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Massage Timing Affects Postexercise Muscle Recovery and Inflammation in a Rabbit Model

Caroline Haas^{1,2}, Timothy A. Butterfield³, Sarah Abshire³, Yi Zhao², Xiaoli Zhang⁴, David Jarjoura⁴, and Thomas M Best^{1,2,5}

¹Division of Sports Medicine, Department of Family Medicine, The Ohio State University, Columbus, OH

²Department of Biomedical Engineering, The Ohio State University, Columbus, OH

³Division of Athletic Training, Department of Rehabilitation Sciences, University of Kentucky, Lexington KY

⁴Center for Biostatistics, The Ohio State University, Columbus, OH

⁵Sports Health and Performance Institute, The Ohio State University, Columbus, OH

Abstract

Purpose—This study compared the effect of immediate versus delayed massage-like compressive loading (MLL) on peak isometric torque recovery and inflammatory cell infiltration following eccentric exercise (EEX).

Methods—Eighteen skeletally mature New Zealand White rabbits were instrumented with peroneal nerve cuffs for stimulation of hindlimb tibialis anterior muscles. Following a bout of EEX, rabbits were randomly assigned to a MLL protocol (0.5Hz, 10N, 15min) started immediately post-EEX, 48 hours post-EEX, or no-MLL control and performed for four consecutive days. A torque-angle (T- θ) relationship was obtained for 21 joint angles pre and post-EEX and post four consecutive days of MLL or no-MLL. Muscle wet weights and immunohistochemical sections were obtained following final treatments.

Results—EEX produced an average 51% ($\pm 13\%$) decrease in peak isometric torque output. Greatest peak torque recovery occurred with immediate application of MLL. There were differences in torque recovery between immediate and delayed MLL ($p=0.0012$), immediate MLL and control ($p<0.0001$), and delayed MLL and control ($p=0.025$). Immunohistochemical analysis showed 39.3% and 366.0% differences in the number of RPN3/57 and CD11b positive cells

Corresponding Author: Thomas Best, OSU Sports Medicine Center, 2050 Kenny Road, Suite 3100, Columbus, Ohio 43221, Phone: (614) 293-3600, Fax: (614) 293-4399, Tom.Best@osumc.edu.

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Author Justification:

Haas: Study design, experiments, analysis, and writing

Butterfield: Experiments, analysis and writing

Abshire: Immunohistochemistry experimentation and analysis

Zhao: Experiments, analysis and writing

Zhang: Study design, analysis, and writing

Jarjoura: Study design, analysis, and writing

Best: Study design, experiments, analysis, and writing

between immediate ($p=0.71$) and delayed MLL ($p=0.12$). Area under the T- Θ curve showed a difference for immediate ($p<0.0001$) and delayed ($p=0.0051$) MLL as compared to control. Exercise produced an average $10^\circ \pm 0.2$ rightward shift from pre exercise peak isometric torque angle. Control, immediate MLL and delayed MLL produced an average leftward angular shift from the post exercise angle ($p=0.28$, $p=0.03$, and $p=0.47$, respectively).

Conclusion—Post-EEX, immediate MLL was more beneficial than delayed MLL in restoring muscle function and modulating inflammatory cell infiltration. These findings invite similar human studies in order to make definitive conclusions on optimal timing of massage-based therapies.

Keywords

massage; inflammation; skeletal muscle; torque-angle properties

INTRODUCTION

Delayed onset muscle soreness (DOMS) is characterized by skeletal muscle strength loss, soreness, swelling, and weakness are known to accompany intense or prolonged physical activity, particularly EEX (3, 4). DOMS is thought to be the result of a delayed secondary inflammatory response to a primary mechanical disruption that arguably leads to further injury (44, 17). A review of both human and animal studies has shown that soreness typically appears between 8 and 24 h post-exercise, peaks at 24 to 48 h and can last for up to 7 days (11, 13, 14). There are multiple theories of the mechanism of injury accompanying intense EEX including: local tissue inflammation, tears in the muscle fibers, disruption of passive, non-contractile components in the sarcomere, sarcoplasmic reticulum, and connective tissue, strain on the sarcomeres causing disruption of Z lines and A bands, and an impairment of plasmalemmal action potential conduction (25, 10, 26, 28).

