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Equine Massage Following Intense Work: Effects On Plasma Creatine Kinase

Brittany Pilat
Otterbein University, brittany.pilat@otterbein.edu

EQUINE MASSAGE FOLLOWING INTENSE WORK: EFFECTS ON PLASMA CREATINE KINASE

by

Brittany N. Pilat

Department of Equine Science

Otterbein University

Westerville, Ohio 43081

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Steffanie V. Burk, Ph. D.
Project Advisor

Advisor's Signature

Sheri S. W. Birmingham, D.V.M.
Second Reader

Second Reader's Signature

Cynthia Laurie- Rose, Ph. D.
Honors Representative

Honor Rep's Signature

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Abstract

Horses are elite athletes, and many of them receive specialized care to keep them operating at their highest potential. Alternative modalities of healing- such as equine massage- have begun to grow in the equine world due to many perceived benefits. Equine massage is thought to decrease inflammation and reduce muscle tension and soreness. The aim of this study was to observe the effect of equine massage on creatine kinase (CK) levels in the blood following intense work. A total of 12 horses were randomly assigned to either the control group (n=6) or the test group (n=6). All horses were exercised heavily. Horses warmed up the same number of laps in the arena following a set walk/trot/canter protocol, galloped continuously at 350 meters per minute for 9 minutes, and then cooled off to the discretion of the rider. Horses in the massage group received a full body massage approximately 2 hours post-exercise. Blood samples were taken from each horse at 24 hours prior to, 24 hours post-, and 48 hours post-exercise. All samples were shipped to Cornell University for analysis of CK. Mean CK levels for the control and experimental groups were compared at each time point using a two-way mixed-design ANOVA. There were no statistical differences or trends observed when comparing mean CK levels between the groups, showing no effect of the equine massage on plasma CK levels. Future studies should evaluate additional time points, inflammatory markers, and behavioral responses.

Introduction

Horses are impressive athletes, and they require a vast amount of care to keep them working at their highest potential. Heavy exercise and training can lead to soreness and muscle fatigue. This soreness can lead to acute or chronic lameness, an unwillingness to work, and even long-term muscle damage. A current, popular treatment for muscle pain and potential injury is the use of non-steroidal anti-inflammatories (Larson, 2012). There are some potential complications that come with the use of NSAIDs -such as toxicity, colic, and ulcers- and many owners are looking for alternative methods for pain relief (Larson, 2012). Along with these complications comes potential behavioral and performance issues as well. Because of this, there has been a surge in the number of owners looking to massage as a means of pain relief and preventative maintenance against injury, and it is important to explore some potential benefits of the modality (Scott et al., 2009). The purpose of this study is to observe the effects of equine massage after intense work on the levels of creatine kinase- an inflammation marker- in blood plasma.

History and Definition of Massage Therapy

Massage therapy is a modality that has been around for thousands of years. It originated in China and India- along with other forms of Eastern medicine (Urtnowska-Joppek et al., 2019). Cave paintings from 15,000 years ago depict healing touch therapy in humans (Hunter & Struve, 1998), and its benefits were later written about by Greeks and Romans, including Hippocrates, Celsus, and Galen (Urtnowska-Joppek et al., 2019). In more recent history, we have seen documented use of massage in many regions of the world, such as Australia, Hawaii, Africa, Cambodia, and Peru (Calvert, 2002; Gann, 1918). It was even included in mainstream medicine in the United States up through the beginning of the 20th century (Rich, 2010). Because it has many origins, techniques, and practices, there are numerous definitions of massage. At the Best Practices Committee Symposium in 2010, the definition of massage was deemed to be, “a patterned and purposeful soft-tissue manipulation accomplished by use of digits, hands, forearms, elbows, knees and/or feet, with or without the use of emollients, liniments, heat and cold, hand-held tools or other external apparatus, for the intent of therapeutic change” (Kennedy et al., 2016). Although now there is great variation within the profession of massage, it all comes from the same basis of healing touch.

Massage Qualifications

An important aspect to mention when discussing massage therapy is who is qualified to perform it. Currently in the United States, both human and equine massage therapies are regulated by state governments. These regulations and legislation are presented through the associations overseeing the massage therapists, such as the American Massage Therapy Association (AMTA) for human massage and the American Veterinary Medical Association (AVMA) for equine massage. In the state of Ohio, equine massage is allowed to be practiced by individuals who are not veterinarians, due to broad wording in the scope of practice (AVMA, 2019). There are many methods by which massage therapists learn their trade. These can include learning knowledge passed down from mentors, self- teaching, and certification classes- both in person and online.

