Effect of Massage-Like Compressive Loading on Muscle Mechanical Properties

DISSERTATION

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Abstract

Acute and chronic skeletal muscle pain and weakness are among the most common ailments treated by physicians. Minimizing this pain and weakness is of critical importance considering high exercise attrition rate in patients with muscle dysfunction, as well as the direct economic burden of physical inactivity, in excess of 76 billion dollars annually in the United States. Although the effectiveness of massage for overcoming muscle pain and weakness following exercise is limited to a few high quality studies, Americans make more than 160 million visits annually to seek relief of musculoskeletal weakness and pain by manipulative, body-based practices. However, neither the mechanisms of actions nor the effectiveness or optimal strategies for massage therapies have been conclusively demonstrated.

In order to achieve an optimal efficacy for any therapy, it is essential to understand the molecular basis of its actions. In this dissertation, *in vivo* mechanisms of massage-mediated biomechanical signals that may limit muscle inflammation, weakness, and damage were explored. We determined a combined magnitude, frequency, and duration of massage (via a customized pneumatic device) that optimized functional recovery following eccentric exercise through quantification of active (peak isometric torque) and passive (stress relaxation) muscle mechanical properties. In particular by: (A) identifying the most effective combination of massage parameters (0.25 or 0.5 Hz, 5 or 10N, 15 or 30 min), (B) quantifying muscle membrane disruption and myofiber damage,
and (C) using the quasi-linear viscoelasticity (QLV) model to study passive time-dependent responses of skeletal muscle to repeated massage-like compressive loading (MLL) following damaging eccentric exercise.

Mechanical properties of the tibialis anterior of New Zealand White rabbits were tested prior to one bout of eccentric exercise, post exercise, and pre and post four consecutive days of massage. The contralateral hind limb served as the non-exercised control. The 0.5Hz, 10N, 15min protocol produced greatest peak torque recovery, values approximately equal to pre-eccentric exercise (EEX). There were no significant interactions between or among the parameters. This is the first evidence of a dose-response effect for magnitude and frequency of massage on recovery of in vivo active muscle properties following EEX. This relationship was also seen for the passive properties, with the 0.5Hz, 10N, 15 min protocol showing the an accelerated recovery of muscle viscoelastic properties. With no significant differences in either instantaneous elastic or reduced relaxation response of the muscle between the 0.5Hz, 10N, 15 and 30 min protocols.

The previous work was extended by comparing the effect of immediate vs. delayed application of massage on peak torque recovery following intense EEX. While there is clinical significance of both immediate and delayed massage producing enhanced recovery compared to non-massaged control animals, massage beginning 48 hours post EEX had a significantly diminished effect in restoring function of EEX muscle compared to immediate massage. These data provide a starting point for linking the mechanical properties of skeletal muscle with physical therapies, and may shed light on
the design and optimization of therapeutic massage based therapies for recovery from EEX in humans.
Dedication

This document is dedicated to my family for their constant support, guidance, and unconditional love.
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Fields of Study

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Chapter 1: Introduction

The human body depends on muscles for all activities that involve movement. The 650 skeletal muscles in the human body make up 40-50% of human body mass. There are three types of muscle in the human body, skeletal, cardiac, and smooth which have four distinct characteristics that differentiate them from other body tissues. They are extensible with the ability to increase in length, elastic with the ability to return to their original length after stretch, excitable with the ability to respond to a stimulus such as an action potential or a mechanical force, and contractile with the ability to develop tension. Skeletal muscle is responsible for all voluntary movements, including contraction for movement and respiration, metabolic activities including glucose metabolism, insulin storage, and glycogen storage, protective functions for the joint and organs, as well as thermoregulation including shivering and dissipation of heat.

1.1 Microstructure

Skeletal muscle is comprised of both structural and contractile elements with the contractile elements pulling on the structural elements to produce movement. A skeletal muscle is ensheathed by a layer of tight connective tissue, the epimysium. Muscle is made up of fascicles, muscle fiber bundles, surrounded by a less dense connective tissue, the perimysium. Muscle fibers, form the basic cellular unit of skeletal muscle, separated from each other by a layer of loose connective tissue, the endomysium. This network of connective tissue forms a continuum that extends from the outside of muscle, the
epimysium to the individual fibers, the endomysium with the spaces filled by muscle fibers (Mense & Gerwin, 2010). The connective tissue network maintains shape and length of the muscle after deformation. While this system does not actively contract, it transmits force and protects the muscle from over lengthening (Mense & Gerwin, 2010). Each muscle fiber is made up of thousands of myofibrils, specialized contractile organelles within the muscle fiber, that are comprised of repeating segments called sarcomeres. The sarcomere is the functional unit of muscle and contains the contractile elements known as the thick and thin molecular filaments. Thick filaments are made up of the protein myosin with a rodlike tail that terminates in two globular heads. The tail is two interwoven heavy polypeptide chains with globular heads on the ends of the chains. The heads link the thick and thin filaments together to form cross bridges during contraction. The cross bridges act like a molecular motor to generate tension during a contraction. Thick filaments contain about 200 myosin molecules bundled together with a smooth center made up of the tails and ends are studded with the heads facing outward and in opposite directions, creating a staggered array that also serve as ATP binding sites and ATPase enzymes that split ATP to generate energy for muscle contraction.

Thin filaments are made up of actin which is comprised of subunits called globular actin that serve as the active sites to which the myosin heads bind during contraction. The backbone of each thin filament is formed by an actin filament that coils back on itself forming a helical structure. Regulatory proteins are present in the thin filament, two strands of tropomyosin, a rod-shaped protein, spiral about the actin core. They are arranged end-to-end along the actin filaments (Figure 1). When a muscle is relaxed, they
block actin’s active sites so that the myosin heads cannot bind. The other major protein in
the thin filament, troponin is a three-polypeptide complex made up of TnI, an inhibitory
subunit that binds to actin, TnT, that binds to tropomyosin and helps position it on actin
and TnC, that binds calcium ions. Both troponin and tropomyosin help to modulate the
myosin-actin interactions involved in contraction (Einhorn et al., 2007, Oatis, 2009 and
Lieber, 2010).

A pattern of bands exists in the sarcomere due to myofilament overlapping. A sarcomere
is the area between two Z lines, a dark line that runs through the center of the lighter I
band. The myosin filament interdigitates with the thin actin filament that is fixed to the Z
disk. The isotropic (I) band on both sides of the Z line contains only actin filaments. The
anisotropic (A) band contains both actin and myosin filaments with the exception of the
middle portion which is free from actin. The M-line consists of proteins that are important for the stability of the sarcomere structure; they crosslink the myosin filaments. During contraction, the actin and myosin filaments slide against each other. The I band becomes narrower, while the A band maintains a constant width. When the muscle is relaxed, actin does not completely overlap myosin. Another important molecule, titin, is the largest protein molecule of the human body and connects the myosin filament with the Z disk. It is long coiled molecule that acts like a spring in order to return the sarcomere back to its original length after stretch and contributes to the elastic stiffness of a muscle. Titin does not resist stretching in the ordinary range of extension, but it stiffens as it uncoils, helping the muscle to resist excessive stretching and is therefore considered important in protection against injury (Einhorn et al., 2007, Oatis, 2009 and Lieber, 2010).

1.2 Sliding Filament Theory

Skeletal muscle has two states, relaxed or contracted. Contracted muscle shortens (or lengthens) while a relaxed muscle will not change length unless acted upon by an external force. Muscular contraction is a result of the myosin heads attaching to the actin at both ends of the sarcomere and pulling the thin filaments toward the sarcomere center by swiveling inward. The myosin head pivots to pull the thin filaments inward toward the center of the sarcomere, then releases to allow the thick and thin filaments to slide past one another. This power stroke, fueled by ATP hydrolysis, is initiated by the release and binding of calcium ions to thin filaments. In the resting state, the binding sites for the myosin heads on the globular molecules of the actin chain are inaccessible due to the
position of tropomyosin. When an action potential releases Ca\textsuperscript{2+} from the sarcoplasmic reticulum and the Ca\textsuperscript{2+} concentration in the cytoplasm reaches a critical concentration, troponin C that covers the binding site for myosin moves aside, and the myosin heads attach to the actin molecules. Subsequently, the neck of the myosin heads makes a power stroke and pulls the actin filament towards the M line. Since the attached myosin heads have the action of an ATPase, energy-rich ATP molecules are cleaved. The released energy is used for separating the myosin heads from the actin. By repeating this sequence of attaching, bending, and separating, the myosin heads make a rowing movement which leads to the sliding movement of the filaments and sarcomere shortening (Figure 2).

Figure 2: Titin is the largest protein molecule of the human body; it consists of approximately 30,000 amino acids. The protein connects the myosin filaments with the Z disks (a) and has also connections with the actin filaments. It has a coiled structure, and functions as a spring that helps brings the sarcomere back to its original length after muscle stretch (b). With kind permission from Springer Science+Business Media: <Muscle Pain: Understanding the Mechanisms, Chapter 2: Functional Anatomy of Muscle: Muscle, Nociceptors, and Afferent Fibers, 2010, Page 20, S. Mense and R.D. Gerwin), Figure 2.2>

1.3 Macroscopic Structure

The macroscopic arrangement of muscle fibers influences muscle architecture and serves as the primary determinant of musculoskeletal function (Lieber and Friden, 2000,
Burkholder et al., 1994 & Gans and Bock, 1965). Muscle architecture is defined by multiple characteristics including muscle length (Lm), fiber length (Lf), pennation angle (Θ), and physiological cross-sectional area (PCSA). Muscle length is the distance from the origin of the most proximal muscle fibers to the insertion of the most distal fibers (Lieber et al., 1992). Fiber length can vary due to some fibers being oriented at an angle relative to the axis of force generation. Muscle PCSA is directly proportional to the maximum tetanic tension generated by the muscle and represents the sum of the cross-sectional areas of all the muscle fibers within the muscle.

\[
\text{PCSA (mm}^2) = \frac{\text{muscle mass (g) } \times \cos \Theta}{\rho \text{ (g/mm}^3) \times \text{fiber length (mm)}}
\]

where \(\rho\) is 1.056 g/cm\(^3\) for mammalian muscle.

Muscle is a highly structured and organized material with distribution of stress and strain in the tissue depending upon fiber orientation (Figure 3). The mechanical properties in the fiber direction are different from those in other directions. Muscles can be arranged as parallel, fusiform, or pennate. Fusiform and parallel have fibers running parallel to the line of action of the entire muscle. Pennate muscles run at an angle to the line of action of the muscle. Since muscle properties depend upon fiber orientation, the degree of anisotropy increases with the degree of pennation (uni, bi, or multi). There is extensive experimental evidence that muscle fibers do not extend the entire length of the muscle and even the entire length of the fascicle (Nigg & Herzog, 1999, Hall, 2003, Nordin & Frankel, 2001, & Chaffin & Andersson, 2006).
Figure 3: Pennate muscles have a fiber arrangement at an angle to the longitudinal axis of the muscle. The greater the angle of pennation, the smaller the amount of effective force transmitted to the tendon. Used with kind permission from Huei-Ming Chai, PT PhD, National Taiwan University.

The physiologic cross sectional area of a pennate muscle will be larger than a non-pennate muscle of the same size. While this allows higher force generation, it is not metabolically ideal. While pennation serves as a space conserving technique for the body, it causes a loss of muscle force as compared to a muscle with the same mass and fiber length with no pennation angle due to shorter muscle fibers and an angular line of action. In a pennate muscle the fascicles form a common angle with the tendon; this causes the muscle not to move the tendon as far as a parallel muscle is capable. However, the muscle contains more muscle fibers allowing more tension to be produced than a parallel muscle of the same size. Therefore a pennate muscle requires more energy to produce the same amount of force as a non-pennate muscle of the same specifications. Because fibers may be oriented at an angle relative to the axis of force generation, not all the fiber tensile force is transmitted to the in-series tendon. Only a component of muscle fiber force will actually be transmitted along the muscle axis, which will be \( F = x \cos \Theta \). The muscle fiber pulls with \( x \) units of force at an angle \( \Theta \) relative to the muscle axis of force generation. The greater the angle of fiber pennation, the smaller the amount of
force transmitted to the tendon; thereby energy is wasted by producing tension in
directions where it cannot be used (Lieber, 2010).

