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***In vivo* passive mechanical properties of skeletal muscle improve with massage-like loading following eccentric exercise**

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Abstract

A quasi-linear viscoelasticity (QLV) model was used to study passive time-dependent responses of skeletal muscle to repeated massage-like compressive loading (MLL) following damaging eccentric exercise. Six skeletally mature rabbits were surgically instrumented with bilateral peroneal nerve cuffs for stimulation of the hindlimb tibialis anterior (TA) muscles. Following the eccentric exercise, rabbits were randomly assigned to a four-day MLL protocol mimicking deep effleurage (0.5 Hz, 10 N for 15 min or for 30 min). The contralateral hindlimb served as the exercised, no-MLL control for both MLL conditions. Viscoelastic properties of the muscle pre-exercise, post-exercise on Day 1, and pre- and post-MLL Day 1 through Day 4 were determined with ramp-and-hold tests. The instantaneous elastic response (AG_0) increased following exercise ($p < 0.05$) and decreased due to both the 15 min and 30 min four-day MLL protocols ($p < 0.05$). Post-four days of MLL the normalized AG_0 decreased from post-exercise (Day 1, 248.5%) to the post-MLL (Day 4, 98.5%) ($p < 0.05$), compared to the no-MLL group (Day 4, 222.0%) ($p < 0.05$). Exercise and four-day MLL showed no acute or cumulative effects on the fast and slow relaxation coefficients ($p > 0.05$). This is the first experimental evidence of the effect of both acute (daily) and cumulative changes in viscoelastic properties of intensely exercised muscle due to *ex vivo* MLL. It provides a starting point for correlating passive muscle properties with mechanical effects of manual therapies, and may shed light on design and optimization of massage protocols.

Keywords

Compressive loading; Skeletal muscle; Viscoelasticity; Manual therapy

1. Introduction

Considerable evidence suggests that both mechanical and functional characteristics of skeletal muscle are influenced by applied mechanical forces (Bosboom et al., 2001; Bryer and Koh, 2007; Butterfield and Herzog, 2006). Evaluation of the muscle's passive mechanical properties provides some insight on the effect of applied loading for both structure and function. For example, Van Loocke et al. (2008) used a transversely isotropic model with strain-dependent Young's moduli (SYM) to *ex vivo* estimate compressive

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Conflict of interest statement

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properties of animal skeletal muscle together with a quasi-linear viscoelastic (QLV) model to explore time-dependent properties. In further studies, higher amplitude compression loading produced nonlinear viscoelastic behavior with a frequency dependent increase (Van Looke et al., 2009). Collectively, these investigations provide important information regarding the nature of skeletal muscle passive mechanical behavioral responses to compression loading and show that the tissue's viscoelastic properties are dependent on compression rate and fiber orientation.

In vivo studies have further characterized skeletal muscle passive responses to mechanical loading. For example, Chaudhry et al. (2008) provided baseline information on the passive properties of the human biceps muscle, observing that muscle is fifteen times stiffer in the direction parallel to the muscle fibers than perpendicular to the fibers. Magnusson et al. (1996) noted that the tensile viscoelastic responses to a single bout of stretching of the human hamstring muscles are dependent upon joint angle. Ylinen et al. (2009) reported that daily passive stretching of the leg over four weeks did not however affect muscle compliance and viscoelastic properties, although the regimen improved the subject's passive straight leg raise. Therefore, there may be a limit on the effects of repeated loading on the muscle's viscoelastic properties.

The evaluation of passive mechanical properties may provide knowledge of muscle behavior and guidance for clinicians utilizing manual therapies for muscle associated injuries and pathologies. According to the American Massage Therapy Association (Kennedy and Blair, 2011, Sherman et al., 2010) the physical benefits of therapeutic massage on muscle include: relief of muscle tension and stiffness, faster healing of strained muscles and sprained ligaments, reduced muscle pain, swelling, and spasm, greater joint flexibility and range of motion, and even enhanced athletic performance. The mechanical load that a therapist applies is partially dependent on the passive muscle properties obtained through hand/palm contact with the subject tissue. If a correlation between mechanical loading, tissue viscoelastic properties, and muscle function can be identified, one would perhaps be able to optimize tissue loading occurring with manual therapies such as massage based on the tissue's passive mechanical responses.

