EFFECTS OF MASSAGE THERAPY AFTER EXHAUSTIVE ENDURANCE EXERCISE

EFFECTS OF MASSAGE THERAPY AFTER EXHAUSTIVE ENDURANCE EXERCISE IN YOUNG HEALTHY MALES

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ABSTRACT

The purpose of this investigation was to examine the physiological effects of massage on indicators of skeletal muscle function following exhaustive endurance exercise. Specifically, we examined the effects of unilateral leg massage therapy on pain, muscle lactate, glycogen and damage, before (BASELINE) and at an early (EARLY, + 30 min), and later (LATE, +2.5 h) time points, following a single bout of exhaustive endurance exercise. Thirteen young, recreationally active males who exercise 2-3 times per week (age: 22.0 ± 2.7 y; weight: 75.2 ± 10.6 kg (mean ± SD)) were recruited to participate in the study. Participants underwent a baseline muscle biopsy of the vastus lateralis of one randomly assigned leg and performed a maximal oxygen uptake test (VO_{2peak}) using an incremental cycle ergometry test to voluntary exhaustion (mean VO_{2peak} = 45.9 ± 7.5 mL/kg/min, peak power = 270 ± 47 W). Two weeks later, the participants returned to the laboratory and completed an exhaustive endurance exercise protocol. Following the cycling protocol, participants were given 10 min of recovery, and were asked to report the pain levels of each of their quadriceps muscles using a visual analog scale (0 = no pain; 10 = worst pain imaginable) at two min post-exercise (post-exercise and pre-intervention (PRE)). A single Registered Massage Therapist randomly selected one leg for each participant to be massaged, while the other leg did not receive any treatment modality. Pain scores were again reported two min post-massage (post-intervention (POST)). Two muscle biopsies were taken from each leg (MAS and CON leg) on the second visit following completion of the endurance exercise protocol at + 30 min and + 2.5 h. There was no difference in post-exercise/pre-massage pain scores between the legs (CON = 7.4 ± 2.0; MAS = 7.4 ± 2.0); however, post-exercise/post-massage pain scores were lower in the massaged leg

(MAS = 2.3 ± 1.8; CON = 3.9 ± 2.3, $P < 0.001$). Massage had no effect on muscle lactate concentration, damage, or glycogen content, at any time point. In conclusion, massage therapy treatment following exhaustive endurance exercise was shown to lower pain scores but had no effect on muscle lactate, damage or glycogen content. These findings support the concept that post-endurance exercise massage therapy lowers pain perception but not through alterations in muscle lactate concentration, damage or glycogen content; consequently, the reduction in pain is more likely due to neurological and/or psychological effects associated with massage.

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TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

1.1 Massage

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" (Galloway & Watt, 2004). A variety of different techniques have been utilized by clinicians for many years to reduce pain and enhance recovery after exercise induced muscle damage. Cafarelli and Flint (1992) reported that massage treatments comprised up to 45 % of the total time in physiotherapy for sport related injuries and performance. Based on personal experience and observed positive outcomes, it is the common belief of coaches, athletes and sport medicine professionals that massage provides a number of benefits to the recipient. Massage therapy has been thought to provide a number of physical benefits to skeletal muscle, including relief of tension and stiffness, improved rate of healing, reduction in muscle pain, swelling and spasm, greater joint range of motion and overall flexibility and enhanced overall athletic performance (Bale & James, 1991; Hemmings, Smith, Graydon & Dyson, 2000; Monedero & Donne, 2000). It has also been reported that massage increases muscle blood flow and lactate clearance (Dubrovsky, 1983, 1990), which could contribute to enhanced muscle recovery.

Despite the extensive use of massage therapy as a therapeutic modality and the commonly held perception of its efficacy for reduction of post-exercise muscle pain, there is little evidence to support or refute its effects on sport performance. There is minimal and conflicting experimental evidence supporting the suggested physiological or

performance benefits of post-exercise massage treatment. Recent studies examining the physiological responses to massage therapy have presented conflicting evidence regarding the proposed mechanisms by which massage elicits changes at the cellular level, which leads to further uncertainty regarding its true effectiveness.

1.1.1 *Acute Effects of Exercise on Skeletal Muscle*

A single high intensity, exhaustive endurance exercise bout results in a number of symptoms and physiological outcomes that have been previously characterized (Warhol, Siegal & Evans 1985; Byrne, Twist & Eston 2004). Subjectively, athletes complain of muscle pain, fatigue and stiffness, which is often accompanied by a reduced ability to perform strong muscular contractions and results in delayed onset muscle soreness (DOMS) (Tee, Bosch & Lambert, 2007). Exercise induced muscle damage (EIMD) is a term used to classify this effect, which is commonly experienced following a bout of unaccustomed physical activity or following activity which is greater than normal intensity (Tee et al, 2007). From a metabolic standpoint, EIMD is often characterized by an increase in the activity of intramuscular enzymes detected in the blood plasma or serum, such as creatine kinase (CK), skeletal troponin I, myoglobin or myosin heavy chains (Tee et al, 2007; Tiidus 1997). Most of the research providing evidence of EIMD has focused on activities that predominately involve eccentric muscle actions, where the muscle is lengthening under tension. However, leg cycling for prolonged periods (>60 min) involves a combination of both concentric and eccentric movements, and also elicits some degree of EIMD (Smith, 1992; Weber, Servedio & Woodall, 1994).

The expected physiological changes following an exhaustive bout of endurance exercise would result in decreases in a number of measures of physical performance including isometric strength for up to two weeks, a decrease in isokinetic strength over different angular velocities (Byrne, Twist & Eston, 2004), and acute decreases in power generation (Byrne, Twist & Eston, 2004; Chambers, Noakes & Lambert, 1998). Other physiological responses expected following a bout of exhaustive endurance exercise include an acute increase in lactate concentration in skeletal muscle and blood plasma (Gleeson, Blannin, Walsh, Field & Pritchard, 1998), and a reduction in muscle glycogen content (Hermansen, Hultman & Saltin, 1967). From a histological perspective, studies have indicated that the initial events of EIMD involve focal disruption of the myofibrils and cytoskeleton, which results in Z-disk streaming. Damage is also seen in the mitochondria (swelling) and in the sacroplasmic reticulum (Hikida, Staron, Hagerman, Sherman & Costill, 1983). The inflammatory response to damaged muscle fibres can cause a shift of fluid and cells to the damaged tissues (Clarkson & Sayers, 1999). The increased fluid produces swelling of the myofiber and limb after injury. Neutrophils and macrophages migrate to the inflammatory sites and play a role in both the damage and repair process (Pizza, 2008; Beaton, Tarnopolsky & Phillips, 2002). The exact mechanism to explain how soreness develops and why there is a delay in pain sensation is not fully understood. (Cheung, Hume & Maxwell, 2003).

Prolonged "submaximal" exercise is typically defined as the intensity of exercise that can be sustained for durations of 30 -180 min and is usually performed at an exercise intensity of 60-85 % of maximal oxygen consumption (VO_{2max}) (Maughan, 2009). In the 1960's, Bergstrom and Hultman (1966) demonstrated a clear relationship between pre-

exercise muscle glycogen content and exercise performance. Typical muscle glycogen stores in healthy humans are approximately 350 mmol kg/dry muscle and are fairly constant in a non-exercised state. Bergstrom and Hultman (1966) showed that the point of voluntary exhaustion in 10 healthy humans biking at an intensity of 80 % of their VO_{2max} for approximately 70 min resulted in near complete depletion of muscle glycogen stores. The rate of glycogen depletion is greatest at the onset of exercise with almost half of the muscle glycogen stores being utilized within the first 15 min of exercise (Stephens & Greenhaff, 2009). The reason for this rapid initial depletion of glycogen is most likely due to the time limitation of mitochondrial ATP production from fat and carbohydrate oxidation to reach a steady state at the exercise onset; consequently, there is a greater contribution from anaerobic glycolysis during this period. Greater levels of anaerobic glycolysis at exercise onset results in greater glycogen degradation and a pronounced elevation in muscle and blood lactate concentration (Maughan, 2009). In theory, glycogen degradation could contribute to muscle damage by limiting the available energy for repair and/or limiting ATP availability for ATPases such as Na^+/K^+- ATPase, or Ca^{++} - ATPase and contribute to cell swelling or calpain activation. Following endurance exercise, glycogen generally begins to resynthesize within a few hours and at a mean restoration rate of 5-6 mmol/h, it can take approximately 24 h to normalize depleted stores (Burke, 2010).

During more prolonged endurance exercise, the rate of ATP hydrolysis is comparatively lower; therefore, the oxidation of fat and carbohydrate become the main contributor to ATP re-synthesis (Spriet, 2002). Fat exhibits a relatively low maximum rate of oxidation and cannot resynthesize ATP at a rate sufficient to maintain exercise at an intensity of more than about 60 % VO_{2max}. Thus, when higher sub-maximal exercise

intensities are achieved, carbohydrates are the preferred substrate for energy supply (Spriet, 2002). Because these stores (muscle and liver glycogen) are relatively small, carbohydrate availability is one of the limiting factors of prolonged sub-maximal exercise performance (Phillips, Green, Tarnopolsky, Heigenhauser, Hill & Grant 1996). Carbohydrate stores are depleted at the point of fatigue, particularly in Type I muscle fibers and the pre-exercise carbohydrate availability is strongly correlated with exercise performance (Maughan, 2009; Jones & Poole, 2009). It is suspected that the mechanisms responsible for fatigue are directly related to an inability to re-phosphorylate ADP at the required rate within the cell (muscle glycogen) and/or systemic hypoglycemia (liver glycogen) (Maughan, 2009).

1.2 Physiological Effects of Post-exercise Massage Therapy

A number of proposed mechanisms have been suggested in the literature to explain how massage therapy can be an effective therapeutic modality. For the purpose of this review, these mechanisms have been classified as physiological, neurological or psychological.

1.2.1 *Effects of Massage on Skin and Muscle Temperature*

Previous literature has shown increases in skin and muscle temperature after massage application, particularly with the use of the effleurage technique (Longworth, 1982; Drust, Atkinson, Gregson, French & Binningsley, 2003). Superficial skin friction increases the local heating in the area of application and consequently, is proposed to induce local hyperaemia. It is believed that this local heating is linked to increases in local microcirculatory blood flow (Weerapong, Hume & Colt, 2005). Longworth (1982)

reported an increase in skin temperature during a six min back massage; however, the skin temperature returned to baseline after 10 min. Drust and colleagues (2003) found an increase in skin and intramuscular temperature at the depths of 1.5 cm and 2.5 cm of the vastus lateralis muscle following effleurage massage interventions lasting 5, 10 and 15 min in duration. Although massage has been shown to increase skin (Longworth, 1982) and intramuscular temperature (Drust et al, 2003), such effects may not be relevant to muscle blood flow.

It is unknown whether massage related increases in skin and intramuscular temperature would result in the enhancement of athletic performance or injury prevention in the absence of changes in muscle blood flow (Shoemaker, Tiidus & Mader 1997). One possible limitation in previous studies which reported increased skin temperature with the effleurage technique was that the skin temperature quickly returned to baseline levels (Longworth, 1983), and the tissue temperature did not increase any deeper in the muscle tissue than 2.5 cm (Drust et al., 2003). These findings imply that effleurage massage techniques may not be suitable as a preparation strategy pre- competition and may not assist in injury prevention as commonly suggested.

1.2.2 *Effects of Massage on Skeletal Muscle Blood Flow and Muscle Lactate Removal*

It has been previously assumed that massage will enhance muscle recovery from intense exercise, principally due to its proposed ability to stimulate increases in muscle blood flow. However, studies to date on the effect of massage on blood flow are contradictory. Cafarelli and Flint (1992) point out that reports on limb blood flow vary

from no effect of massage to as much as a 50 % increase. For example, positive effects were reported by Dubrovsky (1983, 1990) who showed that massage promoted acceleration of muscle and venous blood flow, increased blood volume and reduced muscle tightness. However, Tiidus and Shoemaker (1995) note early studies examining the effects of massage on skeletal muscle blood flow are limited by methodological problems associated with the techniques used to determine blood flow. More specifically, the monitoring of skeletal muscle blood flow by the venous occlusion plethysmography and Xe clearance techniques used in the studies by Cafarelli and Flint (1992) and Dubrovsky (1983, 1990) respectively may limit the findings (Tiidus & Shoemaker, 1995).

Tiidus and Shoemaker (1995) and Shoemaker and colleagues (1997) used a more sensitive Doppler ultrasound method and showed that massage did not elevate muscle blood flow irrespective of massage type or muscle mass receiving treatment. More recently, Hinds and colleagues (2004) compared the effects of massage versus a resting control condition on femoral artery blood flow, skin blood flow, skin and muscle temperature after dynamic quadriceps exercise. Massage did not significantly elevate femoral artery blood flow or muscle temperature over control values. The skin blood flow and skin temperature were elevated, which is probably due to the effects of friction from massage. It was speculated that without an increase in femoral artery blood flow into the massaged leg, an increase in skin blood flow may be diverting blood flow away from the recovering muscle, and this could be deleterious (Hinds, McEwan, Perkes, Dawson, Ball & George, 2004).

A recent study by Wiltshire and colleagues (2010) examined the effects of massage and active recovery on muscle blood flow and lactate removal from exercised muscle after intense exercise. Twelve subjects performed 2 min of strenuous isometric handgrip exercise at 40 % of maximum voluntary contraction to elevate forearm lactate. Forearm blood flow was measured by Doppler and Echo ultrasound of the brachial artery and deep venous forearm blood lactate and proton concentration was measured every ten min in three conditions, passive rest, active rest and massage. The results of the study revealed that massage actually impaired lactic and proton removal from muscle by mechanical impeding the blood flow (Wiltshire, Poitras, Pak, Hong, Rayner & Tschakovsky, 2010). The massage techniques (effleurage and pettrissage), used in this study, which represent common post event techniques resulted in decreased muscle blood flow early in the recovery period after severe exercise. Therefore, sports massage would not be indicated for optimal lactic acid removal from exercised muscle in situations where acute bouts of repeated exercise are occurring.

The exact mechanisms responsible for the development of skeletal muscle fatigue are complex and not fully understood; however, a great deal of evidence exists which suggests that the accumulation of lactate and the associated protons, are partially responsible for slowing recovery from muscle fatigue (Gupta, Goswani, Sadhukhan & Mathur, 1996). Although the majority of lactate accumulated during exercise is removed by direct oxidation locally and in other muscles and through conversion to glycogen (in liver) (Gupta et al, 1996), there has been considerable research investigating whether oxidation can be enhanced by various methods of recovery. Since it has been proposed that massage could increase blood flow to the muscles being treated and blood flow plays

an important role in the removal of lactate following exercise through enhanced oxidation and diffusion out of the muscles (Belcastro & Bonen, 1975; Dodd, Powers, Callender & Brooks, 1984), previous studies have examined whether massage has an effect on lactate removal following exercise.

The more recent research studying the effects of massage on blood flow using more sensitive measurement techniques has found no effect of massage on blood flow in conduit arteries supplying skeletal muscle vascular beds (Tiidus & Shoemaker, 1995; Shoemaker, Tiidus & Mader, 1997). As such, it is not surprising that research findings have not provided much support for a positive effect of massage on lactate removal from skeletal muscle following exercise. One study reported that massage treatment could increase blood lactate removal after strenuous exercise, (Bale & James, 1991), as the post-exercise blood lactate levels were shown to be significantly lower following massage compared with a passive rest condition. However, a cool down intervention was seen to promote the most efficient lactate removal, as it produced a superior blood lactate removal rate than massage therapy. Additionally, Dolgener and Morien (1993) demonstrated superior lactate removal with a light cycling recovery compared with massage and passive recovery conditions following short term intensive exercise. More recently, Gupta and colleagues (1996) reported similar results concluding that short term massage was not effective at enhancing lactate removal and that an active type of recovery is the best modality for enhancing lactate removal after exercise.

In spite of conflicting evidence, there remains inadequate research in this area to render final conclusions. In theory, if blood flow is enhanced with massage, there could be

an increase in the rate of glucose delivery to muscle during recovery that could enhance the rate of glycogen re-synthesis. Furthermore, if lactate removal is enhanced with massage, there would be the potential for an increase in lactate delivery to the liver and gluconeogenesis (Cori cycle) with greater return of glucose back to the muscle for glycogen re-synthesis.

