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Discrete quasi-linear viscoelastic damping analysis of connective tissues, and the biomechanics of stretching

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Abstract

The time- and frequency-dependent properties of connective tissue define their physiological function, but are notoriously difficult to characterize. Well-established tools such as linear viscoelasticity and the Fung quasi-linear viscoelastic (QLV) model impose forms on responses that can mask true tissue behavior. Here, we applied a more general discrete quasi-linear viscoelastic (DQLV) model to identify the static and dynamic time- and frequency-dependent behavior of rabbit medial collateral ligaments. Unlike the Fung QLV approach, the DQLV approach revealed that energy dissipation is elevated at a loading period of ~10 seconds. The fitting algorithm was applied to the entire loading history on each specimen, enabling accurate estimation of the material's viscoelastic relaxation spectrum from data gathered from transient rather than only steady states. The application of the DQLV method to cyclically loading regimens has broad applicability for the characterization of biological tissues, and the results suggest a mechanistic basis for the stretching regimens most favored by athletic trainers.

Keywords

Relaxation spectrum; storage and loss modulus; stress relaxation; ligament; quasi-linear viscoelasticity; discrete quasi-linear viscoelastic model

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1. Introduction

“Stretching” is commonly performed by athletes for the purpose of optimizing body performance, but the mechanisms, optimal conditions, and efficacy of stretching are sources of debate. Stretching has been shown to enhance range of motion (Murphy et al., 2010), but to either decrease or not affect peak athletic performance (Molacek et al., 2010; Behm & Chaouachi, 2011). The time period and character (ramped, ballistic, sustained) of stretching appear to influence its efficacy, with ballistic stretching apparently less effective than lower frequency dynamic stretching (Hough et al., 2009; Pearce et al., 2009; Unick et al., 2005; Behm & Chaouachi, 2011). The mechanisms underlying these effects are hypothesized to be some combination of connective tissue inelasticity, muscle plasticity, paracrine effects, and neural effects, the latter mediated by the Golgi tendon organ and the muscle spindle stretch receptor, and somewhat less long-lasting in effect (Cuissard et al., 1988; Behm & Chaouachi, 2011). Rate-dependent effects are evident from analysis of muscle-tendon units, but the specific nature of these effects are uncertain because a full nonlinear analysis has not yet been performed, and it is not clear whether energy absorption occurs in connective tissue (Taylor et al., 1990; Magnusson et al., 1998).

Although stretching is nearly standard advice for athletes and is often referred to as stretching ones muscles, no correlation seems to exist between stretching and muscle injury, and neither permanent nor transient changes to muscle associated with stretching are clear (Shrier, 1999). However, some stretching regimes appear to be superior to others, with stretches on the order of tens of seconds (~ 0.1 Hz loading cycles) cited as optimal (Behm & Chaouachi, 2011). We therefore focussed on the energy absorption in connective tissues and tested the hypothesis that connective tissues are particularly responsive to stretching in this regime. The metric we used to evaluate this was viscoelastic damping, with the goal of checking whether connective tissues preferentially absorb or transmit energy during stretching on the order of 0.1 Hz.

To test this hypothesis, we extended existing mathematical, computational, and experimental frameworks to improve the nonlinear viscoelastic characterization responses of such tissues. Connective tissues demonstrate viscoelastic damping due to factors such as syneresis and rearrangement of their fibrous structures, both of which elongate the stress-free state of the tissue (Musahl et al., 2003; Fratzl, 2008; Gautieri et al., 2012; Liu et al., 2014; Swedberg et al., 2014). These factors dissipate energy in ways that are rate-dependent (Nekouzadeh et al., 2007; Gupta et al., 2010), and our scheme therefore enabled testing of our hypothesis by characterizing the rate-dependence of energy dissipation.

While linear viscoelastic models exist for predicting the viscoelastic nature of soft tissues under cyclic loadings such as those associated with stretching (Ferry, 1980), these models are not sufficient to describe the complex, non-linear behavior of soft tissues. Specifically, linear viscoelastic constitutive equations describing the relationships between stress, strain, and strain rate are not sufficient to model all aspects of tissue viscoelastic behavior under different loading patterns and stimuli. The most common extension of these linear viscoelastic constitutive relations is Fung's quasi-linear viscoelastic (QLV) model (Fung et al., 1972), but we show here that this model imposes a certain character to viscoelastic

responses that can mask the frequency dependence of tissue hysteresis. We therefore applied the discrete quasi-linear viscoelastic (DQLV) model (Babaei et al., 2015a) to interpret our experiments. The DQLV model is a general constitutive model, and a theoretical framework was derived for characterizing material behavior of nonlinear biological materials using this model in a specimen subjected to a regimen of sinusoidal loadings.