1.4 Types of Contractions

A concentric contraction is the active shortening of muscle where the myosin cross-
bridges attach and draw the actin proteins towards each other (Lieber, 2010), commonly
seen in the upswing of a weight during a bicep curl. An eccentric contraction is the
active lengthening of muscle with the myosin cross-bridges attaching and the actin
proteins moving away from each other as the opposing force is greater than the force of
the muscle, lengthening the sarcomere (Lieber, 2010), commonly seen in downhill
running. Isometric contractions occur when the muscle develops tension equal to the
opposing force; it is activated and held at a constant length. An example of this type of
contraction is carrying an object in front of you with the weight of the object pulling
downward, but hands and arms opposing the motion with equal force going upwards
(Figure 4).

Figure 4: A demonstration of the difference in force responses for between lengthening and non-
lengthening active contractions (isometric vs. eccentric), and between active lengthening (eccentric) vs.
non-active lengthening (passive stretch). With kind permission from Lippincott Williams & Wilkins:
<Skeletal Muscle Structure, Function, and Plasticity: the Physiological Basis of Rehabilitation, Third
Edition, Chapter 6: Skeletal Muscle Response to Injury, 2009, Page 247, Richard L Lieber PhD, Figure 6-23>
1.5 Viscoelasticity

Skeletal muscle is a nonhomogeneous, anisotropic tissue demonstrating nonlinear, viscoelastic properties (Bosboom et al., 2001). Viscoelastic materials have both fluid and solid properties with the elastic portion of their composition (solid like) deforming to a certain extent while the fluid like portion can continuously deform with continual application of force. These materials have time dependent responses that depend on how quickly the load is applied or removed. Another characteristic of a viscoelastic material is energy dissipation. If a viscoelastic material is loaded and unloaded, the unloading curve will follow the loading curve with a phase lag, a property known as hysteresis.

In order to characterize a tissue’s viscoelastic behavior, stress relaxation and creep tests are often performed. A creep test consists of placing a defined load on a specimen and holding the load constant. Viscoelastic behavior is observed if the strain in the specimen increases even though the load is held constant. A stress relaxation test is performed by compressing or stretching a specimen to a known displacement and then holding that level of compression or tension. Viscoelastic behavior is observed if the stress in the specimen decreases even though the displacement is held constant.

By making various combinations of spring and dashpot models, we can model the behavior of a viscoelastic material. The standard linear viscoelastic model is the most commonly used constitutive model for muscle viscoelasticity. It provides a more accurate viscoelastic response than either the Maxwell or the Kelvin-Voigt models (Figure 5). For the standard viscoelastic model, $E_1$ represents the magnitude of the combined modulus and the stress magnitude. The spring and damper in series represents
the creep of the material and the slope of the relaxation response. E2 affects the 
magnitude of the instantaneous stress while the damper (η) affects the relaxation time. 
Stress relaxation behavior is not well captured by the simpler models (Figure 5A&B); 
with the Maxwell model showing the tissue response returning to zero stress and the 
Kelvin-Voigt model not accurately capturing the stress relaxation behavior. However, 
the standard linear viscoelastic model converges to a steady state of stress with the series 
elements describing the slope of the relaxation response with the spring constant (E2) 
controlling the magnitude of the instantaneous stress, the portion that is able to relax over 
time, while the damper (η) affects the relaxation time. Under creep conditions, the 
Maxwell model tissue response does not increase linearly to infinity and the Kelvin-Voigt 
model shows small amounts of strain when load is applied and does not respond 
instantaneously to load due to the spring and damper in series. However by adding the 
spring (E1) in series, the standard linear viscoelastic model is able to have an 
instantaneous response to loading and the model also converges to a steady state of stress.

While the above mentioned constitutive models serve as great mechanical analogs for materials that undergo small strain, soft tissue is known to experience large deformation. Fung observed that the percentage of stress relaxation was linearly related to the applied stress and the stress-strain behavior of soft tissues in nonlinear (1972). Therefore, he proposed the concept of quasi-linear viscoelasticity (QLV) theory in order to account for materials with time dependent viscoelastic behavior that undergo large deformation. The QLV model utilizes a separation of the stress relaxation function into a time dependent reduced relaxation function and an instantaneous elastic response that depends on stretch ratio. While the theory is linear in the relaxation response, it is still able to account for nonlinear large deformation. The QLV theory assumes that the stress relaxation behavior of soft-tissue can be expressed as $\sigma(t) = G(t) * \sigma^e(\epsilon)$ where $\sigma^e(\epsilon)$ represents the instantaneous elastic response, a nonlinear function describing the stress in response to
an instantaneous input of strain and \( G(t) \) is the reduced relaxation function that represents
the time-dependent stress response of the tissue normalized by the peak stress following a
step input of strain [i.e., \( t = 0^+ \), such that \( G(t) = \sigma(t)/\sigma(0^+) \), and \( G(0^+) = 1 \)]
(Abramowitch, 2004).

Skeletal muscle properties are a function of the integrated functions of the structural
Schleip 2006, and Gavronski et al., 2007). The viscoelasticity of skeletal muscle arises
primarily from the series elastic component, \( E_1 \) of the standard linear viscoelastic model
(Figure 5). The muscle membrane structure and fascia serve as the parallel elastic
component, \( E_2 \), while the muscle fiber itself serves as the contractile component, \( \mu \).

Muscle transforms chemical energy into mechanical energy. The makeup of
intramuscular connective tissue is muscle specific with regard to function and therefore,
viscoelastic properties are muscle specific (Mutungi and Ranatunga 1996). Within the
muscle, both connective tissue and muscle fibers absorb strain to prevent injuries.

Anisotropy is the mechanical property of being directionally dependent, showing
differences when measured along different axes. The anisotropy of the tissue is
important for the methodology of testing the rheological properties of muscle and
therefore the interpretation of their results. Studies by Van Loocke et al have shown that
ramp and hold tests at different fiber orientations produced a stiffening effect with
compression rate, especially in directions that approximate fiber direction (2008).
Therefore, viscoelastic behavior is dependent on both compression rate and fiber
orientation. Accordingly, when testing creep and relaxation responses of the muscle, it is
necessary to test in the same location in order to ensure results that can be compared and interpreted correctly (Roylance, 2001, Findley et al., 1976, Conroy, 2004, and Ferry, 1980).

1.6 Eccentric Exercise

Lengthening contractions, commonly referred to as eccentric contractions, have been shown to result in fiber injury in-vitro (Gosselin and Burton, 2002; Yeung et al., 2002a; Talbot and Morgan, 1998;), in-situ (Koh and Brooks, 2001; Brooks and Faulkner, 2001; Best et al., 1998) and in-vivo (Prou et al., 1999; Friden, 1984; Fielding et al., 1993; Butterfield, 2005). Eccentric contractions are physiologically common with a large amount of muscle's normal activity occurring in this manner (Goslow et al. 1973; Hoffer et al. 1989). There are many advantages of eccentric exercise including improved muscle size (Behm, 1995, Enoka, 2002, and Kraemer, 2005), strength, and fiber metabolism (Keogh 1996, Noakes, 2001, Tesch, 2005). The main disadvantages of repeated eccentric muscle contractions include: delayed onset muscle soreness, damaged muscle cells, reduced neural reflexes, acute strength loss and an altered resting state (Allen, 2001). Eccentric exercise results in measurable effects on the torque-angle or force-length relationship (FLR), as peak force is produced at longer muscle lengths following exercise (Jones et al., 1997, Talbot & Morgan, 1990 & 1996). It is well accepted that this rightward shift of the FLR typically mimics a variety of human (Brockett et al., 2004 and Jones et al., 1997) as well as single fiber (Morgan, 1990) and animal (Lieber et al., 1994) muscle fatigue/damage models. Eccentric contractions result in stress on fewer motor units and often lead to an immediate and prolonged reduction in the force- and power-
generating capacity of the muscle (Eston 2003). Eccentric exercise-induced muscle
damage is initiated by mechanical forces (Proske & Morgan 2001; Friden & Lieber 1992;
Jones et al. 1989) which disrupt the contractile component of the muscle tissue,
particularly at the level of the Z line (Friden & Lieber 1992), in addition to more
widespread disruption of the sarcomere structure (Newham et al., 1986) (Figure 6).
Strength loss after a bout of damaging exercise is not uniform across joint angles; there is
a disproportionate loss of strength at short versus optimal or long muscle lengths
(Philippou et al. 2003; McHugh & Tetro 2003; Byrne & Eston 2002a; Byrne et al 2001;
Child et al. 1998; Saxton & Donnelly, 1996). Eccentric exercise results in a redistribution
of sarcomere lengths with muscle lengthening not occurring in a uniform amount for all
sarcomeres and over-extended sarcomeres failing to re-interdigitate upon relaxation
(Proske & Morgan, 2001; Morgan 1990). The injury is therefore primarily a mechanical
disruption to sarcomeres (Warren et al., 1993) followed by a delayed secondary
inflammatory response that arguably leads to further injury (Gleeson et al., 1995).
Following a bout of eccentric exercise, the shift in optimal angle for torque generation
correlates to a rightward shift of the length-tension curve towards longer muscle lengths
(McHugh & Tetro 2003; Whitehead et al. 1998; Jones et al. 1997 & Eston et al., 2003).
1.7 Delayed Onset Muscle Soreness

An injured skeletal muscle heals in a consistent pattern regardless of injury mechanism, a three phase process including destruction, repair, and the remodeling phases (Hurme, 1991), (Kalimo, 1997). The destruction phase includes myofiber rupture and necrosis, formation of a hematoma between the ruptured muscles, and the inflammatory cell infiltration. The repair phase is characterized by phagocytosis of necrotized tissue, myofiber regeneration, and capillary ingrowth into the injured area. Remodeling includes maturation of the regenerated myofibers, contraction and reorganization of scar tissue, and recovery of the muscle’s functional capacity (Kalimo, 1997).

Eccentric exercise causes injury to the cell membrane that 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 (Peake, 2005). Leukotrienes are a potent...
inflammatory mediator that increase vascular permeability and act as neutrophil chemoattractants (Hertel, 1997). The immune system plays a role in the degeneration and regeneration of skeletal muscle and surrounding connective tissue. Immediately after exercise, neutrophils are mobilized into the circulation and invade the damaged muscle tissue within several hours (Beaton et al, 2002, MacIntyre et al., 1996, MacIntyre, 2000, Malm 2000, Raastad, 2003, and Stupka, 2001). Macrophages are present in muscle from 24 h to 14 days after exercise (Beaton, 2002, Hamada, 2005, Peterson, 2003, Round, 1987). Both neutrophils and macrophages contribute to the further degradation of damaged muscle tissue by release of reactive oxygen and nitrogen species (Nguyen 2003), and may also produce pro-inflammatory cytokines (Cannon, 1998). Evidence suggests that reactive oxygen species (ROS) such as superoxide produced by neutrophils and nitric oxide (NO) generated by macrophages contribute to muscle damage (Nguyen, 2003, Tidball, 1991, Hirata, 2003, Chazaud, 2003 and Jarvinen, 2005, Brickson et al 1999).

