

ACUTE MANIPULATION OF TRAINING VOLUME
AND ITS EFFECTS ON CREATINE KINASE
PRODUCTION

by

Justin L. Colf

An Abstract

of a thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
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May, 2017

ABSTRACT

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The purpose of this investigation was to determine the extent and nature of a relationship between creatine kinase (CK) levels and the systematic, acute manipulation of training volume in 12 resistance trained males and females, 18-25 years of age. Each individual participated in two training sessions. Work load and training volume were dependent upon group assignment. For the first training session, the protocol was 2 sets of 10 repetitions of each exercise (hig-bar back squat, leg press, hamstring curls, and calf raises) at 65% of 1-RM (repetition max). This protocol was repeated by the control group during their second training session, while the experimental group completed the same exercises for 3 sets of 10 repetitions at 75% of 1-RM. Blood was collected at four time points in relation to each training session (pre-exercise, 24-, 48-, and 72-hours post-exercise). The two primary findings of the study revealed that lower body resistance training resulted in a significant increase in CK from baseline to peak post-exercise levels, but no significant relationship between training volume manipulation and CK response in resistance trained males and females.

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CHAPTER I

NATURE AND SCOPE
OF THE STUDY

Optimal performance has become an ever increasing focus for elite athletes and recreational fitness enthusiasts across the sports spectrum. The factors that impact performance are nearly limitless, and researchers continue to discover new and improved techniques for measuring and analyzing performance. One main focus for individuals trying to optimize performance is the training protocol itself. Certainly, there are many training protocols, ideas, and strategies within any given sport. While each protocol maintains similar underlying principles (e.g. overload and specificity) to stimulate physiological adaptations, emphasis on particular variables may vary. Training protocol certainly impacts outcome and the protocol should be chosen based on specific goals.

One variable that is recognized to have significant importance is training volume. For resistance training, training volume can be conceptualized as the product of repetitions and weight completed in a given training session for each muscle group (Peterson, Pistilli, Haff, Hoffman, & Gordon, 2011; Baechle & Earle, 2008). Volume must be considered when constructing a training protocol as the resultant adaptations are based on the physiological stressors involved with the different training protocols. Mechanical and metabolic stress achieved is an important stimulating factor that will drive the different adaptations to occur (Mangine, Hoffman, Fukuda, Stout, & Ratamess, 2015). It is traditionally believed that high volume training at lower intensities will result in greater increases in muscular hypertrophy, while high intensities at lower volume will stimulate strength gains (Mangine et al., 2015). Mangine et al (2015) and Herodek, Simonovic, and Rakovic (2012) suggest that while this is true,

the extent to which these adaptations occur is dependent on individual traits (e.g. muscle fiber type or level of ability).

The adaptations that occur via different stimuli are certainly a primary goal for any given training regimen. To drive adaptations, overload must occur. In other words, to achieve specific adaptations, mechanical stress must be applied and the stress must continue to be gradually increased over time. This gradual, but calculated increase in mechanical stress, is referred to as the overload principle. Overload is a cornerstone principle in any properly structured training protocol (Herodek et al., 2012; Issurin, 2014; Koprivica, 2012). Without overload, the musculature will not continue to adapt and the individual will see little to no improvements. When overload is properly applied, damage to the musculoskeletal system occurs by way of microtrauma to the functional unit of the myofibers – the sarcomere. This microtrauma disrupts localized regions of the myofiber and stimulates the repair process to rebuild the area that was damaged. It is through this process that adaptations are achieved. It is important to be cognizant of the amount of microtrauma that is occurring so that adaptations can result from proper overload while excessive damage can be avoided. Investigating how much microtrauma is occurring is beneficial for increasing knowledge about the design of optimal training protocols.

There are a variety of methods used to detect and quantify the presence and severity of microtrauma to muscle. One method is by measuring the amount of creatine kinase (CK) in the blood. Creatine kinase is an intracellular enzyme, so an elevated presence in the blood signifies that muscle damage has occurred (Baird, Graham, Baker, & Bickerstaff, 2012). In essence, the presence of CK denotes the disruption of the cellular membrane.

The function of CK in the cell is to catalyze the hydrolysis of phosphocreatine (PCr) to produce adenosine triphosphate (ATP) (McArdle, Katch, & Katch, 2015). A near-equilibrium

enzyme is identified as an enzyme that is only affected by the concentration of its substrates and products, as opposed to a non-equilibrium enzyme, which can be affected by factors other than substrate and product concentration (Hargreaves & Spriet, 2006). Near-equilibrium enzymes are known to have higher maximal activities and lower activation thresholds than non-equilibrium enzymes and are therefore more active in driving metabolic processes. Being a near-equilibrium enzyme, CK is regulated by the ratio of substrate and product concentrations (Hargreaves & Spriet, 2006). Exercise of an anaerobic nature, i.e. resistance training, uses PCr as a predominant source of energy. Therefore, PCr must be resynthesized at a constant rate in order to match energy demands.

In addition to its direct role in energy production, CK is believed to be an indirect marker of micro trauma and overall muscle damage (Baird et al., 2012; Machado et al., 2012; Machado and Willardson, 2010). Advancements in research techniques have allowed for the ability to analyze physiological responses in new ways. Subcellular focus is now common. Along these lines, there is reason to investigate blood CK levels and how those levels relate to a training period. While it is accepted that CK is an indirect marker of muscle damage, Baird et al (2012) suggests that CK should also be considered as a vital component in muscle performance and recovery, as it may serve to elucidate overtraining, for example. Understanding how CK levels change in response to varying training volume may allow professionals to design individualistic training models for clients.

Purpose of the Study

The purpose of this investigation is to determine the extent and nature of a relationship between creatine kinase levels and the systematic, acute manipulation of training volume.

Significance of the Study

While a significant amount of research has been conducted on CK in the past, very few studies have reached practical conclusions on how CK is affected by training volume. Though it has been determined that CK is an indirect marker of muscle damage, the practical usage of this information and how it can be utilized in everyday training regimens has been undervalued. Evaluating the relationship between CK and training volume can assist in creating a practical base of knowledge on which training variables and future research can be established.

Delimitations

This study was delimited to:

1. Subjects who were recreationally engaging in strength training for a minimum of 1 year and three days/week as this is deemed sufficient activity by ACSM guidelines (ACSM & Pescatello, 2014).
2. Individuals between the ages of 18 and 25.
3. Individuals who verbally identified as being free from use of hormone or steroids.
4. Individuals free from musculoskeletal, pulmonary, or cardiovascular concerns as determined by ACSM guidelines (ACSM & Pescatello, 2014).
5. Training volume was calculated as load completed multiplied by totals reps completed in the training session.
6. A 3-week training timeframe.
7. An exercise protocol based on hypertrophy training recommendations of periodization according to NSCA standards (Baechle & Earle, 2008).

Limitations

The study was limited by:

1. Prior experience; due to each subject having prior experience in strength training, adaptations in CK response may be present, possibly decreasing or increasing response.
2. Diet; each subject was instructed to maintain normal caloric balance throughout the study as well as maintain a euhydrated state. As direct observation outside of training sessions was not viable, subjects may have altered dietary and hydration habits.
3. Additional training; subjects were instructed to not participate in additional strength training outside of scheduled sessions during the duration of the study, any other outside exercise could skew CK levels.
4. Additional activity; recreational activity separate from strength training could possibly affect CK levels.
5. Genetics; fiber type, among other factors, may impact CK responses prior to and after exercise.

Assumptions

It was assumed that:

1. All subjects answered the questionnaire correctly and honestly.
2. Subjects complied with the request to perform at full capacity during each training session.
3. Subjects complied with the request to avoid any additional strength training or strenuous exercise outside of scheduled training sessions.
4. Subjects complied with the request to maintain normal caloric balance throughout the extent of the study.

