Structural and Biomechanical Characteristics After Early Mobilization in an Achilles Tendon Rupture Model: Operative Versus Nonoperative Treatment

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abstract

Full article available online at Healio.com/Orthopedics. Search: 20120822-26

Acute Achilles tendon ruptures are common sports injuries; however, treatment remains a clinical challenge. Studies show a superior effect of early mobilization and full weight bearing on tendon healing and clinical outcome; however, few data exist on structural and biomechanical characteristics in the early healing phase. This study investigated the histological and biomechanical characteristics of early mobilization and full weight bearing in an Achilles tendon rupture model. Eighty rats underwent dissection of a hindpaw Achilles tendon; 40 rats were treated conservatively and 40 underwent open repair of the transected Achilles tendon by suturing. Early mobilization and full weight bearing were allowed in both groups. At 1, 2, 4, and 8 weeks after tenotomy, tensile strength, stiffness, thickness, tissue characteristics (histological analysis), and length were determined. Dissected Achilles tendons healed in all animals during full weight-bearing early mobilization. One and 2 weeks after tenotomy, rats in the operative group showed increased tensile strength and stiffness compared with the nonoperative group. Repair-site diameters were increased at 1, 2, and 8 weeks after tenotomy. Tendon length was decreased in the operative group throughout observation, whereas the nonoperative group showed increased structural characteristics on the cellular level and a more homogeneous collagen distribution. Surgical treatment of dissected rat Achilles tendons showed superior biomechanical characteristics within the first 2 weeks. Conservative treatment resulted in superior histological findings but significant shortening of the tendon in the early healing phase (weeks 1-8).

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The authors thank Ms H. Schaller and C. Pilapil for histology preparation, Dr H. Claesen and Ms E. Krott for assistance during histological examination, Dr L. Duerselen for help during biomechanical testing, and S. Krapf for help during statistical preparation.

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doi: 10.3928/01477447-20120822-26

Figure 1: Effect of nonoperative treatment on the histology of a rat Achilles tendon 8 weeks after transection and suture were performed. The histological pattern is close to a normal Achilles tendon with lined-up fibrocytes (arrows) and big bundles of collagen starting to synchronize (hematoxylin-eosin stain, ×200).

Figure 2: Effect of operative treatment on the histology of a rat Achilles tendon 8 weeks after transection and suture were performed less organized collagen (arrows) and scar tissue and hypercellularity are visible (hematoxylin-eosin stain, ×200).
Achilles tendon ruptures are frequent injuries, with an annual incidence of 10 to 14 per 100,000.\textsuperscript{1-3} Middle-aged recreational athletes are affected most frequently.\textsuperscript{2} In the past, operative and nonoperative treatment normally included the use of orthotic or other stabilization of the injured Achilles tendon (eg, plaster). Clinical data showed increased posttraumatic lengthening with functional loss in conservatively treated patients,\textsuperscript{4,5} with increased rerupture rates compared with surgically treated patients (7%-20% vs 0.6%-3%, respectively).\textsuperscript{6-9} In contrast, surgically treated patients exhibited increased infection rates and wound healing problems.\textsuperscript{6,9-11}

Many studies have reported positive effects of early weight bearing on tendon healing in percutaneous Achilles tendon repair.\textsuperscript{12-19} However, controversy remains regarding whether open repair or nonoperative therapy is best for patients undergoing an early mobilization treatment regimen. A previous animal study showed different functional results in operatively and nonoperatively treated rats; however, only the healing process within the first 2 weeks was examined.\textsuperscript{20} Comprehensive testing during the early and advanced phases of healing was not performed.

The goal of the current study was to investigate the histological and biomechanical characteristics of early mobilization and full weight-bearing treatment in an Achilles tendon rupture model. The authors hypothesized that operatively treated Achilles tendons would show superior biomechanical properties compared to those treated nonoperatively.

**Materials and Methods**

This study was approved by the institutional animal care board. All experiments were performed in accordance with the requirements of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. This animal model is widely used in research.\textsuperscript{20-22}

Eighty adult male Sprague-Dawley rats (400–425 g) were studied. The animals were randomized into 2 groups of 40. At baseline, the right hindpaw Achilles and plantaris tendons of all animals in both groups were dissected. Animals in the operative group underwent Achilles tendon repair using a modified Kessler-type suture. Ten rats in both groups were sacrificed at 1, 2, 4, and 8 weeks following tenotomy. Three sacrificed animals were randomly chosen for histological examination, and specimens from 7 sacrificed rats were used for biomechanical testing. The muscle–Achilles tendon–bone unit was harvested by transecting the middle of the gastrocnemius muscle and the calcaneus.

