Required test duration for group comparisons in ligament viscoelasticity: A statistical approach

Eugene Manley, Jr. [a,b], Paolo P. Provenzano [a,b], Dennis Heisey [c], Roderic Lakes [b,d]
and Ray Vanderby, Jr. [a,b,d,*]

[a] *Department of Orthopedics and Rehabilitation, University of Wisconsin, Madison, WI, USA*

[b] *Department of Biomedical Engineering, University of Wisconsin, Madison, WI, USA*

[c] *Department of Surgery, University of Wisconsin, Madison, WI, USA*

[d] *Department of Engineering Physics, University of Wisconsin, Madison, WI, USA*

Abstract. The goal of this study was to determine the duration of time that ligaments from a study group need to be loaded in order to adequately determine their collective viscoelastic behavior. Rat ligaments were subjected either to creep or stress relaxation for 1,000 s or stress relaxation for 10,000 s to compare estimates of viscoelastic behavior for different test durations. Stresses versus time (relaxation) or strains versus time (creep) were fit with power law models (t^β where β is the rate of creep or relaxation on a log–log scale). Time intervals were separated by logarithmic decade and analyzed using a Random Coefficients approach to compute residual specimen error as a function of the number of decades of data analyzed. Standard Regression was also used for comparison. Results show that by testing for $\leqslant 100$ s (i.e. two logarithmic decades of time) offers 1% less accuracy than testing for 1,000 seconds (i.e. three decades) when estimating the viscoelastic behavior of a specimen. These 100 s power law estimates are far more accurate than the between specimen dispersion of viscoelastic properties. Hence, a better way to compare viscoelastic behavior between study groups is to test more specimens for shorter durations. This reduces experimental time per sample and therefore increases efficiency.

Keywords: Stress relaxation, creep, ligament, random coefficients, standard regression

1. Introduction

Ligaments display viscoelastic behavior, i.e. they have time-dependent and load-history-dependent mechanical behavior. These viscoelastic properties change with age, pathology, damage, healing, etc.; such changes are of functional interest. Many authors have previously studied viscoelasticity in ligaments [7,19,20] and tendons [2,4,6,8,9], including human [9,12], goat [21], rabbit [17,18], swine [21], and rat [14]. The viscoelastic properties of ligaments are useful to understanding normal and abnormal tissue behavior and to aid in the development of graft material that can be used to replace damaged ligament or tendon.

---

*Address for correspondence: Ray Vanderby, Jr., Orthopedic Research Laboratories, 600 Highland Ave, G5/332, University of Wisconsin, Madison, WI 53792-3228, USA. Tel.: +1 608 263 9593; Fax: +1 608 265 9144; E-mail: vanderby@surgery.wisc.edu.

Although numerous studies have examined viscoelastic behavior, a consensus on the testing time required to adequately describe viscoelastic response has not been reached. Hence, a large range of test times (from 100 s to 24 hr) have been applied to ligaments and tendons (Table 1). Table 1 includes three studies that examined viscoelastic effects in ligament over extended time durations [6,10,20]. Woo [20] subjected ligaments to cyclic stretching followed by static stress relaxation for 1,000 minutes and data were fit with the quasi-linear viscoelastic formulation [5]. In a study by King et al. [10], ligaments were cycled in stress relaxation from joint compression to 2 N tension at a frequency of one cycle per minute for 18 hours. They show that during long term cyclic testing, ligaments continue to stress relax and do not achieve a steady-state. Hannafin and Arnockzy [6] subjected canine flexor digitorum tendon to either no load, cyclic load or static tensile loads for times ranging from 5 minutes to 24 hours to look at the effects of testing on water content. They report increased water loss with increased testing time for all samples. Studies such as these are necessary to define fundamental behavior, but they are too time intensive to be used extensively for efficient group comparisons. Frequently, only pseudoelastic comparisons are made to circumvent this issue. If viscoelastic properties could be gained more efficiently, they would be considered more frequently in biomechanical studies.

