ABSTRACT

Determining Related Work-to-Rest Ratios Following a Maximal Effort Isokinetic Leg Extension Bout in Trained and Untrained Males: A Double-Blind Creatine/Placebo Controlled Study

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Purpose: A single 30 second bout of high intensity isokinetic exercise is capable of depleting available skeletal muscle Phosphocreatine (PCr) and adenosine triphosphate (ATP). Within several minutes the body is able to replenish those stores to facilitate further muscular activity. There have been suggested work-to-rest ratios to allow for the optimal recovery period to achieve repletion of the intramuscular PCr and ATP. This study attempted to determine how training status and supplementation with creatine monohydrate (CM) may affect this process. Methods: Subjects in the CM group consumed 20 g of CM (four 5 g doses/day), while subjects in the placebo group consumed 20 g of dextrose (four 5 g doses/day). Five (pre-supplementation, pre-exercise, immediate post exercise, 3 min. post exercise, and 5 min. post exercise) muscle samples were taken from the vastus lateralis of the dominant leg of 32 males [16 resistance trained (8 each receiving CM or placebo), 16 untrained (8 each receiving CM or placebo)]. Samples were measured for Cr, PCr, ATP, glycogen, and lactate, and TCr was calculated by adding Cr and PCr at all time points. Blood lactate was measured via

finger prick prior to and 5 minutes after the exercise bout. Results: Subjects consuming CM increased muscle total creatine content by 10.2%. Neither CM supplementation nor resistance training status significantly affected PCr or ATP repletion rates. Trained subjects had significantly ($p > .05$) higher muscle glycogen content. Trained individuals maintained non-significantly higher muscle ATP and PCr after and at all time points during recovery from the exercise bout compared to untrained individuals. At both 3 and 5 minutes after exercise, none of the groups were able to fully restore resting levels of muscle ATP or PCr. Conclusions: The results indicate that CM and training status did not significantly enhance repletion rates of muscle ATP or PCr. Further, 5 minutes of rest was not long enough to fully recover muscular ATP and PCr content.

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by

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A Dissertation

Approved by the Department of Health, Human Performance, and Recreation

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CHAPTER ONE

Introduction

Strength and conditioning professionals have utilized many techniques in their pursuit of improving their athletes' physical abilities and performance outcomes. One critical aspect of any strength and conditioning program that is transferable to training and competitive environments is the amount of time required during rest periods, both between sets and between workouts. The primary goal of this research protocol is to elucidate the time required between exercise sets to allow for optimal recovery of both phosphocreatine (PCr) stores and adenosine triphosphate (ATP). Furthermore, we are interested in determining how resistance training status and creatine monohydrate (CM) supplementation may affect recovery time. The complete recovery of PCr between exercise sets is of great importance in regards to achieving the maximum amount of benefit in the form of strength gains from any resistance training program.

Human skeletal muscle contraction is dependent upon a sufficient amount of ATP to supply the chemical energy required for functioning of the actin and myosin cross-bridge attachments. Attachment of ATP to the cross bridge of the myosin protein leads to a conformational change that causes a weak binding state which allows the cross bridge to temporarily detach from the actin filament allowing the myosin and actin filaments to slide past one another. Upon release of inorganic phosphate (Pi) from ATP, the cross bridge goes through the power stroke and is then returned to a strong binding state which results in cross bridge cycling and muscular contraction, and the subsequent production of the by-product, adenosine diphosphate (ADP) (Gordon, Homsher, & Regnier, 2000;

Huxley, 1975). One method by which the body supplies the requisite ATP to continue further muscular contraction is the phosphagen energy system. During muscular activity, a reaction catalyzed by the enzyme creatine kinase will allow the ADP by-product to accept Pi from a PCr molecule, if present, to produce a new ATP molecule to allow further muscular activity (Yao, Shen, Terada, Nagamune, & Suzuki, 1999). Following termination of activity, this reaction favors regeneration of PCr from ATP and free creatine (FCr). This freely reversible reaction can be written as:

$$ADP + PCr + H^{+} \xleftrightarrow{\text{creatine kinase}} ATP + FCr$$

In addition to utilizing the phosphate group on the PCr molecule, the phosphagen energy system can produce ATP from two ADP molecules resulting in the production of one ATP and one AMP molecule (Houston, 2006). This reaction is catalyzed by the enzyme AMP kinase and can be written as:

$$ADP + ADP \xrightarrow{\text{AMP\_kinase}} ATP + AMP$$

The phosphagen energy system is primarily utilized in sports activities lasting from 0-15 seconds (sprinting, power-lifting, or a single play in football), while activities that last longer will begin to use glycolysis and aerobic metabolism to provide energy in the form of ATP (Baechle, Earle, & National Strength & Conditioning Association, 2008; Glaister, 2005).

The TAN pool is comprised of intramuscular ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP). These compounds work in concert with select energy production systems to continually supply adequate amounts of ATP to fuel muscular contraction. The TAN pool, and therefore available ATP, has been shown to decrease in response to high intensity exercise with a portion of the intramuscular AMP

being lost in the form of inosine monophosphate (IMP) from the deamination of AMP to IMP via the AMP deaminase reaction (Korzeniewski, 2006). This reaction can be written as:

$$AMP + H_2O \xrightarrow{\text{AMP Deaminase}} IMP + NH_3$$

IMP can further be degraded into inosine, which in turn can be broken down into hypoxanthine (Hasko, Sitkovsky, & Szabo, 2004). Both inosine and hypoxanthine are considered markers of adenine nucleotide metabolism. Following a brief recovery period (10-20 minutes) TAN can be regenerated from IMP with the use of the purine nucleotide cycle, which results in the reamination of IMP to AMP. The decrease in TAN has been associated with fatigue inducing exercise bouts, with trained individuals displaying less of a decrease in the TAN pool (Febbraio & Dancey, 1999b; Green, Jones, Ball-Burnett, Farrance, & Ranney, 1995; Hargreaves et al., 1998). Trained individuals may demonstrate an increased ability to maintain intramuscular ATP compared to untrained individuals who display an increased reliance upon the phosphagen energy system, and therefore degradation of AMP as described above, to provide ATP in the untrained population (Baldwin et al., 2003; Febbraio & Dancey, 1999a).

CM is generally considered a safe and well established method of improving the strength and hypertrophic benefits of resistance training (Greenwood et al., 2003; Kreider, 2003). The primary mechanism by which CM is believed to work is by increasing intramuscular total creatine (FCr + PCr) by approximately 20%, depending on co-ingestion of macronutrients and individual CM response, thus allowing for a higher total volume performed during a resistance training session by enhancing PCr repletion and availability of muscle ATP and PCr stores (Hultman, Soderlund, Timmons,

Cederblad, & Greenhaff, 1996; Kilduff et al., 2002; Kreider et al., 1998; Yquel, Arsac, Thiaudiere, Canioni, & Manier, 2002). The increase in intramuscular creatine content has been linked with an increased total work output during sprint cycling and lifting volume during resistance training, therefore providing the means to achieve improved muscular strength and hypertrophy (Kreider et al., 1998; Preen et al., 2001). CM has also been shown to improve repeated sprint cycling performance compared to placebo when the rest interval between exercise bouts was limited to either 1 or 3 minutes, suggesting a decreased need for a prolonged work-to-rest ratio (Cottrell, Coast, & Herb, 2002).

Another factor that is important to consider when discussing recovery of ATP and PCr from intense exercise is training status. The amount of prior exercise training can affect the recovery time necessary to fully replete PCr and ATP stores. It has been shown that endurance training is capable of improving recovery following aerobic activity through enhanced lactate clearance and faster PCr repletion (Tomlin & Wenger, 2001). Additionally, resistance training can affect PCr repletion through increases in intramuscular enzyme storage and muscle fiber type transformations (Abernethy, Jurimae, Logan, Taylor, & Thayer, 1994). For this reason, we will examine how total body resistance training affects PCr and ATP repletion following high intensity isokinetic exercise.

With these facts being taken into consideration, when training an athlete, a proper rest interval between exercise bouts is essential to allow for optimal recovery of the PCr and ATP within the muscle cells. The ratio between time of activity and length of rest is termed the work to rest ratio (work-to-rest) (Baechle et al., 2008). Previous work investigating the optimum work-to-rest ratios for aerobic activity has been performed;

however, research detailing the most effective work-to-rest ratios for resistance training is limited (Glaister, Stone, Stewart, Hughes, & Moir, 2005; Haseler, Hogan, & Richardson, 1999; McMahon & Jenkins, 2002; Tomlin & Wenger, 2001). Several work-to-rest ratios have been suggested to be effective for adequate recovery during heavy resistance training, including a 2-5 minute rest period between exercise sets suggested by Bompa and Kraemer, and the National Strength and Conditioning Association's (NSCA) recommendation of a 1:12-20 work-to-rest ratio for activity lasting 5-10 seconds, a 1:3-5 work-to-rest ratio for activity lasting 15-30 seconds, and a rest period of 2-5 minutes for resistance training in which the goal is increasing muscular strength or power (Baechle et al., 2008; Bompa & Carrera, 2005; Kraemer et al., 2002). For the purposes of this study, we will investigate the suggested 3-5 minute rest period to determine the appropriateness of this suggested recovery period, and its relationship to training status and creatine supplementation. Research suggests that longer rest intervals have been shown to improve strength gains, primarily by allowing for a higher volume of total work to be performed (Willardson, 2006). One particular study by Robinson et al. concluded that a 3 minute rest period following 5 sets of 10 repetitions of squatting performed twice weekly over the course of five weeks resulted in greater strength gains compared to rest periods of 30 seconds or 90 seconds (Robinson et al., 1995). One goal of this study is to determine whether or not the recommended rest intervals can be further categorized into specific work-to-rest ratios for the purpose of prescribing ideal ratios based on the amount of time spent performing muscular work. This would allow for a relative rest interval to be applied toward exercise protocols that utilize differing amounts of repetitions, and therefore time, during the performance of muscular work. Furthermore,

we are interested in determining what role total body resistance training status may play in this phenomenon, and if supplementation with creatine monohydrate can attenuate the degradation of PCr and ATP stores, or accelerate the time to repletion of PCr and ATP stores.

Purposes of the Study

1.) To investigate the phosphocreatine repletion kinetics following a 30 second bout of high intensity isokinetic exercise in trained and untrained males consuming a placebo or creatine supplementation.

2.) To investigate the adenosine triphosphate repletion kinetics following a 30 second bout of high intensity isokinetic exercise in trained and untrained males consuming a placebo or creatine supplementation.

3.) To determine an appropriate work to rest ratio to allow for repletion of both phosphocreatine and adenosine triphosphate following a 30 second bout of high intensity isokinetic exercise in trained and untrained males consuming a placebo or creatine supplementation.

Hypotheses

H_o: Trained subjects will have a significantly faster phosphocreatine repletion rate compared to subjects who are untrained.

H_1: Subjects consuming supplemental creatine monohydrate will have a significantly faster phosphocreatine repletion rate than those who do not consume supplemental creatine monohydrate.

H_2: Trained subjects will have a significantly faster adenosine triphosphate repletion rate compared to subjects who are untrained.

H_3: Subjects consuming supplemental creatine monohydrate will have a significantly faster adenosine triphosphate repletion rate than those who do not consume supplemental creatine monohydrate.

H_4: Groups receiving creatine supplementation will have significantly faster phosphocreatine and adenosine triphosphate repletion rates than groups receiving placebo only.

H_5: Trained males will have significantly faster phosphocreatine and adenosine triphosphate repletion rates than untrained males.

H_6: The suggested recovery time of 3-5 minutes will be sufficient to allow for total phosphocreatine repletion and total adenosine triphosphate recovery in all groups.

Delimitations

1.) 32 males aged 18-30 [16 resistance trained (8 each receiving creatine supplement or placebo), 16 untrained (8 each receiving creatine supplement or placebo)] were recruited from the Baylor University campus and the surrounding Waco area.

2.) Trained subjects had at least 6 months of total body resistance training prior to participating in this study.

3.) Untrained subjects did not participate in total body resistance training for at least 6 months prior to participating in this study.

4.) Creatine supplementation or placebo was provided to all subjects by the principle investigator in a randomized, double-blind manner.

5.) Pilot work was performed on research procedures to ensure validity and reliability of the research techniques used in this study prior to any data collection for the actual study.

6.) Supplement packets were collected following ingestion in addition to supplementation records to ensure all subjects followed the supplementation protocol correctly.

7.) Food logs were collected prior to all testing sessions to ensure homogenous caloric intake between groups.

8.) Muscle biopsies were obtained from the vastus lateralis muscle of the dominant leg of each subject prior to supplementation, and immediately prior to, immediately following, 3 minutes after, and 5 minutes after the exercise testing session.

9.) Blood lactate levels were analyzed using the finger prick method prior to and 5 minutes after exercise.

10.) All subjects were familiarized to all testing and exercise procedures prior to the exercise testing bout.

11.) All subjects were requested to refrain from participation in any other forms of vigorous physical activity or modification of their diet throughout the duration of the research protocol.

12.) All testing was performed within the Exercise and Sport Nutrition Laboratory, the Exercise and Biochemical Nutrition Laboratory on the Baylor University campus, and Exercise Metabolism Unit, Centre for Ageing, Rehabilitation and Exercise and Sport, at Victoria University following the policies and procedures of each respective laboratory.

Limitations

1.) The subjects were drawn from a convenience sample of the Baylor University campus and surrounding communities, thus limiting the ability to infer results to a larger population.

2.) The study compared the effects of training status and creatine supplementation on phosphocreatine, free creatine, and the adenosine triphosphate repletion kinetics on males aged 18-30.

3.) The motivation of each participant to be present and put forth an appropriate effort for all testing sessions.

4.) The accuracy and reliability of the technologies and protocols utilized to measure the dependent variables.

Assumptions

1.) All participants followed all guidelines set forth for them at the beginning and throughout the entire study.

2.) Participants fasted for 8 hours prior to each testing session.

3.) Participants were apparently healthy with no contraindications for the activities involved.

4.) The training status of each participant was accurately reported.

5.) All participants appropriately exerted themselves during all testing sessions.

6.) All participants accurately reported supplement and food intake on all supplementation and food records.

7.) All assay equipment used in the analysis of blood and muscle samples was accurate and reliable in quantification of dependent variables.

8.) The exercise testing protocols were reliable methods of depleting the phosphocreatine, total creatine, and total adenine nucleotide pool.

Definition of Terms

Actin – One of two primary proteins involved in muscular contraction.

Active recovery – Recovery between exercise bouts that involves some physical activity (walking, cycling, stretching).

Adenosine diphosphate (ADP) – An adenine nucleotide that is produced from the hydrolysis of ATP and is used in reactions to reproduce ATP.

Adenosine monophosphate (AMP) – An adenine nucleotide that is produced from the AMP kinase reaction which caused two ADP molecules to form one ATP and one AMP.

AMP deaminase – An enzyme that catalyzes the deamination of AMP into IMP and NH_3.

AMP kinase – An enzyme that catalyzes the reaction that forms one AMP and one ATP molecule from two ADP molecules.

Adenosine triphosphate (ATP) – An adenine nucleotide that occurs in muscle tissue and is the major source of energy for cellular reactions, including muscular contraction.

Aerobic activity – Physical activity that requires oxygen consumption in order to carry out substrate metabolism.

Anaerobic activity – Physical activity that can be carried out with the absence of oxygen consumption.

Creatine – An amino acid compound that is present within the muscle tissue of vertebrates that can be phosphorylated in order to provide energy for muscular contraction.

Ergogenic aid – Any technique (including dietary habits, nutritional supplementation, use of legal or illegal drugs, or training method) that is employed in order to improve physical or mental function.

Exercise recovery - The return of the muscle to its pre-exercise state following exercise. This means pH levels must return to normal, as well as energy system metabolites (PCr, Cr, ATP) being fully replenished to pre-exercise levels.

Fatigue – The progressive decline in power output, which is largely determined by the intervening rest periods, caused by the depletion of PCr.

Hypertrophy – Growth in muscle tissue size that is not due to increase in cell number.

Hypoxanthine - a purine base formed as an intermediate in the degradation of purines and purine nucleosides.

Inosine - a purine nucleoside containing the base hypoxanthine and the sugar ribose.

Inosine monophosphate (IMP) – A nucleotide produced from the deamination of AMP and used as the precursor for AMP in purine biosynthesis.

Isokinetic – A muscular contraction in which the muscle exerts a variable force throughout a range of motion in which the angular joint speed remains constant.

Isotonic – A muscular contraction in which the muscle exerts a constant force against constant load throughout the entire range of motion of the exercise.

Lactate – a metabolite of glucose metabolism.

Metabolism – A chemical process that occurs within living organisms in which substances are broken down to yield energy.

Metabolite – A product of or required for metabolism.

Myosin – One of two primary proteins involved in muscular contraction.

Passive recovery – Recovery between exercise bouts in which no physical activity is performed.

Percutaneous – Passing through the skin. The muscle biopsy procedure is percutaneous in nature.

Phosphocreatine (PCr) – The phosphorylated form of creatine.

Protein – A polymer of amino acids.

Resistance Trained – For the purpose of this study, a person who has been participating in lower body resistance training at least once per week for at least 6 months prior to participation in this study will be considered trained.

Resistance Untrained – For the purpose of this study, a person who has not been participating in lower body resistance training at least once per week for at least 6 months prior to participation in this study will be considered untrained.

Substrate – A chemical that is acted upon by an enzyme as a function of metabolism.

Work-to-rest ratio – the ratio of time spent performing muscular activity and time spent resting between bouts of muscular activity.

CHAPTER TWO

Review of Literature

Muscle Bioenergetics

The human body has several biochemical pathways available to produce ATP, the high energy phosphate molecule required for muscular contraction. They include the phosphagen system, which is comprised of intramuscular stores of ATP and PCr, which provide for the immediate energy demand during brief (0-10 seconds), high intensity muscular contractions (Glaister, 2005). A second pathway in which ATP is produced is via anaerobic glycolysis, which is the breakdown of glucose into two ATP molecules in the absence of oxygen. This system is capable of maintaining energy production for several minutes at high intensity. A third energy system is known as the aerobic energy system and includes the breakdown of glucose and fats in the presence of oxygen. This energy system is capable of maintaining muscular contraction for very long periods of activity of light to moderate intensity (Baechle et al., 2008; Febbraio & Dancey, 1999a; Hargreaves et al., 1998; Houston, 2006).

During high intensity, brief muscular contraction several events can lead to a decrease or cessation in muscular contractile activity and force production, thus limiting the ability to continue movement. The phosphagen energy system cannot maintain ATP production for more than approximately 10 seconds, which is the first factor leading to fatigue (Glaister, 2005; Hargreaves et al., 1998). Secondly, as anaerobic glycolysis begins breaking down glucose, lactic acid and hydrogen ions, by-products of the biochemical catalysis of glucose, begin to accumulate within the muscle. With the

production of hydrogen ions and thus a decrease in pH, there is a subsequent decrease in the enzyme activity within the muscle that perpetuates muscular contractile activity (Houston, 2006). The decreased ATP production, via the depleted phosphagen system and decreased enzyme activity of anaerobic glycolysis, can eventually lead to failure of the muscle to maintain very high intensity activity. In addition to decreases in PCr, there has been shown to be a decrease in TAN, and specifically ATP, with high intensity exercise, which has been associated with the onset of fatigue (Stathis, Febbraio, Carey, & Snow, 1994; Tullson, Bangsbo, Hellsten, & Richter, 1995; Zhao, Snow, Stathis, Febbraio, & Carey, 2000). Additionally, IMP the degradation product of AMP, has been noted to accumulate during fatigue as a result of an imbalance between ATP production and depletion (Baldwin, Snow, Carey, & Febbraio, 1999; Febbraio & Dancey, 1999a). It is important to note that the elevation in IMP levels with fatiguing exercise has only been shown in untrained individuals and that with training, IMP levels will not become elevated (Baldwin et al., 1999).

When designing a resistance training protocol, the energy sources mentioned above and causes of fatigue must be well understood. This study attempts to further describe what has been discovered up until now while determining whether or not the various suggested work-to-rest ratios are appropriate (Baechle et al., 2008).

Phosphocreatine Depletion and Resynthesis

PCr is the body's most immediate reserve of high energy phosphate to fuel muscular contraction (Hargreaves et al., 1998). Following repeated bouts of high intensity exercise, PCr plays less of a role in energy production as the body relies more upon aerobic pathways to produce ATP (Bogdanis, Nevill, Boobis, Lakomy, & Nevill,

1995; Bogdanis, Nevill, Boobis, & Lakomy, 1996). Concurrent with higher aerobic energy production, depletion of PCr leads to decreased force production in a linear fashion (Bogdanis et al., 1995; Bogdanis et al., 1996; Nevill, Jones, McIntyre, Bogdanis, & Nevill, 1997). This decrease in PCr has been suggested to follow an exponential pattern of decay, with stores being depleted within 10 seconds (Glaister, 2005). Coinciding with the decrease in PCr is the increase in anaerobic glycolytic energy production pathways, the primary by-product of which is lactic acid. As intramuscular lactic acid increases along with a subsequent drop in pH, there is an associated decrease in performance and onset of fatigue (Metzger & Moss, 1987; Spriet, Lindinger, McKelvie, Heigenhauser, & Jones, 1989). Rest periods following high intensity exercise allow the body to return to aerobic energy production resulting in a return of pH to resting levels and the intramuscular stores of ATP/PCr to be replenished. If the rest period following activity is not sufficient, ATP and PCr will not fully recover. As a result, there will be a greater reliance upon anaerobic energy production with subsequent high intensity exercise bouts (Tomlin & Wenger, 2001).

During recovery from exercise, the body will increase oxygen consumption beyond what is necessary. This is termed excess post-exercise oxygen consumption (EPOC). EPOC is induced to allow for regeneration of ATP and PCr stores and has a fast and slow component. The fast component lasts several minutes while the slow component can last as long as several hours (Borsheim & Bahr, 2003). Additionally, it has been shown that trained individuals have a shorter duration of EPOC which suggests an increased ability to regenerate ATP and PCr following exercise (Roussel, Bendahan, Mattei, Le Fur, & Cozzone, 2000; Tomlin & Wenger, 2001). Furthermore, in the absence

of oxygen, PCr regeneration cannot take place. A recent study showed that in hypoxic conditions, the ability of the muscles to regenerate PCr was significantly reduced (Haseler et al., 1999). Also, exercise intensity can have an impact on EPOC and PCr regeneration. As exercise intensity increases, the length of time for PCr recovery and EPOC will increase in a curvilinear fashion (Borsheim & Bahr, 2003). As mentioned previously, if an adequate rest period is not allowed, recovery of PCr will not be complete and performance will be impaired.