EEX induced muscle injury initiates an inflammatory cascade leading to production of pro-inflammatory cytokines within damaged muscle tissue, systemic release of leukocytes and cytokines, as well as alterations in leukocyte receptor expression and functional activity (30). Research has shown inflammation as one of the most prevalent causes for the general decline of physical function and loss of muscle strength in both the young and the elderly (9, 29, 33). Moreover, it has been postulated that the inflammatory process is responsible not only for successful repair but also in extending the damage process and may be coupled with significant loss of muscle mass (23, 40, 47). This progression of injury could occur through a variety of mechanisms, including neutrophil infiltration and activation of the respiratory burst (40). In fact, recent studies suggest that infiltrating neutrophils may play a key role in both injury and repair (42).

A variety of techniques, including therapeutic ultrasound (39), electrical stimulation (43), ice immersion (cryotherapy) (34), static and ballistic stretching (37), therapeutic massage (15), and nonsteroidal anti-inflammatory medications (24), are used clinically to minimize the pain and enhance functional recovery following exercise-induced muscle damage (8, 32). While research on the efficacy of these treatments has increased, there still remains a void in the mechanistic understanding and therefore optimal clinical strategies, especially concerning therapeutic massage, to mitigate muscle pain and weakness associated with EEX.

The science of massage is of interest to athletes, patients presenting with muscle pain, and all personnel associated with medical care. EEX associated muscle pain and loss of function are often the impetus for athletes to receive massage therapies as a means for rapid resolution of pain and recovery of muscle function (20, 45). While evidence to support or

refute its effects on muscle function is insufficient at this time, new reports help formulate an understanding of massage and its possible role in mitigating exercise-related muscle pain (27, 2). It does appear that under certain conditions, massage can reduce muscle soreness associated with eccentric muscle contractions, reduce inflammation and promote mitochondrial biogenesis, although whether muscle force output recovers more quickly is still unclear (21, 27, 12). Despite the lack of conclusive scientific evidence, providers spend a large portion of their time prescribing and utilizing massage based therapies for a variety of soft tissue pathologies (16). Massage treatments can last up to two hours with therapists often advising a series of appointments, without evidence for the efficacy of repeated sessions of this therapy (35). Another important question is the optimal timing for use of massage following EEX for maximal results. The contrast between current scientific understanding of sports massage and its practice is notable, and scientific evidence to corroborate or refute an effect of massage on muscle recovery remains an important area of investigation (27).

Our laboratory has shown in a number of recent studies that MLL incorporated into a laboratory model of EEX can improve the recovery of muscle function and may decrease leukocyte infiltration and myofiber damage (8, 18, 19). In the current study, we wanted to determine if the timing of MLL following EEX could be an important variable in recommending this therapy to individuals to enhance recovery following intense EEX. Based upon our previous work demonstrating an optimal MLL protocol (0.5Hz, 10N, 15min) for recovery of muscle function (isometric joint torque) after a bout of intense EEX (18), we hypothesized that (i) immediate application of MLL post eccentric exercise results in greater recovery of muscle function than delayed MLL and (ii) immediate application of MLL decreases leukocyte infiltration as compared to 48 hour delayed MLL. The primary goals of this study were to 1) utilize an experimental approach that simulated MLL to evaluate the time effects of MLL application on recovery of muscle function from EEX induced muscle damage and 2) evaluate the effects of the different protocols on tissue inflammation (leukocyte infiltration) via quantitative immunohistochemistry; in order to shed further light on the optimal strategy for massage post EEX.

METHODS

Animal Surgery for Nerve Cuff Implantation and Experimental Design

Under a protocol approved by the Institutional Laboratory Animal Care and Use Committee (Ohio State University), eighteen skeletally mature (18–20 weeks) female New Zealand White rabbits (3.66 ± 0.27 kg) were anesthetized and instrumented with peroneal nerve cuffs for stimulation of hindlimb tibialis anterior (TA) muscles as previously described (8, 18).