In spite of a lack of definite mechanistic evidence to support the effectiveness of massage at the muscle level, there is evidence of less fatigue after massage intervention (Weerapong, Hume & Colt, 2005), and the potential link between massage and an effect on metabolites implicated in the fatigue process is worthy of further investigation.

1.2.3 *Effects of Massage on Autonomic Nervous System Regulation*

It has been suggested that massage therapy may provide some of its benefits by shifting the autonomic nervous system (ANS) from a state of sympathetic activation to a state of parasympathetic activation (Field, 1998). Heightened sympathetic activation occurs when an individual prepares to mobilize or defend themselves when faced with a stress. Sympathetic activation is associated with increased cardiovascular activity, stress hormones, and feelings of tension through the systemic and local effects of norepinephrine and epinephrine on β-receptors in a variety of tissues throughout the body. Conversely, a parasympathetic (vagal) response occurs when an individual is at rest and is not faced with a threat and is associated with decreased cardiovascular activity, a decrease in stress hormones and a feeling of calmness and well-being through the effects of acetylcholine on cholinergic receptors (Sarafino, 2004).

It has been proposed that the pressure stimulation associated with massage may stimulate vagal activity (Field, 1998), which in turn lowers physiological arousal and stress hormones (cortisol levels), leading to subsequent elevation of parasympathetic responses by stimulating the sensory receptors of the skin and subcutaneous tissues. The stimuli pass along the afferent fibres of the peripheral nervous system to the spinal cord; from there it is conceivable that the stimuli may disperse through the central and autonomic nervous systems, producing various effects in any zone supplied from the same segment of the spinal cord (Wall & Melzack, 1984). Some of these effects stimulate vagal activity, resulting in a possible feeling of sedation which may be accompanied by reduction in heart rate (Wall & Melzack, 1984). Stimulating this response through physiological means may promote reductions in anxiety, depression, and pain that are consistent with a state of calmness.

1.2.4 *Effects of Massage on Muscle Mechanical Structure*

Health care professionals frequently use massage therapy techniques with the aim of breaking the cycle that causes muscle spasm or cramping with hopes of relieving muscle pain. Muscle spasm is a common, painful, physiological disturbance of skeletal muscle. (Bentley, 1996) in which muscles become tight because of excess neuromuscular stimulation. When the tissue is in a heightened state of contraction, due to an increase in neural activity, the individual muscle fibers are shortened and there is an overlapping of sacromeres (Lowe, 2003). The body has a tendency to maintain this level of contraction in a perpetual state if no other stimulus is introduced.

Disturbances at various levels of the central and peripheral nervous system and skeletal muscle are likely to be involved in the mechanism of muscle spasm and cramps, and may explain the diverse range of conditions in which they occur (Bentley, 1996). The activity of the motor neuron is subject to a multitude of influences including peripheral receptor sensory input, spinal reflexes, inhibitory interneurons in the spinal cord, synaptic and neurotransmitter modulation and descending CNS input (Bentley, 1996). The muscle spindle and golgi tendon organ are sensory proprioceptors and play key roles in controlling muscle length, tone and maintenance of posture. Changes in the activity of these receptors will occur with shortened muscle length, intense exercise and fatigue, which results in increased motor neuron activity and motor unit recruitment. This increased input can "overload" the nervous system and result in tightness in the associated muscles and hence the well known pain/spasm/pain cycle (Travell & Simons, 1983). Muscle spasm causes muscle pain directly by stimulating the mechanosensitive pain receptors or indirectly by compressing the blood vessels thereby causing ischemia and increases in muscle metabolic stimulation of afferent pain fibres (Bentley, 1996; Guyton, 2000).

It is believed that massage may help to physically rearrange muscle fibers and increase microcirculation. The realignment of muscle fibers may help to reduce muscle spasm that stimulates pain receptors and assist in reducing the pressure on the surrounding blood vessels. The improved circulation would help to increase nutritive blood flow to the damaged area. However, there is no strong evidence to support these general speculations, as the current literature suggests that massage is not likely to improve blood flow (Tiidus & Shoemaker 1995; Hinds, McEwan, Perkes, Dawson, Ball & George, 2004; Wiltshire, Poitras, Pak, Hong, Rayner & Tschakovsky, 2010). Furthermore,

there is no published literature examining the effect of massage on the physical realignment of muscle fibers.

1.2.5 *Effects of Massage on Acute Inflammation leading to Delayed Onset Muscle Damage (DOMS)*

Acute inflammation is a generalized response of the body to any kind of tissue injury as a result of chemical, thermal or mechanical stimuli (Hurley, 1973), and is part of the damage-repair process of healing. It is hypothesized that acute inflammation is the underlying mechanism associated with delayed onset muscle soreness (DOMS). DOMS is the sensation of tender, aching muscles, usually felt during movement or palpation, which occurs a day or so after unaccustomed exercise (Armstrong, 1977). A decrease in the ability to generate force and heightened sensitivity to pain are characteristics of DOMS. The soreness associated with DOMS is first sensed approximately 12 – 24 h after an unaccustomed exercise bout containing eccentric muscle contraction. Soreness peaks at about 48 h, but the complete resolution of symptoms may take anywhere from one week to 10 days (Armstrong, 1990). The consequences of damage to muscle function include prolonged loss of muscle strength (Clarkson, Nosaka & Braun, 1992), soreness (Howell, Chleboun & Conaster, 1993), decreased range of motion and increase stiffness (Clarkson, Nosaka & Braun, 1992), increased metabolic rate (Dolezal, Potteiger, Jacobsen & Benedict, 2000) and lowered athletic performance (Smith, 1992).

Histological examination of muscle cells after intense exercise, most typically eccentric in nature, indicates structural damage to the cells and discharge of cellular components, which leads to edema and a localized inflammatory response (Clarkson &

Sayers, 1999). More specifically, the mechanical stress of exercise on the muscle fibers causes disruption of structural proteins in muscle fibers and connective tissue in series between the active cross-bridges and the bony attachments, causing the sarcomeres to rupture, which is followed by a disruption in calcium homeostasis (Appell, Soares & Durate, 1992). The damage of sarcoplasmic reticulum or muscle membrane increases the intracellular calcium and triggers the calcium sensitive pathways such as the calpain proteolytic system that preferentially degrades Z-discs and troponin and tropomyosin (Armstrong 1984). Abnormally high intra-cellular calcium also has a detrimental effect impairing oxidative phosphorylation secondary to mitochondrial swelling from abnormal buffering. Histological and ultrastructural examination of the muscle provides direct evidence of this damage, where Z-disc (Z-line) broadening and streaming is a common characteristic observed (Armstrong, 1984; Ebbeling & Clarkson, 1989).

The physical damage to the muscle fibers stimulates an acute-phase inflammatory response, which includes infiltration into skeletal muscle by neutrophils and macrophages. (Fielding, Violan, Svetkey, Abad, Manfredi, Cosmas & Bean, 2000). Neutrophil infiltration follows a relatively rapid time course, as neutrophils begin to accumulate in skeletal muscle within two to four hours, reaching their peak concentration within a one day period (Pizza, 2002; MacIntryre, Reid, Lyster & McKenzie, 2000). This time period may vary depending on the intensity of the injury (Smith, 1990). They typically return to control levels within seven days after injury induced by lengthening contractions (Koh et al 2003, Pizza et al 2002). Macrophages, on the other hand, generally appear in the injured skeletal muscle after the arrival of neutrophils at approximately 24 h following injury and remain elevated while the neutrophil concentrations are diminishing (Koh et al 2003, Pizza et al 2005).

Their function is to dispose of neurotic tissue and remove foreign bodies, and ultimately assist in the healing process (Smith, 1990). Many studies have investigated the accumulation of inflammatory cells in human skeletal muscle after injury, and investigators have reported conflicted results. Some have shown an elevation in the concentration of neutrophils one to four days after EIMD (Stupka et al 2001, Hellesten et al 1997), while others found no change (Peterson et al 2003, Beaton, Tarnolpolsky & Philips 2002). Conflicting observations may be due to differences in exercise protocol, sampling time points and techniques used to quantify neutrophils in skeletal muscle. Macrophages have consistently been reported to be elevated in human muscle one to four days after exercise inducing injury (Stupka, 2001; Peterson, 2003; Beaton, Tarnopolsky & Phillips 2002).

The exact mechanisms to explain how the pain sensation develops and why there is a delay in soreness is not fully understood. It has been proposed that the presence of noxious chemicals from the structural damage (muscle and connective tissue), enzyme efflux, lactic acid accumulation and the inflammatory cells releasing chemicals like bradykinin can stimulate nerve endings that may be responsible for the pain (Cheung, Hume & Maxwell 2003; Clarkson & Sayers, 1999). Connective tissue damage has also been reported after intense exercise and may also contribute to pain and functional limitations.

Massage therapy is commonly used to alleviate DOMS because it is believed to increase blood and lymph flow and decrease edema and reduce pain. An improved rate of tissue fluid movement through the use of massage may reduce the concentration of noxious chemicals that sensitive nerve endings and reduce the soreness sensation (Moraska, 2005). Another possible mechanism by which massage may reduce DOMS is by accelerating the

delivery of nutrients to damaged tissues, thereby speeding the repair process and enhancing strength recovery. Recent studies investigating the effects of massage on muscle soreness have reported conflicting data. Significant reductions in soreness perception of DOMS after massage has been reported (Tiddus & Shoemaker 1995; Smith, Keating, Holbert et al., 1994; Bale & James, 1991), while other studies have concluded that massage is not beneficial in reducing DOMS (Hasson, Cone, Ellison 1992; Wenos, Brilla, Morrison 1994; Lightfoot, Char, McDermot & Goya, 1997).

1.3 Neurological Effects of Post-exercise Massage Therapy

1.3.1 *Effects of Massage on Pain*

Massage has traditionally been used to relieve pain, although research has only recently provided an acceptable physiological explanation for this observation. Beginning with the work of Melzack and Wall (1965) the concept of a neural "gate" in the region of the dorsal horn of the spinal cord gray matter has been central to research strategies involving pain. Although the original theory has undergone much review and revision, the central concepts remain intact (Wall & Melzack, 1984).

According to the Gate Control Theory of Pain (Melzack & Wall, 1965) stimulation of the skin evokes nerve impulses that are transmitted to three spinal cord systems: the cells of the substantia gelatinosa in the dorsal horn, the dorsal column fibers that project toward the brain, and the first central transmission (T) cells in the dorsal horn. Melzack and Wall (1965) proposed that (1) the substantia gelatinosa functions as a gate control system that modulates the afferent patterns before they influence the T cells, (2) the afferent patterns in the dorsal column system act as a central control trigger which activates selective brain

processes that influence the modulation properties of the gate control system, and (3) the T cells activate neural mechanisms which comprise the action system responsible for response and perception of pain. This theory proposes that pain phenomena are determined by interactions among these three systems.

The substantia gelatinosa acts as a gate control system that modulates the synaptic transmission of nerve impulses from peripheral fibers to central cells. The spinal cord is continually receiving incoming nerve impulses from the internal and external environment, even in the absence of stimulation. This ongoing activity is carried predominately by small myelinated and unmyelinated fibers, which tend to be tonically active and adapt slowly, and these signals serve to hold the gate in a relatively open position. When a stimulus is applied to the skin, it produces an increase in the number of active receptor fiber units, as information about the stimulus is transmitted to the brain. Since many of the large fibers are inactive in the absence of stimulus change, stimulation will produce a disproportionate relative increase in large fiber over small fiber activity. Thus, when the skin is stimulated by a mechanical pressure, the afferent volley contains large fiber impulses which not only fire the T cells but also partially close the presynaptic gate, thereby shortening the barrage generated by the T cells. If the stimulus intensity is increased, more receptor fiber units are recruited and the firing frequency is increased. The resultant positive and negative effects of the large fiber and small fiber inputs tend to counter act each other and therefore the output of the T cells rises slowly. If stimulation is prolonged, the large fibers begin to adapt, producing a relative increase in small fiber activity. As a result, the gate is opened further and the output of T cells rises more steeply.

Thus, the gate control theory proposes that when pain (A-δ and C) fibers are stimulated, pain impulses are passed presynaptically in the substantia gelatinosa (SG) and are transmitted to the brain. They will be perceived and will continue to be felt as long as pain persists. Relief of pain is dependent on the stimulation of the large myelinated A-β fibers that normally transmit the perceptions of touch and pressure. Tactile information from massage is believed to stimulate the A-β fibers, that results in an inhibitory effect setup in the same area of the SG, where pain fibers synapse with a decrease in transmission or "closing the gate" to pain. This effect presumably results from local lateral inhibition in the spinal cord (Guyton, 2000) and explains why massaging the painful area is an effective strategy for relieving pain. Cessation of large fiber stimulation would remove the inhibition of pain in the SG and open the gate to the transmission and perception of pain (Melzack & Wall, 1975). Although this theory is widely accepted and the most commonly cited explanation for pain relief in the area of massage therapy, there is no objective data to support this idea.

1.3.2 *Effects of Massage on Alpha Motorneuron Excitability*

Therapists frequently report empirical changes in reflex activity of muscles resulting from massage treatment. Some authors claim that specific techniques where the muscles are kneaded, such as petrissage, can exert an inhibitory effect on the motorneurons of the muscle being massaged (Tappan & Benjamin, 1998). Massage is believed to stimulate sensory receptors and decrease muscle tension by reducing neuromuscular excitability as measured by changes in the Hoffman reflex (H-reflex) amplitude (Morelli, Seaborne & Sullivan, 1990). The H-reflex is considered to be the

electrical analogue of the muscle stretch reflex (Weerapong, Hume & Colt). Morelli et al (1990) investigated the effects of petrissage on spinal motorneuron activity by measuring changes in H-reflex amplitude, an indirect measure of motorneuron excitability. Short periods of massage (3 and 6 min) were applied to the triceps surae muscle. The H-reflex amplitude was found to be markedly reduced during the period of massage in comparison with that obtained before or following massage. This finding was interpreted as indicating a reduction in (or inhibition) of motorneuron activity with massage.

Belanger and colleagues also reported a reduction in H reflex amplitude recorded from the triceps surae muscle, resulting from application of muscle tapping to other sites on the ipisilateral limb in neurologically non-impaired subjects. The generality of response led to the speculation that massage may also result in a generalized neurophysiological response where its effects may extend beyond the muscle being massaged. Sullivan and colleagues (1991) studied the effects of massage applied to two ipisilateral and two contralateral sites of the lower limbs, on motorneuron excitability of the right triceps surae muscle in the neurologically non-impaired subjects. The principal finding of this study was that there was a reduction in the amplitude of the H reflex recorded from the ITS while it was being manually massaged. The potential inhibitory effects of massage on neuromuscular excitability may be one of the explanations for the reduction of muscle tension or spasm following massage treatment (Sullivan, Williams, Seaborne & Morelli, 1991). Further research is needed to understand the relationship between neurological effects of massage and performance.

1.3.3. *Effects of Massage on Serotonin*

Two studies have linked massage therapy with increased levels of serotonin (Field, Grizzle, Scafidi & Schanberg, 1996; Ironson, Field, Scafidi, Hashimoto et al, 1996), which may inhibit the transmission of noxious nerve signals to the brain (Field, 1998). This pain control concept appears to be mediated in large part by the "descending pain suppression mechanism" (Goats, 1994). According to this theory, unpleasant cutaneous sensations result in stimulation of nuclei within the supraspinal area of the midbrain. In the supraspinal area, the nuclei initiate activity in the descending spinal tracts that release endogenous opiates (inhibitory transmitters) within the spinal segment receiving the painful input. This feedback mechanism thereby results in a reduction of the intensity of pain transmitted to the higher centers. This descending pain suppression mechanism theory supports the belief that massage therapy can reinforce a naturally occurring discomfort which causes a greater release of opiates, subsequently resulting in more profound pain suppression (Andersson & Lundeberg, 1995).

1.4 Psychological Effects of Post-exercise Massage Therapy

One of the most intangible aspects of athletic competition is the psychological component. Athletes understand the importance of being fully prepared to compete, which involves all aspects preparation, including physical and mental and an overall readiness to play. Therefore, the psychological "edge" provided to an athlete by undergoing a massage treatment may be an integral part of their success, regardless of the physiological effects that may or may not be appreciated. Many prominent athletes report that they utilize massage to reduce stress and help get them in the proper mood to perform

(Fleck & Kraemer, 2007), yet little empirical data exist as to the mental health effects of massage. In non-athletic populations, massage therapy has been reported to reduce psychological measures including anxiety, tension, stress, depression and increase mood and quality of life (Moyer, Rounds & Hannum,2004), all of which may contribute to enhancing athletic performance (Hemmings, Smith, Graydon & Dyson 2000).