The goal of this and other research in this area is to determine an adequate rest period to allow for adequate recovery of PCr and ATP. Several previous studies along these lines have been conducted (Greenhaff, Bodin, Soderlund, & Hultman, 1994; Preen et al., 2001; Snow et al., 1998; Yquel et al., 2002). We could not find a specific reference to any studies involving PCr or ATP repletion having been conducted during resistance training activity. Therefore, we felt it particularly important to examine the effects of training and creatine supplementation on ATP and PCr repletion during high intensity resistance training. This information is essential due to the fact that many athletes utilize resistance training activity to improve sports performance, yet there hasn't been, to our knowledge, any information about specific work to rest ratios required for adequate recovery of PCr and ATP following resistance training activity.

Total Adenine Nucleotide Pool Depletion and Resynthesis

In addition to changes in intramuscular PCr, as activity increases, there is a change in the concentration of adenine nucleotides, including ATP, ADP, and AMP, and increases in inosine monophosphate (IMP), a metabolite of AMP deamination (Spencer, Yan, & Katz, 1991; Zhao et al., 2000). As exercise continues there is a slight decrease in

intramuscular ATP as it is hydrolyzed into ADP. In order to generate the necessary ATP for muscular contraction, PCr initially provides the needed high energy phosphate. Upon PCr depletion to around 40 mmol/kg dry weight, a reaction catalyzed by adenylate kinase (AK) causes two ADP molecules to form one ATP and one AMP molecule (Sahlin, Gorski, & Edstrom, 1990; Spencer et al., 1991; Zhao et al., 2000). As AMP levels begin to increase, AMP deaminase causes the formation of IMP and NH_3, in an attempt to maintain the ATP/ADP ratio in order to prevent product inhibition of ATP hydrolysis (Houston, 2006). The resultant formation of IMP has been associated with fatigue following prolonged exercise and glycogen depletion (Norman, Sollevi, Kaijser, & Jansson, 1987; Spencer et al., 1991). Additionally, hypoxanthine and inosine, both of which are metabolites of IMP degradation have been shown to increase markedly following intense exercise (Hargreaves et al., 1998). During recovery from exercise, there is a reamination of IMP into AMP with reactions of the purine nucleotide cycle (Meyer & Terjung, 1979; Meyer & Terjung, 1980). The rate of TAN restoration and IMP removal in recovering human skeletal muscle has been shown to occur from a 1:1 to a 2:1 ratio (Meyer & Terjung, 1979; Sahlin, Palmskog, & Hultman, 1978; Sahlin & Ren, 1989). The differences in the rate of TAN restoration have not been entirely described, but likely occur from a secondary, unknown source of ATP formation (Zhao et al., 2000).

ATP is of primary concern to this line of research, particularly because previous research has shown that activity largely only affects ATP concentrations, whereas ADP and AMP levels, for the most part, remain unchanged during short term, high intensity and long term activity (Hargreaves et al., 1998; Zhao et al., 2000). Furthermore, ATP is directly responsible for providing energy for muscular contraction, whereas ADP and

AMP are only indirectly involved in energy production as substrate for further ATP synthesis via pathways previously mentioned. For these reasons, we chose to specifically measure changes in ATP following our high intensity isokinetic exercise bout.

Muscle Metabolism During Exercise

Previous research in the area of muscle energy metabolism has been performed under several conditions. One such investigation looked at skeletal muscle energy metabolism during prolonged, fatiguing exercise in untrained subjects. In this study, it was shown that fatigue was not caused by a depletion in muscle ATP or PCr, but likely due to limited glycogen availability (Febbraio & Dancey, 1999a). The idea of fatigue, and subsequent recovery, being caused by limited glycogen availability during prolonged, fatiguing exercise is supported by other research as well (Broberg & Sahlin, 1989; Sahlin, Katz, & Broberg, 1990). While interesting to know, the causes of fatigue during prolonged activity is not relevant to our study as we are more concerned with high intensity, resistance activity. It has been shown that during high intensity activity, primarily sprint cycling, ATP is depleted rapidly. One particular study showed that a single 30 bout of "all-out" sprint cycling resulted in a significant ($p < .05$) decline in ATP from 25.9 $\pm$ 1.0 to 16. $\pm$ 1.3 mmol/kg dry mass, with near return to pre-exercise levels within 10 minutes of recovery. In addition, there were no significant changes in muscle ADP or AMP, but significant increases in muscle IMP and ammonia following the 30 second cycling bout. Furthermore, muscle PCr declined significantly, with concomitant increases in FCr, from 84.3 $\pm$ 2.5 to 28.0 $\pm$ 1.2 mmol/kg dry mass following the 30 second bout of sprint cycling. Muscle PCr levels were restored to pre-exercise conditions within 5 minutes of passive recovery with a significant increase beyond resting levels following 10 minutes

of passive recovery (Zhao et al., 2000). Another similar study examined the effects of four repeated 30 second bouts of sprint cycling. The first three bouts of sprint cycling were separated by four minutes of passive recovery. Following the third bout, participants rested for four minutes, then performed a 30 minute recovery exercise bout at 30-35% of VO_{2peak}. Participants then rested for 60 minutes and finally performed the fourth 30 second bout of sprint cycling. Muscle biopsies were collected immediately prior to the first, third and fourth bout of sprint cycling. This study showed significant decreases in muscle ATP prior to bouts 3 and 4 compared to prior to bout 1. As with the previously mentioned study, there were no changes in muscle ADP or AMP content while there were increases in muscle IMP, lactate, and hydrogen ions, all three of which are associated with fatigue. The 30 minute recovery cycling bout did not allow for complete recovery of muscle ATP prior to bout 4, but muscle IMP had returned to baseline levels. Similarly to the previously mentioned study, muscle PCr was significantly reduced from baseline levels prior to bout 3, whereas 30 minutes of active recovery allowed a super-compensation for muscle PCr with a significant increase above baseline levels (Hargreaves et al., 1998). This study suggests that fatigue following repetitive, high intensity exercise, as compared with prolonged exercise, is primarily caused by reductions in PCr and increases in muscle lactate and hydrogen ions, and not related to muscle glycogen levels.

Effect of Creatine Supplementation on Muscle Metabolism

With the understanding of the effect of PCr depletion on fatigue during high intensity exercise coupled with the knowledge of the intramuscular increases in PCr seen with supplementation, several researches have examined the effects of creatine

supplementation of muscle metabolism under different scenarios. One such study examined the effects of supplementation of 20 g/day of creatine monohydrate for 5 days on levels of muscular ATP, PCr, FCr, and lactate during recovery from a intense, electrically invoked muscular contraction in 8 males in a cross-over design. Biopsies were collected at rest and at 0, 20, 60, and 120 seconds of recovery from the electrically induced contractions both prior to supplementation and after supplementation with creatine monohydrate. Results from this study showed significant decreases in muscle ATP and PCr following stimulation with return to resting values after 120 seconds recovery. Participants' total creatine concentration increased by an average of 15% following supplementation. For the first minute of recovery, creatine supplementation showed no difference in muscle PCr recovery, but during the second minute of recovery, creatine supplementation resulted in a 42% increase in rate of PCr resynthesis compared to before supplementation, although the absolute values of PCr were not significantly different (Greenhaff et al., 1994). Another study examined the effects of creatine supplementation (30 g creatine + 30 g dextrose/day) compared to placebo (30 g dextrose/day), in a random, cross-over design separated by four weeks on eight active, untrained males who performed a 20s bout of sprint cycling after five days of supplementation with both creatine or placebo. Muscle biopsies were collected at rest, immediately following exercise and after two minutes of passive recovery. This study resulted in a relatively modest increase (~10%) in total muscle creatine following supplementation when compared to other studies. In addition, the researchers failed to show any significant differences in the TAN pool or muscle PCr during recovery from the 20 second bout of sprint cycling (Snow et al., 1998). A third study investigated the

effects of 5 days of creatine supplementation (20 g/day) on muscle metabolism and performance in 16 triathletes following 4 x 20 second sprints on a cycle ergometer with 20 second rest periods. As with the previous study, this study showed significant increases in muscle total creatine concentration, but failed to show any significant effects on performance. The investigators in this study did not measure PCr during recovery, but showed an increase in average resting total creatine concentration of about 15%. Despite the increase in available creatine, there were no differences in performance between the two treatment groups. A possible reason given for this fact was that the subjects were only allowed a 20 second rest between sprint cycling bouts (Finn et al., 2001). The design of our study was primarily based off of the designs of the previously mentioned studies. To reiterate, we were principally concerned with the effects of creatine supplementation and training on ATP and PCr resynthesis, whereas the previous studies examined other types of activity and did not compare training status.

Effect of Training Status on Recovery from Exercise

Resistance training affects the human body in a number of ways. Of primary concern to strength and conditioning professionals are the increases in muscle mass, muscular strength, and muscular endurance. There are also physiological adaptations that occur as a result of resistance training that prepare the trained individual to undergo further resistance exercise. Some of these changes, dependent upon training modality, include increases in anaerobic enzymes (phosphofructokinase), aerobic enzymes, PCr, FCr, ATP, and glycogen, with a reduction on these enzymes and substrates seen during detraining (Abernethy et al., 1994; Green, Thomson, Daub, & Ranney, 1980; MacDougall, Sale, Ward, & and Sutton, 1977). Due to the changes in muscle

biochemistry as a result of resistance training, and the lack of research done on this specific comparison, we felt it important to delineate any differences in skeletal muscle recovery of PCr and ATP between resistance trained and untrained individuals. This knowledge could potentially be beneficial to strength and conditioning professionals when designing resistance exercise programs for untrained individuals. For instance, a recent article published by Buresh, et al., examined the effects of rest intervals of 1 or 2.5 minutes between sets during a 10-week, whole body resistance training regimen in untrained males. The researchers measured changes in body composition, muscle cross-sectional area, arm cross-sectional area, five repetition maximum squat and bench press, and post-exercise plasma growth hormone, testosterone, and cortisol concentrations. This study demonstrated an increase in plasma testosterone in the 1 minute rest period group, but not he 2.5 minute rest period group following one week of training. Furthermore, they showed that after 10 weeks of training, the differential endocrine response no longer existed (Buresh, Berg, & French, 2009). This study confirms the idea that training status could potentially have an effect on response to and recovery from resistance training.

Training and Work-to-rest Ratios

The purpose of providing an appropriate rest period between exercise sets is to allow an athlete to optimize the amount of weight lifted and number of sets performed in order to gain the most benefit from resistance training. The NSCA recommends a work-to-rest ratio of 1:12 – 1:20 for exercise lasting between 5 and 10 second, and a work-to-rest ratio of 1:3 – 1:5 for exercise lasting between 15 and 30 seconds (Baechle et al., 2008). For example, when an exercise set lasts 10 seconds, the suggested recovery

interval should last between 120 and 200 seconds to allow for adequate recovery of PCr and ATP. Furthermore, the NSCA suggests that a rest period of 2-5 minutes between exercise sets should be utilized for athletes striving to gain muscular strength and power. Additionally, the American College of Sports Medicine (ACSM) recommends a rest interval of 2-3 minutes when training for strength or hypertrophy (Kraemer et al., 2002). Research performed on rest intervals suggests that these recommendations are accurate. The majority of research agrees that longer rest periods result in greater strength gains. A study performed by Hill-Haas and colleagues looked at the effects of three training session per week for five weeks in 18 active females. The subjects were randomly assigned to rest for either 20 or 80 seconds between exercise sets. Results of the study suggest that the 80 second rest interval is more effective at increasing strength than the 20 second rest interval. However, this particular study showed the shorter rest interval resulted in improved repeated sprint ability (Hill-Haas, Bishop, Dawson, Goodman, & Edge, 2007). It is theorized, and supported with research, that the increased strength gains seen with longer rest intervals is due to the ability to finish more repetitions at a higher intensity. Willardson and Burkett have shown in two separate studies that longer rest intervals allow for a higher number of repetitions to be performed in both the squat and bench press exercises (Willardson & Burkett, 2006a; Willardson & Burkett, 2006b). Pincivero et al., have found that longer rest periods improve strength gains in isokinetic resistance training as well. In two separate studies, they found that a 160 second rest interval results in significantly greater leg strength improvement than a 40 second rest interval over the course of a 4-6 week training period (Pincivero, Lephart, & Karunakara, 1997; Pincivero & Campy, 2004). The research in this area seems to agree that the most

benefit, in regards to strength gains, can be seen when rest intervals of around 3 minutes are used. This is due to the fact that a higher training volume can be achieved when adequate rest is provided between exercise sets. It has been shown that training can affect the ability to recover from exercise via multiple adaptations that occur in the body concurrent with training, including hypertrophy of muscle fibers, altered myosin heavy chain expression, increased glycolytic and oxidative enzyme storage, increased intramuscular glycogen storage, and changes in optimum angle of torque production (Abernethy et al., 1994; Brockett, Morgan, & Proske, 2001). These intramuscular changes associated with various types of muscular loading may lead to differential changes in the required work-to-rest ratio for trained and untrained individuals. This study, in part, seeks to clarify any such differences between these two groups.

Creatine Supplementation

Supplementation with creatine monohydrate (CM) has been proven safe and effective at increasing the strength gains seen with resistance training (Greenwood et al., 2003; Kreider et al., 2003; Kreider, 2003). When supplementing with creatine, the most common form ingested is creatine monohydrate. A typical supplementation protocol includes a short term loading dose, usually 20 g/day, followed by a long term maintenance dose of 2-5 g/day (Terjung et al., 2000; van Loon et al., 2003). This supplementation protocol has been shown to result in an average increase of intramuscular total creatine (FCr + PCr) by approximately 20%, depending on co-ingestion of macronutrients and individual CM response (Hultman et al., 1996; Kilduff et al., 2002; Kreider et al., 1998; Yquel et al., 2002). As a result of increased intramuscular creatine, there is enhanced PCr repletion and subsequent ATP availability, which allows

for an increase in work production, all of which is made possible by the increased intramuscular PCr (Casey & Greenhaff, 2000; Yquel et al., 2002). Several studies have shown the supplementation with CM leads to improved recovery times between bouts of high intensity activity (Aaserud, Gramvik, Olsen, & Jensen, 1998; Cottrell et al., 2002; Yquel et al., 2002). One in particular looked at recovery from repeated sprint cycling in thirty males receiving either CM or a placebo. This study showed that those ingesting CM had higher power production after 1 or 3 minutes of recovery compared to those receiving placebo, suggesting that the enhanced recovery during short rest intervals was due to the CM supplementation (Cottrell et al., 2002). In another similar study, subjects received either CM or placebo (15 g/day loading dose for five days, 2 g/day maintenance does for 9 days) and were asked to perform eight 40 meter sprints with 25 seconds of recovery between sprints. This study reported that those subjects consuming CM had reduced run times for the final three sprints compared to subjects consuming placebo. This further substantiates the idea that CM supplementation allows for improved recovery during high intensity, short duration activities (Aaserud et al., 1998).

It is well documented that CM supplementation can lead to enhanced performance and strength gains through improved recovery between exercise bouts in many people. This ergogenic benefit could potentially alter the required work-to-rest ratio during resistance training. One goal of this study is to elucidate what, if any, role supplementation with creatine monohydrate may play in altering PCr repletion kinetics following high intensity resistance exercise.

Response to Creatine Supplementation

Although the majority of research involving CM supplementation supports the reported ergogenic qualities of this nutritional supplement, there is a small proportion of research in this area that does not show evidence of an ergogenic effect from CM supplementation. One proposed reason for this lack of effect is that some people have been observed to not increase intramuscular creatine content following supplementation. These people have been defined as creatine non-responders. There have been several instances reported in the literature that supports the theory that some people do not respond to CM supplementation (Kilduff et al., 2002; Snow et al., 1998; Syrotuik & Bell, 2004a). Creatine uptake across the muscle cell membrane is carried out by a sodium-dependent creatine transporter (Sora et al., 1994). The factors that may affect rate of creatine uptake via this transport system include pre-supplementation muscular TCr content, muscle fiber type, muscle cross sectional area, fat free mass, exercise, and carbohydrate intake as well as several other factors (Snow et al., 1998; Syrotuik & Bell, 2004a). In several studies, the lack of response to CM supplementation has been provided as an explanation for a lack of an ergogenic effect. One recent study, which is very similar to the study we carried out found that following CM supplementation, there was not an enhanced PCr repletion rate, despite a 9.5% increase in muscle TCr content (Snow et al., 1998). However, another similar study was able to show significant increases in muscle power output, increases in PCr repletion, and increased pH when muscle TCr content increases were more substantial (Yquel et al., 2002). It appears that in order for there to be an observed ergogenic effect with CM supplementation, the rise in TCr content must be greater than 20 mmol $\cdot$ kg^{-1} dm (Greenhaff, 1996).

Phosphocreatine Observation Techniques

In order to investigate PCr kinetics and the relationship between PCr resynthesis and exercise performance, several observation techniques have been developed. In order to obtain a complete understanding of this topic, these techniques must be described. The Bergstrom biopsy technique was developed in 1962. It is an invasive procedure in which a small sample of muscle tissue is removed with the use of a 6 mm needle which is inserted into a small incision in the muscle to be sampled. The use of this procedure has led to a vast amount of knowledge about muscle biochemistry and physiology (Alvestrand, Furst, & Bergstrom, 1983; Bergstrom et al., 1981; Bevegard, Castenfors, Danielson, & Bergstrom, 1977; Forsberg, Nilsson, Werneman, Bergstrom, & Hultman, 1991; Larsson & Bergstrom, 1978). In conjunction with the muscle biopsy procedure, the muscle samples can be analyzed for total and FCr, and ATP (Broberg & Sahlin, 1989; Febbraio & Dancey, 1999a; Zhao et al., 2000). The most commonly used technique to analyze FCr and PCr is an enzymatic, fluorometric method described elsewhere by Lowry and Passonneau (Lowry & Passonneau, 1972). This technique has been used in numerous studies to monitor FCr and PCr during exercise and recovery (Hargreaves et al., 1998; Zhao et al., 2000). This observation technique was chosen for use during this investigation.

^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR) is an alternative to the invasive Bergstrom technique. ^{31}P-NMR can be used to measure high energy phosphates and intracellular pH at rest and during exercise, in vivo, providing real-time information about PCr stores during activity (Walter, Vandenborne, McCully, & Leigh, 1997; Yao et al., 1999). Despite the fact that this method is less invasive than the Bergstrom biopsy

technique, the use of the expensive equipment required for the ^{31}P-NMR precluded our lab from utilizing this method.

Total Adenine Nucleotide Pool Observation Techniques

Observation of TAN is typically done using high performance liquid chromatography (HPLC) on whatever tissue is being analyzed, be it muscle or blood serum. This technique involves the use of an HPLC in which the analysis of a single sample can provide measurements for all of the constituents that make up TAN, and some of the metabolites of AMP including, inosine, IMP, and hypoxanthine (Wynants & Van Belle, 1985). Due to the fact that we chose to measure only ATP, we did not use HPLC, but instead an enzymatic, fluorometric technique. This technique involves similar procedures as those used to measure FCr and PCr also described by Lowry and Passonneau (Lowry & Passonneau, 1972). This technique was used to measure ATP in a study comparing ^{31}P-NMR to enzymatic, fluorometric techniques in rat muscle which showed no difference in the two techniques (Madapallimattam, Cross, Nishio, & Jeejeebhoy, 1994).

Isokinetic Exercise Techniques

Isokinetic exercise testing has been used previously in numerous studies as either a training tool or as a means of measuring isokinetic strength (Kerksick, Taylor, Harvey, & Willoughby, 2008; McNeil, Allman, Symons, Vandervoort, & Rice, 2004; Parcell, Sawyer, Tricoli, & Chinevere, 2002). Isokinetic exercise machines are different from traditional and free-weight exercise machines in that the muscular contraction performed is isokinetic in nature. Isokinetic muscular contractions are contractions in which the

angular velocity of contraction is controlled but the force of contraction varies as a function of muscular strength at varying joint angles. This contrasts with typical isotonic contraction seen during traditional machine and free weight exercises in which the force required to lift the weight remains the same but the angular velocity of contraction varies. Although there is a profound difference between the two types activity, several studies have show isokinetic testing to be both a valid and reliable means of measuring muscular strength (Drouin, Valovich-mcLeod, Shultz, Gansneder, & Perrin, 2004; Verdijk, van Loon, Meijer, & Savelberg, 2009). The use of an isokinetic exercise machine, in the case of this study, a Biodex System III (*Biodex Medical Systems, Inc., Shirely, NY*), is ideal for a study such as this. By using isokinetic exercise, it is easier to control for differences in individual strength levels by having each subject perform at the exact same intensity of contraction, for a similar number of repetitions, and for the same length of time. By doing so, we are able to ensure that each subject has reached a similar level of fatigue, while at the same time gather data describing the exact number of repetitions performed, the amount of torque produced by the individual, the amount of work performed, the rate of fatigue, and multiple other variables that cannot be measured via traditional strength testing. In addition to these positive aspects, a timed exercise protocol allows us to more accurately prescribe specific work-to-rest ratios based on time alone.

Summary

A review of the current literature illustrates the importance and benefits associated with both resistance training and CM supplementation. Exercise scientist have made great advances in our understanding of how humans respond to resistance training and how to best utilize the knowledge that has been gained thus far to optimize

performance outcomes that result from this mode of exercise. In order to further understand how best to incorporate work-to-rest ratios into resistance exercise prescription, we feel that it is imperative to understand how training status and creatine supplementation may alter the need for recovery between sets of resistance training.

CHAPTER THREE

Methods

Participants

Thirty-two (32) apparently healthy, college aged males (aged 18-30) were recruited to participate in this study. Sixteen (16) participants had at least 6 months of lower body resistance training (at least 1 day per week) prior to participation in this study, and were designated the trained group. The other sixteen (16) participants had not participated in structured physical activity for at least 6 months prior to participation in this study, and were classified as the un-trained group. Participants were screened to determine whether they meet the entry criteria prior to participation in the study. Participants were not included if they displayed any contraindications to participation in exercise as outlined by the American College of Sports Medicine (ACSM). The ACSM Absolute and Relative Contraindications to Exercise include the following criteria:

Absolute Contraindications
1. Unstable angina.
2. Uncontrolled dysrhythmias.
3. Recent EKG changes and cardiac events.
4. Acute myocarditis or pericarditis.
5. Acute pulmonary embolism or acute myocardial infarction.
6. Severe aortic stenosis.
7. Dissecting aneurysm.
8. Acute infections.

Relative Contraindications
1. Left main coronary stenosis.
2. Severe hypertension (> 200/110).
3. Tachycardia or bradycardia.
4. Uncontrolled metabolic disease.
5. High-degree AV block.
6. Chronic infectious disease.

7. Cardiomyopahty and outflow obstructions.
8. Stenotic valve disease.
9. Ventricular aneurysm.

Additionally, participants were not allowed to participate if they had consumed any nutritional supplements (excluding multi-vitamin/mineral) within 1 month prior to the study. Approval from the Institutional Review Board for Human Subjects of Baylor University was obtained prior to any testing of human participants. After reading and discussing an informed consent outlining the risks and benefits of participation, all eligible participants provided both oral and written consent to all procedures and exercise protocols encountered during participation in this study. Additionally, all experimental procedures involved in the study conformed to the ethical considerations of the Helsinki Code.