To examine the effect of massage-like compressive loading (MLL) on the viscoelastic properties of skeletal muscle, an eccentric exercise model was used to induce muscle damage. Eccentric exercise is known to cause muscle stiffness, swelling, and weakness referred to as delayed onset muscle soreness (Golden and Dudley, 1992; Jones et al., 1987; Newham et al., 1983a,b; Eston et al., 2003). We hypothesized that MLL, delivered at an ideal magnitude, duration, and loading frequency following a bout of eccentric exercise will lead to both acute (daily) and cumulative (over four-day treatment) changes in the muscle's viscoelastic properties, in particular a reduction of both the instantaneous elastic response and apparent viscosity of the tissue. Such findings could pave the way for development of assistive devices implementing manual therapies for targeting recovery following muscle damage and inflammation.

2. Methods

All experiments were approved by Institutional Animal Care and Use Committee (IACUC) at the Ohio State University.

2.1. Animal surgeries and exercise protocol

Six skeletally mature New Zealand White female rabbits (3.46 ± 0.12 kg) were surgically instrumented with bilateral peroneal nerve cuffs for stimulation of the TA muscles using a previously described surgical procedure (Butterfield et al., 2008).

Seven days post-surgery rabbits were sedated with 0.25 ml acepromazine, subsequently anesthetized with 1.5% isoflurane and kept under anesthesia for the entire duration of all experiments. For the eccentric exercise, rabbits were secured supine in a sling with one foot attached to a footplate connected to a torque sensor on the cam of a servo-motor (Fig. 1). The exercise protocol consisted of seven sets of ten cyclic lengthening contractions, with 2 min rest between sets. Cyclic lengthening contractions were performed from a tibiotarsal angle of 95° to 145° of plantar flexion at 150° s⁻¹, with activation preceding the muscle-tendon unit stretch by 100 ms. Previous studies have shown this set of parameters resulted in a reproducible magnitude of muscle damage, approximately 70% loss of torque production measured within 5 min following a bout of eccentric exercise (Butterfield et al., 2008).

2.2. MLL protocol

Rabbits were subjected to a MLL protocol using a custom-designed mechanical device (Fig. 2). This device included two motorized arms that traveled in the directions parallel or normal to the tissue's surface. A mechanical tip was mounted on the lower end of the vertical arm connected to a force sensor. The tip compressed the surface of the TA at 76 µm/s until 10 N compressive force was reached. Given the mechanical tip size, the normal compressive stress was estimated as 88.46 kPa. The tissue was then tilted to zero out the mechanical force in the lateral direction, and to minimize the variation of compression when the mechanical tip traveled along the longitudinal axis of the TA. The mechanical tip moved along the longitudinal axis of the TA at 0.5 Hz, applying a compressive load as well as a transverse load to the TA. The muscle's surface was assumed flat for modeling purposes. The traveling stroke was adjusted to 12.5 mm to avoid direct contact with the bones in the lower extremity. The compressive force was kept at 10 N by dynamically adjusting the vertical position of the mechanical tip through a feedback loop.

Rabbits were randomly assigned to a MLL duration of 15 min or 30 min per day. For each rabbit, one hindlimb was exercised and then immediately received a four-day MLL protocol, while the other hindlimb was exercised but did not receive MLL (no-MLL, control). Experiments on the two hindlimbs were separated by a week to minimize crossover effect. Stress relaxation tests and MLL were both performed on four days (approximately 24 h interval) to evaluate daily effects of massage on muscle's mechanical properties.

2.3. Evaluation of TA muscle viscoelastic properties

Under anesthesia, the hindlimb was secured into the mechanical loading device with the TA muscle facing up. TA's viscoelastic properties were evaluated by recording the vertical compressive force as a function of time and vertical displacement. Evaluation of these properties occurred pre-exercise, post-exercise, and pre- and post-MLL (or post-no-MLL) for all four days using a 300 s ramp-and-hold test (compression) on both the MLL and no-MLL limbs. On Day 5, a final stress relaxation test (compression) was performed to assess the cumulative effects of the four-day MLL protocol.