**Surgical Technique**

Animals were anesthetized with isoflurane in a small animal anesthesia machine. An injection of 20 mg/kg of cefazolin and 0.06 mg/kg of buprenorphine was administered intramuscularly into the left thigh for analgesia and antibiotic prophylaxis. The right hindpaw then was shaved and disinfected 3 times using povidone-iodine and rinsed with 70% alcohol.

In an aseptic manner, the rats were laid on a heated surgery table and a sterile drape was placed, leaving only the limb exposed. A 3-cm skin incision was made longitudinally dorsal to the right Achilles tendon, and the peritendon was split. The Achilles tendon was dissected orthogonal to the visible fibers 5 mm proximal to the calcaneal insertion. The plantaris tendon was cut to prevent an internal splint phenomenon.\textsuperscript{20,22,23} The tendons of animals in the nonoperative group were left unsutured, and animals in the operative underwent Achilles tendon repair using a locking technique (Kessler-type suture, PDS II Ethicon 2-0). The wound was closed in layers in both groups.

**Postoperative Treatment**

A heating pad was used to regain normal body temperature. The cefazolin-buprenorphine injection was repeated twice daily for 3 days. Animals were monitored regularly for signs of pain (eg, lack of locomotion, vocal distress, and tenderness) and infection. Rat chow and water were fed ad libitum. No cast was applied to animals in either group, allowing free range of motion as described by Murrell et al.\textsuperscript{24}

**Mechanical Testing**

Muscle–tendon–bone units were wrapped in cotton gauze soaked with Ringer’s lactated solution and stored at −20°C until testing. Before testing, specimens were put in Ringer’s lactated solution for 4 hours for unfreezing. Tendon thickness at the site of the former transection was measured by fixing the muscle–tendon–bone unit by freezing the muscular segment between the cryo-jaws and the bony segment between the copper clamp.\textsuperscript{25} Tendon length was determined at the starting point, with the tendon tensioned with 0.1 N, by measuring the distance between the cryo-jaws (gastrocnemius) and copper clamp (calcaneus). The clamp was attached to an electrohydraulic materials testing machine (Zwick GmbH & Co. KG, Ulm-Einsingen, Germany). Before testing the tensile strength, the tendons were not stretched or preconditioned. Temperature was maintained at 25°C (77°F) during the entire procedure. Dehydration of the muscle–tendon–bone units was prevented by keeping them covered with Ringer’s lactated solution when dehydration was observed.

After filling the liquid nitrogen reservoirs, the experiment began as soon as the expansion of the freezing zone reached the border of the metal clamp but did not extend into the tendon substance or repair site (registered manually with a metal needle). The displacement rate was set at 1000 mm/min. Force displacement curves were recorded and transferred to a computer for data analysis. Load to failure (N, peak of the curve) and stiffness (N/mm) were measured.
Histology

At 1, 2, 4, and 8 weeks after baseline, 3 specimens of each group were used for histological examination. For dehydration, the specimens were fixed in 4% buffered (pH, 7.4) formalin for 24 hours and embedded in paraffin wax. Sections of 5 µm were taken longitudinally from the midsubstance and stained with hematoxylin-eosin stain. Histological analysis was performed by the semiquantitative score of Bonar, as published by Maffulli et al.26 Collagen ordering, cell number, and tenocyte appearance were evaluated by 2 blinded investigators (D.K., M.M.).

Statistical Analysis

Group comparisons were performed with the nonparametric Mann-Whitney U test. The level of significance concerning a 2-sided alternative hypothesis was set at a P value less than .05.

RESULTS

Mechanical Testing

At 1 and 2 weeks after Achilles tendon dissection, maximum failure load was increased in the operative and nonoperative groups (P<.05) (Figure 1). At 4 and 8 weeks, no significant difference in maximal failure load was observed between the 2 groups.

In the first week, tendon stiffness was significantly increased (P<.01) in the operative group and remained increased in the second week (P<.05). No difference was found at 4 and 8 weeks following tenotomy. Maximum tendon stiffness was found in both groups at 8 weeks following tenotomy (Figure 2).

Thickness at the tendon repair site was increased (P<.05) in the operative group at 1, 2, and 8 weeks after tenotomy. The callus in the operative group showed a constant diameter of approximately 4 mm, whereas the callus in the nonoperative group varied from 1.7 to 3.6 mm, remodeling down to 3.3 mm at week 8 (Figure 3).

The length of the Achilles tendon, measured as the distance between the cryo-jaws (gastrocnemius) and copper clamps (calcaneus), was significantly increased (P<.01) in the nonoperative group throughout the whole observation period (Figure 4).