Weinberg and colleagues (1988) performed a study to assess the relationship between exercise, massage and mood enhancement. Subjects completed the profile of mood states, state anxiety and an activation checklist prior to and 30 min post activity. Results indicated that running and massage conditions consistently produced positive mood enhancement with significant decreases in tension, confusion, fatigue, anxiety, depression and anger while maintaining high levels of vigor, which is representative of positive mental health (Weinberg, Jackson & Kolodny, 1988). A study on amateur boxers examined the effects of massage on perceived recovery and repeated sports performance, while concurrently examining the effect on blood lactate removal after boxing performance (Hemmings et al., 2000). A measure of perceived recovery was made by the subjects rating their feelings on a linear scale for four items ("refreshed", "re-charged", "rested" and "recovered") after an exercise bout that was followed by massage (Hemmings, 2001). While the punching force was not increased by massage (massage was unable to prevent a decrement in repeated performance), the perceived recovery was elevated compared to control subjects. The discovery that perceptions of recovery showed significant changes after massage gives some scientific support for the use of massage as a recovery strategy. Similar findings were observed in a study by Robertson, Watt and Galloway (2004), who examined the effects of leg massage with passive recovery on lactate clearance, muscular

power and fatigue characteristics after repeated high intensity cycling exercise. A significantly lower fatigue index was observed on the second effort in subjects who received a 20 min massage administered between two maximal cycling efforts; despite no difference noted in mean or peak power or blood lactate concentration. Although these studies do not support a direct effect of massage on sports performance, the discovery that perceptions of recovery and reduced fatigue index showed significant changes after massage gives some scientific support for the use of massage as a recovery strategy.

1.5 Effects of Massage on Recovery of Muscle Strength, Performance and Fatigue

Muscular fatigue is a complex physiological process, which cannot usually be attributed to a single mechanism. However, exercise induced alterations in muscle homeostasis, including hydrogen ion accumulation, potassium loss, depletion of high energy phosphates (ATP and creatine phosphate) and glycogen, loss of calcium homeostasis, or local ischemia may be some of the causative factors associated with disruption of the muscle excitation contraction cycle during intense exercise and in post-exercise muscle fatigue (Fitts, 1994). It is suspected that one factor associated with loss of muscle force following very intense exercise is the increased intramuscular concentration of protons (hydrogen ions) (Fitts, 1994). In addition to increased levels of intramuscular lactate, elevations in hydrogen ion concentration are also caused by decreased muscle potassium, lower levels of phosphocreatine (PCr), which limits the potential for PCr buffering capacity and increased carbon dioxide levels (Lindinger 1995). Muscle fatigue can be defined as the inability to sustain a required or expected power or force output (MacIaren, Gibson, Parry-Billings & Edwards (1989)). An integral component of an

athlete's ability to train and perform at the highest level possible is the athlete's ability to recover from intense physical effort. The cause of muscle fatigue is not fully understood and may result from a number of central and peripheral mechanisms (Moraska, 2005).

A limited amount of research studies have examined the role of massage therapy as a recovery intervention. A review of previous literature indicates there are mixed reports regarding the effect of massage on recovery from fatigue. Many of these previous studies employed a design that involved a test, intervention and re-test format. Monedero and Dunne (2000) examined the effects of massage on recovery following a second 5 km maximal effort on a bike ergometer. The cycling time of the second trial was not improved following a 15 min recovery massage as compared to the groups participating in active recovery or passive rest. However, the recovery involving a combined approach of massage treatment and active cycling at 50 % VO_{2max} (7.5 min of each) did significantly improve the cycling time for the second exercise bout (Monedero & Dunne, 2000). The study by Hemmings and colleagues (2000) that was discussed above, measured the effects of massage therapy on a second maximal force effort in eight amateur boxers. The participants underwent two sets of five, two min rounds of simulated boxing, where they were given a one min seated rest between rounds. The average force from each punch round was assessed on a boxing ergometer. Between sets, the participants received either a 20 min massage performed by a Sports Massage Therapist, which consisted of effleurage and petrissage strokes administered to the leg, back, shoulder and arms or a 20 min rest period. The results revealed that punching force was reduced across the second set at an equal rate for both the massage and control groups, indicating that massage did not alter force generation on the second effort. A possible limitation to this study which may have

influenced the results is the complex nature of the act of punching and the difficulty in addressing all muscle groups involved with massage techniques for a long enough period of time to induce effects.

1.6 Overall Conclusions and Hypotheses

Despite the common belief of many coaches, athletes and sport medicine professionals that post-exercise massage provides several benefits that ultimately lead to enhanced performance, there is limited scientific evidence to support these beliefs. Many claims have been made about massage, but few have any empirical data to support them and what little data there are tend to point more to the limitations of massage than to any significant effects. The possible mechanisms of massage usually result from author's speculations and are based on general physiological, neurological and psychological principles and effects. The strongest evidence to date indicates that the effects of massage are mainly neurological and psychological in nature.

Based on the above literature review, our objective and a priori hypotheses were as follows:

Overall Objective

To examine the effects of unilateral leg massage therapy on pain, and muscle lactate, glycogen and damage, before (BASELINE) and at an early (EARLY, + 30 min), and later (LATE, +2.5 h) time points, following a bout of exhaustive endurance exercise.

Hypotheses

1) Acutely following endurance exercise, massage therapy will reduce pain in the treatment leg (MAS) compared to the control leg (CON).
2) At both the EARLY and LATE post-exercise time points, muscle lactate concentration, damage or glycogen content will not be different between the massaged (MAS) versus the control leg (CON).
3) Exhaustive endurance exercise will result in greater indicators of metabolic disruption (higher muscle lactate and glycogen depletion) at 30 min post- exercise compared to 2.5 h post-exercise and greater muscle damage at 2.5 h.

CHAPTER 2

EFFECTS OF MASSAGE ON SUBJECTIVE PAIN, MUSCLE LACTATE, GLYCOGEN AND DAMAGE FOLLOWING EXHAUSTIVE ENDURANCE EXERCISE IN YOUNG HEALTHY MALES

2.1 INTRODUCTION

Massage therapy, the manual manipulation of soft tissue, has been utilized by clinicians for several thousand years to promote health and well being (Moyer, Rounds & Hannum, 2004). A number of theories exist to explain the mechanisms by which mechanical pressure, applied to the body during massage therapy treatment, can lead to changes in physiological responses. Despite the extensive use of massage therapy and the commonly held perception of its efficacy as a therapeutic modality, there is no strong experimental evidence to support the physiological benefits of this intervention. Recent studies examining massage therapy have presented conflicting evidence regarding the proposed mechanisms by which massage elicits changes at the cellular level (Field, 1998; Moyer, Rounds & Hannum, 2004; Tiidus & Shoemaker 1995). The suggested mechanisms of action include up-regulation of parasympathetic activity, increases in levels of hormones, stimulation of endorphin release and improved circulation of blood and lymph in response to therapeutic massage (Field, 1998; Moyer, Rounds & Hannum, 2004). It has also been postulated that massage may enhance skeletal blood flow, enhance lactate clearance and thereby improve oxygen delivery to the tissues (Monedero & Dunne, 2000). If massage truly increases blood flow to the region, then it is also expected that massage will enhance the rate of glycogen re-synthesis. It has also been proposed that massage treatment may

reduce pain by activation of the "pain gate mechanism" and release of inhibitory neurotransmitters, enhance muscle recovery from exercise and thereby stimulate a feeling of overall well-being (Weerapong, Hume & Kolt, 2006, Hemmings et al., 2000). Finally, it is also speculated that massage may have a secondary effect on muscle damage, where the amount of damage may be reduced from the increased rate of glycogen re-synthesis.

Unfortunately, the studies examining the role of massage in eliciting these positive effects have been weak in terms of scientific design, and conflicting findings have emerged. At present, there is little scientific evidence that post-exercise manual massage treatment has any effect on the physiological factors associated with muscle recovery. Despite the lack of evidence, it is generally assumed by the athletes and therapists alike that massage can enhance recovery and reduce muscle soreness after intense exercise. As such, massage therapy is used in a variety of athletic venues and treatment realms.

The purpose of this investigation was, therefore, to examine the physiological effects of massage on pain and muscle metabolites and damage following exhaustive endurance exercise. More specifically, this study examined the effects of unilateral leg massage therapy on pain, and muscle lactate, glycogen and damage, before (BASELINE) and at an early (EARLY, + 30 min), and later (LATE, +2.5 h) time points following a bout of exhaustive endurance exercise. We hypothesized that there would be lower post-exercise pain scores in the treatment leg (MAS) compared to the control leg (CON). It was also hypothesized that in spite of exercise induced higher lactate, lower glycogen and higher muscle damage, massage would not differentially alter these variables following exhaustive endurance exercise.

2.2. METHODS

2.2.1 *Study Design*

This study employed a single group, randomized design. Muscle samples were collected pre-exercise and at two time points, (EARLY, +30 min and LATE, +2.5 h) from both the MAS and CON legs. All testing was conducted in the Neuromuscular and Neurometabolic Centre of Hamilton Health Sciences, under medical supervision. All of the procedures regarding the treatment of human subjects conformed to the Helsinki declaration on the use of human subjects in research.

2.2.2 *Participants*

Thirteen young, recreationally active males who exercise 2-3 times per week (age: 22.0 ± 2.7 y (mean ± SD); weight: 75.2 ± 10.6 kg were recruited to participate in the study. The subject demographics are provided in Table 1. All participants were informed of the purpose of the study and were given a participant information sheet that contained a description of the procedures, and possible risks involved in participation, and of participant's rights. Participants were assessed as being healthy based upon their individual responses to a standardized medical questionnaire, which was used to ensure that they were not taking any medications and had no pre-existing condition that would preclude them from participating.

2.2.3 *Diet and Activity Prior to Trial*

Participants arrived in the laboratory in the morning for their initial visit following an overnight fast. They were requested to adhere to the following requests prior to testing:

abstain from any form of physical exertion for 72 h, abstain from alcohol for 48 h, eat their habitual diet for 48 h and abstain from caffeine for 12 h. Participants were given a 355-kcal defined formula diet (Ensure, Ross Laboratories, Columbus Ohio, USA) to be consumed two h before each experiment.

2.3 General Trial Design

Participants meeting the inclusion criteria were asked to read an information sheet, sign a consent form and complete a medical questionnaire. Participants reported to the laboratory on two separate occasions, separated by a two-week period.

2.3.1 *Initial Visit*

At the initial visit, the participant's height and weight were recorded. The participants then underwent a baseline muscle biopsy procedure, which is outlined below. They then performed a maximal oxygen uptake test (VO_{2peak}) using an incremental cycle ergometry test to voluntary exhaustion. The average peak VO_{2peak} was 45.9 ± 7.5 mL/kg/min and the average peak power was 270 ± 47 W. During the high intensity endurance exercise trial the mean time to test completion was 67.8 ± 5.4 min.

2.3.2 *Intervention Visit*

Approximately two weeks following the baseline testing, the participants returned to the laboratory for their second visit. Participants then completed an exhaustive endurance exercise protocol on the cycle ergometer for approximately 70 min, as described below. Following the cycling protocol, subjects were permitted 10 min of unstructured recovery prior to the start of the treatment intervention. Prior to the treatment

intervention pain scores were recorded, using a visual analog scale, where subjects were asked to report the pain levels of their right and left quadriceps muscles with "0" being no pain and "10" being the worse pain imaginable. These scores were obtained from each participant two min post-exercise (post-exercise and pre- intervention (PRE)) and then repeated two min post-massage (post-exercise and post- intervention (POST)).

2.3.3 *Exhaustive Endurance Exercise Protocol*

The exercise protocol, as previously described by Mahoney and colleagues (2004), consisted of upright cycling exercise on an electrically braked cycle ergometer (Lode Excalibur model, Lode, Amsterdam, The Netherlands) pedaling at a workload calculated to elicit 60 % of their predetermined VO_{2peak} for 30 min at a cycling cadence greater between 70 to 90 rpm. Following the initial 30 min, the intensity was increased to a workload equivalent to 65 % $VO_{2\ peak}$ for 5 min, then dropped back to 60 % for 5 min, increased to 70 % $VO_{2\ peak}$ for 5 min, dropped to 60 % for 5 min, etc., to a maximum of 85 % $VO_{2\ peak}$. 85 % $VO_{2\ peak}$ was attained, then subjects continued with intervals of 85 % $VO_{2\ peak}$ for 2 min followed by 60 % $VO_{2\ peak}$ for 2 min, etc., until test completion. Test completion was the time point when subjects were unable to maintain a cycling cadence of 70 rpm.

2.3.4 *Treatment Intervention*

A single Registered Massage Therapist (RMT) randomly selected a piece of paper with either an "R" or "L" on it, which indicated which leg she would massage. One leg was randomized to massage (MAS), the other leg did not receive massage treatment (CON). With the participant in a supine position on a massage table the RMT commenced the standardized massage protocol on the subject's entire frontal thigh within 10 min after

completion of the exhaustive endurance exercise protocol. The massage intervention lasted a total of 10 min and consisted of a combination of massage techniques. The massage started with 2 min of effleurage, which is a light stroking technique that was delivered with a moderate pressure, (subjective scale ~5/10) as defined by the RMT, with verbal confirmation of the relative pressure with the participant. The next 3 min involved petrissage, which is a firmer, kneading massage technique. During petrissage the objective is to lift the muscle tissue away from the underlying structures, gently knead or compress and then release the muscle (Moraska, 2005). The next 3 min of the massage protocol involved slow muscle stripping, which consisted of longitudinal strokes which lasted for 40 sec intervals up the thigh and then repeated. The final 2 min utilized effleurage techniques delivered with moderate pressure (subjective scale ~3/10) to function as a cool down. Once the massage was completed, the leg was wiped clean and pain scores were reported. With the exception of the massage therapist conducting the treatment, all other members of the study team were blinded as to which leg was massaged.

2.3.5 *Muscle Sampling (Biopsy procedure)*

A percutaneous muscle biopsy of the vastus lateralis of one leg (randomly selected to be used as a baseline) was performed on the first visit. Needle biopsy samples were obtained under local anaesthesia (2% lidocaine) using a modified 5mm Bergstrom needle with manual suction, as described by Mahoney and Tarnopolsky (2005). The incisions were made on the lateral portion of the vastus lateralis starting ~15 cm from the midline of the knee. The biopsy needle was inserted to a depth of ~4 cm, dependent on the depth of subcutaneous fat, and once it was felt to be ~2 cm beyond the fascia, the needle was

advanced proximally along the muscle to ensure that the opening of the needle was essentially parallel with the longitudual axis of the muscle fibres. To obtain the sample of muscle, a single clip of the biopsy needle was used.

Two needle biopsies were taken from each leg (MAS and CON leg) on the second visit at two specific time points following completion of the exhaustive endurance exercise protocol (30 min post-massage invention (EARLY) and 2.5 h post-massage invention (LATE)). All biopsies were between 2 – 3 cm apart, and from separate incisions. Each muscle sample was immediately dissected free from fat and connective tissue and partitioned into four portions. One portion was placed in a chilled (4° C) fixative (2 % glutaraldehyde buffered with 0.1% sodium cacodylate) for staining with toluidine blue, as described previously (Beaton, Tarnopolsky, Phillips 2002). Samples of 30 – 50 mg were immediately placed into polyethylene and plunged into liquid nitrogen and after 2 h, transferred into a -80° C freezer until subsequent analysis.

2.4 Analyses

2.4.1 *Lactate Analyses*

Lactate levels were determined by an enzymatic assay procedure following the principles devised by Hannon and Quinton (1984). Briefly, lactate is enzymatically converted to pyruvate using the enzyme lactate dehydrogenase (LDH). During this reaction the cofactor NAD+ is converted quantitatively to NADH. This conversion can be monitored fluorometrically by observing the increase in fluorescence in the sample over time. The reaction is forced to completion by capturing the reaction product, pyruvate, with hydrazine in an alkanine medium to ensure complete conversion of the lactate. The

lactate assay reagents of concentrated hydrazine, glycine and NAD+ are combined and brought to volume with distilled water. The pH is adjusted to 10.0 in order to create the final buffer solution for the assay. The dilute enzyme, LDH is mixed with the reagent by inversion. The homongenized muscle sample is incubated for 15 min in this solution. The first reading is taken after 15 minutes of incubation. Using a standard curve the assay determines the amount of lactate based on the increase in fluorescence created by the NADH produce in each sample. This assay determines NADH content using an excitation wavelength of 340 nm and an emission wavelength of 460 nm. The sensitivity is approximately 100nm. See Appendices for the lactate analysis technique.

