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The Effects of Pre and Post Exercise Low-Level Laser Therapy on Biochemical Markers of Skeletal Muscle Fatigue in Equines

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Abstract

Our objective was to determine whether administering low-level laser therapy before or after exercise had the greatest effect on biochemical markers of skeletal muscle fatigue in equines such as cortisol and blood lactate. Twelve quarter horses were divided into three groups: Group A received no laser therapy, Group B received laser therapy before exercise, and Group C received laser therapy after exercise. A Class II ERCHONIA ® PL500 handheld low-level laser was utilized for treatment with a wavelength of 635nm. Exercise was utilized using a horse walker system for 30 minutes five days a week for three weeks. Blood was collected via jugular venipuncture at time zero and then once a week for the remainder of the study. According to the results of this study, there is no evidence to suggest that laser therapy had a significant effect on equine cortisol or lactate, regardless if it was performed before or after exercise. However, there was an interaction between group and time for both lactate and cortisol. The results also showed that lactate increased as time increased as a result of lactic acid build up due to exercise, and cortisol decreased over time, which could be due to several possible variables such as weather. Several factors could have altered the results of this study, such as age, gender, weather, and diet of the equine subjects.

Introduction

The training of competition horses has changed a great deal over the last few decades. Arguably, the competition is now tougher, which puts a horse under greater stress, and trainers are presented with ever more information about the condition of their horses- blood tests, food analysis, and so on. (Marlin & Nankervis, 2013). One of the most important aspects of equine sports medicine is the maintenance of these athletes at their highest level of performance.

The biggest impact we can have on how well a horse performs is through training (Marlin & Nankervis, 2013, p.6). Equine athletes endure rigorous training, often on a daily basis, which can result in pain and soreness. The aims of training are to increase the horse's exercise capacity, increase the time to the onset of fatigue, improve overall performance by increasing skill, strength, speed, and endurance, and decrease the risk of injury (Marlin & Nankervis, 2013, p.3-4).

In strenuous physical activity, muscles typically show a progressive decline in performance, which largely recovers after a period of rest. This reversible phenomenon is described as skeletal muscle fatigue (Leal, et al., 2010a, p.1083). Several factors such as the types and intensity of exercise, the muscle groups involved and biochemical environment affect fatigue development (Leal, et al., 2010a, p.1083). This type of fatigue may only last minutes to hours after exercise, but can also persist for several days. Some common features of skeletal muscle fatigue are decreases in muscle strength and motor control as well as muscle pain (dos Reis et al., 2014, p.106). Decreasing skeletal muscle fatigue is now known to be an important way for human athletes to increase their performance levels naturally (dos Reis et al., 2014, p.107).

Low level laser therapy, commonly known as LLLT, is a form of phototherapy which involves the application of low power coherent light to injuries and lesions to stimulate healing (Rerucha, n.d., p.32). Using LLLT as a conservative treatment for human sports injuries developed in the 1960's (Ahmad, et al., n.d., p.281). During the first wave of interest in the use of LLLT for therapeutic benefits in the late 1980s, a limited number of clinical studies were performed with mixed outcomes. Controversy remained and leading medical experts expressed skepticism over the method during the 1990's. By the turn of the century, a renewed interest led to a slowly emerging research activity that identified several potential mechanisms of action, and related dose-response patterns (Leal, et al., 2010b).

Problem Statement: Several animal and human trials have shown positive effects of LLLT on inflammatory disorders both in acute and in chronic phases. However, skeletal muscle fatigue and post-exercise recovery are new areas of research in LLLT and few studies have been performed on this subject (De Marchi et al., 2012, p.235). Studies in humans show a trend toward improvement in muscle performance in response to laser therapy; however, some researchers have reported that applying the laser before the fatigue-inducing exercise provides more satisfactory reduction of fatigue, whereas others have obtained meaningful improvements in performance with laser application after the induction of fatigue. Therefore, the optimal moment to perform irradiation (before, during, or after exercise) still is an open and unanswered issue (dos Reis et al., 2014, p.107).

Objective: The aim of this study was to establish whether administering low-level laser therapy treatment before or after exercise had the greater effect on exercise-induced biochemical markers

that lead to skeletal muscle fatigue and inflammation in equine athletes, such as blood lactate and cortisol.