Selection Criteria

In order to be considered for participation in this study participants must have met the following requirements:

1.) Trained subjects were required to be lower body resistance trained for at least 6 months prior to participation in this study. Trained subjects proved their training status by performing a maximal leg press value that is at 2.5 times their body weight.

2.) All subjects must not have consumed any ergogenic nutritional supplement for at least 1 month prior to participating in this study.

3.) Subjects must not have displayed any absolute or relative contraindications to exercise participation as outline by the ACSM.

4.) Subjects must not have had any known metabolic disorder, orthopedic condition, history of cardiovascular, pulmonary, musculoskeletal, genetic, endocrinological or

psychological disease, or be on medication treating any of these previously mentioned conditions.

Independent and Dependent Variables

The independent variables included creatine supplementation and training status. The dependent variables included phosphocreatine repletion kinetics as assessed via biochemical analysis of the obtained muscle samples. Muscle samples were also analyzed for total creatine, FCr, and phosphocreatine concentrations, ATP, glycogen, and lactate. Additionally, blood samples were analyzed for lactate.

Study Site

All supervised familiarization and testing sessions took place at Baylor University, Waco, Texas, in the Exercise and Sport Nutrition Laboratory (ESNL) and the Exercise and Biochemical Nutrition Laboratory (EBNL). Muscle analyses were performed at the Exercise Metabolism Unit, Centre for Ageing, Rehabilitation and Exercise and Sport, at Victoria University, Melbourne, Australia.

Preliminary Testing and Familiarization

Each subject was interviewed to determine qualification for participation as outlined in the study criteria detailed previously. Subjects believed to meet all participation requirements were invited to come to the ESNL for a familiarization session at which time they were given a verbal and written explanation of study procedures, risks and benefits, and asked to give both written and oral consent to participate. Additionally, height, weight, age, and medical history were determined during the familiarization session. Training status was determined for all subjects with participants who were lower

body resistance trained proving their training status by performing a maximal leg press on a isotonic leg press machine (*Nebula Fitness, Inc., Versailles, OH*) of 2.5 times their body weight. If subjects were unable to perform an isotonic leg press of 2.5 times their body weight, or they did not claim to have trained their lower body, they were deemed untrained. Placement of the leg press sled height was set so as to allow for a complete range of motion for the leg press exercise which is defined as starting at a 90° bend in the knee joint to full knee extension. One repetition maximum testing was performed following NSCA guidelines (Baechle et al., 2008).

Following completion of preliminary testing and familiarization, leg dominance was determined by a maximal, unilateral isokinetic leg extension bout performed on a Biodex System 3 (*Biodex Medical Systems, Shirley, NY*) on each leg, with dominance being defined as the leg that produces the highest maximal torque at $60°\cdot s^{-1}$. After determining leg dominance, a 30 second familiarization exercise bout was performed at $120°\cdot s^{-1}$ concentric extension and $300°\cdot s^{-1}$ concentric flexion on the Biodex machine. After determination of training status, subjects were randomly assigned to the supplement or placebo group.

Prior to receiving and beginning the supplementation protocol, a pre-supplementation biopsy was collected in the non-dominant thigh using the procedures described below in the muscle biopsy section.

Supplementation Protocol

Following successful completion of the familiarization testing session, subjects were randomly assigned using an on-line random number generator (*stattrek.com*), in a double-blind manner, to consume $20\ g\cdot day^{-1}$ (5 g, 4 times per day) for 5 days of either

creatine monohydrate supplement or placebo, with 8 fluid ounces of fruit juice (apple, grape, etc.). To ensure standardization of supplement intake, subjects were asked to consume their supplement or placebo at 8:00 a.m., 12:00 p.m., 4:00 p.m., and 8:00 p.m. daily. Additionally, all empty supplement packages were collected prior to each testing session by the primary investigator in conjunction with a supplementation log to ensure all supplements were ingested.

Isokinetic Exercise Testing Protocol

Each participant reported to the ESNL following 5 days of CM or placebo loading. All subjects provided their 3 day food record and supplementation log at this time point. Upon arrival to the laboratory, subjects' height, weight, and body composition was determined, as well as resting heart rate and blood pressure. Body composition was determined using a seven site skin-fold method (measurements taken at the chest, tricep, sub-scapula, mid-axilla, supra-iliac crest, abdomen, and anterior thigh) with Lange Skin-fold calipers using the Siri body density equation. Following these preliminary procedures, subjects had the lateral aspect of their dominant thigh injected subcutaneously with a 2% Xylocaine with epinephrine into four separate injection sites surrounding the muscle biopsy site superficial to the vastus lateralis muscle. Additionally, blood lactate was measured at rest via the finger prick method. Once the local anesthetic has taken full effect (approximately 10 minutes), a pre-exercise muscle biopsy was collected and immediately flash-frozen in liquid nitrogen to preserve the intramuscular metabolic conditions. Subjects were then seated in the Biodex machine and asked to perform a 30 second bout of isokinetic knee flexion and extension targeting the quadriceps muscle, which includes the vastus lateralis muscle, from which all muscle

biopsies were sampled. During the 30 second bout of isokinetic, concentric knee extension ($120°\cdot s^{-1}$) and knee flexion ($300°\cdot s^{-1}$), subjects averaged 26.8 repetitions, or nearly 1 repetition per second. This protocol was designed to deplete available ATP and PCr in the vastus lateralis muscle in order to allow for the accurate testing of ATP and PCr repletion kinetics. Before any data was collected for the actual study, several rounds of piloting were conducted, on both trained and untrained individuals, in order to determine the ideal isokinetic joint speed and exercise time necessary to achieve optimum depletion of ATP and PCr. Additionally, by using a timed activity, we are able to assign an ideal work to rest ratio for similar resistance training activity, thus allowing us to define a more precise length of time required for ATP and PCr repletion. Following the 30 second maximal isokinetic exercise bout, an immediate post exercise biopsy was collected while the subject remained on the Biodex machine to avoid any time delay in the muscle sampling process. Two further muscle biopsies were collected following 3 and 5 minutes of passive recovery. Also, at 5 minutes of passive recovery another blood lactate sample was taken, again using the finger prick method. Each of the muscle samples were taken from the same incision with the needle aperture being inserted at four different positions within the incision corresponding to the 12, 3, 6, and 9 o'clock positions on a watch dial. All needles were immediately placed in liquid nitrogen in order to preserve intramuscular metabolic conditions. Following submersion in liquid nitrogen, muscle samples were removed from the biopsy needles and stored in micro-centrifuge tubes at -80°C, until analysis could be performed.

Muscle Biopsies

Muscle biopsies (approximately 50-70 mg) were obtained from the vastus lateralis muscle of each participant's exercised thigh using the percutaneous needle biopsy technique with suction. Prior to extraction of muscle tissue, 1.5 mL of a 2% Xylocaine with epinephrine solution was injected subcutaneously at the midway point between the proximal patella and greater trochanter of the femur on the lateral aspect of the thigh. Each muscle tissue sample was collected from the same incision and depth. Subjects were instructed to lie in a supine position on a sterilized table. Following identification of the incision site, the area was shaved and cleaned with rubbing alcohol. For additional sterilization, the site was also cleaned with Betadine and covered with sterile gauze. Once the anesthetic had taken effect (approximately 10 minutes), a 1 cm incision was made through the skin, fat, and muscle fascia. Through this incision, the biopsy needle was inserted approximately 5 cm in depth. Following extraction of the muscle sample, pressure was applied to the wound and covered with a butterfly bandage and overlaying pressure bandage. For each subsequent muscle biopsy, muscle samples were taken from the same incision and depth marking on the biopsy needle. There were five biopsies taken occurring prior to supplementation (PRE), immediately before exercise (REST), immediately after exercise (POST), 3 minutes after exercise (3POST), and 5 minutes after exercise (5POST). Immediately after the muscle tissue sample was collected, the biopsy needle was inserted into a container filled with liquid nitrogen. Following the initial flash-freezing, the muscle sample was extracted from the needle and was placed in a cryogenic storage tube and placed back into the container of liquid nitrogen until being placed into long term storage. Tissue samples were then removed from liquid nitrogen

and stored at -80°C until analysis. Upon completion of the biopsy procedure the scalpel blade and all other contaminated materials were discarded as hazardous waste and placed in an appropriately labeled container. Each stainless steel biopsy needle was rinsed with hot water, washed with a laboratory detergent, and then rinsed in distilled water. Each biopsy needle was then sterilized (autoclaved) for 55 minutes and cooled for future use. Written instructions were given to each participant regarding post biopsy care. Subjects were instructed to leave the bandages in place for 24 hours and were advised to refrain from vigorous physical activity for 48 hours following the biopsy procedure. If required, subjects were advised to take a non-prescription analgesic medicine as needed for pain or discomfort.

Blood Lactate Measurement

Two blood lactate samples were collected using standard finger prick methods. The purpose of blood lactate measurement was twofold. First of all, we wanted to determine whether or not the supplementation or training status would have an effect on blood lactate response following the exercise bout. Secondly, we were interested in providing a means of determining whether or not the subjects were reaching a state of ATP and PCr depletion, as evidences by accumulation of lactate in the blood. One measurement was taken at rest prior to the experimental exercise protocol and the other after 5 minutes of passive recovery following the experimental exercise protocol. Samples were analyzed using a Lactate Plus analyzer (*Nova Biomedical, Waltham, MA, USA*). Prior to being pricked with a spring loaded lancet, the collection site was swabbed with isopropyl alcohol. After the lancet made the incision, the first drop of blood was wiped away using gauze. Then a small sample of blood was placed on the lactate

analysis strip connected to the lactate analyzer and analyzed within 15 seconds. This procedure was used for both blood collections.

Food Records

Subjects were asked to maintain their normal dietary practices for the duration of the study. However, all subjects recorded a 72-hour food record for the 3 days preceding each testing session. All food records were analyzed using the Food Processor dietary assessment software program (*ESHA Research, Salem, OR*) to determine daily macronutrient (carbohydrate, protein, and fat) intake prior to exercise.

Muscle Glycogen and Metabolite Analyses

Approximately 15-30 mg of frozen muscle was lyophilised in a freeze-drier for 48 hours. The metabolite extraction process was performed in accordance with the method of Harris et al. (1992). Each muscle sample was manually crushed to a fine powder with any visible connective tissue or blood removed.

Muscle Glycogen Analysis

The extract was analyzed using a two step enzymatic process requiring readings in triplicate on a fluorimeter (*Turner Digital Filter Fluorometer, Sunnyvale, CA*). Muscle Glycogen was determined according to the methods described by Lowry and Passoneau (Lowry & Passonneau, 1972).

10 µl of Blanks, nicotinamide adenine dinucleotide (NADH) standards (50, 100, 200, & 400 µM), , glucose standards (250 & 500 µM) and samples were added to 1 ml of cocktail reagent (50 mM Tris Buffer, 1 mM MgCl2, 0.5 mM DTT, 0.3 mM ATP, 50 µM NADP+ and 25 µl glucose-6-phosphate-dehydrogenase (G-6-P-DH)/ml) in a test tube.

The first reading taken at 365 absorption, 455 nm emission, gives an indication of any residual endogenous nicotinamide adenine dinucleotide phosphate (NADPH) that may be present

25 µl of dilute hexokinase (diluted 1/40 in cocktail reagent) was then added to each sample, vortexed and placed in the dark for 60 minutes. The final reading is recorded at the same absorption and emission previously used. Subtraction of the first reading from the second gives a change in NADPH concentration. Given that for each glucose molecule broken down from glycogen results in the production of one NADPH molecule; the amount of NADPH gives the value of glycogen. Muscle glycogen was calculated as mmol per kilogram of dry muscle mass (mm · kg-1 dm).

Muscle Lactate Analysis

The extract was analyzed using a two step enzymatic process requiring readings in triplicate on a fluorimeter (*Turner Digital Filter Fluorometer, Sunnyvale, CA*). Muscle Lactate was determined according to the methods described by Lowry and Passoneau (Lowry & Passonneau, 1972).

1 ml of cocktail reagent (100 mM hydrazine, 100 mM glycine, 0.5 mM NAD+ and 5 µl/ml of lactate dehydrogenase (LDH)) was added to all tubes. The first reading at 365 absorption, 455 nm emission gives an indication of any residual NADH that may be present. 10 µl of blanks, NADH standards (50, 100, 200, & 400 µM), lactate standards (50 & 500 µM) and samples were added to each tube, mixed, and placed in the dark for 60 minutes. Following the 60 minutes, the final reading is recorded at the same absorption and emission as previously mentioned. Subtraction of the first reading from the second gives a change in NADH concentration. Based on the stoichiometric

accumulation of NADH from lactate and NAD; the amount of NADH gives the value of lactate. Muscle lactate was determined as mmol per kilogram of dry muscle mass (mm · kg-1 dm).

Muscle Adenosine Triphosphate and Phosphocreatine Analysis

The extract was analyzed using a three step enzymatic process requiring readings in triplicate on a fluorimeter (*Turner Digital Filter Fluorometer, Sunnyvale, CA*). Muscle ATP and PCr was determined according to the methods described by Lowry and Passoneau (Lowry & Passonneau, 1972).

10 µl of blanks, NADH standards (50, 100, 200, & 400 µM), ATP standards (100 & 200 µM), PCr standards (250 & 500 µM) and samples were added to 1 ml of cocktail reagent (50 mM Tris buffer, 1 mM MgCl2, 0.5 mM DTT, 100 µM glucose, 50 µM NADPH+, 5 µl/100ml G-6-P-DH). The first reading at 365 absorption, 455 nm emission gives an indication of any residual NADPH that may be present

25 µl of dilute hexokinase (diluted 1/40 in cocktail reagent) was added to each sample, mixed, and incubated in the dark for 30 minutes. The final reading is recorded at the same absorption and emission previously used. Subtraction of the first reading from the second gives a change in NADPH concentration. Because no CK or ADP are present, the amount of NADPH gives the value of ATP.

To determine PCr, 20 µl of dilute ADP-creatine phosphokinase (CK; 2mg ADP, 2mg CK, 10% BSA diluted in 1 ml of cocktail reagent) was added to each sample, mixed, and incubated for 60 minutes in the dark. The final reading was again taken at the same absorption and emission as previously used. The amount of NADPH produced in this reaction can be directly attributed to the amount of PCr present in the sample. To allow

for variation of connective tissue that may have remained in the muscle samples, ATP and PCr were corrected for total Cr (Cr + PCr) and expressed as mmol per kilogram of dry muscle mass (mm · kg-1 dm).

Muscle Creatine Analysis

The extract was analyzed using a three step enzymatic process requiring readings in triplicate on a fluorimeter (*Turner Digital Filter Fluorometer, Sunnyvale, CA*). Muscle ATP and PCr was determined according to the methods described by Lowry and Passoneau (Lowry & Passonneau, 1972).

30 µl of blanks, NADH standards (50, 100, 200, & 400 µM), Cr standards (200 & 500 µM) and samples were added to 1 ml of cocktail reagent (50 mM imadazole, 5 mM MgCl2, 30 mM KCl, 0.1 mM PEP, 0.2 mM ATP, 20 µl/ 100 ml LDH, 5 µg/ml pyruvate kinase (PK)). The first reading at 365 absorption, 455 nm emission gives an indication of any residual NADH that may be present

20 µl of dilute CK (10 mg/ml diluted in 0.05% BSA) was added to each sample, vortexed and incubated in the dark for 60 minutes. The final reading is recorded at the same absorption and emission previously used. Subtraction of the first reading from the second gives a change in NADH concentration. Creatine concentration was based on the stoichiometric reduction in NADH, as it is used in the formation of ADP and PCr. To allow for variation of connective tissue that may have remained in the muscle samples, final values were corrected for total Cr (PCr + Cr) and expressed as mmol per kilogram of dry muscle mass (mm · kg-1 dm).

Statistical Analyses

One-way univariate analysis of variance (ANOVA) was used to determine if there were any significant differences for baseline variables between the four treatment groups at the beginning of the study. If significant differences existed, Bonferroni post-hoc procedures were used to determine which groups had significant differences. Statistical analyses used to analyze blood lactate and muscle metabolites were performed using two separate repeated measures, two-factor [treatment groups (4) x time points (2)] and [treatment groups (4) x time points (5)] for blood lactate and muscle metabolites, respectively. Blood lactate was analyzed using a two-factor [treatment groups (4) x time points (2)] repeated measures multivariate analysis of variance. Muscle phosphagens (Cr, PCr, and TCr) were also analyzed using a two factor [treatment groups (4) x time points (5)] repeated measures multivariate analysis of variance. All other muscle metabolites (ATP, glycogen, and lactate) were analyzed using individual two-factor [treatment groups (4) x time points (5)] repeated measures analysis of variance. Wilks' Lambda was used to evaluate all multivariate tests. Mauchly's test of sphericity was performed on all dependent variables with the Huynh-Feldt correction factor being utilized for any dependent variable that did not meet the assumption of sphericity. Bonferonni post-hoc procedures were used to compare means for any significant main effects. All reported effect size calculations were performed using the partial Eta squared statistic. All statistical analyses were performed using SPSS 15.0 software for Windows (*SPSS, Inc., Chicago, IL*) with a probability level of $p<0.05$ throughout.

CHAPTER FOUR

Results

Participants

Thirty-two male subjects volunteered for this study. Two subjects were dropped from the study for failure to follow study guidelines. There were no significant differences between groups regarding age ($p = 0.808$), weight ($p = 0.948$), lean mass ($p = 0.796$), or fat mass ($p = 0.349$). The complete demographic data mentioned above is presented in Table 1. There were four groups in this study based on training status and supplement ingestion; untrained/placebo (group 1), untrained/creatine (group 2), trained/placebo (group 3), and trained/creatine (group 4).

Table 1

Demographic Data

Age	21.1 $\pm$ 3.6
Weight (kg)	84.7 $\pm$ 14.9
Lean mass (kg)	70.3 $\pm$ 9.0
Fat mass (kg)	14.4 $\pm$ 7.9

Note: Values are expressed as means $\pm$ standard deviations

72 Hour Dietary Recall Analysis

Subjects were instructed to maintain their normal dietary intake for the duration of the study. In order to verify dietary intake, subjects were required to fill out a food log for 72 hours prior to each testing session. A multivariate ANOVA was used to analyze differences between groups for total caloric content, fat intake, protein intake, protein intake by body mass, and carbohydrate intake. There were no significant main effects for group for any dietary variable (Wilks' Lambda = 0.067, F (45, 39.4) = 1.295, p = 0.205). The 72 hour dietary recall analysis data are presented in Table 2.

Table 2

72 hour Dietary Recall Analysis

Variable	Untrained/Placebo			Untrained/Creatine			Trained/Placebo			Trained/Creatine		
Time Point	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Total Calories (kcal/day)	2367 ±260	2405 ±229	2432 ±220	2920 ±278	2254 ±245	2404 ±235	2512 ±260	2526 ±229	2243 ±220	2408 ±260	2168 ±229	2135 ±220
Fat (g/day)	84 ±14*	93 ±12	86 ±12	127 ±15	76 ±13	91 ±13	94 ±14	93 ±12	77 ±12	76 ±14†	88 ±12	88 ±12
Protein (g/day)	107 ±19	107 ±13	99 ±17	115 ±20	81 ±14	92 ±18	135 ±19	118 ±13	124 ±17	133 ±19	107 ±13	117 ±17
Protein/ Body Mass (g/kg)	1.29 ±.51	1.34 ±.38	1.29 ±.60	1.40 ±.28	0.98 ±.29	1.13 ±.30	1.58 ±.68	1.36 ±.46	1.42 ±.53	1.68 ±1.0	1.34 ±.57	1.47 ±.81
Carbohydrate (g/day)	295 ±35	278 ±31	303 ±33	334 ±38	271 ±33	311 ±36	283 ±35	306 ±31	294 ±33	304 ±35	240 ±31	222 ±33

Note: Values are expressed as means ± standard deviations.

Strength Variables

Prior to participating in this study, all subjects were required to perform a 1RM on the leg press machine. The purpose of this measurement was to determine the resistance

training status of the subjects. The subjects that claimed to be lower body resistance trained were required to correctly perform the 1RM leg press movement with 2.5 times their body mass. The subjects who claimed to not be lower body resistance trained also performed the leg press 1RM so that we could compare the differences in strength between the two groups. Additionally, each participant was asked to perform 5 maximal reps with each leg on the Biodex Isokinetic Dynamometer at 60 °/sec in order to determine their dominant leg based on maximal isokinetic torque production. Following this initial isokinetic testing, the subjects were familiarized to the exercise protocol that would be used during the final testing session using their dominant leg. A MANOVA was used to analyze differences between groups for the lower body strength variables: leg press 1RM, left leg peak torque production, and right leg peak torque production. There was no significant group effect for the lower body strength variables (Wilks' Lambda = 0.723, F (9, 63.42) = 1.006, p = 0.445, effect size = .103, observed power = 0.361). Although there were no significant differences for strength measures between groups, the average leg press 1RM for all trained subjects was 62 kg higher than the average leg press 1RM for all untrained subjects (trained 390 $\pm$ 137 kg, untrained 328 $\pm$ 87 kg) The baseline strength measures are presented in Table 3.

Blood Lactate

Blood lactate was measured for each participant prior to and 5 minutes after the final testing session. Blood lactate was measured via finger prick analysis with a Lactate Plus analyzer (*Nova Biomedical, Waltham, MA, USA*). A two factor [group (4) x time (2)] multivariate ANOVA revealed a main effect for time (Wilks' Lambda = 0.153, F (1, 28) = 155, p = 0.000, effect size = 0.847, observed power = 1.0). There was, however, no

significant group effect or group x time interaction. Table 4 shows values for blood lactate. Although non-significant, there was a difference in blood lactate 5 minutes after exercise for the subjects who consumed creatine compared to those who consumed placebo of 1.0 ± 0.85 mmol/L with the group consuming CM maintaining lower blood lactate levels, as illustrated in Figure 1.

Table 3

Baseline Strength

Variable	Untrained/Placebo	Untrained/Creatine	Trained/Placebo	Trained/Creatine
Leg Press 1RM (kg)	290 ± 76	365 ± 86	394 ± 160	387 ± 108
Left Leg Peak Torque 60 °/sec (N·m)	128 ± 24	162 ± 33	147 ± 49	142 ± 19
Right Leg Peak Torque 60 °/sec (N·m)	132 ± 15	163 ± 38	160 ± 44	163 ± 23

Note: Values are expressed as means ± standard deviations.

Table 4

Blood Lactate

Variable	Untrained/Placebo	Untrained/Creatine	Trained/Placebo	Trained/Creatine
Pre-Exercise (mmol/L)	2.12 ± 1.28	1.08 ± 0.58	1.08 ± 0.44	1.43 ± 0.76
5 minutes Post-Exercise (mmol/L)	8.16 ± 2.25	7.48 ± 1.66	8.35 ± 3.67	7.01 ± 2.54

Note: Values are expressed as means ± standard deviations.