2.4.2 *Muscle Damage Analyses*

The glutaraldehyde fixed muscle was post-fixed in 1% osmium tetroxide, dehydrated in graded alcohol and embedded in plastic resin (Spurr's) with the fibres orientated longitudinally. The longitudinal, semi-thin sections (~1 μm in thickness) were cut with a glass knife and stained with toluidine blue for light microscopic evaluation. Individual fibres from each muscle section were studied under 1000 X magnification and examined for disruption. An average of 61 fibers (range 13-100) per sample were counted and the degree of muscle damage was assessed through visual inspection. Focal damage was defined as encompassing more than two adjacent Z-disks, and extensive damage was defined as encompassed no more than two adjacent Z-disks. We confirmed that the areas reported as Z-disk streaming by toluidine blue did represent Z-disk streaming by cutting ultrathin sections from the same blocks, straining them with uranyl acetate and lead acetate, and examining them using a JEOL 1200 EX transmission electron microscope (n= 3

blocks). Results are expressed as the number of focal or extensive areas of Z-disk steaming per fiber. Samples were blindly assessed by a pathologist with specialized training in electron microscopy.

2.4.3 *Glycogen Analyses*

Glycogen levels are determined using the muscle glucose assay procedure following the principles devised by Passoneau and Lowry (1993). In order to perform this assay, glycogen must first be hydrolysed to glucose. Having done this, the procedure uses a coupled enzyme assay, where hexokinase is used to covert ATP and glucose into ADP and glucose-6-P. The second reaction uses the enzyme G-6-P dehydrogenase to convert glucose-6-P and NADP to gluconolactone and NADPH. In a manner similar to that noted for lactate the amount of glycogen is determine from a standard curve based on a measured increase in fluorescence (excitation = 340nm; emission = 460 nm) that occurs when NADP is converted to NADPH by G-6-P dehydrogenase. See appendences for the full details of the glycogen assay technique.

2.4.4 *Statistics*

All statistical analysis was performed using commercially available software (SPSS, Chicago, IL). This study was a within subject repeated measures design. A repeated measure analyses of variance (ANOVA), using a general linear model was first carried out to determine the effects of exercise by examining the responses in the non-massaged leg at three time points (BASELINE, EARLY and LATE) for the dependant variables of interest, which were muscle lactate, muscle glycogen (total, pro- and macro-glycogen) and muscle damage (total area per mm^2). With findings of $P < 0.05$, we conducted a *post hoc* test for all

pair-wised comparisons using paired t-tests. To account for multiple comparisons, we adjusted the p value by dividing 0.05 by 3 (P value for *post hoc* significance = 0.017) according to the Bonferroni correction method.

Paired t-tests comparisons between the CON and MAS legs were used to examine the effect of post-exercise massage on muscle lactate, glycogen (pro-glycogen and macro-glycogen) and damage (total area per mm^2) at the two time points (EARLY and LATE). The effect of post-exercise massage on pain (leg specific) was analyzed by paired t-tests with pre-planned comparisons including comparison of within each leg changes in pain over time (PRE vs. POST), comparison between legs at each time point (CON vs. MAS) and comparisons of the delta change in pain over time within each leg (CON vs. MAS). For these pair-wise comparisons between CON and MAS legs statistical significance was set at $P < 0.05$. Values are presented as means plus or minus standard deviation (SD).

2.5 Results

2.5.1 *VO_{2peak} and high intensity endurance trial*

The subject demographics are provided in Appendix F. The average peak VO2 was 45.9 ± 7.5 mL/kg/min and the average peak power was 270 ± 47 W. During the high intensity endurance exercise trial the mean time to test completion was 67.9 ± 5 min.

2.5.2 *Effects of exercise on non-massaged control leg (CON)*

There was no difference in muscle lactate concentration at any testing time point ($P = 0.061$). There appeared to be an effect of time on muscle damage, as there was a significant difference in the muscle damage across the three time points ($P = 0.034$). These

differences did not reach significance in the Bonferonni corrected post-hoc pair-wise comparison between the any of the time points, as the paired t-tests revealed P = 0.345 (BASELINE vs. EARLY), P = 0.084 (EARLY vs. LATE) and P = 0.053 (REST vs. LATE) (Figure 1).

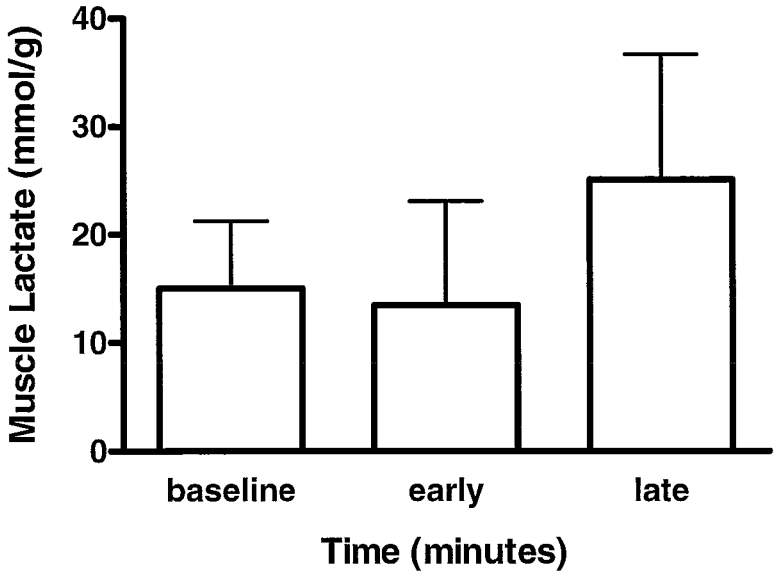
Exercise resulted in a reduction in total muscle glycogen in the CON leg at both the EARLY (P < 0.001) and LATE (P = 0.001) time points compared to BASELINE. No differences were observed between the early and late time points (P = 0.214) (Figure 2). There appeared to be an effect of time on macro-glycogen (P = 0.04). Pair-wise comparisons of the three time points revealed that this difference was not significant (did not reach the corrected P value of P = 0.017) between any of the two time points where P = 0.026 (BASELINE vs. EARLY), P = 0.855 (EARLY vs. LATE), P = 0.026 (BASELINE vs. LATE). There was also an effect of time on pro-glycogen (P = 0.001). Pro-glycogen was lower at LATE (2.5 h) vs. BASELINE where P<0.001 (Figure 2).

2.5.3 *Effects of post-exercise massage*

Endurance exercise resulted in pain scores of 7.4 ± 2.0 in the CON and 7.4 ± 2.0 in the MAS leg at the pre-massage time point. The subjective pain scores reported in both the CON and MAS leg were lower following the massage (P = 0.002 (CON leg PRE vs. POST) and P < 0.001 (MASS leg PRE vs. POST). The pain scores following the massage were lower in the MAS leg (2.3 ± 1.8) than the CON leg (3.9 ± 2.3) where P= 0.001. The difference between the pain scores (PRE –POST) in the MASS leg was greater than the difference in pain in the CON leg (P = 0.001) (Figure 3).

The massage intervention did not differentially affect lactate concentrations. No change was seen from BASELINE at either time points (P=.683 (EARLY time point) and P=.693 (LATE time point)). Similarly, muscle damage was not altered at any time point (P=0.113 (EARLY) and P=0.717 (LATE)) (Figure 4).

In spite of lower glycogen content post-exercise, there were no differences in total glycogen between the MAS and CON leg at either time point, where P = 0 .743 (EARLY) and P = 0.319 (LATE). There was also no effect of massage on macro-glycogen or pro-glycogen, as the difference between the values at EARLY and BASELINE between both legs was not significant (P=0.899 and P= 0.736 respectively). Similarly, there was no difference in the values of macro-glycogen or pro-glycogen noted between legs when comparing BASELINE and LATE time points, where P= 0.450 and P= 0.317 respectively) (Figure 5).



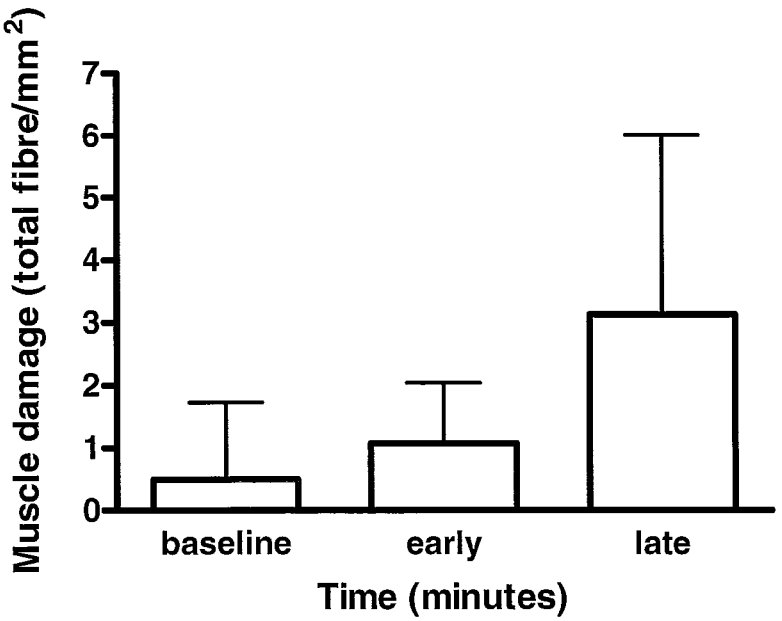


Figure 1: Effect of exercise on muscle lactate and muscle damage at three time points (baseline, early, late). Values are mean ± SD for 6 subjects.

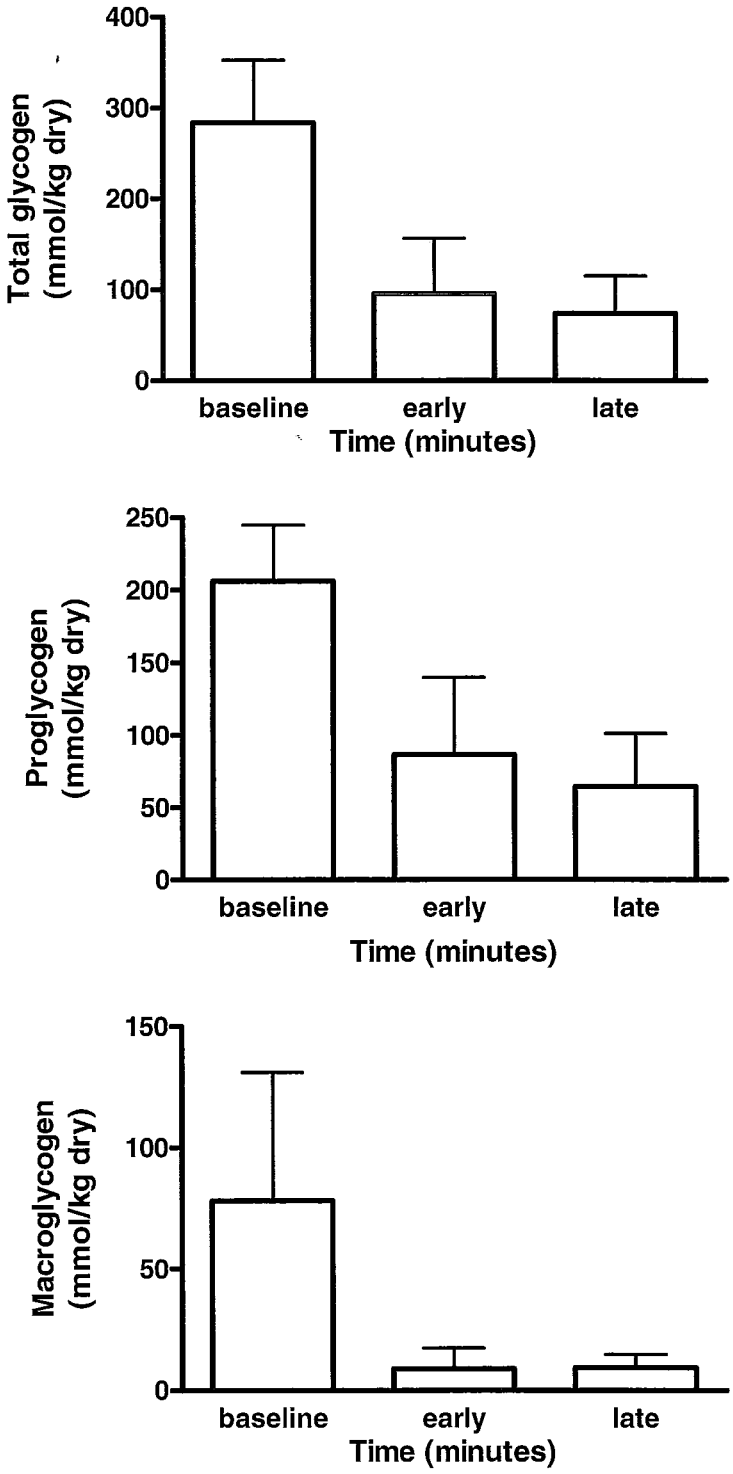


Figure 2: Effect of exercise on muscle glycogen (total glycogen, pro-glycogen, macro-glycogen) at three time points (baseline, early and late). Values are mean ± SD for 6 subjects.

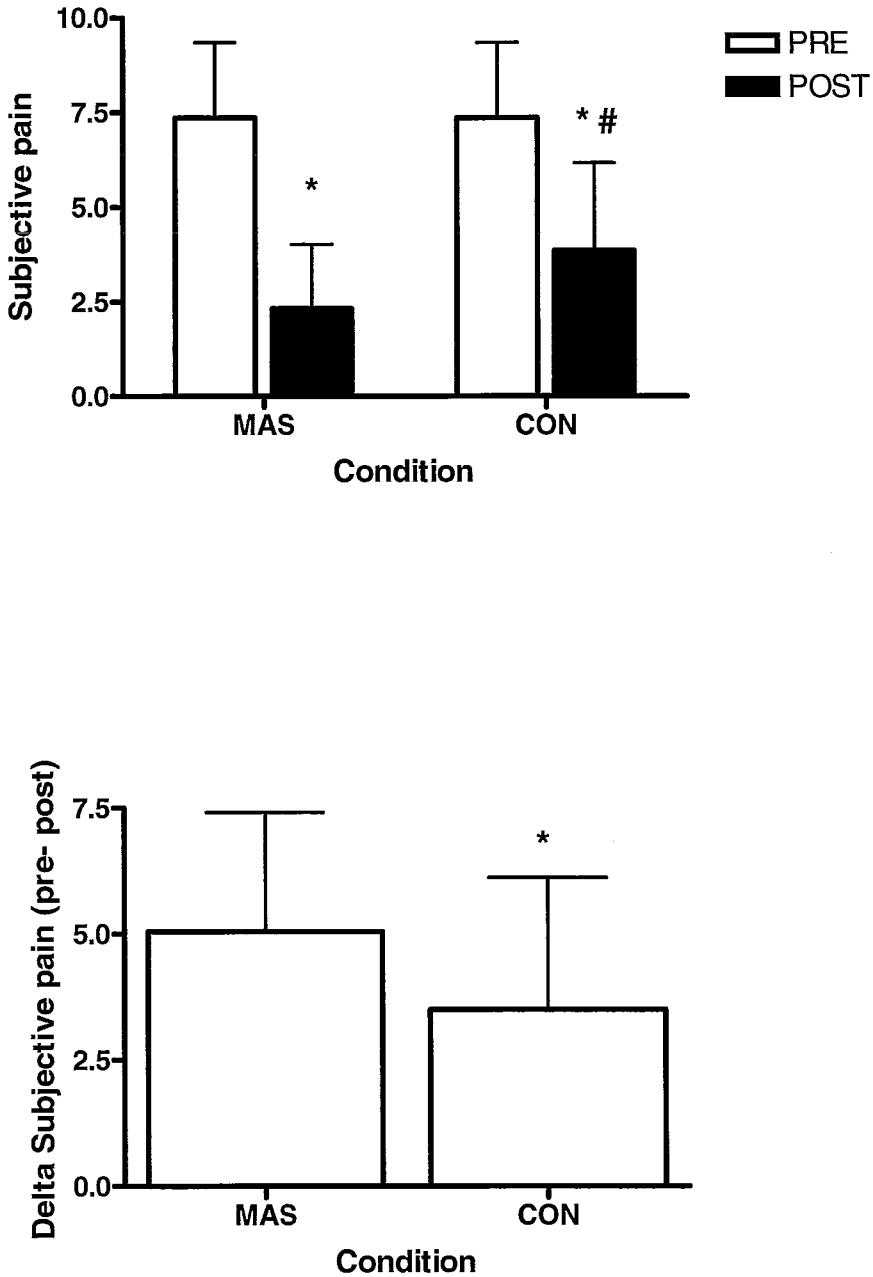
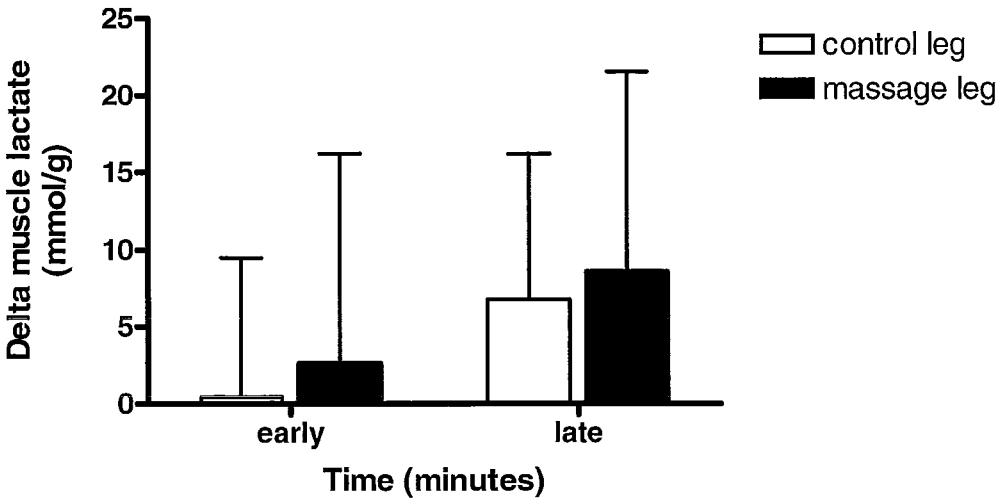