To confirm changes of viscoelastic properties were attributable primarily to MLL and to remove effects of natural healing, the exercised, no-MLL contralateral hind limbs were used as the control group. They were placed in the massage device, kept in the same posture as the MLL hindlimb for the same duration, but did not receive MLL.

2.4. Mathematical modeling

According to the QLV model, the stress relaxation was:

$$\sigma(t) = G(t) * \sigma^e(\lambda) \quad (1)$$

where λ was the stretch ratio, $G(t)$ was the reduced relaxation function, $\sigma^e(\lambda)$ was the instantaneous stress response, and the operator $*$ denotes the convolution of these two factors. Using Boltzmann hereditary integrals, Eq. (1) was formulated as:

$$\sigma(t) = \int_{-\infty}^t G(t-\tau) \frac{\partial \sigma^e(t)}{\partial \varepsilon} \frac{\partial \varepsilon}{\partial \tau} d\tau \quad (2)$$

where ε/τ represented the strain history. The instantaneous elastic stress response was:

$$\sigma^e(\varepsilon) = AG_0(e^{B\varepsilon} - 1) \quad (3)$$

where $\varepsilon = \lambda - 1$ was the engineering strain, AG_0 was a linear parameter with the dimension of stress, and dimensionless B represents the nonlinearity of the elastic response. The reduced relaxation function $G(t)$ was expressed using Prony series expansion:

$$G(t) = 1 - \sum_{i=1}^n g_i^P (1 - e^{-(t/\tau_i)}) \quad (4)$$

where g_i^P was the i th Prony series parameter, and τ was the relaxation time constant at the i th order. According to the obtained relaxation curves, a second order model was chosen. Eq. (4) was rewritten as:

$$G(t) = k_0 + k_1 e^{-t/\tau_1} + k_2 e^{-t/\tau_2} \quad (5)$$

where k_0 , k_1 and k_2 were determined by fitting with the experimental measurements.

Due to the difficulty of fitting the quasi-linear model with a very fast ramping rate, a ramp-and-hold test with a finite ramping period was performed. The strain history was:

$$\forall t \in (0, t_0), \quad \frac{\partial \varepsilon}{\partial \tau} = \gamma \quad (\text{ramping}) \quad (6)$$

$$\forall t \in [t_0, \infty), \quad \varepsilon = \gamma \times t_0 \quad (\text{holding}) \quad (7)$$

where t_0 was a finite value, which was approximately 66 s. The compression depth was 5 mm and the TA thickness was about 10 mm. γ was calculated as 0.0075 s^{-1} . Inserting Eqs. (3), (5)–(7) into Eq. (2), stress relaxation during the ramp-and-hold test was obtained:

$$\sigma(0 \leq t < t_0) = AG_0 B \gamma \left[\left(\frac{k_0}{B\gamma} e^{B\gamma t} + \frac{k_1 \tau_1}{1+B\gamma \tau_1} e^{B\gamma t} + \frac{k_2 \tau_2}{1+B\gamma \tau_2} e^{B\gamma t} \right) - \left(\frac{k_0}{B\gamma} + \frac{k_1 \tau_1}{1+B\gamma \tau_1} e^{-(t/\tau_1)} + \frac{k_2 \tau_2}{1+B\gamma \tau_2} e^{-(t/\tau_2)} \right) \right] \quad (8)$$

$$\sigma(t \geq t_0) = AG_0 B \gamma \left[\left(\frac{k_0}{B\gamma} e^{B\gamma t_0} + \frac{k_1 \tau_1}{1+B\gamma \tau_1} e^{B\gamma t_0} e^{-\frac{t-t_0}{\tau_1}} + \frac{k_2 \tau_2}{1+B\gamma \tau_2} e^{B\gamma t_0} e^{-\frac{t-t_0}{\tau_2}} \right) - \left(\frac{k_0}{B\gamma} + \frac{k_1 \tau_1}{1+B\gamma \tau_1} e^{-\frac{t}{\tau_1}} + \frac{k_2 \tau_2}{1+B\gamma \tau_2} e^{-\frac{t}{\tau_2}} \right) \right] \quad (9)$$

The parameters in Eqs. (8) and (9) were determined from the experimental data using a previously reported approach (Abramowitch and Woo, 2004).