Massage Techniques

Massage has variation in the techniques that are performed, with each providing a certain way of interacting with the muscle, as well as with the components in the vicinity. The movements and techniques that have been seen through more recent history include effleurage, kneading, petrissage, frictions, tapotement, and vibrations and shaking (Goats, 1994). In his 1994 work “Massage- the Scientific Basis of an Ancient Art: Part 1. The Techniques”, Geoffrey C. Goats describes each of these manual techniques well. With great variation in each technique over the years, many individuals were cited in Goats’ work for their input in terms of the modern movements- the compilation of these sources is found in the book *Principles and Practice of Soft Tissue Manipulation*. Effleurage is a slow and rhythmic stroking hand movement that is done along the shape of the skin. Effleurage accelerates blood and lymph flow and improves tissue drainage, therefore reducing swelling (De Domenico, 2007). Kneading is done with slow circulation compression of the soft tissue against the bone, and it promotes flow of tissue fluid, causing vasodilation (De Domenico, 2007). Petrissage is a skin rolling technique, which squeezes, lifts, and rolls the skin against the underlying tissues. Petrissage encourages flow of body fluids and is able to resolve long-standing swelling (De Domenico, 2007). Frictions are usually circular or transverse movements applied directly to the site of damage through the use of the fingertips; they are meant to cause mild tissue damage to incite a local inflammatory response. In "Massage- the Scientific Basis of an Ancient Art: Part 1. The Techniques" (Goats, 1994) it says, "The localized stretching and degradation of collagen caused by frictions can restore fibers to a more normal alignment during the remodeling phase of healing." Tapotement movements are percussive techniques that trigger cutaneous reflexes and cause vasodilation; muscle tone is increased and retained fluid near the muscle is dispersed from the area (Goats, 1994). Vibrations and shaking are similar to tapotement, only more intense. These are done by trembling of the hands while in contact with the skin; these can decrease edema through compression of swollen tissues (Goats, 1994). While these are the basis of techniques that are used, most massage therapists will expand upon and deviate from a certain sequence for the benefit of each individual client. In the case of research, such as this study, a strict sequence was used to avoid bias.

Resurgence of Massage Therapy

The resurgence of massage therapy in recent years can be attributed to many things, including but not limited to a need to alleviate stress and anxiety, decrease pain and inflammation, and improve athletic ability and performance (Gage, 2018). These overarching categories of benefits are only the beginning of the benefits that have been observed over the centuries. A more extensive list includes an increase of immune function, improved attention and cognitive function, improved relaxation, increased blood flow and oxygen circulation, increased lymphatic flow, increased range of motion in joints, improved toxin elimination, increased scar tissue breakdown, decreased muscle spasm and cramping, lowering perceived pain, a reduction of delayed onset muscle soreness (DOMS) and reduced muscle fatigue (Dupuy et al., 2018; Gage, 2018; Kargarfard et al., 2015). With all of the perceived benefits of massage-including those observed benefits noted in the studies above- it would be assumed that all of them are backed with research and are evidence-based claims, but that is not necessarily the case. Perceived benefits are mainly based on common observations over time in those who have been treated with massage. Because there has been a resurgence of interest in the modality, more studies are being conducted on the effects of massage in humans.

Massage Studies in Other Species

While there has been a recent increase in studies on massage therapy in human medicine, studies have also been done in other species to observe effects of massage on animals, as well as act as introductory research for the use of massage therapy in human medicine. Because of similarities between human and rat anatomy and physiology, many studies are done on rats. A study done by Bove et al. looked at the effect of manual therapy on fibrosis due to overuse of the upper extremity. The rats were subjected to repetitive reaching and grabbing that would cause inflammation, leading to replacement of healthy tissue with scar tissue- also known as fibrosis (Bove et al., 2016). The experimental group then was subjected to modeled manual therapy (MMT), similar to what would be performed by a massage therapist (Bove et al., 2016). They observed prevention of functional decline, improved task performance, decreased behavioral changes with exercise, decreased fibrosis, and reduced tissue levels of inflammation marker TGF- β1 in the group receiving MMT (Bove et al., 2016). Another study done on rats observed an increased expression of angiogenesis-stimulating factors and proliferative activity of epidermal cells (Ratajczak-Wielgomas et al., 2018). The animals received massage therapy at the plantar surface of each rear limb for 5 minutes each, and the expression of angiogenesis- initiating factors (VEGF-A, FGF-2, and CD34) were analyzed through the use of immunohistochemical reactions and polymerase chain reaction to amplify the RNA (Ratajczak-Wielgomas et al., 2018). The factors above that were observed in the RNA can stimulate skin and tissue repairing processes (Ratajczak-Wielgomas et al., 2018).