Our goal, is to determine how long ligaments must be tested in order to effectively describe the viscoelastic behavior in an experimental group. This will allow for more efficient study protocols. To accomplish our goal, we test rat medial collateral ligaments in creep and stress relaxation and then apply a Random Coefficients model for various durations of test data from each specimen. The Random Co-

Table 1

Comparison of different viscoelastic testing durations for ligament and tendon by various researchers. The range of testing time varies widely from 100 seconds to as long as 24 hours

Author(s)	Species	Specimen	Type of testing	Test time
Haut and Little, 1972 [8]	Rat	Tail tendon	Stress relaxation	180 sec
Cohen et al., 1976 [2]	Human	Flexor digitorum tendon	Creep	100 sec
Woo, 1982 [20]	Porcine & canine	Digital extension and flexor tendons MCL	Stress relaxation	1000 min
Monléon and Diaz, 1990 [12]	Human	Flexor tendon	Creep	1000 sec
Johnson et al., 1994 [9]	Human	Patellar tendon	Stress relaxation	900 sec
Hannafin and Arnockzy, 1994 [6]	Canine	Flexor digitorum profundus tendon	Creep	5 min–24 hours
Thornton et al., 1997 [17]	Lapine	MCL	Creep and stress relaxation	1200 sec
Atkinson et al., 1999 [1]	Human	Patellar tendon	Stress relaxation	180 sec
King et al., 2000 [10]	Lapine	MCL	Stress relaxation	18 hours
Panjabi et al., 2000 [13]	Lapine	ACL	Stress relaxation	180 sec
Provenzano et al., 2001 [14]	Rat	MCL	Creep and stress relaxation	100 and 1000 sec
Wallace et al., 2001 [18]	Lapine	MCL	Creep	1200 sec

efficients model allows us to establish a protocol with minimal testing duration that adequately defines viscoelastic behavior for a study group.

2. Methods

This study was approved by the Institutional Animal Use and Care Committee and adheres to N.I.H. guidelines for animal welfare. Medial collateral ligaments (MCLs) from male Sprague–Dawley rats (300 ± 50 g) were used as an animal tissue model. Eight ligament specimens were tested for 1,000 seconds of creep, eight were tested for 1,000 seconds of stress relaxation, and six additional specimens were tested for 10,000 seconds of stress relaxation. Tests for 1,000 seconds provided 3 logarithmic temporal decades of data and 10,000 seconds provided 4 decades. For stress relaxation, all 14 samples were pooled for the first 3 decades, whereas only 6 were able to provide 4 temporal decades of data.

Specimen harvesting and testing were adopted from previously described methods [14] and are briefly summarized herein. The MCLs with intact femoral and tibial bone blocks were obtained by careful dissection of the knee and removal of other tissues and other ligaments without disturbing the MCL, leaving a femur–MCL–tibia complex. Tissues were kept hydrated in phosphate buffered saline (1X) and MCL area was measured optically. The femur–MCL–tibia bone complex was loaded axially in a custom designed system starting from an anatomical position that loads the tissue as uniformly as possible. The tibial and femoral insertions of the ligament were marked to allow optical measurement of tissue strain. During testing, tissue elongation was obtained by using video dimensional analysis to measure the change in distance of the centroid of each optical marker. Force and time were measured, recorded, and synchronized with displacement data using video analysis and Labtech Notebook data acquisition software (Laboratory Technologies Corp., Wilmington, MA). A preload of 0.1 N was applied to all tissues to define a uniform starting point. The original (gage) length was taken to be the preloaded state. After preload the tissue was pulled in either displacement or load control to a level below the damage threshold (∼5% strain) for rat MCL [15]. The rise time was 0.32 seconds. This 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. Engineering strain was calculated from initial length and displacement data, and force and area measurements were used to calculate engineering stress. Data from each specimen were plotted and fit with a power law of the form At^β [11,16] to model the physical behavior, with t being time and β being either the rate of stress relaxation or creep.

Statistical analyses (Random Coefficients method [3]) were performed to determine an adequate time frame for testing ligaments in both creep and relaxation in order to accurately predict ligament behavior. Results from the Random Coefficients method were also compared to the more traditional Standard Regression approach. For each specimen, data were divided into intervals that were logarithmic decades of time and analyzed with each of the above approaches. We used SAS PROC MIXED statistical software (SAS Institute Inc., Cary NC) to perform the analyses described in this paper.

