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TEMPERATURE-DEPENDENT VISCOELASTIC PROPERTIES OF THE HUMAN SUPRASPINATUS TENDON

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Abstract

Temperature effects on the viscoelastic properties of the human supraspinatus tendon were investigated using static stress-relaxation experiments and Quasi-Linear Viscoelastic (QLV) theory. Twelve supraspinatus tendons were randomly assigned to one of two test groups for tensile testing using the following sequence of temperatures: (1) 37°C, 27°C, and 17°C (Group I, n=6), or (2) 42°C, 32°C, and 22°C (Group II, n=6). QLV parameter *C* was found to increase at elevated temperatures, suggesting greater viscous mechanical behavior at higher temperatures. Elastic parameters *A* and *B* showed no significant difference among the six temperatures studied, implying that the viscoelastic stress response of the supraspinatus tendon is not sensitive to temperature over shorter testing durations. Using regression analysis, an exponential relationship between parameter *C* and test temperature was implemented into QLV theory to model temperature-dependent viscoelastic behavior. This modified approach facilitates the theoretical determination of the viscoelastic behavior of tendons at arbitrary temperatures.

Keywords

Temperature; Viscoelasticity; Supraspinatus tendon; Rotator cuff

INTRODUCTION

The mechanical properties of tendons are often experimentally characterized at controlled *in vitro* temperatures (e.g. room temperature) which differ from those seen *in vivo*. Quantifying the tissue temperature-mechanics relationship may facilitate scientific and clinical interpretations of *in vitro* mechanical testing data and provide further insight into the *in vivo* mechanical behavior of tendon. The majority of studies examining temperature dependence of tendon and ligament mechanical behavior have focused on elastic (Dorlot *et al.*, 1980, Hasberry and Pearcy, 1986, Rigby *et al.*, 1959, Walker *et al.*, 1976) rather than viscoelastic properties

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(Bass *et al.*, 2007, Lam *et al.*, 1990, Woo *et al.*, 1987), with inconsistent results reported among the former investigations.

Theoretical modeling approaches can facilitate the quantification of transient tissue responses as well as the prediction of biomechanical behavior at varying temperatures. However, very few theoretical studies have modeled temperature effects on tendon and ligament mechanical properties (Bass *et al.*, 2007). The quasi-linear viscoelastic (QLV) theory developed by Fung (1969) has been shown to accurately describe the viscoelastic properties of a variety of tendons and ligaments (Abramowitch *et al.*, 2004a, 2004b, Huang *et al.*, 1997, Johnson *et al.*, 1996, Kwan *et al.*, 1993, Thomopoulos *et al.*, 2003, Woo *et al.*, 1981). In the present investigation, we sought to model the temperature-dependent viscoelastic properties of tendon using QLV theory. The specific tissue studied was the human supraspinatus tendon, which along with the remaining rotator cuff muscles and tendons provides dynamic stability to the glenohumeral joint (Cain *et al.*, 1987, Lippitt *et al.*, 1993, Warner *et al.*, 1993). Tensile property data for the supraspinatus tendon have been reported on the basis of room temperature (Nakajima *et al.*, 1994) and freezing clamp environments (Itoi *et al.*, 1995, Nightingale *et al.*, 2003, Huang *et al.*, 2005). However, prior studies have not addressed thermal effects on the rotator cuff tendons within a spectrum of temperatures (from *in vitro* to *in vivo* conditions). Thus, the objective of the current study was to use QLV theory to investigate temperature effects on the material and viscoelastic properties of the supraspinatus tendon using experimental data from static stress relaxation tests.