Previous work in our laboratory determined an optimal MLL combination of magnitude, frequency, and duration for recovery of peak isometric torque post eccentric exercise (0.5Hz, 10N, 15min) (18). The current study utilizes this protocol in order to study the effects of MLL timing post exercise induced muscle injury on recovery of active muscle properties and magnitude of inflammatory cell infiltration. To this end, three conditions were compared; MLL immediately post EEX, 48 hours post EEX, or no-MLL, exercised control. Seven days post nerve cuff implantation, each rabbit was randomly assigned to a protocol, with all 18 animals initially undergoing an EEX protocol and then receiving MLL or no-MLL (control) either immediately post exercise or 48 hours post exercise. Six rabbits were assigned to each of the three conditions.

Torque-Joint Angle Properties and Eccentric Exercise Protocol

Rabbits were anesthetized and secured supine with one foot attached to a foot pedal that allowed isometric T- θ analysis and implemented a bout of EEX (8, 18). Twenty-one measurements obtained in 5° increments from 55° to 155° were taken to obtain a T- θ relationship. An isometric contraction was elicited by supramaximal stimulation, three times the α -motoneuron threshold voltage of the peroneal nerve, at each 5° increment with a two minute rest between each measurement to minimize fatigue.

Seven sets of ten cyclic lengthening contractions comprised the bout of eccentric exercise. Muscle activation was preceded by stretch of the TA muscle-tendon unit by 100ms for all 70 lengthening contractions. The ankle moved within a tibiotarsal angle of 95° to 145° of plantarflexion at 150°s⁻¹ during each contraction. Two minutes post EEX repeat T- θ measurements were performed to assess the effect of EEX.

MLL Protocol

All protocols were carried out daily (~24 hours apart) for 15 minutes on four consecutive days after initial application (day of injury (day 1) for immediate protocol and two days post injury (day 3) for the delayed protocol). The MLL was applied using a customized device for application of length-wise strokes (19, 48). The self-designed MLL device allowed the capability to produce repeatable deficits in muscle function following eccentric exercise and to apply quantifiable, repeatable compressive loads via a computerized, mechanical device in order to methodically follow recovery and healing. This device has been shown to be far more accurate and precise in quantifying tissue forces (19) than the one used in our initial studies (8). Post daily MLL, animals were recovered from anesthesia, returned to cages, given food and water ad libitum. One day post final MLL or no-MLL bout, a final T- θ relationship was obtained to assess the effects of the four day protocol. Post final T- θ measurements, animals were euthanized with intravenous KCL administered during deep anesthesia (5% Isoflurane) (day 5 for immediate MLL and day 7 for delayed MLL).

Analysis of T- θ Recovery

A recovery index (RI) was calculated to compare the three protocols and their effects on recovery of peak isometric torque. RI was the difference of peak torque at three fixed time points (pre-EEX, immediate post-EEX, and post four days of MLL protocol) (18).

$$RI = \left(1 - \left(\frac{(pre_exercise - post_4days\ MLL)}{pre_exercise} \right) \left(\frac{(pre_exercise - post_exercise)}{pre_exercise} \right) \right) \quad (Eq\ 1)$$

The denominator measured loss of torque post exercise relative to pre-EEX and the numerator measured torque post four day MLL protocol relative to pre-EEX.

In order to investigate the effect of MLL over the whole tested angle range, the area under the curve (AUC) for the entire T- θ relationship (55 angles) was calculated using the trapezoidal method. The RI equation was also used to calculate an RI for AUC. Additionally, the angle corresponding to the peak torque output for pre-EEX, post-EEX, and post-MLL or no-MLL protocol was compared in order to investigate the extent MLL shifted peak torque angle back to that of pre-exercise (18).