Figure 3: Effect of post-exercise massage on subjective pain and the delta change in subjective pain values in the massaged (MAS) and control (CON) leg. Values are mean ± SD for 11 subjects. *denotes a significant difference from PRE within a condition and # denotes a significant difference from massage at same time point ($P<0.05$).



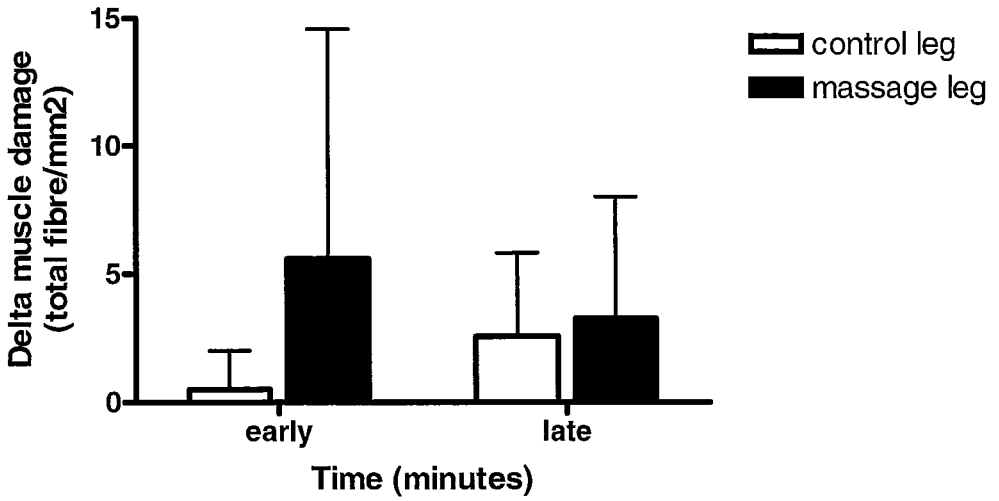


Figure 4: Effect of post-exercise massage on delta muscle lactate and delta muscle damage values in the massaged (MAS) versus control (CON) leg at early and late time points. Values are mean ± SD for 6 subjects for lactate and 9 subjects for muscle damage.

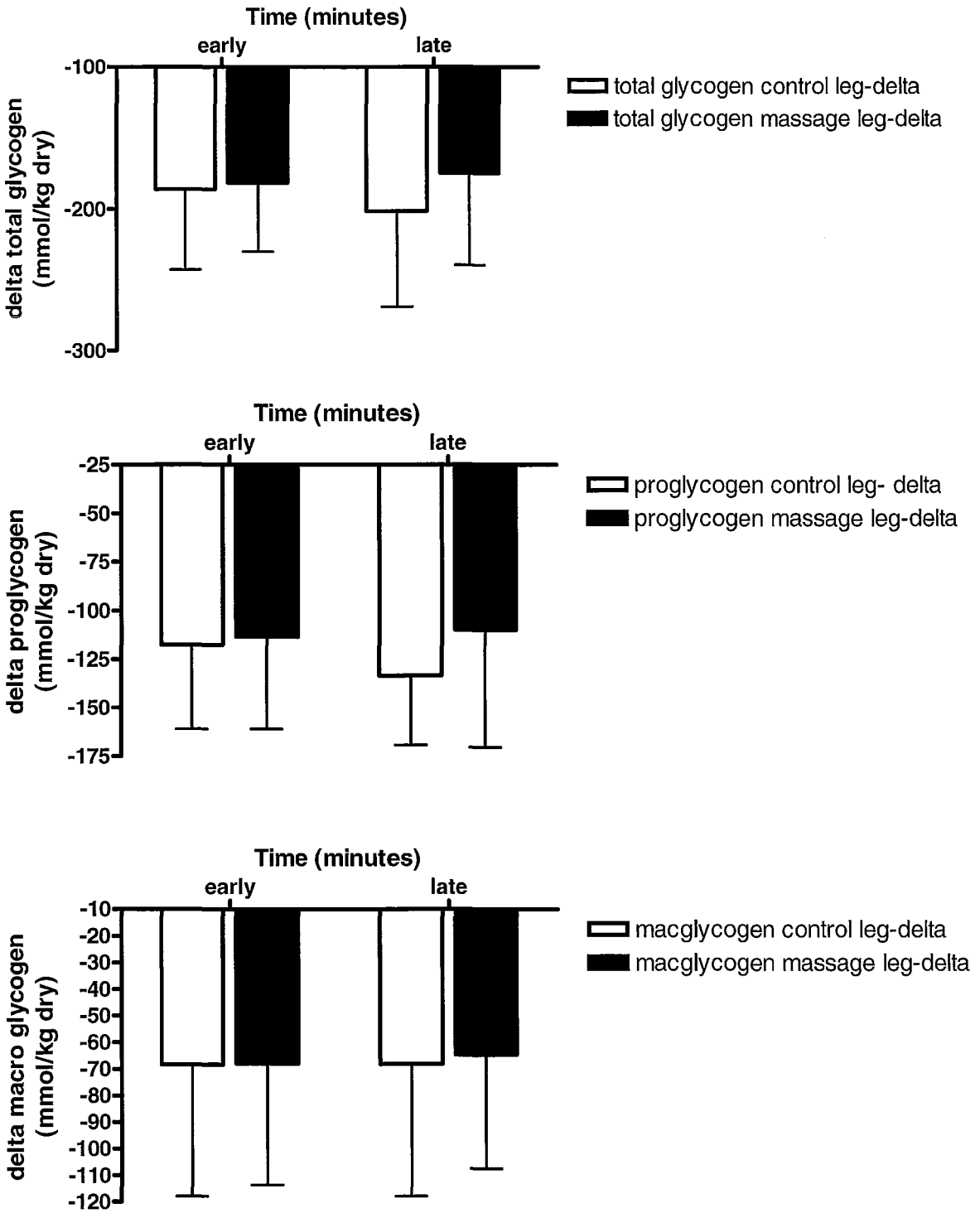


Figure 5: Effect of post-exercise massage on delta muscle glycogen (total glycogen, pro-glycogen, macro-glycogen) values in the massaged (MAS) versus control (CON) leg at early and late time points. Values are mean ± SE for 6 subjects.

2.6 Discussion

The main novel finding from this study was that pain levels were lower in the post-exercise massaged leg. Despite the observed therapeutic effects of massage on pain, the massage intervention had no effect on muscle lactate, damage or glycogen content. Together, these findings support the concept that although post-endurance exercise massage therapy does lessen pain, it must do so with mechanism(s) different than alterations in muscle lactate concentration, damage or glycogen content.

A secondary objective of this study was to examine the acute (up to 2.5 h) time course of skeletal muscle metabolic responses following exhaustive endurance exercise. Interestingly, we found no difference between the EARLY (30 min) and LATE (2.5 h) muscle glycogen content post-exercise and no effect of exercise on muscle lactate or damage.

Despite the fact that massage therapy is recommended for the management of various pain related conditions, especially those that are musculoskeletal in nature, the effectiveness of massage for management of musculoskeletal pain is a matter of debate. Reviews of the literature on the use of massage for low back pain have concluded that the evidence in support of the beneficial effect of massage is inconclusive, mainly due to the weak scientific methodology used in previous trials (Ernst, 1999). Previous investigations have been identified as having a variety of research design weaknesses including ambiguous definitions and descriptions of massage, poor reporting of the protocols used and inconsistency in the various techniques reported (Godrey, Morgan & Schatzker, 1984; Hsieh, Phillips, Adams & Pope (1992).

The reduction in pain, or more appropriately of pain perception that was noted in the current study following massage treatment can potentially be attributed to a few probable mechanisms. The massage specific reduction in post-exercise pain may be associated with an interruption of pain impulses along the pain pathway (Melzack & Wall, 1965). Inhibition through a reflex action is also likely and it is possible that both of the mechanisms were occurring together. An interruption in the transmission of afferent impulses may have taken place at one of the three sites, which are the periphery, where the irritation of the pain receptor occurs, in the spinal cord or at higher levels of the CNS (Wall & Melzack (1984).

One method by which massage may have lead to a pain reduction was through the inhibition of the sensory pain impulses. This mechanism of inhibition is based on the speed of the impulses travelling along the sensory pathways. Applying light touch to the skin during the massage intervention may have stimulated non-pain, low threshold afferent fibers, which are larger and faster A-β fibers (Melzack & Wall, 1975). The impulses travelling along these fibers reach the spinal cord at a faster rate and therefore dominate over the slower stimuli. In doing so, they "blocked" the slower pain impulses travelling along the Class C fibers and other Class A-δ fibers. This proposed mechanism is based on the Gate Control theory (Melzack & Wall, 1967). The "gating mechanism" controls the entry of all upcoming sensory impulses, and in particular those coming from the nociceptors. Because of the "pain gate" mechanism, pain impulses from the fatigued skeletal muscle in the legs may have been modified or prevented from ascending along the cord to the brain due to the manual massage. As a result, the pain intensity in the

massaged leg at any given post-massage time point was reduced and in some cases, not perceived at all.

In the current study, the massage intervention was carried out for a period of 10 min. This provided a continuous flow of sensory impulses along the sensory nerves, which may have helped keep the gates closed or in a partially open position, thereby blocking the pain stimuli from reaching the CNS and reducing the perception of pain in the massaged leg. Another mechanism which may have contributed to the reduction in pain scores in the massaged leg post-massage is a reduction of pain impulses reaching the upper nerve centres in the brain. The sympathetic reaction to pain by the hypothalamus and cortex may have been reduced in response to the massage treatment. This concept has been supported in two previous studies that linked massage therapy with increases in levels of serotonin (Field, Grizzle, Scafidi & Schanberg, 1996; Ironson, Field, Scafidi, Hashimoto et al, 1996), which may inhibit the transmission of noxious nerve signals to the brain (Field, 1998). One outcome is that the descending pathways which emerge from some parts of the thalamus and brain stem can selectively inhibit transmission of impulses originating in the nociceptors. Some of these inhibitory neurons can also release certain endorphins that can exert a weakening influence on the sensory neurons transmitting pain impulses. This inhibition may also help to reduce muscle and tissue tension (Cassar, 2004). This descending pain suppression mechanism theory supports the belief that massage therapy can reinforce a naturally occurring discomfort which causes a greater release of opiates, subsequently resulting in more profound pain suppression.

One of the most probable explanations for the reduction in pain scores reported following the massage intervention is the strong psychological benefits that accompany this form of hands-on treatment. Although extremely difficult to quantify, there is strong anecdotal evidence from both clinical and athletic settings, that manual therapies can result in changes in emotional factors such as expectation, anxiety and fear which can, in turn, influence the perception of pain (Cassar, 2004). It is often seen that the greater the amount of stress and tension felt by the participant, the stronger the perception of pain. The participants in the study reported their "EARLY" pain score approximately two min after the completion of a strenuous exercise regime to voluntary exhaustion. Accompanying all of the physiological aspects of muscle pain and exhaustion was a strong emotional challenge of pushing themselves beyond their normal comfort zone. The stress of the cycling protocol can therefore exacerbate pain, while relaxation, as achieved with the massage treatment, can be said to be instrumental in pain reduction. Therefore, the "POST" pain scores reported approximately two min following the massage may have been greatly influenced not only by the "relaxed state" of the muscles being massaged but also the participant's feeling of an overall relaxation. This theory is supported by the work of Weinberg and colleagues (1988), who performed a study to assess the relationship between exercise, massage and mood enhancement. The results in this study indicated that massage conditions consistently produced positive mood enhancement with significant decreases in tension, confusion, fatigue, anxiety, depression and anger which is representative of positive mental health (Weinberg, Jackson & Kolodny, 1988). It should be considered that if the positive therapeutic effects of massage are related more to a psychological effect, where the participant is feeling an overall sense of relaxation, then we

may not see any significant difference between the legs, but rather a more global effect. Thus, we would expect to see a reduction of pain over time that is more rapid compared to an individual who did not receive any massage. This concept should be considered when determining study design in future investigations.

Hemmings and colleagues (2000) examined the effects of massage on perceived recovery and repeated sports performance after boxing performance. While the punching force was not increased by massage, the perceived recovery was elevated compared to control subjects. Similar results were noted by Robertson, Watt and Galloway (2004), who found a significantly lower fatigue index in subjects who received a 20 min massage between two maximal cycling efforts, despite no difference noted in mean or peak power or blood lactate concentration. Although these studies do not support a direct effect of massage on pain or sports performance, the discovery that perceptions of recovery and reduced fatigue index showed significant changes after massage gives some scientific support to the notion of the psychological benefits of massage for the athletes.

It was proposed that the exhaustive exercise would result in changes in muscle lactate, (where the levels would be higher 30 min post-exercise compared to 2.5 h in the control leg) and damage, (where the levels would be higher 2.5 h post-exercise). We also anticipated greater changes in substrate availability, as it was suspected that the glycogen levels would be more depleted at 30 min post-exercise than at 2.5 h post in the control leg.

The lack of changes in these markers of physiological disruption may be explained by a few probable factors. The muscle samples were taken 30 min post-exercise, which may have given the muscle ample time to clear the excess lactate from muscle. Another

factor was the small sample size of lactate available (N=6), which may have influenced the results. We acknowledge that some of the absolute values for muscle lactate do not fall within the normal range (baseline values of 23 for 1 subject), likely indicating a sample contamination or preparation error. This study was a smaller portion of a larger study with a variety of outcomes and there was not sufficient human muscle tissue available to have complete data sets for all analyses, thereby contributing to the small sample size for some variables at some time points.