Delayed onset muscle soreness (DOMS) is characterized by loss of strength, pain, muscle tenderness, stiffness, swelling and elevated levels of creatine kinase (McHugh et al., 1999). DOMS is considered to be the product of a secondary inflammatory process coupled to the initial injury (Gleeson et al., 1995; Wilmore and Costill, 2004; Connolly et al., 2003) resulting from unaccustomed eccentric contractions (Taleg, 1973; Newman et al., 1983a, b; Armstrong, 1984; Denegar and Perrin, 1992 and Proske & Allen, 2005). It is proposed that the soreness may be the result of mechanical (Newman et al., 1983a, b; Armstrong, 1984; Stauber et al., 1990) or biochemical (Armstrong, 1984; McIntyre et al.,
1995) factors (Curtis et al., 2010). Research suggests that the soreness typically appears between 8 and 24 h post-exercise, peaks at 24 to 48 h and can last for up to 7 days (Cleak and Eston, 1992; Howell et al., 1993 and Balnave and Thompson, 1993). There are multiple theories to explain DOMS including: connective-tissue theory, cellular theory, and excitation-contraction uncoupling. The connective tissue theory proposes that it is the disruption of passive, non-contractile components in the sarcomere, sarcoplasmic reticulum, and connective tissue that causes the symptoms of DOMS (McHugh, 2000). These physical changes include dilation of the transverse tubule system, sarcolemma disruption, distortion of myofibrillar components, fragmentation of the sarcoplasmic reticulum, lesions to the plasma membrane, cytoskeletal damage, swollen mitochondria, and changes in the extracellular myofiber matrix (Enoka 1996). The cellular theory involves strain on the sarcomeres causing disruption of Z lines and A bands (McHugh, 2000). The lengthening of a muscle involves the stretching and ‘popping’ of individual sarcomeres as each attains its yield stress, acting as a mechanical disruption of the chemical actomyosin bond, whereas in normal cross-bridge cycles adenosine triphosphate is bound and detached in an orderly manner (Enoka 2002, Morgan, 1990 and Balnave & Allen, 1995). Excitation-contraction uncoupling is an impairment of plasmalemmal action potential conduction due to eccentric exercise. This causes a failure of excitation-contraction coupling, meaning that while a neuron may be sending an action potential, the muscle is not receiving the signal to contract (Warren et al., 1999).
1.8 Mechatransduction Effects of Skeletal Muscle

Cells are believed to be the transducers of external load, allowing the human body to respond to external forces through changes in stresses and strains. This suggests that mechanosensory cells adapt their activity based on the predominant mechanical environment (Turner, 1999). Biological tissues are responsive to mechanical forces with osteoblasts, osteocytes, endothelial cells, chondrocytes, and fibroblasts all responsive to mechanical loading (Jaasma et al., 2007, Klein et al., 1995, Kreke et al., 2005, Chen et al., 2003, & Breen, 2000).

A variety of loading techniques and modalities have been used to study the effect of applied forces on mechanical changes in human skeletal muscle (Challis et al., 2006; Challis et al., 2007; Zeng et al., 2008) as well as downstream biochemical signals and pathways (Akimau et al., 2005; Zelikovski et al., 1993). Human studies have shown that the in vitro responses of muscle to mechanical loading have been well studied (Tidball, 2005) and systems involving cultured cells and harvested tissue provide the ability to examine the effects of isolated deformation without introducing any other factors that are present in vivo. However, they do not offer insight on the influence of adjacent cells, the extracellular matrix or 3D tissue architecture on cell deformation and damage. Findings from both in vitro and in situ studies and their application to the physical properties of in vivo whole muscle and subsequent recovery response to compression loading are still not well understood.

Compression of skeletal muscle can lead to changes in both mechanical properties and function. Human studies provide the strongest evidence on the sustained influence of
compression on muscle function, stiffness and physiological effects. Animal studies provide the strongest evidence that under certain conditions muscle compression can even lead to tissue damage. The need for further research and the standardization of loading techniques and conditions is apparent due to lack of conclusive results in the literature. Such studies could provide the basis for a meta-analysis and more definitive conclusions on a better understanding of how muscle compression can potentially be used therapeutically. A more complete understanding of the effects of compression and shear loads on muscle mechanical properties is needed in order to better understand tissue tolerance levels to these forces that will ultimately lead to more scientific-based prevention and treatment approaches incorporating manual therapies.

1.8.1 Massage-Based Therapies

Massage therapy is one of the oldest treatments for disease. In 1812, Per Henrik Ling, a Swedish physiologist, systematized massage into the technique now called “Swedish Massage”. In the 1950’s massage was first taught in nursing schools in the United States as a means to “relax muscles, improve muscle tone, increase blood flow, and possibly relieve muscle spasms” (Luckmann & Sorenson, 1987). Americans make more than 160 million visits annually to seek relief of musculoskeletal weakness and pain by manipulative or body-based practices (Galloway et al., 2004, Herman et al., 2005, Nathan, 2005, White et al., 1996).

Massage has been defined as “a mechanical manipulation of body tissues with rhythmical pressure and stroking for the purpose of promoting health and well-being” (Cafarelli & Flint, 1992). It is the manipulation of soft tissue to bring about generalized improvement
in health or specific physical benefits such as the relief of muscular ache and pain (Ernst, 1999, Vickers, 1999, and Richards, 2000). Despite the frequency of their use and self-reported positive effects, there is a paucity of sound scientific evidence for the efficacy of massage therapies to overcome muscle pain and weakness associated with exercise (Galloway, Watt, and Sharp, 1998).

Therapeutic massage can be defined as the assessment of the superficial soft tissues of the skin, muscles, tendons, and ligaments followed by manual manipulation of these structures. The use of the hand, foot, knee, arm, elbow, and forearm can be used for the systematic external application of touch, (effleurage), friction, vibration, kneading (petrissage), stretching, compression, or passive and active joint movements within the normal physiologic range of motion. Effleurage is stroking towards the body or heart with the aim to increase the circulation in the venous blood-vessels and the lymphatics, thereby causing absorption. Friction is firm, circular movements performed over one group of muscles at a time that are always followed by centripetal strokes with the aim to transform pathologically changed parts so that they can be incorporated into the healthy tissues and absorbed by the veins and lymphatics. Vibrations is purported to reduce edema by compressing swollen tissue and thus having less risk of spreading infection (Goats 1994). Petrissage is a kneading that is performed in order to cause a double centripetal pressure on a tissue while also raising it up from its normal point of attachment with the aim of reaching separate muscles. Tapotement is a percussion that is made up of clapping, hacking, and beating performed by striking quickly (Ostrom, 1918).
Eccentric exercise 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 (Herman et al., 2005 and Weerapong et al., 2005). Furthermore, the staggering costs of work-related musculoskeletal disorders that often involve eccentric muscle exertions mandates our attention towards evidence-based prevention and treatment strategies (NRC/IOM, 2001 and NCHS/CDC, 2006). Acute and chronic skeletal muscle pain and weakness are among the most common ailments treated by physicians (American Academy of Family Practitioners, 2005). Minimizing this pain and weakness is of critical importance considering the high exercise attrition rate in patients with muscle dysfunction, as well as the direct economic burden of physical inactivity, in excess of 76 billion dollars annually in the United States alone (Macera et al., 2003, Pratt et al., 2000, White et al., 1996). While treating muscle weakness and pain with pharmaceutical drugs and traditional exercise regimens has met with some success, large numbers of people have turned to alternative approaches including myofacial release and massage to mitigate the effects of intense eccentric exercise (Braun et al., 2005, Gordon et al., 2006, , Moraska, 2007, Sherman et al., 2012).

The science of sports massage interests athletes, athletic trainers, and sports physiologists. Sport massage may help to optimize positive performance factors such as healthy muscle and connective tissues and normal range of motion (Benjamin et al., 1996). Massage treatments can last up to 2 hours with therapists often advising a series of appointments, without evidence for the efficacy of repeated sessions of this therapy (Sherman et al., 2012). While evidence to support or refute its effects on sports
performance is insufficient at this time, new reports help formulate an understanding of massage and its role in exercise-related muscle pain (Moraska, 2007). Furthermore, although the effectiveness of massage for overcoming muscle pain and weakness following exercise is limited to a few high quality studies (Best et al., 2008), it does appear that under certain conditions, massage can reduce muscle soreness associated with eccentric muscle contractions (Butterfield et al., 2008, Jonhagen et al., 2004, Moraska, 2007). Weerapong et al. reviewed the potential mechanisms of massage, suggesting that massage can produce mechanical pressure, which is expected to increase muscle compliance resulting in increased range of joint motion, decreased passive stiffness and decreased active stiffness (biomechanical mechanisms) (2005). Massage is also thought to reduce swelling, oedema and general inflammation that result from muscle injury (Tiidus 1997) which in turn reduces symptoms of pain and aids in recovery of efficient muscle contraction. 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 (Moraska, 2007). However, neither the mechanisms of actions nor the effectiveness or optimal strategies for massage therapies have been conclusively demonstrated. A systematic understanding of the mechanism of action of massage therapies including the effects of magnitude, duration, and frequency is essential to understand the basis of their actions as an important first step to developing innovative approaches to maximize their clinical effectiveness (Galloway et al., 2004).
Not all studies have shown a consistently positive effect for massage-based therapies to enhance recovery of muscle function following eccentric exercise. Zelikovski et al. found 45% improvements in subsequent exercise performances following a 20 minute massage recovery period (1993). Zainuddin et al. noted that massage was effective in reducing the magnitude of DOMS, as well as swelling with 20% - 40% decrease in the severity of soreness compared with no treatment (2005). However, Jõnhagen et al. found that massage does not appear to reduce the symptoms of DOMS, functional loss, and decreased muscle strength following eccentric exercise (2004). Wiktorsson-Moller et al., found that massage of the lower extremities increased only ankle dorsiflexion range of motion, when compared to other activities like warm-up and stretching (1983 & Guest, 2010).

1.8.2 Human Studies

There is limited knowledge of the viscoelastic responses of human skeletal muscle to compressive and shear forces typically applied during manual therapies such as massage. Miyamoto et al. studied the effect of pressure intensity of graduated elastic compression stockings on the active properties of healthy triceps surae muscle following calf-raise exercises (2011). Fourteen healthy young male subjects performed 15 sets of 10 repetitions of calf-raise exercise while wearing one of three stockings; 30, 18, or 0 mm Hg at the ankle, 21–25, 12-14, or 0 mm Hg at the calf, and 10, 7, or 0 mm Hg below the knee. Each subject performed two sessions separated by at least two weeks, with each session performed by a different leg with torque and electromyographic signals of the medial gastrocnemius and soleus muscles recorded before and after exercise. Results
suggested that an elastic compression stocking with higher compression at the calf region (30mmHg at the ankle, 21–25mmHg at the calf, and 10mmHg below the knee) relieves muscle fatigue (torque deficit of the triceps surae) induced by calf-raise exercises. The exercise bout was not a controlled test, introducing inter-individual and inter-bout variability in the amount of muscle activation, causing results to be influenced by the difference in amount of work done by the muscle and the effect of the stocking upon total work. Additionally, there was no measurement to verify if the stocking specified compression forces were equivalent to the actual amount of compression applied to the leg. While this study provided information on skeletal muscles active properties response to the application of constant, graduated compression after fatiguing exercise, a more thorough investigation including the viscoelastic responses of the tissue would guide their translation to clinical practice.

The effect of intermittent pneumatic compression (IPC) on muscle swelling, stiffness, and strength loss resulting from eccentric exercise-induced injury was studied by Chleboun et al. (1995). Utilizing the elbow flexor muscle of twenty-two healthy college women, it was observed that IPC is effective in temporarily decreasing exercise-induced swelling and stiffness in a time dependent fashion. IPC decreased muscle stiffness on days two and three while swelling increased from pre-exercise to post-exercise days one to five before IPC (p<0.05) and days four and five post IPC (p <0.05) with peak change of 4.2% ± 0.9% on day four. Total average circumference of the arm was used to estimate muscle swelling which did not provide the sources and exact location of the swelling. Additionally, static torque angle measurements were not a purely static measurement,
with EMG reporting incidences of muscle activity. While this study provided valuable information on injured human skeletal muscle response to IPC, there still exists the need to understand the effect of cyclic compression on the muscle’s mechanical properties.