The salient differences in the two statistical approaches and the assumptions implicit in our methodology are key to understanding and interpreting our methodology and as such they will be discussed herein. In a statistical sense, whenever a measurement of a material property is taken from a sample, it reflects the contribution of three additive components: the mean material property (M), the individual increment (or decrement) in the material property (I), and measurement error (E). Hence, an individual measurement can be represented as $M + I + E$. The mean material property, M, is usually of importance while the individual increment, I, is generally of little interest because that sample will never be observed

again. In statistical terminology the individual increment (I) is a random effect. However, the variance of the individual increment, $V(I)$, is of great interest because it describes the variability observed in the material property between individuals and hence the expected variability in material behavior. That is, $V(I)$ is itself a material property. In the case of our experiment, $V(I)$ is the between specimen variance of either the creep or stress relaxation behavior. The variance of the measurement error, $V(E)$, is important because it determines how accurately the material properties M and $V(I)$ are estimated, but it is not a material property. Unlike M and $V(I)$, it has no bearing on the physical behavior of a material. It is common practice to estimate a material property from an individual specimen with, for example, regression analysis and then average such estimates over the specimens to obtain an estimate of M. Frequently, the standard deviation is obtained as well. However, the interpretation of the standard deviation is unclear because it combines $V(I)$, a material property, and $V(E)$, a measurement accuracy property.

The distinction between the Standard Regression approach and the Random Coefficients model is illustrated using a creep experiment. Let $\varepsilon_i(t)$ represent the observed strain for the ith experimental unit at time t. Our previous work [14] shows that strain versus time data in rat MCLs subjected to a creep loading can be fit well with a power law of the form $\varepsilon = At^\beta$, where A and β are specimen-specific constants with β representing the rate of creep. Because we have chosen this law to represent material behavior, the variation from this law represents experimental error. The log transformed law for the ith experimental unit is

$$\log_e \varepsilon_i(t) = \alpha_i + \beta_i \log_e(t) \tag{1}$$

with $\alpha_i = \log_e(A)$. The residual portion of the dependent variable (strain) that cannot be explained by the law in Eq. (1) is defined by e. Hence, the actual value of a datum at time t is given by

$$\log_e \varepsilon_i(t) = \alpha_i + \beta_i \log_e(t) + e. \tag{2}$$

The residual, e, is assumed to be the normally distributed random error about the specimen-specific constants of α_i and β_i. Analogously, the statistical model for stress relaxation is

$$\log_e \sigma_i(t) = \alpha_i + \beta_i \log_e(t) + e. \tag{3}$$

Traditionally, the mean of the β_i's receives the most attention, but the variance of the β_i's is also very important because it tells us the variability to expect in the material property of interest from one specimen to the next. Indeed, the variance of β_i, $V(\beta_i)$, is itself an important material property. Using a Standard Regression approach the estimate $V(\beta_i)$ is upwardly biased for the following reasons. The rate parameter calculated for each specimen, β_i, because of random measurement error, is only an estimate of the true β_i. Thus, the variance of the estimates of the material property, $V(\beta_i)$, over-estimates the true variance of the material property $V(\beta_i)$. The extent of this over-estimation depends on the estimation error involved in estimating β_i, which depends on the variance of the random error e. The Random Coefficients approach addresses this problem by using a generalized model that acknowledges that both β_i and e are random variables. The Standard Regression approach by comparison assumes that the β_i's are fixed constants but that values of e are normally distributed random variables with mean zero and variance $V(e)$. In contrast, the Random Coefficients model makes the same assumption about e, but in addition assumes that the β_i's are normally distributed random variables with mean $E[\beta_i]$ and variance $V[\beta_i]$. In a Random Coefficients analysis, all data are used simultaneously to obtain estimates of $E[\beta_i]$, $V[\beta_i]$ and $V(e)$. Random Coefficients models belong to a class of models known as random effects or mixed models.