2.5. Statistical analysis

Given $n=3$ with the variation of data most likely due to difference in inter-animal biological responses and the degree of injury variation by $\pm 6\%$, Mann–Whitney U test was used for

processing non-paired data and Wilcoxon rank sum test was used for processing paired data. These non-parametric tests do not assume certain data distribution nor the normality condition. The alpha level was set to 0.05 for analysis. All statistics are based on Mann–Whitney U test or Wilcoxon rank sum test.

3. Results

Stress–time curves for all muscles exhibited highly non-linear behavior. The ramp-and-hold data were fit with an overall r^2 value greater than 0.97.

3.1. Effect of eccentric exercise on TA viscoelastic properties

Eccentric exercise had a significant effect on the muscle's viscoelastic properties. Immediately following exercise, the viscoelastic behavior had marked differences from the pre-exercise response, as shown by the stress relaxation curves of both the MLL and no-MLL groups. Both peak stress and relaxation response stress were higher than those in the pre-exercise group. Following four-day MLL, the peak stress at the end of ramping exhibited a notable reduction. For a representative test (Fig. 3), the peak stress after four-day MLL reduced to 103.2% of the pre-exercise response. The final stress value after 300 s of holding also decreased. In contrast, the no-MLL muscle showed a further increase in both the peak stress and final stress after four days, indicating a monotonic change of viscoelastic properties without mechanical intervention.

3.2. Effect of eccentric exercise and MLL on the instantaneous elastic response—

To quantify the effects of exercise and MLL on the muscle viscoelastic properties, the parameters representing the instantaneous elastic responses, *i.e.*, AG_0 and B , and the parameters representing the reduced relaxation function, *i.e.*, g_1^p , g_2^p , τ_1 , and τ_2 were obtained by fitting the measured stress curves. The instantaneous elastic response $\sigma^p(\epsilon) = AG_0(e^{Be}-1)$ showed both the immediate effect of exercise (comparison of curves I and II) and the cumulative effects of MLL (comparison of curves II and III) over four days. As shown, exercise increased the elastic response while four-day MLL reduced elastic response to near pre-exercise values (Fig. 4A). For the group with 0.5 Hz, 10 N, 15 min condition ($n=3$), the AG_0 over the entire MLL period was determined. Since the initial elastic response among individual animals had a large variation, normalized AG_0 were used for analysis: the AG_0 values of pre-MLL groups were set as the references (100%), and changes of AG_0 values were expressed as percentile (Fig. 4B). It was showed that eccentric exercise significantly increased the normalized AG_0 ($p<0.05$). The post-exercise normalized AG_0 values between the MLL and no-MLL groups did not have significant difference ($p>0.05$). This indicated that muscle damage increased normalized AG_0 in both MLL and no-MLL groups. After the four-day MLL protocol, normalized AG_0 significantly reduced ($p<0.05$). However, the normalized AG_0 did not exhibited a significant change after four-day no-MLL protocol ($p>0.05$), indicating that natural healing was not sufficient to induce significant change in normalized AG_0 . No definitive conclusion could be drawn for parameter B based on statistical analysis.

The effect of daily MLL duration was examined by comparing AG_0 obtained from both the 15 min and 30 min MLL groups. The results showed no difference between the normalized AG_0 for both the 15 min and 30 min MLL protocols ($p>0.05$), implying that the longer duration (30 min) of MLL did not significantly affect the post-MLL AG_0 (Fig. 4C).