1.8.2 Image Based Modeling

Mathematical modeling is a valuable tool that utilizes information from non-invasive techniques such as magnetic resonance imaging (MRI) to estimate in vivo properties of tissue to provide further insight on tissue behavior under a variety of loading conditions. Chaudhry et al. developed a mathematical model to assess the in vivo elastic and viscoelastic properties of the biceps brachii subjected to compression, shear, and extension forces similar to those utilized in massage based therapies. The model was comprised of two components, a simplified elastic model that provided input data for the dynamic viscoelastic model. The combined model estimated loads of 7% less pressure were needed to produce 10% deformation of the muscle as compared with the purely elastic model. In the viscoelastic model, there was stress relaxation of 18% of maximum pressure when muscle was deformed by 10% over 60 seconds and maintained in that state for 200 seconds. While these results provide insight on the necessary compressive forces to alter the rheological properties of the biceps muscle, the model used a linear theory of elasticity and may therefore not estimate the true viscoleastic responses of human soft tissues.
1.8.3 Animal Studies

Animal studies allow for both in vivo and in vitro investigations that can provide valuable basic science knowledge as well as information necessary for the development of mathematical models that can be used to predict the mechanical responses of human skeletal muscle. Utilizing a model of deep pressure sores, Palevski et al. characterized the in vitro viscoelastic stress relaxation and shear moduli of muscle in response to chronic, intense compression (2006). Indentation experiments (4mm over 2000ms) were conducted on eight gluteus muscles from adult pigs using a custom-made pneumatic device. Each muscle was indented transversely at 3 different sites, 7 times per site, to obtain non-preconditioned and preconditioned data that mimic compression of bony prominences. Short and long-term shear moduli data were fit to a biexponential equation. Short-term shear moduli were greater than long-term moduli by an order of magnitude with preconditioning shown to significantly decrease mean short term shear moduli 0.67-fold. Results indicate stress relaxation of muscle in the transverse direction begins within less than 1 min and continues for an extended period of time. This study highlights the need to understand the role of acute and cumulative changes in tissue viscoelasticity that occur due to massage-like compressive loading and their effect on muscle behavior and physiology.

The majority of experimental work on muscle mechanical responses to compressive loading has been performed by the Van Loocke laboratory (2006, 2008, 2009). Initially, a quasi-static uniaxial unconfined compression test was performed on fresh porcine muscle tissue to investigate the in vitro passive elastic properties skeletal muscle (2006).
Muscle elastic behavior was shown to be nonlinear and transversely isotropic with the cross-fiber orientation. A transversely isotropic model including strain-dependent Young’s moduli was developed which successfully fit experimental data and predicted muscle compressive behavior in various muscle fiber orientations. In further studies, three-dimensional square samples oriented in fiber, cross-fiber, 45° and 60° from the fiber direction were obtained (2008). Samples were compressed up to 30% strain at rates of 0.5% s⁻¹, 1% s⁻¹, 5% s⁻¹, and 10% s⁻¹. Above a very small compression rate, the viscoelastic component represents approximately 50% of the total stress reached at a compression rate of 0.5% s⁻¹. A stiffening effect with compression rate was observed particularly in directions parallel to the muscle fibers. With stress relaxation data modeled using a quasi-linear constitutive approach, skeletal muscle viscoelastic behavior was found to be dependent on both compression rate and fiber orientation.

The anisotropic behavior of skeletal muscle at higher loading rates has also been studied by Van Loocke et al. (2009). Low and high amplitude cyclic compression tests were conducted on six porcine muscle samples up to 25% compression with triangular wave cycles of 2% or 10% amplitude for 250s. Muscle samples were cyclically tested at 5, 20 and 80 Hz. Fiber and cross-fiber results were qualitatively similar, but stiffer in the cross-fiber direction. In higher amplitude tests, nonlinear viscoelastic behavior with a frequency dependent increase in the stress cycles amplitude was observed (factor of 4.1 from 0.2 to 80Hz). An anisotropic quasi-linear viscoelastic model predicted results with a high correlation for low amplitude cycles but differences were observed in the stress cycle amplitudes. A nonlinear model was developed for higher amplitude cycles that
provided a good fit and predicted high amplitude and low frequency cyclic tests performed in the fiber and cross-fiber directions. However, it was not a good fit for high frequency cyclic tests nor relaxation tests. Neither model adequately predicted the stiffness increase observed at frequencies above 5Hz.

Bosboom et al. studied the passive transverse properties of skeletal muscle. In vivo compression experiments were performed on the rat tibialis anterior (TA) muscle (2001). A 0.15 mm displacement was applied to each TA and successive compression tests were performed with incremental displacements. Compression loading experiments were repeated three times from the lowest to the highest force level separated by three minutes. The material parameters were obtained by utilizing a mixed numerical–experimental method. A plane stress model of the cross section was developed for each muscle. An incompressible viscoelastic Ogden model permitted analysis of both the elastic and viscous relaxation responses. When applying the estimated material parameters in a three-dimensional finite element model, the measured behavior was accurately simulated. However for clinical application, the estimated material properties of the muscle are unknown and therefore this model can only be used to estimate baseline responses.

There is abundant experimental evidence suggesting that both form and function of skeletal muscle can also be influenced by applied mechanical forces (Bosboom et al., 2001; Butterfiled and Herzog, 2006; Bryer and Koh, 2007). Moreover, studies show that under physiological conditions, muscle can undergo large deformations due to both compressive/shear loads and tension (stretch) in vivo (Bosboom et al., 2001; Ceelen et al., 2008; Cronin et al., 2009). However, under certain conditions, muscle cell death can
result when the compressive forces exceed the tissue’s tolerance (Linder and Gefen, 2004).

1.9 Purpose

Recognizing the importance of muscle function in everyday human health and treatment and prevention of musculoskeletal disorders, we have initiated studies attempting to accelerate recovery of muscle function following eccentric exercise. Using our well established animal model, we are currently investigating possible strategies to reduce tissue inflammation, enhance recovery of function, and promote muscle regeneration and repair following exercise-induced damage. More specifically, we are interested in testing the efficacy of massage-like compressive forces to improve muscle function following a controlled bout of eccentric exercise. Our primary outcome measure will be muscle active properties, peak isometric torque, although we will also examine the torque-angle (T-Θ) relationship as a useful indicator of the alteration in muscle function over its entire working range. We have found both parameters to be useful indicators of exercise specific function in the physiological range (Butterfield & Herzog, 2005, Butterfield & Herzog, 2005, Butterfield & Herzog, 2006). Since there is no single best measure of muscle functional deficit (Warren et al., 1999), we will also quantify muscle stiffness and compliance as secondary measures of muscle function. It has been seen that mechanical signals can profoundly regulate biochemical events affecting muscle inflammation (Chandran et al., 2007). It has been postulated that the inflammatory process is responsible not only for successful repair but also in extending the damage process (Ley, 2002 and Tidball, 2005). This progression of injury could occur through a variety of
mechanisms including neutrophil infiltration and activation of the respiratory burst (Tidball, 2005). In fact, recent studies from our lab suggest that infiltrating neutrophils may play a key role in both injury and repair (Toumi et al., 2006).

It is our hypothesis that biomechanical signals generated during massage therapies are (i) able to enhance peak torque recovery following exercise induced muscle injury and (ii) potent anti-inflammatory signals that inhibit leukocyte infiltration. We will critically explore the relationship between magnitude, duration, and frequency of loading (massage) and their interactive effects to restore muscle function following eccentric exercise and their effect on muscle inflammation (Figure 7). Understanding the mechanisms of actions of massage in suppressing the targeted disruption of muscle structure and function may provide a scientific rationale for development of strategies that are presently advocated and practiced, yet poorly understood.

![Figure 7: Black box model of skeletal muscle. Forces applied to muscle can be modulated and information about the muscle is learned through the mechanical, structural, and biomarker outputs.](image-url)
2.0 Document organization

This dissertation is based on a collection of three stand alone manuscripts, and therefore has some redundancy in the introduction and methods sections of chapters two through four. Chapter one serves as the introduction to muscle anatomy and mechanical properties, how those properties can be altered by eccentric exercise, and how massage-like loading may mediate these changes. Chapter two determines an optimal combination of massage-like compressive loading parameters (magnitude, frequency, and duration) that facilitates the greatest recovery of peak isometric torque following exercise induced muscle damage. Chapter three explores the acute and accumulated effect of a four day massage-like loading protocol on the passive rheological properties of skeletal muscle. Chapter four compares the effect of immediate versus 48 hour post exercise delayed massage-like compressive loading on muscle active properties and inflammatory response. In Chapter five, ideas for future studies and pilot data are presented.
Chapter 2: Dose-Dependency of Massage-Like Compressive Loading on Recovery of Active Muscle Properties Following Eccentric Exercise: Rabbit Study with Clinical Relevance

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2.1 Abstract

Background: Optimal strategies for massage and its use in athletes have not been conclusively demonstrated.

Purpose/Study Design: Effects of varying duration, frequency, and magnitude of massage-like compressive loading (MLL) on recovery of skeletal muscle active properties (torque-angle (T-Θ) relationship) following exercise induced muscle injury were studied.

Methods: Twenty-four New Zealand White rabbits were surgically instrumented with bilateral peroneal nerve cuffs for stimulation of hindlimb tibialis anterior muscles.
Following a bout of eccentric exercise (EEX), rabbits were randomly assigned to a MLL protocol of 0.25 or 0.5Hz at 5 or 10N for 15 or 30min. T-Θ was obtained for 21 tibiotarsal joint angles pre and post EEX and post four consecutive days of MLL. Muscle wet weight and hematoxylin and eosin sections were obtained following final treatments. Results: EEX produced an average 61.8%±2.1 decrease in peak isometric torque output. Differences in torque recovery were found between magnitudes (5 and 10N; p=0.004, n=12) and frequencies (0.25 and 0.5Hz; p=0.012, n=12), but no difference for durations (15 and 30min) with the 0.5Hz, 10N, 15min protocol showing greatest recovery four days post EEX. MLL muscle (n=12) wet weight was 3.22±0.18g, while no MLL tissue (n=9) weighed 3.74±0.22g (p=0.029). Histological analysis showed a difference in torn fibers between low parameter and high parameter MLL (6.5±1.04 vs. 0.5±0.29 per 0.59 mm², p=0.005).

Conclusions: Results showed a dose-response effect for magnitude and frequency of MLL on recovery of active muscle properties following EEX. Future studies will investigate underlying biologic mechanisms for this enhanced recovery of muscle function.

2.2 Introduction

Muscle soreness and weakness accompany intense or prolonged physical activity, particularly EEX with symptoms typically peaking 48 hours post exercise and lasting for up to one week resulting in reduction of physical activity and time away from sport¹-⁴. Although there is interest in adaptive properties of skeletal muscle following EEX in
humans, studies have focused on attenuation of muscle soreness and symptoms of delayed onset muscle soreness\textsuperscript{5-8}.

Massage is a popular complementary and alternative medicine (CAM) that is being increasingly utilized by the general public and athletes. Up to 45\% of time in physiotherapy for sport-related injury and performance consists of massage treatments\textsuperscript{9}. Despite the frequency of massage use by athletes and self-reported positive effects, there is a paucity of sound evidence for efficacy of this therapy to mitigate muscle pain and weakness associated with exercise\textsuperscript{10}. A systematic review concluded that the effects of massage-based therapies on recovery from intense EEX are inconsistent across studies\textsuperscript{11}. The authors were unable to synthesize the data into definitive conclusions from case series and randomized controlled trials given the variability in type of massage as well as differences in timing of massage and rate, magnitude, and duration of loading\textsuperscript{11}. Previous work in our laboratory highlights the role of inflammation in post exercise muscle damage, suggesting that modulating certain aspects of inflammation may effect the repair processes\textsuperscript{12}. A recent clinical trial in humans supports the idea that massage down-regulates pro-inflammatory cytokine expression following eccentric exercise\textsuperscript{13}. Taken together, these studies suggest that further work is needed to clarify the role of inflammation following muscle injury and intense exercise. Moreover, the effects of mechanical tissue loading, such as that which occurs with massage, may have important mechanistic and clinical implications that should be considered when prescribing this modality.
Questions regarding the use of manual therapies have recently been highlighted in mathematical modeling studies quantifying the effects of applied loads to the human biceps brachii\textsuperscript{14}. Findings showed that the biceps is surrounded by a fascia much stiffer than the muscle itself illustrating that while relatively small loads can deform the muscle, larger forces, often outside the normal physiologic range, are required to produce even small deformations of the overlying fascia\textsuperscript{14}. The different stiffness of the various soft tissues is difficult to account for in the clinical setting and may help to explain the conflicting observations on the benefit of manual therapies for treatment and prevention of soft tissue injuries associated with sport and exercise\textsuperscript{9,15,16}.