Hypotheses

It was hypothesized that:

1. Compared with pre-exercise, participants would have an increase in CK levels in the blood in at least one of the three (24, 48, or 72 hours) post-exercise time points.
2. The higher training volume group would have a greater increase in CK in the blood from baseline to post-exercise peak values when compared to the low training volume group.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Energy systems contain regulatory enzymes that control flux and adjust production of energy based on the availability of its primary substrates. In the PCr system, also referred to as the phosphagen system, CK serves as the regulatory enzyme for ATP production. Specifically, CK catalyzes the hydrolysis of PCr. In the reaction, the transfer of a phosphoryl group from PCr to adenosine diphosphate (ADP) occurs. The PCr molecule is an important energy buffer used to phosphorylate ADP to ATP (McArdle, Katch, & Katch, 2015). At the onset of exercise, the PCr system is vital as it helps maintain the ATP:ADP ratio. It is vital because the other two primary metabolic pathways, glycolysis and oxidative phosphorylation, are more slowly activated (Hargreaves & Spreit, 2006; McArdle et al., 2015)

When the reaction moves in the direction favoring ATP production, it assists in maintaining cellular energy needs. However, it is important to remember that the role of CK as a near equilibrium enzyme results in this particular reaction being a reversible process (McLeish & Kenyon, 2005). Figure 2.1 illustrates the basic role of CK in the mitochondria and cytosol.

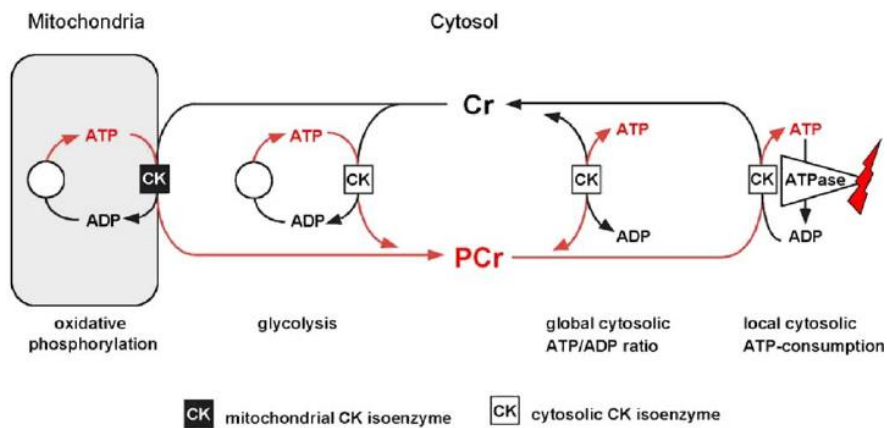


Figure 2.1—The connection of the mitochondrial and cytosolic (i.e. soluble) fraction of creatine kinase in relationship to bioenergetics is shown. Mitochondrial CK uses ATP generated by oxidative phosphorylation to synthesize PCr. In less oxidative tissues (i.e. fast-twitch muscle fibers) PCr is mainly synthesized by the cytosolic fraction of CK. Synthesis results in a buildup of PCr which acts as an energy buffer to maintain the ATP:ADP ratio over a wide range of workloads. Figured adapted from Baird et al., 2012.

While CK serves as a primary enzyme for energy production in skeletal muscle, it has been shown to function in other tissues as well. Creatine kinase exists as three tissue specific enzymes known as CK-MB (cardiac muscle), CK-MM (skeletal muscle), and CK-BB (brain tissue) (Baird, Graham, Baker, & Bickerstaff, 2012). The CK enzyme plays a similar role in each of these tissues—it supports energy needs by buffering the loss of ATP (Wu & Beard, 2009). Overall, CK-MM accounts for 98% of the isoenzyme concentration in skeletal muscle, making it the most prominent source of CK in the body (Baird et al., 2012).

Damage to Muscle and Creatine Kinase in the Blood

Creatine kinase is an intracellular enzyme. Accordingly, it is only found in the blood when it is expelled from the cell or when damage to the cell membrane occurs. The mechanisms are complex, and debated, so the present section will only focus on how calcium ions (Ca^{2+}), AMP-activated protein kinase (AMPK), mechanical movement, and the inflammatory response may result in an increase in plasma CK. It is also important to note that the presence of CK in the

blood might occur in acute fashion due to Ca^{2+} , AMPK, and mechanical movement, whereas, the presence of CK in the blood due to the inflammatory process may occur several days after the exercise (Evans & Cannon 1991).

Baird and colleagues (2012) recently wrote a review on CK and exercise-induced muscle damage (EIMD). One thought is that metabolic disturbances to the muscle may result in the release of cellular components into the blood through a Ca^{2+} dependent mechanism. There is evidence to support this assertion. As ATP is depleted, Ca^{2+} is leaked into the intracellular space due to the dysfunction of both the Na-K-ATPase and Ca^{2+} -ATPase pumps (Baird et al., 2012). When intact, isolated muscle fibers were incubated with bupivacaine after exercise, the fibers exhibited a large efflux of CK into the incubation media (Steer, Mastaglia, Papadimitriou, & Van Bruggen, 1986). However, when the culture was free of Ca^{2+} , CK efflux was greatly reduced during the first hour of incubation. This data suggests that Ca^{2+} is an important stimulant for skeletal muscle breakdown (Steer et al., 1986).

Baird et al. (2012) have also proposed that AMPK, an important energy-sensing enzyme, may directly or indirectly impact the presence of CK in the cell and in the blood. As activities occur that deplete ATP, AMPK is activated and stimulates processes that increase ATP production and inhibits pathways that consume ATP (Baird et al., 2012). Due to its role in stimulating ATP production, it is likely that AMPK has a role in controlling CK activity. Given that CK is used in a reversible reaction (to both produce ATP and resynthesize PCr), AMPK may limit the use of ATP by CK to produce PCr during ongoing activity (Baird et al., 2012). It is postulated that AMPK could initiate processes to expel CK from the cytosol in order to preserve ATP (Baird et al., 2012). Accordingly, CK release into the blood may be a result of regular metabolic functions as well as EIMD.

A third mechanism that may lead to increases in CK in the blood is mechanical movement—EIMD. During the mechanical movement of a contraction, especially eccentric contractions, muscle fibers are stretched, altered, and potentially destroyed. These actions often result in increased permeability of the muscle fiber and create an environment in which enzyme efflux may occur (Armstrong, Ogilvie, & Schwane, 1983). Armstrong et al. (1983) suggest that cellular destruction may be a result of the shearing of membranes or myofilaments by excessive loads during exercise. Although trauma and shearing of muscle fibers is necessary to stimulate adaptations, the modality in which this damage occurs has a significant impact on the health of the muscle and the release of enzymes into circulation. Significantly larger increases in CK circulation have been seen following eccentric exercise protocols when compared to concentric exercise. Schwane, Johnson, and Vandenaeker (1983) observed a 351% increase in circulating CK following downhill running (45 min at 57% of VO_2 max, -10% incline), but no change was seen following an equal bout of level running at 0%. However, a bout of downhill running (-10% grade, 30 min, heart rate of ~170BPM) was shown to protect against future EIMD and CK spikes in circulation when compared to uphill running (Byrnes et al., 1985). These data suggest that while eccentric exercise protocols result in significantly higher levels of CK in the blood, they also provide lasting adaptations to protect against further EIMD.

Finally, it is likely that the process of inflammation at the site of muscular injury may increase plasma CK. Within hours of injury or muscle damage, circulating neutrophils can increase several fold (Evans & Cannon, 1991). Neutrophils are the responsible cells for phagocytosis of the tissue debris. Neutrophils also release cytotoxic factors (Evans & Cannon, 1991). While essential for repair, the activity of the cytotoxic factors (elastase, collagenase, and oxygen radicals) increase vascular permeability by breaking down cell membranes of the

microvasculature near the site of damage (Movat, Cybulsky, Colditz, Chan, & Dinarello, 1987). In addition to indirectly increasing cell permeability, neutrophil activity during and following exercise, likely contributes to increased cell volume and swelling from the intracellular accumulation of sodium and water. Cell swelling may contribute to the prolonged release of soluble CK fraction into circulation as a result of protein degradation (Evans & Cannon, 1991).

