Tendon pathology in long-standing achillodynia

Biopsy findings in 40 patients

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We evaluated biopsy specimens from the Achilles tendon in 40 patients with long-standing achillodynia and an ultrasonographic widened tendon with hypoechogenic areas. We used a standardized protocol to assess the general tendon pathology score of paraffin-embedded specimens stained with HE. Stereologic measurement of the volume density of glycosaminoglycan (GAG)-rich areas, stained with the Alcian blue (pH 2.5)/periodic acid Schiff method (AB/PAS) was performed. 14 specimens obtained at autopsy served as reference material. Abnormal fiber structure and arrangement, focal variations in cellularity, rounded nuclei, decreased collagen stainability and increased non-collagenous extracellular matrix were seen in all biopsy specimens. Slight histopathological changes were noted in half of the controls. Increased vascularity was present in two thirds of the patient specimens and in one third of the controls, and signs of perivascular hemorrhage, as evidenced by hemosiderin deposition in 6/40 of the patients, but in none of the controls. The volume density of GAG-rich areas was higher in the patients 0.47 (0–0.86) than in the controls 0 (0–0.07).

Changes in the fiber structure and arrangement, as well as increased amounts of interfibrillar GAG, appear to be characteristic morphological features in Achilles tendons with long-standing achillodynia and ultrasonographic widening. These findings may indicate that achillodynia is due to local disturbances in connective tissue metabolism or circulation or to both.

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Chronic pain syndromes involving the Achilles tendon region (achillodynia) are common: partial ruptures (Ljungqvist 1968, Denstad and Roaas 1979, Skeoch 1981, Kälebo et al. 1990), tendinopathy (Fox et al.1975, Williams 1986, Åström and Rausing 1995) and paratendinous pathology (Snook 1972, Harms et al. 1977, Kvist and Kvist 1980, Kvist et al. 1985, 1987, Kvist 1991) have been suggested as main causes of the symptoms. In a series of 461 Achilles tendon patients (Williams 1986), mixed lesions were seen in most of the cases. Clinical studies of Achilles pain suggest that excessive physical activity, especially running, is a major triggering factor (Kvist 1991, Åström and Rausing 1995).

In so-called chronic Achilles tendinitis or tendinopathy, ultrasonography shows widening of the tendon and areas of lucencies within it (O'Reilly and Massouh 1993). Mucoid degeneration has been reported in the Achilles tendon in both ruptures (Arner et al. 1959, Kannus and Józsa 1991) and tendinosis, mostly among athletes (Clancy et al. 1976, Puddu et al. 1976, Merkel et al. 1982, Schepsis and Leach 1987, Nelen et al. 1989).

During the last three decades, stereological techniques have been developed for morphometric studies. Stereologic volume density estimation from sections is a simple and efficient technique largely free from bias (Gundersen et al. 1988). This technique can be used to quantify tissue compartments, such as glycosaminoglycan (GAG)-rich areas in tendons.

We describe the histopathological changes, including quantification of GAG-rich areas, in a surgical biopsy material from patients with long-standing achillodynia.

Material and methods

Patients

Tendon biopsy specimens were obtained from 40 patients (26 men) treated surgically for long-standing achillodynia—in this study defined as pain in the mid-part of the tendon for more than 3 months. The median age was 41 (16–65) years and the median duration of symptoms was 14 (4–190) months. 6 patients did not perform any special physical activity, while 6 other patients were competitive runners. Treatment without surgery was unsuccessful and the patients complained of daily pain. On physical examination, localized pain and swelling in the mid-part of

the tendon were found. In all cases, preoperative ultrasonography showed increased tendon width and hypoechogenic areas in the mid-part of the tendon, while 7 also had low echogenic findings closely around the tendon indicating peritendinous fluid or thickening. The clinical signs and symptoms combined with these ultrasonographic findings were the basic criteria for surgery and inclusion in the study.

28 patients were operated on under local anesthesia and 12 patients were operated on under regional anesthesia. A straight medial longitudinal incision was performed. The peritendinous tissue was incised, adhesions were freed and excised (19 patients) if macroscopically abnormal. The paratenon was left open. Macroscopically, tendons characteristically showed a slight dull-greyish discoloration with altered fiber structure. Specimens were excised from the macroscopically abnormal-looking regions, but not from neighboring areas. Each specimen was 10–15 mm long, 2–6 mm wide and 3–8 mm thick.

