

Age-related changes in human tendo calcaneus collagen fibrils

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ABSTRACT

Objectives: The ruptures of tendo calcaneus often occur between the age of 30 - 45 years as described by several textbooks. It is also described that some diseases and drugs are said to be responsible in the etiology; however, there are no studies related with the detailed histological structure of collagen fibrils found in the tendon in the age groups of humans. In view thereof, this study was aimed to obtain further information on the etiology and to find an answer regarding the frequency of the ruptures occurring between the age of 30 - 45 years in humans.

Methods: In the study, the biopsy specimens taken from 28 patients (ages 1 - 68 year) who had undergone surgery due to tendo calcaneus ruptures or achilloglasy operation were examined by transmission electron microscope. All the specimens were prepared according to routine electron microscopic tissue preparation technique. The patients were divided into 7 age groups (1 - 9, 10 - 19, 20 - 29, 30 - 39, 40 - 49, 50 - 59, >60 years) and there were 4 patients in each group. The transverse diameters of collagen fibers were measured from the ultra thin sections and statistical analysis of the results were performed. The study was carried out in the electron microscopy laboratory of the Anatomy Department of

Hacettepe University, Ankara, Turkey between January 2004 and September 2004.

Results: The diameters of the collagen fibers were higher in the 20 - 29 year-old group compared to other groups and it showed a statistically significant difference. In patients who were in the 30 - 39 year-old group or older, the diameters of the collagen fibers were lesser than the 20 - 29 year-old group. However, an increase was observed in the collagen fibril concentration of these groups. In examination of the specimens of patients who were under 20-year-old, the diameter of the collagen fibers were less than the 20 - 29 year-old group. The electron microscopic appearance of the tissue sample of a one-year-old patient had a specific organization and in this patient, both the diameters and concentration of collagen fibers were less.

Conclusion: We believe that the decrease in the diameters of collagen fibers of 30 - 45 year-old patients who are in the active period of their life, can play a role in the etiology of the frequency of tendo calcaneus ruptures similar to other etiologic factors.

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The tendo calcaneus or Achilles tendon is the thickest and strongest tendon of the human body. It is approximately 15 cm long and begins near the middle of the calf.¹ According to the classical textbooks and literature, the rupture of this tendon often occur in ages between 30 - 45 year.²⁻⁴ Sports injuries, drugs (steroids, levofloxacin, ciprofloxacin, androstenediol, quinolones),

granulomatous vasculitis, alkaptonuria, rheumatoid arthritis and systemic lupus erythematosus are said to be responsible in the rupture etiology;⁵⁻¹³ however, there are no studies related with the detailed histological structure of the collagen fibrils found in the tendon, in the age groups of humans. Depending upon all these knowledge, this study was

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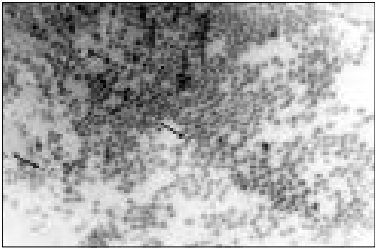


Figure 1 - Electron micrograph showing the transverse diameters of collagen fibers (arrows) in 1 - 9 years group. (Original magnification x 15000).

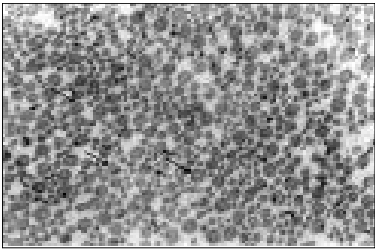


Figure 2 - Electron micrograph showing the transverse diameters of collagen fibers (arrows) in 20 - 29 years group. (Original magnification x 15000).

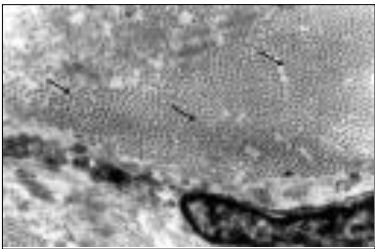


Figure 3 - Electron micrograph showing the transverse diameters of collagen fibers (arrows) in over 60 years age group. (Original magnification x 15000)

aimed to obtain further information on the etiology and to find an answer to the frequency of the ruptures in ages between 30 - 45 years.

Methods. In this study, the biopsy specimens taken from 28 patients (aged 1 - 68 years) who had undergone surgery, due to tendo calcaneus ruptures or achilloplasty operation were examined by transmission electron microscope. Informed consent was obtained from the patients prior to surgery, for taking approximately 1 mm³ of tissue specimen. The patients were divided into 7 age groups with 4 patients in each group. The age groups of the patients were 1 - 9 years (mean 5.3 years), 10 - 19 years (mean 16.9 years), 20 - 29 years (mean 24.6 years), 30 - 39 years (mean 34.9 years), 40 - 49 years (mean 46.3 years), 50 - 59 years (mean 55.8 years), 60 years and over (mean 65.3 years). In these groups, the transverse diameters of collagen fibers were measured from the ultra thin sections. Fifty measurements from each patient were carried out during the study, which totaled to 200 measurements from each group from which statistical analysis of the results was performed. The study was carried out in the electron microscopy laboratory of the Anatomy Department, Hacettepe University, Ankara, Turkey between January 2004 and September 2004.