MATERIALS AND METHODS

Tissue Preparation

Supraspinatus tendons obtained from twelve fresh frozen human cadaveric shoulders were randomly assigned to one of two experimental groups which differed only with regard to temperature treatments: Group I (62±9 years old) and Group II (65±19 years old). For material testing, a rectangular specimen (1.5mm thick, 6.5mm, wide, 20mm long) was prepared from the posterior side of each tendon using a sledge microtome (Leica Instruments, Nussloch, Germany). Width and thickness measurements were taken at five locations along the length of each specimen using a stereomicroscope and a digital micrometer (Akizuki *et al.*, 1986). The cross-sectional area of each specimen was calculated by multiplying the average width and thickness. In order to prevent specimen slippage during testing, 400 grit sandpaper was affixed to both ends of each specimen prior to its placement in custom clamps. The clamps and specimen were mounted in a chamber filled with physiologic saline containing enzymatic inhibitors, while temperature changes and stabilization (±1°C) of the saline bath were controlled by a circulator with a heating unit and cooler (Neslab Instruments Inc., Portsmouth, NH, USA).

Tensile Testing

Material testing was performed using a servo-hydraulic MTS machine (Model 858 Bionix, Eden Prairie, MN, USA). The potential degradation of tendon properties due to prolonged exposure to the saline bath precluded the examination of all six test temperatures for each tendon sample. Hence, biomechanical testing was performed on each specimen using the following sequences of temperatures (Fig. 1): 37°C, 27°C, and 17°C (Group I); and 42°C, 32°C, and 22°C (Group II). This range of temperatures is relevant both to laboratory testing (e.g., use of cryogenic grips [Riemersa and Schamhardt, 1982]) as well as clinical scenarios (Wilson and Goodship, 1994). Each specimen was subjected to a 0.5-Newton pre-load to establish its initial length prior to testing. At each temperature, 20 cycles of preconditioning were performed from 0% to 5% strain at 0.01 sec⁻¹ followed by a 30 minute recovery period. Specimens were then ramped from 0% to 5% strain at 0.01 sec⁻¹, and the displacement was subsequently held

constant for 30 minutes during the stress relaxation phase. One hour of recovery time was allocated between changes of temperature to achieve thermal equilibrium of the tendon as well as to allow viscoelastic effects to dissipate in a fully unloaded state. Hence, the total soak time for each specimen was approximately 5.5 hours.

Theoretical analyses

QLV theory was used to model the viscoelastic behavior of the supraspinatus tendon. As described previously (Kwan *et al.*, 1993), for a specimen stretched to strain level ε , at a constant strain rate γ , the stress response $\sigma(t)$ over a time interval α , can be determined by equations (1a) and (1b).

Ramp-up phase:

$$\sigma(t) = \int_0^t G(t-\tau) \frac{\partial \sigma^e}{\partial \varepsilon} \frac{\partial \varepsilon}{\partial \tau} d\tau, \text{ for } 0 < t < \alpha, \quad (1a)$$

Stress-relaxation phase:

$$\sigma(t) = \int_0^\alpha G(t-\tau) \frac{\partial \sigma^e}{\partial \varepsilon} \frac{\partial \varepsilon}{\partial \tau} d\tau, \text{ for } \alpha < t < \infty, \quad (1b)$$

where

$$G(t) = \frac{1+C[E_1(t/\tau_2)-E_1(t/\tau_1)]}{1+C\ln(\tau_2/\tau_1)}, \quad E_1(y) = \int_y^\infty e^{-z}/z dz, \\ \sigma^e(\varepsilon) = A(e^{B\varepsilon} - 1), \quad \varepsilon = \begin{cases} \gamma t, & 0 \leq t < \alpha \\ \gamma \alpha, & \alpha \leq t < \infty \end{cases}.$$

The physiological significance of the parameters C , τ_1 , and τ_2 has been described previously by Sauren and Rousseau (1983) who summarized that parameter C indicates the extent of viscous behavior while parameters τ_1 and τ_2 represent, respectively, the fast and slow viscous characteristics. The experimental stress versus time data were normalized by the peak stress to eliminate parameter A , and the four remaining parameters (B , C , τ_1 , and τ_2) were obtained by curve-fitting equation (1) to the normalized data (Kwan *et al.*, 1993). After B , C , τ_1 , and τ_2 were determined, A was calculated using equation (1) and the peak stress. The quality of curve-fitting was assessed using the coefficient of determination r^2 (Kvalseth, 1985; Ateshian *et al.*, 1997).