Immunohistochemical Analysis

Immediately after sacrifice, the tissue was harvested, weighed, flash frozen in liquid nitrogen, and carefully mounted on cork and oriented perpendicular to the long axis of the muscle. TA muscles were sectioned at 8 μ m thickness for immunohistochemistry staining. Sectioned tissues were fixed for 10 minutes in ice cold acetone, washed in 0.1M PBS then

quenched for 10 minutes in 3% H₂O₂ and washed again in 0.1M PBS. Sections were then blocked for 1h at room temperature (RT) with 2% bovine serum albumin (BSA) in PBS. Muscle sections were incubated with the primary antibody (Mouse anti-Rabbit Macrophage Clone RAM11 (1:50) Dako, Carpinteria, CA or Mouse anti-Rabbit CD11b (1:50) or Mouse anti-Rabbit T-Cells and Neutrophils RPN3/57 (1:50) AbD Serotec, Raleigh, NC) for 90 minutes at RT. Sections were then washed with 0.1M PBS with 0.1% Tween-20 before incubation in ImmPRESS anti-mouse Ig Detection Kit (Vector Labs, Burlingame, CA) for 30 minutes at RT. Sections were then washed in 0.1M PBS with 0.1% Tween-20 before incubation in TSA amplification kit for 20min at room temperature. After a final wash in 0.1M PBS with 0.1% Tween-20, sections were stained with Dapi and coverslipped with Vectashield (Vector Labs, Burlingame, CA).

Muscles were randomly sectioned within the MLL area and viewed with a Zeiss Axio Imager M.1 microscope (Carl Zeiss, Thornwood, NY) at 20x. Five random fields for each muscle with each of the three antibodies were photographed for cell quantification. Positively labeled cells were then counted for each of the five photos and the numbers were averaged for each animal limb by two blinded individuals. Only stained areas that co-localized with Dapi stained nuclei were counted as positively stained cells. CD11b antibody was used primarily to identify neutrophils, however it also recognizes other granulocytes, macrophages, blood monocytes, lymphocytes, and bone marrow cell (36) and the RPN3/57 antibody detects an uncharacterized antigen on neutrophils, T lymphocytes, thymocytes, and platelets. However, RAM11 is specific to the detection of rabbit macrophages (46).

Statistical Analysis

Recovery index for both peak torque relationship and area under the curve as well as joint angle shift and muscle wet weight were described with a sample mean and SE and were compared between protocols using Analysis of variance (ANOVA) to assess the effects of MLL protocol on muscle function, with the level of significance set at $P < 0.05$. For the immunohistochemical data, as the observations from the same rabbit are correlated, the linear mixed models were used with the level of significance set at $P < 0.05$. PS: Power and Sample Size Calculation version 3.0, 2009 was used to perform a *post hoc power analysis* to differentiate significant effects with a statistical power of 0.87 for torque output analyses. All other analyses were performed using SAS/STAT software, Version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

EEX produced an average 51% ($\pm 13\%$) decrease in peak isometric torque ($n=18$; 6 immediate, 6 delayed, and 6 no-MLL). Both immediate and delayed application of four consecutive days of MLL showed significant improvement in the recovery of peak torque output as compared to the EEX, no-MLL control ($p < 0.0001$ and $p = 0.02$, respectively). Immediate application of MLL produced an average 129% difference in peak torque recovery as compared to the exercised, no-MLL control ($p < 0.0001$). The 48 hour delay of the same four day MLL protocol produced an average 82% difference of peak torque recovery compared to control ($p = 0.02$). There was a 64% difference in peak torque recovery between immediate and delayed application of MLL in favor of immediate ($p = 0.0012$) (Figure 1).

Area under curve

Area under the T- θ curve (AUC) for pre- exercise, post- exercise, and post MLL protocol was examined as a secondary indicator of recovery. Similar to peak torque analysis, application of MLL beginning immediately post EEX showed the highest AUC recovery

index at 0.86 ± 0.08 with a 119% difference from control (Figure 2). There was a significant difference for both applications of MLL as compared to control ($RI = 0.86 \pm 0.08$ vs. 0.22 ± 0.06 , $p < 0.0001$ immediate and $RI = 0.22 \pm 0.06$ vs. 0.61 ± 0.10 , $p = 0.0051$ delayed). There was greater recovery for the immediate MLL as compared to the delayed MLL with a 34% difference of RI_AUC between the two groups, however not significant ($p = 0.0526$).

