Other possible influencing factors that were studied but that did not show a significant relationship with the morphological characteristics of the connective tissue were running surface (38), running performance (39) and dominant side (40). The studies that have evaluated these aspects are few, so the scientific evidence is limited.

Running biomechanics variables

The two main running biomechanics variables that were analysed were the FSP (45, 46) and the footwear (44, 49). However, the results of these studies are controversial. FSP and footwear are closely related as it is known that runners wearing minimalist shoes typically show a forefoot strike pattern while runners using traditional footwear usually collide with the ground with the heel first (59). However, there is no consensus about the relation of these variables and the AT morphologic characteristics. While some studies supported that minimalist runners showed larger AT-CSA (44) and AT-Thickness (49), other studies did not find any differences between AT characteristics and foot strike pattern (45, 46). The fact that minimalist runners demand more the AT (60) could explain why these tendons are larger. Again, the loading repetition would result in tendon adaptations. One study that did not found any differences between foot strike pattern and AT characteristics was the study of Kubo *et al.* (46), where forefoot, midfoot and rearfoot strike patterns where compared. This fact could be explained because all the subjects of this study (46) were high level runners (*i.e.*, best official 5000 m record faster than 15'). Probably, the AT of all these highly trained subjects have been exposed to high repetitive loadings, regardless of the foot strike pattern, which could explain that no differences were found in relation to the foot strike pattern. The other study that did not found AT differences in relation with the foot strike pattern was Kernozek *et al.* (45). In this study, the sample was formed only by females. This fact could be key to explain this lack of differences, since in other study, Joseph *et al.* (61), found that greater AT adaptations, after a 6-month adaptation from traditional shod to minimalist shod, appeared only in males.
It seems that the chronic adaptations to the repetitive loading mentioned above also occurs acutely. It has been found that professional cross-country runners showed larger AT-CSA during cross country season, compared to pre-season and post-season (3-5 week after) (48). Therefore, these tendon adaptations could occur in a chronic way between subjects demanded to different loads (*i.e.*, runners *vs* untrained people), but acute intra-subject differences would also appear in response to moments of the season with greater loads. However, when the immediate response to the load is evaluated, the results corresponding to the tendon morphology are again contradictory. While, Neves *et al.* (47) found a decrease in the AT-CSA after 10´run, no changes were found by Farris *et al.* (43) after 30' run. More research seems necessary in this regard. It would also be interesting, when assessing the immediate adaptations of the tendon to the load, to take into account a factor that seems to influence such as the type of footwear.
Other running biomechanics variable, that showed a relation with tendon morphology is the lower limb stiffness, although this variable has been less studied. It seems that vertical stiffness (Kvert) has a positive correlation with AT-CSA (19) although further research is needed in this line. Finally only two studies (49, 50) analysed the relation between the PF morphologic characteristics and running biomechanics variables. The main finding in relation with this structure was that PF was thinner among runners using minimalist running shoes (49).
An outstanding finding in relation with running biomechanics variables is that none of the studies considered here analysed the influence of the PT characteristics. The lack of studies on PT is striking as it is a basic structure in running biomechanics. It is known that during running the lower limb behaves such as a spring that continually compresses and decompresses (9). During such mechanism, a proper function of the knee is needed and consequently the PT characteristics would be decisive.