The effect of massage on lymph flow was investigated in pigs. In this study, the experimental subjects were injected into the dermis with three different isotope- labelled tracers (I-human serum albumin, colloidal goal, and Tc-labelled rhenium sulphide colloid) (Mortimer et al., 1990). Gentle local massage using a hand-held device was performed on each experimental subject, and the changes in radioactive counts were observed, with clearance being defined as half clearance (time taken for 50% of isotope to be cleared from the injection site) (Mortimer et al., 1990). This study observed significantly enhanced clearance of tracers due to local massage, confirming its effect on lymph movement (Mortimer et al., 1990).

Studies have also been done to investigate the effect of massage on heart rate and overall relaxation. One study done on Arabians investigated the effect of relaxing massage on heart rate (HR) and heart rate variability (HRV) (Kowalik et al., 2017). Seventy-two Arabian racehorses were subjected to massage 3 days a week throughout their racing season, and measurements of their HR and HRV were taken six times every 4-5 weeks (Kowalik et al., 2017). The measurements were taken using a telemetric device mounted on the horse’s body, as well as an elastic belt to strapped around their chest (Kowalik et al., 2017) This study observed decreased HR at rest, saddling, and warm-up after 2 months of receiving the treatment (Kowalik et al., 2017). They also observed significantly lower HRV values in the experimental groups (Kowalik et al., 2017).

The studies done on other species have investigated the effect of massage on scar tissue formation due to injury, the expression of angiogenesis-stimulating factors, cell proliferation activity, lymphatic flow, and heart rate activity. While these are only a handful from the list of perceived benefits, studies are continuing to be done in hopes of further understanding the effects of massage therapy. The hope for this study is to continue to add to the knowledge surrounding the use of this modality in horses.

Importance of Creatine Kinase

For this study, creatine kinase (CK) was chosen as the measurable component due to its involvement in muscle damage. Creatine kinase is a dimeric globular protein containing two subunits that buffers ADP and ATP concentrations by catalyzing the reversible exchange of high-energy phosphate bonds between phosphocreatine and ADP being produced during contraction (Brancaccio et al., 2007). There are five isoforms of CK, with three existing in cytoplasm (CK-MM, CK-MB, and CK-BB) and two in mitochondria (non-sarcomeric and sarcomeric) (Brancaccio et al., 2007). The isoform used in this study is CK-MM, which is bound to the M-line structure of sarcomeres in muscle fibers (Brancaccio et al., 2007). This muscle enzyme is normally low in plasma, but its levels increase after exercise due to muscle damage (Wessely- Szponder, 2015). Creatine kinase is measured in units of enzyme activity per liter of serum (U/L). Levels of CK can return to normal within 24 to 48 hours due to it having a short half-life of 2 to 4 hours, which makes it an easy marker to observe (IDEXX, 2013). Although there are many inflammation markers in the blood, such as cytokine Interleukin-1, Interleukin-6, and tumor necrosis factor alpha (Farinelli de Siquiera et. al., 2016), CK was chosen to observe for effects of equine massage due to its ease of use, length of half-life, and cost. This marker was successfully observed as an indicator of muscle damage in other studies as well, including *Effects of Daily Administration of an Amino Acid- Based Supplement on Muscle and Exercise Metabolism in Working Horses, Post-ride Inflammatory Markers in Endurance Horses, Transport Induced Inflammatory Responses in Horses,* and others (Vineyard et al., 2013; Farinelli de Siqueira et al., 2016; Wessely- Szponder et al., 2015). The hypothesis of this study is that a decrease in levels of CK will accompany equine massage after intense work.

Materials and Methods

Otterbein University Institutional Animal Care and Use Committee (IACUC) approval was granted prior to the start of this research. A total of 12 horses were randomly allocated to either the control group (n=6) or the experimental group (n=6) for the trial that took place during the week of May 13-18. They remained at Otterbein University’s Austin E. Knowlton Center for the duration of the trial, and they continued to be used in the lesson program as well as recreational riding sessions. The amount of riding time and intensity of each lesson or free riding session varied.

Horses used in this trial ranged in age from 9 to 18 years old and were of varying breeds, genders, and backgrounds (Appendix 1). All horses remained on their normal diets for the duration of the study, including grain, hay, and various supplements, along with free access to fresh water at all times (Appendix 2). Horses were stalled for approximately 18 hours per day and turned out in pasture or dry lot for approximately 6 hours, weather permitting.