A daily decrease of the pre-MLL AG_0 was observed (Fig. 5A). In addition, MLL produced an additional decline of AG_0 . Notably, the acute AG_0 reduction due to MLL was significant in the first two days (Day 1 and Day 2) following the exercise ($p<0.05$). However on Day 3 and Day 4, pre- and post-MLL AG_0 did not show a significant difference ($p>0.05$). For

better illustration, post-MLL AG_0 was normalized using the pre-MLL group of the same day as the reference (Fig. 5B). The greatest reduction of averaged normalized AG_0 was observed on Day 1 with percentile reduced to 41.24%. On each successive day, the percentile reduction in AG_0 upon MLL decreased monotonically. On Day 4, the averaged normalized AG_0 after MLL reached 98.52%, suggesting that MLL did not have a substantial impact on instantaneous elastic response at this time point.

3.3. Effect of eccentric exercise and MLL on the reduced relaxation response

Fig. 6 is a typical reduced relaxation response showing the effects of exercise, MLL and no-MLL. The reduced relaxation curve moved to the left immediately following the eccentric exercise, as indicated by the left shift of its intercept with a horizontal line. This suggested that the muscle became less viscous upon eccentric exercise. After four-day MLL, the intercept had a significant shift to the right (Fig. 6A). In no-MLL group, the intercept also shifted to the right, while not as great as that in the MLL group (Fig. 6B). This suggested that both natural healing and MLL increased muscle viscosity, and MLL did at a much faster rate. The estimates of viscosity change referred to the fast relaxation component.

In this QLV model, g_1^p and τ_1 represented fast relaxation, while g_2^p and τ_2 represented slow relaxation, *i.e.* τ_1 was smaller than τ_2 . The changes in g_1^p and g_2^p pre- and post-MLL on each day were obtained (Fig. 7A). The g_1^p and g_2^p after daily MLL were normalized using the pre-MLL group of the same day as reference (Fig. 7B). This analysis revealed that on Day 1 the average normalized g_1^p post-MLL increased to 112.18%, while the average normalized g_2^p post-MLL decreased to 64.90%. On the following days, the averaged normalized g_1^p post-MLL varied between 100% and 140%. However, no definitive conclusion can be drawn for g_1^p and g_2^p ($p > 0.05$ for all daily comparisons).

4. Discussion

This study tested the hypothesis that repeated bouts of MLL following eccentric exercise change the viscoelastic properties of skeletal muscle. Passive mechanical behavior of the rabbit TA muscle was evaluated and modeled following a controlled bout of eccentric exercise and over the course of four consecutive days of MLL. This resulted in the first experimental evidence of the effect of MLL on both acute (daily) and cumulative (over four days) changes in viscoelastic responses of the muscle. The QLV model was selected to identify the dominating viscoelastic parameters that are most correlated with the massage action. It should be noted that such correlation could be dynamic and varying with the range of loading parameters. The correlations reported herein only refer to the magnitudes, frequencies, and durations used in this study.

Results showed that eccentric exercise produced an immediate increase in the muscle's instantaneous elastic response, which is consistent to previous studies showing a large increase in passive tension post-exercise and the increase sustained over four days (Whitehead et al., 2001, Pousson et al., 1990). This could occur due to a number of factors. It was proposed an uneven distribution of the muscle's length change during exercise with some sarcomeres taking up most of the stretch while others lengthening much less (Whitehead et al., 2001). The increase of instantaneous elastic response may indicate problems with realignment of sarcomere interdigitation when the tissue returns to its relaxed state as some sarcomeres extend beyond myofilament overlap.

The acute effect of loading on the instantaneous elastic response was seen by daily pre- and post-MLL analysis, which showed decline of post-MLL AG_0 with the greatest reduction on

Day 1. The reduction of AG_0 upon MLL decreased daily, with the post-MLL average AG_0 reaching 98.5% of the pre-MLL value on Day 4. This suggested that after three days of MLL, the instantaneous elastic behavior had approached near pre-exercise status so that subsequent loading (on and after Day 4) may not produce a further substantial effect.

The cumulative effect of daily MLL on muscle's viscoelastic properties was best seen from the instantaneous elastic response. Analysis showed a daily decrease of pre-MLL AG_0 and a cumulative 112.3% reduction of averaged normalized AG_0 after four-day MLL, while control tissues showed 51.5% averaged normalized AG_0 increase.

