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Nonlinear Ligament Viscoelasticity

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Abstract—Ligaments display time-dependent behavior, characteristic of a viscoelastic solid, and are nonlinear in their stress–strain response. Recent experiments[25] reveal that stress relaxation proceeds more rapidly than creep in medial collateral ligaments, a fact not explained by linear viscoelastic theory but shown by Lakes and Vanderby[17] to be consistent with nonlinear theory. This study tests the following hypothesis: nonlinear viscoelasticity of ligament requires a description more general than the separable quasilinear viscoelasticity (QLV) formulation commonly used. The experimental test for this hypothesis involves performing both creep and relaxation studies at various loads and deformations below the damage threshold. Freshly harvested, rat medial collateral ligaments (MCLs) were used as a model. Results consistently show a nonlinear behavior in which the rate of creep is dependent upon stress level and the rate of relaxation is dependent upon strain level. Furthermore, relaxation proceeds faster than creep; consistent with the experimental observations of Thornton *et al.*[25] The above results from rat MCLs are not consistent with a separable QLV theory. Inclusion of these nonlinearities would require a more general formulation. © *2001 Biomedical Engineering Society.* [DOI: 10.1114/1.1408926]

Keywords—Stress relaxation, Creep, Ligament, Quasilinear viscoelasticity (QLV).

INTRODUCTION

Ligaments are viscoelastic and thus, display time-dependent and load-history-dependent mechanical behavior. Viscoelasticity has been studied in numerous biological materials such as bone,[18,19] articular cartilage,[31] skeletal muscle,[5] ligament,[11,32] tendon,[3,12] and cardiovascular tissues.[22,23] Recently, ligament viscoelasticity has been studied in healing,[27] damaged,[20] grafted,[16] and prosthetic[1] ligaments. It is axiomatic that a repaired or replaced ligament must possess the same viscoelastic characteristics as a normal ligament to provide the same function. It is also of interest to know the difference in performance between healthy and damaged ligaments. For these reasons it is important to understand viscoelastic behavior throughout its functional range.

Prior studies of ligament viscoelasticity often consist of a creep or relaxation test at one load or strain level. Force versus displacement curves at constant strain rates demonstrate that ligament is nonlinear. The reason is that collagen fibers are recruited as load increases.[30] The stress–strain curves of ligament display a ''toe'' region where fibers straighten and elongate in a strain-stiffening fashion until the fibers are no longer crimped. At that point the fibers elongate, giving rise to the linear segment of the stress–strain curve. It is this toe region and the lower strain portion of the linear region that is addressed in this study. Arms *et al.*[2] reported strains in the human medial collateral ligament (MCL) of 4% at 120° of flexion during passive knee motion and Hull *et al.*[13] reported human MCL strains up to $7.7\% \pm 2.9\%$ under combined loading. Strains in these studies are based upon *in situ* changes in length. The onset of mechanical damage in the rat MCL has been shown by Provenzano *et al.*[21] to be at 5.1% strain after experimental preload *ex vivo*. At strains below this threshold the tissue will return to its original, preloaded length after a recovery time equal to ten times the duration of tissue loading during the test.[21] Previous studies have not studied viscoelastic behavior at multiple deformation or load levels throughout the physiologic domain of recoverable loading.

Researchers have tried to quantify fundamental viscoelastic behavior with phenomenological models including linear,[29] quasilinear,[12] and nonlinear.[15,17] Linear viscoelasticity is expressed in terms of the Boltzmann integral:

$$\sigma(t) = \int_0^t E(t-\tau)\frac{d\varepsilon(\tau)}{d\tau}d\tau, \tag{1}$$

in which $E(t)$ is a relaxation function, which in the present context refers to deformation in the axial direction, as it depends on time t, $\sigma(t)$ is stress, $\varepsilon(t)$ is strain, and τ is a time variable of integration. Nonlinear-

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ity may be described by a single-integral form called nonlinear superposition, which allows the relaxation function to depend on strain level:

$$\sigma(\varepsilon,t)=\int_0^t E[t-\tau,\varepsilon(\tau)]\frac{d\varepsilon(\tau)}{d\tau}d\tau. \tag{2}$$

In the quasilinear viscoelasticity (QLV) formulation of Fung,[8] the relaxation function, which depends on strain, is *separable* into the product of a function of time and a function of strain: $E(t,\varepsilon)=E_t(t)g(\varepsilon)$, so

$$\sigma(\varepsilon,t)=\int_0^t E_t(t-\tau)\frac{d\sigma}{d\varepsilon}\frac{d\varepsilon(\tau)}{d\tau}d\tau. \tag{3}$$

As an illustration, suppose the strain history is controlled as a Heaviside step function of magnitude ε_0 in time. The derivative becomes a delta function, so the stress relaxation given by Eq. (3) is: $\sigma(\varepsilon,t)=\varepsilon_0 E_t(t)g(\varepsilon)$. The stress is clearly dependent on strain level, but its time dependence does not depend on strain. Thus, time dependence is assumed to be independent of strain. Analogously, a *separable* assumption in a creep formulation would imply that time dependence in creep is independent of stress. Elastic nonlinearity and time dependence can be easily discriminated in a log–log plot of stress versus time in a relaxation experiment. Purely strain-dependent elastic nonlinearity $g(\varepsilon)$ in QLV manifests itself in the overall height of the relaxation curve. The time dependence manifests itself in the shape of the curve. Since in QLV, the time dependence is decoupled from the strain dependence, all the relaxation curves must have the same shape (on this log–log plot), in general, or the same slope if they are power law. In a more general single-integral nonlinear formulation, the shape or slope of the relaxation curves can depend on strain level.

In the past, the most common phenomenological model of the viscoelastic behavior of ligaments has been the QLV model. This model has been useful in describing experiments with ligaments and tendons (see Refs. 32 and 33, and others). In the above studies, the experiments were performed only in deformation control, and at a limited number of strain levels. The quasilinear viscoelastic formulation described the relaxation response of the tissue in these experiments very well.

In a study by Thornton *et al.*,[25] both creep and relaxation were investigated. They observed that relaxation proceeded faster than creep and showed that linear viscoelastic theory was not able to phenomenologically model both behaviors with interrelated constitutive coefficients. This rate difference between creep and relaxation was also observed in the more clinically focused experiments of Graf *et al.*[9] A single linear or QLV model is likely to predict ligament response poorly when components of both creep and relaxation are included in the load history. In consequence, models may be unable to simulate complex joint behavior with fidelity, and the desired time-dependent behavior for graft or replacement ligaments may be poorly defined.

In this study two viscoelastic properties are considered: the increase in tissue deformation over time with a constant load (creep) and the decrease in load with time at a constant tissue elongation (stress relaxation). The authors' hypothesis is that nonlinear viscoelasticity of ligament requires a description more general than the separable quasilinear viscoelasticity (QLV) formulation commonly used. To test this hypothesis both creep and relaxation experiments were performed at multiple levels in the physiologic region of recoverable loading.

METHODS

This study was approved by the Institutional Animal Use and Care Committee and meets the National Institute of Health (NIH) guidelines for animal welfare. Eighteen medial collateral ligaments (MCLs) from euthanized Sprague–Dawley male rats (weight $=250\pm25$ g) were used. Each MCL was exposed by carefully dissecting away all extraneous tissue. The MCLs including intact femoral and tibial bone sections were carefully excised for *ex vivo* testing with care taken not to disturb the ligament insertion sites. The tissues were kept hydrated in Hank's physiologic solution.

Ligaments were divided into three groups: (1) stress relaxation, (2) creep, and (3) stress relaxation and creep on contralateral ligaments. Group 1 ($n=6$ ligaments) consists of MCLs subjected to stress relaxation testing for 100 s at varying levels of strain below the damage threshold of $\sim5\%$ for this method of testing.[21] Group 2 ($n=6$ ligaments) is made up of tissues tested in creep for 100 s at varying levels of load below the loads seen near the damage threshold. None of the group 2 specimens exceeded 5% strain when loaded. The order of the tests was random. A brief test period was chosen for these series to allow sufficient time for recovery between repeated tests and to minimize time spent by the specimen in the bath. For group 3 ($n=4$ pairs of ligaments) stress relaxation and creep were tested on contralateral ligaments (20 min tests).

Similar methods were used for all ligaments. Ligament cross-sectional area was calculated by optically measuring the width and thickness of the ligament and assuming an elliptical cross section. Histological cross sections of the Sprague–Dawley MCL showed that an elliptical cross section is a reasonable approximation for calculating area. The experimental system (load frame-camera–image processor) has a resolution of at least 10

μm and repeated measures at a fixed length of optical markers are consistently reproducible to at least 10 μm. Specimens were tested in a custom-designed load frame with special structures to hold the femur and tibial end sections of the sample in an anatomical position that loads the fibers as uniformly as possible. Strain in relaxation specimens was measured by placing graphite-impregnated silicon grease markers on the specimen and using video dimensional analysis to measure displacement. Strain was measured grip to grip in creep testing. Force was measured and recorded by Labtech Notebook data acquisition software (Laboratory Technologies Corp., Wilmington, MA). Each test was video taped with time, load, and displacement from the data acquisition software written and recorded on the video tape in real time. Afterward, the video images of the stretching ligament were digitized and evaluated to calculate strain for a particular load and time in the tissue with N.I.H. Image software (http://rsb.info.nih.gov/nih-image). Gage length [7.25 ± 0.41 mm (mean $\pm$ s.d.)] was measured at 0.1 N of preload before each test in order to obtain a uniform zero point. After preload the tissue was pulled in either displacement or load control with a rise time of 0.32 s. The rise time was selected based upon stability considerations in load control in our testing apparatus. Displacement-controlled tests used the same rise time for uniformity. Other than the preload, no preconditioning was done, to eliminate the possibility of history effects. For contralateral stress relaxation and creep tests, the relaxation test was performed first and the load history recorded, a creep test was then performed on the contralateral MCL in load control at the maximum load obtained during the relaxation test. For tissue recovery the ligaments were unloaded and allowed to recover for at least ten times the length of the test[28] while remaining hydrated in Hank's physiologic solution. After recovery the gage length of the MCL was measured again at the preload loading of 0.1 N and another viscoelastic test was performed on the ligament. Data were plotted on a log–log scale with the first time point being ten times larger than the rise time of the load or displacement to prevent transient effects from a loading that is not truly a step function.[17,28] A power law, t^n, was used for curve fitting. The parameter n, which is the slope on a log–log plot of stress or strain versus time, was considered as the ''rate'' of relaxation or creep, respectively.

Statistical analyses were performed on the data in order to determine if the rate of stress relaxation or creep is strain or stress dependent, respectively, and whether a significant difference in rate of stress relaxation or creep exists for group 3. To account for the subsampling within individual specimens in groups 1 and 2, the data were analyzed with repeated measures analysis of covariance. The null hypothesis being tested is that there is no association between rate and stress or strain. This hypothesis is rejected if the p value is less than 0.05. A paired students t-test was performed on group 3 to determine if a significant rate difference exists. A significant rate difference exists at $p < 0.05$. All analyses were performed with SAS PROC MIXED (SAS Institute, Inc., Cary, NC).

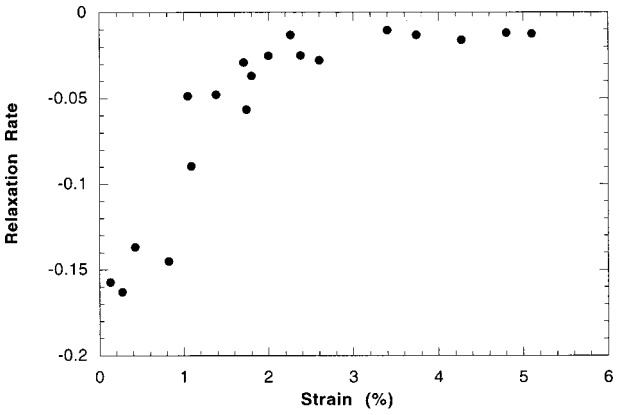

FIGURE 1. Stress relaxation is seen to be nonlinear with respect to strain for group 1. Multiple stress relaxation tests were performed on each of the ligaments in group 1. Results from all tests in group 1 are represented. The relaxation rate n is the slope obtained from curve fitting the data with a single-term power law t^n in time, n is dimensionless. Statistical analysis indicates that the rate of stress relaxation is strongly dependent upon strain ($p = 0.0001$). A linear time dependence as in linear viscoelasticity or a quasilinear time dependence in QLV would predict the same slope (rate) for each relaxation test and would therefore be represented by a horizontal line.

RESULTS

Stress relaxation and creep were both nonlinear in that their rates (slopes on log–log plots) are dependent on strain and stress, respectively (Figs. 1 and 2). In both stress relaxation and creep, rates changed by approximately an order of magnitude throughout this low load region (Figs. 1 and 2). Statistical analysis for group 1 indicated that the rate n (slope in a log–log plot) of stress relaxation is strongly dependent upon strain ($p = 0.0001$). Statistical analysis for group 2 indicated that the rate of creep is strongly dependent upon stress ($p = 0.0078$). To ensure specimen recovery from serial testing, the preloaded gage length was measured before each test. Each ligament subjected to multiple tests showed gage length differences less than 0.5% for stress relaxation and less than 0.75% for creep. Multiple stress relaxation tests from group 1 demonstrate a decrease in stress with time and a decrease in the rate of stress relaxation with increasing strain (Fig. 3). In addition, the elastic moduli at 10 s for increasing strain are 129.6, 290.0, 396.0, and 396.4 MPa, respectively (Fig. 3). Creep data at multiple levels of stress from group 2

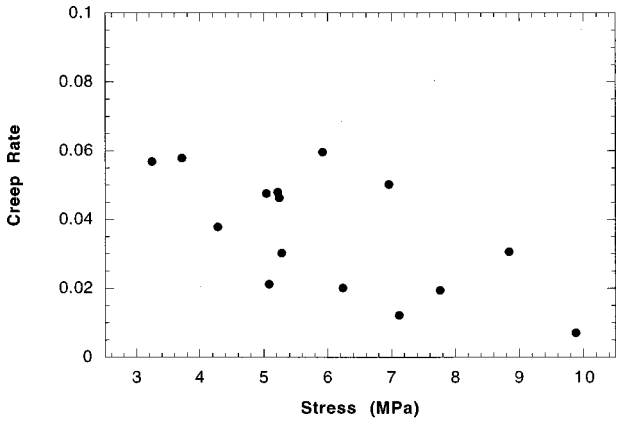

FIGURE 2. Creep is seen to be nonlinear with respect to applied stress for group 2. Multiple creep tests were performed on each of the ligaments in group 2. Results from all tests in group 2 are represented. The creep rate n is the slope obtained from curve fitting the data with a single-term power law t^n in time, n is dimensionless. Statistical analysis indicates that the rate of creep is strongly dependent upon stress ($p=0.0078$). A linear time dependence as in linear viscoelasticity or a quasilinear time dependence in QLV would predict the same slope (rate) for each creep test and would therefore be represented by a horizontal line.

display increasing strain with time and a reduction in creep rate with increasing levels of stress. For one of the creep samples, three tests were run within 0.1 N of each other, revealing rate changes less than 3% when fit ($R^2 > 0.95$) with a power law of the form $\varepsilon = At^n$. A single-exponential curve fit was not used because its sigmoidal shape in a log–log plot does not correspond to the data.

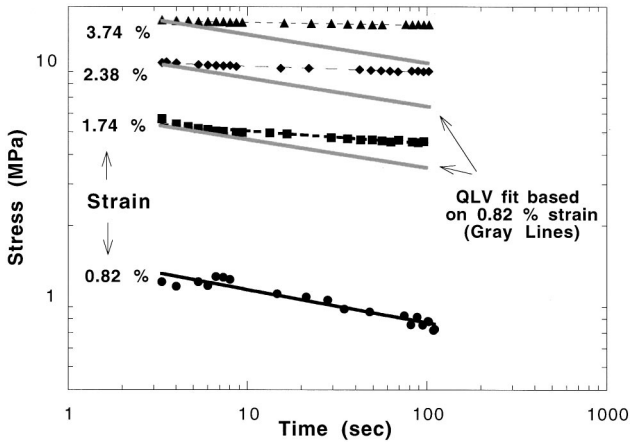

FIGURE 3. Stress relaxation at multiple levels of strain. Stress and time are plotted on log scales. Note the decrease in the rate of stress relaxation with increasing strain as seen from the slope of a single-term power law in time ($R^2 \geq 0.91$): ● ($\varepsilon=0.82\%$) $\sigma=1.474t^{-0.141}$, ■ ($\varepsilon=1.74\%$) $\sigma=5.700t^{-0.053}$, ♦ ($\varepsilon=2.38\%$) $\sigma=10.103t^{-0.025}$, and ▲ ($\varepsilon=3.74\%$) $\sigma=15.015t^{-0.012}$. A QLV fit (gray lines) based on 0.82% strain would predict the same rate for all strains, and so QLV does not capture the dependence of rate on strain.

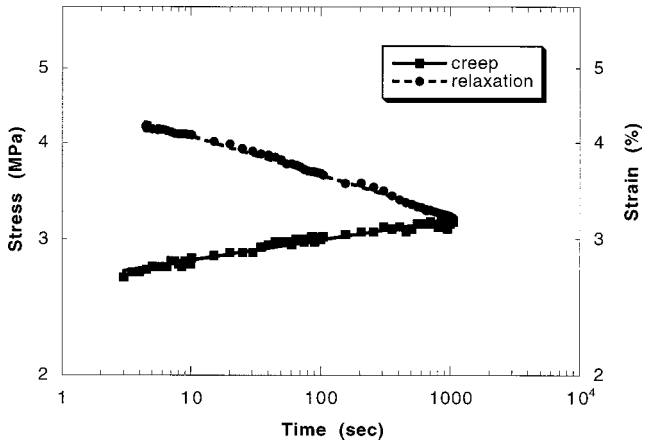

FIGURE 4. Stress relaxation and creep on contralateral ligaments. Stress, strain, and time are plotted on log scales. Note the rate of stress relaxation proceeds faster than the rate of creep. Curves fit with a single-curve power law in time: ● (relaxation) $\sigma=4.590t^{-0.051}$, $R^2 \geq 0.99$, and ■ (creep) $\varepsilon=2.641t^{0.027}$, $R^2 \geq 0.95$.

Group 3 data (stress relaxation and creep on contralateral ligaments) demonstrate a statistically significant difference in the rate of stress relaxation and creep ($p = 0.0009$) and that the rate of stress relaxation proceeds faster than creep by 1.9 ± 0.57 times (Fig. 4).

The separable form of the nonlinear constitutive equation (QLV) does not describe the above results. For these data the rate of creep depends on load level and the rate of relaxation depends on strain level. The rate of relaxation varied by more that an order of magnitude from -0.163 for 0.27% strain to -0.0125 for 5.10% strain. Creep rate also varied by nearly an order of magnitude between 0.058 for 3.72 MPa and 0.007 for 9.88 MPa stress. In QLV the time dependence and stress or strain dependence are assumed to be independent, and the same (separable) function of time is used regardless of applied stress (in creep) or applied strain (in relaxation) (Fig. 3).

DISCUSSION

The results from groups 1 and 2 show that stress relaxation and creep in rat MCLs are nonlinear in time and strain or stress, respectively, within physiologic range. This observed nonlinear viscoelastic behavior indicates stress dependence for rate of creep and strain dependence for rate of stress relaxation, a result which, to our knowledge, has not been previously observed or reported. These findings support the authors' hypothesis that nonlinear viscoelasticity of ligament requires a description more general than the separable quasilinear viscoelasticity (QLV) formulation commonly used. However, it is worth noting that the rate of relaxation in Fig. 1 appears to be approaching an asymptotic value at strains which lead into the linear region of the stress–

strain curve. The separable QLV formulation would be adequate to describe the rate of relaxation for strains in this subregion. However, most normal ligament loading occurs at low strains in daily life making nonlinearities in rate of relaxation and creep physiologically relevant.

Results from group 3 (stress relaxation and creep performed on contralateral ligaments) show stress relaxation to proceed faster than creep by about a factor of about 1.5–2.5, a result similar to the ~2.5 seen by Thornton *et al.*[25] These creep and relaxation behaviors cannot be described by an interrelated QLV model but can be described by interrelating descriptions of Lakes and Vanderby.[17] The work of Thornton *et al.*,[25] and experimental group 3 of this study, are predicted by continuum concepts, which demonstrate that stress relaxation will proceed faster than creep for this type of nonlinear stress–strain behavior (strain-stiffening behavior).[17] This strain-stiffening behavior can be obtained using a fiber recruitment model.[26]

A power law was used to fit our data because it is well suited for linear data points on a log–log plot, which correspond to our data. An exponential curve fit has a sigmoidal shape on a log–log plot which does not correspond as well to our data. One can, of course, model any legitimate relaxation function as an integral of a distribution $H(\tau)$ of exponentials of time constant τ:

$$E(t)-E_e=\int_{-\infty}^{\infty} H(\tau)e^{-t/\tau}d\ln\tau. \tag{4}$$

This is sensible if the underlying causal processes are exponential in nature. However, analysis of this type is more complex and obfuscates our basic point that a separable relaxation function (such as QLV) does not admit strain-dependent differences in the rate of relaxation.

Several limitations must be borne in mind when considering the results of this study. First, strain reported in this study is measured from optical markers that span from origin to insertion on the ligament. This represents average tissue strain and does not reflect regional variations in strain. The zero strain (reference) is taken to be when the ligament is preloaded. The relationship between this preloaded position and the *in situ* reference position requires further elucidation. Second, ligament stress relaxation and creep data were typically gathered over 100 s (groups 1 and 2). Although test times of 100 s may not describe the entire temporal behavior of the tissue, this time scale gives a good indication of initial ligament behavior in the physiologic range and facilitates serial testing on a specimen due to acceptable recovery times (ten times the length of the test). When group 3 specimens were tested for 1000 s the additional decade of test time had little effect on the rates of creep and relaxation, i.e., the power-law trend continued. Third, both the creep and the relaxation data may be affected by the temporal behaviors of the insertions and bone blocks. The independent variable (load or displacement) was controlled on the ligament in both experimental modalities, but the behavior of the structural complex may have entered the dependent variable measurements. That is, grip displacements were used to measure creep and were held fixed for relaxation. Fourth, group 3 only contains eight ligaments (four pairs). However, statistical significance is achieved for this experimental group. Fifth, symmetry was assumed between left and right MCLs in the same animal for group 3. This is supported by published work in which no statistical difference in stiffness, failure force, failure deformation, and the energy absorbed has been reported between contralateral rabbit ACLs.[6] Last, this study relies upon a rat MCL model with a small ligament compared to the human knee. Because of its size, the rat MCL may be more susceptible to load-induced changes in hydration, which would, in turn, alter its viscoelastic response. Fluid present in ligament tissue has been shown to play a role in the mechanical response.[4,7,24] As for tendon, when they are loaded, water content decreases with static and cyclic loading.[10] Chimich *et al.*[7] showed that ligaments with higher water content demonstrated greater relaxation than ligaments with lower water content and stated that water content has a significant effect on viscoelastic behavior. In this regard, similar testing on a larger model is recommended.

The authors speculate that the decrease in relaxation rate with increasing strain could be the result of larger strains causing greater water loss (wringing out effect) which causes the tissue to be more elastic (less viscous) than tissues subjected to lower strains. Chimich *et al.*[7] showed that rabbit MCLs with larger water content showed greater relaxation. Hannafin and Arnoczky[10] reported that as tendons are loaded to 100 g water content decreases with static and cyclic loading, probably due to fluid being driven out of the ligament during loading.[24] In combination these studies show that load influences hydration and that differences in the rate of stress relaxation with strain are due in part to water content. Further study of fluid content under varying levels of strain could add insight into the mechanism by which stress relaxation varies with strain.

Thornton *et al.*[25] speculated that differences in stress relaxation and creep behavior are due to progressive recruitment of collagen fibers during creep[26] and that this microstructural behavior is unlikely to have as significant an effect on stress relaxation as on creep. If this concept is correct, then the progressive recruitment of collagen fibers could also explain the decrease in the rate of creep with increasing load. As larger loads are applied to the ligament more fibers are recruited leaving fewer fibers to be progressively recruited after initial loading and there-

fore decreasing the creep response. Creep behavior may be significant in daily life.[25,27] Load control by muscles is more representative of joint position than displacement control. Such loading may induce creep in ligaments as they help maintain joint position. Moreover, ligaments are subjected to repetitive loads *in vivo*[14] that may have a static component. Stress relaxation may also be important in athletic stretching exercises in which the athlete holds a limb at a prescribed angle for a period of time. Considering the above concepts, examining and attempting to understand the mechanisms of creep behavior may be of equal importance as stress relaxation, although it has received less emphasis in the biomechanical literature. Studying the relationship between creep and stress relaxation at multiple levels is beneficial in understanding the nature of nonlinear viscoelasticity in ligament.

Quasilinear viscoelasticity is a valuable and powerful tool to describe viscoelastic behavior in biologic tissue. It has been successfully used to describe experimental results in many tissues. However, this paper shows that there can be nonlinearities in rates of relaxation or creep which are not admitted in the QLV formulation. Understanding these nonlinearities allows one to better understand the appropriate application of QLV and is essential in order to formulate a robust constitutive representation.

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