Tendon pathology

Although eight studies assessed the connective tissue morphologic characteristics in relation with tendon pathology variables, only three of these studies (53, 54, 57) analysed the direct relation between these variables and the connective tissue characteristics. It was found that symptomatic AT were thicker compared to healthy AT (53, 57). Contrary to this finding, Lieberthal *et al.* (54) showed that the AT with abnormal ultrasound findings were thinner than normal AT. Probably, the variable used in each of these studies to consider the tendon as a "pathological tendon" can explain the reason for these contradictory results. In relation with the pathology model of the tendinopathy, Cook *et al.* (20) claimed that is a continuum. Thus, following this continuum, the tendinopathy process would comprise three states or phases: reactive tendinopathy, tendon disrepair and degenerative tendinopathy (20). In the first two phases, the presence of a thickening of the tendon structure is observed, while in the last of them the clinic of this tendon may show a thickening but also not occur (20). It would be worth knowing in which of these three phases were the subjects of the mentioned studies (53, 54, 57) as this could better explain why this thickening occurs or not. Additionally, it should be noted that one of the inclusion criteria of Lieberthal *et al.* study was that the subjects must have run a marathon or half marathon in the last two years (54). The volume of kilometres necessary to complete the training of such efforts is usually quite important. In fact, the number of weekly kilometres run by the participants in the study of Lieberthal *et al.* (54) is almost double than that of the studies of Hirschmuller *et al.* (53) as Tillander *et al.* (57). It may happen that the participants of the study by Lieberthal *et al.* (54), having run a greater number of kilometres and therefore a greater load on the tendon, will be in a more advanced stage of tendinopathy. This could correspond to that phase of degenerative tendinopathy in which the tendon does not necessarily appear thickened. As is explained by Cook *et al.* (20), this last tendon pathology phase is characteristic of younger subjects or elite athletes with a chronically overloaded tendon, which is also a characteristic of the study sample of Lieberthal *et al.* (54). Furthermore, as Cook *et al.* explain in their recent update to their continuum model of tendon (59), not always all the pathologic and painful tendons show an altered ultrasound image.

Finally, only two studies (52, 58) analysed the PT and the PF in relation with pathology variables, without any significant result. Again, as in the previous sections, the main research focus was the AT. It would be interesting for future researchers to delve deeper into the influences and relationships of these structures, PT and PF.

From a practical point of view, it seems clear that runners present larger tendons as adaptations to the repetitive loading stimulus. These adaptations might lead to better running performance, so coaches should consider how the training load affects the tendon in order to optimize that adaptation. Especially, this attention to tendon adaptation should be a fundamental aspect in the training of amateur runners, whose tendons do not show such adaptations. However, when the running repetitive load is excessive, tendons might not be able to adapt properly increasing, thus, the likelihood of running related injuries. The ultrasonography is reliable method to control these morphologic tendon changes, and consequently assess these adaptations. Nevertheless, the clinicians should not consider this diagnostic imaging technique as an essential criteria, but rather as a complementary test, in the diagnosis of tendon pathology in runners. Given that tendon pathology may or may not present with morphological changes, detectable with ultrasound, the diagnosis of tendinopathy should be based on the tendon symptoms as well as its functional alteration.

CONCLUSIONS

The present systematic review about the morphologic characteristic of lower limb connective tissue and its influence in running biomechanics and tendon pathology variables of runners reveals that: 1) runners have higher AT and PT than control subjects (*i.e.*, untrained subjects) whose tendons are demanded at lower loads. When the characteristics of the sample are not homogeneous, it seems important to analyse the differences with the normalized connective tissue values in relation to body mass. 2) The footwear and Kvert seems to be the main running biomechanics variables that relate with the morphologic characteristics of the connective tissue. 3) In relation to the tendons of runners that present tendinopathy, there is no consensus regarding whether these tendons are thicker or thinner. Scientific evidence seems to indicate that this fact will depend on the phase of tendinopathy in which it is found.

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DATA AVAILABILITY

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LERS, ARP: study design. LERS, ARP, FGP: study analysis. LERS, DJC, ACL, ARP: data collection. ARP, FGP: results interpretation. All authors contributed to the manuscript writing and have read and approved the final version.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Kannus P. Structure of the tendon connective tissue. Scand J Med Sci Sports 2000;10(6):312-20.
2. Kubo K, Ikebukuro T, Yata H, Tomita M, Okada M. Morphological and mechanical properties of muscle and tendon in highly trained sprinters. J Appl Biomech 2011;27(4):336-44.
3. Kubo K, Miyazaki D, Ikebukuro T, Yata H, Okada M, Tsunoda N. Active muscle and tendon stiffness of plantar flexors in sprinters. J Sports Sci 2017;35(8):742-8.
4. Del Baño-Aledo ME, Martínez-Payá JJ, Ríos-Díaz J, Mejías-Suárez S, Serrano-Carmona S, de Groot-Ferrando A. Ultra-