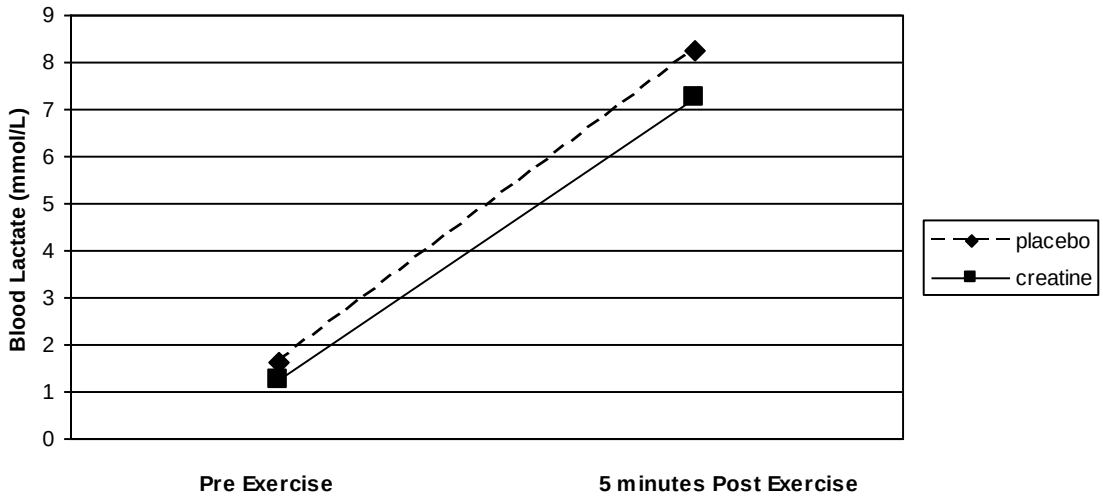

Figure 1. This graph illustrates the non-significant difference in blood lactate between the creatine and placebo groups.

Muscle Phosphagens

A two-way [treatment group (4) x time point (5)] MANOVA with repeated measures on the second factor was used to evaluate the effects of training status and CM supplementation on muscle phosphagens. These included free creatine (Cr), phosphocreatine (PCr), and total creatine (TCr). Additionally, two-way [treatment groups (4) x time points (5)] univariate ANOVAs with repeated measures on the second factor were used to analyze the remaining muscle metabolites. These included adenosine triphosphate (ATP), glycogen, and lactate. The within-subjects factor was time with five levels [pre-supplementation (PRE), pre-exercise (REST), immediately post-exercise (POST), 3 minutes post-exercise (3POST), and 5 minutes post-exercise (5POST)]. The between-subjects factor was group with four levels [group 1 (untrained/placebo), group 2 (untrained/creatine), group 3 (trained/placebo), and group 4 (trained/creatine)].

Additional tests were performed with the between-subjects factor being supplementation status with two levels (placebo and creatine monohydrate) and training status with two levels (untrained and trained). The main effects for group and time, and the group x time interaction were analyzed using the Wilks' Lambda multivariate statistic.

For the overall MANOVA on muscle phosphagens there was a significant main effect for time (Wilks' Lambda = 0.069, F (12, 17) = 19.24, p = 0.000, effect size = 0.931, observed power = 1.0). There was not a significant main effect for group (Wilks' Lambda = 0.802, F (9, 63.4) = 0.669, p = 0.734, effect size = 0.071, observed power = 0.240) or a significant group x time interaction (Wilks' Lambda = 0.231, F (36, 50.96) = 0.908, p = 0.615, effect size = 0.386, observed power = 0.682). As a result of the multivariate tests, univariate ANOVAs were performed with subsequent Bonferroni post-hoc tests to determine time differences for all muscle phosphagens. The assumption of sphericity was not met for Cr (0.043) or TCr (0.005). However, the assumption of sphericity was met for PCr (0.795). As a result, Huynh-Feldt corrections were used for variables that did not meet the assumption of sphericity.

Additionally, when comparing all subjects consuming CM to all subjects consuming placebo, there were no significant main effects for supplement (Wilks' Lambda = 0.954, F (3, 28) = 0.446, p = 0.722, effect size = 0.046, observed power = 0.128) or supplement x time interaction (Wilks' Lambda = 0.628, F (12, 19) = 0.937, p = 0.533, effect size = 0.372, observed power = 0.356). As was expected, there was a significant main effect for time (Wilks' Lambda = 0.074, F (12, 19) = 19.75, p = 0.000, effect size = 0.926, observed power = 1.0). As a result of the multivariate tests, univariate ANOVAs were performed with subsequent Bonferroni post-hoc tests to

determine time differences for all muscle phosphagens. For this analysis, the assumptions of sphericity were also not met for Cr (0.020) or TCr (0.005), while the assumption of sphericity was met for PCr (0.677). As a result, Huynh-Feldt corrections were used for variables that did not meet the assumption of sphericity.

Finally, comparisons of muscle phosphagens between the trained and untrained subjects showed a significant main effect for time for training status (Wilks' Lambda = 0.073, F (12, 19) = 20.08, p = 0.000, effect size = 0.927, observed power = 1.0). There was no significant main effects for training status (Wilks' Lambda = 0.901, F (3, 28) = 1.021, p = 0.398, effect size = 0.0991, observed power = 0.247). Furthermore, there was no significant training status x time interaction (Wilks' Lambda = 0.628, F (12, 19) = 0.938, p = 0.533, effect size = 0.372, observed power = 0.356). As a result of the multivariate tests, univariate ANOVAs were performed with subsequent Bonferroni post-hoc tests to determine time effects for all muscle phosphagens. For this analysis, the assumption of sphericity was not met for Cr (p = 0.023) or TCr (p = 0.006). However, they assumption of sphericity was met for PCr (p = 0.662. As a result, Huynh-Feldt corrections were used for variables that did not meet the assumption of sphericity.

Muscle Free Creatine

There was a significant main effect for time for muscle Cr (F (4, 112) = 33.8, p = 0.000, effect size 0.547, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant time point differences for Cr. Muscle Cr was significantly higher at POST compared to all other time points (p = 0.000 for all comparisons). At 3POST, muscle Cr was significantly higher than PRE (p = 0.000) and REST (p = 0.004).

There were no significant group differences for muscle Cr ($p = 0.595$) or group x time interaction ($p = 0.699$). Muscle Cr values are shown in Table 5.

Table 5

Muscle Free Creatine

Variable	PRE	REST	POST	3POST	5POST
Untrained/Placebo	57.6 ± 8.24	67.2 ± 11.3	103.2 ± 13.8	78.4 ± 6.64	73.2 ± 25.0
Untrained/Creatine	63.6 ± 9.31	69.4 ± 7.85	89.2 ± 11.9	76.9 ± 8.09	73.8 ± 11.1
Trained/Placebo	60.1 ± 21.9	61.0 ± 11.4	96.7 ± 34.1	76.4 ± 37.3	63.1 ± 15.2
Trained/Creatine	63.6 ± 8.96	71.0 ± 13.3	104.0 ± 25.6	90.5 ± 17.1	74.3 ± 26.4

Note: Values are expressed as means ± standard deviations (n = 8/group). All Cr values are expressed as mmol per kilogram of dry muscle mass (mmol · kg^{-1} dm).

The comparison of Cr by supplement group revealed a significant main effect for time ($F (3.73, 112.1) = 34.31$, $p = 0.000$, effect size = 0.553, observed power = 1.0). Bonferroni post-hoc tests revealed Cr was significantly higher at POST compared to all other time points ($p = 0.000$ for all comparisons). At 3POST, muscle Cr was significantly higher than PRE ($p = 0.000$) and REST ($p = 0.003$). There was no significant group difference for muscle Cr ($p = 0.411$) or supplement group x time interaction ($p = 0.59$).

The third comparison of Cr by training status also revealed a significant main effect for time ($F (3.76, 112.8) = 34.41$, $p = 0.000$, effect size = 0.534, observed power = 1.0). Bonferroni post-hoc tests revealed Cr was significantly higher at POST compared to all other time points ($p = 0.000$ for all comparisons). At 3POST, muscle Cr was significantly higher than PRE ($p = 0.000$) and REST ($p = 0.002$). There was no

significant group difference (p = 0.865) or training status x time interaction (p = 0.533) for muscle Cr.

Muscle Phosphocreatine

We observed a significant main effect for time for PCr values (F (4, 112) = 54.180, p = 0.000, effect size = 0.659, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant time point differences for PCr. Muscle PCr was significantly lower at POST compared to all other time points (p = 0.000 for all comparisons). At 3POST, muscle PCr was significantly lower than PRE (p = 0.000) and REST (p = 0.004). At 5POST PCr was also significantly lower than PRE (p = 0.000) and REST (p = 0.000). There was no significant group difference for PCr (p = 0.341). Although not significant, there was a trend towards a group x time interaction for PCr (F (12, 112) = 1.669, p = 0.083). Muscle PCr values are shown in table 6.

Table 6

Muscle Phosphocreatine

Variable	PRE	REST	POST	3POST	5POST
Untrained/Placebo	66.6 ± 19.8	76.0 ± 17.6	31.6 ± 24.4	42.9 ± 26.9	40.2 ± 19.2
Untrained/Creatine	68.9 ± 20.6	70.9 ± 22.5	15.1 ± 8.15	31.5 ± 11.5	41.3 ± 17.5
Trained/Placebo	65.6 ± 23.4	73.8 ± 21.2	38.9 ± 19.6	59.1 ± 23.6	58.4 ± 20.9
Trained/Creatine	72.2 ± 18.4	84.5 ± 14.2	36.3 ± 8.88	45.0 ± 32.5	45.9 ± 20.9

Note: Values are expressed as means ± standard deviations (n = 8/group). All PCr values are expressed as mmol per kilogram of dry muscle mass (mmol · kg^{-1} dm).

The comparison of PCr by supplement group revealed a significant main effect for time (F (4, 120) = 52.798, p = 0.000, effect size = 0.638, observed power = 1.0).

Bonferroni post-hoc comparisons revealed the following significant time point differences. Muscle PCr was significantly lower at POST compared to all other time points ($p = 0.000$ for PRE and REST, $p = 0.010$ for 3POST, and $p = 0.001$ for 5POST). At 3POST, muscle PCr was significantly lower than PRE ($p = 0.000$) and REST ($p = 0.000$). At 5POST PCr was also still significantly lower than PRE ($p = 0.000$) and REST ($p = 0.000$). There were no significant main effects for supplement group ($p = 0.470$). There was a non-significant trend towards a supplement group x time interaction ($p = 0.077$).

The third comparison by training status also revealed a significant main effect for time ($F = (4, 120) = 51.349$, $p = 0.000$, effect size = 0.631, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant time point differences. Muscle PCr was significantly lower at POST compared to all other time points ($p = 0.000$ for PRE and REST, $p = 0.010$ for 3POST, and $p = 0.001$ for 5POST). At 3POST, muscle PCr was significantly lower than PRE ($p = 0.000$) and REST ($p = 0.000$). At 5POST PCr was also significantly lower than PRE ($p = 0.000$) and REST ($p = 0.000$). There was a trend towards a significant main effect for training status ($F (1, 30) = 2.969$, $p = 0.095$, effect size = 0.090, observed power = 0.385). This weak trend suggested that the trained group had higher PCr at all time points than the untrained group, as illustrated in figure 2. There was no significant training status x time interaction for PCr ($p = 0.281$)

As observed from the statistical measures reported above, we failed to show support for our hypotheses, H_0, H_1, H_4, and H_5, which stated that trained subjects and subjects who consumed CM would have greater PCr repletion rates. In an attempt to comprehend these outcomes, and in light of previous research that supports those

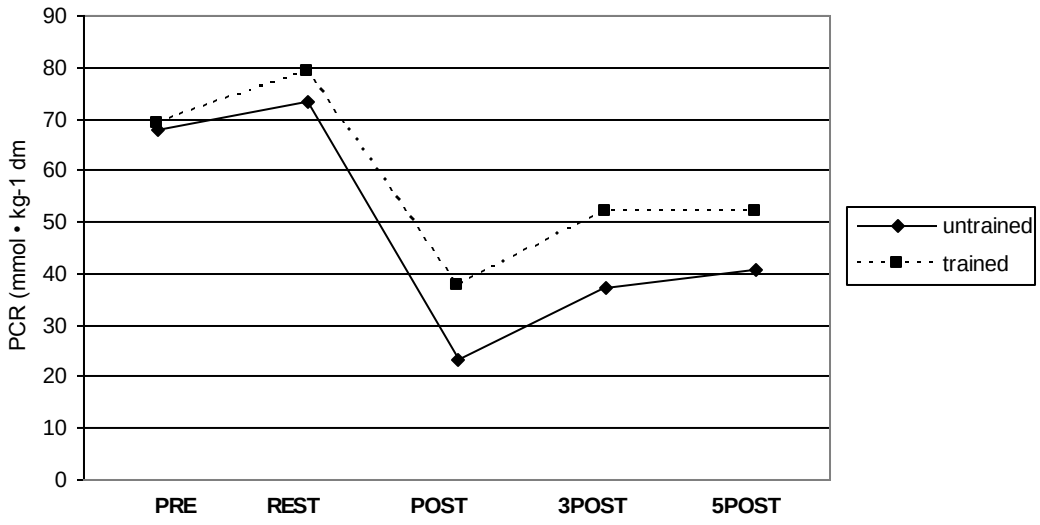

Figure 2. This graph illustrates the non-significant difference (p = 0.281) in muscle PCr between the trained and untrained group at all time points.

hypotheses, we re-examined the data by only using the subjects who appeared to respond to CM supplementation (Greenhaff et al., 1994; Snow et al., 1998; Yquel et al., 2002). For those subjects (n = 8) who showed a net increase in TCr of approximately 20%, we included their data. Those subjects who failed to show an increase in TCr were considered non-responders and their data was not used relative to this statistical approach. Additionally, several subjects who consumed placebo showed increases in TCr. These subjects' (n = 8) data were also removed for this re-analysis. Two-way repeated measures ANOVAs were used for this re-analysis. The re-analysis still failed to show any significant main effects for group for any of the muscle phosphagens. There was however a non-significant trend for a group effect for PCr ($F(3, 12) = 2.957$, $p = .075$). A discussion of these results will follow in the subsequent chapter.

Muscle Total Creatine

Analysis of muscle TCr showed a significant main effect for time (F (3.53, 98.79) = 6.937, p = 0.000, effect size = 0.199, observed power = 0.987). Bonferroni post-hoc analysis revealed that TCr was significantly higher at REST than all other time points (PRE (p = 0.006), POST (p = 0.021), 3POST (p = 0.001), 5POST (p = 0.000). There was no significant main effect for group (p = 0.324) or group x time interaction (p = 0.138). Values for TCr are shown in table 7.

Table 7

Muscle Total Creatine

Variable	PRE	REST	POST	3POST	5POST
Untrained/Placebo	124.3 ± 21.0	143.2 ± 18.4	134.9 ± 27.9	121.4 ± 25.5	113.4 ± 36.2
Untrained/Creatine	132.6 ± 14.4	140.3 ± 21.3	104.4 ± 16.7	108.4 ± 18.15	115.1 ± 23.7
Trained/Placebo	125.7 ± 35.2	134.8 ± 24.2	135.6 ± 32.3	135.5 ± 41.8	121.6 ± 26.7
Trained/Creatine	135.8 ± 18.2	155.6 ± 11.3	140.3 ± 20.8	135.5 ± 29.1	120.2 ± 30.4

Note: Values are expressed as means ± standard deviations (n = 8/group). All TCr values are expressed as mmol per kilogram of dry muscle mass (mmol · kg^{-1} dm).

Further analysis by supplementation status also revealed a significant main effect for time (F (3.42, 102.56) = 6.79, p = 0.000, effect size = 0.185, observed power = 0.983). Bonferroni post-hoc analysis revealed that TCr was significantly higher at REST than at all other time points (PRE (p = 0.006), POST (p = 0.024), 3POST (p = 0.002), 5POST (p = 0.000). There was no significant main effect for supplement group (p = 0.974) or supplement group x time interaction (p = 0.356).

Finally, analysis by training status also revealed a significant main effect for time (F (3.42, 102.57) = 6.71, p = 0.000, effect size = 0.183, observe power = 0.982).

Bonferroni post-hoc analysis revealed that TCr was significantly higher at REST than all other time points (PRE (p = 0.006), POST (p = 0.037), 3POST (p = 0.001) 5POST (p = 0.000). There was also no significant main effect for training status (p = p = .122) or training status x time interaction (p = 0.246).

Other Muscle Metabolites

Muscle Adenosine Triphosphate

A two-way [treatment group (4) x time point (5)] ANOVA with repeated measures on the second factor revealed a significant a significant main effect for time (Wilks' Lambda = 0.145, F (4, 25) = 36.95, p = 0.000, effect size = 0.855, observed power = 1.0). There was no significant main effect for group (Wilks' Lambda = 0.073, F (12, 19) = 20.08, p = 0.000, effect size = 0.927, observed power = 1.0) or group x time interaction (Wilks' Lambda = 0.491, F (12, 66.4) = 1.709, p = 0.085, effect size = 0.211, observed power = 0.735). Bonferroni post-hoc analysis revealed that ATP was significantly higher at PRE and REST compared to POST, 3POST, and 5POST (p = 0.000 for all pair wise comparisons). There was no significant main effect for group (p = 0.73). Muscle ATP values are shown in table 8.

When analyzed by supplement group, there was a significant main effect for time (Wilks' Lambda = 0.151, F (4, 27) = 38.0, p = 0.000, effect size = 0.849, observed power = 1.0). Bonferroni post-hoc analysis revealed that ATP was higher at PRE and REST compared to POST, 3POST, and 5POST (p = 0.000 for all pair wise comparisons). There was no significant main effect for supplement group (p = 0.537) or supplement group x time interaction (p = 0.101)

Table 8

Muscle Adenosine Triphosphate

Variable	PRE	REST	POST	3POST	5POST
Untrained/Placebo	21.4 ± 2.78	21.6 ± 1.96	14.5 ± 4.27	14.0 ± 2.81	16.5 ± 4.47
Untrained/Creatine	22.2 ± 3.29	20.9 ± 1.94	14.1 ± 2.59	13.2 ± 3.19	14.9 ± 3.33
Trained/Placebo	20.1 ± 2.56	21.8 ± 2.91	16.3 ± 2.06	17.2 ± 2.75	16.1 ± 2.85
Trained/Creatine	21.9 ± 2.44	22.2 ± 2.69	17.1 ± 3.48	13.4 ± 5.36	14.5 ± 3.74

Note: Values are expressed as means ± standard deviations (n = 8/group). All ATP values are expressed as mmol per kilogram of dry muscle mass (mmol · kg^{-1} dm).

The final analysis by training status also revealed a significant main effect for time (Wilks' Lambda = 0.157, F (4, 27) = 36.36, p = 0.000, effect size = 0.843, observed power = 1.0). Bonferroni post-hoc analysis revealed that ATP was higher at PRE and REST compared to POST, 3POST, and 5POST (p = 0.000 for all pair wise comparisons). There were no significant main effects for training status (p = 0.322) or training status x time interaction (p = 0.069). Although non-significant the trained group was able to maintain higher ATP levels within the muscle following exercise, as illustrated in figure 3.

In regards to the re-analysis of subjects who responded to CM supplementation, there were also no significant main effects for group (p = 0.316) or group x time interaction (p = 0.211) for ATP.

Muscle Glycogen

Analysis of muscle glycogen using a two-way [treatment group (4) x time point (5)] ANOVA with repeated measures on the second factor showed a significant main

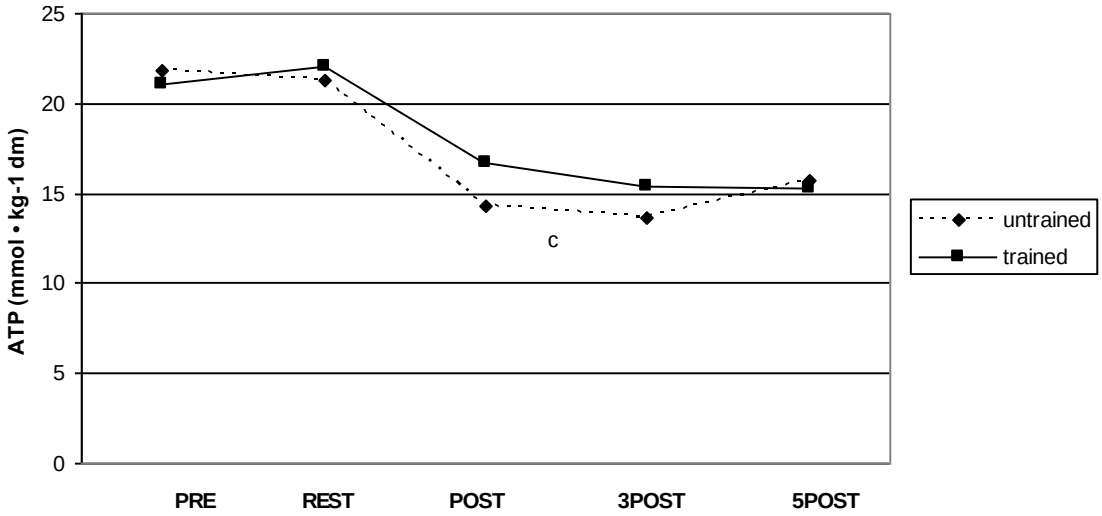

Figure 3. This graph illustrates the non-significant difference (p = 0.322) in muscle ATP between the trained and untrained group at POST and 3POST.

effect for time (Wilks' Lambda = 0.298, F (4, 25) = 14.73, p = 0.000, effect size = 0.702, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant differences in glycogen. Muscle glycogen was significantly higher at PRE than POST (p = 0.032), 3POST (p = 0.000), and 5POST (p = 0.000). Muscle glycogen was also significantly higher at REST than POST (p = 0.001), 3POST (p = 0.000), and 5POST (p = 0.000). Finally, muscle glycogen was higher at POST than 3POST (p = 0.036). There was also a significant main effect for group found for glycogen averaged across all time points (F (3, 28) = 12.54, p = 0.000, effect size = 0.573, observed power = 0.999). Bonferroni post-hoc analysis revealed that glycogen was lower in group 1 compared to group 4 (p = 0.037), and that group 2 was significantly lower than group 1 (p = 0.027), group 3 (p = 0.004), and group 4 (p = 0.000). This seems to suggest that the trained groups on average maintained higher muscle glycogen that the untrained groups. There

was no significant group x time interaction (p = 0.126). Muscle glycogen values are shown in table 9.