Using regression analysis of the experimental data, a quantitative relationship was obtained to express the dependence of parameter C on the tendon temperature T . The effect of test temperature on each of the five QLV parameters was determined using a one-way ANOVA (significance assumed for $p < 0.05$) followed by a Student-Newman-Keuls post-hoc test.

RESULTS

Typical temperature-dependent stress-relaxation responses together with QLV curvefits for the supraspinatus tendon are shown in Figures 2a (Group I) and 2b (Group II). The nonlinear coefficient of determination r^2 averaged across all twelve specimens was 0.97 ± 0.02 , demonstrating strong agreement between experimental and theoretical results. For Group I specimens, the effect of temperature was more pronounced during the stress-relaxation phase

than the ramp-up phase, while thermal effects were more evident between 37°C and 27°C than between 27°C and 17°C (Fig. 2a). A similar pattern of results among the three temperatures of Group II was observed (Fig. 2b).

A comparison of the computed QLV parameters across different temperatures is provided in Table 1. Parameter C was found to be significantly greater at 42°C than at 32°C, 27°C, 22°C and 17°C ($p=0.001$, statistical power of 0.81); and at 37°C compared with 22°C and 17°C ($p=0.003$). However, no significant differences in parameter C were noted among temperatures below 32°C. There were no significant differences in parameters A , B , E , τ_1 , and τ_2 among the six temperatures.

Regression analysis revealed an exponential relationship between parameter C and tendon temperature T (Fig. 3):

$$C=0.0021 e^{0.089T}+0.08, (r^2=0.99) \quad (2)$$

DISCUSSION

The present study characterizes the subfailure, material and viscoelastic properties of human rotator cuff tendon across a range of temperatures. Results from QLV modeling of stress-relaxation data revealed a statistically greater value for parameter C at the two highest temperatures studied. As the relative magnitude of C is indicative of the extent of viscous material behavior (Sauren and Rousseau, 1983), our findings suggest that the supraspinatus tendon is less viscous (i.e., more elastic) at lower temperatures. These findings are consistent with the studies of canine patellar tendon (Walker *et al.*, 1976) and canine knee ligaments (Woo *et al.*, 1987).

The lack of a statistically significant effect of temperature on parameters A and B (which appear in the elastic response term) demonstrates that the instantaneous stress response of tendon to applied strain is not temperature dependent. This result further suggests that the viscoelastic stress response of the supraspinatus tendon may not be sensitive to temperature over shorter testing durations, and may potentially explain why previous studies observed minimal temperature effects on the load-elongation relationship of tendon and ligament (Dorlot *et al.*, 1980, Hasbery and Percy, 1986, Rigby *et al.* 1959). This dependency of viscoelastic behavior on test duration, at different temperatures, is similar to the results reported by Lam *et al.* (1990) for rabbit ligaments. However, our results disagree with those reported by Bass *et al.* (2007) who found that elastic, rather than viscoelastic, modeling coefficients were strongly dependent on temperature. The dissimilar results of Bass and coworkers, relative to those of the present study, are likely attributable to multiple methodological differences (e.g., strain rate and theoretical model).

A high correlation coefficient was calculated from the regression analysis relating parameter C to test temperature (equation 2 in Results). Hence, this equation can be used to determine the viscous parameter C at arbitrary temperatures. Since parameters A , B , τ_1 , and τ_2 , were insensitive to temperature, substituting the regression equation for parameter C into the QLV formulation enables the prediction of temperature-dependent viscoelastic behavior of the supraspinatus tendon. Therefore, this novel approach can facilitate prediction of the transient and steady-state tensile response of tendons at *in vivo* temperatures when experimental results obtained from *in vitro* temperatures are available.