3. Results

All creep and relaxation data are well-fit by straight lines on a log–log scale and therefore physical behavior is described well with a power law (At^β). The R^2 values are all >0.93. A representative curve of a 1,000 s stress relaxation test (Fig. 1) is fit with an R^2 of 0.9949. A representative curve of a 1000 s creep test (Fig. 2) is fit with an R^2 of 0.9703. A representative curve of a 10,000 s test (Fig. 3) is fit with an R^2 of 0.9811.
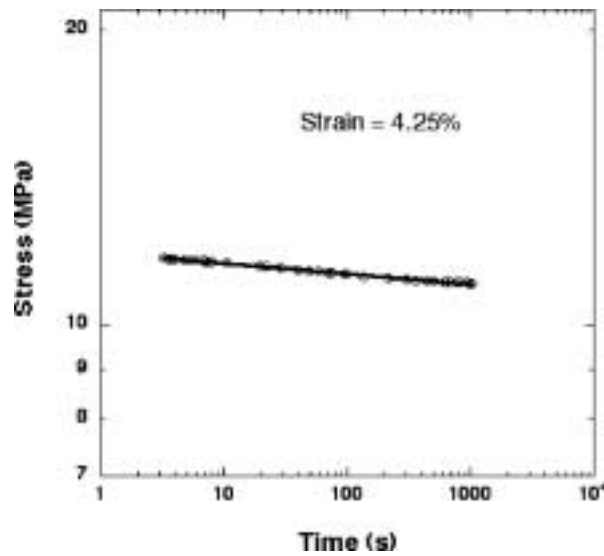

Fig. 1. Typical stress relaxation for 1,000 seconds. The data were fit with the power law At^β, with β as the rate of stress relaxation ($\sigma = 11.85t^{-0.0108}$). The strain was 4.25% and the rate of stress relaxation was -0.0108 with an R^2 of 0.9949.
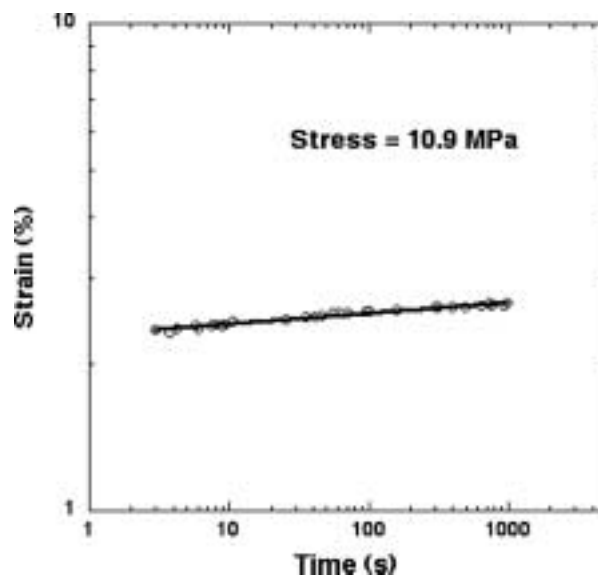

Fig. 2. Typical creep test for 1,000 seconds. The data were fit with the power law At^β, with β being the rate of creep ($\varepsilon = 2.30t^{0.022}$). The stress was 10.9 MPa the rate of creep was 0.022 with an R^2 of 0.9703.
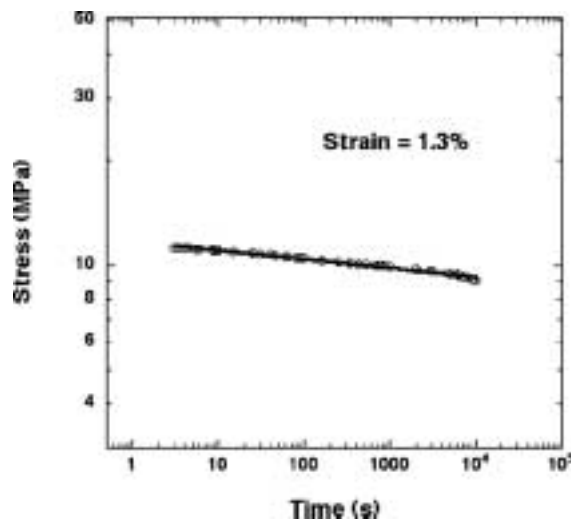
Fig. 3. Typical stress relaxation test for 10,000 seconds. The data were fit with the power law At^β, with β as the rate of stress relaxation ($\sigma = 11.701t^{-0.0254}$). The strain was 1.3% and the rate of stress relaxation was -0.0254 with an R^2 of 0.9811.