When you combine the potential for an acute release of CK (due to Ca^{2+} , AMPK, and EIMD) with the potential for a prolonged release of CK into circulation, there is the chance to see a biphasic representation of CK levels in the blood. Initial spikes in CK have been shown to fall around the 48hr post-exercise mark and then rise again near 95hrs post-exercise (Magal et al., 2010). Bottom line, the release of CK is evident due to multiple mechanisms and factors.

Clinical Investigations of Creatine Kinase in the Blood

The cause of cell membrane damage is the presentation of some stimulus that disrupts homeostasis (Baird et al., 2012). With knowledge of this information, plasma CK has historically been used to diagnosis an acute myocardial infarction due to the elevation of CK-MB present in the blood following cardiac events (Baird et al., 2012). Over the last 20 years, however, troponin, not CK-MB, has become the more useful enzyme to investigate damage to the heart (Baird et al., 2012). While the initial interest in blood levels of CK began from damage to myocardium, researchers began to expand the scope of investigation to include skeletal muscle CK—CK-MM.

Exercise and Sport Investigations and Creatine Kinase in the Blood

In more recent years, CK profiles have been used to investigate acute muscle damage. Truthfully, the story that unfolds from research is confusing, at times. It appears that duration of exercise, intensity of exercise, and type of exercise all influence serum CK levels. Along these lines, Clarkson and Ebbeling (1988) reported that CK levels were elevated following general

exercise which varied in mode, intensity, and duration. It also appears that training status, fiber type of muscle, and genetics influence the response after exercise as well. Details about these studies will be discussed by topic.

Running, Stepping, Cycling and Creatine Kinase

Aerobic exercise and exercise training can have a significant influence on the accumulation of CK in the blood. In one of the earliest investigations, Siegel, Silverman, & Evans (1983) measured resting CK in 25 marathon trained male runners and compared those values to ten sedentary males. According to results, CK-MB accounted for a only 7.9% (skeletal muscle) and 8.9% (serum) of total CK activity in the blood. This data suggests elevated CK levels in the blood are largely contributed by non-cardiac sources, such as CK-MM (Siegel et al., 1983).

That same year, Newham and colleagues published work investigating the impact of 20 min of stepping exercise on CK levels in eight male and eight female subjects (Newham, Jones, & Edwards, 1983). The eccentric action of the stepping was intended to cause muscle damage. Indeed, all subjects showed a rise in blood CK levels (~400 IU/liter) shortly after the exercise. Tenderness in the muscle was also reported. Interestingly, about half of the subjects (both male and female) showed a much greater response (up to 34,500 IU/liter) which took 4-5 days to peak. The authors speculated that eccentric contractions from the exercise result in some “particular form of muscle damage which, in susceptible subjects, may initiate changes giving rise to a large delayed release of muscle enzymes.” This coincides with the fact that there are mechanisms that can result in short term (Ca^{2+} , AMPK, and mechanical movement) increases in CK in the blood and also longer acting (inflammatory process) increases in CK several days after the exercise. These variables likely explain the authors findings.

Warhol and colleagues (1985) examined muscle biopsies taken from the gastrocnemius muscle of marathon runners immediately after and up to 12 weeks after a marathon. Examination of the fibers at both time points, immediately after the marathon and 12 weeks after, showed significant tearing of the sarcomeres at the Z-band level, which accompanied increased cell permeability. Compromised cell structure (i.e. cell permeability) may result in the efflux of intracellular CK.

It was discovered that CK efflux from the cell is related to endurance exercise as well as physical characteristics (Totsuka, Nakaji, Suzuki, Sugawara, & Sato, 2002). Fifteen healthy young men performed 90 min of bicycle exercise for 3 consecutive days, during which 22 blood samples were collected. According to results, plasma CK was elevated from 3 hrs post-exercise and gradually increased thereafter. This increase in serum CK appeared to be correlated to the cross sectional area of the quadriceps femoris muscle (QFM), which was measured prior to exercise in order to determine the extent of a relationship between CK and physical characteristics. Overall, plasma CK increases were found to be two to three times higher than when measured at rest in all individuals.

In short distance, high intensity running events, the duration of the activity appears to impact CK levels in the blood. The CK levels of sprinters were analyzed for the 110m and 400m hurdles (Krysciak, Podgorski, & Eichler, 2015). When eight well-trained athletes completed both distances, results indicated that while CK levels increased beyond baseline in both exercise tests, higher levels were recorded following the 400-m hurdle race.

Recently, Buzala et al. (2015) reported that the mean increase in CK activity in race horses is directly proportional to the duration of effort, with endurance rides producing significantly higher CK levels. This supports the applied human research (Newham et al., 1983;

Siegel et al., 1983; Warhol et al., 1985; Totsuka et al., 2002; Krysciak et al., 2015) and extends the outcomes to equine.

Sport Applications and Creatine Kinase in the Blood

In addition to being used as a marker of muscle damage, CK can also be used to help gauge the recovery status of an individual. Researchers evaluated CK responses of elite rugby players (McLellan, Lovell, & Gass, 2010). McLellan et al. (2010) measured CK levels of 17 rugby players at eight different intervals: 24 hrs pre-match, 30 min pre-match, 30 min post-match, and then at 24 hr intervals for a period of 5 days post-match. Creatine kinase increased significantly post-match, with peak CK levels measured at 24 hr post-match and remained elevated despite 120 total hours of recovery post-match. McLellan et al. (2010) stated that CK levels could be useful in determining the recovery status of individuals after acute muscle damage.

In support of this finding, Russell et al., 2015 investigated 14 professional soccer players, Russell et al. (2015) analyzed peak power output (PPO) and CK levels following each of the four, 90-min matches that were played. These measurements were taken at 24 and 48 hrs post-match and results showed that there was a significant, negative correlation between PPO and CK levels. In comparison to baseline levels, CK accumulation was approximately 343 ± 150 uL higher at the 24 hr mark, but these values significantly decreased at the 48 hr mark as the levels measured at this time were $26.3 \pm 20.0\%$ lower than those recorded at 24 hrs post-match. Overall, these results indicate that CK can be used to effectively monitor the recovery status of individuals following a performance or training session.

Concepts Related to Resistance Exercise

Resistance training is primarily anaerobic in nature (Gasten, 2001; Walsh et al., 2001). Adequate rest is needed between sets to facilitate recovery. Separated by periods of rest, the PCr system has ample time to replenish PCr stores in between working sets. Recovery allows for reliance on the metabolic system for subsequent energy production. Recovery influences volume and training volume is one of the most vital components in any training protocol. It has been viewed as one of the primary manipulative variables in training protocols since the 1950's (Herodek, Simonovic, & Rakovic, 2012).

The amount of volume used may be an indication of training protocol. For example, individuals seeking hypertrophy of muscle fibers require significantly more overload, in terms of volume, during training sessions when compared to those seeking increases in strength or power (Goldberg, Etlinger, Goldspink, & Jablecki, 1975). Athletes and coaches often alter training for off-season, pre-season, or in-season time periods. The volume used during the off-season training period will be higher than the volume used during the in-season period (Plisk & Stone, 2003). This change is not only in response to the alteration of primary goals, but also the increased level of cellular disruption that occurs with high-volume training.

Resistance Exercise and Creatine Kinase in the Blood

When the stimulus from a single bout of resistance training disrupts the homeostasis of the cell, the cell's structure and function is compromised (Baird et al., 2012). This is when CK becomes more evident in the blood. Research suggests that duration, volume, and rest intervals are all likely to play a role in the presence of CK in the blood (Tiidus and Ianuzzo, 1983; Machado & Willardson, 2010; Evangelista, Pereira, Hackney, & Machado, 2011; Machado et al., 2012).