The focus of our work was the mechanics of connective tissue. To ensure that connective tissue independent of muscle was targeted, the experiments focused on quantifying the time- and frequency-dependent viscoelastic properties of rabbit medial collateral ligaments (MCLs). The model stretching regimen was a combined static-dynamic (ramp-hold-sinusoidal) testing procedure applied over a range of frequencies (from 0.01 to 50 Hz), with three levels of baseline strain levels (1.25%, 2.5% and 5%). This pre-strain was required to ensure that, as in the body, the ligament was never slack during a sinusoidal stretching cycle and to assess the nonlinearity of the response. We applied the framework to identify the DQLV spectra associated with the rabbit MCL, and compared these to linear and QLV fits of the same data. Results supported our hypothesis, and further suggested a mechanical basis for the stretching regimens most favored by athletic trainers.

2. Background

2.1. Quasi-linear viscoelasticity

The rabbit MCLs tested showed rate-dependence and non-linearity. The usual model of choice for quantifying the behavior of such a tissue is Fung's QLV model, in which the stress $\sigma(\epsilon, t)$ at time strain ϵ and time t is obtained by solving the following convolution integral (Fung et al., 1972):

$$\sigma(\epsilon, t) = \int_{-\infty}^t G(t-u) \frac{\partial \sigma^{(e)}}{\partial \epsilon} \frac{\partial \epsilon}{\partial u} du, \quad (2.1)$$

where $\sigma^{(e)}(\epsilon)$ represents the (possibly nonlinear) elastic stress response of the tissue, and $G(t)$ is the relaxation function, normalized so that $\lim_{t \rightarrow 0} G = 1$, and $\lim_{t \rightarrow \infty} G = c$, where c is a constant for a specific material (Figure 1a). For $\sigma^{(e)}(\epsilon)$, Fung proposed the exponential relationship he attributed to Kenedi et al. (1965) (Fung et al., 1972):

$$\sigma^{(e)} = A(e^{B\epsilon} - 1), \quad (2.2)$$

where A and B are material constants. For $G(t)$, Fung proposed the following form:

$$G(t) = \frac{1 + \int_{\tau_1}^{\tau_2} (C/\tau) e^{-t/\tau} d\tau}{1 + \int_{\tau_1}^{\tau_2} (C/\tau) d\tau} \quad (2.3)$$

where C , τ_1 and τ_2 are material constants to be determined experimentally.

Many viscoelastic biomaterials exhibit frequency-dependent dissipation that is not represented well by Equation (2.3) (Pryse et al., 2003; Babaei et al., 2015b, 2016). A more general choice for $G(t)$ is the discrete DQLV form of Equation (2.3) (Babaei et al., 2015a):

$$G(t) = \frac{1 + T \sum_{i=1}^n H_i e^{-t/\tau_i}}{1 + T \sum_{i=1}^n H_i}, \quad (2.4)$$

where τ_i are the n time constants, typically chosen in advance and spaced equidistantly on a temporal logarithmic axis, T is the logarithmic distance between two adjacent time constants on this temporal logarithmic axis (Babaei et al., 2015a), H_i are dimensionless spectrum amplitudes, and n is a number sufficiently large to ensure that the spectrum has converged to a one that varies little with subsequent increases in n (typically, $n=100-1000$). For the case of a uniform spectrum, with all H_i equal, the DQLV model is analogous to the Puso-Weiss (Puso & Weiss, 1998) discrete form of the Fung QLV model.

We note that, the form of Equation 2.4 is analogous to that arising in a Maxwell-Wiechert (“Prony series”) model, several important differences exist to make it suitable for discrete spectral analysis of a QLV material. First, for a Maxwell-Wiechert material, $G(t)$ has the form:

$$G(t) = \frac{A_0 + \sum_{i=1}^n A_i e^{-t/\tau_i}}{A_0 + \sum_{i=1}^n A_i}, \quad (2.5)$$

with time constants A_i that are not normalized by the time constant spacing as a function of the spacing of time constants τ_i . In the absence of this normalization the values of A_i approach zero as the number of trial time constants n is increased. The spacing of time constants is prescribed to be logarithmic in the DQLV spectrum, and the scaling factor T for H_i scales with n , enabling meaningful convergence of the series to a spectral representation that is invariant with respect to the number of trial time constants chosen.