Interestingly, no statistical differences among QLV parameters were found among temperatures ranging from 17–32°C, implying that temperature effects do not have a significant effect on the subfailure mechanical response of the supraspinatus tendon when the tissue is

maintained within these temperatures. Elastic parameters A and B were not significantly different above 32°C, suggesting that the *in vivo* mechanical behavior of the human supraspinatus tendon is not sensitive to small changes in temperature under short duration loading. However, a significant increase in parameter C (i.e., viscous behavior) was noted at temperatures above 32°C, indicating the potential of this tissue to absorb more energy due to its increasing viscosity at elevated temperatures. The latter results further imply that, in general, the tendon's *in vivo* mechanical properties are better approximated in an environment approximating body temperature. However, maintenance of supraspinatus tendon temperature within this higher thermal range can be challenging if testing equipment such as freezing grips are utilized (Itoi *et al.*, 1995, Nightingale *et al.*, 2003, Rincón *et al.*, 2001, Huang *et al.*, 2005).

Potential study limitations include the use of actuator displacements (rather than an optical method) for strain measurement as well as the fact that the temperature test sequence was not randomized during testing. However, in pilot experiments the mechanical response of the supraspinatus tendon was repeatable (up to 5% strain) both at the highest and lowest temperatures examined. The reproducibility of tensile properties at 42°C further implies that the duration of tendon exposure to the heated saline bath did not lead to irreversible specimen degradation.

In summary, the present investigation quantified temperature effects on the subfailure material and viscoelastic properties of the human supraspinatus tendon. Thermal effects were more pronounced with regard to viscoelastic behavior in comparison to elastic behavior. Hence, as noted by previous investigators, temperature should be considered as an important factor when the mechanical properties of soft tissues are determined at temperatures that differ from *in vivo* conditions. QLV theory was found to accurately model the temperature-dependent tensile material properties of tendon. The exponential relationship between viscoelastic parameter C and temperature derived from regression analysis can be implemented into QLV theory to predict the temperature-dependent viscoelastic behavior of the supraspinatus tendon. The combined experimental and analytical approaches presented in the current study can also be applied to other tendons and ligaments in order to compare the temperature sensitivity of the mechanical behavior of different tissues. Our methodology may be implemented into computational simulations in which mechanical properties of connective tissues in response to thermal variations (e.g., due to clinical treatments and/or exercise, as noted in additional replies below) may be calculated.

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References

- Abramowitch SD, Woo SL. An improved method to analyze the stress relaxation of ligaments following a finite ramp time based on the quasi-linear viscoelastic theory. *Journal of Biomechanical Engineering* 2004;126:92–97. [PubMed: 15171134]
- Abramowitch SD, Woo SL, Clineff TD, Debski RE. An evaluation of the quasi-linear viscoelastic properties of the healing medial collateral ligament in a goat model. *Annals of Biomedical Engineering* 2004;32:329–335. [PubMed: 15098537]
- Akizuki S, Mow VC, Muller F, Pita JC, Howell DS, Manicourt DH. Tensile properties of knee joint cartilage. I. Influence of ionic conditions, weight bearing and fibrillation on the tensile modulus. *Journal of Orthopaedic Research* 1986;4:379–392. [PubMed: 3783297]