During the trial, each horse took part in an intense exercise routine which remained consistent for all horses. All six riders were verbally instructed on the sequence of gaits for the horses they were riding, and the directions were also posted on the arena wall in case any questions arose. Five of the riders were experienced members of the Otterbein Equestrian team, and the sixth rider was an experienced staff member. The sequence of the exercise was as follows: walk 3 laps to the left, trot 4 laps to the left, canter 2 laps to the left, walk 1 lap, reverse direction, walk 3 laps to the right, trot 4 laps to the right, canter 2 laps to the right, walk 2 laps to the right, controlled gallop at 350 meters per minute (mpm) for 9 minutes, walk as many laps as needed to properly cool down the horse. A proper cool down was determined by a decreased breathing rate, normal surface body temperature, and decrease in sweat production. The 9- minute controlled gallop was timed using a cross country watch (The Optimum Time Eventing Watch, United Kingdom). The number of laps walked at the end of the exercise varied but was recorded (Appendix 3). This was done to ensure the health and safety of the horses involved in the project, assuming each individual horse has a different tolerance to exercise.

Each day, 4 horses to took part in the exercise regimen, with 2 of the 4 horses receiving a massage following exercise. Horses were scheduled for exercise at different times throughout the day (Appendix 4) to allow adequate time for exercise, massage, and blood sample collection. There was an alternation in riding slots of horses in the control group and horses in the experimental group, which allowed enough time to properly cool out and massage each horse. Blood samples were collected at the beginning of each day at 12:15 PM for the indicated groups for 24- hours prior to and 24- and 48-hours post exercise. Massages were done within two hours after the exercise period for each given horse.

Samples were collected via jugular venipuncture using sodium-heparin coated vacutainer tubes and 1 inch 20-gauge needles. Almost a full tube of blood was collected, then inverted 8 to 10 times before being placed in the centrifuge. The samples were centrifuged for 10 minutes at 1040xG, and the plasma was pipetted using disposable plastic pipettes into labelled 1.5mL microcentrifuge tubes. The microcentrifuge tubes were stored at -20° C until shipment to Cornell University- they were mailed to Cornell's Animal Health and Diagnostic Center within two weeks of collection.

All massages were performed by the same massage therapist who was certified through Therasage Equine Massage Certification. It was instructed by Greg Gage, who is both a human and animal massage therapist. The classes were taught at Ohio University Southern, and they were taught over a span of 4 full days in October of 2018.

Data Analysis

A two-way mixed-design ANOVA was performed using SPSS (v. 25) to compare mean values for each group over time. The between-groups factor was control versus treatment, and the repeated-measures factor was time. Assumptions for the two-way mixed-design ANOVA were checked using the Shapiro-Wilk test of normality, Levene's test for homogeneity of variances, and Mauchly's test of sphericity.

Results

The mean and standard deviation of the baseline CK levels at 24 hours prior to the exercise period were 294.00±65.96 U/L in the control group and 277.33±51.05 U/L in the massage group (Table 1). The mean and standard deviation of the levels of CK for 24 hours post exercise were 279.17±58.04 U/L for the control group and 277.33±81.72 U/L for the massage group (Table 1). There was an observed decrease in CK levels in the control group, and no change was observed in the massage group within the first 24 hours. The mean and standard deviation of the levels of CK for 48 hours post exercise were 267.83±60.53 U/L for the control group and 278.67±52.33 U/L for the massage group (Table 1). Mauchly's test of sphericity met the assumption that the variances of the differences between all possible pairs of the groups are equal (p=.850). The Wilke's Lambda portion of the multivariate test for both the effect of time and time*treatment were not statistically significant. This means that there were no differences in means of the groups due to time or time*treatment being applied. The tests of within-subjects effects were insignificant (F=.353, p=.71) for time and for time*treatment (F=.428, p=.66), which states that effects of the treatment on the individuals were not significant. The tests of between-subjects effects did not present as significant either (p=.937), indicating that the massage treatment did not have a statistically significant effect on the CK levels of the horses (Table 2). Figure 3 displays the means and standard errors of the massage and control groups at 24-hours prior to and 24- and 48- hours post exercise. A visual representation of the effect of the treatment on the estimated marginal means showed the variability between the control and the experimental groups, even during the 24-hour pre-workout blood draw (Figure 4). This observation shows a discrepancy, starting even before the experiment took place.