For the common viscoelastic test consisting of a linear ramp in strain followed by a sustained, isometric strain at the ramp's peak strain, a simple analytical solution for Equation (2.1) can often be found. However, for more complex strain profiles, such as the ramp-hold-sinusoidal loading protocol considered here, numerical approaches are preferred. Although when solving Equation (2.1) numerically the choices for $\sigma^{(e)}(\epsilon)$ and $G(t)$ need not be restricted to those that admit simple analytical solutions, simple forms like those described above are often desirable for interpreting fits to experimental data.

2.2. Memory effects in viscoelastic materials

The experimental protocol we applied in this work involved sinusoidal straining of a viscoelastic tissue. A challenge for such a loading is that applying a complete cycle of tension and compression is impractical for connective tissues due to their tendency to buckle. Therefore, application of a sinusoidal loading require that the sample first be placed in tension with a pre-stress greater than the amplitude of the sinusoidal loading. Because the

instantaneous response of a viscoelastic material depends upon the history of loadings it has experienced, the history must be factored into material characterization. To clarify this point, consider a sinusoidal straining of a material:

$$\varepsilon(t) = \varepsilon_0 \sin(\omega t), \quad (2.6)$$

where ε_0 is a defined amplitude and ω is angular frequency. The stress response to this straining of a linear viscoelastic material that was fully relaxed at $t = 0$ (Figure 1b) shows, as expected from (2.1)–(2.4), that $\varepsilon(t = 0) = \sigma(t = 0) = 0$. However, as the sinusoidal response approaches a steady state, the peak stress changes and the stress profile falls behind the strain profile by a phase lag ϕ :

$$\sigma(t) = \sigma_0 \sin(\omega t + \phi), \quad (2.7)$$

where σ_0 is the stress amplitude. A central focus of the current work is therefore taking proper account of strain history when interpreting experimental data.

2.3. Complex modulus of a Fung QLV material

The viscoelastic response of a linear viscoelastic material is often represented by a complex modulus $G^*(\omega)$, defined by a one-sided Fourier transform (Kamath & Mackley, 1989):

$$G^*(\omega) = j\omega \int_0^\infty G(u) e^{-j\omega u} du = G'(\omega) + jG''(\omega), \quad (2.8)$$

where $j = \sqrt{-1}$ and $G'(\omega)$ and $G''(\omega)$ are the storage and loss moduli, respectively. The storage modulus represents the material response of the elastic portion (corresponding to the stored energy), and the loss modulus represents the viscous portion (corresponding the dissipated energy).

For the reduced relaxation function of Equation (2.3), the normalized storage and loss moduli are (Fung et al., 1972):

$$G' = \frac{1 + \frac{C}{2} [\ln(1 + \omega^2 \tau_2^2) - \ln(1 + \omega^2 \tau_1^2)]}{1 + C \ln(\tau_2/\tau_1)} \quad \text{and} \quad G'' = \frac{C [\tan^{-1}(\omega \tau_2) - \tan^{-1}(\omega \tau_1)]}{1 + C \ln(\tau_2/\tau_1)}. \quad (2.9)$$

3. Materials and methods

3.1. Characterization of MCL relaxation and damping responses

MCLs were dissected and cut free at the insertion sites of $N=6$ rabbit knees. The geometry was standardized by cutting the ligaments into a dog-bone shape with a length-to-width ratio of 6.8 ± 0.8 (width 1.6 ± 0.2 mm). The tissue samples were affixed to custom-made soft tissue clamps, and the cross-sectional area was determined with a laser micrometer system (1.0 ± 0.3 mm²). Measurements were taken at three locations along the length of the tissue

sample and averaged for stress calculations. Then, the tissue sample-clamp construct was mounted on an Enduratec Elf 3200 testing machine (Bose, Framingham, MA). Reflective markers were placed on the tissue sample to track strain using a Motion Analysis Corporation strain tracking system (Santa Rosa, CA). A heated saline drip was utilized to hydrate the tissue sample and maintain temperature at a constant 37°C. The tissue sample was aligned along the loading axis using an $x - y$ table and then loaded with five preconditioning triangle waves of strain at 50 Hz and 5% strain amplitude. Thereafter, it was left unloaded for 30 min to acclimate. A 0.5 MPa pre-stress was placed on the tissue sample and this position served as the starting point for all subsequent tests.