- Ateshian GA, Warden WH, Kim RP, Grelsamer RP, Mow VC. Finite deformation biphasic material properties of bovine articular cartilage from confined compression experiments. *Journal of Biomechanics* 1997;30:1157–1164. [PubMed: 9456384]
- Bass CR, Planchak CJ, Salzar RS, Lucas SR, Rafaels KA, Shender BS, Paskoff G. The temperature-dependent viscoelasticity of porcine lumbar spine ligaments. *Spine* 2007;32:E436–442. [PubMed: 17632382]
- Bigliani LU, Pollock RG, Soslowsky LJ, Flatow EL, Pawluk RJ, Mow VC. Tensile properties of the inferior glenohumeral ligament. *Journal of Orthopaedic Research* 1992;10:187–197. [PubMed: 1740736]
- Cain PR, Mutschler TA, Fu FH, Lee SK. Anterior stability of the glenohumeral joint - A dynamic model. *The American Journal of Sports Medicine* 1987;15:144–148. [PubMed: 3578636]
- Clarke RSJ, Hellon RF, Lind AR. The duration of sustained contractions of the human forearm at different muscle temperature. *Journal of Physiology (Lond)* 1958;143:454–473. [PubMed: 13588567]
- Cofield RH. Rotator cuff disease of the shoulder. *Journal of Bone and Joint Surgery* 1985;67A:974–979. [PubMed: 4019548]
- Cohen RE, Hooley CJ, McCrum NJ. Viscoelastic creep of collagenous tissue. *Journal of Biomechanics* 1976;9:175–184. [PubMed: 1262352]
- Dorlot JM, Ait Ba Sidi M, Tremblay GM, Drouin G. Load-elongation behavior of the canine anterior cruciate ligament. *Journal of Biomechanical Engineering* 1980;102:190–193.
- Fung, YCB. Stress-strain history relation of soft tissues in simple elongation. In: Fung, YC.; Peronne, N.; Anliker, M., editors. *Biomechanics: Its foundations and objectives*. Prentice Hall: Englewood Cliffs; 1972. p. 181–208.
- Hasberry S, Percy MJ. Temperature dependence of the tensile properties of interspinous ligaments of sheep. *Journal of Biomedical Engineering* 1986;8:62–66. [PubMed: 3951211]
- Huang CY, Wang VM, Cohen NP, Bucchieri JS, Pawluk RJ, Pollock RG, Mow VC. Nonlinear viscoelastic properties of the inferior glenohumeral ligament. *Proceedings of the ASME International Mechanical Engineering Congress: Advances in Bioengineering* 1997;36:195–196.
- Huang CY, Wang VM, Pawluk RJ, Bucchieri JS, Levine WN, Bigliani LU, Mow VC, Flatow EL. Inhomogeneous mechanical behavior of the human supraspinatus tendon under uniaxial loading. *Journal of Orthopaedic Research* 2005;23:924–930. [PubMed: 16023009]
- Itoi E, Berglund LJ, Grabowski JJ, Schultz FM, Growney ES, Morrey BF, An KN. Tensile properties of the supraspinatus tendon. *Journal of Orthopaedic Research* 1995;13:578–584. [PubMed: 7674074]
- Johnson GA, Tramaglini DM, Levine RE, Ohno K, Choi NY, Woo SLY. Tensile and viscoelastic properties of human patellar tendon. *Journal of Orthopaedic Research* 1994;12:796–803. [PubMed: 7983555]
- Ker RF. Dynamic tensile properties of the plantaris tendon of sheep. *Journal of Experimental Biology* 1981;93:283–302. [PubMed: 7288354]
- Kvalseth TO. Cautionary note about R^2 . *The American Statistician* 1985;39:279–285.
- Kwan MK, Lin TH, Woo SLY. On the viscoelastic properties of the anteromedial bundle of the anterior cruciate ligament. *Journal of Biomechanics* 1993;26:447–452. [PubMed: 8478348]
- Lam TC, Thomas CG, Shrive NG, Frank CB, Sabiston CP. The effects of temperature on the viscoelastic properties of the rabbit medial collateral ligament. *Journal of Biomechanical Engineering* 1990;112:147–152. [PubMed: 2345444]
- Lippitt SB, Vanderhooft JE, Harris SL, Sidles JA, Harryman DT, Matsen FA. Glenohumeral stability from concavity-compression: A quantitative analysis. *Journal of Shoulder and Elbow Surgery* 1993;2:27–35.
- Nakajima T, Rokuuma N, Hamada K, Tomatsu T, Fukuda H. Histologic and biomechanical characteristics of the supraspinatus tendon: Reference to rotator cuff tearing. *Journal of Shoulder and Elbow Surgery* 1994;3:79–87.
- Nightingale EJ, Allen CP, Sonnabend DH, Goldberg J, Walsh WR. Mechanical properties of the rotator cuff: response to cyclic loading at varying abduction angles. *Knee Surgery Sports Traumatology Arthroscopy* 2003;11:389–92.