Histology

Histological examination of the nonoperatively treated tendons showed a higher degree of organization and a more homogeneous pattern of collagen fibers at every time point. Collagen formation and arrangement was seen earlier, and matura-
tion of fibroblasts to fibrocytes was faster. At week 8, the appearance of the nonoperatively treated tendon resembled a normal histological pattern of Achilles tendons compared with the left side. Tendon fibers and collagen crimp started to synchronize within big bundles, and fibrocytes were lined up (Figure 5). In contrast, the sutured tendons had fewer organized collagen fibers, and scar formation was seen around the sutures. At week 8, more cells were visible compared with the normal tendon; only partial organized fiber bundles with a lower grade of synchronization could be seen (Figure 6). The mean histological Bonar score showed better results in the nonoperatively treated group (Figure 7).

**DISCUSSION**

In the current study, histological and biomechanical characteristics of early mobilization and full weight-bearing treatment in an Achilles tendon rupture model were assessed. Operatively and nonoperatively treated animals were observed for 8 weeks. Animals in both groups were allowed unlimited mobility. No cast or orthotic fixation was applied. The recent literature reports the effectiveness of early functional therapy compared with cast immobilization after Achilles tendon rupture. The key component of modern rehabilitation is early functional therapy that allows foot and ankle movement in the first week after rupture. The improved outcome after early mobilization has ignited debate among orthopedic surgeons as to which approach is best. Operative treatment results in less maximum force reduction, lower rerupture rates, and earlier return to work or sport. Nonoperative treatment exhibits fewer local complications, especially infections, and generally lower costs. However, the final outcome appears to be comparable between operative and nonoperative treatment regimens.

In the current study, full weight-bearing mobilization allowed Achilles tendon healing within 8 weeks in animals in both groups. The main finding was that open repair of ruptured Achilles tendons leads to superior biomechanical results compared to nonoperative treatment. Tendons in the operative group showed a significantly thicker and more stable tendon construct compared with those in the nonoperative group. Biomechanical results of both groups differed only 1 and 2 weeks following tenotomy. The superior biomechanical properties after open repair only occurred during the early phase of healing. At 4 weeks after tenotomy, animals in both groups showed indifferent results in stiffness and tensile strength. This is
surprising because conservatively treated Achilles tendons were expected to show decreased load capacity throughout the entire observation period.

Similar results were reported by Murrell et al. They focused on biomechanical, functional, and morphological advantages of operative vs nonoperative treatment. Functional performance was determined by the measurement of hind-paw prints using the Achilles Functional Index. On day 15, the animals were sacrificed, and biochemical and histological evaluations were performed. By day 15, no functional or failure load impairments were observed after operative or nonoperative treatment.

The second important finding of the current study is that conservative treatment of dissected Achilles tendons led to increased length and decreased diameter at the defect site. This finding is supported by a study by Konerding et al., who showed that sutured Achilles tendons were shorter and thicker in appearance, whereas the nonsutured tendons appeared elongated and more slender. Furthermore, nearly identical maximum tensile strengths (median, 245 vs 253.4 N) were reported 3 months after tenotomy in operatively and nonoperatively treated animals. Indifferent maximum tensile strength was found in both groups when compared with the healthy contralateral Achilles tendon (86% to 96%). In a third group, an external fixator made of 3 Kirschner wires was applied to prevent early functional mobilization. In this group, dissected tendons reached only 60% of tensile strength compared with the contralateral side. In the current study, tendon length was found to be significantly elongated after nonoperative treatment compared with suture repair.

These results underline the importance of early functional therapy after Achilles tendon rupture. A third finding of the current study is that positive effects on tissue remodeling were shown by nonoperatively treated rats. Histological examination showed a higher grade of organization of collagen fibers at all time points in this group. Collagen formation following nonoperative therapy was observed to occur earlier, maturation of fibroblasts to fibrocytes was faster, cellularity was lower, and the specimens showed a typical tendon-like appearance compared with more scar-like tissue in the operative group.

After 12 weeks, Thermann et al. found no significant difference between operative and nonoperative groups regarding histological appearance, collagen III content, or tendon thickness. After 1 to 4 weeks, the current authors found that the nonoperatively treated tendon showed a much higher degree of collagen organization and fibroblast maturation, and after 8 weeks, it appeared far more similar to a normal Achilles tendon than the operatively treated Achilles tendons.

The current authors are aware of the difficulty of applying such results to humans. Besides the difference in healing itself, surgery was not performed using a traumatic-
degenerative tendon within this study, as is normally done in humans. Furthermore, the current authors did not use postoperative immobilization in a cast or shoe; the rats immobilized themselves due to pain. The effect of freezing tendons on structural integrity and biomechanical characteristics is still disputed.

**Conclusion**

This study showed that full weight-bearing mobilization allowed Achilles tendon healing within 8 weeks in a rat model. Surgical treatment of dissected rat Achilles tendons showed superior biomechanical characteristics during the first 2 weeks. Nonoperative treatment resulted in superior or histological findings but significant lengthening of the tendon in the early and advanced healing phase (weeks 1 to 8).

**References**