sound measures of tendon thickness: Intra-rater, Inter-rater and Inter-machine reliability. Muscles Ligaments Tendons J 2017;7(1):192-9.
5. Stenroth L, Sefa S, Arokoski J, Töyräs J. Does Magnetic Resonance Imaging Provide Superior Reliability for Achilles and Patellar Tendon Cross-Sectional Area Measurements Compared with Ultrasound Imaging? Ultrasound Med Biol 2019;45(12):3186-98.
6. Wang JH, Guo Q, Li B. Tendon biomechanics and mechanobiology--a minireview of basic concepts and recent advancements. J Hand Ther 2012;25(2):133-40; quiz 141.
7. Shaw HM, Vázquez OT, McGonagle D, Bydder G, Santer RM, Benjamin M. Development of the human Achilles tendon enthesis organ. J Anat 2008;213(6):718-24.
8. Gruber M, Kramer A, Mulder E, Rittweger J. The Importance of Impact Loading and the Stretch Shortening Cycle for Spaceflight Countermeasures. Front Physiol 2019;10:311.
9. McMahon TA, Cheng GC. The mechanics of running: how does stiffness couple with speed? J Biomech 1990;23 Suppl 1:65-78.
10. Vogt M, Hoppeler HH. Eccentric exercise: mechanisms and effects when used as training regime or training adjunct. J Appl Physiol (1985) 2014;116(11):1446-54.
11. Fletcher JR, MacIntosh BR. Running Economy from a Muscle Energetics Perspective. Front Physiol 2017;8:433.
12. Ishikawa M, Pakaslahti J, Komi PV. Medial gastrocnemius muscle behavior during human running and walking. Gait Posture 2007;25(3):380-4.
13. Hunter GR, McCarthy JP, Carter SJ, *et al*. Muscle fiber type, Achilles tendon length, potentiation, and running economy. J Strength Cond Res 2015;29(5):1302-9.
14. Ueno H, Suga T, Takao K, *et al*. Relationship between Achilles tendon length and running performance in well-trained male endurance runners. Scand J Med Sci Sports 2018;28(2):446-51.
15. Gabaldon AM, Nelson FE, Roberts TJ. Relative shortening velocity in locomotor muscles: turkey ankle extensors operate at low V/V(max). Am J Physiol Regul Integr Comp Physiol 2008;294(1):R200-10.
16. Kubo K, Miyazaki D, Yamada K, Yata H, Shimoju S, Tsunoda N. Passive and active muscle stiffness in plantar flexors of long distance runners. J Biomech 2015;48(10):1937-43.
17. Kubo K, Tabata T, Ikebukuro T, Igarashi K, Yata H, Tsunoda N. Effects of mechanical properties of muscle and tendon on performance in long distance runners. Eur J Appl Physiol 2010;110(3):507-14.
18. Stanley LE, Lucero A, Mauntel TC, *et al*. Achilles tendon adaptation in cross-country runners across a competitive season. Scand J Med Sci Sports 2018;28(1):303-10.
19. Monte A, Nardello F, Pavei G, Moro S, Festa L, Tarperi C, Schena F, Zamparo P. Mechanical determinants of the energy cost of running at the half-marathon pace. J Sports Med Phys Fitness 2020;60(2):198-205.
20. 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(6):409-16.
21. Romero-Morales C, Martin-Llantino PJ, Calvo-Lobo C, *et al*. Comparison of the sonographic features of the Achilles Tendon complex in patients with and without achilles tendinopathy: A case-control study. Phys Ther Sport 2019;35:122-6.