The biopsy specimens were fixed in 2.5% glutaraldehyde for 24 hours, washed in phosphate buffer (pH: 7.4) and post-fixed in 1% osmium tetroxide in phosphate buffer (pH: 7.4). Then, the third fixative; 10% formaldehyde was applied to the specimens for one hour and the samples were dehydrated in increasing concentrations of alcohol. Following this procedure, the tissues were washed with propylene oxide and embedded in epoxy-resin embedding media. Semi-thin sections approximately 2 μ m in thickness and ultra thin sections approximately 60 nm in thickness were cut with a glass knife on a LKB-Nova (Sweden) ultramicrotome. Ultra thin sections were collected on copper grids, stained with uranyl acetate and lead citrate and examined with a Jeol JEM 1200 EX (Japan) transmission electron microscope.

Results. Transmission electron microscopic examination of the Achilles tendons of those aged 1 - 9 years showed collagen fibers with very small diameters. Additionally, the concentration of the collagen fibrils was very low, in this group (Figure 1). In measuring the transverse diameters of 200 collagen fibers in this group, the mean diameter was 69.80 ± 20.60 nm (25 - 110). The Achilles tendons of those in the 10 - 19 years age group had collagen fibers, with a larger transverse diameter than the previous group. The mean diameter was 96.10 ± 17.65 nm (65 - 150). Transmission electron

microscopic examination of the Achilles tendons in those aged 20 - 29 years revealed collagen fibers with the largest transverse diameters (Figure 2). In this group, the mean diameter was 222 ± 63.04 nm (75 - 300). The transverse diameters of the collagen fibers of those in the 30 - 39 year group were smaller than the individuals in the 20 - 29 year group. The mean diameter was 159.10 ± 47.23 nm (50 - 265). The ultrastructure of the Achilles tendons of those in the 40 - 49 year group was very similar to that of individuals in the 30 - 39 year group. In this group, the mean diameter of the collagen fibers was 148.3 ± 51.6 nm (50 - 260). The collagen fibers in the age group of 50 - 59 years showed no marked ultrastructural differences from those in the age group of over 60 years (Figure 3). Additionally, the collagen fiber concentrations in these 2 groups were higher than the other groups and the maximum increase in the collagen fibril concentration was seen in the over 60 years group. The mean diameters of the collagen fibers in the 50 - 59 was 76.40 ± 24.85 nm (50 - 165) while for the over 60 years groups was 68.70 ± 19.74 nm (45 - 135).

Differences were statistically significant between the group aged 1 - 9 years and those 10 - 19, 20 - 29, 30 - 39 and 40 - 49 years old ($p < 0.05$); between the group aged 10 - 19 years and those 20 - 29, 30 - 39, 40 - 49, 50 - 59, over 60 years old ($p < 0.05$); between the group aged 20 - 29 years and those 30 - 39, 40 - 49, 50 - 59, over 60 years old ($p < 0.05$); between the group aged 30 - 39 years and those 50 - 59, over 60 years old ($p < 0.05$); between the group aged 40 - 49 years and those 50 - 59, over 60 years old ($p < 0.05$). No significant differences were found between the group aged 1 - 9 years and those 50 - 59, over 60 years old ($p > 0.05$); between the group aged 30 - 39 years and those 40 - 49 years ($p > 0.05$) and between the group aged 50 - 59 years and those over 60 years old ($p > 0.05$).

Discussion. The tendo calcaneus may be particularly susceptible to complete rupture¹⁴ and it is certain that its incidence is increasing.¹⁵ However, it remains unknown if the ruptures of this tendon are associated with predisposing morphological changes, which can be observed in the age groups. Magnusson et al¹⁶ examined collagen fibril crimp angle and diameter in age and gender matched intact and ruptured Achilles tendons. The main findings were that there were no intra-tendon region-specific differences in fibril diameter in intact or ruptured tendons. In another study, Maffulli et al¹⁷ described the light microscopic histology of Achilles tendon ruptures and compared the ruptured and unruptured tendons. They found a significantly higher degeneration in the ruptured tendons. Strocchi et al¹⁸ studied the age related morphometric changes in human Achilles

tendon and these changes included decrease in the average, maximum diameter and density of collagen fibrils and an increase of fibril concentration. Nakagawa et al¹⁹ investigated age related changes in the mechanical properties of rabbit Achilles tendon. The animals used were immature, young adult and old rabbits. They found that the cross-sectional area of the tendon was increased with growth and the tensile strength of the young adult and old tendon was significantly higher than that of the immature tendon. Jozsa et al²⁰ reported that intact human Achilles tendons consisted of fibrils with a diameter of 30 - 80 nm. In contrast, human ruptured and degenerated tendons displayed 2 distinct populations of fibrils of 40 - 60 and 10 - 20 nm. However, in our measurements, the diameters of the collagen fibrils were in between 25 - 300 nm. Additionally, our results were in accordance with the study of Strocchi et al¹⁸ generally, but we examined the diameters of the collagen fibrils in age groups and compared these diameters with each other statistically. Therefore, to our knowledge, this is the first quantitative study, examining the diameters of human Achilles tendon in different age groups. Secondly, we believe that the decrease observed in the diameters of collagen fibers of 30 - 45 year-old patients, who are in an active period of their life, can play a role in the etiology of the frequency of tendo calcaneus ruptures in this age group.

References

- Williams PL, Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, et al. Gray's Anatomy, 38th ed. London (UK): Churchill Livingstone; 1995. p. 886.
- Moore KL, Dalley AF. Clinically Oriented Anatomy, 4th ed. Philadelphia (PA): Lippincott Williams and Wilkins; 1999. p. 587.
- Houshian S, Tscherning T, Riegels-Nielsen P. The epidemiology of Achilles tendon rupture in Danish county. *Injury* 1998; 29: 651-654.
- Leppilahti J, Puranen J, Orava S. Incidence of Achilles tendon rupture. *Acta Orthop Scand* 1996; 67: 277-279.
- Fisher P. Role of steroids in tendon rupture or disintegration known for decades. *Arch Intern Med* 2004; 164: 678.
- Gold L, Igra H. Levofloxacin-induced tendon rupture: a case report and review of the literature. *J Am Board Fam Pract* 2003; 16: 458-460.
- Ozaras R, Mert A, Tahan V, Uraz S, Ozaydin I, Yilmaz MH, et al. Ciprofloxacin and Achilles' tendon rupture: a causal relationship. *Clin Rheumatol* 2003; 22: 500-501.
- Battista V, Combs J, Warme WJ. Asynchronous bilateral Achilles tendon ruptures and androstenediol use. *Am J Sports Med* 2003; 31: 1007-1009.
- Casparian JM, Luchi M, Moffat RE, Hinthorn D. Quinolones and tendon ruptures. *South Med J* 2000; 93: 488-491.
- Benthien JP, Delling G, Ruther W. Spontaneous Achilles tendon rupture in granulomatous vasculitis. *Z Rheumatol* 2003; 62: 402-405.
- Manoj Kumar RV, Rajasekaran S. Spontaneous tendon ruptures in alkaptonuria. *J Bone Joint Surg Br* 2003; 85: 883-886.

12. Matsumoto K, Hukuda S, Nishioka J, Asajima S. Rupture of the Achilles tendon in rheumatoid arthritis with histologic evidence of enthesitis: A case report. *Clin Orthop* 1992; 280: 235-240.
13. Kissel CG, Sundareson AS, Unroe BJ. Spontaneous Achilles tendon rupture in a patient with systemic lupus erythematosus. *J Foot Surg* 1991; 30: 390-397.
14. Jozsa L, Kannus P. Histopathological findings in spontaneous tendon ruptures. *Scand J Med Sci Sports* 1997; 7: 113-118.
15. Maffulli N, Waterston SW, Squair J, Reaper J, Douglas AS. Changing incidence of Achilles tendon rupture in Scotland: a 15-year study. *Clin J Sport Med* 1999; 9: 157-160.
16. Magnusson SP, Qvortrup K, Larsen JO, Rosager S, Hanson P, Aagaard P, et al. Collagen fibril size and crimp morphology in ruptured and intact Achilles tendons. *Matrix Biology* 2002; 21: 369-377.
17. Maffulli N, Barrass V, Ewen SW. Light microscopic histology of Achilles tendon ruptures. A comparison with unruptured tendons. *Am J Sports Med* 2000; 28: 857-863.
18. Strocchi R, De Pasquale V, Guizzardi S, Govoni P, Facchini A, Raspanti M, et al. Human Achilles tendon: morphological and morphometric variations as a function of age. *Foot Ankle* 1991; 12: 100-104.
19. Nakagawa Y, Hayashi K, Yamamoto N, Nagashima K. Age-related changes in biomechanical properties of the Achilles tendon in rabbits. *Eur J Appl Physiol Occup Physiol* 1996; 73: 7-10.
20. Jozsa L, Reffy A, Balint JB. Polarization and electron microscopic studies on the collagen of intact and ruptured human tendons. *Acta Histochem* 1984; 74: 209-215.