Discussion

This study showed no significant changes of the creatine kinase levels in the blood due to an hour-long massage after intense exercise, and no trends were noted for either group. No negative effects were noted, and the horses overall reacted well to the treatment. It is important to note that the normal range for CK in horses is between 90 and 275 U/L (Buzala et al., 2015). The CK values observed in this study- even the baseline (24-hour prior to treatment) CK values- were higher than normal. This could be due to the work the horses were in at the beginning of the summer- especially the few days before and throughout the study. The results of this study differed from those obtained in a study observing the effect of massage on muscle soreness, perceived recovery, physiological restoration and physical performance in male bodybuilders (Kargarfard et. al., 2016). They observed slightly elevated CK levels in the experimental group compared to the control at baseline, pre-massage, and 0 hours post massage. They observed a change, though, and saw a decrease in CK levels in the experimental group as compared to the control at 24, 48, and 72 hours post massage. Results from the current study also differed from those of a study conducted on individuals partaking in upper arm resistance training (Zainuddin et al., 2005). In the study 5 males and 5 females performed identical, maximal eccentric exercise of the elbow flexors with each arm. Blood samples were taken on 7 of the 14 total days of the study, and they were tested on the spot for their CK plasma concentrations. The study saw significantly decreased CK plasma concentrations in the massaged arm when compared to the control arm, and they concluded this was due to the massage treatment (Zainuddin et al., 2005). While there are various studies done in humans relating to massage and its effect on CK levels, there have been few studies done on horses that would allow for comparison with the current study that was conducted. Further research will need to be done.

There were several limitations that should be noted in this study. The major limitation was the variation of exercise each horse received in the day or two prior to the start of the trial, as well as the variation of exercise the horses were receiving throughout the week of the trial. The horses that were used in this study were considered "upper level" and more advanced, so they were continuously used for lessons, leasing, and free riding throughout the trial. The large difference between the baseline of the two groups could be due to a few things, such as intensity of work done the previous day, amount of turnout the previous day, or potential muscle strain due to being stalled or worked on by the farrier or veterinarian. Another potential limitation in this study is the use of multiple riders for the exercise portion of the trial. Each rider is unique and has a different riding style, but the use of a speed suggestion (350 meters per minute) was implemented in hopes of eliminating skewedness due to rider variation. Another possible limitation in this study was the variation of feed and supplements given to each horse. Some of them, having had previous injury, were taking supplements to combat muscle injury, tendon and ligament injury, decreased circulation, colic and digestion complications, as well as joint pain; however, each horse remained on the same diet for the duration of the trial (Appendix 5).

Future research is required for clarification of the effects of equine massage on the levels of CK in the blood after intense exercise. Along with altering the previously mentioned limitations of the study, an increase in sample size could prove to be beneficial to analysis of the results. A larger sample size would allow for an assumption of normality that was not able to be assumed in this current study. It would also be beneficial to model a new study after the one Purina conducted when testing the effectiveness of their SuperSport Supplement (Vineyard et. al., 2013). They looked at creatine kinase levels in the blood at two time intervals following exercise: four hours and twenty four hours, observing both the immediate and long-term effects on the levels of CK in the blood. The time frame used in this current study was based on a study done on human bodybuilders, looking at creatine kinase levels pre-massage and at 0, 24, 48, and 72 hours post-massage (Kargarfard et. al., 2016). The time frame used in this study was potentially not on par with the action of creatine kinase levels in the equid- it observed only the long- term effects of equine massage on levels of CK in the blood. In other studies that were looking to observe changes in creatine kinase in horses, the time frame in which they drew blood was within 24 hours of the work or exercise (Brioschi Soares et. al., 2013; de Miranda et. al., 2009; Klobučar et. al., 2019;

Vineyard et. al., 2013). This would be a beneficial change to the protocol if this study were to be repeated in the future.

Other interesting topics to consider when studying equine massage in the future include each individual horse's reactions, as well as body surface temperature and other inflammation markers found in the blood (tumor necrosis factor alpha or serum amyloid A protein). In performing the massages on the experimental group of horses at the Austin E. Knowlton Center, all but one displayed signs of relaxation during the massage portion. Licking and chewing was observed in most of the horses in the experimental group, along with stretching of the neck and head and yawning, all of which are signs of relaxation and comfort (Gage, 2018). Only one horse in the group was irritable, offered to kick, and would not remain still for the duration of the massage. A potential solution to that problem would be an introduction of massage to each horse being used prior to a study being done to acclimate the horse to the feel of massage and human touch for long periods of time. Another topic that would be interesting to investigate would be the surface body temperature distribution of the horses before and after receiving a massage using a thermal camera. Muscles could be isolated and massaged, then compared to the muscles that had not received the massage to study their differences. Massage increases the body temperature due to the physical manipulation of the tissues, so one would expect a slight increase in temperature to the muscles being massaged (Gage, 2018). The study of body surface temperature could also have potential benefits for observing the effects of massage over time, such as the potential of decreased swelling and inflammation in the long run. Another way to measure the effects of massage both short- and long-term is the use of different blood inflammation markers, such as tumor necrosis factor alpha, serum amyloid A protein, and others. These markers can be isolated from blood samples and analyzed in a similar fashion to creatine kinase, as was observed in this study.