Tiidus and Ianuzzo (1983) found that higher intensity exercise resulted in greater indices of muscle damage (CK and blood lactate) when compared to low intensity exercise. They also found that when the intensity was matched at 70% of the 10RM, but volume was increased to 200-300 reps per session (from 150 reps per session), that serum indices of muscle damage increased. These results suggest that increased training volume, while effective at stimulating hypertrophy, also results in higher CK levels in the blood (Tiidus & Ianuzzo, 1983).

Machado and Willardson (2010) sought to determine the relationship between CK activity and rest intervals in between working sets. Thirty-two trained men performed two resistance exercise bouts, separated by a week, with either 1-min or 3-min rest intervals between sets. Blood samples were obtained and CK measurements were recorded prior to each exercise bout as well as 24, 48, 72, and 168 hrs post-exercise. The results showed indicated that greater volume was completed per exercise bout by subjects using a 3-min rest interval. Furthermore, it was found that CK activity was approximately 70% higher in the 1-min rest interval group versus the 3-min rest at the 48 and 72 hr time points. These findings suggest that rest intervals play a key role in the presence of CK in the blood by affecting the amount of volume completed in a given exercise bout.

Evangelista et al. (2011) investigated the impact of rest intervals on CK activity, volume completed, and muscle soreness. Twenty-eight healthy sedentary men were divided into two groups, 1-min or 3-min rest intervals between sets. Each participant performed three sets of bicep curls at 40% of their maximal voluntary isometric contraction strength until voluntary failure. Results showed that individuals in the 3-min rest interval group achieved greater volume, but presented no differences in serum CK or muscle soreness when compared to those individuals in the 1-min rest interval group. These findings dispute what was found by Machado and

Willardson (2010) in that rest intervals do not appear to affect CK serum activity following exercise.

Research by Machado et al. (2012) aimed to determine the extent of relationship between upper body resistance exercise and volume completed during each respective session. Twenty healthy men with at least two prior years of resistance training volunteered for the study and served as their own controls. Each subject completed an upper body resistance training session using 1-min and 3-min rest intervals; these sessions were separated by one week and performed at the same time of day to negate circadian variation. Subjects were verbally encouraged to complete each working set to voluntary exhaustion. Results showed that the total volume for all exercises was significantly greater for the session with the 3-min rest intervals versus the 1-min rest intervals and there was a weak correlation between CK accumulation and total volume completed in a given session.

As presented above, duration, volume, and rest intervals are all likely to influence the presence of CK in the blood (Tiidus and Ianuzzo, 1983,; Machado & Willardson, 2010; Evangelista et al., 2011; Machado et al., 2012).

Nutrition, Supplementation, and Creatine Kinase in the Blood

Nutrition and supplementation play an integral role in athletic performance, particularly among more elite trained individuals. It is widespread knowledge that protein powder and carbohydrate drinks are two of the most commonly used supplements to improve performance. The choice to use supplementation may impact the accumulation of CK in the blood after exercise. Skillen et al. (2008) evaluated the relationship between CHO (4.6% CHO) or CHO plus amino acid (CHO-AA; 3.6% CHO and 1.0% AA) ingestion on cycling time to exhaustion and presence of CK in the blood. Twelve male athletes volunteered for the crossover designed study

with a two-week washout period. The CHO-AA solution was found to reduce fatigue and decrease post-exercise measurements of CK—both favorable outcomes. In one of the more recent works on this topic, Hansen et al. (2015) analyzed the effects of ingesting protein (PRO), protein-carbohydrate (PRO-CHO), or carbohydrate (CHO) only beverages on 4-km run and presence of CK in the blood. Eighteen elite orienteers volunteered. Pre- and post-tests were conducted at the beginning and end of the week to investigate the influence of the different beverages. Results showed that the PRO-CHO drink consistently reduced CK levels during the one-week training camp when compared with the CHO drink. Therefore, not only did the PRO-CHO drink improve performance on the 4-km run test after a one-week training camp, but it also mitigated muscle damage based on the interpretation of CK levels (Hansen et al., 2015). Consuming these beverages likely aided the body through provision of a readily available energy source. Drinks with AAs, such as PRO-CHO or CHO-AA, help increase the blood glucose concentration and increase the free amino acid pool to expand and spare protein metabolism (Wagenmakers, Coakley, & Edwards, 1990; Gibala, MacLean, Graham, & Saltin, 1997).

Genetic Predispositions and Creatine Kinase in the Blood

As with many aspects of anatomy and physiology, genetics play a role in the CK response to exercise. Three factors that appear to influence CK responses to exercise are sex, predisposition to enzymatic activity, and distribution of fiber type. Evidence from multiple studies indicate that while some variation may be present, there is little to no difference between males and females in their response to exercise (Rinard, Clarkson, Smith, & Grossman, 2000; Clarkson & Hubal, 2002). When the effect of sex is removed, predispositions to enzymatic activity still impact the CK accumulation in the blood. Since the 1980's, individuals have been classified as either “high responders” (HRCK) or “low responders” (LRCK) based on the

accumulation of CK in the blood in response to exercise (Newham, Jones, & Edwards, 1986; Magal et al., 2010; Machado et al., 2012). The primary difference between HRCK and LRCK has yet to be solidified, but individual genotype and muscle fiber composition are implicated. Results have shown that individuals with the CK-MM Ncol AA genotype presented a six fold higher prevalence of being classified as a HRCK when compared to those with other phenotypes (Heled, Bloom, Wu, Stephens, & Deuster, 2007). The presence of the CK-MM Ncol AA genotype allows for the CK enzyme to be increasingly active which may influence the response (Heled et al., 2007)

Summary

There is some consensus that CK can be used to quantify muscle damage in both skeletal muscle and myocardium (Clarkson and Ebbeling, 1988; Friden & Lieber, 2001; McLeish & Kenyon, 2005; Machado & Willardson, 2010). While research concerning CK and its relationship with training, nutrition, and genetics has grown, there is still a large amount of knowledge to be gained. Being able to quantify muscle damage by measuring CK gives coaches, trainers, and athletes the ability to track recovery and adjust workloads based on the stress induced on individuals in a given match or training session (McLellan et al., 2010; Russell et al., 2015). With this information, multiple variables can be altered to improve performance and promote more efficient training regimens and subsequent recovery.

CHAPTER III

METHODOLOGY

Overview

The purpose of this research was to determine the extent of a relationship between acute manipulation of training volume and plasma CK in recreationally-trained male and female resistance exercisers. Participants completed three visits for testing and data collection. During the one repetition maximum session, the individual's 1-RM was determined for the high-bar back squat (HBBS), leg press (LP), hamstring curl (HC), and calf raise (CR). The baseline protocol for obtaining the 1-RM followed the directives of the NSCA in which the following order of repetitions was utilized to obtain a true 1-RM: 7-5-3-1-1-1 (Baechle & Earle, 2008). Based on total weight lifted at the maximal testing, participants were pair-matched and randomly assigned to either the volume control group (called "control"—CON) or a volume increase group (called "experimental"—EXP). At the first training session, the CON and EXP groups completed an identical warmup, four exercise (same relative load) training session, and cool down. Each of these aspects are described below. To decipher the effect of acute manipulation of volume on plasma CK levels, the CON group replicated the exact procedures of session #1 at session #2, while the EXP group completed an additional set of the exercises and at an increased relative load during session #2. Figure 3.1 illustrates the general research design.