Hypotheses:

Null hypothesis: There will be no significant difference between the three groups.

Alternative hypothesis: There will be a significant difference between the three groups.

Definition of Terms:

Skeletal muscle fatigue- reversible phenomenon in which muscles show a progressive decline in performance, which largely recovers after a period of rest following strenuous physical activity (Leal, et al., 2010a, p.1083)

Low-level laser therapy (LLLT)- a form of phototherapy that involves the application of low-power, monochromatic, coherent light to injuries and lesions (Sussai et al., 2010, p.116)

Limitations of Study: There are several factors that can limit the accuracy of this study, such as the age, sex, breed, relative fitness, and athletic discipline of each horse. Other factors such as the horse's diet, weather, and the time of day the horses were exercised may also limit the study.

Literature Review

The Physiology of Fatigue: In strenuous physical activity, muscles typically show a progressive decline in performance, which largely recovers after a period of rest. This reversible phenomenon is described as skeletal muscle fatigue (Leal, et al., 2010a, p.1083). Skeletal muscle fatigue is characterized by impairment of muscle ability to generate and maintain force

production during exercise (De Marchi et al., 2012, p.231-232). Other features of skeletal muscle fatigue include decreases in muscle strength and motor control as well as increased muscle pain (dos Reis, et al., 2014, p.106). Several factors such as the types and intensity of exercise, the muscle groups involved, and biochemical environment affect fatigue development (Leal, et al., 2010a). The type of damage caused by skeletal muscle fatigue may be transient, lasting only minutes to hours after exercise, but can also persist for several days (dos Reis, et al., 2014, p.106).

Equine Exercise Physiology: Much of what we currently understand about equine exercise physiology has been established in the last 20-30 years, largely as a result from an increased scientific and veterinary interest in exercise physiology, improvements in technology, and availability of equipment (Marlin & Nankervis, 2013, p.3). Equine exercise physiology research has focused on assessing the physiological capacity and adaptability of horses to specific exercise loads (Buza, Krumrych, & Janicki, 2015, p.267). During the last few decades, there has been an ever increasing popularity of equestrian competitions (Linden, et al., 1991, p.391). The training of competition horses has also changed a great deal over the last few decades (Marlin & Nankervis, 2013, p.1). Training is a longer process of many repeated bouts of exercise that brings about an increase in fitness (Marlin & Nankervis, 2013, p.6). The aims of training are to increase the horse's exercise capacity, increase the time to the onset of fatigue, improve overall performance by increasing skill, strength, speed, and endurance, and decrease the risk of injury (Marlin & Nankervis, 2013, p.3-4). Sport horses are submitted to intensive training which can cause important physiological and biochemical fluctuations (Linden, et al., 1991, p.391).

Blood Lactate: High-intensity muscle activity increases blood lactate levels, which is associated with an increase in hydrogen ion (H+) concentration and consequently reduction of intracellular pH or lactic acidosis (Leal, et al., 2010a, p.1083). Lactic acid is formed via anaerobic energy pathways and quickly dissociates into lactate ions and hydrogen ions (Marlin & Nankervis, 2013, p.128). The hydrogen ions pose a threat to the muscle cell because they decrease the pH of the cell (Marlin & Nankervis, 2013, p.128). The decrease in pH known as lactic acidosis leads to a decrease in the aerobic capacity of muscle. The most common cause of lactic acidosis occurs during intense exercise (Cayman Chemical Company, 2016, p. 6). Therefore, monitoring lactate levels is a good indicator of the balance between tissue oxygen demand and utilization (Cayman Chemical Company, 2016, p. 6). Measurement of the blood lactate level is valuable in planning sports training and supervision as it closely related to the development of muscle fatigue (Leal, et al., 2010a).