Based upon previous studies in our lab showing that four days of MLL for 30 minutes daily resulted in accelerated recovery of tibialis anterior (TA) isometric torque production, we hypothesized that (i) there exists an optimal MLL protocol (combination of magnitude, frequency, and duration) that enhances recovery of muscle function (isometric joint torque) after a bout of intense EEX and (ii) the optimal protocol of MLL of the exercised muscle results in decreased muscle wet weight, myofiber damage, and cell infiltration\textsuperscript{1}. The primary goals of this study were to 1) utilize an experimental approach that approximates massage to evaluate the effect of varying parameters of MLL on recovery of muscle function from EEX induced muscle damage and 2) evaluate the effect of MLL on muscle fiber damage and muscle wet weight.
2.3 Methods

2.3.1 Animal Surgery for Nerve Cuff Implantation and Experimental Design

Following Institutional Laboratory Animal Care and Use protocol approval (Ohio State University), twenty-four skeletally mature female New Zealand White rabbits (3.42±0.32 kg) were anesthetized using Isoflurane (IsoSol, Vedco St. Joseph MO) and surgically instrumented with bilateral peroneal nerve cuffs for stimulation of hindlimb tibialis anterior (TA) muscles of as previously described¹,¹⁷,¹⁸.

In order to compare the effects of varying MLL duration, magnitude, and frequency on recovery of active muscle properties, a 2 X 2 X 2 design was used to create 8 different MLL protocols (Table 1).

### Table 1. MLL Protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Magnitude (N)</th>
<th>Frequency (Hz)</th>
<th>Duration (min)</th>
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Seven days post nerve cuff implantation, each rabbit was randomly assigned to an MLL protocol defined as a frequency of 0.25 or 0.5Hz, compressive force of 5 or 10N and duration of 15 or 30min. Three rabbits were assigned to each protocol, providing 12 rabbits to compare each pair of conditions, e.g. n=12 for 5N and n=12 for 10N. Loading
parameters were chosen after careful scaling calculations that allowed forces used to be estimated from human massage studies\textsuperscript{1}.

2.3.2 Eccentric Exercise Bout and Evaluation of Torque-Joint Angle Properties

Rabbits were anesthetized and secured supine in a sling with one foot attached to a foot pedal\textsuperscript{1}. The hindlimb underwent an isometric T-Θ analysis and a bout of EEX\textsuperscript{5}. The T-Θ relationship was obtained in 5° increments from 55° to 155° (21 measurements). At each 5° increment, the foot pedal was locked in a fixed position and an isometric contraction was elicited by supramaximal stimulation (three times the α–motoneuron threshold voltage, pulse duration = 0.1ms, frequency =150 Hz, train duration =1000msec) of the peroneal nerve. Two minutes of rest with the foot at 55° tibiotarsal angle were allowed between each measurement to minimize fatigue. The EEX bout consisted of seven sets of ten cyclic lengthening contractions with a two minute rest between sets. For each contraction, the ankle moved within a tibiotarsal angle of 95° to 145° of plantarflexion at 150°s\textsuperscript{-1}. Muscle activation preceded stretch of the TA muscle-tendon unit by 100ms (total stimulus train duration=433 ms). Immediately following exercise bout a repeat T-Θ analysis was performed to assess for EEX effect.

2.3.3 Massage (MLL) Protocol

All MLL protocols began immediately after exercise and occurred daily for four consecutive days. The MLL bouts were approximately 24 hours apart using a customized device for application of length-wise strokes of MLL protocol\textsuperscript{19}. Following daily MLL, the animals were recovered from anesthesia, returned to cages, given food and water ad
libitum, and weighed every other day to monitor for any signs of nutritional deficiency. A
final T-Θ relationship was obtained one day post last MLL bout to assess the effects of
the four day MLL protocol. After completion of final T-Θ measurements, the animals
were euthanized with intravenous KCL administered during deep anesthesia (5% Isoflurane).

2.3.4 Statistical Analysis

Using this 2x2x2 design, three rabbits were tested under each of the eight MLL protocols,
which provided n=12 for comparison of the high and low condition of each parameter
(magnitude, duration, and frequency). A saturated linear model (ANOVA) was used to
estimate residual variance and focused on the overall effects of the three parameters.
The two way interactions and the three-way interaction of parameters were explored in a
sensitivity analysis. The main measure of interest was the recovery index that a MLL
condition produced, defined as a contrast of peak torque at three fixed time points (pre-
exercise, immediate post-exercise, and post four days of MLL)

\[
RI_{-MLL} = 1 - \left( \frac{(pre\_exercise - post\_MLL)}{pre\_exercise} \right) \left( \frac{pre\_exercise - post\_exercise)}{pre\_exercise} \right)
\]  
(Eq 1)

The denominator measured loss of torque post exercise relative to pre-exercise and the
numerator measured torque post four day MLL protocol relative to pre-exercise. When
the pre-exercise and post-MLL torques were the same, the recovery was 100% and the
RI=1.0, however an RI=0 indicated no recovery from post-exercise torque value.
Peak torque was also measured in the contralateral hindlimb, which received no MLL serving as a measurement control for RI. Each rabbit was used as its own control by comparing the RI for the two hindlimbs; one exercised and MLL (RI_MLL) and the other exercised, but no MLL (RI_noMLL). For the no MLL hindlimb, the same four day protocol (including anesthesia) was used as for the MLL protocol, except no MLL was applied. A washout period of seven days occurred between the completion of testing for the initial hindlimb and the exercise of the contralateral hindlimb. Twelve animals received exercise and MLL first, followed by exercise and no MLL for the contralateral hindlimb and in the other twelve animals the order was reversed. Measuring the contralateral hindlimb at the same point in time post-exercise allowed for correction for natural recovery over the four day protocol. This adjustment was not needed to make relative comparisons of RIs across conditions, but necessary to provide an absolute estimator of recovery for each condition. A RI_adjusted value was determined by subtracting the RI of the no MLL hindlimb from the RI of the MLL hindlimb

\[ RI_{\text{adjusted}} = RI_{\text{MLL}} - RI_{\text{noMLL}} \]  

(Eq 2)

In order to investigate the MLL effect over the whole tested angle range, the area under the curve (AUC) for the corresponding torques was calculated using the trapezoidal method. The same recovery index was used by substituting AUC for peak torque. In addition, the angle corresponding to the peak torque output for each condition was also compared to investigate to what extent MLL shifted the peak torque angle back to that of pre-exercise.
2.3.5 Histological analysis

Although both hindlimbs of each rabbit were tested, only the muscle tissue that was tested after the washout period was used for analysis to allow comparisons to be made at the same time point. There were twelve MLL tissues and nine no MLL, control tissues due to three animals without testing of the contralateral control hindlimb. 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. Samples were later sectioned at 8μm thickness, and stained with hematoxylin and eosin to assess myofiber damage and cellular infiltration. Sections were viewed at 200x magnification (Nikon light microscope; Fryer Company, Inc) in order to quantitatively measure torn fiber and leukocyte infiltration. In both longitudinal and cross sectional images, fibers were counted as damaged if there was any evidence of membrane discontinuity. In the longitudinal sections, all cells were counted, while in the cross sections, infiltrating leukocytes were counted as those cells that completely infiltrated the fiber\(^1\). Quantitative analysis was performed by two blinded individuals in order to test for repeatability. Two randomly selected equal0.59mm\(^2\) (0.89 mm x 0.67 mm) areas of the specimen (midbelly of MLL muscle area) in both the longitudinal and cross sections were assessed for leukocyte infiltration of myofibers.

2.4 Results

All animals survived the entire study (approximately one month for each animal) without greater than 10% loss of body weight. In three animals, there were no data for the
contralateral control (no MLL condition) hindlimb due to nerve cuff disruption at various stages of testing.

2.4.1 Torque Angle Analysis

The EEX protocol produced an average peak isometric torque deficit of 61.8% (±2.1). There was a significant difference for the RI of the MLL hindlimb between the two magnitudes in favor of 10 N (5N vs. 10N: RI=0.28 vs. 0.76, p=0.004, n=12). The two frequencies also showed a significant difference in favor of 0.5 Hz (0.25 Hz vs. 0.5 Hz: RI = 0.32 vs. 0.73, p=0.012, n=12). No significant difference for the two durations was found (15min vs. 30min: RI=0.47 vs. 0.58, p=0.48, n=12) (Table 2).

| Table 2. Comparison of RI_MLL between each of the three parameters |
|----------------------|-----------|----------------|----------------|--------------|----------------|----------------|
| Effect               | Condition1| Condition2    | Estimate       | SE           | Pr > |t|      | 95% CI of the diff |
| Frequency            | 0.25      | 0.5           | -0.4082        | 0.1438       | 0.0118       | -0.713         | ~0.1034         |
| Magnitude            | 5         | 10            | -0.4824        | 0.1438       | 0.004        | ~0.7872        | ~0.1775         |
| Duration             | 15        | 30            | -0.1053        | 0.1438       | 0.4745       | ~0.4101        | 0.1995          |

There were no significant interactions between or among the frequency, magnitude, and duration conditions eliminating concerns about combining results across conditions to test the effects of magnitude, frequency, and duration (Figure 8). Comparison of RI across the conditions showed that the 0.5Hz, 10N, 15min combination of MLL parameters showed highest RI (1.08).
Figure 8: Recovery index for massage (RI_MLL) showed a dose dependent effect of magnitude (a) frequency (p-value=0.012) and (b) magnitude (p-value=0.004), but no dependence on (c) duration.

The above relative comparisons across conditions required no adjustment of RI with the no MLL hindlimb in order to identify an ideal condition for recovery of peak torque (Figure 9). Analysis of RI_adjusted confirmed that the most effective MLL condition was at the 0.5Hz, 10N, 15min combination. Table 3 provides the RI_MLL (MLL only) and RI_adjusted (RI_MLL relative to RI_no MLL) means and standard errors. Using this adjustment, the combination of the same three conditions as seen with the RI_MLL showed 97% recovery. Two of the remaining seven conditions resulted in at least 50% adjusted recovery (0.25Hz, 10N, 15min and 0.5Hz, 10N, 30min).
Figure 9: Representative T-Θ plots demonstrating that the combinations of low conditions produces approximately the same degree of recovery as the non-massaged control, while the combination of high conditions of magnitude and frequency facilitated recovery back to and beyond the baseline (pre exercise). These plots represent the smallest effect and greatest effect range for the massage conditions.

<table>
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<th>Frequency</th>
<th>Magnitude</th>
<th>Duration</th>
<th>Mean RI_MLL</th>
<th>SE RI_MLL</th>
<th>Mean RI_adjusted</th>
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2.4.2 Area under curve

Area under the T-Θ curve (AUC) for pre-exercise, post-exercise, post MLL and no-MLL measurements was examined as a secondary indicator of recovery. Similar to peak torque analysis, the combination of 0.5Hz, 10N, 15min gave the highest AUC recovery.
index at 1.05. The correlation between peak torque and AUC was 0.91 showing high redundancy between the two measures.

2.4.3 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 an RI for joint-angle shift was calculated.

Exercise produced a 4.4° ± 0.6 rightward shift from pre exercise peak isometric torque angle. Four days of MLL produced an average -2.9°± 0.5 leftward angular shift from the post exercise peak isometric torque angle while control, no MLL hindlimbs produced a 1.8°± 0.5 rightward angular shift from post exercise peak isometric torque angle (Figure 10).

![Figure 10: The angle where peak torque is produced moves rightward due to exercise and even further rightward for the non-massaged, control condition. Conversely, massage produced a leftward shift of peak torque angle back towards pre exercise angle.](image)

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2.4.4 Muscle Wet Weight & Histology

Average hindlimb muscle wet weight for no MLL (n=9) was 3.7 ±0.22g and MLL (n=12) was 3.22±0.18g (p=0.029). The difference of peak torque output between the two magnitudes in favor of 10N and two frequencies in favor of 0.5Hz was further explored via histological analysis. The MLL protocols that included the combination of low and high parameter values for both magnitude and frequency were analyzed and compared for torn muscle fibers and cell infiltration (0.25Hz, 5N, 15min vs. 0.5Hz, 10N, 15min). Longitudinal sections showed that the combination of low parameter MLL resulted in minimal effect on muscle fiber damage and extent of cellular infiltration, while the combination of high magnitude and frequency MLL resulted in decreased myofiber damage and cellular infiltration (Figure 11).