Table 2

Standard Regression (columns 2 and 3) and Random Coefficients (columns 4–7) analysis of creep data over 3 logarithmic decades (1–1,000 sec) to estimate rate of creep based upon a power law model

Time (s)	Mean[1]	SD[2]	$E[M]$[3]	SD(I)[4]	SD($E[M]$)[5]	CV[6]
<10	0.031	0.019	0.031	0.017	0.006	19%
<100	0.032	0.012	0.032	0.012	0.004	13%
<1000	0.027	0.010	0.027	0.010	0.003	12%

[1]Mean rate of creep from Standard Regression coefficients for each sample;
[2]Standard deviation of Standard Regression coefficients;
[3]Random Coefficients estimate of material property (rate of creep);
[4]Random Coefficients estimate of standard deviation of rates of creep;
[5]Standard error of the estimated rate of creep: $E[M]$;
[6]Percent coefficient of variation: $100 \times$ SD($E[M]$)$/E[M]$. Note, a decrease in the last column indicates an increase in accuracy, therefore, testing for 1,000 seconds only increased the accuracy obtained by testing for 100 seconds by 1%.

The properties associated with the rates of creep or relaxation are listed in Tables 2, 3 and 4. Column 1 indicates the time over which data are analyzed. One logarithmic decade of time is indicated by <10 s, two logarithmic decades of time are indicated by <100 s, etc. Columns 2 and 3 are results based on the Standard Regression approach. Columns 4–7 represent the Random Coefficients approach. Column 2 is simply the mean of the rates of creep (Table 2) and stress relaxation (Tables 3 and 4) for all specimens, and column 3 is their standard deviation. Column 4 ($E[M]$) is the Random Coefficients mean of the desired material property (rate of stress relaxation or creep). In statistical terms, $E[M]$ is the estimate of the expectation of the rate parameters β_i. Column 5, SD(I), is the variability in true material property from individual to individual. It is an estimate of the standard deviation of the rate parameters, that is, it estimates the square root of $V(\beta_i)$. For example, SD(I) is an estimate of the variation between individual MCLs. Column 6, SD($E[M]$) is a measure of confidence in the estimate of the rate of creep or stress relaxation. Column 7 is the percent coefficient of variation (CV) or relative error, $100 \times$ SD($E[M]$)$/E[M]$.

Table 3

Standard Regression (columns 2 and 3) and Random Coefficients (columns 4–7) analysis of the pooled stress relaxation data ($n = 14$ ligaments) over 3 logarithmic decades (1–1,000 sec) to estimate the rate of stress relaxation based upon a power law model

Time (s)	Mean[1]	SD[2]	$E[M]$[3]	SD(I)[4]	SD($E[M]$)[5]	CV[6]
<10	−0.029	0.019	−0.029	0.019	0.005	18%
<100	−0.033	0.019	−0.033	0.019	0.005	15%
<1000	−0.032	0.017	−0.032	0.017	0.004	14%
<10000	−0.033	0.018	−0.033	0.017	0.005	14%

[1]Mean rate of stress relaxation from Standard Regression coefficients for each sample;
[2]Standard deviation of Standard Regression coefficients;
[3]Random Coefficients estimate of material property (rate of stress relaxation);
[4]Random Coefficients estimate of standard deviation of rates of stress relaxation;
[5]Standard error of the estimated rate of stress relaxation: $E[M]$;
[6]Percent coefficient of variation: $100 \times$ SD($E[M]$)/$E[M]$. Note, a decrease in the last column indicates an increase in accuracy, therefore, testing for 1,000 seconds only increased the accuracy obtained by testing for 100 seconds by 1%.

Table 4

Standard Regression (columns 2 and 3) and Random Coefficients (columns 4–7) analysis of stress relaxation data ($n = 6$ ligaments) over 4 logarithmic decades (1–10,000 sec) to estimate the rate of stress relaxation based upon a power law model

Time (s)	Mean[1]	SD[2]	$E[M]$[3]	SD(I)[4]	SD($E[M]$)[5]	CV[6]
<10	−0.022	0.008	−0.022	0.008	0.003	15%
<100	−0.030	0.006	−0.030	0.006	0.002	8%
<1000	−0.031	0.009	−0.031	0.009	0.004	12%
<10000	−0.035	0.013	−0.035	0.013	0.006	16%

[1]Mean rate of stress relaxation from Standard Regression coefficients for each sample;
[2]Standard deviation of Standard Regression coefficients;
[3]Random Coefficients estimate of material property (rate of stress relaxation);
[4]Random Coefficients estimate of standard deviation of rates of stress relaxation;
[5]Standard error of the estimated rate of stress relaxation: $E[M]$;
[6]Percent coefficient of variation: $100 \times$ SD($E[M]$)/$E[M]$. Note, a smaller CV in the last column indicates increased accuracy.