Certainly one of the most noted and proclaimed effects of massage in the athletic domain, is its ability to "flush out" and remove lactate from the muscle. Therapists and athletics alike believe that the manipulation of soft tissue through the use of popular massage techniques like those used in this study, will clear lactate from the area and expedite the recovery process. The current literature and our data clearly show that by the EARLY post-exercise period, most of the lactate generated in the first portion of the exercise (i.e. first 15 min of the 70 min bout) is gone 30 min after exercise completion. This notion has been supported by previous studies (Bergstrom & Hultman, 1966; Spriet, Howlett & Heigenhauser, 2000). Since the lactate would be naturally cleared and back to normal baseline values by this time period, regardless of any intervention post-exercise, it is not surprising that massage did not have an effect on lactate. Nevertheless, it is clear from the current findings that any effect of massage on pain or any other variables at or beyond the + 30 min time-point is not due to lactate differences.

The results of this study clearly revealed that massage had no effect on post-exercise glycogen re-synthesis as no significant differences were seen in glycogen levels at

either of the time-points following exercise. Previous work from Dr. Tarnopolsky's laboratory showed that there was no glycogen re-synthesis in the four hours following exhaustive endurance exercise in non-fed men (Tarnopolsky, Bosman, MacDonald, Vandeputte, Martin & Roy, 1997); however, it was possible that massage could have enhanced this for the reasons suggested above (increased delivery of glucose to muscle). Given the lack of increase in post-exercise glycogen, any potential increase in blood flow that may have been created by massage, did not lead to an increased delivery of glucose to the muscle. In spite of the theory that massage will lead to enhanced lactate clearance, which could result in an increase in the rate of glycogen re-synthesis (indirectly via liver gluconeogenesis), our data on lactate provided further support for the lack of an effect on on glycogen. Furthermore, glycogen concentration could not be related in any mechanistic way to the lower pain scores and could not likely enhance recovery.

Finally, massage treatment post-exercise had no effect on muscle damage in this study, as no significant difference in muscle damage was noted at either time point. We had expected to see some evidence of damage based upon earlier work showing damage at four hours post-exercise using the identical technique (Beaton, Tarnopolsky & Phillips, 2002). Although the current study used an endurance exercise protocol, the degree of muscle damage at four hours post-exercise using the exact same protocol was identical between the two modes of exercise (eccentric vs. endurance)(Parise, Mahoney and Tarnopolsky, unpublished observations, 2000). Some believe that muscle damage actually occurs during exercise, as a result of structural damage and enzymes "breaking down" the muscle, in which case, massage could have an immediate benefit on damage. However, more recent research has revealed that it may take hours for this structural damage to

occur (Stupka, 2001; Peterson, 2003; Beaton, Tarnopolsky & Phillips 2002). Although not significant, the trend was certainly present that there was more evidence for structural damage to the muscle at the LATE vs. EARLY post-exercise time point. In spite of the trend, there was no differential effect of exercise that was apparent. As mentioned earlier, there is an inflammatory response in muscle after unaccustomed exercise that is seen at 4 h post-exercise, with a significant increase in muscle neutrophils (Stupka et al 2001, Hellesten et al 1997). Because our study examined the immediate effects of massage on damage (up to 2.5 h post-exercise), we don't know if there is any influence of massage on inflammation.

2.6.1 *Limitations*

Similar to previous massage studies, the timing and duration of the massage intervention, as well as the amount of pressure applied with each type of massage was difficult to standardize (Callaghan, 1993). The use of an experienced RMT provided consistency in treatment techniques and assisted with the consistent replication of the massage techniques that would be provided within a field/sport setting. The amount of pressure and timing of each phase of treatment was clearly defined throughout each specialized technique, where the RMT used an objective 0-10 rating to best attempt to standardize the massage intervention between subjects. The massage techniques chosen for this study were based on those previously used in past studies (Godrey, Morgan & Schatzker, 1984); Jonhagen, Ackermann, Erikson, Saartok & Renstrom, 2004) and those which are used commonly in clinical practice. The type of technique utilized and the length of time taken for each technique may have influenced the results, as different techniques

may elicit different mechanical and physiological effects. In this study, the participants were used as their own controls. This approach reduced the chances for confounding variables such as individual differences in response to massage or rest (control). However, there can be limitations to using a within subjects design alone, as it could result in effects that might be explained otherwise by spontaneous recovery or a placebo effect. Addressing this issue by having additional treatment groups may have strengthened the findings that massage alone explained the beneficial effects observed.

Another obvious limitation in this study was the sample size. Originally 13 participants were recruited and attended the initial study visit, but only 11 subjects returned and completed the intervention visit. Unfortunately, due to the multi-pronged nature of the investigation, the muscle samples obtained were used for a number of different analyses prior to this study. As a result, this study did not have the necessary amount of muscle needed for all subjects to obtain full data for the lactate and glycogen analyses, which resulted in missing data for some subjects at different time points. Consequently, some of the statistical tests were run with a small sample size (n=6), which made it difficult to obtain significant findings. Another important observation was noted when examining the lactate values at baseline. Normal resting values of muscle lactate have been reported at levels between 5-10 mmol/kg. One of our BASELINE values was 23mmol/kg, which may be classified as an outlier.

2.6.2 *Future Considerations*

The potential for massage therapy to aid in muscle recovery and enhance athletic performance has garnered great interest from coaches, athletes and medical professionals, particularly those specializing in the field of sport medicine. A common feature in this study and many of the others performed examining the effects of massage report the use of a single massage session administered after exercise. In many of these instances, only one limb or body part received the massage. Future massage studies could experiment with the application of additional massage sessions at different times post-exercise and may lead to different results. Furthermore, the application of massage to more than just one limb is another possible strategy that could be employed. It would be useful to design a follow up study that looks at massage intervention with different trials, looking at massage of one limb at one intervention and both limbs during a second intervention. This would allow the author to investigate whether the reports of perceived pain would be different in the different conditions. Past studies have linked the reduction in pain reports following massage treatment to positive mood enhancement with significant decreases in tension, confusion, fatigue, anxiety, depression and anger, leading to a feeling of positive mental health (Weinberg, Jackson & Kolodny, 1988). If this is truly the case, it would be interesting to investigate if the results would be different based on whether one or both limbs were massaged. Alternatively, in future investigations where the subject is being used as their own control, it may be useful to apply a sham physiotherapy treatment on the non-massaged leg to diminish the chance of a placebo effect. Careful consideration must be made in selecting this treatment, as it must not involve any light touch, stimulation or true intervention technique that would influence the results. Another important consideration

for future research is the need for standardization of massage therapy techniques and their application. This includes factors such as time of treatment after exercise, duration of treatment and time of data collection following the treatment intervention.

2.6.3 ***Conclusion***

In summary, massage therapy intervention following exhaustive endurance exercise was shown to have a therapeutic effect on pain. Despite the observed effects of massage on pain, the massage intervention had no effect on muscle lactate, damage or glycogen content. This latter finding supports concept that post endurance exercise massage therapy does not impact perceived pain levels through alterations in muscle lactate concentration, damage or glycogen content, however the reduction in pain is more likely due to neurological and psychological effects associated with massage.

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McMaster University
Health Sciences

Hamilton Health Sciences

APPENDIX A: LETTER OF INFORMED CONSENT

Title of Study:

Does massage therapy alter metabolites and gene expression profile in muscle?

Principal Investigator: *Dr. Mark A. Tarnopolsky, Departments of Pediatrics and Medicine, McMaster University*

Co-Investigators: *Dr. Brian W. Timmons, Departments of Pediatrics, McMaster University*

Colleen Cupido, Physiotherapist, Department of Athletics and Recreation; MSc student, Department of Kinesiology, McMaster University

Sponsor: *NSERC, New Investigator Fund (awarded to Dr. Brian Timmons)*

INTRODUCTION

You are being invited to participate in a research study conducted by Colleen Cupido, Dr. M. Tarnopolsky and Dr. Brian Timmons because you are a healthy man. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once

you understand the study, you will be asked to sign this form if you wish to participate. Please take your time to make your decision. Feel free to discuss it with your friends and family or your family physician.

WHY IS THIS RESEARCH BEING DONE?

Massage therapy is widely used by different health care professionals to reduce symptoms of muscle pain and to help muscle recovery following exercise. However, we do not know the effect of massage on muscle damage and the genes within the muscle. Understanding these effects will allow for increased knowledge in the field and will benefit both clinicians and patients.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to determine the effects of massage therapy after exercise to see if it will reduce muscle damage and change the genes found in muscle.

WHAT IS MASSAGE THERAPY?

Massage is a type of therapy that is based on the use of hands to treat specific problems in an individual's soft tissue of the body, which includes skin, fascia and muscle. This hands-on treatment applies mechanical pressure to the tissues and is believed to reduce muscle pain, improve range of motion, increase blood flow and improve overall function. Different techniques are used in massage to move the tissues in hopes of gaining positive effects. Effleurage describes the gliding or stroking techniques used to gain the mechanical effects of massage.

WHAT WILL MY RESPONSIBILITIES BE IF I TAKE PART IN THE STUDY?

If you volunteer to participate in this study, we will ask you to do the following things:

1) Visit our laboratory for an initial visit for approximately 2 h

- During your initial visit, we will explain the study to you and will be asked some questions about your general health. We will also take some basic

measurements of your height and weight. You will be asked to review and sign the informed consent form. We will collect some blood through a very small, flexible plastic tube placed in a vein in your arm. At the same time, we will take one muscle biopsy of ~150 mg (about the size of the eraser on the end of a pencil) of muscle from the outer thigh of both of your legs. The procedure will take about 15 min. You can walk and exercise immediately after the muscle biopsy. You will then ride a stationary bike for a minumum of 15 min, and no more than 30 min. The load on the bike will increase every 2 min. We will put a monitor on you to record your heart rate while you cycle. You will be breathing into a mouthpiece (with a nose clip sealing off your ose) so we can collect all of the air you breath out. The breathing apparatus is equiped with a sterile disposable filter which will be disgarded after your test. You will be encouraged to ride as long as you can (VO2 max). You will remain in the laboratory until you are fully rested and recovered from the bicycle test.

2) Return back to the laboratory two weeks following your initial visit for approximately 3 ½ h

- You will be performing an endurance test on the stationary bicycle, which lasts ~75 min. It will get harder as you go along. You will undergo a 10 min massage therapy treatment performed by a Registered Massage Therapist (RMT). This treatment will be performed on one of your upper thighs, as you lie comfortably on your back on a treatment table. This treatment will involve hands-on actions in the form of muscle gliding and stroking (effleurage) in a comfortable, controlled manner. Blood samples and muscle biopsies will be taken on both legs (as above) both at 30 min and 3 h after the massage treatment.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

i) *Cycling.* The exercise you are asked to complete will require a maximal effort. There is a risk of major heart problems during endurance exercise. However, in order to minimize these risks you will undergo an exercise stress test prior to beginning the study. Also, all exercise sessions will be supervised by individuals trained in CPR and there will be a complete "crash cart" on site. The design of the exercise protocol is progressive in order to further decrease the risk of heart problems. We will monitor your heart rate during the cycling session. You will experience feeling of extreme fatigue and exhaustion, and may experience aching pain and weakness in your leg muscles. Our lab has not had a significant problem in over a decade of studies aside from the occasional ice pack use. Physical assessment will be available from Dr. Tarnopolsky and if appropriate, consultation will be arranged.

ii) *Blood sampling.* A single needle will be used to place the small, flexible plastic tube. A small bruise might appear where the needle goes through your skin. A total of 50 ml (about 5 tablespoons) will be taken over a 1 hour period. Because we will give you a drink throughout this visit, taking this amount of blood will have no negative effects.

iii) *Muscle biopsies.* Dr. Tarnopolsky has performed over 14,000 muscle biopsies in patients and healthy research subjects ranging in age between 1 week and 90 years. A dull ache at the site of biopsy occurs for 24-48 h, which can be reduced with ice and mild

analgesics. As with any cut to your skin, there is the possibility of a small scar to the site of biopsy. There is a slight risk of local skin infection (4/10,000), developing a fibrous lump at the site of biopsy (6/10,000; which disappeared with massaging the muscle for < 1 week), and developing a small patch of numbness just past the biopsy incision (full recovery was reported in < 3 months for all cases).

iv) *Massage therapy.* Massage treatment is a hands-on type of therapy. It is non-invasive and should not create any significant pain during treatment. At times, some discomfort may be felt due to the mechanical pressure of the therapists' hands during the treatment, but this discomfort should resolve once the stroking techniques are finished and the hands are removed.

HOW MANY PEOPLE WILL BE IN THIS STUDY?

We are asking 13 young men to participate in this study. Your participation is voluntary.

WHAT ARE THE POSSIBLE BENEFITS FOR ME AND/OR FOR SOCIETY?

We cannot promise any personal benefits to you from your participation in this study. Your participation will help us to see if massage therapy does in fact promote muscle recovery and alter genes in muscle. This information will benefit health care professionals and patients who use massage therapy as their choice of treatment as it may provide the evidence to support its use clinically. This information will also be important for future research in this area.

WHAT INFORMATION WILL BE KEPT PRIVATE?

All of your information will be stored in locked filing cabinets under the supervision of Dr. Mark A. Tarnopolsky and Dr. Brian W. Timmons for 5 years. We will supervise access to your information by other people in our group, if necessary. You will be assigned a subject number, and this number will be used to identify you. Records identifying you will be kept confidential. If the results of the study are published, your identity will remain confidential.

CAN PARTICIPATION IN THE STUDY END EARLY?

If you volunteer to be in this study, you may withdraw at any time. The investigator may withdraw you from this research if circumstances arise which warrant doing so.

WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

You will receive $200 for participating in the study. If you quit the study for personal reasons, we will change the amount for the time spent in the study. If you choose to quit because of a complication from the study, we will give you the full amount.

IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CALL?

If you have any questions about the research now or later, or if you think you have a research-related injury, please contact Colleen Cupido: 905 525 9140 x20344 or Dr. Brian Timmons: 521-2100 x76930 (Daytime) or 318-4754 (Nighttime) or Dr. Mark Tarnopolsky: 905-2100 x76593 or 521-2100 x76443 (#2888). If you have any questions regarding your rights as a research participant, you may contact Office of the Chair of the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board at 905-521-2100, ext. 42013.

CONSENT STATEMENT

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in this study entitled: *"Does massage therapy alter metabolites and gene expression profile in muscle?"* I understand that I will receive a signed copy of this form.

Name of Participant

Signature of Participant Date

Consent form administered and explained in person by:

Name and title

Signature Date

SIGNATURE OF INVESTIGATOR:

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

Name and title

Signature of Investigator Date

FUTURE RESEARCH

At the end of the study, we may wish to store leftover sample for use in a future study. We will not store your sample longer than 5 years. All records identifying you will remain confidential. Information about you will not be released. If the results of the study are published, your identity will remain confidential.

CONSENT STATEMENT FOR STORAGE OF SAMPLES (BLOOD AND MUSCLE)

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to have my blood and muscle stored so they can be used in future research studies approved by the Research Ethics Board other than the one described in this information form.

_____

Name of Participant

_____

Signature of Participant

_____

Date

Consent form administered and explained in person by:

_____

Name and title

_____

Signature

_____

Date

SIGNATURE OF INVESTIGATOR:

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to have their blood and muscle stored so they can be used in future research studies approved by the Research Ethics Board other than the one described in this information form.

Name and title

Signature of Investigator

Date

Appendix B: Freeze Drying Protocol

Tarnopolsky Lab
Revised Feb 2010

Equipment:	Buschi vacuum pump (V-500) Thermo Sasvant Micro Modulyo Freeze dryer

1.	Transfer samples from storage (-80°C) to a styrofoam box of liquid nitrogen.
2.	Turn on the freeze dryer to allow it to go to the proper temperature. Make sure the port valves are turned to *vent* (as opposed to *vac).* Do not start the vacuum at this time.
3.	Using an 18G needle (1.5 length), poke 3 or 4 holes in the top of a fresh 1.5ml eppendorf tube. Label each tube and transfer muscle to tubes, keeping samples frozen. Samples should weigh 15-25mg when wet. *Note:* It is easier to poke holes in room temperature tubes than frozen tubes.
4.	In a beaker, mix dry ice chunks and acetone to make a cold slurry. Be cautious of acetone spillover when adding dry ice.
5.	Turn on the vacuum only if the freeze dryer temperature indicator light is now on.
6.	Place a freeze dryer flask in the dry ice slurry about halfway up the side. When the acetone has settled from the warm flask insertion, add the samples to the flask. Do not add more than 10-15 samples per flask. Split samples evenly over several flasks. The cold solution should keep the samples cool for transfer to the freeze dryer, yet minimize condensation within the flask that would harm the samples.
7.	With the flask still in the slurry, place the beaker under the freeze dryer port. Quickly attach the flask and make sure the lip is sealed around the flask. Minimize time spent out of the slurry. Turn the port valve to *vac* as soon as the flask is properly attached. A seuction sound and some tube movement in the flask is normal.
8.	Repeat this process for any additional flasks.
9.	Typically 18-24 h is enough to fully dry samples. Usually a test sample is placed amongst actual study samples that is first checked for dryness. This is often a rodent piece of muscle that is expendable. The tester is quickly removed from the freeze dryer and then the flask is placed back on the dryer to continue until dryness is confirmed.
10.	When samples are dry, turn the port valve to *vent* and remove the flask. Place tubes in Ziploc bag of drierite and put in -80°C until powdering and extraction.
11.	Turn off the vacuum.
12.	Turn off the freeze dryer.