Blood lactate is one of the prominent physiological responses of exercise in equines and is the biochemical signature of muscular fatigue (Kumar, 2015, p.201-202). The metabolic changes seen within horse muscles are generally more extreme than those of even elite human athletes (Marlin & Nankervis, 2013, p.75). Horses have very high maximal oxygen uptake, very high levels of the enzymes in muscles associated with energy generation and metabolism, and they are able both to produce and tolerate high levels of muscle lactate (Marlin & Nankervis, 2013, p.75). An increase of concentration of lactate ions due to exercise leads to- despite good ability of horses to accumulate them- disintegration of the cytoplasmic membrane structures, including decomposition of mitochondria, and even the destruction of entire muscle cells (Janicki, et al., 2012, p.325). Blood lactate concentration at rest in healthy horses is usually close to 1-2 mmol/L (Marlin & Nankervis, 2013, p.234). If exercise has been of sufficient intensity to

increase blood lactate concentration above 10-12 mmol/L, the peak blood lactate level will generally occur at the end of exercise: the higher the concentration, the later the peak of concentration will generally be. The lactate has been observed to rise as high as 38.5 mM/L in untrained horses during instant high intensity exercise (Kumar, 2015, p.304.) High lactate production above the threshold causes accumulation in the cells, resulting in lactate being released into the blood stream (Kumar, 2015, p.304). This causes fatigue in the cells that have resorted to anaerobic respiration and reduction in the performance (Kumar, 2015, p.304). In trained equine athletes, the increase in blood lactate is transient and would return back to near normal values in 30 minutes of rest post exercise (Kumar, 2015, p.304).

Cortisol: Cortisol is a glucocorticoid produced by the adrenal cortex in response to adrenocorticotropic hormone (Cayman Chemical Company, 2017, p. 6). Cortisol is secreted with a circardian periodicity and peaks early in the morning. The production of glucocorticoids is increased by stressed; therefore, cortisol is used as a biomarker of stress (Cayman Chemical Company, 2017, p. 6). Exercise results in a 2-3 fold increase in cortisol, with the peak usually occurring 15-30 minutes following exercise, and going back to pre-exercise levels within the hour (Marlin & Nankervis, 2013, p.131). However, recovery time seems to be related to the duration of exercise, in that prolonged submaximal exercise shows a later peak in plasma cortisol and a slower return to pre-exercise levels (Marlin & Nankervis, 2013, p.131). Cortisol levels also usually increase with age (Cayman Chemical Company, 2017, p. 6). Increased cortisol concentration in blood can lead to increased glycogen deposition, an increase in mobilization of fat stores, stimulation of protein synthesis for repair of tissue damage, increased sensitivity to adrenaline, and increased storage of liver glycogen (Marlin & Nankervis, 2013, p.131).

The resting cortisol level in horses is about 3.5 μg/dL or 35,000 pg/mL. In sport horses, the exercise-induced changes in plasma corticosteroids have been shown to be closely related to physiological stress (Linden, et al., 1991, p.391). Moderate exercise causes little alteration in plasma cortisol concentration, while strenuous and exhaustive exercise produces a marked increase (Linden, et al., 1991, p.394). In a study conducted by Linden (1991), cortisol levels were tested after five different types of equine events which all resulted in physiological stress. In the study, all the disciplines produced a significant increase in cortisol concentration.

Introduction to Low-Level Laser Therapy: Low-level laser therapy has now been investigated and used clinically for over thirty years (Houreld & Abrahamse, 2008, p.11). Although LLLT is both an active research topic and an expanding clinical therapeutic technique, it is still necessary acquire more evidence on therapy results and to clarify the cellular mechanisms mediated by LLLT (Mantineo, Pinheiro, & Morgado, 2014, p.098002-1).

The laser that was utilized in this study was a Class II Pl-Touch Laser from Erchonia. Erchonia LLLT is a universal method of treating muscle, tendon, ligament, connective tissue, bone, neurological dysfunction or damage, and skin tissue with one simple piece of equipment (Rerucha, n.d., p.33). Erchonia lasers are being used by those in chiropractic, medical, dental, acupuncture, podiatry, osteopathic, veterinary, physiotherapy, acupuncture practice and cosmetic applications (Rerucha, n.d., p.33). The effects of LLLT are photochemical (cold), not thermal. Hot lasers in the medical world are used for surgical precision while cold lasers are used for healing precision (Rerucha, n.d., p.32). Low-level laser therapy can be used to increase the speed, quality, and tensile strength of tissue repair, resolve inflammation, increase range of motion and provide pain relief (Rerucha, n.d., p.32). The portability and diversity of battery

powered diode laser systems allows treatment to be carried out in clinical and field locations. This opens up possibilities for the immediate and therefore more effective treatment of sporting and athletic injuries, such as sprains, muscle tears, and inflammatory conditions (Rerucha, n.d., p.33).