As CV is a measure of the relative precision of the $E[M]$, for all data an increase in CV represents a decrease in accuracy for the rate of creep or relaxation. A decrease in CV represents an increase in accuracy of estimate for the rate of creep or stress relaxation.

Using the model in Eq. (1), creep rate is estimated by the coefficient β (Table 2). It should be noted that the Random Coefficients approach produced the same mean value of β (i.e. the group estimate for rate of creep) as the Standard Regression approach. A difference in the techniques does occur in their <10 second estimates for the standard deviation of the mean, i.e. SD versus SD(I). Within-specimen sampling error has inflated the Standard Regression estimate of dispersion of the creep rates for <10 seconds. This dispersion estimate is obtained as the standard deviation of the individual estimated regression slopes. These estimates do not just reflect the variability of the true underlying β_i's, but are contaminated by within-specimen sampling error as well. These components are separated in the random coefficients model, for which the standard deviation of the β_i's is estimated to be 0.017, compared to 0.019 from the standard regression approach. The coefficient of variation from the Random Coefficients model gives a general indication the of within-specimen measurement error, and relative large value for <10 seconds

suggests that within-specimen measurement error is indeed inflating the Standard Regression estimate of dispersion. Thus, using the Random Coefficients approach, testing for 10 seconds produces 19% accuracy in the measurement of creep rate for the group. Testing for 100 seconds decreases the CV (6%), thereby increasing the accuracy to 13%. Testing for 1,000 seconds further decreases the CV 1%, thus increasing (to 12%) the accuracy of the estimate of group viscoelastic behavior. Hence, testing for 1,000 seconds, as opposed to 100 seconds, only increases the accuracy by one percent.

For statistical analysis of the relaxation data, the model is Eq. (3). Results for the entire group of 14 ligaments tested under stress relaxation are shown in Table 3. Random Coefficients estimates for mean and standard deviation of the rates of stress relaxation are very similar to the Standard Regression estimates for these data. Testing for 100 seconds, instead of 10 seconds, confers a 3% increase in the accuracy of the estimated rate of relaxation for the group from 18% to 15%. Testing for 1000 seconds, as opposed to 100 seconds, is shown to only increase the accuracy by 1% from 15% to 14%. Testing for a fourth decade offers no increase in accuracy as the values remained at 14%. Table 4 shows the Standard Regression and Random Coefficients approaches applied solely to the six stress relaxation specimens tested for 10,000 seconds. Testing for 100 seconds substantially improves the accuracy obtained from the first decade from 15% to 8%. There is a decrease in accuracy when including data from the third decade (up to 12%) and an even further decrease in the fourth decade to 16%.

4. Discussion

Our goal was to determine how long individual ligaments must be tested in order to effectively describe the viscoelastic behavior in a group of ligaments. Results from a Random Coefficients approach show that we are able to successfully estimate the rate of creep or stress relaxation (as quantified by a power law model) by testing individual ligaments for relatively short time spans. That is, there appears to be no statistical advantage to test fewer specimens for longer periods of time when the goal is to describe the behavior of a group of specimens. These results reflect the accuracy of experimental data to fit our power law model. Hence, there must be an a priori knowledge and confidence that the law describing material behavior is valid and robust. This study used a power law formulation but a similar Random Coefficient approach could be applied to other models shown to describe the data well over the entire experimental time window.