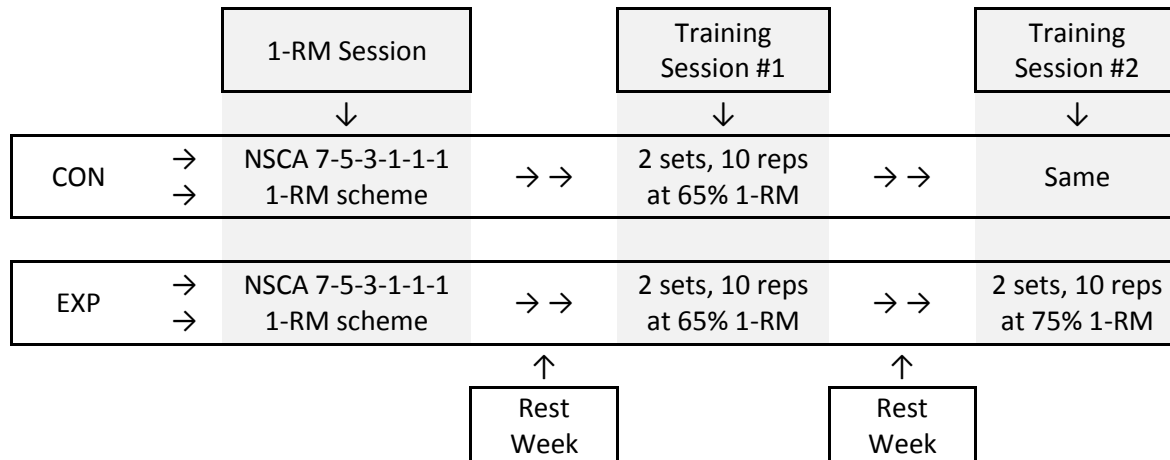


Figure 3.1—The general study protocol is depicted in a series of boxes and shaded regions. Flowing from left to right, the order of the sessions and the CON and EXP groups are defined. Flowing from top to bottom are the explanation of the training session characteristics. A two-week data collection period was utilized. The 1-RM session could be considered Day 0. Training Session #1 and #2 occurred on days 7 and 14, respectively. The 1-RM and training session exercises were the high-bar back squat, leg press, hamstring curl, and calf raise.

Participants

The research was open to males and females between the ages of 18 and 25. Primary inclusion criteria for the volunteers were: a) affiliation with the University of Central Missouri, b) sufficient activity to achieve ACSM requirements for health (Pescatello & ACSM, 2014), c) engaged in resistance training for the previous year at least 3 times per week; and, d) proficient in the movements of HBBS, LP, HC, and CR with proficiency defined as the subjects' ability to achieve full range of motion based on NSCA standards (Baechle & Earle, 2008).

Explanation of 1-RM and Training Sessions

A total of 12 volunteers reported to the UCM Human Performance Lab and signed the informed consent prior to any testing. Thereafter, participants were guided through an aerobic warm-up consisting of a preferred pace between 2.5 and 3.5mph treadmill walking for five minutes at a 6% incline. The 1-RM session was conducted in accordance to guidelines from NSCA in which a 7-5-3-1-1-1 scheme was used to help the subject warm-up, approach their

maximal weight, and obtain their 1-RM within three lifts (Baechle & Earle, 2008). This procedure was used for all four exercises which were completed in the following order: HBBS, LP, HC, and CR. Volunteers were given between two and five minutes of rest between lifts based on rating of perceived exertion (RPE) on a 0-10 scale. Two-min of rest was given when RPE was between 0-2, 3-min given when RPE was between 3-5, 4-min given when RPE was between 6-8, and 5-min given when RPE was between 9-10. Based on the summed total weight lifted at 1-RM, participants were ordered and pair-matched with the closest volunteer who shared their strength. Those pairs were then split, randomly, into either the CON or EXP groups. The 1-RM Session lasted approximately 90 min.

At Training Session #1, CON and EXP volunteers were guided through a general warm-up consisting of a preferred pace between 2.5 and 3.5mph for 5-min at a 6% incline followed by an exercise-specific warm-up of 3 sets of HBBS. The first set of HBBS was 10 reps at 45% of 1-RM, the second set was 5 reps at 55% of 1-RM, and the third set was 3 reps at 75% of 1-RM. Two minutes of rest were provided between the warmup sets. Thereafter, the exercises (HBBS, LP, HC, and CR) began. They were performed, at each training session, in the following order: HBBS, LP, HC, and CR. Both the CON and EXP groups lifted utilizing relative loads of 65% of 1-RM, for 2 sets, 10 reps each set, with 3-min of rest between sets. When the sessions concluded, subjects were guided through a general cool-down consisting of preferred-pace treadmill walking for five minutes at a 1% incline. Training session #1 lasted approximately 90 min. At Training Session #2, CON and EXP volunteers were guided through an identical general and exercise-specific warm-up which replicated that of Training Session #1. While both groups utilized the same exercises, 10 reps for each set, and had 3-min of rest between sets, there was a difference in number of sets and relative load. The CON had no change in volume, thus they lifted a relative

load of 65% of 1-RM, for 2 sets, 10 reps each set, with 3-min rest between sets. The EXP, however, lifted a relative load of 80% of 1-RM, for 3 sets, for 10 reps each set, with 3-min rest between sets. When participants were not able to complete all 10 reps in a set, aid was given so that the desired numbers of reps were achieved. This volume increase (relative load and additional set), was used to determine the effect of acute manipulation of volume on plasma CK levels. Readers are again referred to figure 1. When the sessions concluded, subjects were guided through a general cool-down consisting of preferred-pace treadmill walking for 5-min at a 1% incline. Training Session #2 lasted between 90-120 min with the varying length of time being tied to group (CON = approximately 90 min and EXP = approximately 120 min). Training Sessions #1 and #2 were completed at approximately the same time of the day to negate potential impacts of circadian variation.

Blood Collection and Analysis

As the primary outcome, measures of CK were recorded at 4 different time points around Training Sessions #1 and #2. Specifically, venous puncture draws from the antecubital vein were taken at 15-min pre-exercise and 24hr, 48hr, and 72hr post-exercise. To mitigate undue influence of exercise, participants were required to abstain from training in the 48 hrs prior to the scheduled training session and 72 hrs after. Exercise is a primary variable known to affect CK levels. Collection occurred in the Human Performance Laboratory and participants were seated during collection. Blood was collected into an untreated 7mL vacutainer at each time point.

Blood samples were immediately centrifuged at 1500 RPM for 15-min. The plasma was removed via pipet and placed in a freezer and stored at -20°C. For analysis, blood samples were removed, allowed to thaw for 20 min, and a 1mL sample of plasma was placed into a 1.5mL micro centrifuge tube and analyzed by an enzymatic method (CK-UV NAC optimized,

Awareness Technology Inc, Palm City, FL) at 37°C via the ChemWell model 2902 automated chemistry analyzer (Awareness Technology Inc, Palm City, FL).

Diet, Drink, and Supplement Regulation

Participants were instructed to continue his/her customary dietary regimen throughout the course of the study. To assess nutrition, participants completed dietary and drink logs for the 48 hrs prior to each lab visit. In addition, to mitigate confounding influences of supplementation, participants were instructed to cease any supplementation one week prior to completing the 1-RM session and refrain from supplementation throughout the course of the study. Restricted supplements included: any kind of supplemental protein, creatine powders or pills, pre-workout powders or pills, beta-alanine, nitric oxide, and anabolic steroids. When questions arose, subjects were asked to consult with study personnel as to what may or may not have been appropriate to consume in relation to scheduled training session. The documentation of subject compliance in this inclusion criterion was limited to verbal confirmation. As an added precaution, researchers supplied participants with a 500mL bottle of water which was consumed in the 20-min timeframe between arrival and initiation of the session.

Statistical Analysis

It was hypothesized that compared with baseline CK levels, the mean peak CK level post-exercise would be significantly higher in both groups. This was tested by separate dependent T-tests; one for the CON group and one for the EXP group. It was also hypothesized that compared with the CON group, the EXP group would have a greater change in CK from baseline to mean peak CK level post-exercise in Training Session #2 when compared with Training Session #1. An independent *t*-test was utilized to compare the change in peak values between groups for Training Session #1 and Training Session #2. The α level was set at 0.05 for

all analyses. All statistical analyses was completed using IBM SPSS Statistics 24.0 (IBM Corporation, Armonk, New York) for Windows.