22. Lopes AD, Hespanhol Júnior LC, Yeung SS, Costa LO. What are the main running-related musculoskeletal injuries? A Systematic Review. Sports Med 2012;42(10):891-905.
23. Knobloch K, Yoon U, Vogt PM. Acute and overuse injuries correlated to hours of training in master running athletes. Foot Ankle Int 2008;29(7):671-6.
24. McKean KA, Manson NA, Stanish WD. Musculoskeletal injury in the masters runners. Clin J Sport Med 2006;16(2):149-54.
25. Lorimer AV, Hume PA. Achilles tendon injury risk factors associated with running. Sport Med 2014;44(10):1459-72.
26. Magnan B, Bondi M, Pierantoni S, Samaila E. The pathogenesis of Achilles tendinopathy: a systematic review. Foot Ankle Surg 2014;20(3):154-9.
27. Kovács B, Kóbor I, Gyimes Z, Sebestyén Ö, Tihanyi J. Lower leg muscle-tendon unit characteristics are related to marathon running performance. Sci Rep 2020;10(1):17870.
28. Munteanu SE, Barton CJ. Lower limb biomechanics during running in individuals with achilles tendinopathy: a systematic review. J Foot Ankle Res 2011;4:15.
29. Liberati A, Altman DG, Tetzlaff J, *et al*. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med 2009;151(4):W65-94.
30. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 1998;52(6):377-84.
31. Devaprakash D, Obst SJ, Lloyd DG, *et al*. The Free Achilles Tendon Is Shorter, Stiffer, Has Larger Cross-Sectional Area and Longer T2(*) Relaxation Time in Trained Middle-Distance Runners Compared to Healthy Controls. Front Physiol 2020;11:965.
32. Hullfish TJ, Hagan KL, Casey E, Baxter JR. Achilles tendon structure differs between competitive distance runners and nonrunners despite no clinical signs or symptoms of midsubstance tendinopathy. J Appl Physiol 2018;125(2):453-8.
33. Magnusson SP, Kjaer M. Region-specific differences in Achilles tendon cross-sectional area in runners and non-runners. Eur J Appl Physiol 2003;90(5-6):549-53.
34. Rosager S, Aagaard P, Dyhre-Poulsen P, Neergaard K, Kjaer M, Magnusson SP. Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. Scand J Med Sci Sports 2002;12(2):90-8.
35. Salinero JJ, Lara B, Gutierrez-Hellin J, *et al*. Thickness And Cross-Sectional Area Of The Achilles Tendon In Marathon Runners: A Cross-Sectional Study. Rev Brasil Med Esporte 2020;26:391-5.
36. Wiesinger HP, Rieder F, Kosters A, Muller E, Seynnes OR. Are Sport-Specific Profiles of Tendon Stiffness and Cross-Sectional Area Determined by Structural or Functional Integrity? PloS One 2016;11(6):e0158441.
37. Kongsgaard M, Aagaard P, Kjaer M, Magnusson SP. Structural Achilles tendon properties in athletes subjected to different exercise modes and in Achilles tendon rupture patients. J Appl Physiol (1985) 2005;99(5):1965-71.
38. Dar G, Waddington G, Stern M, Dotan N, Steinberg N. Differences Between Long Distance Road Runners and Trail Runners in Achilles Tendon Structure and Jumping and Balance Performance. PM R 2020;12(8):794-804.

39. Kubo K MD, Shimoju S, Tsunoda N. Relationship between elastic properties of tendon structures and performance in long distance runners. European J Appl Physiol 2015;115(8):1725-33.
40. Kudron C, Carlson MJ, Meron A, Sridhar B, Brakke Holman R. Using Ultrasound Measurement of the Achilles Tendon in Asymptomatic Runners to Assist in Predicting Tendinopathy. J Ultrasound Med 2020;39(3):491-6.
41. Kunimasa Y, Sano K, Oda T, Nicol C, Komi PV, Locatelli E, *et al*. Specific muscle-tendon architecture in elite Kenyan distance runners. Scand J Med Sci Sports 2014;24(4):e269-74.
42. Shiotani H, Mizokuchi T, Yamashita R, Naito M, Kawakami Y. Acute effects of long-distance running on mechanical and morphological properties of the human plantar fascia. Scand J Med Sci Sports 2020;30(8):1360-8.
43. Farris DJ, Trewartha G, McGuigan MP. The effects of a 30-min run on the mechanics of the human Achilles tendon. Eur J Appl Physiol 2012;112(2):653-60.
44. Histen K, Arntsen J, L'Hereux L, *et al*. Achilles Tendon Properties of Minimalist and Traditionally Shod Runners. J Sport Rehabil 2017;26(2):159-64.
45. Kernozek TW, Knaus A, Rademaker T, Almonroeder TG. The effects of habitual foot strike patterns on Achilles tendon loading in female runners. Gait Posture 2018;66:283-7.
46. Kubo K, Miyazaki D, Tanaka S, Shimoju S, Tsunoda N. Relationship between Achilles tendon properties and foot strike patterns in long-distance runners. J Sports Sci 2015;33(7):665-9.
47. Neves KA, Johnson AW, Hunter I, Myrer JW. Does achilles tendon cross sectional area differ after downhill, level and uphill running in trained runners? J Sports Sci Med 2014;13(4):823-8.
48. Sponbeck JK, Perkins CL, Berg MJ, Rigby JH. Achilles Tendon Cross Sectional Area Changes Over a Division I NCAA Cross Country Season. Int J Exerc Sci 2017;10(8):1226-123.
49. Zhang X, Delabastita T, Lissens J, De Beenhouwer F, Vanwanseele B. The morphology of foot soft tissues is associated with running shoe type in healthy recreational runners. J Sci Med Sport 2018;21(7):686-90.
50. Chen TL, Agresta CE, Lipps DB, *et al*. Ultrasound elastographic assessment of plantar fascia in runners using rearfoot strike and forefoot strike. J Biomech 2019;89:65-71.
51. Freund W, Weber F, Billich C, Schuetz UH. The foot in multistage ultra-marathon runners: experience in a cohort study of 22 participants of the Trans Europe Footrace Project with mobile MRI. BMJ Open 2012;2(3):e001118.
52. Hagan KL, Hullfish TJ, Casey E, Baxter JR. Tendon Structure Quantified using Ultrasound Imaging Differs Based on Location and Training Type. J Appl Physiol (1985) 2018.
53. Hirschmüller A, Frey V, Konstantinidis L, *et al*. Prognostic value of Achilles tendon Doppler sonography in asymptomatic runners. Med Sci Sports Exerc 2012;44(2):199-205.
54. Lieberthal K, Paterson KL, Cook J, Kiss Z, Girdwood M, Bradshaw EJ. Prevalence and factors associated with asymptomatic Achilles tendon pathology in male distance runners. Phys Ther Sport 2019;39:64-8.
55. Ooi CC, Schneider ME, Malliaras P, Counsel P, Connell DA. Prevalence of morphological and mechanical stiffness alterations of mid Achilles tendons in asymptomatic marathon runners before and after a competition. Skeletal Radiol 2015;44(8):1119-27.
56. Shaikh Z, Perry M, Morrissey D, Ahmad M, Del Buono A, Maffulli N. Achilles tendinopathy in club runners. Int J Sports Med 2012;33(5):390-4.
57. Tillander B, Gauffin H, Lyth J, Knutsson A, Timpka T. Symptomatic Achilles Tendons are Thicker than Asymptomatic Tendons on Ultrasound Examination in Recreational Long-Distance Runners. Sports (Basel) 2019;7(12):245.
58. Hall MM, Finnoff JT, Sayeed YA, Smith J. Sonographic Evaluation of the Plantar Heel in Asymptomatic Endurance Runners. J Ultrasound Med 2015;34(10):1861-71.
59. Cook JL, Rio E, Purdam CR, Docking SI. Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? Br J Sports Med 2016;50(19):1187-91.