The overall effects of LLLT are decreased pain and inflammation, and increased range of motion (ERCHONIA, n.d.). When a tissue is injured or diseased, the metabolism of that tissue decreases. The laser creates an increase in metabolism and cell communication immediately (Rerucha, n.d., p.33). Low-level laser therapy also suppresses the mechanism that up-regulates inflammation (Erchonia Laser Healthcare, n.d.). The effect at the cellular level following laser irradiation reveals an extensive mechanism capable of suppressing cell apoptosis and is capable of promoting the proliferation of healthy, viable cells (Erchonia Laser Healthcare, n.d.).

Mechanism of Low-Level Laser Therapy: The unique approach of laser therapy works on two different fronts. First, the application of laser therapy at the site of the injury will address the localized elevation of inflammatory agents and will also help repair the damaged tissue (Erchonia Laser Healthcare, n.d., p.12). Secondly, the application of laser therapy to the first cervical vertebrae and below will reestablish proper nerve function by stimulating specific nerve tissue within the spinal cord (Erchonia Laser Healthcare, n.d., p.12).

Those experienced in the scientific process will be aware that aspects of LLLT research continue to be controversial, as is the case in all branches of science. However, when the results are taken together, an inescapable conclusion emerges: The cells in the human body, and the body as a whole, both emit and absorb coherent biophotons; these phototonic emissions and absorptions play key roles in the regulation of cellular and physiological processes, including the

healing injuries and diseases (Oschman, 2006, p.3) The primary mechanism of laser therapy is coupled with the basic principles of quantum mechanics: stimulating electrons to transition from a ground state to an excited one (Erchonia Laser Healthcare, n.d., p.6).

The photobiological nature of LLLT effects means that a molecule (photo-acceptor) must first absorb the light used for irradiation (Sussai, et al., 2010, p.116). After the promotion of electronically excited states, primary molecular processes from these states may lead to a measurable biological effect at the cellular level (Sussai, et al., 2010, p.116).

Investigators have determined that the light absorbing centers, chromophores, of specific enzymes found in eukaryotic cells contain transition metals capable of generating electronically excited states (Erchonia Laser Healthcare, n.d., p.6). Transition metals, such as copper or iron, are more susceptible to an electron shift because of their unique electron configuration (Erchonia Laser Healthcare, n.d., p.6). With transition metals more susceptible to electron excitation, the literature has identified these unique metals as the primary target for laser therapy (Erchonia Laser Healthcare, n.d., p.6).

The enzyme within the mitochondria that is stimulated the most by LLLT is cytochrome c oxidase. Cytochrome c oxidase is the terminal enzyme in the mitochondrial electron transport chain that is responsible for mediating the transfer of electrons from cytochrome c to molecular oxygen within the mitochondria (Erchonia Laser Healthcare, n.d., p.6). Cytochrome oxidase c is capable, due to the presence of transition metals, of absorbing photonic energy: thus, identifying cytochrome c oxidase as a photo-acceptor molecule responsible for the various cellular responses following LLLT (Erchonia Laser Healthcare, n.d., p.6). When this enzyme is affected by LLLT, electron transfer across the mitochondrial membrane is increased resulting in increased oxidative metabolism and ATP production that are imperative for cellular function, metabolism, and

proliferation (Whitfield, Jann, & Bartels, n.d., p.5). The photostimulation of cytochrome oxidase c and subsequent production of ATP following laser irradiation is referred to as the primary reaction (Erchonia Laser Healthcare, n.d., p.7). Immediately following the primary reaction is a cascade of chemical reactions referred to as secondary reactions, such as the reduction of oxidative stress and restored mitochondrial function, and a cascade of effects promoting tissue repair and reducing inflammation (Mantineo, Pinheiro, & Morgado, 2014, p.098002-1).