CHAPTER IV

RESULTS

Introduction

The purpose of this research was to determine the extent of a relationship between acute manipulation of training volume and plasma CK in recreationally-trained male and female resistance exercisers. Each individual participated in two training sessions with specified workloads throughout each session which were dependent on group assignment. Participants in the CON group completed identical training sessions which consisted of 2 sets of 10 reps at 65% of their 1-RM for each given exercise. Participants in the EXP group completed their first training session with the same prescription as the CON group; however, participants in the EXP group experienced an increase in volume (from 2 sets of 10 reps, to 3 sets of 10 reps) and intensity (65% 1-RM to 75% 1-RM) during their second training session. It was hypothesized that when compared to baseline, participants would have an increase in CK levels in the blood during post-exercise time points. It was also hypothesized that the EXP group would have a greater change in peak plasma CK level when compared to the CON group.

Descriptive Statistics

A total of 12 subjects (six male, six female) participated in the study and were randomly assigned to either the CON or EXP group. Table 4.1 provides descriptive statistics for the twelve individuals and the data for the CON and EXP groups.

Table 4.1. Descriptive statistics of age, height, weight and body composition (N=12)

Variable	Overall Mean (SD)	Control Group Mean (SD)	Experimental Group Mean (SD)
Age (yrs)	22.4 (1.8)	22.4 (2.1)	22.5 (1.9)
Height (cm)	172.2 (8.1)	171.2 (10.4)	172.5 (8.1)
Weight (kg)	71.1 (12.7)	69.9 (28.9)	72.4 (12.4)
Body Fat (%)	24.4 (10.2)	24.2 (11.2)	23.0 (9.5)

Yrs = years, cm = centimeters, kg = kilograms

Quantitative Results

Creatine Kinase Levels Prior to and in Response to Training Session 1

In the CON group, the individual responses for CK were variable, ranging from 27.0 to 161.5 U/L at baseline. The average CK for the CON at baseline was 64.2 U/L (± 50.4). The range and average for the EXP group were numerically lower. The average was 48.7 U/L (± 20.5) with individual levels between 26.4 and 71.2 U/L at baseline.

Overall, the peak response for both groups was found 24 hrs after the first training session. The peak ranged from 33.6 to 236.0 U/L in the CON group and 31.6 to 180.6 U/L in the EXP group. The peak response in the CON and EXP groups averaged 141.3 U/L (± 83.3) and 98.4 U/L (± 64.1), respectively. Figure 4.1a provides a visual representation of the CK response at the four test points for each individual subject in relation to the first training session for the CON group while 4.1b provides the same visual representation of the CK response at the four test points for the EXP group. The scaling has been chosen to simplify the comparison of CK values at the first and second training sessions (second training session to be discussed below).

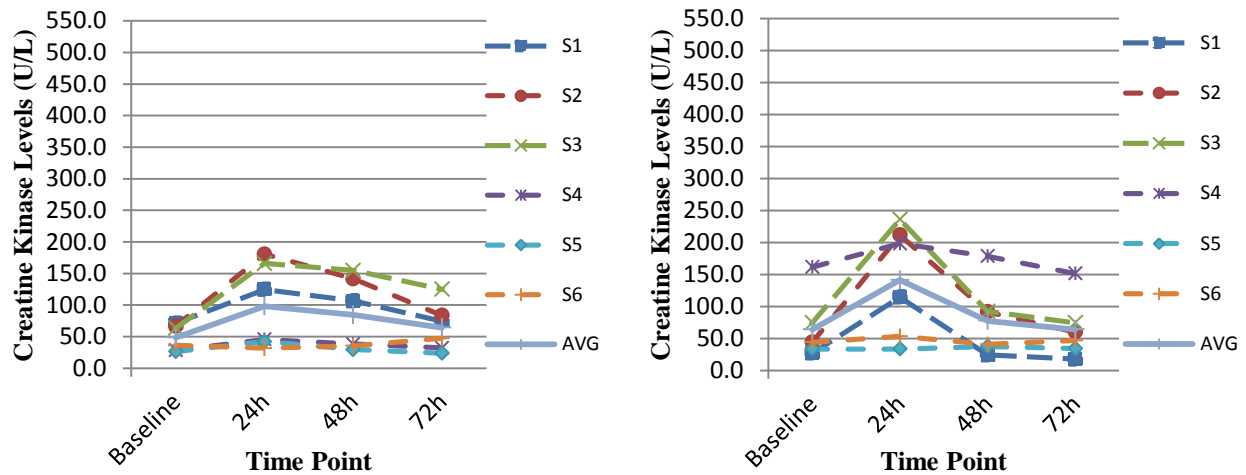


Figure 4.1a and 4.1b: The CK response measured in U/L at baseline (just before exercise) and 24, 48 and 72 hours after the first training session in the control (a, left side) and experimental groups (b, right side).

Testing the Pre- and Post-Exercise Creatine Kinase Levels at Training Session 1

To test the first hypothesis, that participants in both groups would experience an increase of CK found in the blood following exercise when compared to pre-exercise levels, separate paired samples *t*-Tests were conducted for the CON and EXP groups. Table 4.2 provides the statistical results from both *t*-Tests. The results indicate that peak post-exercise blood CK levels in both groups were significantly different compared with baseline levels. The null hypothesis was rejected in the CON [$t(5) = -2.595, p = .049$] and EXP [$t(5) = -2.786, p = .039$] groups. In the CON group, CK levels increased by an average of 121.2% from baseline, while in the EXP group they increased by an average of 107.6%.

Table 4.2. Group Means and the Paired Samples t-Tests Results for CK levels (U/L) at Baseline and Peak for Training Session #1 (N=12)

	Mean CK Pre-Exercise (U/L) (SD)	Mean CK Post-Exercise (U/L) (SD)	Absolute Difference (U/L)Mean	<i>t</i> -ratio	df	P Value
<i>Control</i> (<i>n</i> =6)	64.2 (50.4)	141.9 (83.3)	77.8	-2.595	5	0.049*
<i>Experimental</i> (<i>n</i> =6)	48.7 (20.5)	101.1 (64.1)	52.4	-2.786	5	0.039*

*= significant at $p < .05$; U/L= microliters

Creatine Kinase Levels Prior to and in Response to Training Session 2

In the CON group, the individual responses for CK ranged from 33.0 to 122.0 U/L at baseline. Overall, the average CK for the CON at baseline was 56.9 U/L (± 33.7), down from the 64.2 U/L found at Training Session #1. The average for the EXP group at baseline was 65.8 U/L (± 42.2) with individuals' levels between 21.8 and 123.3 U/L.

Overall, the peak response for both groups was found 24 hours after the second training session. The peak ranged from 31.6 to 207.0 U/L in the CON group and 31.6 to 497.0 U/L in the EXP group. The peak response after the second training session in the CON and EXP groups were 73.3 U/L (± 62.4) and 197.3 U/L (± 184.6), respectively. Figure 4.2a provides a visual representation of the CK response of each individual subject at the four test points in relation to the second training session for the CON group while 4.2b provides the same visual representation of the CK response at the four test points for the EXP group.

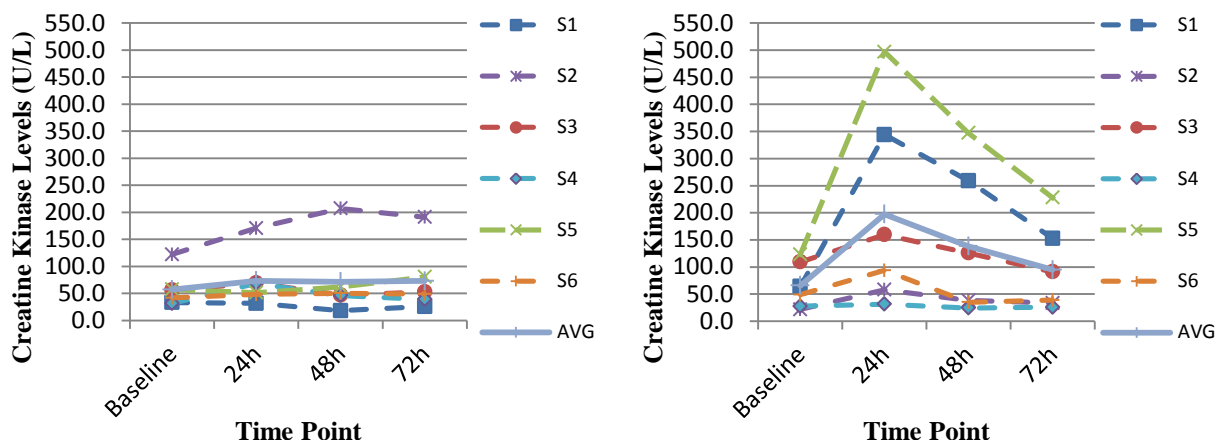


Figure 4.2a and 4.2b: The CK response measured in U/L at baseline (just before exercise) and 24, 48 and 72 hours after the first training session in the control (a, left side) and experimental groups (b, right side).