In the end, this study found no effect of equine massage after intense exercise on the levels of CK in the blood at 24- hours prior to and 24- and 48- hours post massage. Equine massage as a therapy has not been validated, and much more research is needed to further understand and quantify the effects of the modality. Future research on the effectiveness of equine massage is needed due to the increased use of it in the industry, the increasing demand for equine massage therapists, and the observed benefits that have been reported thus far.

Figures and Tables

	Treatment	Mean	Std. Deviation
24 PRE	Control	294.00	65.96
	Massage	277.33	51.05
24 POST	Control	279.17	58.04
	Massage	277.33	81.72
48 POST	Control	267.83	60.53
	Massage	278.67	52.33

Table 1. Descriptive statistics showing mean and standard deviation creatine kinase levels measured in units of enzyme activity per liter of serum (U/L) for each treatment group for the three time periods

Test	Effect	P Value
Multivariate Test-Wilkes Lambda	Time	.710
	Time*Treatment	.657
Mauchly's Test of Sphericity	N/A	.850
Tests of Within-Subjects Effects	Time	.353
	Time*Treatment	.428
Tests of Within-Subjects Contrasts	Time	.395
	Time*Treatment	.349
Tests of Between-Subjects Effects	N/A	.937

Table 2. SPSS two-way mixed design repeated measures ANOVA test values

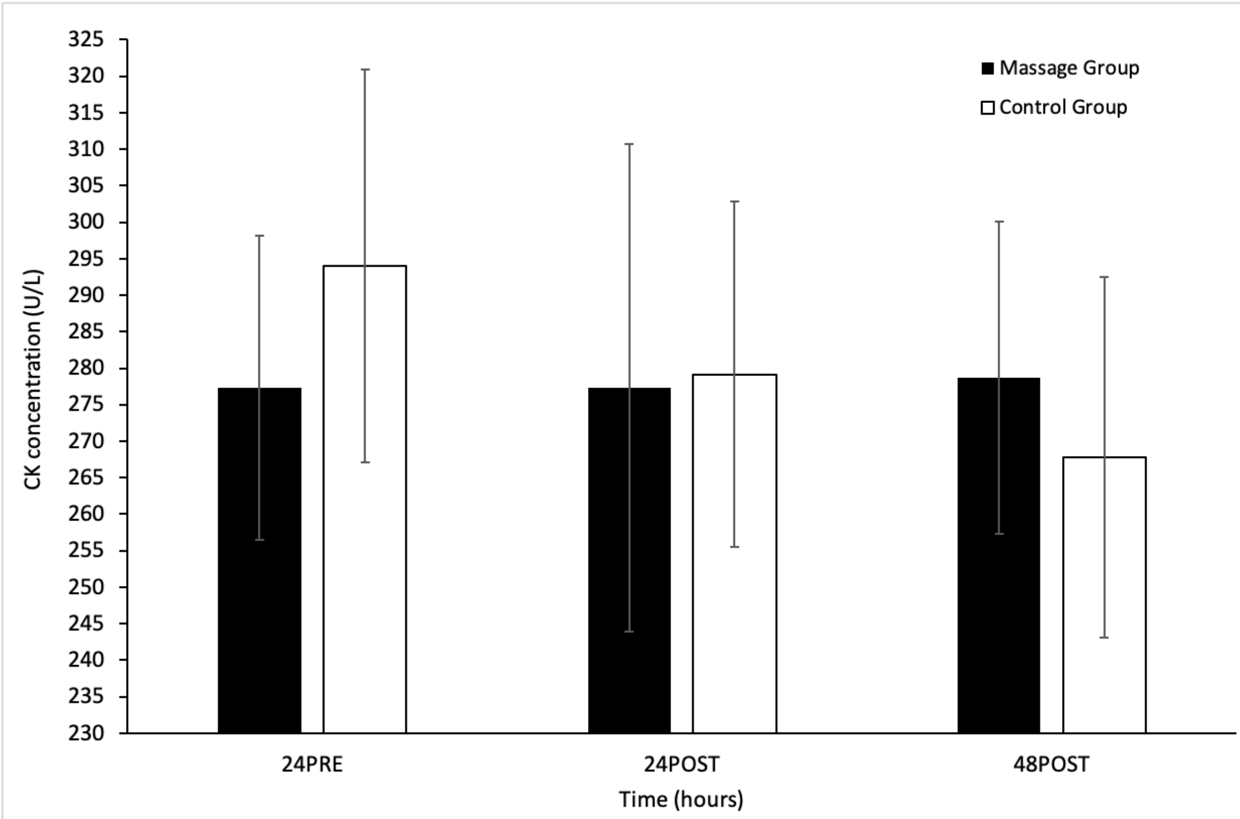

Figure 3. Mean and standard error CK concentrations for the massage and control groups at 24-hours prior to and 24- and 48- hours post exercise.