When analyzed by supplement group, there was a significant main effect for time (Wilks' Lambda = 0.299, F (4, 27) = 15.81, p = 0.000, effect size = 0.701, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant

Table 9

Muscle Glycogen

Variable	PRE	REST	POST	3POST	5POST
Untrained/Placebo*	271 ± 31.0	333 ± 67.9	267 ± 82.1	265 ± 54.8	209 ± 34.3
Untrained/Creatine†	273 ± 76.9	251 ± 35.9	200 ± 43.1	166 ± 30.2	211 ± 52.5
Trained/Placebo	339 ± 57.8	297 ± 86.3	263 ± 59.7	243 ± 25.1	266 ± 65.5
Trained/Creatine	348 ± 34.0	360± 21.1	329 ± 38.8	264 ± 26.8	280 ± 60.0

Note: Values are expressed as means ± standard deviations (n = 8/group). All glycogen values are expressed as mmol per kilogram of dry muscle mass (mmol · kg^{-1} dm). * indicates significant difference from group 4 (p = 0.034) overall. † indicates significant difference from group 1 (p = 0.027), 3 (p = 0.004), and 4 (p = 0.000) overall.

differences in glycogen. Muscle glycogen was significantly higher at PRE than POST (p = 0.049), 3POST (p = 0.000), and 5POST (p = 0.000). Muscle glycogen was also significantly higher at REST than POST (p = 0.001), 3POST (p = 0.000), and 5POST (p = 0.001). Finally, muscle glycogen was higher at POST than 3POST (p = 0.033). There was no significant main effect for supplement group (p = 0.664) or supplement group x time interaction (p = 0.234).

Finally, analysis by training status also revealed a significant main effect for time (Wilks' Lambda = 0.310, F (4, 27) = 15.0, p = 0.000, effect size = 0.690, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant differences in

glycogen. Muscle glycogen was significantly higher at PRE than POST (p = 0.049), 3POST (p = 0.000), and 5POST (p = 0.000). Muscle glycogen was also significantly higher at REST than POST (p = 0.001), 3POST (p = 0.000), and 5POST (p = 0.001). Finally, muscle glycogen was higher at POST than 3POST (p = 0.046). There was a significant univariate difference for glycogen by training status (F (1, 30) = 16.63, p = 0.000, effect size = 0.357, observed power = 0.976). Bonferroni post-hoc analysis revealed that the trained group had significantly higher muscle glycogen that the untrained group (p = 0.000). There was no significant training group x time interaction (p = 0.57). Figure 4 shows the significant difference between trained subjects compared to untrained subjects.

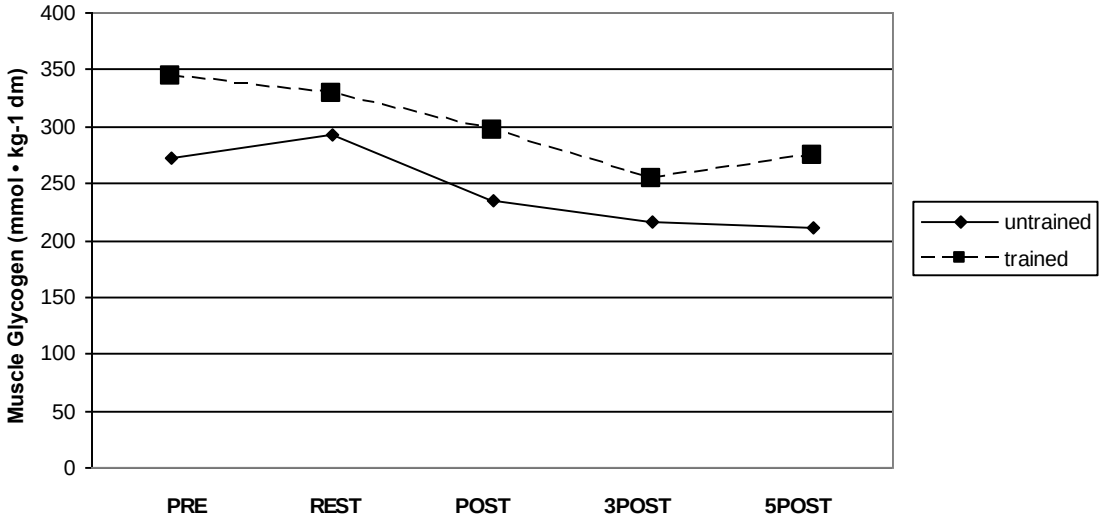

Figure 4. This graph illustrates the significant difference (p =0.000) in muscle glycogen between the trained and untrained groups at all time points.

Muscle Lactate

Analyses of muscle lactate revealed a significant main effect for time (Wilks' Lambda = 0.093, F (4, 25) = 36.95, p = 0.000, effect size = 0.907, observed power = 1.0).

Bonferroni post-hoc analysis revealed the following significant differences for lactate. Muscle lactate at PRE was significantly lower than POST, 3POST, and 5POST ($p = 0.000$ for all comparisons). At REST, muscle lactate was significantly lower than POST, 3POST, and 5POST ($p = 0.000$ for all comparisons). Finally, at POST, lactate was significantly higher than 3POST ($p = 0.003$) and 5POST ($p = 0.000$). There was no significant main effect for group ($p = 0.596$) or group x time interaction ($p = 0.471$) for muscle lactate. Muscle lactate values are shown in table 10.

Table 10

Muscle Lactate

Variable	PRE	REST	POST	3POST	5POST
Untrained/Placebo	25.7± 21.4	22.3 ± 14.6	83.6 ± 25.0	72.8 ± 38.2	62.4 ± 18.0
Untrained/Creatine	25.1 ± 19.4	23.5 ± 19.5	92.5 ± 23.8	65.4 ± 19.0	53.2 ± 16.5
Trained/Placebo	36.5 ± 25.6	18.3 ± 12.9	79.1 ± 22.1	57.0 ± 18.7	44.5 ± 13.0
Trained/Creatine	19.2 ± 11.8	12.2 ± 3.72	79.3 ± 23.3	67.7 ± 3.1	54.6 ± 13.7

Note: Values are expressed as means ± standard deviations (n = 8/group). All lactate values are expressed as mmol per kilogram of dry muscle mass (mmol · kg^{-1} dm).

The analysis by supplement group revealed a significant main effect for time for muscle lactate (Wilks' Lambda = 0.096, F (4, 27) = 63.52, p = 0.000, effect size = 0.904, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant differences for lactate. Muscle lactate at PRE was significantly lower than POST, 3POST, and 5POST ($p = 0.000$ for all comparisons). At REST, muscle lactate was significantly lower than POST, 3POST, and 5POST ($p = 0.000$ for all comparisons). Finally, at POST, lactate was significantly higher than 3POST ($p = 0.003$) and 5POST (p

= 0.000). There was no significant main effect for group (p = 0.825) or group x time interaction (p = 0.846) for muscle lactate when compared across supplement groups.

The analysis by training status revealed a significant main effect for time for muscle lactate (Wilks' Lambda = 0.098, F (4, 27) = 62.29, p = 0.000, effect size = 0.902, observed power = 1.0). Bonferroni post-hoc analysis revealed the following significant differences for lactate. Muscle lactate at PRE was significantly lower than POST, 3POST, and 5POST (p = 0.000 for all comparisons). At REST, muscle lactate was significantly lower than POST, 3POST, and 5POST (p = 0.000 for all comparisons). Finally, at POST, lactate was significantly higher than 3POST (p = 0.003) and 5POST (p = 0.000). There was no significant main effect for group (p = 0.169) or group x time interaction (p = 0.796) for muscle lactate when compared across training groups.

CHAPTER FIVE

Discussion

Creatine monohydrate is one of the most studied and well understood nutritional supplements being sold today. It has been shown in numerous studies to benefit athletes of sports requiring repeated bouts of high intensity exercise, such as football, basketball, and soccer, as well as individuals interested in gaining lean body mass and strength (Aaserud et al., 1998; Casey & Greenhaff, 2000; Greenhaff et al., 1993; Grindstaff et al., 1997; Kreider et al., 1998; Kreider, 2003). The purpose of this study was threefold. First, we wanted to examine the effects CM supplementation would have on PCr and ATP repletion following a 30 second bout of high intensity isokinetic exercise. Secondly, we wanted to examine the role resistance training status played in the recovery of these variables. Our third issue we wanted to investigate was the validity of the NSCA's work to rest ratios for resistance training, and how CM and training status might alter the suggested work to rest ratios. Several studies have examined the effects of CM supplementation or training status on recovery from exercise, but to our knowledge, no studies have reported muscle ATP or PCr recovery using a resistance training model (Snow et al., 1998; Soderlund & Hultman, 1991; Stathis et al., 1994; Yquel et al., 2002). This particular study is unique in that we looked at both resistance trained and untrained individuals using a resistance training model to examine the effects of CM supplementation on recovery of muscle metabolites following a bout of isokinetic exercise.

72 Hour Dietary Recall

As described above, subjects were asked to maintain normal dietary practices for the duration of this study. All subjects were also screened prior to participation in this study to ensure that none of them had used any nutritional supplements for at least 4 weeks prior to participation in this study. Furthermore, we determined if any of the subjects may have been eating a diet that was lacking in animal proteins (vegetarian or vegan diet). Diets that are not high in animal proteins may lead to lower endogenous levels of muscle creatine content, as this nutrient is found in high quantities in animal products, thus resulting in a greater response to CM supplementation. It has been shown that 1.1 kg of raw beef steak contains approximately 5 g of creatine (Harris et al., 1992). Analysis of the 72 hour dietary recalls showed no significant differences in dietary intake between the four groups for total calories consumed, carbohydrate, fat, protein, or protein by body mass. These findings ensured that this study controlled for the effects dietary intake may have on muscle creatine content and served as one confirmation that a homogenous population was evaluated.

Strength Variables

Prior to participation in this study, subjects performed a 1RM for the leg press exercise. This particular test was performed to determine training status of the individuals who claimed to be resistance trained as well as to ensure the homogeneity of the subjects within each group. In order to be considered resistance trained, subjects had to correctly perform the leg press with a weight equal to at least 2.5 times their body weight. There were no significant differences between groups for leg press 1RM, although the trained group had an average 1RM that was 138 pounds greater than the

untrained group. This lack of significant difference in lower body strength based off of training status was an unexpected result. However, several of the trained individuals had not been resistance trained for a long period of time; while several of the untrained individuals were former athletes who had simply not been resistance training recently, yet maintained some degree of lower body strength. This may be expected, as the musculature of the lower body is frequently used during ambulation and daily functional activities, thus lower body strength may be maintained in previously trained, sedentary subjects despite a decrease in activity (Trappe, Costill, Goodpaster, & Pearson, 1996).

Subjects were also required to perform a 5RM at 60 °/second on the Biodex isokinetic dynamometer to determine leg dominance based off of maximal torque production. The leg that was able to produce the largest amount of torque was considered to be the dominant leg and was used for the subsequent 30 second isokinetic exercise bout and muscle biopsies. There were also no significant group differences for isokinetic torque production on either the left or right legs. This lack of difference in lower body torque production between trained and untrained subjects is likely similar to the lack in difference in lower body strength mentioned above.

Blood Lactate

Blood lactate was measured in all subjects participating in this study prior to beginning the isokinetic resistance exercise bout and again five minutes following the exercise bout. Lactate is produced by muscle cells during times of high intensity muscular activity when the anaerobic energy production system known as glycolysis breaks down glucose to produce ATP for continued muscular contraction. As a result of this process taking place anaerobically, lactate and hydrogen are produced along with the

ATP (Spriet, Howlett, & Heigenhauser, 2000). The hydrogen that is produced along with hydrogen that is released during the hydrolysis of ATP into ADP and Pi can build up in muscle tissue decreasing the rate of enzyme activity that can eventually lead to a decrease in muscle power production and cross bridge cycling. When muscle lactate levels rise dramatically, much of the lactate is released into the blood stream to be taken up by other working muscle tissue or the liver via lactate shuttles (Brooks, 2007). Lactate taken up by the liver is then converted back into glucose via the Cori cycle to be released into the blood stream for other body tissue to utilize as energy or stored as glycogen. This rise in lactate seen in the blood has been used as an indicator of exercise intensity and the reliance upon anaerobic glycolysis. One potential result of CM supplementation may be a decreased reliance upon anaerobic glycolytic energy production and therefore a lower rate of lactate and hydrogen production, as more ATP can be produced via the creatine kinase reaction following increases in muscle TCr content, especially during repeated bouts of high intensity activity (Greenhaff et al., 1993). Furthermore, exercise training may also decrease this reliance on anaerobic glycolysis and/or increase the body's ability to utilize lactate as an energy source.

Results from this study showed that neither CM supplementation nor resistance training status significantly affected the amount of lactate found in the blood. There was however a non-significant difference in blood lactate 5 minutes after exercise for individuals who consumed CM compared to those who did not of 1.0 ± 0.85 mmol/L, with the group consuming CM maintaining lower blood lactate levels. This difference is illustrated in Figure 1. This trend may suggest a small difference in the rate of lactate production and therefore a smaller reliance upon anaerobic glycolysis for energy

production. The increase in blood lactate levels seen in this study were similar to previous research (Snow et al., 1998; Zhao et al., 2000). Similar, researchers also found no significant changes in post-exercise blood lactate levels following creatine supplementation (Aaserud et al., 1998; Snow et al., 1998).

Muscle Metabolite Recovery Following Creatine Monohydrate Supplementation

Muscle Free Creatine, Phosphocreatine, and Total Creatine Content

Muscular contraction is carried out using the energy stored in ATP. When a motor neuron carries a nerve impulse to a motor unit, calcium is released from the sarcoplasmic reticulum of the muscle fibers in a single motor unit. The released calcium then binds to a protein known as troponin causing a conformational change in a second protein known as tropomyosin that uncovers the myosin binding site on the actin myofilimant allowing the cross bridge of the myosin myofilimant to attach. In order for muscular contraction, also known as cross-bridge cycling, to take place, ATP must be present. During brief, high intensity muscular contractions, ATP stored within the cytosol can fuel contraction for several seconds, beyond which ATP must be produced via the various energy systems available. The phosphagen energy system provides substrate to produce ATP by giving the phosphate group from PCr to an ADP molecule. PCr is stored in skeletal muscle as a short-term supplier of phosphate for the production of ATP from the chemical reaction catalyzed by the enzyme creatine kinase that produces one ATP molecule from PCr and ADP leaving behind a Cr molecule which can be re-phosphorylated during rest. In an attempt to increase the TCr content of muscle tissue, supplementation of CM has been utilized. Generally, there is an increase in muscular

TCr of as much as 50%, depending on pre-supplementation TCr content. (Harris et al., 1992) However, in some instances, CM supplementation fails to result in an adequate increase TCr. People who do not show an increase of approximately 20% in TCr following CM supplementation are known as non-responders.

The results from this study failed to support our hypotheses, H_0, H_1, H_4, and H_5, which state that subjects consuming CM and subjects considered to be resistance trained would show enhanced PCr repletion rates. We found no significant main effects for group, supplement group, or training status in regards to Cr, PCr, or TCr. These findings are contrary to results of previously published articles suggesting that CM supplementation increases the rate of PCr repletion ((Greenhaff et al., 1994; Yquel et al., 2002). Greenhaff and colleagues found that CM supplementation increased the rate of PCr resynthesis following electrically invoked muscular contraction. However, this was only shown to occur in subjects who had a significant increase in TCr content following CM supplementation, suggesting that creatine non-responders would not have an increase in PCr repletion rates (Greenhaff et al., 1994). Another study performed by Yquel and colleagues found similar results when subjects who supplemented their diets with 20 g of CM per day for 6 days performed maximal plantar flexion. This study also showed increased PCr repletion rates and suggested that the increased rate of PCr repletion led to better maintenance of muscle power output (Yquel et al., 2002). A third study, which looked at PCr repletion rates after sprint cycling found no increase in PCr repletion rates following CM supplementation. The subjects in that particular study showed only moderate increases in TCr (9.5 %) following CM supplementation, and the failure of CM supplementation to produce increased performance or increased PCr repletion rates was

suggested to be caused by the lack of response to CM supplementation (Snow et al., 1998). The results of the previously mentioned research suggests that in order for there to be an effective ergogenic response, increased in TCr following CM supplementation must be substantial.

The subjects in our study consuming CM showed an average increase in TCr of 13.7 mmol · kg^{-1} dm, or 10.2%. According to previous literature, this group as a whole would be considered non-responders (Greenhaff et al., 1994; Syrotuik & Bell, 2004b). In addition, the subjects consuming placebo also showed an increase in TCr on average of 14.0 mmol · kg^{-1} dm, or 11.2%. Previous literature has not shown similar findings. There may have potentially been complications with the assays performed on the muscle tissue samples. Another potential explanation could be that some subjects may have been taking supplements containing CM that they failed to report, despite our instructions for them to refrain from consuming any other nutritional supplements during participation in this study. In attempt to explain why the placebo group showed an increase in TCr, we analyzed the group dietary protein intakes to determine if certain individuals may have been ingesting unusually high amounts of that particular macronutrient, as creatine is found in meat products and may have provided large amounts of dietary creatine. This dietary analysis failed to show any significant differences in overall protein intake or protein intake relative to body mass. Therefore, dietary differences aren't likely the cause of this discrepancy. Furthermore, we performed subsequent statistical analyses on 8 individuals within the CM supplementation group who responded to CM supplementation with increases of approximately 20% in TCr and 8 individuals in the placebo group who did not have an increase in TCr. These further statistical analyses still

failed to show a significant main effect for PCr repletion rates despite our attempts to observe only CM responders.

There was a significant main effect for time for muscle Cr, PCr, and TCr observed with this study. Following the 30 second isokinetic exercise bout, the average PCr depletion for all groups was 60.5%. This data suggests that a large portion of ATP production was provided by the phosphagen energy system during the exercise bout. There was no difference in PCr recovery between supplementation groups with each group achieving PCr repletion of approximately 66% of resting levels. The likely reason for this similarity between supplementation groups being that the group given CM did not have a substantial response to supplementation. These results are very similar to those found by Snow et al. (1998). In their study PCr returned to within 65% of resting levels following 2 minutes of recovery.

One of our hypotheses, H_0, stated that resistance trained individuals would have faster PCr repletion than sedentary individuals. We failed to show statistical support for this hypothesis, but on average, the trained group had higher muscular PCr content at all time points (Figure 2). Resistance exercise training alone has been shown to increase PCr content by 39%, which is a greater response than what is typically seen with CM supplementation (MacDougall, Ward, Sale, & Sutton, 1977). This may be a further explanation as to why the trained subjects in this particular study failed to respond to CM supplementation. On average, the trained group had a pre-supplementation TCr content of 130.8 $\pm$ 27.6 mmol $\cdot$ kg^{-1} dm. It has been suggested that in order for a substantial response to be seen with CM supplementation, pre-supplementation TCr content should be below 120 mmol $\cdot$ kg^{-1} dm (Greenhaff et al., 1994). This may potentially suggest that

supplementation with CM may be more beneficial for untrained individuals than trained individuals.

Muscle Adenosine Triphosphate

Adenosine triphosphate availability is vital to continued muscular contraction at any intensity. The human body is extremely well prepared to handle decreases in ATP due to muscular contraction by producing ATP through various energy systems. During high intensity muscular activity, the phosphagen system is able to supply ATP for approximately 15 seconds before anaerobic glycolysis begins to create ATP by catalyzing glucose. It has been suggested that supplementation with CM may help in recovery of ATP from repeated bouts of high intensity activity by increasing the availability of PCr within the muscle during rest (Balsom, Soderlund, Sjodin, & Ekblom, 1995; Greenhaff et al., 1994).

In regards to ATP, we hypothesized that both the trained individuals and subjects who consumed CM would have higher ATP repletion rates. We failed to show support for this hypothesis, H_2, as there was no significant main effect for group. There was a significant main effect for time indicating that muscular ATP levels fell by approximately 28% from rest to immediately post exercise. Previous research has shown somewhat larger decreases in ATP following high intensity exercise, but similar resynthesis of ATP during recovery (Snow et al., 1998; Zhao et al., 2000). After both 3 and 5 minutes of passive rest, muscle ATP levels did not increase above immediate post exercise levels. This would seem to suggest that the 30 second bout of isokinetic exercise was quite taxing and that 5 minutes of rest was not adequate to allow for repletion of muscular ATP. As with PCr, we found a non-significant difference in ATP between the trained

and untrained groups, with the trained groups being able to maintain higher levels of ATP both after and during recovery from exercise (Figure 3). Previous research has also shown that it may take longer than 5 minutes of rest following extremely high intensity activity to allow for complete recovery of muscle ATP (Snow et al., 1998; Zhao et al., 2000). Zhao and colleagues examined the effects of 30 seconds of sprint cycling on muscle metabolites, including ATP and PCr among others. They found that after five minutes of rest, muscle ATP levels had increased to above immediate post exercise levels, but were still far from resting levels. Furthermore, following ten minutes of rest, muscle ATP levels were still significantly lower than resting levels (Zhao et al., 2000). According to our research, it appears that neither supplementation with CM nor resistance exercise training are able to significantly enhance recovery of muscle ATP following intense exercise.

Muscle Glycogen and Lactate

During high intensity exercise, after muscle PCr stores become depleted, further ATP production is reliant upon availability of muscle glycogen (Hargreaves et al., 1998). Glycogen is broken down to produce glucose for entry into glycolysis. The by-products of anaerobic glycolysis are lactate and ATP. Examining the intramuscular levels of glycogen and lactate before and after intense exercise provides information regarding how much ATP is being provided by this energy system.

Results from our study showed a significant main effect for time for both muscle glycogen and lactate. Muscle glycogen content decreased by approximately 14.5 % across all groups suggesting a substantial amount of ATP was produced via anaerobic glycolysis during the 30 second isokinetic exercise bout. Along with the decrease in

glycogen, there was a significant increase in muscle lactate of 366% across all groups. Furthermore, there was a significant difference in muscle glycogen in the trained group compared to the untrained group with the trained group having a much larger amount of stored muscle glycogen at rest. Additionally, although not significant, the trained individuals maintained lower lactate levels at all time points after exercise suggesting greater clearance rates for lactate. Supplementation with CM had no effect on muscle glycogen or lactate levels.

Work to Rest Ratios

The NSCA suggests that for work lasting 5-10 seconds, relying primarily upon the phosphagen energy system, the rest period should last between 12 and 20 times longer than the work period. Also, the NSCA suggests that for work lasting between 15 and 30 seconds, relying primarily upon the anaerobic glycolysis energy system, the rest period should last between 3 and 5 times longer than the work period. For example, when an exercise set lasts 10 seconds, the suggested recovery interval should last between 120 and 200 seconds to allow for adequate recovery of PCr and ATP. The NSCA also suggests that for the training goals of increasing strength and power, rest periods should last for 2-5 minutes (Baechle et al., 2008).

