Aging Leads to Inferior Achilles Tendon Mechanics and Altered Ankle Function in Rodents

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Introduction

Achilles tendon injuries are most common in middle-aged men, especially those involved in recreational sports. Increased tendon stiffness, decreased blood flow, and lack of regular physical activity have been suggested as potential causes for the higher incidence of ruptures in this age group, although clinical evidence is conflicting[1-3]. Animal models offer a highly controlled system to study Achilles tendon biomechanics, and have demonstrated a potential explanation for the disparate incidence in Achilles tendon rupture across sex[4]. However, it is unknown if effects of aging could help explain, at least in part, the particularly high frequency of Achilles tendon ruptures in middle-aged men. Therefore, the objective of this study was to identify functional, mechanical, and structural differences among Achilles tendons from young, middle aged, and old male rats. We hypothesized that middle aged and old rats would exhibit increased joint stiffness and decreased Achilles tendon tissue quality as compared to young rats.

Materials and Methods

Design: Young (7 mo), middle aged (18 mo), and old (27 mo) male F344XBN rats, approximating respective human ages of 18, 41, and 60 years, were acquired from the National Institute of Aging (n = 16/group) (IACUC approved) and euthanized three weeks after arriving[5]. *Gait analysis:* Animals (n = 12-16/group) were acclimated to an instrumented walkway, and spatial, temporal, and kinetic parameters were quantified during autonomous locomotion[6]. *Passive joint function:* Ankle range of motion (ROM) and stiffness were measured using a custom device while animals (n = 16/group) were anesthetized[6]. *Sample preparation:* Following euthanasia, Achilles tendon-foot units were harvested and either processed for histological assays or frozen until preparation for structural and mechanical analysis. *High frequency ultrasound (HFUS):* B-mode images of tendons (n = 11-12/group) were captured and analyzed to determine tendon matrix alignment and density[6]. *Mechanical testing:* Samples (n=11-12/group/protocol) were tested to evaluate failure properties (ramp to failure with optical strain tracking) or viscoelastic and fatigue properties (stress relaxation, frequency-sweep, fatigue testing). *Statistics:* One-way ANOVAs were used to compare groups, and significant relationships ($p < 0.05$) were further evaluated using post hoc Student's t-tests with Bonferroni corrections, except for cycles completed (where non-parametric Kruskal-Wallis test with Dunn's post hoc tests were used).

Results

Gait analysis revealed that propulsion force decreased and lateral force increased with increasing age (Figure 1A-B). Animals also took slower, wider, and shorter steps as they aged (not shown). Aging resulted in increased plantarflexion stiffness and decreased range of motion (Figure 1C-D). HFUS analysis showed no differences in tendon organization or density (not shown). Although tendon area was significantly increased in middle aged and old compared to young animals (Figure 2A), stiffness and max load were not different between groups (not shown). Conversely, Achilles tendon max stress and modulus were superior in young animals (Figure 2B-C). Dynamic modulus was greater in the young group as compared to the middle aged and the old animals at all frequencies tested (not shown). Viscoelastic properties (percent relaxation, tan (δ)) were not different between groups, as well as otherfatigue properties (peak strain, laxity, secant stiffness) (not shown). Young animals exhibited decreased fatigue life, but increased hysteresis (trend, 1500 cycles) and secant modulus (50 cycles) (Figure 2D-F).

Discussion

This study identified numerous functional and mechanical differences in the Achilles tendons of young, middle aged, and old rats. Most notably, the young tendons exhibited the greatest mechanical property (max stress, modulus), which could help explain why younger individuals experience fewer Achilles tendon ruptures than middle aged adults. Interestingly, older rats demonstrated impairments in ankle joint function similar to those observed in humans[7-8]. Increased calf muscle activation and metabolic cost of walking during gait in the elderly may be a compensatory mechanism to overcome a tight heel cord that subsequently contributes to the injury of an

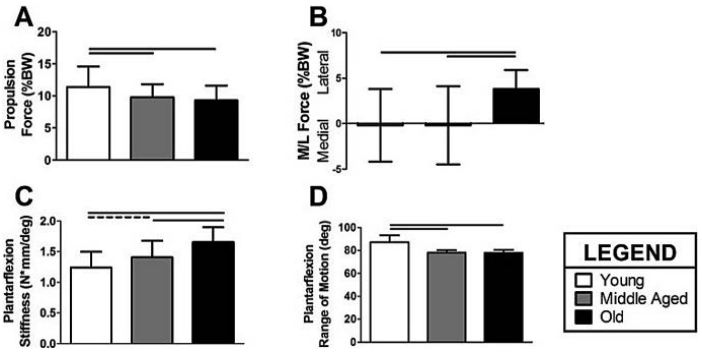

Figure 1. Ankle function. **(A)** Propulsion force decreased and **(B)** lateral force increased with increasing age during gait. Passive motion analysis revealed greater ankle joint **(C)** stiffness and inferior **(D)** range of motion in middle-aged and old rats. Data presented as mean and standard deviation. Solid lines indicate significant differences ($p < 0.017$), dashed lines indicate trends ($p < 0.10$).

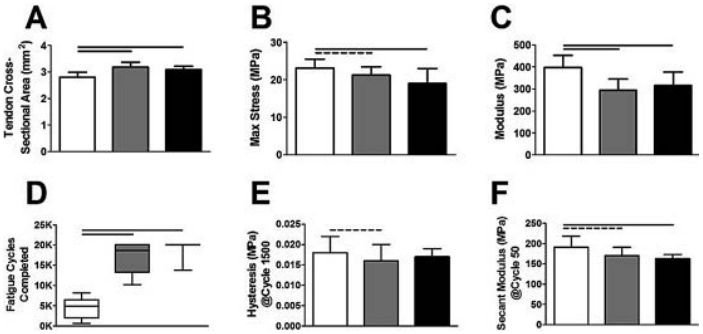

Figure 2. Achilles tendon mechanical properties. Young animals had the smallest **(A)** tendon area and greatest **(B-C)** quasi-static material properties, though they also had the shortest **(D)** fatigue life. Tendons from the young group increased **(E)** hysteresis after 1500 cycles and **(F)** secant modulus after 50 cycles. Data presented as mean and standard deviation, except fatigue cycles completed (five number summary box plot). Solid lines indicate significant differences ($p < 0.017$), dashed lines indicate trends ($p < 0.10$).

already mechanically inferior tendon[9-11]. However, this requires further investigation in a more clinically relevant age group and during more challenging physical activity. We did not detect age-related tendon differences in low-strain viscoelastic properties, although such differences may be present in the entire Achilles tendon-muscle unit[5]. The similar tendon macrostructure observed by HFUS in the current study indicates that age-specific mechanical differences may primarily be the result of altered composition or microstructure, rather than matrix degeneration as a result of increased MMP activity as previously suggested[12]. Lastly, the decreased fatigue life of the younger tendons is likely explained by their decreased cross-sectional area, as they consequently experienced greater peak cyclical stress during load-controlled fatigue testing. We are currently investigating Achilles tendon histological properties and muscle composition in this aging model.

Conclusions

This study supports a potential explanation of the increased incidence of Achilles tendon rupture in the active middle-aged population. Additionally, our results provide a foundation for future studies on Achilles tendon-muscle unit's age-specific responses to loading or injury.

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