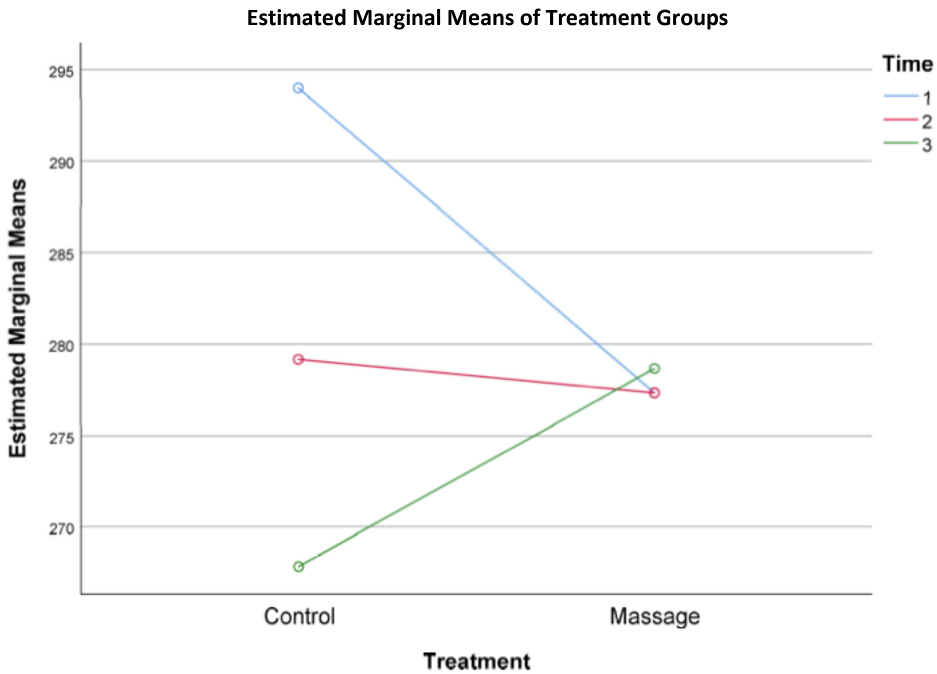

Figure 4. Estimated Marginal Means of Treatment Groups (SPSS Version 25, 2019)

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Appendices

Horse Name	Year of Birth	Breed	Gender	Former Job
Ace	2005	Hanoverian	Gelding	Dressage
Annabelle	2008	Holsteiner Draft cross	Mare	Eventing
Big Leo	2007	Thoroughbred Holsteiner cross	Gelding	Eventing
Blake	2005	Thoroughbred	Gelding	Hunter
Danny	2003	Oldenburg	Gelding	Hunter/Jumper
Harp	2010	Thoroughbred	Gelding	Eventing
Indigo	2010	Belgian Warmblood	Gelding	Hunter/Jumper
Luna	2010	American Warmblood	Mare	Hunter/Jumper
Paco	2002	Quarter Horse	Gelding	Eventing
Tanner	2010	Belgian Warmblood	Gelding	Eventing
Taz	2011	Warmblood	Gelding	Hunter/Jumper
Wizz	2004	Warmblood	Gelding	Jumper

Appendix 1. Horse histories

Horse Name	Grain Type	Hay Type	Supplements
Ace	Purina Ultium	Grass Hay	Vitamin E
Annabelle	Purina Enrich	Grass Hay	None
Big Leo	Purina Ultium	Grass Hay	Vitamin E
Blake	Purina Ultium	Grass Hay (2^{nd} cut)	Equioxx, Amplify, Cimetidine
Danny	Purina Senior	Grass Hay	Equioxx, Cosequin, Isoxsuprine
Harp	Purina Ultium	Grass Hay	None
Indigo	Purina Senior	Grass Hay	None
Luna	Purina Ultium	Grass Hay	Cosequin
Paco	Purina Enrich	Grass Hay	SmartPak tendon, Smartflex Senior
Tanner	Purina Enrich	Grass Hay	None
Taz	Purina Senior	Grass Hay	None
Wizz	Purina Senior	Grass Hay	Purina SuperSport