Controls

Specimens were obtained from the left Achilles tendon during autopsies of 8 men and 6 women, median age 44 (37–55) years. No tendon was widened on visual inspection or palpation. Specimens were excised dorsomedially in the mid-part within 3 days after death. The history of these persons was obtained from medical records. The cause of death was cancer in 6, infectious disease in 4 (of whom 2 had uremia), neurological disease in 2 and cardiac disease in 2. 3 persons had done heavy manual work and the others had light work, especially during the last year of life. The clinical history of these individuals so far as achillodynia is concerned was not known. The control group was similar to the surgically treated group regarding sex and age. They had presumably worked less than the patients.

Histology

The specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 4–5 µm, according to routine procedures. Sections from all patients and controls were stained with HE and Alcian blue (pH 2.5)/periodic acid-Schiff (AB/PAS) for detection of GAG-rich areas. Sections from 32 cases and all controls were also stained with phosphotungistic acid hematoxylin (PTAH) for fibrin, Perl's method for iron and with immunoperoxidase techniques for S-100 protein as a marker for nerve tissue.

Sections stained with HE, PTAH, S-100 protein and Perl's method were evaluated by conventional light microscopy. Sections stained with HE were evaluated according to a modified standardized protocol used by Åström and Rausing (1995). In a 4-point scoring system, where 0 is normal, 1 slightly abnormal, 2 moderately abnormal and 3 markedly abnormal, the following parameters were assessed: fiber structure, fiber arrangement, roundness of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability, non-collagenous extracellular matrix and fibrosis or hyalinization. The protocol gave a maximum total score of 24 for each specimen. Scores between 1 and 8 were classified as slightly abnormal, 9–16 as moderately abnormal and 17–24 as markedly abnormal.

The sections stained with AB/PAS were examined stereologically to assess the volume density of GAG-rich areas, using a Reichert Jung projection microscope. Areas stained blue (GAG-rich area) and red (collagen) were measured, using a square lattice of 70 points placed on the projection screen with the intersecting lines at an angle of 20 degrees to the longitudinal axis of the tendon fibers. The intersection points on GAG-rich areas and collagen were recorded. If there were less than a total of 500 hits, the whole section area was evaluated, otherwise every other field of vision was sampled. The number of hits in each specimen ranged from 300–700. With this morphometric technique (Collan et al. 1983, Romppanen and Collan 1983, Gundersen et al. 1988) we were able to calculate the volume fraction of GAG-rich matrix areas in the specimens.

Statistics

Non-parametric statistical methods were used for ordinal data and for continuous variables, as it cannot be assumed that a parametric distribution is specific for the population. Kappa statistics (Svanholm et al. 1989) was used to analyze the intra-observer reproducibility of the classification of tendon lesions. The surgically treated patients were compared to the control group with respect to sex and age using the chi-2 test and Mann-Whitney U-test, respectively. Hypotheses concerning differences in the assessed histopathological parameters, the total tendon score and the volume density of GAG-rich areas in the operated group, compared to the controls, were analyzed by using the Mann-Whitney U-test. The total histopathology scores and the volume density of GAG-rich areas in the operated patients were then correlated with age, duration of symptoms and activity level, using the Spearman rank correlation coefficient. Gender differences were analyzed with the chi-2 test. A probability level of < 0.05 was considered significant.

Table 1. Summary of histopathological findings in 40 patients and 14 controls. Median (range)

	Patients	Controls	P-value
Total tendon score	21 (6–24)	0 (0–13)	<0.001
Volume density of GAG-rich areas	0.47 (0–0.86)	0 (0–0.07)	<0.001

Results

The total tendon score and the volume density of GAG-rich areas were higher in the patients (Table 1).

Semi-quantitative investigations

The area of the specimens showing the most advanced pathological changes was assessed by semi-quantitative evaluation of the HE-stained sections (Table 2). The total score represented the overall severity of the pathological changes. Every other section was reassessed after 2 months and the intra-observer reproducibility was evaluated. The kappa coefficient was 0.67, which is regarded as satisfactory.

Fiber structure. The normal tendon showed straight parallel packed fibers, some of which had a slight waviness. With slight and moderate changes, there was separation of the fibers as well as increased waviness (Figure) and with severe changes there was a total loss of fine fiber structure and hyalinization. The patients' score was a median of 3, compared to 0.5 for the controls.