Appendix C: Proglycogen and Macroglycogen Extraction from Freeze Dried Muscle Tissue

Adapted from Devries (2005) and Adamo & Graham (1998)

1.	Freeze dry tissue for 24 h. Powder and dissect free of connective tissue, etc.
2.	Add 200uL of ice cold 1.5M PCA to 1.5-2.5mg tissue.
3.	Press with a plastic inoculation loop or glass rod for 20 min on ice to make sure all the tissue is exposed to the acid. Press the first sample for about a min, then move on to the next sample. When all samples are finished, go back through each one again. An inoculation loop is needed for each sample.
4.	Spin for 15 min at 3000rpm, at 4 degrees.
5.	Remove 100uL of supernatant for MG.
6.	Aspirate remaining supernatant and discard, but keep pellet.
7.	Add 1mL of 1M HCl to each sample (100uL MG and pellet for PG).
8.	Briefly press PG samples and briefly vortex MG samples then weigh tubes to make sure no liquid is lost to evaporation.
9.	Hydrolyze samples for two h at 100°C in a water bath. Let tubes cool and then weigh. Use 5mL Pyrex tubes with glass stoppers (marbles). If there is a difference of greater than 50uL in pre/post weighing, add deionized water to match the amount of water that has evaporated.
10.	Neutralize reactions with 2 M tris base. Determine the amount of tris base needed prior to starting the procedure, bu adding base to I M HCl to determine how much you need to add to neutralize the HCl in the sample. I'd do it with 10mL HCl and then add base by the mL to start. I've found that usually .7mL base neutralizes 1mL HCl.
11.	You can now transfer the samples to labeled eppendorf tubes. Vortex and spin at 3000rpm for 5 min room temperature. Store at -80°C.
12.	

Appendix D: Muscle Lactate Assay for Fluorometer

(LDH)

Lactate + NAD^+ ----------→ Pyruvate + NADH + H^+

Pyruvate + Hydrazine ----------→ Pyruvate Hydrazone

Reagent	Stock Con.	Final Con.	Volume (250 mL)
1. Hydrazine	1.00 M	100.0 mM	25 mL
2. Glycine	1.00 M	100.0 mM	25 mL
3. NAD^+	100.0 mM	0.5 mM	1250 µL
LDH (Sigma L2500)			See procedure

1. Hydrazine Buffer (1.0M) – Add 3.15 g Hydrazine Dihydrochloride to 24 ml of dH20, slowly add 2400 mg NaOH, bring to final volume of 30 ml and FILTER.
Store at 0-4 degrees C for up to 3 months (Sigma 216208-200g).

2. Glycine Buffer (1.0M) – Add 2.25 g glycine to 30 ml dH20.

3. NAD^+ – Add 132.7 mg NAD^+ to 2 ml dH20 (Sigma N1511-5g FW 663.4)
Store at -80 degrees C (small aliquots) for 3 months.

Note: Mix reagents 1-3 together. Bring to volume with distilled water and adjust to pH 10.0. (this is the buffer solution for the assay)

Preparation of Dilute Enzyme

Add 17.25 µL of LDH to 1.0 mL of reagent. Mix by inversion.
(for 265 cuvettes, add 172.5 ul of LDH to 10 ml of buffer)

Assay Procedure:

Part 1:

1. Add 555 ul of buffer to each cuvette
2. Add 30 ul of H20 (blank) or standard. *Note for muscle samples add 15 ul of PCA extract & 15 ul of buffer to cuvette directly
3. Incubate for 15 min
4. Take your first reading (excitation setting 340; emission setting 460; sensitivity 100)

Part 2:

1. Add 30 µL of dilute LDH to all cuvettes
2. Place in dark for 120 min
3. Take a second reading

Lactate Standard Curve
(premade lactate standard 4.44 mM – stored in 1.0 ml aliquots in freezer)

Conc (uM)	Stock (µL)	Distilled water (µL)
25	5.6	994.4
50	11.25	988.25
100	22.5	978.5
200	45	955
800	180	820

Appendix E: Muscle Glucose Assay

Passoneau & Lowry (1993)

Principle
Hexokinase ATP + glucose ----------ADP + Glucose-6-P G-6-P dehydrogenase Glucose-6-P + NADP----------Gluconolactone + NADPH

Reagents	
1.	Tris pH 8.1 (1M stock solution) [fridge]
2.	Magnesium Chloride (1M stock solution) [freezer] 203.3mg/mL
3.	DTT (0.5 M stock solution) [freezer]
4.	ATP, disodium salt, Sigma A-2383 (MW=551.1) 33mg/200uL
5.	NADP, Roche 128 058 (MW = 787.4) 39.4mg/mL
6.	D-(+)-glucose; dextrose; corn sugar, Sigma G-8270 (MW=180.2)
7.	Glucose-6-P Dehydrogenase: from yeast, grade 1 Roche 127 035 (1mg in 1 mL ~ 350 U/mg) 10 127 655 001 5mg/mL ~ 350U/mg
8.	Hexokinase (from yeast), Roche 1 426 362 (1mL; ~1500U/mL)

Stock Solutions	
1.	ATP, 300mM: dissolve 33.0mg in 200uL dH_2O
2.	NADP, 50mM: dissolve 39.4mg in 1mL dH_2O

Buffer	**Concentration**	**50mL**	**100mL**	**150mL**	**200mL**
Tris, pH 8.1	1M	2.5mL	5.0mL	1.5mL	10mL
$MgCl_2$	1M	50uL	100uL	150uL	200uL
DTT	0.5M	50uL	100uL	150uL	200uL
ATP	300mM	50uL	100uL	150uL	200uL
NADP	50mM	50uL	100uL	150uL	200uL
pH to 8.1 with NaOH, bring to volume then add G-6-P-DH					
G-6-DH	350U/ml	5uL	2uL	15uL	6uL

Enzyme	
1.	Dilute 10uL of hexokinase in 1000uL of assay buffer. Mix by inversion. 10uL + 1000uL = 1010uL. To make 4mL HlC, 4mL buffer + 40uL HK

Concentrations in Assay Mixture:	
Tris	50mM
$MgCl_2$	1mM
DTT	0.5mM
ATP	300uM
NADP	50uM
G-6-P-DH	0.02 U/mL
Hexokinase	0.3 U/mL
Glucose	~0.15-18.0 uM

Standards				
Glucose (1.0mM): Dissolve 36.04 glucose in 200mL dH_2O (may be stored frozen at -80°C in 1 mL aliquots)				
Standard	Concentration	Stock B	dH_2O	Glucose concentration in buffer
1.	5uM	10 uL	1990 uL	0.15 uM
2.	10 uM	20 uL	1980 uL	0.30 uM
3.	20 uM	40 uL	1960 uL	0.60 uM
4.	40 uM	80 uL	1920 uL	1.20 uM
5.	60 uM	120 uL	1880 uL	1.80 uM
6.	80 uM	160 uL	1840 uL	2.40 uM
7.	100 uM	200 uL	1800 uL	3.00 uM
8.	200 uM	400 uL	1600 uL	6.00 uM
9.	400 uM	800 uL	1200 uL	12.0 uM
10.	600 uM	1200 uL	800 uL	18.0 uM

Procedure:	
Settings for L555: Ex=340nm, Em=450nm, voltage = 700, delay = 5 seconds, int = 5 seconds	
1.	Prepare buffer and pipette 1mL into each cuvette.
2.	Add 30uL dH_2 (blank), standard or muscle extract to cuvette. *NB:* for muscle glycogen assay, use 10uL extract + 20uL dH_2O
3.	Mix, wait 5 min for equilibriation, then take first reading at 30 second intervals (can do 20 second intervals when comfortable)
4.	Add 20uL of hexokinase to each tube.
5.	Mix, wait 15 min then take second reading.

Recipes for reagents than can be stored in the freezer	
Glucose	See above

Tris (1M)	60.55 g TRIS base (FW 121.1) Add 250mL dH_2), pH with HCl bring to 500mL with dH_20 Store at 0-4 degrees
$MgCl_2$ (1M)	0.2033g $MgCl_2.6H_2O$ (FW 197.9) Add 1.0 mL H_2O Make fresh daily
DTT (0.5M)	0.7715g Dithiolthreitol (FW 154.3) Sigma D8255 Add 10mL H_2O Store -50°, 3 months

Appendix F: Participant Demographic Information and Maximal Oxygen Uptake Values

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age_(years)	13	19	29	22.00	2.646
Height_(cm)	13	142	180	173.69	10.610
Weight_(kg)	13	63.6	88.6	75.146	8.0442
BSA	13	1.71	2.08	1.9046	.10890
Oxygen (max)	13	30.8	64.2	44.931	8.2374
Valid N (listwise)	13				

Appendix G1: Lactate ANOVA

Effects of exercise on non–massaged leg:

Lactate

Within-Subjects Factors

time	Dependent Variable
1	Lacaterest
2	lactate30
3	lactate2.5

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	476.106	2	238.053	3.746	.061
	Greenhouse-Geisser	476.106	1.618	294.251	3.746	.077
	Huynh-Feldt	476.106	2.000	238.053	3.746	.061
	Lower-bound	476.106	1.000	476.106	3.746	.111
Error(time)	Sphericity Assumed	635.566	10	63.557		
	Greenhouse-Geisser	635.566	8.090	78.561		
	Huynh-Feldt	635.566	10.000	63.557		
	Lower-bound	635.566	5.000	127.113		

Tests of Within-Subjects Contrasts

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	302.141	1	302.141	3.618	.116
	Quadratic	173.965	1	173.965	3.990	.102
Error(time)	Linear	417.567	5	83.513		
	Quadratic	217.998	5	43.600		

Tests of Between-Subjects Effects

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5727.008	1	5727.008	41.576	.001
Error	688.739	5	137.748		

Descriptive Statistics

	Mean	Std. Deviation	N
Lacaterest	15.0177	6.18039	6
lactate30	13.4407	9.61117	6
Lactate2.5	25.0533	11.58832	6

Appendix G2: Muscle Damage ANOVA

Effects of exercise on non–massaged leg:

Muscle Damage

Within-Subjects Factors

time	Dependent Variable
1	musdamrest
2	musdam30
3	musdam2.5

Descriptive Statistics

	Mean	Std. Deviation	N
Musdamres	.4999	1.22650	8
musdam30	1.0714	.96816	8
musdam2.5	3.1322	2.88128	8

Tests of Within-Subjects Contrasts

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	27.718	1	27.718	5.417	.053
	Quadratic	2.958	1	2.958	1.522	.257
Errortime	Linear	35.820	7	5.117		
	Quadratic	13.602	7	1.943		

Tests of Between-Subjects Effects

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	58.994	1	58.994	16.018	.005
Error	25.782	7	3.683		

Appendix G3: Pro-glycogen ANOVA

Effects of exercise on non–massaged leg:

Pro-glycogen

Within-Subjects Factors

Measure:MEASURE

time	Dependent Variable
1	progrest
2	prog30
3	prog2.5

Descriptive Statistics

	Mean	Std. Deviation	N
Progres	205.868	38.83576	6
prog30	86.3491	53.36421	6
prog2.5	64.4330	36.72556	6

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	69538.658	2	34769.329	47.409	.000
	Greenhouse-Geisser	69538.658	1.570	44283.812	47.409	.000
	Huynh-Feldt	69538.658	2.000	34769.329	47.409	.000
	Lower-bound	69538.658	1.000	69538.658	47.409	.001
Error (time)	Sphericity Assumed	7333.868	10	733.387		
	Greenhouse-Geisser	7333.868	7.851	934.075		
	Huynh-Feldt	7333.868	10.000	733.387		
	Lower-bound	7333.868	5.000	1466.774		

Tests of Within-Subjects Contrasts

Source	time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	60012.210	1	60012.210	120.075	.000
	Quadratic	9526.448	1	9526.448	9.852	.026
Error time	Linear	2498.944	5	499.789		
	Quadratic	4834.925	5	966.985		

Appendix G4: Macro-glycogen ANOVA

Effects of exercise on non–massaged leg: Macro-glycogen

Within-Subjects Factors

time	Dependent Variable
1	macglyrest
2	macgly30
3	macgly2.5

Descriptive Statistics

	Mean	Std. Deviation	N
Macglyrest	78.1246	52.99369	6
macgly30	8.8666	8.41607	6
macgly2.5	9.2590	5.40786	6

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	19078.571	2	9539.285	9.749	.004
	Greenhouse-Geisser	19078.571	1.013	18841.607	9.749	.026
	Huynh-Feldt	19078.571	1.022	18666.444	9.749	.025
	Lower-bound	19078.571	1.000	19078.571	9.749	.026
Error(time)	Sphericity Assumed	9784.966	10	978.497		
	Greenhouse-Geisser	9784.966	5.063	1932.687		
	Huynh-Feldt	9784.966	5.110	1914.719		
	Lower-bound	9784.966	5.000	1956.993		

Tests of Within-Subjects Contrasts

Source	time	Type III Sum of Squares	Df	Mean Square	F	Sig.
Time	Linear	14227.397	1	14227.397	9.862	.026
	Quadratic	4851.173	1	4851.173	9.432	.028
Error(time)	Linear	7213.355	5	1442.671		
	Quadratic	2571.611	5	514.322		

Tests of Between-Subjects Effects

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	18528.218	1	18528.218	19.474	.007
Error	4757.065	5	951.413		

Appendix G5: Total Glycogen ANOVA

Within-Subjects Factors

Measure:MEASURE_1

factor1	Dependent Variable
dimension1 1	totglyrest
2	totgly30
3	totgly2.5

Descriptive Statistics

	Mean	Std. Deviation	N
totglyrest	283.9933	68.03789	6
totgly30	95.2155	60.74871	6
totgly2.5	73.6920	40.66205	6

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
factor1	Sphericity Assumed	160653.928	2	80326.964	48.126	.000
	Greenhouse-Geisser	160653.928	1.456	110343.111	48.126	.000
	Huynh-Feldt	160653.928	1.901	84529.642	48.126	.000
	Lower-bound	160653.928	1.000	160653.928	48.126	.001
Error(factor1)	Sphericity Assumed	16690.927	10	1669.093		
	Greenhouse-Geisser	16690.927	7.280	2292.790		
	Huynh-Feldt	16690.927	9.503	1756.419		
	Lower-bound	16690.927	5.000	3338.185		

Tests of Between-Subjects Effects

Measure:MEASURE_1

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	410238.288	1	410238.288	61.832	.001
Error	33173.884	5	6634.777		

Appendix H1: Lactate Post-Hoc T-Tests

Lactate Post Hoc Test – T-tests

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	lacaterest	15.0177	6	6.18039	2.52313
	lactate30	13.4407	6	9.61117	3.92374
Pair 2	lactate30	13.4407	6	9.61117	3.92374
	lactate2.5	25.0533	6	11.58832	4.73091
Pair 3	lacaterest	15.0177	6	6.18039	2.52313
	lactate2.5	25.0533	6	11.58832	4.73091

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	lacaterest & lactate30	6	.541	.268
Pair 2	lactate30 & lactate2.5	6	.353	.492
Pair 3	lacaterest & lactate2.5	6	.038	.943

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	lacaterest - lactate30	1.57698	8.14492	3.32515	-6.97059	10.12454	.474	5	.655
Pair 2	lactate30 - lactate2.5	11.6126	12.16441	4.96610	-24.37836	1.15317	-2.338	5	.067
Pair 3	lacaterest - lactate2.5	10.0356	12.92389	5.27616	-23.59841	3.52717	-1.902	5	.116