Figure 11: For the 0.25Hz, 5N, 15min condition there was little difference between the massage and non-massed muscles. In contrast, massage lead to decreased myofiber damage and cellular infiltration for the 0.5Hz, 10N, and 15min condition.
Longitudinal sections showed a difference of average leukocytes between no MLL tissue and both low parameter and high parameter MLL (447.5±36.28 vs. 121±12.13, p= 0.001 and 81.3±20.64, p= 0.0005, respectively). Cross sectional images showed no difference (p = 0.18) for leukocytes within the interstitial space between the low parameter and the high parameter MLL (52.5±10.36 vs. 77±6.28 per area, respectively), however a difference in infiltrating leukocytes was found (p = 0.002) (36±3.18 vs. 10.3±1.44 per area, respectively). Analysis of both cross sectional and longitudinal sections showed a significant difference in torn fibers between the low and the high parameter conditions MLL (5±0.41 vs. 0, p =0.001 and 6.5±1.04 vs. 0.5±0.29, p =0.005, respectively). Longitudinal sections showed no significant difference in torn fibers between the no MLL and the low parameter MLL (6.75±1.03 vs. 6.5±1.04, p =0.9) (Figure 12).

![Figure 12: Longitudinal sections showed no significant difference in torn fibers for the no MLL and the 0.25Hz, 5N, 15min MLL protocol. In contrast, the 0.5Hz, 10N, 15min MLL protocol resulted in a significantly decreased number of torn fibers.](image)
2.5 Discussion

This study shows the ability of MLL to accelerate recovery of muscle and joint function following EEX. Moreover, it is shown for the first time that there is a dose-dependent effect of MLL on the recovery of mechanical properties as well as histological evidence for MLL to decrease muscle fiber damage. Our *in vivo* animal model and self-designed device permitted loading frequency, magnitude, and duration to be varied in order to examine dose-response effects of MLL on recovery of muscle function. Similar controlled experiments in humans will be important when designing interventions and determining an optimal magnitude, duration, and frequency of massage in order to optimize its clinical indications for athletes.

The ability to quantify the effect of MLL parameters allowed for a detailed investigation of the effect of each parameter individually. Frequency and magnitude were shown to be important loading variables for promoting recovery of joint torque, while loading duration, at the parameters chosen (15 or 30min), provided no significant effect. These findings suggest that greatest recovery of peak isometric torque is produced by the high condition for both magnitude and frequency (10N and 0.5Hz), with the low conditions (5N and 0.25Hz) producing significantly less recovery.

Histological analysis confirmed that the optimal MLL protocol (0.5Hz, 10N, 15min) resulted in a significant reduction in both torn muscle fibers and cellular infiltration as compared to both the low parameter MLL and the no MLL protocols. Torn muscle fibers could result in lower torque production due to less cross bridge attachment and therefore
force production. Moreover, the same number of torn muscle fibers for both the control and the low parameter MLL conditions correlated with the equal effects of these two conditions on recovery of torque production. The significant increase in number of infiltrating cells in the low parameter MLL as compared to the high parameter MLL may be an indicator of further inflammation and perhaps the increase in number of torn muscle fibers. On the other hand, the decrease in both cell infiltration and muscle fiber damage correlated with the greater recovery of peak torque observed with the high parameter MLL condition.

The current findings support our previous work where we observed a beneficial effect of 4 days of MLL following EEX. The optimal MLL protocol (0.5Hz, 10N, and 15min) showed not only the largest RI_adjusted, but also greatest RI_AUC. Area under the T-Θ curve was shown to be a secondary indicator of enhanced recovery and took into account all 21 joint angles measures, as opposed to only the joint angle at which the highest peak isometric torque was produced.

The rightward shift in peak torque angle produced by the bout of EEX is consistent with previous studies that showed an increased series compliance in muscle due to EEX\textsuperscript{1,6,20}. Over the four day no MLL protocol, the exercised, control limb produced a further rightward shift from the post exercise peak torque angle suggesting further tissue damage. Conversely, MLL produced a leftward shift from the post exercise torque angle suggesting recovery from muscle damage associated with the bout of EEX. This recovery is further emphasized by the return of peak isometric torque values to pre exercise level\textsuperscript{1,21,22}.
We acknowledge that the injury created in our animal model may not be completely analogous to that produced in humans with EEX, with higher torque deficits than could necessarily be tolerated in humans produced by our protocol. However, limited objective data exist that quantify torque deficits experienced by humans following a single bout of EEX. Moreover, the deficits produced by our protocol allowed for differentiation between natural recovery and recovery due to the applied MLL protocol. The potential therapeutic effects of touch with massage cannot be directly addressed in our animal model, particularly given that the actual MLL and evaluation of torque production were carried out under general anesthesia. Nevertheless, the highly reproducible conditions allowed testing of MLL effects using a minimum number of animals and systematic investigation of various loading parameters. Our results demonstrate the effects of loading on recovery of muscle function, irrespective of motor unit recruitment and potential confounding variables such as pain and motivation. There is a significant difference in recovery of function at the basic level of muscle force production, which most likely could not be achieved in a human study.

It has been postulated that the inflammatory process is responsible not only for successful repair but may also contribute to extending the initial damage process. This progression of injury could occur through a variety of mechanisms, including neutrophil infiltration and activation of the respiratory burst. In fact, studies from our lab suggest that infiltrating neutrophils may play a key role in both injury and repair. Collectively, these studies suggest that inflammation may play an integral role in the pathogenesis of both acute and chronic muscle dysfunction and pain and loss of muscle strength.
Despite the popular use of massage by athletes for a variety of reasons, its optimal use and indications are still not well known. The current study provides the first evidence of the effect of varying MLL parameters [magnitude, duration, and frequency] on the recovery of muscle active properties following EEX induced damage. Massage has been hypothesized to moderate inflammation, improve blood flow, and reduce tissue stiffness, amongst other possible mechanisms. Indeed, a recent study in humans showed that a single bout of massage down-regulated the expression of pro-inflammatory cytokines following eccentric exercise\textsuperscript{13}. Our data showed MLL reduced muscle wet weight and fiber damage and therefore support the findings of Crane et al\textsuperscript{13}. Future investigations will explore the effects of delayed versus immediate massage on functional recovery and inflammation as well as local blood flow to help further define the mechanism for this therapy. Our eventual goal is translating findings from our animal model to human trials in order to develop cost-effective optimal indications and strategies for the use of massage-based therapies.

2.6 Contributorship

Haas: Study design, experiments, analysis, and writing
Butterfield: Experiments, analysis and writing
Zhao: Experiments, analysis and writing
Zhang: Study design, analysis, and writing
Jarjoura: Study design, analysis, and writing
Best: Study design, experiments, analysis, and writing
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2.8 Competing interests

None of the authors have any professional relationships with companies or manufacturers.
Chapter 3: In Vivo Passive Mechanical Properties of Skeletal Muscle Improve With Massage-Like Loading Following Eccentric Exercise

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3.1 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 (0.5Hz, 10N for 15min or for 30min). 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 15min and 30min four-day MLL protocols (p<0.05). Post four days of MLL the normalized \(AG_\theta\) 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 \textit{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

3.2 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 and colleagues used a transversely isotropic model with strain-dependent Young’s moduli (SYM) to \textit{ex vivo} estimate compressive properties of animal skeletal muscle together with a quasi-linear viscoelastic (QLV) model to explore time-dependent properties (Van Loocke et al., 2008). In further studies, higher amplitude compression loading produced nonlinear viscoelastic behavior with a frequency dependent increase (Van Loocke 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 and colleagues 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 (Chaudhry et al., 2008). Magnusson and colleagues noted that the tensile viscoelastic responses to a single bout of stretching of the human hamstring muscles are dependent upon joint angle (Magnusson et al., 1996). Ylinen et al. 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 (Ylinen et al., 2009). 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) 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 & 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 and viscous responses 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.

3.3 Methods

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

Six skeletally mature New Zealand White female rabbits (3.46±0.12kg) 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 0.25ml acepromazine, subsequently anesthetized with 1.5% isoflourane 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 (Figure 13). The exercise protocol consisted of seven sets of ten cyclic lengthening contractions, with 2min rest between sets. Cyclic lengthening contractions were performed from a tibiotarsal angle of 95° to 145° of plantar flexion at 150°sec⁻¹, with activation preceding the muscle-tendon unit stretch by 100msec. Previous studies have shown this set of parameters resulted in a reproducible magnitude of muscle damage, approximately 70% loss of torque production measured within 5min following a bout of eccentric exercise (Butterfield et al., 2008).
3.3.2 MLL Protocol

Rabbits were subjected to a MLL protocol using a custom-designed mechanical device (Figure 14). This device included two motorized arms that travelled 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 76μm/s until 10N compressive force was reached. Given the mechanical tip size, the normal compressive stress was estimated as 88.46kPa. 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 travelled along the longitudinal axis of the TA. The mechanical tip moved along the longitudinal axis of the TA at 0.5Hz, 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 12.5mm to

Figure 13: 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.
avoid direct contact with the bones in the lower extremity. The compressive force was kept at 10N by dynamically adjusting the vertical position of the mechanical tip through a feedback loop.

Rabbits were randomly assigned to a MLL duration of 15min or 30min 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 (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 24hr interval) to evaluate daily effects of massage on muscle mechanical properties.

Figure 14: Customized MLL device. The resolution of motion along each axis was 25μm. 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.
3.3.3 Evaluation of TA muscle viscoelastic properties

Under anesthesia, the hindlimb was secured into the mechanical loading device with the TA muscle facing up. TA 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 300sec 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.

3.3.4 Mathematical Modeling

According to the QLV model, the stress relaxation was:

$$\sigma(t) = G(t) * \sigma^\varepsilon(\lambda)$$

(1)

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

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

(2)
where $\frac{\partial \varepsilon}{\partial \tau}$ represented the strain history. The instantaneous elastic stress response was:

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

(3)

where $\varepsilon = \dot{\lambda} - 1$ 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:

$$G(t) = 1 - \sum_{i=1}^{n} g_i^p \left( 1 - e^{-\frac{t}{\tau_i}} \right)$$

(4)

where $g_i^p$ was the $i^{th}$ Prony series parameter, and $\tau_i$ was the relaxation time constant at the $i^{th}$ order. According to the obtained relaxation curves, a second order model was chosen. Equation (4) was rewritten as:

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

(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:

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

(6)

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

(7)

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

\[ \forall t \in (0,t_0) \]

\[ \sigma(t) = AG_0B\gamma \left[ \frac{k_0}{B\gamma} e^{\beta t} + \frac{k_1r_1}{1+B\gamma r_1} e^{\alpha t} + \frac{k_2r_2}{1+B\gamma r_2} e^{\beta t} \right] \left( \frac{k_0}{B\gamma} + \frac{k_1r_1}{1+B\gamma r_1} e^{\alpha t} + \frac{k_2r_2}{1+B\gamma r_2} e^{\beta t} \right) \] (8)

\[ \forall t \in [t_0, \infty) \]

\[ \sigma(t) = AG_0B\gamma \left[ \frac{k_0}{B\gamma} e^{\beta t} + \frac{k_1r_1}{1+B\gamma r_1} e^{\alpha t} + \frac{k_2r_2}{1+B\gamma r_2} e^{\beta t} \right] \left( \frac{k_0}{B\gamma} + \frac{k_1r_1}{1+B\gamma r_1} e^{\alpha t} + \frac{k_2r_2}{1+B\gamma r_2} e^{\beta t} \right) \] (9)

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

3.3.5 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 ±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.

3.4 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.

3.4.1 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 (Figure 15), the peak stress after four-day MLL reduced to 103.2% of the pre-exercise response. The final stress value after 300sec 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.

![Figure 15: 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&C showed the raw data, while B&D showed the fitted curves. A&B were subjected to the MLL with the peak compressive loads of 10N. C&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.](image)
3.4.2 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.* $g^p_1$, $g^p_2$, $\tau_1$, and $\tau_2$ were obtained by fitting the measured stress curves. The instantaneous elastic response $\sigma^e(\varepsilon) = AG_0(e^{B\varepsilon} - 1)$ 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 (Figure 16A). For the group with 0.5Hz, 10N, 15min 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 (Figure 16B). 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 15min and 30min MLL groups. The results showed no difference between the normalized $AG_0$ for both the 15min and 30min MLL protocols ($p>0.05$), implying that the longer duration (30 min) of MLL did not significantly affect the post-MLL $AG_0$ (Figure 16C).