One goal of this study was to determine the appropriateness of the NSCA's recommended work to rest ratios. We hypothesized, in hypothesis H_6, that the 3 to 5 minute rest period following the 30 second isokinetic bout would be a sufficient amount of time to allow for recovery of muscular PCr and ATP levels. Once again, we failed to show support for this hypothesis. For our study, the work lasted 30 seconds, meaning that the suggested rest period following activities of this duration should last between 90

and 150 seconds. As mentioned previously in this chapter, after both 3 and 5 minutes of rest, neither PCr nor ATP had returned to resting levels. A large amount of research in this area suggests that longer rest intervals result in greater strength increases (Hill-Haas et al., 2007; Pincivero et al., 1997; Willardson, 2006). A recent study examining the effect of rest intervals on the ability to perform repeated exercise sets found that rest intervals of 1, 3, or 5 minutes were not long enough to allow for complete recovery. This study revealed that fewer repetitions were performed for subsequent sets for all rest intervals, indicating that rest intervals lasting up to 5 minutes did not allow enough time to perform the same amount of work as was performed on the first set (Richmond & Godard, 2004). Therefore, in agreement with previous research, our research suggests that longer rest intervals than what are currently recommended may be beneficial for athletes attempting to develop strength and power, although more research in this area should be conducted. By allowing longer rest intervals, athletes would be more likely to increase intramuscular PCr and ATP content, thus allowing greater force and power production during exercise sets. One drawback to lengthening rest intervals would be an overall lengthening of any given training session. With rest intervals lasting longer than 5 minutes, it may take an athlete several hours to complete a typical training session. This would obviously put a large constraint on the number of exercises and sets of each exercise that could be performed during that training session.

Conclusions

The results from this study revealed CM supplementation did not significantly affect repletion rates of ATP or PCr. This was likely due to the fact that there were several non-responders in the CM groups, as has been shown in previous research (Snow

et al., 1998). Furthermore, training status did not significantly affect ATP or PCr repletion rates, although the trained subjects maintained higher levels of PCr and ATP at all time points compared to the untrained subjects. Based on these results, it appears that chronic resistance training has a greater effect on energy substrates than supplementation with CM when response to supplementation is not substantial. Additionally, due to the fact that 5 minutes of rest was not adequate to allow for complete recovery of muscle ATP and PCr, continued research regarding the most effective work to rest ratios should be performed to determine if rest intervals that are longer than what are currently recommended by the NSCA might result in greater gains in muscular strength or power.

APPENDICES

APPENDIX A

Informed Consent

BAYLOR UNIVERSITY

Department of Health, Human Performance, & Recreation
Informed Consent Form

Title of Investigation: Determining related work-to-rest ratios following a maximal effort isokinetic leg extension bout in trained and untrained males: A double-blind creatine/placebo controlled study

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Rationale:

Numerous studies have shown the effectiveness of resistance training in providing maximal strength gains, improved neuromuscular communication, and overall increases in athletic performance. The ultimate goals of resistance training are to prevent injury and maximize neuromuscular adaptations to develop athletes into the best competitors their genetic framework will allow. Strength and conditioning professionals are

ultimately responsible for designing progressive programs that will produce optimal gains in physical aptitude and overall athletic performance.

In order to properly train an athlete, the sport in which they compete must be thoroughly analyzed in order to determine which energy system is principally utilized for energy production. Fatigued muscles are unable to generate maximum force so it is essential to allow complete recovery of the phosphagen system in order to make maximum strength gains. In addition to identifying the primary energy system used, a proper rest interval between exercise bouts must be established. The length of an exercise bout, followed by the length of rest between these bouts can be termed as the work: rest ratio.

Previous studies have investigated effective work: rest ratios in order to maximize training of the aerobic oxidative system. However, research in resistance training work-to-rest ratios is limited. Heavy and intense resistance exercise has been associated with a high rate of energy utilization via phosphagen breakdown and activation of glycogenolysis. This means the high energy ATP-PC system is the predominating energy system. According to Fleck (1983), a 1:3 work: rest ratio is suggested when training for maximum strength. Periodization guru, Tudor Bompa, advises a rest period of 3-5 minutes when training the immediate energy system. The National Strength and Conditioning Association (NSCA) suggests a 1:12-20 work: rest ratio for complete ATP-PC energy system recovery. These numbers have been the norm; however, it remains to be seen if these ratios allow for complete recovery of the ATP-PC system or the total adenine nucleotide pool (TAN). Because of the wide range of time covered in a 1:12-20 recovery period, it may well be implied that the conditioning level of the athlete in training will determine whether a 1:12 or a 1:20 (and anything between) work-to-rest ratio will be used. Age is an additional factor that could potentially influence the work-to-rest ratio needed to adequately recover from a high intensity exercise bout.

Work-to-rest ratios may be further effected by creatine supplementation. Creatine, a nitrogen-containing compound known as an amine, is a derivative of three amino acids. Creatine is found in some foods, particularly meats, and may be formed endogenously in the kidney & liver. In the muscle, creatine combines readily with phosphate to form phosphocreatine (PCr). As previously discussed, PCr is a high-energy phosphagen in the immediate energy system. Creatine has been shown to be a safe and effective ergogenic aid. By supplementing the body with creatine ATP regeneration is able to take place more quickly via the creatine kinase reaction:

$$ADP + PCr + H^{+} \leftarrow^{\text{creatine kinase}} \rightarrow ATP$$

More creatine available in the muscles allows ATP to be regenerated more rapidly. Quicker ATP repletion allows a quicker recovery from high intensity exercise, thus resulting in a lower suggested work-to-rest ratio time. Creatine has been shown to promote gains in fat/bone-free mass, isotonic lifting volume, and sprint performance during intense resistance/agility training. Short-term creatine supplementation has been reported to improve maximal power & strength (5–15%), work performed during sets of maximal effort muscle contractions (5–15%), single-effort sprint performance (1–5%), and work performed during repetitive sprint performance (5–15%). The purpose of this

study is to see if the NSCA work-to-rest ratios are adequate times for PCr and TAN recovery following a maximal isokinetic leg extension bout in trained and untrained males with and without creatine supplementation.

Description of the Study:

I will be one of 40 apparently healthy untrained or trained college aged males who will participate in this study. During an initial familiarization session, I will be informed of the requirements of the study and sign an informed consent statement in compliance with the Human Subjects Guidelines of Baylor University and the American College of Sports Medicine. A trained individual will examine me to determine if I am qualified to participate in this study. If I am cleared to participate in the study, I will be familiarized to the testing procedures

Demographic information, medical history, lifting history, height, weight, skinfold testing (to estimate body composition), thigh circumference, bioelectrical impedance analysis (BIA) (to measure total body water), will all take place during the familiarization session. During the first testing sesstion, a Biodex isokinetic test, height, weight, BIA, a baseline blood draw, and a baseline muscle biopsy will be performed. I understand that I will have the biopsy location identified on the thigh of my dominant leg. The biopsy area will be shaved clean of leg hair, washed with antiseptic soap and cleaned with rubbing alcohol. In addition, the biopsy site will be further cleansed by swabbing the area with Betadine (fluid antiseptic). I understand that a small area of the cleaned skin approximately 2 cm in diameter will be anesthetized with a 1.0 mL subcutaneous injection of the topical anesthetic Lidocaine. Once the local anesthesia has taken effect (approximately 2-3 minutes) the biopsy procedure will only take 15-20 seconds. Once anesthetized, I understand that a scalpel point will be used to make an incision approximately 1 cm in length through the skin. Due to the localized effects of the anesthetic, however, I should feel no pain during this process. At this point, I understand that the biopsy needle will be advanced into the incision approximately 1 cm and during this part of the procedure I may feel pressure in my thigh area. Once the muscle sample has been obtained, pressure will be immediately applied to the incision. I understand that five biopsies will be taken during the testing session, 1) baseline measurement during the first testing session and 4) at the third testing session at pre exercise, post exercise and the time point corresponding with a 1:12 and a 1:20 work-to-rest ratio. With the incision being so small bleeding is slight; therefore, only a butterfly bandage is needed to close the incision, which will then be covered with a pressure bandage. I understand that I will be provided verbal and written instructions for post-biopsy care. I understand that I will be instructed to leave the butterfly bandage in place for 72 hours. However, I understand that I will be asked to report back to the lab 24 hours after the biopsy (unless unexpected bleeding or pain occurs) to have the old bandage removed, the incision inspected and new bandages applied, and that I will also report back to the lab for the same reason at 48 hours after the biopsy. I will be further advised to refrain from vigorous physical activity with my leg during the first 24 hours post-biopsy. I understand that if I feel it necessary I may take a non-prescription analgesic medication such as Tylenol to relieve pain if

needed and that some soreness of the area may occur for about 24 hours after the biopsy. I will also be advised to avoid such medications such as aspirin, Advil, Bufferin, or Nuprin, as they may lead to bruising at the biopsy site. I understand that five biopsies will be taken between two occasions during the study; one during testing session 1 and four (4) during testing session 3. The first familiarization session will take approximately 1.5 hours to complete. Once I complete the familiarization session, I will be scheduled for the first testing session.
Following the familiarization session, I will be instructed to refrain from resistance exercise for the duration of the study. I will also be instructed to fast for 8 hours prior to the first testing session. I will be provided with a 3 day dietary analysis form that I am to complete for 3 days, not necessarily directly prior to testing. Once I report to the lab for each testing session, I will turn in my dietary analysis and supplement forms, along with empty supplement packet. I also understand that I will be asked to provide a total of 3 food logs and 3 supplement logs over the course of the study.

I understand that during a blood draw I will donate about 6-12 milliliters (3-4 teaspoons) of venous blood from a vein in my arm. Blood samples will be obtained using standard/sterile procedures using a needle inserted into a vein in my arm. I understand that personnel who will be taking my blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5 minutes and I understand that I will be asked to donate the same volume of blood on five separate occasions throughout the study corresponding to the same time points as the biopsy collections.

I will be matched by my age, lean body mass, thigh circumference & training history, and then randomly assigned to ingest in a double blind manner supplement powder containing either a: 1) dextrose placebo, or 2) creatine monohydrate. I will be asked to orally ingest my selected supplement at a daily dose of 20g/day (4 x 5 g/day) for 4 days in the loading phase and, immediately following the loading phase, a daily dose of 5 g/day during the remainder of the study. I will be instructed not to change my routine dietary intake and to ingest the supplements in equal intervals at 8:00 am, 12:00 pm, 4:00 pm, and 8:00 pm each day during the loading phase, and at 8:00 am during the maintenance phase. Compliance to the supplementation protocol will be monitored by having me return the empty supplement container at the beginning of the following lab session, at which point I will be given the required supplement dosage for the time remaining until the third testing session (T_3). I understand that if I do not take my supplements I will be removed from the study, and I also understand that I will cease supplementation on the last day of the study, testing session three (T_3).

During the second testing session, I will complete a 1 repetition maximum leg press lift (1-RM). I will sit in the leg press apparatus with my feet about shoulder width apart. After the investigator completes verbal instructions, I will then complete a 1-RM leg press test according to NSCA guidelines. I understand that this test is to be performed in order to determine resistance training status of my lower body. In order to be considered resistance trained I will need to be able to lift a weight that is greater than or equal to 2.5 times my body weight.

Testing Session
Four days after the third, and final, familiarization session I will return to the lab after an 8 hour fast, to complete the final testing session. Before testing begins, I will turn in the final completed food log and supplementation log. Before I am prepared for the testing day leg press bout I will have a final BIA completed. Once these tasks are completed I will be prepared for a pre-exercise biopsy and blood draw.

I understand that following 4-day loading phase, I will immediately begin maintenance dosages of supplementation, to end on the last day of the study. I understand that on the last lab session I will have four biopsies and blood draws performed before and after I complete an isokinetic leg extension bout I understand that I am expected to maintain my normal dietary habits as well as record 3 day food logs before each lab session. I understand that before I report to the lab for any familiarization sessions or testing sessions I am expected to be fasted for a minimum of 8 hours. I understand that I am expected to complete a supplement log to record the times I ingested the supplement provided to me. I agree to do my best to: 1) follow the instructions outlined by the investigators; 2) show up to all scheduled testing times; and 3) take supplements as instructed. I agree not to engage in any resistance training for the duration of the study. I agree not to take any other nutritional supplements or performance enhancing aids during this study (ie vitamins/minerals, creatine, HMB, androstenedione, DHEA, etc). In addition, I agree not to take any non-medically prescribed medications and to report any medication that is prescribed for me to take during this study. I understand that if I take any other nutritional supplements or medications during the course of the study that I may be removed from the study.

Exclusionary Criteria
I understand that in order to participate in the study, a trained individual will examine me to determine whether I qualify to participate. I understand that I will not be allowed to participate in this study if: 1.) I have any known metabolic disorder including heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; 2.) I have a history of pulmonary disease, hypertension, liver or kidney disease, musculoskeletal disorders, neuromuscular or neurological diseases, autoimmune disease, cancer, peptic ulcers, or anemia; 3.) I am taking any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (ie, thyroid, insulin, etc), psychotropic, neuromuscular/neurological, or androgenic medications; 4) I have any bleeding disorders.

I have reported all nutritional supplements, medically prescribed drugs, and non-medically prescribed drugs that I am presently taking. I have reported whether I have had any prior allergic reactions to topical anesthetics. I have completed medical history questionnaires and am not aware of any additional medical problems that would prevent me from participating in this study. I agree to report all changes in medical status, nutritional and/or pharmacological agents (drugs) that I take during the course of the investigation to Maureen Head (515-556-4434). I understand that if I experience any unexpected problems or adverse events from participating in this study I may be referred to discuss the problem with Lori Greenwood, PhD, LAT, ATC. Upon her discretion, I may be referred to discuss the matter with Dr. Ronald Wilson to determine whether any medical treatment is needed and/or whether I can continue in the study.

Risks and Benefits
I understand that the supplement used in the study has various medical uses in humans and that research has demonstrated that oral administration of creatine is not associated with any significant medical side effects. Creatine monohydrate is currently available in over the counter nutritional supplements sold in United States and Europe. As with any food or nutritional supplement, possible side effects may include stomach upset, gastrointestinal distress, allergic reactions, changes in mood and vigor, and/or changes in training adaptations. However, as with the vast majority of nutritional supplements, I understand that the FDA may not have evaluated the safety or marketing claims of creatine.

On five separate occasions during this study, I understand that I will have about 3-4 teaspoons (6-12 milliliters) of blood drawn from a vein in my forearm using a sterile needle and blood tubes by an experienced phlebotomist following an 8-hour fast. This procedure may cause a small amount of pain when the needle is inserted into my vein as well as some bleeding and bruising. I may also experience some dizziness, nausea, and/or faint if I am unaccustomed to having blood drawn.

On five occasions during this study, I understand that I will undergo muscle biopsies in which a small sample of muscle will be obtained from the thigh of my dominant/exercised leg. I will have one baseline biopsy during the first testing session and four biopsies during the third testing session. I understand that Matthew Cooke, Ph.D. will perform all of the biopsies and that a local anesthetic (Lidocaine) will be injected into the skin of my thigh prior to the biopsy which will help prevent pain and discomfort during the procedure. I understand that I will have a small incision made in my skin and a biopsy needle introduced 1 cm into the incision. I also understand that the incision is so small that it will not require any stitches and will be simply closed with a butterfly bandage and then covered with an adhesive bandage (band-aid). After the anesthetic wears off within 3-4 hours, I understand that the sensation at the biopsy site is comparable to that of a bruise and may persist for 24-36 hours after the procedure. I understand that I am required to inform the study's investigators if I have had any prior allergic reactions to anesthesia (e.g. while in the hospital or during a dental visit).

I understand that the main benefits that may be obtained from this study are the potential ergogenic benefits of creatine supplementation, which may include increased muscular strength, power, and reduced recovery time from resistance training. I also understand that all of my analyzed muscle and blood samples will be discarded in an appropriately-labeled biohazard waste disposal container.

Alternative Treatments
This is not a medical treatment. Therefore, if medical treatment is needed, I must continue to obtain treatment for any medical problem I might have from my personal physician.

Costs and Payments
If I am a Baylor University student, I will not receive any academic credit for participating in this study. I understand that if I am an intercollegiate scholarship athlete I may not be eligible to receive payment to participate in this study. Eligible subjects will be paid $100 for completing the familiarization and experimental testing sessions. I also understand that I may request the results of any tests performed on me.

New Information
Any new information obtained during the course of this research that may affect my willingness to continue participation in this study will be provided to me. In addition, I will be informed of any unusual/abnormal clinical findings in which medical referral to my personal physician may be warranted. If I desire, I may request that this information be provided to my physician.

Confidentiality
I understand that any information obtained about me in this research, including medical history, laboratory findings, or physical examination will be kept confidential to the extent permitted by law. In addition, I understand that my records of this research may be subpoenaed by court order or may be inspected by federal regulatory authorities. I understand that data derived may be used in reports, presentations, and publications. However, I will not be individually identified unless my consent is granted in writing. Additionally, that confidentiality will be maintained by assigning code numbers to my files, limiting access to data to research assistants, locking cabinets that store data, and providing passwords to limit access to computer files to authorized personnel only. I understand that once blood and muscle samples are analyzed that they will be discarded.

Right to Withdrawal
I understand that I am not required to participate in this study and I am free to refuse to participate or to withdraw from the study at any time. Further, that my decision to withdraw from the study will not affect my care at this institution or cause a loss of benefits to which I might be otherwise entitled. If there is concern about my medical safety, I may be referred to seek medical attention.

Compensation for Illness or Injury
I understand that if I am injured as a direct result of taking part in this study, I should consult my personal physician to obtain treatment. I understand that the cost associated with the care and treatment of such injury will be the responsibility of me or my insurance carrier. In some cases, insurers may not reimburse claims submitted for a research-related injury resulting from medical procedures or treatments performed as part of a research study. I understand that Baylor University, the investigator's institutions, and the grant sponsor have not budgeted funds to compensate me for injury or illness that may result from my participation in this study and thus will not be accountable for illness

or injury acquired during the course of this study. However, I may be referred to my personal physician if any clinically significant medical/psychological findings are observed during the course of this study.

I agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss I suffer as a result of my participation in this study regardless of the cause of my injury, damage or loss.

Statement on Conflict of Interest
I understand that researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

Voluntary Consent
I certify that I have read this consent form or it has been read to me and that I understand the contents and that any questions that I have pertaining to the research have been, or will be answered by Mike Greenwood, Ph.D. (principal investigator, Department of Health, Human Performance & Recreation, 109 Marrs McLean Gymnasium, Baylor University, phone: 254-710-7987) or Adam Parker, MS (Doctoral Level Graduate Assistant, Department of Health, Human Performance & Recreation, 122 Marrs McLean Gymnasium, Baylor University, phone: 254-733-3286) or one of the research associates. My signature below means that I am at least 18 years of age and that I freely agree to participate in this investigation. I understand that I will be given a copy of this consent form for my records. If I have any questions regarding my rights as a research participant in this study, I may contact Baylor's University Committee for Protection of Human Subjects in Research. The chairman is Dr. Matt Stanford, Professor, Department of Psychology and Neuroscience, P.O. Box 97334, Waco, TX 76798-7334, phone number 254-710-2961.

Date _____ Participant's Signature _____

I certify that I have explained to the above individual the nature and purpose of the potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature. I have explained the above to the volunteer on the date stated on this consent form.

Date _____ Investigator's Signature _____

APPENDIX B

IRB Approval

Part 2: Introduction & Rationale

Numerous studies have shown the effectiveness of resistance training in providing maximal strength gains, improved neuromuscular communication, and overall increases in athletic performance (4, 11, 12, 13, 21, 22). The ultimate goals of resistance training are to prevent injury and maximize neuromuscular adaptations to develop athletes into the best competitors their genetic framework will allow. Strength and conditioning professionals are ultimately responsible for designing progressive programs that will produce optimal gains in physical aptitude and overall athletic performance.

In order to properly train an athlete, the sport in which they compete must be thoroughly analyzed in order to determine which energy system is principally utilized for energy production (23). Fatigued muscles are unable to generate maximum force so it is essential to allow complete recovery of the phosphagen system in order to make maximum strength gains (33). In addition to identifying the primary energy system used, a proper rest interval between exercise bouts must be established. The length of an exercise bout, followed by the length of rest between these bouts can be termed as the work: rest ratio (5).

Previous studies have investigated effective work: rest ratios in order to maximize training of the aerobic oxidative system (6, 8, 20, 30). However, research in resistance training work-to-rest ratios is limited. Heavy and intense resistance exercise has been associated with a high rate of energy utilization via phosphagen breakdown and activation of glycogenolysis (29). This means the high energy ATP-PC system is the predominating energy system. According to Fleck (1983), a 1:3 work: rest ratio is suggested when training for maximum strength. Periodization guru, Tudor Bompa, advises a rest period of 3-5 minutes when training the immediate energy system (2). The National Strength and Conditioning Association (NSCA) suggests a 1:12-20 work: rest ratio for complete ATP-PC energy system recovery (21). These numbers have been the norm; however, it remains to be seen if these ratios allow for complete recovery of the ATP-PC system and the total adenine nucleotide pool (TAN), which consists of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Because of the wide range of time covered in a 1:12-20 recovery period, it may well be implied that the conditioning level of the athlete in training will determine whether a 1:12 or a 1:20 (and anything between) work-to-rest ratio will be used. Age is an additional factor that could potentially influence the work-to-rest ratio needed to adequately recover from a high intensity exercise bout.

Work-to-rest ratios may be further effected by creatine supplementation. Creatine, a nitrogen-containing compound known as an amine, is a derivative of three amino acids. Creatine is found in some foods, particularly meats, and may be formed endogenously in the kidney & liver. In the muscle, creatine combines readily with phosphate to form phosphocreatine (PCr). As previously discussed, PCr is a high-energy phosphagen in the immediate energy system (35). Creatine has been shown to be a safe and effective ergogenic aid (1,7,14,16,17,19,24,28,31,34,35). By supplementing the body with creatine, ATP regeneration is able to take place more quickly via the creatine kinase reaction:

$$ADP + PCr + H^{+} \xleftarrow{\text{creatine kinase}} \rightarrow ATP \quad (9)$$

More creatine available in the muscles allows ATP to be regenerated more rapidly. Quicker ATP repletion allows a quicker recovery from high intensity exercise, thus resulting in a lower suggested work-to-rest ratio time. Creatine has been shown to promote gains in fat/bone-free mass, isotonic lifting volume, and sprint performance during intense resistance/agility training (15,17). Short-term creatine supplementation has been reported to improve maximal power & strength (5–15%), work performed during sets of maximal effort muscle contractions (5–15%), single-effort sprint performance (1–5%), and work performed during repetitive sprint performance (5–15%) (17). It remains to be seen if creatine supplementation improves recovery following a maximum lift using the NSCA's 1:12-20 work-to-rest ratio guidelines for training of the phosphagen system.

