**Objective:** Augmented soft tissue mobilization (ASTM) has been used to treat Achilles tendinopathy and is thought to promote collagen fiber realignment and hasten tendon regeneration. The objective of this study was to evaluate the biomechanical and histological effects of ASTM therapy on rabbit Achilles tendons after enzymatically induced injury.

**Methods:** This study was a non-human bench controlled research study using a rabbit model. Both Achilles tendons of 12 rabbits were injected with collagenase to produce tendon injury simulating Achilles tendinopathy. One side was then randomly allocated to receive ASTM, while the other received no treatment (control). ASTM was performed on the Achilles tendon on postoperative days 21, 24, 28, 31, 35, and 38. Tendons were harvested 10 days after treatment and examined with dynamic viscoelasticity and light microscopy.

**Results:** Cross-sectional area in the treated tendons was significantly greater than in controls. Storage modulus tended to be lower in the treated tendons but elasticity was not significantly increased. Loss modulus was significantly lower in the treated tendons. There was no significant difference found in tangent delta (loss modulus/storage modulus). Microscopy of control tendons showed that the tendon fibers were wavy and type III collagen was well stained. The tendon fibers of the augmented soft tissue mobilization treated tendons were not wavy and type III collagen was not prevalent.

**Conclusion:** Biomechanical and histological findings showed that the Achilles tendons treated with ASTM had better recovery of biomechanical function than did control tendons. (J Manipulative Physiol Ther 2015;xx:1-7)

**Key Indexing Terms:** Achilles Tendon; Tendon Injuries; Tendinopathy; Massage

Achilles tendinopathy is a common overuse syndrome, especially among runners.\(^1\) In a cohort study with 11 years of follow-up, Kujala et al.\(^3\) reported that 29% of 269 male orienteering runners and 4% of 188 controls had experienced an Achilles tendon overuse injury; the age-adjusted odds ratio was 10.0 in runners compared with controls. Achilles tendinopathy causes many patients to decrease their physical activity, with a potentially negative effect on their overall health and well-being.\(^4\) Despite rest from sports activities and various conservative treatments, many patients continue to have symptoms. The need for surgery increases with patient age, duration of symptoms, and changes in tendinopathy.\(^5\) The results of operative treatment for Achilles tendinopathy are usually good.\(^7\)–\(^11\) However, postoperative complications often contribute to impairment and delayed recovery.\(^9\)–\(^11\)

Massage is one of the oldest forms of therapy for musculoskeletal disorders. Several forms of massage or soft tissue mobilization techniques have been developed and used for chronic Achilles tendon overuse injuries. Augmented soft tissue mobilization (ASTM) is a modification of traditional soft tissue mobilization. It uses specifically designed solid instruments to provide the contact force on the tendon. In the only published clinical trial of ASTM,
Wilson et al.\textsuperscript{12} compared ASTM with a traditional physical therapy regime for patellar tendinitis. ASTM treatment was superior (100% vs 60% symptom resolution). ASTM is commonly used for Achilles tendinitis although no clinical trials have been reported. It has been shown to result in morphologic changes in the tendon in at least 2 animal studies.\textsuperscript{13,14} Davidson et al.\textsuperscript{15} suggested that ASTM may promote healing via increased fibroblast recruitment. Gehlsen et al.\textsuperscript{14} suggested that applying heavy pressure promotes the healing process to a degree greater than use of light or moderate pressure. Both studies showed that ASTM increased the number of fibroblasts and activated fibroblasts. Thus, ASTM theoretically increases the synthesis of collagen and might change the biomechanical properties of the tendon. However, there are no reports of the effect of ASTM on the biomechanical properties of injured Achilles tendons.