Fiber arrangement. The loss of parallel arrangement and the deterioration of fibers was graded. The patients' score was a median of 3 versus 0.5 for the controls.

Cell nuclei. Normally, the tenocyte nucleus is flattened or spindle-shaped and the cells are located between collagen fibers in rows. In the specimens, the nuclei had either a normal or a more rounded shape (Figure). Both a reduced and an increased number of nuclei were classified as slight changes. If the number of rounded nuclei in a high-power field was less than 10, the changes were called slight and, if more than 20, they were large.

The variation in cellularity was graded in the whole section. The patients' median score was markedly abnormal (3), compared to normal (0) in the controls.

Collagen stainability. Normally, the collagen stains deep rosy-red in HE. The stainability was often reduced and the pallor was graded. The patients' median score was in 3, versus 0 for the controls.

Extracellular matrix. An increased amount of extracellular matrix in-between the fibrils and fibers, sometimes seen as vacuolated lakes, was graded. This pathological change was also studied with AB/PAS and the volume density calculated as described below. The patients' median score in the semi-quantitative assessment was 2, compared to 0 for the controls.

Vessels. Normally, most of the vessels and nerves run parallel to the collagen fiber bundles. Increased vascularity (Figure) was seen in 26/40 of the patient specimens and in 4/14 of the controls. In 5 of 32 patients, but in none of the controls, perivascular hemosiderin, demonstrated with Perl's method, was seen around nests of vessels. In some patients, the microscopic picture suggested an ingrowth of vessels from the peritendinous tissue.

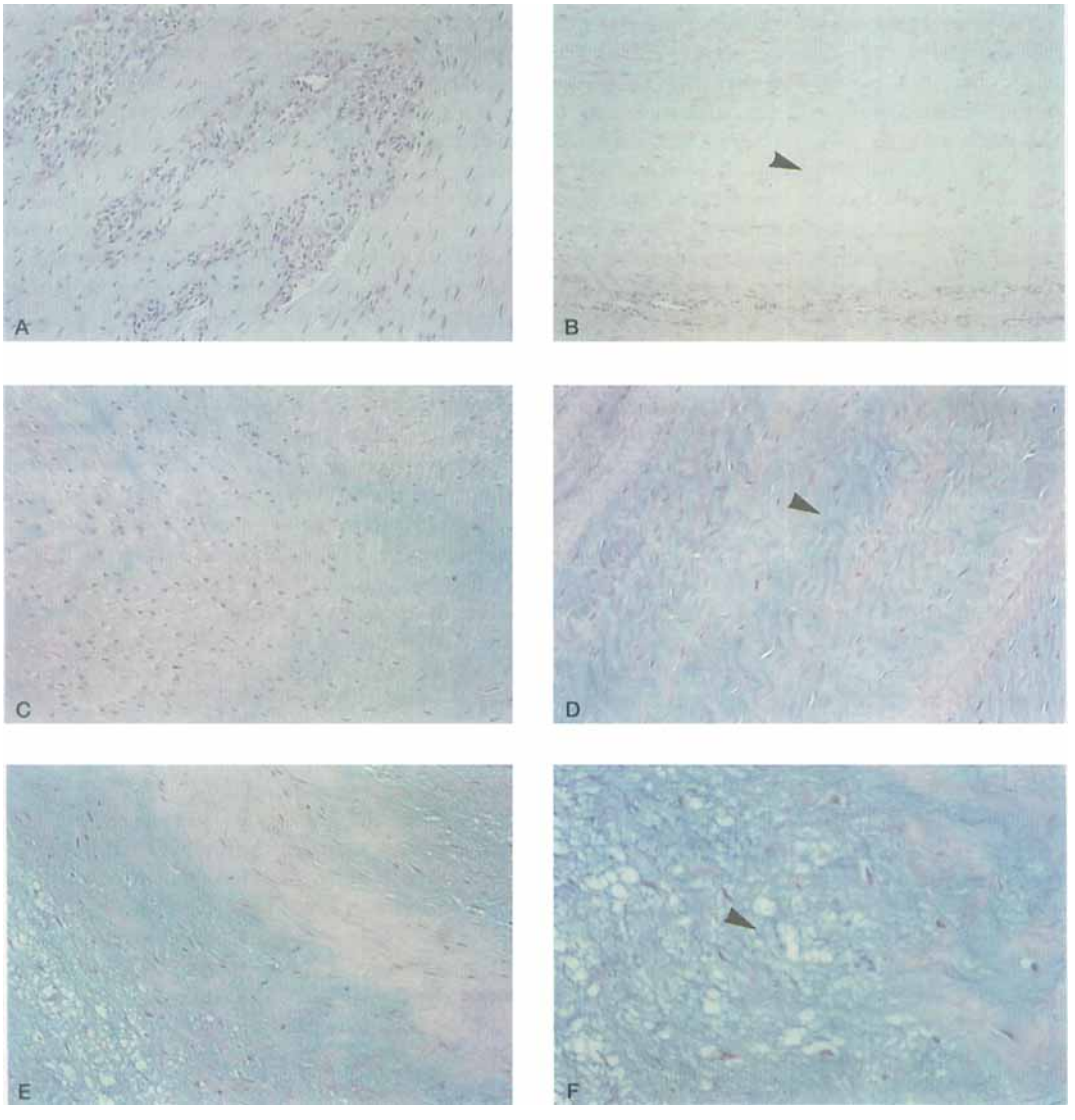
Table 2. Distribution of the tendon scores versus the controls