Random Coefficients analysis accounts for the variability in individual samples by separating out the material property (either creep rate or relaxation rate) from the testing variability. The between-specimen variability, SD(I), should not change with sample size although SD($E[M]$) depends on the quality of data relevant to the model. That is, SD($E[M]$) is a measure of the portion of the dependent variable (i.e. rate of creep or relaxation) at each time measurement that cannot be explained by the model. Our data clearly show that in a statistical sense, testing for longer than 100 seconds offers no significant increase in accuracy in the group estimate for rate of creep or stress relaxation. If long term behavior of an individual sample is desired or higher order effects are of interest such as biologic adaptation or degradation, longer test times are then required. Looking at the standard deviations, it is conceivable that only testing for 10 seconds offers an accurate description of ligament behavior if a large number of samples are tested. For our stress relaxation data, testing for a fourth decade offers no increase in accuracy. Shorter test times, on the other hand, do not allow for the observation of possible biological changes in the specimen or behavior that required a higher order model. With our experimental data and our power law model, the only advantage for testing longer periods of time ($>$100 seconds) would be to confirm that the specimen conforms to our law.

Considering creep results (Table 2), the standard deviation of the individual increments SD(I) closely matched the standard deviation of the regression slopes, except in the first decade. The regression slopes include variance of both the material property as well as estimation error E. This estimation error is large enough in the first decade to inflate the SD of the creep rates. In fitting data to our model, there was little improvement in the relative precision of $E[M]$ (as measured by the CV) by extending testing beyond 100 seconds. Considering the extent of individual variability SD(I), there is little statistical advantage in testing samples beyond 100 seconds, especially if a shorter duration of testing allows more samples to be tested. Again, if long term biologic behavior (i.e. adaptation or degradation), is desired within one ligament or a small number of ligaments (i.e. the specimen specific model for creep behavior remains in doubt), then longer test times must be applied.

Results for stress relaxation are very similar to those obtained for creep. There is little improvement in the CV after 100 seconds. In fact, residual variability in the data (for 10,000 second tests) substantially increased the CV of the relaxation rates. Results indicate that residuals, e, in Eq. (3) were larger in the fourth logarithmic decade of time. This may be due to increased error in the testing system (e.g., thermal drift, vibrations, etc.) over the long term or due to biological degradation of the tissue, or this may indicate that the power law model is less accurate in the fourth decade. These results requires further investigation to understand. Even in this 10,000 second analysis, however, most of the variation in the rates of relaxation is still due to variation in the material property.

It should be noted that in the Woo study [20], ligament specimens had already been subjected to a prior load (load history) before they were tested for 1,000 minutes of stress relaxation. Thus, comparisons between his data and our long term data are difficult. He first subjected his specimens to 10 cycles of strain at a rate of 0.1%/second in the range of 1.5 to 2.5%, then they were stretched to 2.5% strain at a rate of 10%/sec, and then relaxation was recorded for 16 hours. Our ligaments, on the other hand, were subjected to only stress relaxation for a shorter time and hence have a different load history and test duration.

Our results, it should be recognized, are only valid for the time window that we have examined (1–1,000 or to 10,000 seconds). Statistical analysis over this window does not allow us to predict what might or might not happen over a wider window. This would be true even for an inert material, but ligaments are more complex. Long term in vivo behavior, which may include biological changes (e.g., remodeling), is not predictable from short *ex vivo* tests. Additionally, our experiments do not predict outcomes during and immediately after high rates of loading (e.g., an impact during a sporting event) which occur more rapidly than the shortest times explored in the current series of tests. Elucidation of viscous ligament behavior at high rates of loading as well as long term behavior will require additional testing.

In conclusion, this study shows that the variation between individual rat specimens (inter-specimen dispersion) is dominant when collecting creep or relaxation data. Therefore when attempting to quantify differences or similarities between study groups (clinical treatments, age, species, etc.) more samples tested over shorter times provides better accuracy than a few samples at longer times. In a more general sense, this study shows a method that can determine an optimal duration for viscoelastic testing to improve the experimental efficiency.

Acknowledgements

References

[1] T.S. Atkinson, B.J. Ewers and R.C. Haut, The tensile and stress relaxation responses of human patellar tendon varies with specimen cross-sectional area, *J. Biomech.* **32** (1999), 907–914.

[2] R. Cohen, C. Hooley and N. McGrum, Viscoelastic creep of collagenous tissue, *J. Biomech.* **9** (1976), 175–184.

[3] P.J. Diggle, K.Y. Liang and S.L. Zeger, *Analysis of Longitudinal Data*, Clarendon Press, Oxford, 1994.

