Structural and In Vivo Functional Measures Predict Achilles Tendon Fatigue Mechanics During Healing

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Introduction

The Achilles tendon is one of the most commonly injured tendons, affecting approximately 31 in 100,000 people each year[1]. Previous laboratory studies have shown that specific mechanical and material properties of the rat Achilles tendon in the post-injury period may be used to assess healing quality. However, direct clinical measurement of these mechanical properties is not yet possible[2-5]. Further, clinical functional measures, such as the "hop test," are not capable of isolating specific tendon properties[6]. Therefore, the purpose of this study was to use multiple regression analyses to identify clinically measurable functional capable of predicting the mechanical properties of healing Achilles tendon. We hypothesized that tendon properties, including cross sectional area (CSA), echogenicity, collagen fiber alignment, weight-bearing, and ankle range of motion would be strongly predictive of post-injury tendon fatigue mechanical properties.

Materials and Methods

Study Design

Data used in this multiple regression statistical analysis study were obtained from previous studies using rat Achilles tendon blunt transection injury models, which incorporated post-injury immobilization and gradual return to treadmill activity ($n = 110$). Complete data sets needed for developing a robust regression model were acquired from October to November 2016. Studies #1[2] and #2[3] investigated the role of surgical treatment (repaired [R] vs. non-repaired [NR]) and return to activity timing at 3 and 6 weeks post-injury in male rats. Study #3[5] evaluated tendon healing at 3 and 6 weeks post-injury on injured female and ovariectomized (OVX) rats that underwent NR treatment.

Regression Modeling

The predictor variables were: measures of collagen structure (echogenicity and circular standard deviation (CSD) (*high frequency ultrasound*)); active functional limb assessment (vertical, braking, propulsion, and lateral ground reaction force magnitudes (*gait analysis*)); tendon morphology (CSA (*laser-based measurement*)); and passive functional limb assessment (total ankle ROM, toe, and linear stiffness in dorsiflexion and plantarflexion (*passive ankle manipulation*)). Sex (M = [1,0], F = [0,1], OVX = [0,0]) and surgical treatment (NR = 1, R = 0) were inputted as categorical variables. The response variables were secant stiffness, hysteresis, laxity, secant modulus, and secondary phase slope (fatigue testing). Assumptions required for linear regression analysis were general linearity between single predictor and dependent variables, normality, non-multicollinearity (Durbin-Watson), neutrality of the dependent variables, and lack of outliers. Step-wise backward elimination linear regression analysis was performed to select the best structural and functional variables for predicting mechanical properties. The resulting regression coefficients were then used to predict mechanical properties for all groups.

Statistical Significance Evaluation

Coefficients of determination and two-tailed p-values were calculated for each regression model and significance was set at $p < 0.05$ for all tests (SPSS, IBM, Inc., Version 24, Armonk, NY).

Results

All assumptions for multiple regression were satisfied and Durbin-Watson scores were greater than 1.62 for all models presented (Table 1). The chosen independent variables strongly predicted secant modulus and hysteresis ($R^2 > 0.74$, $p < 0.001$). Secant stiffness and laxity were moderately predicted by the chosen independent variables, with R^2 values of 0.34 and 0.46, respectively. Although statistically significant, the slope of the secondary phase was only weakly predicted by the independent variables. Repair type and plantarflexion linear stiffness were involved in predicting four of the five response variables. Sex was a contributing factor in predicting hysteresis, laxity, and secondary phase slope. Dorsiflexion linear stiffness, plantarflexion toe stiffness, lateral force, echogenicity, and total ankle ROM were not significantly predictive in any regression model.

Discussion

The Achilles tendon typically operates under high and cyclic loading scenarios, which can result

Table 1. Significant coefficient results using multiple regression analysis.

Response variable	R^2	*p*	CSA (mm^2)	CSD (*)	Dorsi.Toe Stiffness (N*mm/deg)	Plantar Lin Stiffness (N*mm/deg)	Vertical Force (%BW)	Propulsion Force (%BW)	Braking Force (%BW)	Ankle ROM(*)	Sex	Repair Type
Secant Stiffness (N/mm)	0.3	<0.001	–	–	–	−34.3	0.1	0.8	–	–	–	–
Hysteresis (MPa mm/mm)	0.74	<0.001	0.0003	–	−0.01	−0.04	–	–	–	−0.00006	0.008	0.002
Laxity (mm/mm)	0.46	<0.001	–	–	−19 .9	2.3	–	–	–	–	2.3	−18.6
Secant Modulus (N/mm)	0.76	<0.001	−2.9	−3.3	–	−53.1	0.3		−0.9	–	–	−5.8
2nd Phase Slope (mm/mm /% fatigue life)	0.11	0.036	–	–	–	–	–	–	–	–	0.017	0.016

in tendinopathy and acute rupture. Following treatment, injured tendons are expected to perform under similar physiologic cyclic loading conditions. Clinical assays such as ultrasound, gait analysis, and passive joint assessment cannot directly evaluate tendon fatigue properties. However, since many of these assays are generally simple to perform, even if not used routinely in clinics, defining their relative importance would be important to clinicians. Our regression results suggest that clinical evaluation of ankle joint stiffness into plantarflexion may serve as a viable metric for estimating and tracking tendon strength after injury. Further, CSA measurements allow clinicians to predict the healing tendon's efficiency in storing elastic energy (hysteresis) and ability in bearing load (secant modulus). Both parameters are crucial for allowing the tendon to withstand cyclic loading. Surprisingly, unlike ankle stiffness and cross-sectional area, ultrasound parameters (echogenicity, CSD) were not predictive of tendon fatigue properties, in contrast to a previous study[7]. Although both studies had similar coefficients of determination between echogenicity and secant stiffness, the other functional measures included in our model were more predictive. Finally, in this study, CSA values were obtained using a laser-based technique not performed in humans; however, transverse plane ultrasound methods are in use clinically, and can provide similar area measurements that correlate very well to laboratory-based measurements[8]. Overall, clinically relevant in vivo functional measures assessed healing tendon quality by accurately predicting tendon fatigue mechanical properties.

Conclusions

This study highlighted specific clinical measurements of tendon properties that reliably and significantly predicted the quality of tendon healing. This was achieved through controlled and rigorous laboratory experiments not generally feasible in the clinical setting.

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