There are no published guidelines regarding the recommended dose of ASTM for Achilles tendinitis in humans. Proponents of ASTM have noted that an average of 8 treatments resulted in 77% excellent results (at least 90% symptom resolution) and 15% good results (80%-90% symptom resolution).\textsuperscript{15} Histological changes have been reported in a rat model after 4 to 6 treatments\textsuperscript{13,14} and the amount of pressure applied during treatment was noted to correlate with the amount of organized rough endoplasmic reticulum in the healing tendon (indicative of protein synthesis).\textsuperscript{14}

During rehabilitation after Achilles tendon overuse injury, the tendon is subjected to both static and dynamic loads during walking, running, and jumping. Therefore, it is necessary to assess not only static but dynamic biomechanical properties. Studies assessing viscoelastic properties of soft tissues have primarily used static testing,\textsuperscript{16–19} but we have not found reports assessing the biomechanical properties of Achilles tendon by dynamic viscoelastic testing. While creep\textsuperscript{17} and stress-strain\textsuperscript{20,21} have often been used as static viscoelastic tests, the rate of change with stress and strain is slow, and there are no reports of chronological change.

Dynamic mechanical analysis measures viscoelastic properties by repeatedly applying sine wave stress at various frequencies to a tendon sample while measuring the dynamic strain response. The storage modulus ($E'$) is an indicator for the elastic component representing energy storage, while the loss modulus ($E''$) is an indicator for the viscous component of the tissue which dissipates the mechanical energy and converts it to heat. Tangent delta ($\tan \delta$) is calculated by dividing $E''$ by $E'$.\textsuperscript{22,23} Thus, dynamic viscoelastic testing can ascertain the dynamic mechanical response of the Achilles tendon to mechanical perturbations that reflect activities of daily living.

The purpose of this study was to evaluate the effect of ASTM therapy on healing in a rabbit model of Achilles tendinopathy by quantifying dynamic biomechanical properties and examining histological features.

**Methods**

Both Achilles tendons of 12 male New Zealand white rabbits (mean weight 3.53 +/- 0.06 kg) were injected with collagenase to produce tendon injury simulating Achilles tendinopathy. One hind leg of each rabbit was randomly assigned to treatment with ASTM while the other served as a control. This protocol was approved by the Mayo Clinic Institutional Animal Care and Use Committee. All animals were monitored for adverse effects by animal care staff.

**Achilles Tendon Injury Model**

The Achilles tendons of the 12 rabbits were injected with collagenase to induce tendinopathy.\textsuperscript{24,25} The direct injection of collagenase provided a good model of tendinosis because connective tissue natively contains collagen and levels of collagenase rise after injury. Prior to the injection, the animals were anesthetized intramuscularly with a cocktail solution of ketamine (70 mg/mL), acepromazine (2 mg/mL), and xylazine (10 mg/mL) at a dosage of 0.6 mL/kg of body mass. A longitudinal incision was made slightly medial to the outline of the Achilles tendon. The Achilles tendon was exposed 1 cm proximal to the calcaneal insertion. Under direct visualization, 30 μL of collagenase (10 mg/mL) were injected into the center of the tendon. The incision was closed with simple sutures. The rabbits were returned to cage activity after surgery. The surgical site was allowed to heal for 3 weeks before beginning treatment.

Although the treatment was expected to produce minimal if any discomfort, the animals were monitored for signs of pain before, during, and after the collagenase injection and throughout the study using published guidelines.\textsuperscript{26}

**Augmented Soft Tissue Mobilization**

The animals underwent ASTM using a custom-made stainless steel instrument (Graston Technique, Indianapolis, Indiana) to apply mobilization force to the Achilles tendon. To administer ASTM, animals were placed in a prone position with the foot elevated to allow access to the Achilles tendon. An assistant gently held the animals body and steadied the lower limbs while the investigator applying the treatment held the foot of the side being treated to assure minimal movement of the animal. Prior to administering treatment, the treating person practiced consistently applying 1.5 N/mm$^2$ pressure to tendon as measured by a force sensor (K-Scan System sensor model 6900; Tekscan, Inc, South Boston, Massachusetts), with real-time feedback to help maintain the target contact force. The animals were awake during the procedure so normal passive tension was present in the Achilles tendon. Pressure was applied to one Achilles tendon of each rabbit using 3 strokes in each direction with approximately 1.5 N/mm$^2$ pressure. Each treatment lasted approximately 3 minutes. The tendon was massaged moving distal to proximal, then proximal to distal.
along the length of the tendon. Care was taken not to break the overlying skin. ASTM was performed on postoperative days 21, 24, 28, 31, 35, and 38 for 6 treatments. The rabbits were allowed to move normally within their cages between treatments.

