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

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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 applied loading for both structure and function. For example, Chaudhry et al. (2008) provided baseline information on the passive proper-

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.

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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 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:

\[ \sigma(t) = G(t) \omega \sigma'(t) \]

where \( \omega \) was the stretch ratio, \( G(t) \) was the reduced relaxation function, \( \sigma'(t) \) was the instantaneous stress response, and the operator \( \omega \) denotes the convolution of these two factors. According to the obtained relaxation curves, a second order model was chosen. Eq. (4) was rewritten as:

\[ G(t) = k_0 + k_1 e^{-t/\tau_1} + k_2 e^{-t/\tau_2} \]

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:

\[ \forall t \in (0, t_0), \frac{\Delta \varepsilon}{\tau} = \gamma \] (ramping)

\[ \forall t \in [t_0, \infty), \Delta \varepsilon = \gamma \times t_0 \] (holding)

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. \( \gamma \) 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:

\[ \sigma(0 \leq t < t_0) = AG_0 \left( \frac{k_0}{B_1} e^{t_0/\tau_1} + \frac{k_1 \tau_1}{B_1 \tau_2} e^{t_0/\tau_2} + \frac{k_2 \tau_2}{B_1 \tau_2} e^{t_0/\tau_2} \right) \]

\[ \forall t \in [t_0, \infty), \sigma(t \geq t_0) = AG_0 \left( \frac{k_0}{B_1} e^{t/\tau_1} + \frac{k_1 \tau_1}{B_1 \tau_2} e^{t/\tau_2} + \frac{k_2 \tau_2}{B_1 \tau_2} e^{t/\tau_2} \right) \]

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

![Graphs and Tables](https://example.com/fig3.png)

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.
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.

![Stress-time curves](image.png)

Fig. 4. Effects of exercise and MLL action on the instantaneous elastic response. (A) Instantaneous elastic response \( \sigma'(t) \); (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.

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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., $g_1^p$, $g_2^p$, $\tau_1$, and $\tau_2$ were obtained by fitting the measured stress curves. The instantaneous elastic response $\sigma^e(t) = AG_0(e^{\frac{t}{\tau_1}} - 1)$ 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 shown 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. 

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.
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, \(g^p_1\) and \(\tau_1\) represented fast relaxation, while \(g^p_2\) and \(\tau_2\) represented slow relaxation, i.e. \(\tau_1\) was smaller than \(\tau_2\). The changes in \(g^p_1\) and \(g^p_2\) pre- and post-MLL on each day were obtained (Fig. 7A). The \(g^p_1\) and \(g^p_2\) 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 \(g^p_1\) post-MLL increased to 112.18%, while the average normalized \(g^p_2\) post-MLL decreased to 64.90%. On the following days, the averaged normalized \(g^p_1\) post-MLL varied between 100% and 140%. However, no definitive conclusion can be drawn for \(g^p_1\) and \(g^p_2\) (\(p > 0.05\) 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) passive mechanical properties of skeletal 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.

**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).

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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 $g^{p}_{1}$ and $g^{p}_{2}$ ($p > 0.05$). 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 $g^{p}_{1}$ and $g^{p}_{2}$.

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.

Conflict of interest statement

All authors confirm they have no financial or other conflicts of interest relevant to this study.

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