In a 2014 LLLT study (dos Reis, et.al.,p.111), reductions in the levels of blood lactate in the pre-fatigue and post-fatigue laser groups were observed in humans. Laser treatment significantly reduced the blood lactate levels (in the pre-fatigue and post-fatigue laser groups) measured 10-15 minutes after exercise, indicating that LLLT can be effective for improving muscle performance.

Materials and Methods

Research Design: This study utilized a true experimental quantitative design to reach its purpose. The experiment consisted of two experimental groups and one control group. Each group consisted of four quarter horses. The subjects used in this study ranged in age from 3-8 years old and varied in relative fitness. The experiment was conducted at a training facility in southeast Missouri for three weeks. Verbal permission was obtained from the owners of each horse used as subjects in this study.

Purpose: The purpose of this study was to determine whether the effect of low-level laser therapy on exercise induced biochemical markers are greater when treated before or after exercise.

Objective 1: Group A horses did not receive any low-level laser therapy while still being subjected to exercise.

Objective 2: Group B horses received low-level laser therapy treatment before exercise.

Objective 3: Group C horses received low-level laser therapy after exercise.

Objective 4: Results from Groups A, B, and C were compared to determine the effects of low-level laser therapy before and after exercise.

Participants: Twelve equine subjects were selected for this study. The subjects were all Quarter Horse breed between the ages of 3 and 8 years old. Only mares and geldings of moderate fitness were used in this study. No subjects had any known prior health conditions or lameness issues.

Sampling: The sampling method used in this study was a non-probability convenience sampling, selecting subjects based on availability.

Treatments:

Group A (Control): Four subjects were exercised for 30 minutes and did not receive any low-level laser treatment before or after exercise.

Group B: Four subjects received low-level laser treatment of both stifle areas at a wavelength of 635nm for 6 minutes immediately before exercise. This group did not receive any laser treatment after exercise. Exercise lasted 30 minutes.

Group C: Four subjects received low-level laser treatment of both stifle areas at a wavelength of 635nm for 6 minutes immediately following exercise. This group did not receive any laser treatment before exercise. Exercise lasted 30 minutes.

Instruments: A Class II ERCHONIA ® PL500 handheld low-level laser was utilized for treatment. A wavelength of 635nm was performed with a constant wave modulation.

Exercise was provided by utilizing a walker. Horse walkers are an ideal installation to balance out movement deficits and to allow controlled training of horses through walking or trotting. An entire group was hooked up to the walker and exercised at trot of the same speed for 30 minutes.

Sample Collection and Analysis: Approximately 3.0 mL of blood was collected from the subjects on a weekly basis via jugular venipuncture. The blood was immediately placed on ice. The blood was centrifuged at 2,000 x g for 15 minutes at room temperature within 4 hours of collection. The top serum layer was then pipetted off and placed in a separate test tube. The serum was then frozen for approximately five months until it was tested in the laboratory.

A Cayman Chemical L-Lactate Assay Kit was utilized for lactate testing in this study. The assay provided a fluorescence-based method for detecting L-lactate in the serum samples. In the assay, lactate dehydrogenase catalyzed the oxidation of lactate to pyruvate, along with the reduction of NAD+ to NADH. The NADH then reacted with the fluorescent substrate to yield a highly fluorescent product. That product was then analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm (Cayman Chemical Company, 2016, p.7).

The serum to be tested for lactate was quick thawed in 45°C water and placed on ice. Five hundred μL of serum was deprotonated at a 1:7 ratio by adding 500 μL of cold 0.5 M MPA. The tube was then vortexed and placed on ice for another five minutes. The deprotonated serum

was centrifuged at 10,000 x g for five minutes at 4°C to pellet the proteins. The supernatant was removed and 50 μL of Potassium Carbonate was added to neutralize the acid. The serum was centrifuged again at 10,000 x g for five minutes at 4°C to remove any precipitated salts. The supernatant was removed to be used for assaying.