Appendix 2. Horse Diets

Horse Name	Laps Until Cooled Down
Blake	2
Big Leo	5
Danny	5
Luna	5
Harp	2.5
Ace	3
Paco	2.5
Taz	3
Indigo	4
Annabelle	3
Wizz	3
Tanner	5

Appendix 3. Laps walked until cooled down properly

Daily Schedule

Week: May 13-May 18 Start Time: 12:15PM

	Mon	Tue	Wed	Thu	Fri	Sat	Su
12:00PM	Will need Dr. Birmingham	Will need riders (Jordan and possibly Lily) and Dr. Birmingham or Kari	Will need riders (Megan and Wendy) and Kari	Will need riders (probably Jordan Golen and Lily, but may need someone else if Jordan can't make it) and Kari	Will need Kari	Will need Dr. Birmingham or Kari	
12:15 PM		Go over schedule for the day	Go over schedule for the day	Go over schedule for the day			
12:30 PM		Do blood draw A on Group 2	Do blood draw B on Group 1	Do blood draw C on Group 1	Do blood draw C on Group 2	Do blood draw C on Group 3	
12:45 PM		CENTRIFUGE AND PIPETTE BLOOD SAMPLES	Do blood draw A on Group 3	Do blood draw B on Group 2	Do blood draw B on Group 3	CENTRIFUGE AND PIPETTE BLOOD SAMPLES	
1:00 PM		CENTRIFUGE AND PIPETTE BLOOD SAMPLES	CENTRIFUGE AND PIPETTE BLOOD SAMPLES	CENTRIFUGE AND PIPETTE BLOOD SAMPLES	CENTRIFUGE AND PIPETTE BLOOD SAMPLES		
1:15 PM		**Exercise Blake**		Exercise Indigo			
1:30 PM	Do blood draw A on Group 1						
1:45 PM	CENTRIFUGE AND PIPETTE BLOOD SAMPLES						
2:00 PM			Exercise **Harp**				
2:15 PM		Exercise Big Leo		Exercise **Annabelle**			
2:30 PM							
2:45 PM							
3:00 PM		Start Massaging Blake 1 hr after exercise	Exercise Ace				
3:15 PM		Exercise **Danny**		Exercise Wizz			
3:30 PM							
3:45 PM			Start massaging Harp 1 hr after exercise				
4:00 PM			Exercise **Paco**	Start massaging Annabelle 1 hr after exercise			
4:15 PM		Exercise Luna		Exercise **Tanner**			
4:30 PM							
4:45 PM							
5:00 PM		Start massaging Danny 1 hr after exercise	Exercise Taz				
5:15 PM							
5:30 PM							
5:45 PM			Start massaging Paco 1 hr after exercise				
6:00 PM				Start massaging Tanner 1 hr after exercise			

Appendix 4. Exercise and Massage Schedule

Horse	Supplements	Supplement Advantage
Ace	Vitamin E	Antioxidant to improve cell activity and protect cell membranes
Annabelle	None	N/A
Big Leo	Vitamin E	Antioxidant to improve cell activity and protect cell membranes
Blake	Equioxx	Non-steroidal anti-inflammatory to control pain/inflammation due to osteoarthritis
	Amplify	High-fat supplement that provides extra calories and supports immune function
	Cimetidine	Reduces stomach acid production and may aid in preventing or treating stomach ulcers
Danny	Equioxx	Non-steroidal anti-inflammatory to control pain/inflammation due to osteoarthritis
	Cosequin	Joint supplement that protects cartilage function
	Isoxsuprine	Vasodilator that aids in circulation; used to treat laminitis and navicular disease
Harp	None	N/A
Indigo	None	N/A
Luna	Cosequin	Joint supplement that protects cartilage function
Paco	SmartPak Tendon	Contains hydrolyzed collagen, silica, MSM, and vitamin C intended to support tendons and ligaments
	Smartflex Senior	Contains glucosamine, hyaluronic acid, and MSM intended to support joint health in senior horses
Tanner	None	N/A
Taz	None	N/A
Wizz	Purina SuperSport	Contains essential amino acids to support muscle development, protection, and repair

Appendix 5. Horse Supplements and their advantages; information courtesy of Purina, SmartPak, Cosequin, and FarmVet (see References)

IACUC Approval

This project was approved by the Institutional Animal Care and Use Committee on April 17, 2019. The reference number assigned to this project is #2019-04-01-02