	Patients (n 40)				Controls (n 14)			
	0	1	2	3	0	1	2	3
Fiber structure	0	4	4	32	7	3	4	0
Fiber arrangement	0	3	5	32	7	2	5	0
Rounding of the nuclei	2	6	12	20	11	2	1	0
Regional variations in cellularity	0	4	14	22	11	2	1	0
Increased vascularity	1	13	14	12	10	3	1	0
Decreased collagen stainability	0	2	6	32	8	3	3	0
Hyalinization	1	2	10	27	9	1	4	0
GAG content	1	7	14	18	14	0	0	0
General description [a]		1	6	33		11	3	0

0 normal, 1 slightly abnormal, 2 moderately abnormal and 3 markedly abnormal.

[a] 1 normal or slight tendon changes (0–8), 2 moderate changes (9–16), 3 severe changes (17–24).

$P < 0.001$ for all comparisons between patients and controls.



Specimens from patients with long-standing achillodynia.

A. Tendon tissue with increased vascularity, HE ×200.

B-F. Tendon tissue with increasing amount of extracellular GAGs, indicated by arrowheads, stained with HE in B and AB/PAS in C–F. The increased amount of GAGs, separating the red-stained tendon fibers, form blue GAG-rich areas. Larger GAG-rich areas, with a markedly increased ratio to the red tendon fibers, are shown in E and F. The number of nuclei in tendon tissue is increased and their shape varies (C).

Miscellaneous. No inflammatory cell infiltrate, calcium deposits or tendolipomatosis were noted. Nerves, mostly accompanying the vessels, were sparse in the tendon and more frequent in peritendinous tissue. In some patients, nerves were surrounded by stroma rich in GAGs. Staining with PTAH revealed no areas of extracellular fibrin, indicative of microruptures or vessel wall damage.

Quantification of areas rich in acid glycosaminoglycans

The volume density estimation of the Alcian blue-stained sections for GAGs was higher ($p < 0.001$) in patients, median 0.47 (0–0.86), than in the controls, 0 (0–0.07). The Alcian blue-stained areas were located extracellularly between the collagen fibers. Regions with intensive staining were seen only in sections from the patients (Figure).

The general tendon score was higher ($p < 0.001$) in the surgically treated patients, median 21 (6-24), than in the controls, 0 (0-13). The total score for the patients correlated well with the volume density of GAG-rich areas ($r = 0.74$; $p = 0.04$). Correlation analysis of the contributing factors in the patients, such as age, time between onset of symptoms and operation, level of physical activity and the total tendon score showed an association between age and the score ($r = 0.33$; $p = 0.04$), and low physical activity and the score ($r = -0.54$; $p < 0.001$). The patient's age was not significantly correlated to the volume density of the GAG-rich areas ($r = 0.12$; $p = 0.44$). Neither the duration of symptoms nor gender showed a significant correlation to this total score of tendon pathology or volume density of GAG-rich areas in the tendon.