Samples were assayed in duplicate and performed at room temperature. Standard wells were prepared with 20 μL of standard, and 20 μL of sample was added to the sample wells. One hundred microliters of Assay Buffer, 20 μL of Cofactor Mixture, and 20 μL of Fluorometric Substrate were added to all wells. The reactions were initiated by adding 40 μL of Enzyme Mixture to all wells. The plate was incubated for 20 minutes at room temperature. The fluorescence was then read using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

A Cayman's Cortisol Express ELISA Kit was utilized to prepare cortisol samples to be tested in this study. The serum to be tested for cortisol was quick thawed in 45°C water and placed on ice. The samples were deprotonated at a ratio of 1:8. A pool of 10 samples was then created for parallelism. The samples were diluted with PBS (phosphate buffered saline) with serial dilution at ratios of 1:10, 1:20, 1:40, 1:80. Once the samples were prepared, they were refrozen and shipped to a lab at Louisiana State University to be tested for cortisol.

Data Analysis: Results from the blood tests were compared between each group of the study using correlation and mixed model procedures of Statistical Analysis Software (SAS) was utilized to analyze quantitative data. Tukey's adjustment and multiple t-tests were performed when F tests for main affects were significant ($P<0.05$). Performance of laser therapy was

utilized as the independent variable, while cortisol and lactate levels were used as independent variables.

Results

The data from this study shows that lactate levels and time were positively correlated ($r= 0.71$; $p= 0.0001$, Figure 2), while cortisol levels and time were negatively correlated ($r= -0.033$; $p= 0.03$, Figure 1). Therefore, lactate and cortisol levels have an inverse relationship with one another and are negatively correlated ($r= -0.32$; $p= 0.035$, Figure 3). As lactate increased during the three-week period, cortisol decreased. There was also an interaction between cortisol, group, and time ($p= 0.0034$, Figure 5), as well as an interaction between lactate, group, and time ($p= 0.014$, Figure 4).

Discussion

According to the results of this study, there is no evidence to suggest that laser therapy had a significant effect on equine cortisol or lactate, regardless if it was performed before or after exercise. In accordance with the literature, lactate increased as time increased as a result of lactic acid build up due to exercise. Cortisol decreased over time, which could be due to several possible variables. For example, the subjects could have grown more comfortable to the trial routine and became less stressed as the study went on, resulting in lower cortisol levels.

While laser therapy did not appear to make a significant impact on cortisol or lactate in this study, it is widely noted in other studies that low-level laser therapy did have significant effects on other biochemical markers of fatigue such as creatine kinase. One possible explanation

for this is that cortisol and blood lactate tend to return to resting levels in a relatively short amount of time.