Dynamic Viscoelasticity Testing

Ten days after the last ASTM treatment the animals were sacrificed and the lower limbs amputated superior to the knee joint. The area of tendon damage was excised along with margins of normal tissue at each end and each was prepared for biomechanical testing with ample margins of proximal and distal normal tendon. Viscoelasticity of each tendon was measured using dynamic mechanical analysis (Bose Electro-Force Systems Group, Eden Prairie, Minnesota). The normal ends of tendon were grasped with clamps and mounted in the tester (Fig 1). The initial length of the tendon was defined as the grip length at a static tensile pre-load of 1 N. A cyclic tensile strain of 1% was applied to measure the strain ($\varepsilon$) and stress ($\sigma$) by varying the frequency of dynamic load at 0.1, 0.5, 1.0, 5.0, and 10 Hz. Storage modulus ($E'$), loss modulus ($E''$), and loss tangent (tan $\delta$) were derived using the following formula:

$$\varepsilon = \varepsilon_0 \cdot \sin(\omega t) \quad (\varepsilon_0 \text{ : peak strain})$$

$$\sigma = \sigma_0 \cdot \sin(\omega t - \delta) \quad (\sigma_0 \text{ : maximal stress}, \delta \text{ : phase difference})$$

$$|E| = \sigma_0 / \varepsilon_0 \text{ (Pa)}$$

$$E' = |E| \cos \delta \text{ (Pa)} \quad (E' \text{ : dynamic storage modulus})$$

$$E'' = |E| \sin \delta \text{ (Pa)} \quad (E'' \text{ : dynamic loss modulus})$$

$$\tan \delta = E'' / E'$$

Cross-Sectional Area

The diameter of the center part of the injured Achilles tendon was measured, and the cross-sectional area calculated. To calculate the cross-sectional area of each tendon we assumed an elliptical shape and measured the long and short axis of each tendon with digital calipers. Those measurements were then multiplied.

Light Microscopy Examination

Two treated specimens and 2 control specimens were examined with light microscopy to determine the alignment of collagen and quantify the number of tenocytes present. The specimens were fixed with 10% formaldehyde (pH 7.4) and embedded in paraffin. Sections were cut at a thickness of 3 $\mu$m in a plane parallel to the longitudinal axis of the tendon. The sections were stained with hematoxylin-eosin (HE) and type I and III collagen primer antibody.

Statistical Analysis

The $E'$, $E''$, tan $\delta$ and tendon cross-sectional area of the treated tendons were compared with those of the untreated tendons using paired t tests. A P value of less than 0.05 was considered statistically significant.

RESULTS

Cross-Sectional Area

The mean ± SD cross-sectional area of the treated tendons was greater than the control tendons and was 12.30 ± 5.47 mm$^2$ and 9.57 ± 8.36 mm$^2$, respectively ($P < .01$) (Fig 2).

Dynamic Viscoelastic Testing

At all dynamic loading frequencies, the storage modulus ($E'$) in the control tendons was higher than in the treated tendons but it was statistically significant only at 0.1 Hz and 10 Hz ($P = .05$) (Fig 3). The loss modulus ($E''$) was significantly lower in the treated tendons than in the control tendons ($P < .05$) (Fig 4). There was no significant difference in loss tangent (tan $\delta$) between the treated and control tendons (Fig 5).

Light Microscopy

HE staining showed that the tendon fibers in the control tendons were wavy and kinking and displayed a disordered collagen arrangement. In contrast, the fibers of the tendons treated with ASTM were well aligned (Fig 6). Immunohistochemical staining demonstrated higher color intensity of type III collagen in the control tendons than in the ASTM treated tendons (Fig 7).

DISCUSSION

Achilles tendinopathy is a chronic degenerative condition that frequently does not respond to conservative
treatments such as rest, training modification, traditional physical therapy, nonsteroidal anti-inflammatory medications, and corticosteroid injections. Numerous treatments have been proposed to promote healing of this injury. Cell therapy and mesenchymal stem cell therapy have been described. Several studies in animals and in humans have shown that extracorporeal shockwave therapy may be an effective treatment. Low laser therapy has been suggested as it has modulatory effects on inflammatory markers, reducing the inflammatory process, and modulating leukocyte activity in both animals and humans. The mechanical effects of treatments for Achilles tendinopathy have not been routinely studied. Nagasawa et al. measured viscoelasticity of Achilles tendons 3 weeks after tenotomy and found the Young modulus and tangent delta to be lower in tenotomy specimens than in control specimens. We found no reports of dynamic mechanical analysis after tenotomy or surgical repair.

ASTM therapy has been advocated for Achilles tendinopathy with a few reports of its histological effects in animal models. To our knowledge, there have been no reports of its effect on the dynamic behavior of Achilles tendon. We therefore sought to determine its effects on the biomechanical behavior of healing Achilles tendon. We found that ASTM resulted in greater viscosity in treated tendons compared with the control tendons in a rabbit model. Elasticity increased also but it was not statistically significant. Because both elasticity and viscosity increased there was no significant difference in tangent delta between the treated and control tendons. Nonetheless, the treated tendons may be stronger than the untreated tendons because of the increases observed in the cross-sectional area and dynamic viscoelasticity.

We used collagenase to induce an injury and simulate a chronic degenerative clinical condition. There may be other models that better demonstrate aspects of the pathology seen in chronic tendinopathy. The ASTM therapy started 3 weeks after injection, as done by Chen et al. The results indicated that rabbits injected with collagenase had clear degenerative changes consistent with advancing tendinopathy, including fiber disruption, fiber mal arrangement, angiogenesis, and micronucleation by 10 weeks. Waiting longer than 3 weeks before starting treatment might have resulted in more degenerative change reflective of the clinical condition.
We performed exploratory HE staining in a few specimens which showed that the tendon fiber in the control tendons was wavy and kinking with a disordered collagen arrangement. The tendon fibers were well aligned in the tendons treated with ASTM. Type III collagen color intensity in the specimens examined in ASTM group was lower compared to the control tendons examined. This suggests that the remodeling phase with conversion of Type III to Type I collagen occurred quicker in the ASTM treated tendons with less granulation tissue formation. These observations are similar to the findings in previous studies. Chen et al. used histologic evaluation scores to assess the effect of treatment in an Achilles tendinopathy rabbit model. In that study HE staining showed that collagen fibers in treated tendons were oriented in a parallel pattern, with slight splitting between bundles and immunochemical staining found the type III collagen intensity index to be similar among 3 treatment groups.

The combined biomechanical and histologic results of the current study suggest that the tendons treated with ASTM were in the process of healing and the untreated control tendons remained in a state consistent with chronic inflammation.

**LIMITATIONS**

The present study has several limitations. We performed viscoelasticity testing.
And histology at 10 days after completion of therapy as done in previous studies by Chen et al.13,14 Nagasawa et al.36 measured these characteristics at 3, 6, and 12 weeks and showed the cross-sectional area was smaller and the Young modulus was larger with each week that passed. Although we found differences suggesting benefit 10 days after stopping treatment, the effects of ASTM should be evaluated over a longer time period in future studies.

Although we completed microscopy on a few specimens, we did not do it on all specimens and did not grade the findings as an outcome measure. Our histological results are exploratory and must be weighed accordingly. Future studies of ASTM should use an objective score or index27 to judge histological results.

CONCLUSION

We found that ASTM had a measureable beneficial effect on the dynamic biomechanical behavior of healing Achilles tendons in a rabbit model of Achilles tendinopathy.

Practical Applications

- Augmented soft tissue mobilization (ASTM) of injured Achilles tendons in a rabbit model resulted in better recovery of biomechanical function than did no treatment.
- Loss modulus of the treated tendons was significantly lower and cross-sectional area of the treated tendons was significantly greater than in controls.
- ASTM may result in improved clinical results in chronic Achilles tendinopathy.

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