Discussion

In a controlled study of 891 cases, Kannus and Józsa (1991) reported the histopathological changes preceding a spontaneous tendon rupture. Concerning the Achilles tendon, 397 patients having a tendon rupture versus 220 autopsy controls were examined histopathologically. Hypoxic degeneration was found in half of the patients vs 9% of the subjects, mucoid degeneration in 19% vs 12%, tendolipomatosis in 6% vs 4%, and calcifying tendinopathy in 3% vs 1%. Multiple changes were seen in one fifth of the cases of tendon rupture and in 4 % of the controls. All ruptured tendons were classified as pathologic by their microstructure as compared to 31% of the control tendons.

In a normal tendon, about 70–80% of the dry weight of the tissue is collagen and about 1% is noncollagenous extracellular matrix. Water accounts for 65–75% of the total wet weight of a tendon and a substantial part of it is associated with the proteoglycans of the extracellular matrix. The water and proteoglycans probably provide the lubrication and spacing that are crucial to the gliding function of the tendon (Woo and Tkach 1989). Proteoglycans are also supposed to provide mechanical support due to the fixed negative charge and to regulate cell migration and aggregation (Kjellén and Lindahl 1991). The proteoglycans consist of a protein core with glycosaminoglycan side chains. The GAGs chondroitin sulfate, dermatan sulfate and keratan sulfate are very negatively charged. The small interstitial proteoglycans decorin (dermatan sulfate proteoglycan) and fibromodulin (keratan sulfate proteoglycan) have been shown to bind to collagen I and II and inhibit fibrillogenesis in vitro (Hedbom and Heinegård 1989). In the human tibialis posterior tendon, the predominant small proteoglycan of the proximal/tensional region is decorin, whereas two types of small proteoglycans (decorin and biglycan) and large proteoglycans are present in the region passing under the medial malleolus and presumably subjected to compressive and shear forces in addition to tension (Vogel et al. 1993).

An increased ratio of proteoglycans and GAGs versus collagen may interfere with their normal function and could explain the reduced interfiber cohesion of the collagen bundles. However, the so-called mucoid degeneration of the tendon has received little attention in the literature (Kannus and Józsa 1991). The degenerative tendon changes in chronic Achilles tendinopathy were described by Åström and Rausing (1995) as abnormal fiber structure, focal hypercellularity and vascular proliferation. They noted metachromatic fiber staining in small lesions and increased ground substance in larger lesions. They also noted that age was directly correlated to the histopathology score and inversely correlated to the activity score. In our study, the tendon alterations were more pronounced in older patients, while the volume density of GAG-rich areas did not correlate significantly to age or gender.

Abnormal tendon fiber structure and a substantial increase in the volume density of the GAG-rich areas are the two major histopathological findings of this study. These changes may be interrelated, but it remains unclear which of them precedes the other. It seems probable that the fundamental pathology of achillodynia with widening of the tendon is a quantitative imbalance between the two main structural components of the tendon tissue. Tendon fibers, on the one hand, may lose resilience, elasticity and durability by thickening and loss of fine structure. On the other hand, the increase of the amount of the interstitial GAGs inaggerates the disturbance in tendon function and may ultimately lead to partial or complete rupture. The structural balance between collagen and matrix is seemingly of great importance for proper function.

Our hypothesis is that the increased GAG content may be a reactive cell response to tendon insult, the GAG reaction probably being a result of mechanical overloading, with or without collagen fiber disruption, or a result of an adverse reaction to a drug (Movin et al. 1997). The altered internal milieu in the tendon affects the fiber structure and arrangement leading to a reparative response with activation of cells as well as neovascularization and subsequent increase in synthesis of GAGs. The healing response with the imbalance between the injury and repair may fail, leading to tissue damage.

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References

Arner O, Lindholm Å, Orell S R. Histologic changes in subcutanous rupture of the Achilles tendon. A study of 74 cases. Acta Chir Scand 1958; 116: 484-90.

Clancy Jr W G, Neidhart D, Brand R L. Achilles tendonitis in runners: a report of five cases. Am J Sports Med 1976; 4: 46-57.

Collan Y, Oja E, Whimster W F. Mathematical background to stereology and morphometry for diagnostic pathologists. In: Stereology and morphometry in pathology (Eds Collan Y et al). Kuopio University Press, Kuopio 1983; 2: 214-38.

Denstad T F, Roaas A. Surgical treatment of partial Achilles tendon rupture. Am J Sports Med 1979; 7: 15-7.