Testing for Difference in Creatine Kinase Response between Control and Experimental Groups

To test the second hypothesis, that participants in the EXP group would have a greater increase in CK accumulation following the second training session when compared to those in the CON group, an independent samples *t*-Test was utilized. Table 4.3 provides group outcomes and statistical results for analysis. Results indicate that there was not a significant difference in the changes in peak CK values between the CON and EXP groups. The null hypothesis was not rejected [$t(10) = -1.948, p = .189$].

Table 4.3. Group Outcomes and Statistical Results for the Independent Sample *t*-Test Comparing Pre- and Post-Exercise CK Change Scores between the CON and EXP Groups (N=12)

	Change Score (U/L) Training Session 1	Change Score (U/L) Training Session 2	Change Score (U/L) Mean	<i>t</i> -ratio	df	<i>p</i> value
<i>Control</i> (n=6)	77.8	27.6	-50.217	-1.948	10	0.189
<i>Experimental</i> (n=6)	52.4	131.5	79.083			

CHAPTER V

DISCUSSION

The first prominent finding of this research was that lower body resistance exercise (high bar back squat, leg press, hamstring curls, and calf raises) at a set volume of 80 repetitions (reps) at 65% of 1-RM caused an acute increase in plasma CK of +77.7 (121.2%) and + 52.4 U/L (107.6%) from baseline to post-exercise in the CON and EXP groups, respectively. The second prominent finding was that volume manipulation for the EXP group from 80 total reps at 65% of 1-RM to 120 total reps at 75% of 1-RM did not result in a significant difference in CK accumulation compared with the CON group. These primary findings offer support for the first but not the second hypothesis posed for this research. The first hypothesis stated that compared with baseline CK levels, the mean peak CK level post-exercise would be significantly higher in both groups. However, support for the second hypothesis, which stated that compared with the lower training volume group, the higher training volume group would have a greater change in CK from baseline to mean peak CK level post-exercise in Training Session #2 when compared with Training Session #1, was not evident. How these findings align with published literature and the implications of the results are discussed below.

Findings Relating to the First Hypothesis

The CK levels of the CON and EXP groups rose from 64.2 to 141.9 U/L and 48.7 to 101.1 U/L, respectively, after the first training session. This direction and magnitude of change is in agreement with published literature (Clarkson & Ebbeling, 1988; Magal et al., 2010; Baird et al., 2012).

In relation to the first training session, 10 of 12 individuals presented their peak CK level at the 24-hr post-exercise time point. This timing is consistent with research by McLellan et al.

(2010) in which CK was measured at eight different intervals following match play in elite rugby players. Results showed that peak CK levels occurred 24-hrs post-match. In addition, Russell et al. (2015) conducted a similar study with professional soccer players and found that CK accumulation peaked at 24-hrs post-match.

In terms of magnitude of increase, there was some variability among individuals at Training Session #1. From baseline to peak post-exercise CK, levels ranged from a negligible change of 4.0 U/L to 167.4 U/L. Again, the average for the CON was 141.3 U/L, which was numerically higher than the 98.4 U/L average found for the EXP group at Training Session #1. This finding may reflect differences in genetic predispositions as has been suggested by previous work (Newham et al., 1986; Magal et al., 2010; Machado et al., 2012). Since the 1990's, individuals have been classified as either HRCK or LRCK based on the accumulation of CK in the blood following exercise (Newham et al., 1986; Magal et al., 2010; Machado et al., 2012). It is plausible that some individuals in this study were LRCK to exercise and others were HRCK based on the variation of CK response.

Aside from individual differences, it is practical to propose that the CK response after the first training session was the result of multiple mechanisms. One primary mechanism involved was likely exercise induced muscle damage (EIMD). There was controlled movement through the concentric and eccentric phases of all four exercises, thus eccentric damage was expected. During exercise, especially eccentric exercise, muscle fibers are put under tremendous stress as they are stretched, sheared, and potentially destroyed during the stages of contraction. Armstrong et al. (1983) suggests that myofibril destruction may be a result of this shearing and contributes to the increased permeability of the muscle fiber, which would then allow for enzyme and ion efflux from the cell, into the blood.

A second mechanism could involve AMPK-mediated expulsion of CK from the cell. During exercise, maintenance of ATP is regulated by AMPK, which has the role of stimulating processes for ATP production as well as inhibiting other pathways that may consume ATP (Baird et al., 2012). As CK is a near-equilibrium enzyme, it poses a threat to ATP maintenance during periods of rapid energy demands. It has been conjectured by Baird and colleagues (2012) that AMPK would actively expel CK from the cell and into the blood. This would result in a direct increase of CK in the blood during and after exercise, regardless of the presence of muscle damage.

The final mechanism contributing to the acute accumulation of CK in the blood is linked with Ca^{2+} concentration. As ATP is depleted, Ca^{2+} leaks into the intracellular space and causes dysfunction to both the Na-K-ATPase and Ca^{2+} -ATPase pumps (Baird et al., 2012). Given that Ca^{2+} is a stimulant for skeletal muscle breakdown, its accumulation in the muscle fiber results in the leaking of CK and other muscle damage indices into the blood (Baird et al., 2012). Each of these mechanisms may have acted to cause the acute accumulation of CK in the blood that was observed at 24-hrs post-exercise.

It was beyond the scope of this research to include muscle biopsies or monitoring of calcium. Thus, there is no ability to make specific guesses or offer insight into which of these mechanisms was most responsible for the acute accumulation of CK in the blood in response to exercise.

In addition to mechanisms which result in acute increases in CK release into the bloodstream, the inflammatory response has been postulated to result in an increase in CK levels at time points of 72-hrs post-exercise or later (Evans & Cannon, 1991). If this was the case, there was a lack of evidence for its onset in the subjects within this research, as CK levels rose and

peaked at 24-hrs, but steadily decreased thereafter. If there was an inflammatory-mediated change, it had to have occurred at or after 72-hrs with little influence on the 72hr CK level measurements.

Findings Relating to the Second Hypothesis

Musculoskeletal trauma is a result of mechanical overload. Although this overload is a direct cause of muscle damage, it is also a pivotal stimulus for adaptations in the form of muscle hypertrophy and strength (Goldberg et al., 1975). If workloads, intensity, and volume were to remain the same over a training cycle, the body's musculature would adapt and as a result, damage, strength gains, and growth would be mitigated (Goldberg et al., 1975; Plisk & Stone, 2003).

In a study focused on downhill running, Schwane and colleagues (1983) observed a 351% increase in circulating CK following a bout of downhill running, yet no change was seen following a bout of level running. This suggests that eccentric muscle damage results in significantly higher CK release into the bloodstream. However, Byrnes et al. (1985) discovered that previous muscle damage, in the form of downhill running, protected against future EIMD and CK spikes. This protective effect in response to previous exercise is a key idea to consider and it may explain the results related to the second hypothesis.