Appendix H2: Muscle Damage Post-Hoc T-Tests

Post Hoc test – T-tests (Muscle Damage)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	musdamrest	.4999	8	1.22650	.43363
	musdam30	1.0714	8	.96816	.34230
Pair 2	musdam30	1.0714	8	.96816	.34230
	musdam2.5	3.1323	8	2.88128	1.01868
Pair 3	musdamrest	.4999	8	1.22650	.43363
	musdam2.5	3.1323	8	2.88128	1.01868

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	musdamrest & musdam30	8	-.047	.912
Pair 2	musdam30 & musdam2.5	8	.152	.720
Pair 3	musdamrest & musdam2.5	8	-.061	.887

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	musdamrest - musdam30	-.57150	1.59786	.56493	-1.90735	.76435	-1.012	7	.345
Pair 2	musdam30 - musdam2.5	-2.06087	2.89712	1.02429	-4.48293	.36118	-2.012	7	.084
Pair 3	musdamrest - musdam2.5	-2.63238	3.19912	1.13106	-5.30690	.04215	-2.327	7	.053

Appendix H3: Pro-glycogen Post-Hoc T-Tests

Post Hoc Test - T-tests (Pro-glycogen)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	progrest	205.868	6	38.83576	15.85463
	prog30	86.3491	6	53.36421	21.78585
Pair 2	prog30	86.3491	6	53.36421	21.78585
	prog2.5	64.4330	6	36.72556	14.99315
Pair 3	progrest	205.868	6	38.83576	15.85463
	prog2.5	64.4330	6	36.72556	14.99315

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	progrest & prog30	6	.514	.297
Pair 2	prog30 & prog2.5	6	.771	.072
Pair 3	progrest & prog2.5	6	.651	.161

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	progrest - prog30	119.51964	47.19618	19.26776	69.99028	169.04899	6.203	5	.002
Pair 2	prog30 - prog2.5	21.91611	34.25295	13.98371	-14.03015	57.86237	1.567	5	.178
Pair 3	progrest - prog2.5	141.43575	31.61609	12.90722	108.25669	174.61480	10.958	5	.000

Appendix H4: Mac-glycogen Post-Hoc T-Tests

Post Hoc – T-tests (Macro- glycogen)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	macglyrest	78.1246	6	52.99369	21.63458
	macgly30	8.8666	6	8.41607	3.43585
Pair 2	macgly30	8.8666	6	8.41607	3.43585
	macgly2.5	9.2590	6	5.40786	2.20775
Pair 3	macglyrest	78.1246	6	52.99369	21.63458
	macgly2.5	9.2590	6	5.40786	2.20775

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	macglyrest & macgly30	6	-.091	.863
Pair 2	macgly30 & macgly2.5	6	.825	.043
Pair 3	macglyrest & macgly2.5	6	-.083	.875

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	macglyrest - macgly30	69.25796	54.41221	22.21369	12.15585	126.36008	3.118	5	.026
Pair 2	macgly30 - macgly2.5	-.3924	4.99491	2.03916	-5.63424	4.84944	-.192	5	.855
Pair 3	macglyrest - macgly2.5	68.86556	53.71538	21.92921	12.49473	125.23640	3.140	5	.026

Appendix H5: Total Glycogen Post-Hoc T-Tests

Post Hoc – T-tests

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	totglyrest	288.9883	8	59.70371	21.10845
	totgly30	107.7854	8	56.91914	20.12395
Pair 2	totgly30	95.2155	6	60.74871	24.80056
	totgly2.5	73.6920	6	40.66205	16.60021
Pair 3	totglyrest	283.9933	6	68.03789	27.77635
	totgly2.5	73.6920	6	40.66205	16.60021

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	totglyrest & totgly30	8	.564	.145
Pair 2	totgly30 & totgly2.5	6	.804	.054
Pair 3	totglyrest & totgly2.5	6	.268	.607

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	totglyrest - totgly30	181.20292	54.49198	19.26583	135.64648	226.75936	9.405	7	.000
Pair 2	totgly30 - totgly2.5	21.52349	37.06054	15.12990	-17.36915	60.41614	1.423	5	.214
Pair 3	totglyrest - totgly2.5	210.30131	69.26771	28.27842	137.60930	282.99331	7.437	5	.001

Appendix I1: Lactate Paired T-Test

Paired t-tests comparisons: Control and massaged leg (two time pts)

Lactate

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	laccon30	.4504	8	9.04144	3.19663
	lacmass30	2.5871	8	13.62056	4.81559
Pair 2	laccon2.5	6.7686	6	9.40955	3.84143
	lacmass2.5	8.5777	6	12.96183	5.29165

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	laccon30 & lacmass30	8	.268	.522
Pair 2	laccon2.5 & lacmass2.5	6	.591	.217

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	laccon30 - lacmass30	-2.13667	14.19054	5.01711	-14.00026	9.72693	-.426	7	.683
Pair 2	lacont2.5 - lacmass2.5	-1.80917	10.59879	4.32694	-12.93192	9.31358	-.418	5	.693

Appendix I2: Muscle Damage Paired T-Test

Paired t-tests comparisons: Control and massaged leg (two time pts)

Muscle Damage (#Total/mm2)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	mdmass30	5.6224	9	8.95397	2.98466
	mdcon30	.5080	9	1.50658	.50219
Pair 2	mdmas2.5	3.2741	10	4.73438	1.49714
	mdcon2.5	2.5634	10	3.27714	1.03632

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	mdmass30 & mdcon30	9	.293	.444
Pair 2	mdmass2.5 & mdcon2.5	10	-.099	.785

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	mdmass30 - mdcon30	5.11440	8.63354	2.87785	-1.52193	11.75073	1.777	8	.113
Pair 2	mdmass2.5 - mdcon2.5	.71079	6.01894	1.90336	-3.59490	5.01648	.373	9	.717

Appendix I3: Macro-glycogen Paired T-Test

Paired t-tests comparisons: Control and massaged leg (two time pts) Macro-glycogen

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	macgmass30	-67.7469	8	46.07274	16.28917
	macgcon30	-66.9548	8	42.51289	15.03058
Pair 2	macgmass2.5	-68.8656	6	53.71528	21.92917
	macgcon2.5	-65.4363	6	46.92536	19.15720

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	macgmass30 & macgcon30	8	.992	.000
Pair 2	macgmass2.5 & macgcon2.5	6	.978	.001

Appendix J: Pain Stats Paired T-Test

Pain Stats

Paired T-Tests

1) Control leg – post-exercise values vs. post-massage
2) Massage leg – post-exercise vs. post-massage
3) Difference in pain scores between massage leg and control leg

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	precontrol	7.3636	11	1.97599	.59578
	postcontrol	3.8636	11	2.30316	.69443
Pair 2	premassage	7.3636	11	1.97599	.59578
	postmassage	2.3182	11	1.79266	.54051
Pair 3	diffmassage	5.0455	11	2.37123	.71495
	diffcontrol	3.5000	11	2.61725	.78913

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	precontrol - postcontrol	3.5000	2.61725	.78913	1.74171	5.25829	4.435	10	.001
Pair 2	premassage - postmassage	5.0454	2.37123	.71495	3.45244	6.63847	7.057	10	.000
Pair 3	diffmassage - diffcontrol	1.5454	1.12815	.34015	.78755	2.30336	4.543	10	.001

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	painpostexmassageleg	7.3636[a]	11	1.97599	.59578
	painpostexcontrolleg	7.3636[a]	11	1.97599	.59578
Pair 2	painpostmassagerxleg	2.3182	11	1.79266	.54051
	painpostcontrolleg	3.8636	11	2.30316	.69443
Pair 3	diffmassage	5.0455	11	2.37123	.71495
	diffcontrol	3.5000	11	2.61725	.78913

a. The correlation and t cannot be computed because the standard error of the difference is 0.

Paired Samples Correlations

		N	Correlation	Sig.
Pair 2	painpostmassagerxleg & painpostcontrolleg	11	.877	.000
Pair 3	diffmassage & diffcontrol	11	.902	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 2	painpostmassagerxleg - painpostcontrolleg	-1.54545	1.12815	.34015	-2.30336	-.78755	-4.543	10	.001
Pair 3	diffmassage - diffcontrol	1.54545	1.12815	.34015	.78755	2.30336	4.543	10	.001

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	macgmass30 - macgcon30	-.79205	6.67918	2.36145	-6.37598	4.79189	-.335	7	.747
Pair 2	macgmass2.5 - macgcon2.5	3.4293	12.52179	5.11200	-16.57014	9.71148	-.671	5	.532

Appendix K: Raw Data

Pain Scores

Sub	PM	PC	mas	con	diff	diff(control)
1.00	4.00	4.00	1.00	1.00	3.00	3.00
2.00	7.00	7.00	5.00	7.00	2.00	.00
3.00	9.50	9.50	2.50	5.50	7.00	4.00
4.00	7.00	7.00	2.00	2.00	5.00	5.00
5.00	8.00	8.00	4.00	6.00	4.00	2.00
6.00	9.00	9.00	1.00	3.00	8.00	6.00
7.00	4.00	4.00	.00	3.00	4.00	1.00
8.00	7.00	7.00	2.00	2.00	5.00	5.00
9.00	10.00	10.00	.00	1.00	10.00	9.00
10.00	7.00	7.00	3.00	5.00	4.00	2.00
11.00	8.50	8.50	5.00	7.00	3.50	1.50

Demographic Information and Maximal oxygen uptake Values

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
age_yr	13	19	29	22.00	2.646
height_cm	13	142	180	173.69	10.610
weight_kg	13	63.6	88.6	75.146	8.0442
BSA	13	1.71	2.08	1.9046	.10890
Oxygenmax	13	30.8	64.2	44.931	8.2374
Valid N (listwise)	13				

Summary Raw Data

Proglycogen					
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	253.8391634	166.3984	148.185		
	189.8400604	98.65431	104.1881	42.35583	120.5271
	212.0154306			127.1397	143.8001
	162.5124214	12.58366	35.9722	9.952571	40.22628
	189.8741216	128.7716	82.69692	63.34383	53.30185
	243.0527396	49.53318	43.92164	11.38638	73.68762
	186.0017628	104.4763	117.2047	87.77422	104.8368
	263.9311019	163.0326	106.9937	102.4632	211.6295

Macroglycogen					
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	74.70208104	2.690726	12.04822		
	147.1540528	3.608534	10.6112	3.384163	15.15891
	67.39013575			3.617384	7.468916
	23.67756367	3.080827	2.597033	5.561796	0.288242
	132.3370151	14.49826	5.85578	15.7431	12.56233
	47.07610508	8.560518	2.096627	6.120685	4.358695

LACTATE	
S1-1	5.913778
S1-2L	20.86908
S1-2R	12.86391
S3-1	8.544608
S3-2L	15.73606
S3-2R	3.29852
S3-3L	19.0908
S3-3R	30.21035
S6-1	23.48924
S6-2L	5.711189
S6-2R	11.21375
S6-3L	11.74364
S6-3R	15.73445
S8-1	18.58831
S8-2L	16.20141
S8-2R	47.07901
S8-3L	24.4539
S8-3R	31.70778
S4-1	15.08581
S4-2L	6.094533

	30.12483989	10.31541	16.01418	2.227702	15.45445
	88.37807981	22.54321	18.45909	14.23275	36.5915

S4-2R	13.19478
S9-1	7.14978
S9-2L	3.028482
S9-2R	6.68151
S9-3L	11.71706
S9-3R	12.76678
S10-1	16.05224
S10-2L	27.78649
S10-2R	14.87815
S10-3L	23.70902
S10-3R	21.76843
S13-1	16.28199
S13-2L	22.7581
S13-2R	19.11663
S13-3L	24.89279
S13-3R	44.49516

Summary of Glycogen Data

sample	mmol/kg dry wgt
s1-1 PG	253.8392
s1-2L PG	148.185
s1-2R PG	166.3984
s1-1 MG	74.70208
s1-2L MG	12.04822
s1-2R MG	2.690726
s3-1 PG	189.8401
s3-2L PG	104.1881
s3-2R PG	98.65431
s3-3L PG	120.5271
s3-3R PG	42.35583
s3-1 MG	147.1541
s3-2L MG	10.6112

PG samples	
s1-1 PG	253.8392
s1-2L PG	148.185
s1-2R PG	166.3984
s3-1 PG	189.8401
s3-2L PG	104.1881
s3-2R PG	98.65431
s3-3L PG	120.5271
s3-3R PG	42.35583
s8-1 PG	189.8741
s8-2L PG	82.69692
s8-2R PG	128.7716
s8-3L PG	53.30185
s8-3R PG	63.34383

s3-2R MG	3.608534	s6-1 PG	162.5124
s3-3L MG	15.15891	s6-2L PG	35.9722
s3-3R MG	3.384163	s6-2R PG	12.58366
s8-1 PG	189.8741	s6-3L PG	40.22628
s8-2L PG	82.69692	s6-3R PG	9.952571
s8-2R PG	128.7716	s10-1 PG	186.0018
s8-3L PG	53.30185	10-2L PG	117.2047
s8-3R PG	63.34383	10-2R PG	104.4763
s8-1 MG	132.337	10-3L PG	104.8368
s8-2L MG	5.85578	10-3R PG	87.77422
s8-2R MG	14.49826	9-1 PG	243.0527
s8-3L MG	12.56233	9-2L PG	43.92164
s8-3R MG	15.7431	9-2R PG	49.53318
s6-1 PG	162.5124	9-3L PG	73.68762
s6-2L PG	35.9722	9-3R PG	11.38638

s6-2R PG	12.58366	s4-1pg	212.0154
s6-3L PG	40.22628	S4-3l pg	143.8001
s6-3R PG	9.952571	4-3R PG	127.1397
s6-1 MG	23.67756	13-1 PG	263.9311
s6-2L MG	2.597033	13-2L PG	106.9937
s6-2R MG	3.080827	13-2R PG	163.0326
s6-3L MG	0.288242	13-3L Pg	211.6295
s6-3R MG	5.561796	13-3r PG	102.4632
s10-1 PG	186.0018		
10-2L PG	117.2047		
10-2R PG	104.4763		
10-3L PG	104.8368		
10-3R PG	87.77422		
9-1 MG	47.07611		
9-2L MG	2.096627		
9-2R	8.560518		

MG	
9-3L MG	4.358695
9-3R MG	6.120685
9-1 PG	243.0527
9-2L PG	43.92164
9-2R PG	49.53318
9-3L PG	73.68762
9-3R PG	11.38638
s4-1 MG	67.39014
s4-3L MG	7.468916
s4-3R MG	3.617384
s4-1pg	212.0154
S4-3l pg	143.8001
4-3R PG	127.1397
10-1 MG	30.12484
10-2L MG	16.01418

10-2R	
MG	10.31541
10-3L	
MG	15.45445
10-3R	
MG	2.227702
13-1	
MG	88.37808
13-2L	
MG	18.45909
13-2R	
MG	22.54321
13-3L	
MG	36.5915
13-3R	
MG	14.23275
13-1	
PG	263.9311
13-2L	
PG	106.9937
13-2R	
PG	163.0326
13-3L	
Pg	211.6295
13-3r	
PG	102.4632

MACROGLYCOGEN

MG samples

s1-1
MG 74.70208

s1-2L
MG 12.04822

s1-2R
MG 2.690726

s3-1
MG 147.1541

s3-2L
MG 10.6112

s3-2R
MG 3.608534

s3-3L
MG 15.15891

s3-3R
MG 3.384163

s8-1
MG 132.337

s8-2L
MG 5.85578

s8-2R
MG 14.49826

s8-3L
MG 12.56233

s8-3R
MG 15.7431

s6-1
MG 23.67756

s6-2L
MG 2.597033

s6-2R
MG 3.080827

s6-3L
MG 0.288242

s6-3R
MG 5.561796

10-1
MG 30.12484

10-2L
MG 16.01418

10-2R
MG 10.31541

10-3L
MG 15.45445

10-3R
MG 2.227702

9-1 MG 47.07611

9-2L
MG 2.096627

9-2R
MG 8.560518

9-3L
MG 4.358695

9-3R
MG 6.120685

s4-1 67.39014

MG

s4-3L
MG 7.468916

s4-3R
MG 3.617384

13-1
MG 88.37808

13-2L
MG 18.45909

13-2R
MG 22.54321

13-3L
MG 36.5915

13-3R
MG 14.23275