The subsequent testing protocol (Figures 3 and 2) began with a ramp to a baseline level of strain ($0 \rightarrow t_p \rightarrow t_p = 9.2 \text{ s}$) followed by an isometric hold of 2100 s ($t_p \rightarrow t_f \rightarrow t_f = 2100 \text{ s}$) at this baseline strain to enable the specimen to reach a steady state stress. Specimens were elongated to three different levels of baseline strain (1.25%, 2.5%, or 5%). Afterwards, the baseline strain level was maintained, and each specimen was subjected to sinusoidal perturbations in strain (amplitude 0.25% strain) at frequencies of $(\omega/2\pi) = \{0.01 \text{ Hz}, 0.1 \text{ Hz}, 1 \text{ Hz}, 10 \text{ Hz}, 25 \text{ Hz}, 50 \text{ Hz}\}$. This protocol was repeated for each baseline strain level with 1 hour of recovery between tests at each baseline strain level (Figure 2). In addition, the order of testing was randomized for strain level. For each frequency, 10 cycles of stress and strain versus time data were collected following the first 10 cycles of loading and unloading.

3.2. Numerical fitting algorithm

The DQLV and QLV models were applied to fit the entire loading histories applied to each specimen, including the ramp, the hold/relaxation, the sinusoidal oscillations, and all of the unintended features of the loading history such as minor overshoot by the control system. As discussed below, this enabled analysis of the transition between the first sinusoidal loading and the steady state, obviating the need to achieve steady state before fitting. A simple regularizing criterion was used to penalize unwanted states and to ensure that the fitting algorithm did not become trapped in local minima. In the absence of this regularization, optimizations during inverse fitting of spectra are prone to become trapped in local minima, yielding irreproducible estimates (Provencher, 1982). For the DQLV model, H_i were regularized and identified by minimizing:

$$MSE = \sum_{k=1}^m \left(\sigma_k^{(model)} - \sigma_k^{(exp)} \right)^2 + \alpha \sum_{i=1}^{n-1} (H_i - H_{i+1})^2, \quad (3.1)$$

where m is the number of data points, $\sigma_k^{(exp)}$ are known stress data or a calculable relationship, α is a regulating factor and $\sigma_k^{(model)}$ is the model estimate of $\sigma_k^{(exp)}$. For the QLV approach, $\alpha = 0$ and $H_i = C$.

As shown in Figure 2, the strain profiles were treated as:

$$\varepsilon(\omega_l, t_k) = \begin{cases} \gamma t_k & k=0, 1, 2, \dots, p \\ \gamma t_p & k=p+1, p+2, \dots, f \\ \gamma t_p + \varepsilon_0 \sin(\omega_l t_k) & k=f+1, f+2, \dots, q \end{cases} \quad (3.2)$$

where p , f and q , respectively, represent the time when the ramp, hold and sinusoidal loading phases are ended, and $(\omega/2\pi) = \{.01 \text{ Hz}, 0.1 \text{ Hz}, 1 \text{ Hz}, 10 \text{ Hz}, 25 \text{ Hz}, 50 \text{ Hz}\}$ represents the frequency of sinusoidal loading.

The parameters were fit to experimental data using the direct downhill simplex method of Nelder & Mead (1965), which avoided the need for estimating numerical gradients. Data were fit using the Matlab (The MathWorks, Natick, MA) environment and the Matlab implementation of the Nelder-Mead algorithm.

3.3. Complex modulus of a DQLV material

Results are presented in terms of complex moduli. After the terms H_i were fit, normalized storage and loss moduli for the DQLV fitting were obtained by substituting Equation (2.5) into Equation (2.8):

$$G'(\omega) = \frac{1 + T \sum_{i=1}^n H_i \frac{\omega^2 \tau_i^2}{1 + \omega^2 \tau_i^2}}{1 + T \sum_{i=1}^n H_i} \quad \text{and} \quad G''(\omega) = \frac{T \sum_{i=1}^n H_i \frac{\omega \tau_i}{1 + \omega^2 \tau_i^2}}{1 + T \sum_{i=1}^n H_i}. \quad (3.3)$$

As is evident from Equation (3.3), each term H_i contributes to both the storage and loss moduli at all frequencies. At relatively high frequencies ($\omega \tau_i \gg 1$), a particular H_i will make a larger contribution to the storage modulus than to the loss modulus.

3.4. Statistics

Multiple factor ANOVA was conducted to assess whether damping was frequency dependent. Responses at all frequencies and strain levels were compared. A false discovery rate of 0.05 was assigned for statistical significance (McDonald, 2009).

4. Results

The stress-relaxation data for rabbit MCLs at the three baseline strain levels of 1.25%, 2.5%, and 5% exhibited a characteristic non-linear rise during stretching, then a gradual decrease to a plateau by ~1500 s (Figure 3(a)). The responses exhibited two hallmarks of non-linear viscoelastic behavior. First, whereas linear viscoelastic models such as the standard linear solid model (Findley & Davis, 2013) have linear dashpots that relax the linear proportionality of stress and strain to produce concave-down stress-strain curves, the stress-strain curves for the MCLs were concave up (Figure 3). Second, whereas linear viscoelasticity requires that stress levels be proportional to strain levels, the stress peaks were 1.5 MPa, 4.2 MPa and 12.7 MPa, for slow ramps up to 1.25%, 2.5% and 5% strain,

respectively. When normalized versus peak stress, the relaxation curves did not quite collapse to a single master curve (Figure 3).

The DQLV approach was applied to fit ramp-hold-sinusoidal experimental data at the three baseline strain levels using equations (3.1) and (3.2). The DQLV spectra that approximated the time-dependent properties of the MCLs at 1.25% and 2.5% baseline strain showed four peaks at about $\tau = 0.002$ s, 0.2 s, 20 s and 1000 – 2000 s (Figure 4a). These peaks shifted slightly with straining, so that at 5% the baseline strain they appeared at about $\tau = 0.001$ s, 0.02 s, 20 s and 1000 s (Figure 4a).

The QLV approach was also used to fit these data, but the tissue was clearly not a perfect Fung QLV material (Figure 4b). The upper estimated time constants (τ_2) were fairly consistent at all three levels of baseline strain, but the damping coefficients, C (plateau level in Figure 4b), decreased with strain, and the lower estimated time constants (τ_1) varied non monotonically (Figure 4b).

To achieve the DQLV fits shown in Figure 4, a careful convergence study had to be performed to ensure that a sufficient number n of prospective time constants had been considered. Too few time constants led to a misrepresentation of the spectrum ($n=10$, Figure 5). The nature of the spectrum was captured by $n=30$ time constants. However, further refinement was needed to ensure that the character of the spectrum did not change with further refinement; in this case, the refinement was stopped with $n=100$ time constants. Note that, as described in the discussion, additional time constants might be needed for application of the spectrum in finite element analyses. The mean standard error from the Nelder-Mead algorithm converged after approximately 10 iterations in a typical analysis (Figure 5(b)).

The elastic parameter A and non-linearity parameter B for each strain level, estimated using equation (2.5), were similar for QLV and DQLV estimates (Tables 1 and 2). However, the DQLV fits matched the cyclic strain data more closely than the Fung QLV fits, with the differences most pronounced at the 1 Hz loading (Figure 6).

As represented in equations (2.9) and (3.3), the normalized storage and loss moduli depend on ω and the relaxation spectra (including τ_i , T and H_i). For the DQLV model, the frequency-dependent storage and loss moduli for MCLs at all three baseline strains increased monotonically with respect to frequency from $\omega/2\pi=0.01$ Hz to $\omega/2\pi=50$ Hz (Figure 7, the right column). The loss moduli, however, showed a substantial and statistically significant increase in damping at a frequency of $\omega/2\pi=0.1$ Hz (Figure 7, the left column): multi-factor ANOVA tests revealed that the damping response at 0.1 Hz differed substantially from the responses at all other frequencies, and that the responses at all other frequencies were not significantly different. The QLV approach predicted a similar monotonic increase in storage modulus (Figure 8), although for 1.25% baseline strain data, the modulus estimate was about 25% lower than for the 2.5 and 5% baseline strains. The QLV approach predicted a frequency-independent loss modulus at each strain level.

5. Discussion

Based on QLV theory, the time-dependent and elastic responses of a viscoelastic material are separable, with nonlinearity arising only from the nonlinear elastic nature of biomaterials. The relaxation functions in QLV theory are purely time-dependent. This serves as the workhorse for modeling of viscoelastic biological materials, and is effective in many ways, especially compared to linear viscoelasticity. For example, the definition of complex modulus based upon linear viscoelasticity permits no strain dependence for the frequency response spectrum of a material, and is therefore inappropriate for nearly all biological materials.

However, although it provides a strong foundation, the Fung QLV model is not adequate for identifying the frequency-dependent hysteretic behavior of the MCL at different strain-levels (Figure 4b). The Fung QLV model predicts a constant loss modulus over a wide range of frequencies; that is, it predicts that the hysteresis of a quasi-linear viscoelastic materials is frequency-independent (Fung et al., 1972). This is contradicted by many experiments (Iatridis et al., 1997; Hatami-Marbini & Rahimi, 2015; Lujan et al., 2009; Anderson et al., 1991). As shown in Figure 4d, the QLV approach predicts a frequency-independent hysteresis for MCLs as well. This observation is consistent with a well-known series of papers on nonlinear viscoelasticity of ligaments (Provenzano et al., 2001, 2002; Hingorani et al., 2004) that definitively characterized the inability of the Fung QLV model to capture nonlinear viscoelastic ligament behavior. These articles showed a strain-dependence of stress relaxation that is inconsistent with the Fung QLV model: at lower strains (below approximately 2%), the exponent of the power law that described the rate of stress relaxation was a strong function of strain. Similarly, in the current work, we observed that the normalized stress relaxation data do not collapse onto a single master curve, as would be required for rigorous application of the Fung QLV model. The viscoelastic behavior of the rabbit ligament was clearly strain-dependent (Figure 3). Fung's QLV theory requires that the time-dependent and strain-dependent behaviors of a material be separable. This is not the case, but these are far closer to being separable when the normalized DQLV model was applied. Like (Provenzano et al., 2001), we observed that relaxation rates become more similar at higher strains: the relaxation spectra for 2.5% and 5% baseline strains were closer to each other at the faster time constants than to the 1.25% baseline strain relaxation spectrum.

The DQLV approach provides a partial resolution to this problem. It identified robust, temporal relaxation spectra (Figure 4a) and complex moduli (Figures 4a and b) from ramp-hold-sinusoidal data, including transient data, that were, while strain dependent, much closer to one another than were the Fung QLV spectra. The more refined relaxation spectra led to substantially improved predictions of the stress versus time responses of the specimens at each strain level (Figure 6). Note that the degree of refinement of the spectrum of n time constants considered may need to be increased beyond what is needed for reproducing hysteresis in applications such as finite element analyses (Puso & Weiss, 1998). Practical considerations in such cases can require either the use of more time constants in DQLV fitting, or the interpolation of additional time constants onto a DQLV spectrum. In all cases, the procedures we applied, which involved fitting to the entire loading history, had the distinct advantage of enabling fitting to transient rather than just steady state data.

Although the DQLV model is better than the Fung QLV model in predicting relaxation spectra, it is, in the implementation used here, identical to the Fung QLV model in estimating elastic parameters (Table 1). This is because we used exactly the exponential relationship that Fung applied, with all of its strengths and limitations. Although suggesting a new elastic relationship was beyond the scope of this paper's focus, finding an elastic stress-strain relationship is warranted for ligaments.

The ramp-hold-sinusoidal protocol analyzed here provided a fuller picture of the relaxation spectra of rabbit MCLs than is available through other means. In an analysis of stress-relaxation data alone for rabbit MCLs presented elsewhere (Babaei et al., 2015a), only a portion of the time-dependent material properties were accessible, (over the range of $10^0 < \tau < 10^4$). Here, we showed that by applying high frequency sinusoidal loading, the fast time constants of MCLs can be estimated as well. In other words, high frequency loading excited very fast time constants, which were not detectable in a simple stress-relaxation test.

From the perspective of material damping, the DQLV interpretation of the data revealed an interesting increase in damping at a loading rate of 0.1 Hz. This frequency-dependence of the MCL's mechanical response is not evident in the QLV interpretation, again because the QLV model by its nature enforces a prediction of frequency-independent hysteresis. All three loss modulus ($G''(\omega)$) curves collapse onto a single curve; note that the strain energy loss per cycle is linearly proportional to $\langle G''(\omega) \rangle$.

Although stretching protocols prior to exercise typically target muscles and tendons rather than ligaments (Behm & Chaouachi, 2011), this result is interesting in that it shows increased energy absorption of a collagenous tissue at a critical loading rate of 10s of seconds, which is in the range of loading times recommended for stretching. For example, Bandy et al. (1997) showed that the optimal stretching period for increasing hamstring flexibility is a 30 s cycle. Although a great many hypotheses exist about what stretching accomplishes physiologically for those who subscribe to the hypothesis that the rearrangement of collagenous tissues is a beneficial aspect of stretching, the data presented here suggest that loading over 10s of seconds appears to be optimum for applying energy inelastically to such tissues.

6. Conclusions

The DQLV approach, when applied to the entire history of ramp-hold-sinusoidal data for a specimen, provided a robust spectral characterization of rabbit MCL tissues that enabled more accurate prediction of mechanical response than the QLV approach. Data showed for the first time that the rabbit MCL damped energy most effectively over 10 second loading cycles. These results have implications for stretching protocols prior to athletic events. More broadly, the protocol presented provides a straightforward mechanism for characterizing the high frequency responses of biological tissues, and for overcoming limitations of the Fung QLV model when studying the frequency-dependence of hysteresis.

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Figure 1.

Relaxation function, input strain and stress response of a viscoelastic material under sinusoidal excitation using a simulated experiment. (a) Relaxation function $G(t)$, which is normalized so that $G(0) = 1$. The rate of relaxation to a constant c depends on the viscoelastic response of a material. (b) In response to a sinusoidal straining, the stress response requires several cycles to reach a steady state amplitude and phase delay with respect to the strain.

Figure 2.

Specimens were elongated to one of three baseline strain levels {1.25%, 2.5%, 5%} over an interval of $t_p=9.2$ s and were then held isometrically for 2100 s as specimens relaxed to a steady state at time t_f cycles at each of six frequencies (0.01, 0.1, 1, 10, 25, and 50 Hz). The sequence of frequencies was randomized.

Figure 3.

(a) Representative data for force relaxation during application of baseline strain. The DQLV fits shown represent the parameter that best fit not only the data for ramp loading and relaxation, but also the subsequent sinusoidal oscillation of the specimens. (b) When normalized versus peak stress, the relaxation curves did not quite collapse to a single master curve.

Figure 4.

Representative DQLV and Fung QLV relaxation spectra of a rabbit MCL specimen (replicate data in the Supplementary document). (a) The DQLV spectra were similar at all three strain levels, a key requirement for application of quasi-linear theory. The spectra showed four dominant peaks (around $\tau = 0.002$ s; 0.2 s; 20 s and $1000 - 2000$ s), which shifted only slightly as a function of strain. (b) The Fung box spectra overstated minor variations of the spectrum with respect to strain, and were not strain independent.

Figure 5.

Convergence studies. (a) Results of a convergence study performed to ensure that a sufficient number n of prospective time constants had been considered. (b) The mean standard error from the Nelder-Mead algorithm converged after approximately 10 iterations in a typical analysis.

Figure 6.

Data for the final sinusoidal oscillation of a specimen at each of the six frequencies, with DQLV and QLV fits. The DQLV fit captured the small differences between loading and unloading responses better than the QLV fit.

Figure 7.

Storage and loss moduli estimates from the DQLV fitting of the data, as a function of frequency, for all three strain levels. Right column: Storage moduli estimated using the DQLV model increased with loading frequency, and did not change significantly as a function of strain. As load increased, more energy was stored in rabbit MCL. Left column: Loss moduli estimated using the DQLV model were highest at $\omega/2\pi=0.1$ Hz for all three baseline strain levels, indicating that inelastic energy absorption was highest for stretching on the order of tens of seconds.

Figure 8.

Storage and loss moduli obtained using the Fung QLV fittings of the data. Right column: Storage moduli estimated using the Fung QLV model were similar to those estimated using the DQLV model. Left column: Loss moduli estimated using the Fung QLV model were independent of strain and frequency.

Table 1

Estimated elastic parameters using the DQLV model

	1.25 % strain	2.5 % strain	5% strain
A(MPa)	1.505 ± 0.26	2.17 ± 0.31	10.43 ± 1.71
B	56.57 ± 5.4	43.57 ± 7.8	18.22 ± 3.3

Table 2

Estimated elastic parameters using the Fung QLV model

	1.25 % strain	2.5 % strain	5% strain
A(MPa)	1.395 ± 0.21	2.31 ± 0.29	11.03 ± 1.89
B	42.62 ± 8.18	37.59 ± 5.81	20.42 ± 8.43