Joint-angle shift

The effect of the EEX bout on the torque-angle curve was calculated by subtracting the mean peak isometric torque angle pre exercise bout from the corresponding post exercise angle and from each MLL protocol. Exercise produced an average $10^\circ \pm 0.2$ rightward shift from pre exercise peak isometric torque angle ($n = 18$, $p = .006$). Four consecutive days of MLL applied immediately produced an average $-5.6^\circ \pm 2.8$ leftward angular shift from the post exercise peak isometric torque angle (day 5) while delayed MLL produced a $-9.7^\circ \pm 0.8$ leftward angular shift from post exercise peak isometric torque angle (day 7) ($p = 0.28$ and $p = 0.03$, respectively). Exercised, no-MLL control produced a $-3.8^\circ \pm 3.9$ leftward angular shift from post exercise peak isometric torque angle (day 5, $p = 0.47$) (Figure 3).

Muscle Wet Weight & Immunohistochemistry

Average muscle wet weight for the exercised, control group ($3.77 \pm 0.09g$) was higher than for both the immediate ($2.47 \pm 0.12g$) and delayed ($3.19 \pm 0.19g$) MLL groups ($p < 0.0001$ and 0.012 , respectively). There was also a difference in wet weight between the immediate and delayed MLL groups ($p = 0.002$) (Figure 4).

There was a 0.94 correlation between the two blinded counters. Immediate MLL showed a 53.4% and 69.8% decrease in the respective number of RPN3/57 and CD11b positive cells infiltrating the muscle compared to EEX, no-MLL animals (30.9 ± 7.7 vs. 14.4 ± 2.4 for RPN3/57 and 29.3 ± 9.2 vs. 8.8 ± 2.9 for CD11b, $p = 0.28$ and 0.33 , respectively) (Figure 5). Delayed MLL showed a 35.09% decrease and a 40.8% increase in the number of RPN3/57 and CD11b positive cells, respectively, infiltrating the muscle compared to EEX, no-MLL animals (30.9 ± 7.7 vs. 14.4 ± 2.4 for RPN3/57 and 29.3 ± 9.2 vs. 8.8 ± 2.9 for CD11b, $p = 0.28$ and 0.33 , respectively). There was a 39.3% and 366.0% increase in the number of RPN3/57 and CD11b positive cells from immediate to delayed MLL protocols ($p = 0.71$ and 0.12 , respectively) (Figure 6A). There was a 38.9% and 7.9% decrease in the number of RAM11 positive macrophages between the EEX, no-MLL (11.1 ± 2.6) and the immediate (6.8 ± 1.9 , $p = 0.45$) and delayed MLL (10.3 ± 2.5 , $p = 0.88$) protocols, respectively. Delayed MLL produced a 50.9% increase in the number of RAM11 positive macrophages as compared to immediate MLL, $p = 0.54$ (Figure 6B).

DISCUSSION

This study shows using an *in vivo* animal model, the ability of both immediate and 48 hour delayed MLL to accelerate recovery of muscle and joint function following a controlled bout of EEX. To our knowledge, we showed for the first time however that there is a time - dependent effect of MLL on the recovery of mechanical properties as well as evidence for MLL to decrease muscle edema and modulate inflammatory cell infiltration. The immediate MLL protocol produced the greatest recovery of peak isometric torque as compared to both delayed MLL and EEX, no-MLL protocols. Our animal model and self-designed device allowed the capability to produce repeatable deficits in muscle function following eccentric exercise and to apply quantifiable, repeatable compressive loads via a computerized, mechanical device in order to methodically follow recovery and healing. Similar experiments in humans will be important in determining the optimal use and indications for MLL and recovery of muscle function following intense eccentric exercise.