Figure 16: Effects of exercise and MLL action on the instantaneous elastic response. I: Instantaneous elastic response $\sigma^e(\varepsilon)$; II: the cumulative effects on $AG_0$ for 15min MLL at 0.5Hz and 10N over 4 days (n=3); III: Comparison of the cumulative effects on $AG_0$ for 15min and 30min MLL over 4 days (n=3). Both are at 0.5Hz and 10N.
A daily decrease of the pre-MLL $AG_0$ was observed (Figure 17A). 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 ($p>0.05$) (Figure 17B). For better illustration, post-MLL $AG_0$ was normalized using the pre-MLL group of the same day as the reference. 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.
3.4.3 Effect of Eccentric Exercise and MLL on the Reduced Relaxation Response

Figure 18 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 (Figure 18A). In no MLL group, the intercept also shifted to the right, while not as great as that in the MLL group (Figure 18B). 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.
Figure 18: Reduced relaxation response upon exercise and MLL. (A) MLL group under 0.5Hz, 10N, 15min; and (B) Control no MLL group.

In this QLV model, $g_1^p$ and $\tau_1$ represented fast relaxation, while $g_2^p$ and $\tau_2$ represented slow relaxation, i.e. $\tau_1$ was smaller than $\tau_2$. The changes in $g_1^p$ and $g_2^p$ pre- and post-MLL on each day were obtained (Figure 19A). The average $g_1^p$ and $g_2^p$ after daily MLL were normalized using the pre-MLL group of the same day as reference (Figure 19B). 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).
3.5 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.

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). 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 $AG_0$ 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 0.5Hz, 10N, 15min MLL to the 0.5Hz, 10N, 30min MLL. This comparison showed that such a longer loading duration (30min) 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 0.5Hz, 10N, 15min 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 (Paleveski et al., 2006). The discrepancy could be due to different loading parameters (indentation v.s. MLL) and different holding durations (60s vs. >200s). 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.

3.6 Conflict of Interest Statement

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

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4.1 Abstract

Purpose: This study extends our previous work by comparing 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 the hindlimb tibialis anterior muscles. Following a controlled bout of EEX, rabbits were randomly assigned to a MLL protocol (0.5Hz, 10N, 15min) started immediately post EEX, 48 hours post EXX, or no MLL control. Six rabbits were tested under each of the three protocols, each receiving four consecutive days of MLL or no MLL. A torque-angle (T-Θ) relationship was obtained for 21
tibiotarsal 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: Eccentric exercise produced an average 51% (±13%) decrease in peak isometric torque output (n=18). Greatest peak torque recovery occurred (near 100% of the pre-exercise value) with the immediate application of MLL for four consecutive days. There were significant differences in torque recovery between the two application times (immediate and delay; p=0.0012), immediate MLL and control (p<0.0001), and delayed MLL and control (p=0.0253). Immunohistochemical analysis showed immediate MLL protocol decreased the average number of infiltrating neutrophils as compared to both no MLL and delayed MLL protocols, p=0.0403 and 0.0027, respectively.

Conclusion:
Post EEX, immediate MLL was more beneficial than delayed MLL in restoring muscle function (peak isometric torque) and decreasing inflammatory cell infiltration. These findings invite a similar study in humans in order to make definitive conclusions on the optimal timing of massage-based therapies for recovery from eccentric exercise.

Key Words: massage, inflammation, mechanical properties, skeletal muscle.

4.2 Introduction

Skeletal muscle strength loss, soreness, swelling, and weakness are known to accompany intense or prolonged physical activity, particularly EEX (Agarwal, 2003, Bechhofer, 1995, Best, 1998, Bowers, 2004). A review of both human and animal studies has shown
that these symptoms occur immediately post exercise, typically peak between 24-72 hours, and can last for up to seven days (Cleak & Eston, 1992a&b, Friden et al., 1984 and 1998, Ebbeling et al., 1989). The symptoms accompanying intense EEX are thought to be caused by local tissue inflammation, tears in the muscle fibers, and unaccustomed bouts of training (Miles et al., 1997). Research has shown inflammation as one of the underlying causes responsible for the general decline of physical function and loss of muscle strength in both the young and the elderly and has been proposed as a treatment target for pain syndromes (Omogiui, 2007, Cesari, 2004, Schaap, 2006). 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 (Ley, 2003, Tidball, 2005, Yumet, 2002). This progression of injury could occur through a variety of mechanisms, including neutrophil infiltration and activation of the respiratory burst (Tidball, 2005). In fact, recent studies suggest that infiltrating neutrophils may play a key role in both injury and repair (Toumi, 2006). Collectively, these studies suggest that inflammation plays an integral role in the pathogenesis of both acute and chronic muscle dysfunction and pain and loss of muscle strength as well as both the injury and repair processes. Minimizing the pain and weakness associated with EEX is of great importance considering the high exercise attrition rate in patients with muscle dysfunction, as well as the direct economic burden of physical inactivity, in excess of 76 billion dollars annually in the United States alone (Macera et al., 2003, Pratt et al., 2000, White et al., 2996). A
variety of techniques, including therapeutic ultrasound (Stay et al., 1998), electrical stimulation (Tourville et al., 2006), ice immersion (Sellwood et al., 2007), static and ballistic stretching (Smith et al., 1993), therapeutic massage (Friden et al., 1981), and nonsteroidal anti-inflammatory medications (McAnulty et al., 2007), are used clinically to minimize the symptoms and enhance recovery following exercise-induced muscle damage (Butterfield et al., 2008). While research on the efficacy of these treatments has increased, there still remains a void in the phenomenological understanding and therefore optimal clinical strategies, especially concerning therapeutic massage, to overcome muscle pain and weakness associated with EEX.

The science of sports massage is of interest to athletes, coaches, and all personnel associated with medical care and performance. While evidence to support or refute its effects on sports performance is insufficient at this time, new reports help formulate an understanding of massage and its possible role in mitigating exercise-related muscle pain (Moraska, 2007). 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 (Jonhagen et al., 2004, Moraska, 2007, Crane et al., 2012). 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 (Galloway et al., 2004). 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 (Moraska, 2007).
Based upon previous efforts in our lab demonstrating an optimal massage-like loading (MLL) protocol (0.5Hz, 10N, 15min) that enhances recovery of muscle function (isometric joint torque) after a bout of intense EEX (Haas et al., 2012), we hypothesized that (i) immediate application of MLL post eccentric exercise causes 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 approximates 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).

4.3 Methods

4.3.1 Animal Surgery for Nerve Cuff Implantation and Experimental Design

Following Institutional Laboratory Animal Care and Use protocol approval (Ohio State University), eighteen skeletally mature 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,(Butterfield et al., 2008, Koh, Haas et al., 2012).

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) (Haas et al., 2012). 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 (Table 4).

<table>
<thead>
<tr>
<th>Protocol</th>
<th>MLL</th>
<th>Application</th>
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<tbody>
<tr>
<td>Immediate</td>
<td>0.5Hz, 10N, 15min</td>
<td>Immediately Post EEX</td>
</tr>
<tr>
<td>Delay</td>
<td>0.5Hz, 10N, 15min</td>
<td>48 Hours Post EEX</td>
</tr>
<tr>
<td>Control (Exercised)</td>
<td>None</td>
<td>N/A</td>
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Table 4: Protocols

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.

4.3.2 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, as previously described (Butterfield et al., 2008, Haas et al., 2012). 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 EEX. 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. Immediately post EEX repeat T-Θ measurements were performed to assess the effect of EEX.

4.3.3 MLL Protocol

All protocols were carried out daily (~24 hours apart) for four consecutive days after initial application (day 1 for immediate protocol and day 3 for the delayed protocol). The MLL was applied using a customized device for application of length-wise strokes (Zeng et al., 2008). 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).

4.3.4 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) (Haas et al., 2012).
\[
RI = \left( 1 - \frac{(pre\_exercise - post\_MLL)}{pre\_exercise} \right) \frac{1}{\left( \frac{(pre\_exercise - post\_exercise)}{pre\_exercise} \right)} \]  \hspace{1cm} (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 (Haas et al., 2012).

4.3.5 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 8μm 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). 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) was incubated 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 limb 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 recognizes not only neutrophils, but also other granulocytes, macrophages, blood monocytes, lymphocytes, and bone marrow cell (Smet et al., 1986) 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 (Wilkinson et al., 1992).

4.3.6 Statistical Analysis

Recovery index for both peak torque relationship and area under the curve as well as immunohistochemistry counts 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, muscle wet weight, and immunohistochemical data with the level of significance set at \( P < 0.05 \). A post hoc power analysis was used to
differentiate significant effects with a statistical power of 0.87 for torque output analyses. Microsoft Excel 2010 and PS: Power and Sample Size Calculation version 3.0, 2009 were used for all analyses.

4.4 Results

4.4.1 Torque – Angle Analysis

EEX produced an average 51% (±13%) decrease in peak isometric torque (n=18; 6 immediate, 6 delay, 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=.0012) (Figure 20).
Figure 20: Immediate application of MLL produced greatest recovery as compared to delayed MLL (RI=0.934 ±0.083 vs. 0.481 ± 0.089, respectively). However both applications of MLL produced a significantly greater recovery than control (RI=0.200 ± 0.064).

4.4.2 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. There was a significant difference for both applications of MLL as compared to control (RI = 0.86±0.08 vs. 0.22±0.06, p<0.0001 immediate and RI = 0.22±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) (Figure 21).
Figure 21: RI_AUC served as a secondary indicator of recovery with immediate application of MLL producing greatest recovery of peak torque and AUC as compared to both delayed MLL and control.

4.4.3 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 ($n=18$). Four days of immediate MLL 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). Exercised, no MLL control produced a $-3.8^\circ \pm 3.9$ leftward angular shift from post exercise peak isometric torque angle (day 5) (Figure 22).
Figure 22: The angle where peak torque is produced moves rightward due to exercise and even further rightward for the non-massaged, control condition. While all three protocols, control, immediate, and delayed MLL, produced a leftward shift of peak torque angle back towards pre exercise angle.

4.4.4 Muscle Wet Weight & Immunohistochemistry

Average muscle wet weight for the exercised, control group (3.77 ± 0.09g) was higher than for both the immediate (2.47 ± 0.12g) and delayed (3.19 ± 0.19g) MLL groups (p<0.0001 and 0.0116, respectively). There was also a difference in wet weight between the immediate and delay MLL groups (p=0.0024) (Figure 23).

Figure 23: Muscle wet weight for the control was higher than both the immediate and delayed MLL. There was also a difference in wet weight between immediate and delayed.
There was a .94 correlation between the two blinded counters. Results showed the EEX, no MLL animals had a significantly higher number of RPN3/57 and Cd11b positive cells infiltrating the muscle (30.9 ± 7.7 and 29.3 ± 9.2, respectively) compared to immediate MLL (14.4 ± 2.4 and 8.8 ± 2.9, respectively), (p=0.046 and 0.040, respectively) (Figure 24). While there were no difference between the number of RPN3/57 and Cd11b positive cells between the EEX, no MLL and delayed MLL protocols (20.1 ± 4.1 and 41.1 ± 9.9, respectively), p=0.226 and 0.382, respectively. There was a difference in number of Cd11b positive cells between immediate and delayed MLL protocols (p=0.002) (Figure 25A). There was no difference in number of RAM11 positive macrophages between the EEX, no MLL (11.1 ± 2.6) and immediate MLL (6.8 ± 1.9) nor the delayed MLL (10.3 ± 2.5) protocols, (p=0.193 and 0.814, respectively) (Figure 25B).

![Figure 24: Representative Immunohistochemistry Images for the control, delayed, and immediate protocols, showing florescence for both macrophage and neutrophil presence.](image-url)
4.5 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. Moreover, to our knowledge we showed for the first time that there is an application time-dependent effect of MLL on the recovery of mechanical properties as well as immunohistochemical evidence for MLL to decrease muscle edema and inflammatory cell infiltration. 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. The immediate MLL protocol produced the greatest recovery of peak isometric torque as compared to both delayed MLL and EEX, no MLL protocols. Similar controlled experiments in humans will be important in determining the optimal use and
indications for MLL and recovery of muscle function following intense eccentric exercise.