The lack of change in volume and the protective effect of previous muscle damage likely explains the CK response seen in the control group after Session #2. While CK levels increased significantly from baseline to post-exercise in Training Session #1 (64.2 U/L vs 141.9 U/L), this was not the case following the second training session (56.9 U/L vs 73.3 U/L). This conceivably indicates that a muscular adaptation had occurred and given that workload, intensity, and volume remained the same, muscle damage was mitigated, and the CK response followed accordingly.

Those in the EXP group underwent an acute manipulation of both training volume and intensity and therefore they experienced greater stimulus for muscle damage and the other mechanisms which increase CK levels. In line with this statement, those in the EXP group presented with an increase in CK levels from baseline to post-exercise in relation to Training Session #2 (65.8 U/L vs 197.3 U/L).

Change scores (calculated as difference in CK change from baseline to peak level in Training Session #2 minus change from baseline to peak level in Training Session #1) were -50.2 in the CON and +79.1 in the EXP groups (see Table 4.3). Despite an increase of +79.1 U/L of CK in the EXP and a -50.217 U/L decrease in the CON group, the second hypothesis was not supported. While the change scores were distant from each other, standard deviations in the EXP (± 136.4) and CON (± 88.5) rendered this difference insignificant.

As mentioned previously, a variety of mechanisms such as EIMD, AMPK activity, and Ca^{2+} concentration likely served as stimulus for CK release as an acute response to exercise. While several individuals presented large increases in post-exercise CK levels when compared to baseline, others did not.

Genetic predispositions are likely a significant contributor to these individual differences. The classification of individuals as HRCK or LRCK should not be overlooked as it could have significant implications on CK response to exercise. If an individual is classified as a HRCK, they will likely show large increase in CK from baseline to post-exercise, as is seen in several individuals (Newham et al., 1986; Magal et al., 2010). However, individuals classified as LRCK may not experience abrupt increases in CK despite changes in volume and intensity as was done in this study. This predisposition alone could impact the overall results of the study. While there has not been much research conducted on the matter, Magal et al. (2010) also included that it

could be reasonably assumed that muscle fiber type could impact CK response. If individuals were classified with primarily Type I muscle fibers, then exposing them to anaerobic resistance exercise could result in significantly higher CK levels post-exercise than if aerobic exercise was administered. The same can be said for individuals with primarily Type IIx and their response to anaerobic resistance exercise, which may be mitigated due to the capacity of the muscle to withstand this type of stress as opposed to aerobic exercise.

Beyond genetic predispositions, individual habits may have also impacted CK response following the second training session. Supplementation in conjunction with the training sessions or between training sessions could have blunted CK response. There was verbal confirmation of compliance, but there is certainly the possibility that noncompliance occurred. Research has shown that beverages containing whey protein, amino acids, and carbohydrate have a significant impact on CK levels and may mitigate muscle damage following exercise (Skillen et al., 2008; Hansen et al., 2015). If individuals supplemented with these beverages or other means prior to or after the training session, it is likely CK response would have been blunted. Another potential factor would be outside exercise. Any sort of outside exercise following the training session could have served as “active rest” and aided in the recovery of the muscle and clearing of CK from the blood. Lastly, the training base and experience of each individual cannot be ignored. While each participant met the minimum requirements and activity for the study, training style and base differed between subjects. Regularly taking part in periodized training programs as opposed to general resistance exercise has been shown to result in substantial muscular adaptations which would allow the individual to tolerate higher anaerobic stresses (Herodek et al., 2012). This finding indicates that training style may have protected subjects in the study from muscle damage or left them vulnerable to incur more muscle damage.

Limitations

While critical variables in this research were controlled via inclusion criteria, exclusion criteria, and monitoring of study training by trained staff, there are several limitations that must be acknowledged: small sample size, heterogeneous training background, outside participation in exercise, and potential supplementation around scheduled training sessions.

Despite having statistical power for detecting a difference, the small sample size opened the study for influence by individual variations in CK response. These outcomes impacted the statistical testing. While the differences between groups appeared large, large variation in response (reflected as a large standard deviation) rendered the results insignificant. This realization connects with the second major limitation of the research—that training style and program were not homogenous. Each individual met the minimum requirements in regards to training activity, but varying backgrounds in training methodology, such as periodized hypertrophy training vs strength training, may have influenced individual variation more than was originally anticipated. In retrospect, a more intentional and selective inclusion criteria focused on training style could have been utilized to protect against the confounding influence of CK response by training style. Finally, individual habits outside of the scheduled training sessions may have skewed results. While exclusion standards regarding outside exercise or supplementation were in place, compliance cannot be feasibly monitored. Compliance was based solely on self-report. If noncompliance did occur, CK response would have been altered. This could have resulted in exaggerated CK responses or more rapid recovery.

Recommendations for Future Research

Based on the results of the study, the following recommendations should be considered for future research regarding the relationship between training volume and CK response, if employing resistance training. A larger sample size is needed in order to protect against individual variation and outliers that may influence the mean and standard deviation. In addition, a more homogenous group should be utilized in terms of training status and practice. While training volume was manipulated, it is possible that a more distinct change in workload was required. For instance, perhaps a greater change in intensity is required. It is also possible that the addition of one or two more exercises would have promoted a significant change in the CK levels between the groups when comparing the CON and EXP groups.

Conclusion

The findings of the present study reinforce the conclusion that EIMD increases CK accumulation in the blood in an acute timeframe. However, there was no significant difference in CK response among individuals who trained at a slightly higher volume (additional 40 reps at 10% greater 1-RM intensity for 4 exercises) when compared to those training at a lower volume to which they had been accustomed (80 reps at 65% of 1-RM). The individual differences in CK response vary so widely that other factors known to impact CK response—training volume, intensity, mode, duration, etc.—the magnitude of their relationship with CK response is not easily identified. The need for strongly controlled research to determine whether or not any of these factors impact CK response more than others is needed.

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APPENDIX A

Physical Activity Readiness
Questionnaire-PAR-Q
(revised 2003)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want—as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those that are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

If you answer "Yes" to any of the above questions, the Health & Fitness Lab staff requires that you provide a written physician's consent to participate in the service prior to scheduling an appointment.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active—begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal—this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of temporary illness such as a cold or a fever—wait until you feel better; or
- If you are or may be pregnant—talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME _____

SIGNATURE _____

SIGNATURE OF PARENT _____

Or GUARDIAN (for participants under the age of majority)

DATE _____

WITNESS _____

APPENDIX B

Participant # _____

Medical History Questionnaire

	YES	NO
1. History of heart problems, chest pain, or stroke?	_____	_____
2. Increased blood pressure?	_____	_____
3. Any chronic illness or condition?	_____	_____
4. Difficulty with physical exercise?	_____	_____
5. Advice from a physician not to exercise?	_____	_____
6. Recent surgery? (Last 12 months)	_____	_____
7. Pregnancy? (Now or within the last 3 months)	_____	_____
8. History of breathing or lung problems?	_____	_____
9. Muscle, joint, back disorder, or any previous injury still affecting you?	_____	_____
10. Diabetes or thyroid conditions?	_____	_____
11. Cigarette smoking habit?	_____	_____
12. Increased blood cholesterol?	_____	_____
13. History of heart problems in your immediate family?	_____	_____
14. Hernia or any condition that may be aggravated by lifting weights?	_____	_____
15. Do you have any condition limiting your movement?	_____	_____
16. Are you aware of being allergic to any drugs or insect bites?	_____	_____
17. Do you have asthma?	_____	_____
18. Do you have epilepsy, convulsions, or seizures of any kind?	_____	_____
19. Do you follow any specific diet?	_____	_____

Please explain in detail any "YES" answers:

Family History

Has any member of you family had any of those listed above?

Medication	YES	NO
Are you currently taking:	_____	_____
1. Cholesterol-lowering medications?	_____	_____
2. Psychiatric medication that may alter weight?	_____	_____
3. Appetite suppressants?	_____	_____
4. Contraceptive or hormone replacement medications?	_____	_____
5. Birth control?	_____	_____
6. Blood thinning medication (NSAIDs)?	_____	_____

Please list any medications you are currently taking: