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Structural and Functional Evaluation of Achilles Tendons in Rabbits: A Biomechanical Perspective

Imdat Orhan[1], Aydın Alan[1], Emel Alan[2], Ayhan Düzler[1]

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ABSTRACT

Background: The Achilles tendon (tendo calcaneus communis) is a critical structure for movement, transmitting force from muscle contraction to the skeletal system. Understanding its biomechanical and histological properties is essential for veterinary medicine, biomechanics and tendon injury research. While previous studies have examined Achilles tendon adaptation in various species, limited research has compared the structural and functional differences between right and left tendons. This study aims to analyze and compare the biomechanical properties of right and left Achilles tendons in rabbits, focusing on tensile strength, elongation and stiffness.

Methods: Six adult, healthy male New Zealand rabbits were used in this study. A total of 12 Achilles tendons (both right and left) were dissected and analyzed. Biomechanical testing was performed using a SHIMADZU AG-X 50 kN Autograph device, measuring failure force, elongation and stiffness. Additionally, histological analysis was conducted using Crossman's triple staining technique to examine structural differences between the tendons.

Result: The results indicated that right Achilles tendon exhibited significantly higher tensile strength than left tendon. The mean failure force for right tendons was 408.53 N, while left tendon had a lower mean failure force of 337.45 N. Similarly, right tendon showed greater elongation and stiffness, suggesting higher mechanical resilience. These findings align with previous research on tendon adaptation to mechanical stress and provide insights into functional differences between tendon structures. This study highlights the biomechanical asymmetry of Achilles tendons in rabbits, which may be relevant for tendon rehabilitation strategies and experimental tendon models.

Key words: Achilles tendon, Biomechanics, Histological analysis, Rabbit model, Tendon adaptation, Tensile strength.

INTRODUCTION

In domestic mammals, the Achilles tendon is formed by the muscles m. gastrocnemius, m. soleus, m. biceps femoris, m. semitendinosus and m. flexor digitorum superficialis, along with their extensions (Dursun, 2008; NAV, 2012). However, in rabbits, the Achilles tendon formation differs as the M. semimembranosus replaces the M. semitendinosus in this structure (Nisbet, 1960; Bensley, 1910).

Tendons are fibrous connective tissue that transmit the force of muscle contractions to bones, enabling effective limb movement (Buchanan and Marsh, 2002; Ker *et al*., 2000; Merrilees and Flint, 1980; Curwin, 1997). These structures consist of a complex composition, primarily made up of 70-80% type I collagen for tensile strength and 10-40% elastin for elasticity (Merrilees and Flint, 1980; Curwin, 1997).

The evaluation of the structural and mechanical properties of tendons provides important insights into soft tissue health in both clinical and experimental studies. In particular, studies utilizing advanced imaging techniques such as elastography have made it possible to characterize tendon lesions and monitor mechanical changes (Jani *et al*., 2020).

The Achilles tendon is notable for its biomechanical properties, which vary with age. Nakagawa *et al*. (1996) conducted a study on the Achilles tendon in rabbits and demonstrated that young rabbit tendons exhibit high tensile strength and elasticity as they age. These findings indicate that while the Achilles tendon develops structural strength during growth, its durability remains stable throughout aging. It has been reported that age and a history of physical activity play an important role in the prevention of tendon injuries (Nakagawa *et al*., 1996).

Detailed anatomical analyses of the musculoskeletal structures, such as those performed on the ostrich foot locomotor system, provide valuable insights into the mechanical roles of tendons and ligaments in weight-bearing and movement (Zhang *et al*., 2016). Similar biomechanical underst anding is essential when evaluating

[1]Department of Anatomy, Faculty of Veterinary Medicine, Erciyes University, Türkiye.
[2]Department of Histology, Faculty of Veterinary Medicine, Erciyes University, Türkiye.

Corresponding Author: Imdat Orhan, Department of Anatomy, Faculty of Veterinary Medicine, Erciyes University, Türkiye.
Email: imdatorhan@erciyes.edu.tr
ORCIDs: 0009-0000-4450-0371, 0000-0003-0428-578X, 0000-0003-4990-3991, 0000-0001-9515-574X

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the structural and functional properties of the Achilles tendon in rabbits.

Collagen-based structural evaluations, such as those conducted on chicken shank tendons, have highlighted the relationship between age and fiber density, underscoring the importance of morphological integrity in biomedical applications (Rana *et al*., 2024). Similar principles apply to the study of structural and functional properties of the Achilles tendon in rabbits.

Numerous studies have explored the properties of the Achilles tendon, including stiffness, resilience, elongation, maximum load and breaking load, across various animal species (Matsumoto *et al*., 2003; Almeida-Silveria *et al*., 2000; Diehl *et al*., 2006; Doral *et al*., 2010; Buchanan and Marsh, 2002; Han *et al*., 2000; Jielile *et al*., 2010; Nagasawa *et al*., 2008; Szaro *et al*., 2011; Nakagawa *et al*., 1996; Legerlotz *et al*., 2007; Buchanan and Marsh, 2001; Vidik, 1969; Maganaris and Narici, 2005; Wang, 2006; Nisbet, 1960).

This study introduces a rabbit Achilles tendon model using a full transection method to examine two major challenges in tendon healing: re-rupture and adhesion formation (Meier Bürgisser *et al*., 2016). Evaluating the biomechanical properties of the Achilles tendon highlights the importance of appropriate immobilization techniques to prevent adhesion to surrounding tissues. Studies have shown that adhesion formation can be controlled by varying immobilization angles (Meier Bürgisser *et al*., 2016).

Achilles tendon injuries or ruptures are among the most common cases of tendon damage in clinical practice (Matsumoto *et al*., 2003; Doral *et al*., 2010; Jielile *et al*., 2010; Nagasawa *et al*., 2008; Nakagawa *et al*., 1996; Legerlotz *et al*., 2007).

Additionally, studies comparing the biomechanical properties of tendons in different animal species have aimed to identify the most suitable model for simulating human tendons. Rabbit, horse, dog, and pig tendons have been examined for their mechanical resistance, elastic modulus, and ultimate tensile strength (Burgio *et al*., 2022). These findings contribute to underst anding tendon load-bearing capabilities and provide valuable insights for biomechanical testing of human tendons.

The Achilles tendon plays a vital role in transmitting muscle contraction force to bones, ensuring effective movement (Merrilees and Flint, 1980; Curwin, 1997). Its biomechanical properties, primarily determined by its type I collagen composition, allow it to withst and high tensile loads and resist injuries. Additionally, studies on rabbits have demonstrated that specific loading frequencies positively impact tendon strength (Wang *et al*., 2013).Wang *et al*. (2013) found that low-frequency loading at 6% strain for 8 hours daily significantly increased collagen production and enhanced tendon strength.

This study aims to investigate the mechanical and histological properties of the common calcaneal tendon (tendo calcaneus communis) in rabbits.

MATERIALS AND METHODS

This study was conducted between 2013 and 2015 at Erciyes University Faculty of Veterinary Medicine and Erciyes University Research Center. In the study, six healthy, adult male New Zeal and rabbits weighing between 2.8 and 3.2 kg were used. A total of 12 Achilles tendons, both right and left, were obtained from rabbits sacrificed under anesthesia. From these fresh specimens, three were sectioned from the midline for histological examination. These sections were fixed in a 10% formaldehyde-ethanol solution for 12 hours, followed by processing through graded alcohols, methyl benzoate and benzene and then embedded in paraplast blocks. Thin sections of 5 µm were prepared from these blocks and three histological slides were generated. The slides were stained using Crossman's triple staining technique to examine the general structure.

For mechanical testing, the tendons were prepared by immersing them in 10% formalin for 10 days. Testing was conducted using the SHIMADZU AG-X 50 kN Autograph device.

Fig 1: Mechanical testing of tendons.

The distal end of each tendon was clamped at the calcaneus attachment, while the proximal end was secured at the muscle-tendon junction (Fig 1). A tensile force of 5 N/s was applied to all specimens and the pulling process continued until tendon rupture. The obtained data were visualized using graphs.

RESULTS AND DISCUSSION

Structures of tendons that consist of much collagen and few elastin fibers were determined in histological investigation. As shown in the Fig 2-3, in longitudinal and cross-section, fibroblast nuclei were observed to be squeezed between collagenous fiber bundles (Fig 2,3).

The thickness of the tendons used in the study was measured as an average of 5 mm at the midpoint. The cross-sectional area was described as oval in shape. The tendon length from the muscle-tendon junction to the calcaneus was measured as an average of 27.8 mm.

According to the tensile test values shown in the Table 1, the average breaking force of all analyzed right and left Achilles tendons was 361.147 N, the average elongation percentage was 62%, and the average elongation length was 9.524 mm. The average breaking force for the left tendons was measured as 337.455 N, while the right tendons had a higher average breaking force of 408.530 N. All the obtained values were visualized using Table 1 and Graph 1.

The results indicated that the right tendons were more resistant than the left tendons. Additionally, the failure force and elongation rate for the right tendons were found to be higher than the average. Considering all the tendons used in the study, they were observed to withst and an average

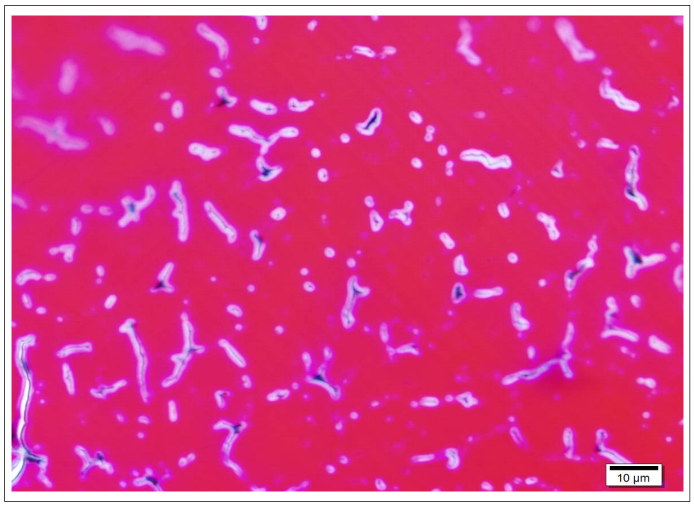


Fig 2: Cross section of tendon. A: fibroblast nucleus.

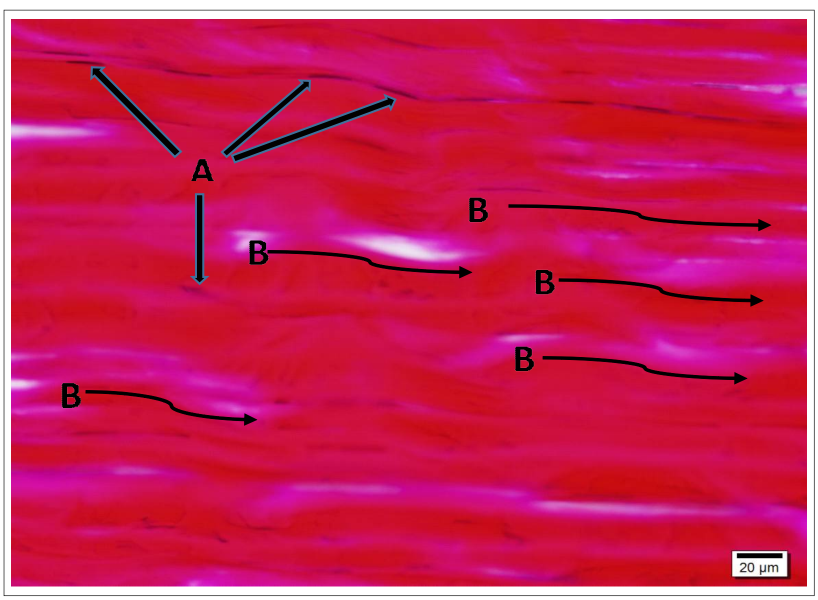


Fig 3: Longitudinal section of tendon. A: fibroblast nuclei, B: Collagenous fiber bundles.

force of 361.14 N and exhibit an average elongation of 62.37%. These findings indicate that tendons can stretch beyond half of their original length under normal conditions.

Merrilees and Flint (1980) reported that the presence of 70-80% type I collagen in tendon composition contributes significantly to its strength. Similarly, Curwin (1997) emphasized that immobilization negatively impacts the biomechanical properties of tendons, a finding consistent with the importance of mechanical stress for optimal tendon resilience, as observed in our study.

The higher tensile strength observed in the right tendons compared to the left tendons is comparable to the findings of Viidik *et al*. (1965), who demonstrated that tendons subjected to greater loads exhibit increased tensile capacity. This observation suggests that the right tendons in our study may have been exposed to higher stress, leading to greater resilience.

The results also align with Nakagawa *et al*. (1996), who studied age-related changes in the biomechanical properties of rabbit Achilles tendons. They found that young adult tendons had the highest tensile strength, which was attributed to increased collagen fibril dimensions during growth. In our study, no age distinction was made and adult rabbits were used. Therefore, no conclusions can be drawn regarding the relationship between aging and tendon injury.

The mechanical properties of the rabbit Achilles tendons in our study are also consistent with Burgio *et al*. (2022), who compared tendons from different animal species. Rabbit tendons demonstrated biomechanical properties, such as elastic modulus and tensile strength, that closely resemble those of human tendons, making them a suitable model for biomechanical studies. Our findings support the notion that variables like applied stress rates contribute to variations in mechanical properties.

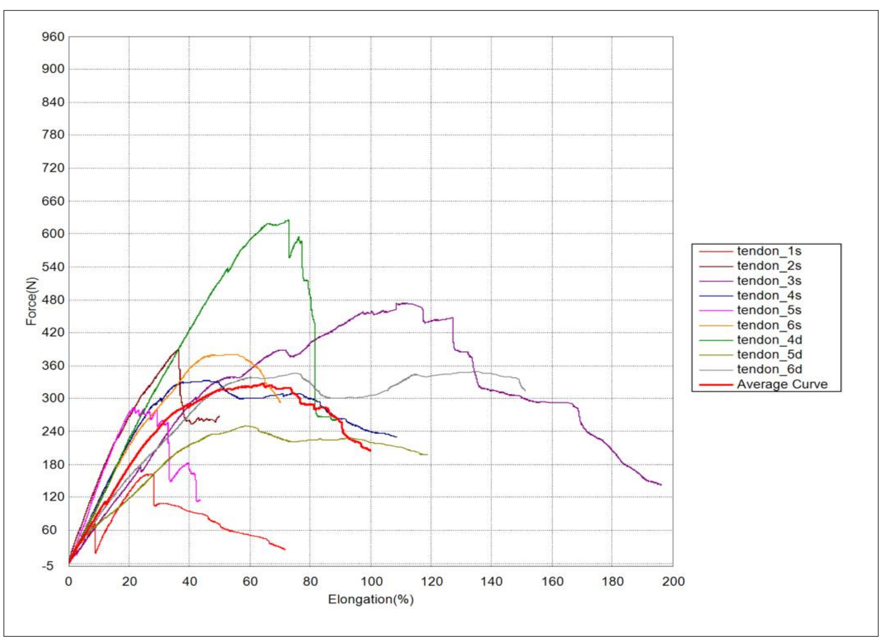
Our results also parallel the findings of Meier Bürgisser *et al*. (2016), who showed that the biomechanical properties of Achilles tendons improved over time in a full transection healing model, although they did not fully recover to pre-injury levels. Their study highlighted the significance of immobilization techniques in reducing adhesion formation and optimizing tendon healing strategies.

Furthermore, studies by Bürgisser and Buschmann (2014) suggest that elongation during tendon healing may lead to decreased force transmission and functional impairment, findings consistent with our observations on tendon properties.

Compared to our study, Matsumoto *et al*. (2003) reported higher stiffness (125.1) and tensile strength (549.2

Table 1: This table presents a comparative overview of the biomechanical properties of right and left Achilles tendons, including their maximum, minimum, and mean values for failure force, elongation and stiffness.

Tendons	Right			Left		
Measurement	Tendon (Max)	Tendon (Min)	Tendon (Mean)	Tendon (Max)	Tendon (Min)	Left tendon (Mean)
Failure force (N)	625.88	249.68	408.53	474.58	162.99	337.45
Elongation (%)	135.26	58.72	88.95	111.03	21.32	49.09
Stiffness (N/mm²)	31.87	12.71	20.81	24.17	8.31	17.18



Graph 1: All tendons are showed graphically with force-elongation rate.

N) in a study of 20 adult female rabbits. Similarly, Nakagawa *et al*. (1996) observed a higher tensile strength (average: 919.6 N) but lower elongation rate (16.3%) in adult Japanese white rabbits, though the sex of the animals was unspecified. Viidik (1969) examined Achilles tendons in 30 male rabbits and found similar results (mean tensile strength: 377.4 N) in the control group, consistent with the average tensile strength in our study (361.14 N).

CONCLUSION

Since the aim of previous studies was not to determine the fundamental properties of Achilles tendons in rabbits, some characteristic could not be compared. We used control group values from earlier studies for comparisons. Variations may arise due to differences in sex, fixation and storage methods, or the breeds of rabbits used. No prior study has compared left and right Achilles tendons within the same animal. Despite the limited sample size, determined that the right Achilles tendon is more resistant than the left in this study.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and h andling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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