There is a rather ubiquitous host inflammatory response to various types of skeletal muscle injury. Research has suggested that the inflammatory process is responsible not only for successful repair but may also act to extend the damage process (Ley, 2003, Tidball, 2005). This progression of injury could occur through a variety of mechanisms, including neutrophil infiltration and activation of the respiratory burst (Tidball, 2005, Toumi et al., 2006). 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 (Best et al., 199, Toumi and Best, 2003 & Pizza, 2002).

Interestingly, it has been shown that treatment with an antibody that blocks the neutrophil’s respiratory burst can attenuate the degree of myofiber damage following a single stretch injury (Brickson et al., 2003). Smith et al. studied the effect of massage on inflammation and DOMS, with results indicating that a 30 minute massage protocol applied two hours after the exercise interfered with neutrophil emigration (1994). This may reduce the intensity of pain due to inflammation and help to reduce DOMS. While it is known that mechanical signaling can regulate biochemical events modulating muscle inflammation (Chandran et al., 2007), this is the first study to show that MLL acts on muscle cells to inhibit inflammatory effects of eccentric exercise while at the same time enhance recovery of peak torque.
Crane et al. found that 10 minute massage post a controlled bout of upright cycling eccentric exercise in humans reduced tissue inflammation and promoted mitochondrial biogenesis (2012). Our results build on this study and our previous work by demonstrating that massage carried out immediately following a bout of intense eccentric exercise favors a quicker recovery of muscle and joint function along with a decreased tissue inflammatory response. Interestingly, in the current study the immediate application of 0.5Hz, 10N, 30min protocol produced an average recovery index of RI=0.94, similar to our previous study in which this condition produced an RI= 1.08. Area under the T-Θ curve was shown 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 delay and control in order of average RI value. This study, in combination with previous studies in our laboratory, shows a highly reproducible exercise and MLL protocol that can allow a systematic understanding of therapeutic massage and its effects on mechanical, cellular, and molecular properties (Butterfield, et al., 2008, Haas et al., 2012). The rightward shift in peak torque angle produced by the bout of eccentric exercise is consistent with previous studies (Agarwal et al., 2004, Borckett et al., 2001, Bryer & Koh, 2007, Haas et al., 2012). All MLL protocols resulted in a leftward shift from the post exercise peak torque angle, indicating some degree of muscle recovery from EEX (Butterfield & Hertzog, 2005). 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 (Agarwal et al., 2004). 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 enhanced leftward shift further suggests a beneficial effect of MLL post EEX.

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.

In conclusion, the current study provides the first evidence of the effect of varying the time of application of MLL post EEX. We have shown that skeletal muscle responds to MLL in a time dependent fashion with application of MLL immediately following damaging EEX attenuated the infiltration of neutrophils and macrophages in skeletal muscle, and facilitated 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 injury and
disease alters human muscle function as well as the optimal indications for manual therapies such as massage.

4.6 Acknowledgements

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Chapter 5: Future Work

5.1 Vascularization of Injured Muscle

Vascularization of an injured muscle is necessary for healing (Snow, 1973, Jarvinen, 1976, Jozsa, 1980). Restoring vascular supply to the injury site allows the regeneration process to begin, being considered a prerequisite for morphological and functional recovery of injured muscle (Jarvinen, 1976). Blood vessels in the center of the injury sprout new capillaries in order to provide the area with an adequate supply of oxygen in order to allow aerobic energy metabolism for the regenerating myofibers (Jozsa et al., 1980). During the final stages of regeneration, aerobic metabolism is considered to be the main energy pathway for the multinucleated myofibers (Jarvinen et al, 1978 & 2005). The revascularization of injured muscle is necessary in order for newly formed thin myotubes to progress and allow for sufficient healing (Jarvinen et al, 2005). Testing for biomarkers of angiogenesis of massaged tissue can allow a better understanding of one mechanism by which massage causes restoration of mechanical function.

5.1.1 Blood Flow

Exercise is a complex process involving the brain, the spine, the nerves, and the muscles. Activity of a muscle produces a constant change in the circulation. When circulation is disrupted due to an injury, the muscles in the neighborhood must be made to act, so that the blood will circulate more freely in the part injured (Brummitt, 2008). Mechanical
pressure might help to increase blood flow by increasing arteriolar pressure. Massage has been proposed as a modality to modulate local circulatory changes however evidence for this hypothesis is lacking (Travell, 1983). Increases in blood microcirculation increases nutrition to the damaged area and allows an increase in muscle blood flow that could hasten oxygen delivery, increase muscle temperature and buffer blood pH, all events that would then aid in exercise performance (Cafarelli and Flint, 1992 and Weerapong, et al., 2005). However, there is mixed evidence of the effect of massage on blood flow (Shoemaker et al., 1997, Hinds et al., 2004, Tiidus et al., 1995). Caferelli et al. and Tiidus et al. both found that massage had positive effect on blood flow, increasing circulation. A decrease in girth in conjunction with increased blood flow was seen by Hart et al., indicating that increased blood flow may help decrease swelling (1992,1995, 2005, Brummitt., 2008).

5.1.2 Pilot Data on Blood Flow

Transdermal measurements of hindlimb femoral artery blood flow (cm/s) were taken pre and post massage using a Terason ultrasound doppler system at 4.8MHz. This vessel was chosen as it is easily located directly under the skin and it was a local vessel without being in the lower hindlimb so it would not cause a positional change of the hindlimb for testing. There is an n=2 for each condition (immediate MLL and delayed MLL) (Figure 26A&B).
While the pilot data has a small sample size and therefore no definitive conclusions can be drawn however, it appears that MLL may cause perturbations of blood flow based on timing of MLL application and provides a starting point for exploring the effect of MLL on blood flow. Increase in blood flow could potentially play a role in the decrease of tissue edema due to MLL as well as clearance of inflammatory cells.

5.2 Compressive and Shear Forces due to Massage

Massage of skeletal muscle produces not only compressive forces, but shear forces that act upon the tissue. The effects of shear forces on skeletal muscle have been largely neglected to date in both human and animal studies. It has been shown however that when a threshold value of maximum shear strain is exceeded the relative damaged area increases monotonically with increasing strain (Ceelen et al., 2008). To develop a better constitutive model that relates complex tissue structure to its mechanical response, it may be important to study the effect of both compression and shear forces (Ceelen et al., 2008). While the compressive force of massage may only be sensed by the local area
where massage is applied, we hypothesize that the entire muscle body and local vessels are affected by the shear forces involved in massage (Figure 27).

![Local vs. Remote Effects]

Figure 27: Forces of MLL on muscle and area of application.

5.2.1 Effect of Frequency and Magnitude on Resultant Shear

Sixteen New Zealand White rabbits (from chapter 2) were surgically instrumented with peroneal nerve cuffs and subcutaneous interfaces for stimulation of the hindlimb tibialis anterior muscles. Following a bout of eccentric exercise, rabbits were randomly assigned to a protocol with MLL frequency of 0.25 or 0.5 Hz at a constant compressive force of 5 or 10N (15 and 30min data was combined due to findings in chapter 2, showing no dependence upon duration of MLL). This design allowed an n=4 for each frequency and compressive force combination. MLL was applied over a period of four consecutive days immediately following exercise and occurring daily (24 hours apart) via a customized device that allowed quantifiable, repeatable loading. The device also provides for the
simultaneous recording of shear forces produced in the muscle due to the applied compressive loading (Figure 28A&B).

Both frequency and magnitude of compressive force showed a significant effect on the magnitude of shear force (p<0.05 for each parameter). Shear force was higher for 0.25Hz at both the 5 and 10N conditions as compared to the 0.5Hz condition (8.8 ± 0.2 and 13.6 ± 0.4 N vs. 5.1 ± 0.1 and 9.1± 0.2 N, respectively, p<0.05). Compressive force produced an average 39.4 ± 0.4% increase in shear force within the 0.25 and 0.5Hz conditions (p<0.05). Frequency produced an average 37.4 ± 0.4% increase in shear force between the 5 and 10N conditions (p<0.05). Therefore, the changes in shear force caused by varying the frequency and magnitude of MLL may cause differential changes in blood flow and therefore in the edema, swelling, and stiffness of the muscle itself.

Figure 28: Magnitude effect on resultant shear force. A) Resultant shear for 5N MLL. B) Resultant shear for the 10N MLL.
5.2.2 Pilot Study: Effect of immediate versus 48 hour post exercise MLL on resultant shear force.

Twenty-four New Zealand White rabbits were surgically instrumented with peroneal nerve cuffs and subcutaneous interfaces for stimulation of the hindlimb tibialis anterior muscles. Following a bout of eccentric exercise, rabbits were randomly assigned to a protocol with MLL (0.5Hz, 10N, 15min) applied immediate post EEX (0.5Hz, 10N, 15min) or 48 hours post EEX. This design allowed an n=12 for each protocol. MLL was applied over a period of four consecutive days immediately following or 48 hours post exercise and occurring daily (24 hours apart) via a customized device that allowed quantifiable, repeatable loading.

Shear force was higher daily for the immediate protocol as compared to the delayed protocol with significant differences seen on days three and four (p=0.04 and 0.01, respectively) (Figure 29). For each protocol, there was a significant difference in the
change from day one to day two (p=0.02 for immediate and p= 0.04 for delayed MLL), which could be due to dissipation of fluid and inflammatory cells present due to injury and thereby, reducing their downstream signaling of reactive oxygen species that cause the secondary inflammatory response. The analysis of the shear values was performed at approximately the half way time point of MLL (~7 minutes). Analysis within the first five minutes of MLL may show greater differences between the two protocols as that time period would have the greatest effect on initial fluid movement and our results indicate a statistical heavier wet weight for delayed MLL as compared to immediate MLL. Wet weight is believed to correlate to tissue edema, indicating a more fluid filled tissue.

5.3 Utilizing Multiple Massage Techniques

The research presented in this document has focused on Swedish massage, while many other types of manipulations, such as friction, vibration, petrissage, stretching, compression, or passive and active joint movements, are used in clinical practice. Developing a systematic understanding of these methods would allow an objective understanding of the physiological benefits of each manipulation and invite further studies of combinations of these treatments for optimal therapeutic effect. Current research shows conflicting evidence of the beneficial effect of combined treatments; however no preliminary studies were performed to determine optimal dosages of each type of massage (Shoemaker et al., 1997, Dolgener et al., 1993, Robertson et al., 2004, and Hinds et al., 2004).
5.4 Long Term Goals

Despite the fact that massage has been around for thousands of years, scientists and clinicians still do not fully understand what changes occur in the body during massage, whether they influence health, and, if so, how (Sherman, 2012). A dramatic resurgence of interest in massage, particularly among athletes, for exercise associated muscle pain, relief of anxiety, and performance enhancement (Sherman, 2012) warrants a detailed and systematic evaluation of the efficacy of this therapy. These data provide a starting point for better elucidating structure-function and for a better understanding of the effect of compressive and shear loading on muscle mechanical properties. This will lead to further understanding of tissue tolerance levels to these forces that may ultimately lead to more scientific-based prevention and treatment approaches. The long term goal of massage-like compressive loading research is to develop a detailed understanding of the scientific basis of massage in order to utilize the therapy in a prescriptive fashion clinically. The hypothesized relationship between tissue inflammation and pain in chronic conditions such as neck and back pain and even conditions such as fibromyalgia and muscle spasticity suggest the utility and potential importance of this research and its application to a wide variety of musculoskeletal and neuromuscular disorders (Omoigui, 2007).
Chapter 1


Lieber, RL. Skeletal muscle structure, function, and plasticity: the physiological basis of rehabilitation Baltimore, MD: Lippincott Williams & Wilkins, c2010


Mutungi G and RanatungaK W. The viscous, viscoelastic and elastic characteristics of resting fast and slow mammalian (rat) muscle fibres J. Physiol. 496 827–36, 1996.


Nigg, B. and Herzog, W. Biomechanics of the musculo-skeletal system. Chichester; New York: Wiley, c1999


Chapter 2


Chapter 3


Golden, C.L. and Dudley, G.A. (1992) Strength after bout of eccentric or concentric actions. MSSE 24, 926-933


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