Fox J M, Blazina M E, Jobe F W, Kerlan R K, Carter V S, Shields Jr C L, et al. Degeneration and rupture of the Achilles tendon. Clin Orthop 1975; 221-4.

Gundersen H J, Bendtsen T F, Korbo L, Marcussen N, Moller A, Nielsen K, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988; 96: 379-94.

Harms J, Biehl G, von Hohbach G. Pathology of paratenonitis achilleae in athletes. Arch Orthop Unfallchir 1977; 88: 65-74.

Hedbom E, Heinegård D. Interaction of a 59-kDa connective tissue matrix protein with collagen I and collagen II. J Biol Chem 1989; 264: 6898-905.

Kannus P, Józsa L. Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg (Am) 1991; 73: 1507-25.

Kjellén L, Lindahl U. Proteoglycans: structures and interactions. Annu Rev Biochem 1991; 60: 443-75.

Kvist M. Achilles tendon overuse injuries. A clinical and pathophysiological study in athletes with special reference to Achilles paratenonitis. Thesis, Universitatis Turkuensis, Finland 1991.

Kvist H, Kvist M. The operative treatment of chronic calcaneal paratenonitis. J Bone Joint Surg (Br) 1980; 62: 353-7.

Kvist M, Józsa L, Jarvinen M, Kvist H. Fine structural alterations in chronic Achilles paratenonitis in athletes. Pathol Res Pract 1985; 180: 416-23.

Kvist M, Józsa L, Jarvinen M J, Kvist H. Chronic Achilles paratenonitis in athletes: a histological and histochemical study. Pathology 1987; 19: 1-11.

Kälebo P, Goksor L A, Swärd L, Peterson L. Soft-tissue radiography, computed tomography, and ultrasonography of partial Achilles tendon ruptures. Acta Radiol 1990; 31: 565-70.

Ljungqvist R. Subcutaneous partial rupture of the Achilles tendon. Acta Orthop Scand (Suppl 113) 1968.

Merkel K H, Hess H, Kunz M. Insertion tendopathy in athletes. A light microscopic, histochemical and electron microscopic examination. Pathol Res Pract 1982; 173: 303-9.

Movin T, Gad A, Güntner P, Földhazy Z, Rolf C. The pathology in the Achilles tendon in association with ciprofloxacin treatment. Foot Ankle Int 1997, in press.

Nelen G, Martens M, Burssens A. Surgical treatment of chronic Achilles tendinitis. Am J Sports Med 1989; 17: 754-9.

O'Reilly M A, Massouh H. Pictorial review: the sonographic diagnosis of pathology in the Achilles tendon. Clin Radiol 1993; 48: 202-6.

Puddu G, Ippolito E, Postacchini F. A classification of Achilles tendon disease. Am J Sports Med 1976; 4: 145-50.

Romppanen T, Collan Y. Practical guidelines for a morphometric study. In: Stereology and morphometry in pathology (Eds. Collan Y et al). Kuopio University Press. Kuopio 1983; 2: 274-97.

Schepsis A A, Leach R E. Surgical management of Achilles tendinitis. Am J Sports Med 1987; 15: 308-15.

Skeoch D U. Spontaneous partial subcutaneous ruptures of the tendo achillis. Review of the literature and evaluation of 16 involved tendons. Am J Sports Med 1981; 9: 20-2.

Snook G A. Achilles tendon tenosynovitis in long-distance runners. Med Sci Sports 1972; 4: 155-8.

Svanholm H, Starklint H, Gundersen H J G, Fabricius J, Barlebo H, Olsen S. Reproducibility of histomorphologic diagnoses with special reference to the kappa statistic. APMIS 1989; 97: 689-98.

Vogel K G, Ordog A, Pogany G, Olah J. Proteoglycans in the compressed region of human tibialis posterior tendon and in ligaments. J Orthop Res 1993; 11: 68-77.

Williams J G. Achilles tendon lesions in sport. Sports Med 1986; 3: 114-35.

Woo S L-Y, Tkach L V. The cellular and matrix response of ligaments and tendons to mechanical injury. In: Sports-induced inflammation: Clinical and Basic Science Concepts (Eds. Leadbetter W B, Buckwalter J A, Gordon S L). American Academy of Orthopaedic Surgeons, Park Ridge 1989; 189-204.

Åstrom M, Rausing A. Chronic achilles tendinopathy—a survey of surgical and histopathologic findings. Clin Orthop 1995; 316: 151-164.