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Tables, Figures, and Graphs

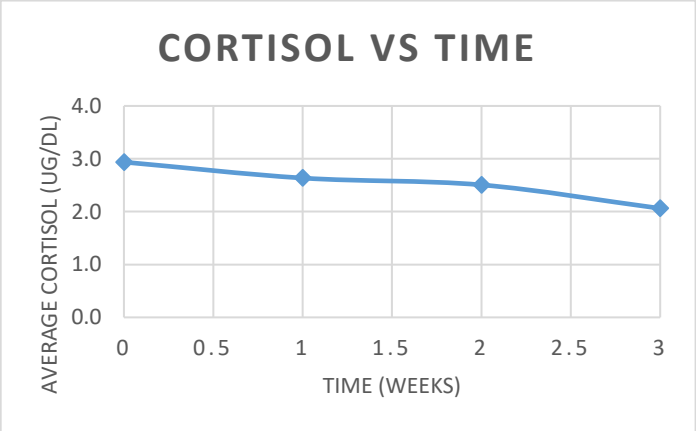

FIGURE 1: CORTISOL VS TIME

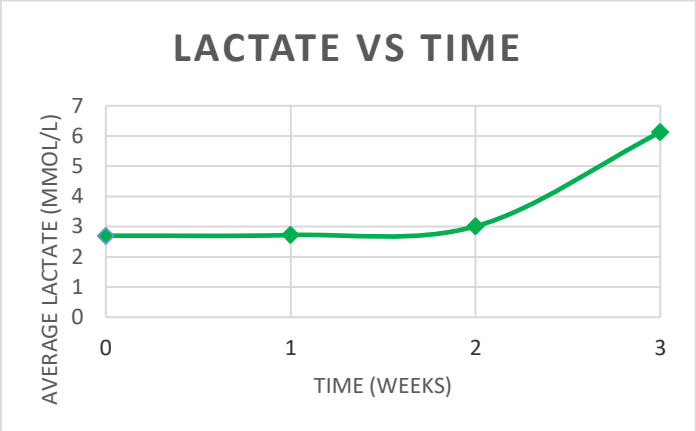

FIGURE 2: LACTATE VS TIME

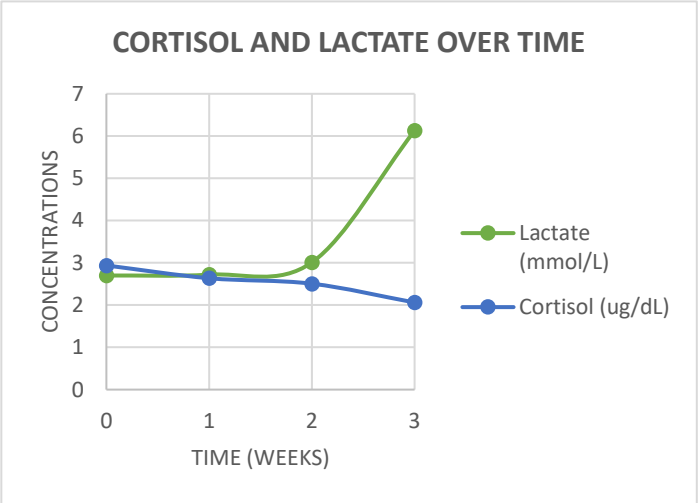

FIGURE 3: CORTISOL AND LACTATE VS TIME

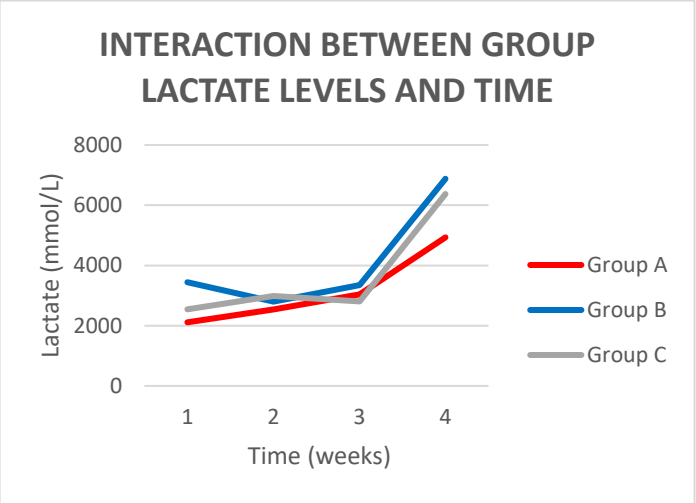

FIGURE 4: LACTATE- INTERACTION BETWEEN GROUP AND TIME

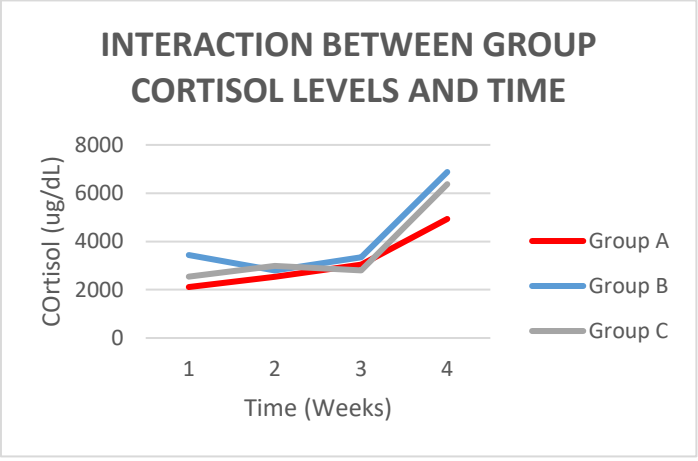

FIGURE 5: CORTISOL- INTERACTION BETWEEN GROUP AND TIME

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