Our findings build on our previous work (8, 18) by demonstrating that massage carried out immediately following a bout of intense eccentric exercise favors a quicker recovery of muscle and joint function than massage delayed by 48 hours. Interestingly, in the current study the immediate application of 0.5Hz, 10N, 15min protocol produced an average recovery index of $RI=0.94$, similar to our previous study in which this set of loading conditions produced an $RI=1.08$. The RI takes into account the loss of torque post-exercise relative to pre-exercise and therefore is a more complete measure of recovery than peak isometric torque alone, building on our initial work where we only evaluated peak torque as a measure of recovery (8). Area under the T- Θ curve was shown in the present study to be a secondary indicator of recovery with MLL from the bout of exercise. The same trend as was seen for peak torque recovery was seen using this measure with immediate MLL producing the greatest RI followed by delayed and control in order of average RI value. The current study, in combination with previous investigations in our laboratory, shows a highly reproducible exercise and injury protocol that allows us to systematically investigate a number of dependent variables using the smallest sample size possible (8, 18).

Research has suggested that the inflammatory process is responsible not only for successful repair but may also act to extend the damage process (23, 40). This progression of injury could occur through a variety of mechanisms, including neutrophil infiltration and activation of the respiratory burst (40, 42). Invading leucocytes, neutrophils and macrophages, can exacerbate the initial mechanical damage and elicit unwanted tissue destruction to healing muscle through the release of oxygen-derived free radicals and proteases which potentially cause injury (31, 41). In the current study, muscle wet weight was measured as an indicator of tissue edema. Results showed a significant difference between the three protocols, with weight of the exercised, control group higher than both the immediate and delayed MLL groups. There was also a significant difference in wet weight between the immediate and delayed MLL protocols, suggesting a difference in fluid dissipation from the exercised muscle dependent upon timing of MLL application. Surprisingly, we did not observe statistical differences in leukocyte infiltration for either the immediate or delayed MLL conditions when compared to the non-massaged controls. Despite lack of statistical differences, the large percent differences (up to 366%) between the immediate MLL and control conditions may still be of clinical significance, however this is challenging to test using our animal model. Immediate MLL also produced a modulation of the immune response as compared to control (53.4% and 69.7% decrease for neutrophils and 38.9% for macrophages), again suggesting an effect on the host inflammatory response. It may be noteworthy that we used quantitative immunohistochemistry to definitely characterize the presence of both neutrophils and macrophages rather than simple H&E staining used in our previous work (8).

Smith et al. studied the effect of massage on inflammation and delayed onset muscle soreness in humans, with results indicating that a 30 minute massage protocol applied two hours after the exercise interfered with neutrophil emigration (38). Crane et al. found that 10 minute massage following a controlled bout of upright cycling in humans reduced pro-inflammatory cytokine expression while promoting mitochondrial biogenesis (12). Previous work in our lab has shown that four consecutive days MLL led to an accelerated recovery of isometric torque production in addition to a decrease in the number of torn fibers (18). Additional studies have shown that MLL can modulate the passive stiffness of skeletal muscle with both an acute (daily) and a cumulative (over four-day treatment) change in the muscle's viscoelastic properties (19). These observations provide, to our knowledge, the first objective, quantifiable evidence to support the various assertions about massage and its physical effect on tissues (22). Evidence suggests that massage may work by affecting multiple mechanisms that cause delayed onset muscle soreness and it may be a combination of these factors that allow for the beneficial effect of this modality. In the current work, we

chose to compare the 0hr (immediate) and 48hr (delayed) strategies as a starting point to determine an optimal timing for use of massage. Given the findings noted herein, future studies may examine additional time points such as massage-like loading commenced 24hours after exercise to determine its effect on recovery of muscle function and tissue inflammation and whether or not this strategy provides for an even more enhanced recovery. Moreover, possible biologic mechanisms to account for the accelerated recovery of muscle function such as reduced pro-inflammatory cytokine expression and myogenic upregulation should be explored in our model.

The rightward shift in peak torque angle produced by the bout of eccentric exercise is consistent with previous studies from our lab and others (1, 6, 18). Herein, both MLL protocols resulted in a leftward shift from the post exercise peak torque angle, indicating some degree of muscle recovery from EEX (7). However, shift in peak torque has been shown not to simply be an indicator of muscle damage, but possibly a combination of both muscle damage and fatigue (1). While delayed MLL produced the greatest leftward angular shift in the current study, there was no statistically significant difference from the immediate MLL angular shift. The clinical significance of this finding is not certain but does suggest a benefit to MLL.

While we acknowledge that the injury created in our animal model may not be completely analogous to the injury produced in humans with eccentric exercise, the high torque deficits produced by the exercise in our model allow for differentiation of the effects of MLL and natural healing. Additionally, in our model we utilize maximum motor unit recruitment by stimulating the muscle to tetanus in order to determine the full extent of muscle damage by activating any motor unit that is capable of firing. While this is not how human muscle functions, our results demonstrate the effects of timing of MLL on recovery of muscle function, irrespective of motor unit recruitment and potential confounding variables such as pain and motivation. Moreover, there may be a difference between statistical and clinical implications of our findings. It is well documented that regional strains within exercised muscle result in focal damage and therefore, regional and localized tissue inflammation (15). While muscle tissue is a 3D structure, the immunohistochemical analysis in the present study is based on a set of samples from a single slice of the muscle which may not be representative of the entire muscle and therefore may not accurately characterize the non-normal distribution of injury occurring throughout that 3D structure. Additionally, the non-normal distribution of injury may cause the appearance of substantial variation within animal, thereby making statistical significance difficult to serve as a true representation of the biological response.

In conclusion, we have shown that skeletal muscle responds to MLL in a time dependent fashion with application of MLL immediately following damaging EEX attenuating the infiltration of neutrophils and macrophages in skeletal muscle, and facilitating recovery of function to near pre-exercise levels. Delayed application of MLL following EEX was less effective in restoring function, and was associated with more edema and greater infiltration of immune cells. These data provide support for the immunomodulating effects of MLL and its effect on recovery of function following muscle injury. This study allows greater understanding of how eccentric exercise induced injury alters muscle function and the ability of quantifiable massage-like loading to both enhance muscle recovery and influence the host inflammatory response, suggesting further the need for human studies to confirm the efficacy of this modality in recovery from eccentric exercise.

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Figure 1.

Immediate application of MLL produced greatest recovery as compared to delayed MLL (RI=0.93 ± 0.08 vs. 0.48 ± 0.09, respectively). However both applications of MLL produced a significantly greater recovery than control (RI=0.20 ± 0.06).

Figure 2.

RI_AUC served as a secondary indicator of muscle functional recovery with immediate application of MLL producing greatest recovery of peak torque and AUC as compared to both delayed MLL and control.

Figure 3.

The angle corresponding to peak torque output shifted rightward due to eccentric exercise. All three protocols, control, immediate MLL, and delayed MLL, produced a leftward shift of peak torque angle back towards pre exercise angle indicating recovery from muscle injury.

Figure 4.

Muscle wet weight for the control was higher than both the immediate ($p<0.0001$) and delayed MLL ($p=0.012$). There was also a difference in wet weight between immediate and delayed ($p=0.002$).

Figure 5.

Representative immunohistochemistry images for the control, delayed, and immediate protocols, showing fluorescence for both macrophage and neutrophil presence. Both control and delayed muscle showed a greater presence of RPN3/57 & CD11b positive neutrophils and RAM11 positive macrophages as compared to immediate MLL.

Figure 6.

A&B: Neutrophil (RPN3/57 and CD11b) infiltration per protocol and macrophage (RAM11) infiltration per protocol. Delayed MLL showed an increase in the number of RPN3/57 and CD11b positive cells as compared to immediate MLL. There was a decrease in the number of RAM11 positive macrophages between the EEX, no-MLL and immediate and delayed MLL protocols. Delayed MLL showed an increase in the number of RAM11 positive macrophages as compared to immediate MLL.