The effect of MLL duration on the muscle's instantaneous elastic response was examined by comparing the 0.5 Hz, 10 N, 15 min MLL to the 0.5 Hz, 10 N, 30 min MLL. This comparison showed that such a longer loading duration (30 min) did not significantly affect muscle's elastic properties. Further work is needed to explore whether the loading duration is a critical factor of affecting passive muscle properties.

Results also showed that the 0.5 Hz, 10 N, 15 min MLL protocol produced no acute or cumulative changes in the relaxation coefficients g_1^P and g_2^P ($p>0.05$). This is not consistent to the previous study which showed G2 coefficient was affected by the mechanical preconditioning, and G1 was not (Palevski et al., 2006). The discrepancy could be due to different loading parameters (indentation vs. MLL) and different holding durations (60 s vs. >200 s). Also, the eccentric exercise induced damage model may contribute. The inflammatory response to damaged muscle fibers caused a transfer of fluid and cells to the damaged tissue, causing swelling after the injury (Smith, 1991) which would affect relaxation coefficients g_1^P and g_2^P .

The large variation of the fitted coefficients obtained from the QLV model is attributed to inter-animal variation of initial mechanical properties, the degree of induced injury, and the resultant disruption of calcium channels and inflammatory response (Butterfield and Best, 2009; Butterfield and Herzog, 2006; Weerapong et al., 2005). Normalized coefficients were used for statistical analysis to minimize the effect of inter-animal variation.

In conclusion, this study provides the first evidence of acute and cumulative effects of a multiple-day MLL on changing muscle's viscoelastic properties following eccentric exercise. These findings provide objective evidence that is valuable to clinicians utilizing massage therapies. The translation of our findings to humans remains an avenue for further investigation.

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Fig. 1. Customized exercise device performed eccentric exercise. The apparatus systematically altered muscle fiber dynamics, joint torque production and muscle damage through altered activation timing and joint range of motion.

Fig. 2. Customized MLL device. The resolution of motion along each axis was 25 μm . Data acquisition device within Labview software bundle (National Instruments, NI PCI 6133) was used to control the traveling direction, speed, duration of loading, and the magnitude of compressive loads.

Fig. 3.

A representative set of experimental data and the fitted curves comparing the *in vivo* passive mechanical response of pre-exercise, post-exercise, and post-four-day MLL protocols. (A) and (C) showed the raw data, while (B) and (D) showed the fitted curves. (A) and (B) were subjected to the MLL with the peak compressive loads of 10 N. (C) and (D) were the control groups without MLL. Note that in (A) and (B) MLL led to near complete recovery to the pre-exercise relaxation response, while in (C) and (D) relaxation response showed a further deviation from the pre-exercise group after four-day MLL.

Fig. 4. Effects of exercise and MLL action on the instantaneous elastic response. (A) Instantaneous elastic response $\sigma^e(\epsilon)$; (B) the cumulative effects on AG_0 for 15 min MLL at 0.5 Hz and 10 N over 4 days ($n=3$); (C) comparison of the cumulative effects on AG_0 for 15 min and 30 min MLL over 4 days ($n=3$). Both were at 0.5 Hz and 10 N.

Fig. 5. Day-to-day effects of MLL (0.5 Hz, 10 N, 15 min condition) on AG_0 illustrating that the acute reduction of AG_0 was the greatest on Day 1 and decreased with each following day. (A) AG_0 of the pre-MLL and post-MLL groups on each day; and (B) normalized AG_0 change upon MLL (normalized against pre-MLL group each day).

Fig. 6. Reduced relaxation response upon exercise and MLL. (A) MLL group under 0.5 Hz, 10 N, 15 min; and (B) control no-MLL group.

Fig. 7. Day-to-day effects of MLL (0.5 Hz, 10 N, 15 min condition) on the relaxation coefficients. (A) Prony series parameters of fast relaxation and slow relaxation of pre-MLL and post-MLL groups on each day; and (B) normalized change of the relaxation coefficients upon MLL (normalized against pre-MLL group each day).