Part 3: Methodology

Methods

Participants

Forty (40) college aged males will volunteer to participate in this study. Twenty (20) of these participants will be resistance trained in the lower body, and Twenty (20) untrained. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) will be allowed to participate. Participants will not be allowed to take part in the study if they have ingested any type of nutritional supplement within the past six months (excluding a multi-vitamin). All eligible participants will be asked to provide verbal and informed written consent before any data collection occurs. The following events will be explained to the participants: 1) purpose of the study, and 2) the protocol to be followed throughout the study. All experimental procedures involved in the study will conform to the ethical consideration of the Helsinki Code. All participants who complete the study will receive a monetary compensation of $100 due to the invasive nature of the muscle biopsies they are required to undergo.

Study Site
All supervised testing and supplement assignment will be conducted in the Exercise & Sport Nutrition Laboratory (ESNL) at Baylor University. All sample analyses will be completed in the Exercise and Biochemical Nutrition Laboratory (EBNL) at Baylor University.

Independent and Dependent Variables
Independent variables include trained or untrained status, creatine supplementation, placebo supplementation, intensity of the 15 second isokinetic exercise bout, work-to-rest ratios, and the number of muscle biopsy samples taken before and after the isokinetic resistance training bout. The dependent variables include phosphocreatine repletion and adenine nucleotide changes which will be assessed using biochemical analysis of obtained muscle and blood samples. Muscle samples will be analyzed for total creatine, FCr, and phosphocreatine levels, ATP, ADP, AMP, inosine monophosphate, hypoxanthine, and inosine. Blood samples will be analyzed for hypoxanthine and inosine.

Entry and Familiarization Session
Each participant will be interviewed by telephone to ensure they meet the criteria of the study. Interviewers will verify that trained participants have indeed lifted weights at least one day per week with their legs, for at least six months prior to the study. Interviewers will also be responsible for making sure participants have not taken any type of supplements at least six months prior to the study. Once participants meet the criterion of the study, they will come to the lab and fill out informed consent papers. During the familiarization session (FAM) they will be given a thorough written and oral explanation of the requirements of the study and the testing protocol they will undergo.

Following the completion of the informed consent papers, descriptive information will be acquired (ie. height, weight, fat-free mass, bioelectrical impedance analysis, thigh circumference, training status, medical history). The initial food log for participants to complete will be handed out at this time as well. Participants will be instructed to follow their normal diet for the duration of the study. Food logs will be recorded for one weekend day and two weekdays prior to the first testing session (T_1). Three total days should be recorded in their food log. Participants will also be asked to refrain from resistance exercise for the remaining time they are involved in the study.

During the first testing session, total mass and height of all participants will be measured using a standard dual beam balance scale with height rod attached (Detecto, Webb City, MO). Fat-free mas will be determined by a skilled technician using Lange calipers (Beta Technology, Inc., Santa Cruz, CA). A 7 site skin fold test will be used with measurements taken at the tricep, chest, thigh, abdominal, midaxillary, subscapular, and suprailiac locations. A 7 site prediction equation will be used to estimate body fat percentage, and fat free mass (10). Total body water will be measured using bioelectrical impedance analysis (BIA). By determining the electrical impedance of body tissues, an

estimate of total body water (TBW) is possible. BIA will be measured using the Omron HBF-306 Bioelectrical Impedance Analyzer (Omron Healthcare Inc., Vernon Hills, IL). A trained research assistant will perform this procedure. Thigh circumference will be obtained using a tape measure. Participants will stand with one foot placed on a bench so the knee is flexed to 90 degrees. A measure will be taken midway between the inguinal crease and the proximal border of the patella, perpendicular to the long axis. The tape measure will be placed on the skin without compressing the subcutaneous adipose tissue. Duplicate measures at each site will be taken and retested if the duplicate measurements are not within 5 mm (32). Next, participants will be tested for leg dominance using the Biodex System 3 Pro Isokinetic Dynamometer (Biodex Medical Systems, Shirley,NY). Leg dominance will be decided by a Biodex System 3 Pro Isokinetic Dynamometer (Biodex Medical Systems, Shirley,NY). Peak torque will be measured and the leg with the highest torque generation will be deemed the dominant leg. Both legs will be tested for torque production using a speed of 60° degrees per second for 5 repetitions. The order in which legs will be tested will be randomized. Participants will be required to cross their arms across their chest while performing the isokinetic test. The leg with the most torque production ability will be the dominant leg.

Following the determination of leg dominance participants will then have a baseline biopsy taken from the dominant leg. Wound care instructions will be given to subjects following this biopsy so they are familiar with how to properly treat the incision. Additionally, a baseline blood draw will be collected at this time. Following this last testing procedure, participants will be given their supplement packets to last until the next testing session.

A supplement or placebo dose will be pre-measured for each participant. The pre-measured supplement or placebo doses will be given to participants with written instructions on the loading phase. For the loading phase participants will ingest 5 g/day of supplement or placebo, 4 times per day (20 g/day). Preferably doses will be ingested at 8am, noon, 4pm, and 8pm to maximize absorption, as well as to regulate supplement ingestion among all participants. A supplementation log will be given to subjects for the recording of supplement ingestion and times of ingestion. Participants will be asked to return empty supplement packets for additional monitoring of supplement ingestion.

Four days after T_1 when the creatine loading phase is complete, participants will return to the lab for the second testing session (T_2). Participants will turn in completed food and supplementation logs. New food and supplementation logs will be handed out at this time, as well as a pre-measured maintenance dose of supplement. Participants will be instructed to consume 5 g of supplement once daily (8 am) for the remainder of the maintenance dosage period.

Participants will then complete a 1 repetition maximum leg press lift (1-RM) to determine their training status. When participants are seated in the leg press apparatus, (Nebula Fitness, Inc., Versailles, OH) foot placement will be such that the participants' feet are about shoulder width apart. This placement will be recorded by using numbers placed on the leg press foot plate to ensure consistency between lifting bouts. Placement

of the leg press sled height will also be recorded to ensure the correct range of motion for each participant. Proper technique will be described to the participant and technique will be monitored throughout the testing session to ensure internal validity and consistency between participants. Proper technique includes lowering the leg press sled until the knees are at a ninety degree (90°) angle, placing the hands on the handle bars placed at the side of the leg press to prevent the arms from pushing on the legs, keeping the knees in line with the feet and keeping the back and glutes in constant contact with the leg press seat. Participants will then complete a 1-RM leg press test according to the following NSCA guidelines (21):

1) Primary investigator will instruct the participant to warm up with a light resistance that easily allows 5-10 repetitions.
2) Provide 1 minute rest period.
3) Estimate a warm up load that will allow the participant to complete 3-5 repetitions by adding
    a. 30-40 pounds or 10-20% for lower body exercise
4) Provide a 2 minute rest period
5) Estimate a conservative, near maximum load that will allow the participant to complete 2-3 repetitions by adding
    a. 30-40 pounds or 10-20% for lower body exercise
6) Provide a 2-4 minute rest period
7) Make a load increase
    a. 30-40 pounds or 10-20% for lower body exercise
8) Instruct the participant to attempt a 1RM.
9) If the participant is successful, provide a 2-4 minute rest period and go back to step 7. If the athlete failed provide a 2-4 minute rest period and decrease the load by 15-20 pounds or 5-10% for lower body exercise and then go back to step 8.

Continue increasing or decreasing the load until the participant can complete one repetition. Ideally, the participant's 1RM will be measured within 5 testing sets. Investigators will be assured that trained participants are truly trained if they achieve a leg press max of at least 2.5 times their body weight (18).

Following the leg press testing protocol, completed food and supplementation logs will be turned in to the investigator and new food and supplementation logs will be handed out for completion before (T_3). Additional supplement (pre-measured doses of 5 g per day) will be handed out at this time as well. Participants will be instructed to continue the maintenance dose and supplementation logs until their next session.

Testing Session

Participants will return to the lab three days after T_2 to complete T_3 to ensure that symptoms of DOMS are not negatively influencing leg press performance (3,27). Before testing begins, participants will turn in a completed food log and supplementation log to ensure participants have not deviated from their normal diet, in addition to making sure the creatine or placebo supplement was ingested correctly.

Before participants receive a local anesthetic they will have a final BIA completed. Once these tasks are completed Lidocaine will be administered at four points around the biopsy site in the vastus lateralis to numb the area to be biopsied. Once the local anesthetic takes effect, a baseline biopsy and blood draw will be collected to determine resting PCr and TAN levels. Following the baseline biopsy a bandage will be placed over the biopsy site. Participants will then move to the Biodex isokinetic exercise apparatus to complete a 15 second isokinetic leg extension bout (60 °/sec).

Immediately post exercise participants will undergo the second (post exercise) biopsy and blood draw. Third and fourth biopsies and blood draws will be taken at time points that are commensurate with the 1:12 (3 min) and 1:20 (5 min) work-to-rest ratios.

Muscle Biopsies and Venous Blood Sampling

Percutaneous muscle biopsies (50-70 mg) will be obtained from the middle portion of the vastus lateralis muscle of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. For the remaining four biopsies, attempts will be made to extract tissue from approximately the same location as the initial biopsy by using the pre-biopsy incision and depth markings on the needle. After removal, muscle specimens will be immediately frozen in liquid nitrogen and then stored at -70°C for later analysis. A total of five muscle samples will be obtained: 1) a baseline biopsy will be collected during T_1 2) prior to the isokinetic leg extension bout on testing day; 3) immediately post exercise; 4) at the time point corresponding to a 1:12 work-to-rest ratio (3 minutes post exercise); 5) at the time point corresponding to a 1:20 work-to-rest ratio (5 minutes post exercise).

Venous blood samples will be obtained from the antecubital vein into a 10 ml collection tubes using a standard Vacutainer^{TM} apparatus. Blood samples will be allowed to stand at room temperature for 10 min and then centrifuged. The serum will be removed and frozen at -70°C for later analysis. Five (5) blood samples will be obtained: 1) a baseline blood draw will be collected during T_1; 2) prior to the isokinetic leg extension bout on testing day; 3) immediately post exercise; 4) at the time point corresponding to a 1:12 work-to-rest ratio (3 minutes post exercise); 5) at the time point corresponding to a 1:20 work-to-rest ratio (5 minutes post exercise).

Serum creatine levels will be assessed using an enzymatic, spectrophotometeric assay. Using the same assay, the skeletal muscle levels of free creatine (FCr), total creatine, and phosphocreatine (PCr) will also be assessed. This assay will evaluate muscle FCr, total creatine & PCr levels at different time points before supplementation and following the isokinetic leg extension bout. Additionally, skeletal muscle levels of ATP, ADP, AMP, IMP, inosine, and hypoxanthine, and blood levels of inosine and hypoxanthine will be assayed using high performance liquid chromatography.

Anthropometric and Body Composition Testing Procedures

Total body mass (kg) will be determined on a standard dual beam balance scale (Detecto, Webb City, MO). Percent body fat, fat mass, and fat-free mass, will be determined using a Lange skinfold calipers. A skilled skinfold technician will perform this procedure. Total body water (total, intracellular, and extracellular) will be determined with bioelectrical impedance (BIA). Thigh circumference will be obtained through the measurement of thigh circumference using a tape measure. Participants will stand with one foot placed on a bench so the knee is flexed to 90 degrees. A measure will be taken midway between the inguinal crease and the proximal border of the patella, perpendicular to the long axis. The tape measure will be placed on the skin without compressing the subcutaneous adipose tissue. Duplicate measures at each site will be taken and retested if the duplicate measurements are not within 5 mm (32). Participants will be weighed, have thigh circumference measured and receive a skinfold measurement only during the first familiarization session. However, BIA will be taken during all three familiarization sessions as well as the final testing session.

Dietary Analysis

Participants will be required to record their dietary intake for three days prior to familiarization sessions two and three, and, prior to the testing session. The participants' diets will not be standardized, however, participants will be asked not to change their dietary habits during the course of the study. The three day food logs will be evaluated with the Food Processor dietary assessment software program to determine the average daily macronutrient consumption of fat, carbohydrate, and protein in the diet for the duration of the study.

Supplementation Protocol

Participants will be randomly assigned to ingest in a double blind manner powder supplement containing dextrose placebo or creatine monohydrate. After baseline testing procedures, participants will ingest creatine or the placebo at a daily dose of 20 g/day (4 x 5 g/day) for 4 days in the loading phase and, immediately following the loading phase, a daily dose of 5 g/day will be ingested during the maintenance phase. The maintenance phase will last for 6 days, long enough for participants to complete the study. All groups will cease supplementation on T_3.

In order to standardize supplement intake throughout the study, participants will be instructed to ingest the supplements in equal intervals at 8:00 am, 12:00 pm, 4:00 pm, and 8:00 pm each day during the 4 day loading phase. During the maintenance phase participants will be instructed to ingest the supplement at 12:00 pm. Dextrose and creatine powders will be comprised of similar mesh size, texture, taste, and appearance and will be prepared for distribution by an objective third-party company (AST Sport Science, Colorado Springs, CO). Compliance to the supplementation protocol will be monitored by having the participants return empty supplement containers when they return to the lab for their next familiarization or testing session. In addition, participant's compliance will be verified by weekly verbal communication. After completing the

compliance procedures the participants will be given the required supplement dosage to continue supplementation until the next lab session.

Isokinetic Strength Assessment
Leg dominance will be decided by a Biodex System 3 Pro Isokinetic Dynamometer (Biodex Medical Systems, Shirley,NY). Additionally, the 15 second isokinetic exercise bout (60 °/sec) will be performed on this machine.

Creatine Analysis
Serum and muscle tissue samples will be analyzed spectrophotometrically for total creatine, free creatine, and phosphocreatine by the diacetyl/α-napthtol reaction. Serum and muscle creatine levels will be determined from the five blood and muscle samples provided. Additionally, from each muscle sample obtained, free creatine and phosphocreatine will also be determined.

Total Adenine Nucleotide Pool Analysis.
Muscle tissue samples will be analyzed for adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, inosine monophosphate, inosine, and hypoxanthine using high performance liquid chromatography, and serum samples will be analyzed for inosine and hypoxanthine.

Research Team

Mike Greenwood, PhD, FNSCA, CSCS*D, FACSM, FISSN. Dr. Greenwood is Professor and Graduate/Research Coordinator in the Department of HHPR at Baylor University. Dr. Greenwood's research expertise is in exercise nutrition predominately creatine supplementation and strength related exercise programming. Dr. Greenwood will serve as the primary investigator in this study.

Matthew Cooke, PhD. Dr. Cooke is an Assistant Professor in the Department of Health, Human Performance, & Recreation at Baylor University. Dr. Cooke himself will perform all of the muscle biopsies as he has extensive training and experience with this technique. Dr. Cooke will also assist with the biochemical and clinical chemistry assays involved in the project.

Lori Greenwood, PhD, LAT, ATC. Dr. Greenwood currently serves as Associate Professor in the Department of HHPR at Baylor University. Dr. Greenwood will assist in data collection as well as provide medical supervision for subjects involved in the study.

Richard Kreider, PhD, FACSM, EPC, FASEP. Dr. Kreider is a Professor and Chair of the Department of Health, Human Performance, and Recreation at Baylor University. Dr. Kreider, is a prominent exercise nutrition scientist and has performed numerous studies on the effects of sports supplements and exercise. Dr. Kreider will assist with study design and analysis.

Matthew Stanford, PhD. Dr. Stanford is a professor of Psychology, Neuroscience, and Biomedical studies.

Adam Parker, MS, CSCS. Mr. Parker is a doctoral student in the Exercise, Nutrition, and Preventive Health program in the Department of Health, Human Performance, & Recreation at Baylor University. Mr. Parker will be using the data generated during this study for his doctoral dissertation. He will recruit and familiarize subjects, administer the supplements, assist with the muscle biopsies, and perform the majority of the biochemical and clinical chemistry assays involved in the project.

Ronald Wilson, MD. Dr. Wilson serves as medical supervisor for the ESNL, EBNL, and Center for Exercise, Nutrition & Preventive Health Research (CENPHR).

Additional Graduate Assistants. The assistance of Baylor University graduate assistants in the department of Health, Human Performance, and Recreation will be utilized when necessary. Graduate assistants involved in any part of data collection will be familiarized with all aspects of the study.

Procedures

Medical Monitoring. Interested participants will be contacted by telephone and asked preliminary questions to determine if they are eligible for the study. If the investigator finds that preliminary requirements of the study are met, an appointment will be made for participants to come to the lab for the first familiarization session. During this time, participants will sign informed consent papers and complete medical history information. Participants will then undergo a general exam by the research nurse to determine whether the participant meets entry criteria to participate in the study. This exam will include evaluating the medical and training history questionnaires and performing a general physical examination according to ACSM exercise testing guidelines. Based on this examination, participants will be assessed for their risk of cardiovascular disease and contraindications to exercise and then a recommendation will be made on whether the participant meets entry criteria and may therefore participate in the study. Trained, non-physician exercise specialists certified in CPR will supervise participants undergoing

testing and assessments. A telephone is in the laboratory in case of any emergencies, and there will be no less than two researchers working with each participant during testing sessions. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance. Participants will be informed to report any unexpected problems or adverse events they may encounter during the course of the study to Dr. Lori Greenwood, PhD. If clinically significant side effects are reported, the participants will be referred to discuss the problem with the laboratory nurse, and if deemed necessary the participant will be referred to Ronald Wilson, MD for medical follow-up. Dr. Wilson is one of the Sports Medicine physicians for Baylor University and is an adjunct Professor in the Department of HHPR. He has agreed to provide medical support and consultation for this study and to Exercise and Sport Nutrition Laboratory lab. Dr. Wilson will evaluate the complaint and make a recommendation as to whether any medical treatment is needed and/or whether the participant can continue in the study. If Dr. Wilson feels medical follow-up is necessary, the participant will be referred to obtain medical treatment from their personal physician. This is a similar referral/medical follow-up system that Baylor athletes are provided with the exception that participants in this study will not be provided medical care. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the participants research file, and reported to the Baylor IRB committee.

Screening for Cardiopulmonary Disease Risk and Exercise Contraindications. All participants will have their risk of cardiopulmonary disease and their possible contraindications to exercise assessed by Certified Exercise Physiologists and the laboratory nurse in accordance to standard procedures described by the American College of Sports Medicine (ACSM) (*32)*. Only those participants considered as low risk for cardiovascular disease with no contraindications to exercise will be considered as eligible to participate in the study. These guidelines are outlined and presented below:

ACSM Risk Stratification Criteria for Cardiovascular Disease

Low Risk

Younger individuals (men < 45 years of age; women < 55 years of age) who are asymptomatic for
cardiovascular disease and possess no more than one positive cardiovascular disease risk factor.

Moderate Risk

Older individuals and/or those who are asymptomatic for cardiovascular disease and possess two or more cardiovascular disease risk factors.

High Risk

Individuals with one or more signs/symptoms suggestive or cardiovascular disease.

ACSM Criteria for Signs and Symptoms Suggestive of Cardiovascular Disease

1. Pain, discomfort in the chest, neck, jaw, arms, or other areas that may be due to myocardial ischemia.
2. Shortness of breath at rest or with mild exertion.
3. Dizziness or syncope.
4. Orthopnea or paroxysmal nocturnal dyspnea.
5. Ankle edema.
6. Palpitations or tachycardia.
7. Intermittent claudication.
8. Known heart murmur.
9. Unusual fatigue or shortness of breath with usual activities.

ACSM Absolute and Relative Contraindications to Exercise

Absolute Contraindications
1. Unstable angina.
2. Uncontrolled dysrhythmias.
3. Recent EKG changes and cardiac events.
4. Acute myocarditis or pericarditis.
5. Acute pulmonary embolism or acute myocardial infarction.
6. Severe aortic stenosis.
7. Dissecting aneurysm.
8. Acute infections.

Relative Contraindications
1. Left main coronary stenosis.
2. Severe hypertension (> 200/110).
3. Tachycardia or bradycardia.
4. Uncontrolled metabolic disease.
5. High-degree AV block.
6. Chronic infectious disease.
7. Cardiomyopahty and outflow obstructions.
8. Stenotic valve disease.
9. Ventricular aneurysm.

Food Logs. Participants will be required to record their dietary intake for three days prior to testing sessions one, two, and three. The participants' diets will not be standardized, however, participants will be asked not to change their dietary habits during the course of the study. These three day food logs will be evaluated with the Food Processor dietary assessment software program to determine the average daily macronutrient consumption of fat, carbohydrate, and protein in the diet for the duration of the study.

Body Composition Assessments. Participants will undergo body composition tests in the ESNL. Prior to the first familiarization session, height will be measured using standard anthropometry and total body weight will be measured using a calibrated electronic scale with a precision of +/- 0.02 kg. Total body water will then be estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio-resistance of water and body tissues based on a minute low energy, high frequency current (500 micro-amps at a frequency of 50 kHz) transmitted through the body. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe. This is measured through four electrodes placed on the body. Participants will lie on a table in the supine position and electrodes will be connected to the analyzer. After the participant is connected, age, gender, weight, height, and activity level are entered into the unit by the technician. After the unit has measured the resistance, which takes approximately 30 seconds, the unit then calculates total body water and body water percent.

Body composition will be determined using a Lange skinfold calipers (Beta Technology, Inc., Santa Cruz, CA). A 7 site skin fold test will be used with measurements taken at the tricep, chest, thigh, abdominal, midaxillary, subscapular, and suprailiac locations. A 7 site prediction equation will be used to estimate body fat percentage, and fat free mass (10).

Creatine Analysis. Blood serum and muscle tissue samples will be analyzed for total creatine, free creatine, and phosphocreatine by the diacetyl/α-napthtol reaction. Unmodified serum samples will be ready for analysis, while muscle tissue must be prepared. Muscle tissue will be homogenized in a 0.5 M perchloric acid/1 mM EDTA solution, spun in a centrifuge at 10,000 rpm for 2 minutes, and the supernatant will neutralized with 2.1 M potassium bicarbonate/0.3 M MOPS solution, at which point the supernatant is ready for analysis. Total creatine of samples will be determined by reaction with 6.94 mM α-napthtol and a 1:2500 dilution of diacetyl. The reaction will incubate for 40 minutes at room temperature in the dark, and color formation will be detected by spectrophotometer at 520 nm. The samples will be run against a standard curve of known creatine concentrations. Free creatine will be determined by combining homogenate with 0.4 N hydrochloric acid and heating at 65˚C for 10 minutes. The homogenate will then be neutralized with 2.0 N sodium hydroxide, and subsequently participanted to the diacetyl/α-napthtol reaction as previously described. Phosphocreatine concentration may be calculated as free creatine concentration subtracted from total creatine concentration.

Total Adenine Nucleotide Pool Analysis. Muscle tissue samples will be analyzed for adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, inosine monophosphate, inosine, and hypoxanthine using high performance liquid chromatography, and serum samples will be analyzed for inosine and hypoxanthine. All

muscle samples used for HPLC analysis will be freeze dried, weighed, dissected free of connective tissue, and powdered. The samples will then be analyzed using reverse phase HPLC on a BioRad Biologic Duo Flow HPLC analyzer (. For this analysis approximately 10 mg of muscle sample will be dried, weighed, and homogenized with a mortar and pestle using ice cold 0.6 N $HCLO_4$ (1 mL/1.5 mg dry weight). The solution will then be centrifuged and 300 µl of supernatant will be extracted and kept in ice water. 200 µl of ice-cold $KHCO_3$ will be added dropwise for neutralization. After centrifugation, 20 µl of supernatant will be injected into the HPLC for analysis. Additionally, plasma hypoxanthine and inosine will be analyzed using the same HPLC method as muscle tissue samples. Before analysis, the plasma will be deproteinized with 1.5 mol/l perchloric acid and neutralized with 2.1 mol/l potassium bicarbonate.