[4] T.L.H. Donahue, C. Gregersen, M.L. Hull and S.M. Howell, Comparison of viscoelastic, structural, and material properties of double-looped anterior cruciate ligament grafts made from bovine digital extensor and human hamstring tendons, *J. Biomech. Eng.* **123** (2001), 162–169.

[5] Y.C.B. Fung, Stress–strain history relations of soft tissue in simple elongation, in: *Biomechanics: Its Foundations and Objectives*, Y.C.B. Fung, N. Perrowe and M. Anliker, eds, Prentice Hall, Englewood Cliffs, NJ, 1972, pp. 181–208.

[6] J.A. Hannafin and S.P. Arnockzy, Effect of cyclic and static tensile loading on water content and solute diffusion in canine flexor tendons: an *in vitro* study, *J. Orthop. Res.* **12** (1994), 350–356.

[7] R.C. Haut and R.W. Little, Rheological properties of canine anterior cruciate ligaments, *J. Orthop. Res.* **2** (1969), 289–298.

[8] R.C. Haut and R.W. Little, A constitutive equation for collagen fibers, *J. Biomech.* **5** (1972), 423–430.

[9] G.A. Johnson, D.M. Tramaglini, R.E. Levine, K. Ohno, N.Y. Choi and S.L. Woo, Tensile and viscoelastic properties of human patellar tendon, *J. Orthop. Res.* **12** (1994), 796–803.

[10] G.J.W. King, C.J. Pillon and J.A. Johnson, Effect of *in vitro* testing over extended periods on the low-load mechanical behaviour of dense connective tissues, *J. Orthop. Res.* **18** (2000), 678–681.

[11] R.S. Lakes and R. Vanderby, Interrelation of creep and relaxation: a modeling approach for ligaments, *J. Biomech. Eng.* **121** (1999), 612–615.

[12] P.M. Monléon and C.R. Diaz, Nonlinear viscoelastic behaviour of the flexor tendon of the human hand, *J. Biomech.* **23** (1990), 773–781.

[13] M. Panjabi, R. Huang and J. Cholewicki, Equivalence of single and incremental subfailure stretches of rabbit anterior cruciate ligament, *J. Orthop. Res.* **18** (2000), 841–848.

[14] P. Provenzano, R.S. Lakes, T. Keenan and R.J. Vanderby, Nonlinear ligament viscoelasticity, *Ann. Biomed. Eng.* **29** (2001), 908–914.

[15] P.P. Provenzano, D. Heisey, K. Hayashi, R. Lakes and R. Vanderby, Jr., Subfailure damage in ligament: a structural and cellular evaluation, *J. Appl. Physiol.* **92** (2002), 362–371.

[16] P.P. Provenzano, R.S. Lakes, D.T. Corr and R. Vanderby, Jr., Application of nonlinear viscoelastic models to describe ligament behavior, *Biomechanics and Modeling in Mechanobiology* **1** (2002), 45–57.

[17] G.M. Thornton, A. Oliynyk, C.B. Frank and N.G. Shrive, Ligament creep cannot be predicted from stress relaxation at low stress: a biomechanical study of the rabbit medial collateral ligament, *J. Orthop. Res.* **15** (1997), 652–656.

[18] A.L. Wallace, R. Hollinshead and F. CB, Electrothermal shrinkage reduces laxity but alters creep behavior in a lapine ligament model, *J. Shoulder & Elbow Surg.* **10** (2001), 1–6.

[19] S.L.-Y. Woo, M.A. Gomez and W.H. Akeson, The time and history-dependent viscoelastic properties of the canine medial collateral ligament, *J. Biomech. Eng.* **103** (1981), 293–298.

[20] S.L.-Y. Woo, Mechanical properties of living tissues I. Quasistatic and nonlinear viscoelastic properties, *Biorheology* **19** (1982), 385–396.

[21] J.W. Xerogeanes, R.J. Fox, Y. Takeda, H.-S. Kim, I. Ishibashi, G.J. Carlin and S.L.-Y. Woo, A functional comparison of animal anterior cruciate ligament models to the human anterior cruciate ligament, *Ann. Biomed. Eng.* **26** (1998), 345–352.