- Pollock, RG.; Flatow, EL.; Bigliani, LU.; Kelkar, R.; Mow, VC. Shoulder biomechanics and repetitive motion. In: Gordon, SL., editor. Repetitive Motion Syndromes of the Upper Extremity. American Academy of Orthopaedic Surgeons Publishing; Rosemont: 1995. p. 144-155.
- Riemersa DJ, Schamhardt HC. The cryo-jaw, a clamp designed for in vitro rheology studies of horse digital flexor tendons. *Journal of Biomechanics* 1982;15:619–620. [PubMed: 7142228]
- Rincón L, Schatzmann L, Brunner P, Stäubli HU, Ferguson SJ, Oxland TR, Nolte LP. Design and evaluation of a cryogenic soft tissue fixation device –load tolerances and thermal aspects. *Journal of Biomechanics* 2001;34:393–397. [PubMed: 11182132]
- Rigby BJ, Hirai N, Spikes JD, Eyring H. The mechanical properties of rat tail tendon. *The Journal of General Physiology* 1959;43:265–283.
- Sauren HJ, Rousseau EPM. A concise sensitivity analysis of quasi-linear model proposed by Fung. *Journal of Biomechanical Engineering* 1983;105:92–95. [PubMed: 6843108]
- Thomopoulos S, Williams GR, Gimbel JA, Favata M, Soslowsky LJ. Variation of biomechanical, structural, and compositional properties along the tendon to bone insertion site. *Journal of Orthopaedic Research* 2003;21:413–419. [PubMed: 12706013]
- Walker P, Amstutz HC, Rubinfeld M. Canine tendon studies. II. Biomechanical evaluation of normal and regrown canine tendons. *Journal of Biomedical Materials Research* 1976;10:61–76. [PubMed: 1249090]
- Wilson AM, Goodship AE. Exercise-induced hyperthermia as a possible mechanism for tendon degeneration. *Journal of Biomechanics* 1994;27:899–905. [PubMed: 8063840]
- Woo SLY, Gomez MA, Akeson WH. The time and history-dependent viscoelastic properties of canine medial collateral ligament. *Journal of Biomechanical Engineering* 1981;103:293–298. [PubMed: 7311496]
- Woo SLY, Lee TQ, Gomez MA, Sato S, Field FP. Temperature dependent behavior of the canine medial collateral ligament. *Journal of Biomechanical Engineering* 1987;109:68–71. [PubMed: 3560883]
- Woo, SLY.; Livesay, GA.; Runco, TJ.; Young, E. Structure and function of tendons and ligaments. In: Mow, VC.; Hayes, WC., editors. *Basic Orthopaedic Biomechanics*. Lippincott-Raven; Philadelphia: 1997. p. 209-251.

Figure 1.

Schematic of the experimental testing protocol. A 60 minute recovery period was incorporated between tests conducted at different temperatures to achieve thermal equilibrium of the tendon and for dissipation of viscoelastic effects.

Figure 2. Typical stress-relaxation responses and the QLV curve-fitting for supraspinatus tendon specimens in (a) Group I and (b) in Group II.

Figure 3. Exponential relationship between QLV parameter C and test temperature T as obtained by regression analysis of experimental data.

Table 1
Computed QLV parameters for the human supraspinatus tendon at different test temperatures.

	17°C	22°C	27°C	32°C	37°C	42°C
C (MPa)	0.089±0.012	0.093±0.015	0.107±0.014	0.112±0.023	0.143±0.027 [§]	0.165±0.058 [¶]
τ_1 (×10 ⁻³ sec)	2.07±0.86	1.75±0.95	2.52±1.27	1.87±1.31	2.3±0.74	1.92±0.24
τ_2 (×10 ² sec)	4.26±0.66	3.30±0.58	4.01±0.71	3.81±0.91	3.25±0.84	3.71±0.14
A (MPa)	0.21±0.09	0.32±0.13	0.27±0.10	0.31±0.13	0.24±0.12	0.28±0.13
B	31.0±3.6	23.7±3.6	27.4±5.3	23.4±3.9	27.0±4.4	26.1±7.2
E (=A×B)	6.36±2.21	7.25±2.05	7.07±1.96	6.94±2.38	6.2±2.34	6.76±2.19

[§] >17°C and 22°C (p=0.003);
[¶] >17°C, 22°C, 27°C, and 32°C (p=0.001)