Blood Samples. Participants will donate approximately 3-4 teaspoons of fasting venous blood (6 – 12 milliliters) during each blood draw. Blood samples will be obtained using standard phlebotomy procedures using standard sterile venipuncture of an antecubital vein by Adam Parker, MS., or laboratory technician's trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. Up to this point, Mr. Parker has successfully performed several hundred blood draws without any complications. The phlebotomists and lab technicians will wear personal protective clothing (gloves, lab coats, etc.) when handling blood samples. Participants will be seated in a phlebotomy chair. Their arm will be cleaned with a sterile alcohol wipe. A standard rubber tourniquet will then be placed on the brachium. An antecubital vein will be palpated and then a 21 gauge sterile needle attached to a plastic vacutainer holder will be inserted into the vein using standard procedures. Once samples are obtained, the vacutainer holder and needle will be removed. The needle will be discarded as hazardous waste in an appropriately-labeled plastic sharps container. The site of the blood draw will then be cleaned with a sterile alcohol wipe and gauze and a sterile Band-Aid will be placed on the site. The alcohol wipe and gauze then will be discarded in an appropriately-labeled biohazard waste receptacle. The blood collection tubes will be labeled and placed in a test tube rack. Laboratory technicians (who have received blood borne pathogen training and will be wearing personal protective clothing) will centrifuge the serum samples, transfer serum into labeled serum storage containers, and store at -20°C for later analysis.

Muscle Biopsies. Percutaneous muscle biopsies (approximately 50-70 mg) will be obtained from the vastus lateralis of each participant's thigh. Samples will be extracted under local anesthesia of 2% Lidocaine with epinephrine from the middle portion of the muscle at the midway between the patella and the greater trochanter of the femur. For each biopsy, muscle tissue will be extracted from the same location by using the previous incision and depth markings on the needle. First, the participant will lie supine or assume a comfortable reclining position on a sterilized table. Once the extraction point is identified, the area will be shaved clean of leg hair, washed with a antiseptic soap and cleaned with rubbing alcohol. In addition, the biopsy site will further be cleansed by swabbing the area with Betadine (fluid antiseptic) and then draped. A small area of the

skin approximately 2 cm in diameter will be anesthetized with a 1.0 mL subcutaneous injection of Lidocaine. Once anesthetized, a scalpel point will be used to produce the initial biopsy site by making an incision approximately 1 cm in length through the skin, subcutaneous fat, and fascia lata. Due to the localized effects of the anesthetic, the participant should feel no pain during this process. The biopsy needle will be advanced into the incision approximately 1 cm and during this part of the procedure the participant may feel pressure to the thigh area. Once the muscle sample has been obtained, pressure will be immediately applied and the wound will immediately be bandaged. With the incision being so small bleeding is slight; therefore, only a butterfly bandage is needed to close the incision, which is then covered with a pressure bandage. The needle and scalpel blade will be discarded as hazardous waste in an appropriately-labeled plastic sharps container. The site of the biopsy will be cleaned with a sterile alcohol wipe and gauze. The alcohol wipe and gauze then will be discarded in a appropriately labeled biohazard waste receptacle. The tissue sample will be stored at -70°C for future analyses. Once the local anesthesia has taken effect (approximately 2-3 minutes) the biopsy procedure will take approximately 15-20 seconds. Written instructions for post-biopsy care will be given to the participants. The participant will be instructed to leave the bandages on for 24 hours (unless unexpected bleeding or pain occurs) and asked to report back to the lab within 24 hours to have the old bandages removed, the incision inspected and new bandages applied. The participant will be further advised to refrain from vigorous physical activity during the first 48 hours post-biopsy. These suggestions will minimize pain and possible bleeding of the area. If needed, the participant may take non-prescription analgesic medication such as Acetaminophen to relieve pain if needed. However, medications such as aspirin, Nuprin, Bufferin, or Advil will be discouraged as these medications may lead to increased bruising at the biopsy site. Soreness of the area may occur for about 24 hours post-biopsy.

Equipment

***Digital Scale*.** Total body weight will be determined using a digital scale accurate to ±0.02 kg. The scale is calibrated by placing certified 25-kg weights and balancing the scale. Other than general instructions, special skills are not required to measure body weight.

Bioelectrical Impedance Analyzer (BIA). The Omron HBF-306 Bioelectrical Impedance Analyzer (*Omron Healthcare Inc., Vernon Hills, IL*) measures bio-resistance and body composition based on a minute low energy, high frequency current transmitted through the body from surface electrodes.

Lange Skinfold Calipers. The Lange Skinfold Caliper (Beta Technology Inc., Santa Cruz, CA)provides accurate measurement of subcutaneous tissue using pivoted tips that adjust for parallel measurement of skinfolds. Spring-loaded levers provide a substantially

constant standard pressure of 10 gm./ sq. mm. over the entire operating range. A skilled technician will perform skinfold measurements.

Resistance Exercise Machines. Maximum strength (1-RM strength) tests will be performed on a isotonic leg/hip sled (Nebula Fitness, Inc., Versailles, OH). Equipment and testing will be located within the ESNL and/or EBNL.

Compensation or Incentives

Participants completing all familiarization and testing sessions as well as turning in all required materials (i.e., dietary & supplement logs and unused supplements) in the study will be paid $100. Participants may receive information regarding results of these tests if they desire. If participants are Baylor students, they will not receive any academic credit for participating in this study.

Potential Risks

Participants who meet eligibility criteria will be exposed to a very low level of electrical current that will be passed through each participant's body using a bioelectrical impedance analyzer. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of the BIA analyzer has been shown to be a safe method of assessing body composition and total body water and is approved by the FDA.

Creatine monohydrate has been studied for various uses, both medical and non medical in humans. Numerous investigations have found no negative effects in connection with creatine supplementation. Furthermore, these supplements are currently available in over-the-counter nutritional supplements sold in United States. As with the vast majority of nutritional supplements, however, the FDA may not have evaluated the safety or marketing claims of these supplements.

Participants who meet eligibility criteria will engage in strength testing sessions involving dynamic muscle contractions. Half of the participants in this study will be not be experienced resistance trainers. As a result of the 1- and 10-RM testing protocol, non-trained participants will most likely experience short-term muscle fatigue. In addition, they will likely experience muscle soreness in their thigh area for up to 24 to 48 hours after the leg press exercise. This soreness is normal and should correspond with the type of muscle soreness participants may have felt after doing unaccustomed physical activity. Muscle strains/pulls resulting from 1-RM testing, specifically the eccentric phase of the leg press, are possible. However, potential injury due to exercise will be minimized by ensuring that all participants adhere to correct lifting technique. In addition, only Dr. Mike Greenwood, Ph.D. and/or trained graduate student study personnel will conduct the testing and exercise procedures. Participants will be made aware of the intensity and duration of the expected soreness due to the exercise sessions. Participants will donate 3-4 teaspoons (6-12 milliliters) of venous blood four times during the study using standard phlebotomy procedures. This procedure may cause a small amount of pain when the

needle is inserted into the vein as well as some bleeding and bruising. The participant may also experience some dizziness, nausea, and/or faint if they are unaccustomed to having blood drawn.

Complications resulting from the muscle biopsy are rare, especially in this case where the biopsy is similar to receiving a routine intramuscular injection. As with the blood draw, however, there is a risk of infection if the participant does not adequately cleanse the area for approximately 48-72 hours post biopsy. While leaving the butterfly bandage in place, participants will be instructed to cleanse the biopsy area with soap and water every 4-6 hours, pat the area dry and reapply a fresh adhesive bandage. The participant will be instructed to leave the bandages on for 24 hours (unless unexpected bleeding or pain occurs) and asked to report back to the lab within 24 hours to have the old bandages removed, the incision inspected and new bandages applied. The participant will be further advised to refrain from vigorous physical activity with the affected leg for 24 hours after the biopsy. There is a potential risk of an allergic reaction to the Lidocaine. All participants will be asked if they have known allergies to local anesthetics (e.g. Lidocaine, Xylocaine, etc.) that they may have been previously given during dental or hospital visits. Participants with known allergies to anesthesia medications will not be allowed to participate in the study. Matthew Cooke, Ph.D. will perform all muscle biopsies. Up to this point in his professional career, Dr. Cooke has successfully performed dozens of muscle biopsies on both male and female participants without any complications. Researchers involved in collecting data represent trained, non-physician, certified exercise specialists (American Society of Exercise Physiologies Certified Exercise Physiologist, Certified Strength & Conditioning Specialists, and/or American College of Sports Medicine Health Fitness Instructor$_{SM}$, Exercise Technologist$_{SM}$, or Exercise Specialists$_{SM}$). All personnel involved in collecting data will be certified in CPR, which is also a condition to holding these professional certifications. A telephone and automated electronic defibrillator (AED) is located in the laboratory in case of any emergencies and there will be no less than two researchers working with each participant during testing. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance.

Potential Benefits

Participants will receive free body composition testing and free creatine supplementation. These two factors will give participants insight about their health and fitness status. Depending on the findings of the study, if creatine supplementation is in fact an effective way to increase PCr recovery participants will have a method to improve their workouts. Participants finishing the study will earn $100. However, even if no individual benefit is obtained, participating in this study will help to determine whether NSCA work-to-rest ratios are indeed the best way to train the immediate energy system and if creatine supplementation has a positive effect on recovery. This information will be helpful to athletes and non-athletes alike who use nutritional creatine supplements during training with the intent of improving intra-workout recovery and workout intensity.

Assessment of Risk

Resistance training has the potential to cause muscle strains and/or soreness. Creatine monohydrate has been used in both clinical and non-clinical settings, with no negative side effects reported. The greatest risk associated with participating in this study will likely be from the muscle soreness participants will experience from participating in the resistance exercise protocol. However, since the intensity of the exercise protocol will be no more than when individuals engaged heavily in a new or different form of physical activity. Therefore, the potential benefits of participants participating in this study outweigh the potential risks.

Compensation for Illness or Injury

Each participant will agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss suffered as a result of participation in this study regardless of the cause of injury, damage, or loss.

Confidentiality

Information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential to the extent permitted by law. However, records of the research may be subpoenaed by court order or may be inspected by federal regulatory authorities. Data derived from this study may be used in reports, presentations and publications. Participants in this study will not be individually identified unless they give their written consent. All participants will have a number to identify their results. Only the study personnel will know the participant numbers. Only study personnel will have access to the data. All data will be stored in a locked cabinet in the Exercise and Sport Nutrition Laboratory and only Mike Greenwood, Ph.D. will have access to the key. All evidence of primary data will be stored for exactly three years after the completion of the study. At this time data will be destroyed in a manner that instills complete privacy to all participants of the study. Analyzed muscle and blood samples will be discarded in an appropriately-labeled biohazard waste disposal container. However, unused muscle and blood samples will be kept in a locked freezer for no longer than one year. If any subsequent analysis occurs with the samples, they will be re-coded to further instill confidentiality.

Data Presentation, Analysis, & Publication

Statistical analysis will be performed using a repeated measures multivariate factorial design (2x2x4) to analyze the dependent variables determined from the obtained muscle biopsy and blood samples. If significant difference is found ($p < 0.05$) an LSD post-hoc analysis test will be used. All statistical procedures shall be performed using SPSS 11.0 software and a probability level of <0.05 used throughout.

Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, International Society of Sports Nutrition, Experimental Biology, etc.) and published in a peer reviewed scientific journal (e.g., Medicine & Science in Sport and Exercise, Journal of Sport Science and Medicine, International Journal of Sport Nutrition and Exercise Metabolism, etc.).

Statement on Conflict of Interest

Researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

References

1. Almada, A.L. et al. (2000). Long-term creatine supplementation does not affect markers of renal stress in athletes. *The Journal of Strength and Conditioning Research*, 14, 359.
2. Bompa & Carrera. (2005). Periodization training for sports: Science based strength and conditioning plans for 20 sports, 2nd ed. Champaign: Human Kinetics.
3. Cheung, K., Hume, P., & L. Maxwell. (2003). Delayed onset muscle soreness: Treatment strategies and performance factors: Review article. *Sports Medicine*, 33(2), 145-164.
4. Deschenes, M.R. & W.J. Kraemer. (2002). Performance and physiological adaptations to resistance training. *American Journal of Physiology Medicine and Rehabilitations*, 81 (suppl) 53-516.
5. Fleck, S. (1983). Bridging the Gap: Interval training physiological basis. *NSCA Journal*, 5, 40 & 57-63.
6. Glaister, M. (2005). Multiple Sprint Work: Physiological responses, mechanisms of fatigue and the influence of aerobic fitness. *Sports Medicine*, 35, 757-777.
7. Greenwood et al. (2003). Creatine supplementation during college football training does not increase the incidence of cramping or injury. *Molecular and Cellular Biochemistry*, 244, 83-88.
8. Haseler, L.J., M.C. Hogan, & R.S. Richardson. (1999). Skeletal muscle phosphocreatine recovery in exercise-trained humans is dependent on O_2 availability [Electronic version]. *Journal of Applied Physiology*, 86, 2013-2018.
9. Houston, M.E. (2001). Biochemistry primer for exercise science, 2nd ed. Champaign: Human Kinetics.
10. Jackson & Pollock. (1985). Practical assessment of body composition. *Physician and Sports Medicine*, 13, 76-90.
11. Kraemer, et al. (2000). Influence of resistance training volume and periodization on physiological and performance adaptations in collegiate women tennis players. *The American Journal of Sports Medicine*, 28, 247-252.
12. Kraemer et al. (2002). American College of Sports Medicine Position Statement: progressive models in resistance training for healthy adults. *Medicine Science Sport & Exercise*, 34, 364-380.
13. Kraemer, W.J., Ratamess, N., & D. French. (2002). Resistance training for health and performance. *Current Sports Medicine Reports*, 1, 165-171.
14. Kreider, R. (1998). Creatine supplementation: Analysis of ergogenic value, medical safety and concerns. Journal of Exercise Physiology Online 1: 7-18. Available: http://www.css.edu/users/tboone2/asep/jan3/htm.
15. Kreider, R. et al. (1998). Effects of creatine supplementation on body composition, strength and sprint performance. *Medicine & Science in Sports & Exercise*, 30 (1), 73-82.
16. Kreider, et al. (2003). Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. *Molecular and Cellular Biochemistry*, 244, 95-104.
17. Kreider, R. (2003). Effects of creatine supplementation on performance and training adaptations. *Molecular and Cellular Biochemistry*, 244, 89-94.

18. Kutz, M. (2003). Theoretical and practical issues for plyometric training. *NSCA Performance Training Journal,* 2 (2), 10-12.
19. Larson-Meyer, et al. (2000). The effect of creatine supplementation on muscle strength and body composition during off-season training in female soccer players. *Journal of Strength and Conditioning Research*, 14 (4), 434-442.
20. McMahon, S. & D. Jenkins. (2002). Factors affecting the rate of phosphocreatine resynthesis following intense exercise. *Sports Medicine*, 32, 761-784.
21. National Strength and Conditioning Association. (2000). Essentials of Strength and Conditioning (2nd ed.) Champaign: Human Kinetics. [Editors: Baechle, T.R. & R.W. Earle].
22. O'Connor, T.E., & K.L. Lamb. (2003). The effects of bodymax high-repetition training measures of body composition and muscular strength in active adult women. *Journal of Strength and Conditioning Research*, 17, 614-620.
23. Parcell, A.C., Sawyer, R.D., Tricoli, V.A., & T.D. Chinever. (2002), Minimum rest period for strength recovery during a common isokinetic testing protocol**.** *Medicine & Science in Sports & Exercise, 34*(6):1018-1022.
24. Prevost, M., Nelson, A., & G. Morris. (1997). Creatine supplementation enhances intermittent work performance. *Research Quarterly in Exercise & Sports*, 68 (3), 233-240.
25. Rahimi, R. (2005). Effect of different rest intervals on the exercise volume completed during squat bouts. *Journal of Sports Science and Medicine*, 4, 361-366.
26. Reynolds, Gordon, & Robergs. (2006). Predicition of 1 repetition maximum strength from multiple repetition maximum testing and anthropometry. *The Journal of Strength and Conditioning Research*, 20(3), 584-592.
27. Savvas, P. et al. (2003). The effects of ibuprofen on delayed muscle soreness and muscular performance after eccentric exercise. *The Journal of Strength and Conditioning Research*, 17(1), 53-59.
28. Stout, J., Eckerson, J., Housh, T., & K. Ebersole. (1999). The effects of creatine supplementation on anaerobic working capacity. *The Journal of Strength and Conditioning Research*, 13 (2), 135-138.
29. Tesch, Colliander & Kaiser. (1986) Muscle metabolism during intense, heavy-resistance training exercise. *European Journal of Applied Physiology*,55 (4), 362-366.
30. Tomlin, D. & H. Wenger. (2001). The relationship between aerobic fitness and recovery from high intensity intermittent exercise. *Sports Medicine*, 31, 1-11.
31. Vandenberghe, K., Van Hecke, P., Van Leemputte, M., Vanstapel, F., & P. Hespel. (1999). Phosphocreatine resynthesis is not affected by creatine loading. Medicine and Science in Sports and Exercise, 31 (2), 236-242.
32. Whaley, Brubaker, & Otto. (2005). ACSMs guidelines for exercise testing and prescription, 7th ed. New York: Lippincott Williams & Wilkins.
33. Willardson & Burkett. (2005). A comparison of 3 different rest intervals on the exercise volume completed during a workout. *Journal of Strength and Conditioning Research*, 19, 23-26.

34. Willoughby, D.S. & Rosene, J. (2001). Effects of oral creatine and resistance training on myosin heavy chain expression. *Medicine and Science in Sports and Exercise*, 33, 1674-1681.
35. Ziegenfuss, T. (2002). Effect of creatine loading on anaerobic performance and skeletal muscle volume in NCAA division I athletes. *Nutrition*, 18(5), 397-402.

Study Overview

Participant Qualification Interview (FAM) Day 1	**Baseline Measurements and Leg dominance (T_1) Day 4**	**One Repetition Maximum/Leg Press Volitional Exhaustion (T_2) Day 8**	**Test Day (T_3) Day 12**
Phone interview - prior supplement use - prior resistance training history - requirements of study *If participant meets requirements over the phone they will report to the lab for the initial familiarization session* Demographic Form Medical & Fitness History Completion of Informed Consent Skinfold Measurement Height/Weight Thigh circumference measurement BIA Food Log 1 handed out	Collect Food Log 1 Hand out food log 2 and supplement log 1 Height/Weight Thigh circumference measurement BIA Baseline blood draw – initial serum creatine and adenine nucleotide levels Baseline biopsy – initial muscle creatine and adenine nucleotide levels Participants randomly assigned (block randomization) based on Age/Mass/Body composition/ thigh circumference/Training History Preload Supplement or Placebo with 8 oz of water each ingestion - 5g, 4 times per day preferably at 8am, noon, 4pm, 8pm	Collect food log 2, supplement log 1; hand out food log 3 and supplement log 2 BIA Determine leg press 1-RM (NSCA guidelines) Distribution of maintenance dose of creatine or placebo 5 g/ day until T3	Hand in final food/supplement logs BIA Lidocaine biopsy site – wait at least 7-10 minutes Blood and Biopsy (1) Isokinetic Bout Blood and Biopsy (2) immediately post exercise Blood and Biopsy (3) 3 minutes post exercise Blood and Biopsy (4) 5 minutes post exercise

Part 5: Informed Consent Form Checklist

When using humans as participants in research you must obtain their informed consent. Check each of the following items as they appear on your Informed Consent Form and include this checklist with your protocol:

__x_(a) A statement explaining the purpose of the research.

__x_(b) A statement of the expected duration of the participant's participation.

__x_(c) A description of the procedures to be followed.

__x_(d) A description of any reasonable foreseeable risks or discomforts to the participant, including
invasion of privacy.

__x_(e) A description of any benefits resulting from the research, either to the participant or to others.

__x_(f) A statement that informs participant of his/her right not to be a participant in a research project that
is also a teaching exercise.

__x_(g) A statement informing participant about how his/her anonymity will be guarded; i.e., that their
confidentiality will be protected by assigned code numbers, by limitations of who has access to
data, by data storage in locked cabinets, by locked computer files, etc.

__x_(h) A statement that the participant's participation is voluntary, and that his/her refusal to participate
will involve no penalty or loss of benefits to which the participant is otherwise entitled, and that the
participant may discontinue participation at any time without penalty or loss of benefits to which the
participant is otherwise entitled.

_na_(i) A disclaimer, if applicable, regarding the use of the Internet to collect data.

__x_(j) For research involving more than minimal risk, an explanation regarding the availability of any
compensation or any medical treatments if injury occurs (if applicable, see OHRP Reports).

__x_(k) If written informed consent is required, a place for the participant to sign and date the form and a
statement that a copy of the signed consent form will be given to the participant for his/her records.

_na_(l) If the participant is a minor, a statement of parental responsibility in consenting to the child's
participation in the study with a place for the parent to sign and date the form in addition to the
participant's signature.

__x_(m) Include a short summary of your expertise related to this research proposal.

__x_(n) The name, address, and telephone number of the principal investigator of the research project,
and his/her affiliation with Baylor University. If the principal investigator is a graduate student,
the name and telephone number of the faculty advisor is also required.

__x_(o) A statement informing participant that inquiries regarding his/her rights as a participant, or any
other aspect of the research as it relates to his/her participation as a participant, can be directed to Baylor's University Committee for Protection of Human Participants in Research. The chairman is Dr. Matt Stanford, Professor Psychology and Neuroscience, PO Box 97334, Waco, Texas 76798-7334, phone number 254-710-2236.

APPENDIX C

Advertisement Flyer

Want to get PAID $100 in ten days?

Men Needed for a Weight Lifting & Nutritional Supplement Study

*Researchers in the Exercise & Sport Nutrition Lab at Baylor University are recruiting 40 healthy men between the ages of **18-30** to participate in a study designed to evaluate the effects of creatine supplement and training status on physiological markers associated with energy production. Subjects will be required to ingest supplements and undergo muscle biopsies and blood sampling. Eligible subjects will receive **$100** for completing the study.*

For more information call:

Adam Parker at (254) 733-3286

Or send email to:

Adam_Parker1@baylor.edu

APPENDIX D

Biopsy Wound Care Flyer

Wound Care for the Muscle Biopsy Procedure

Complications resulting from the muscle biopsy procedure are rare. Furthermore, after the procedure, you can reduce your risk of chance of infection by adhering to the following course of action for wound care:

For approximately 24 hours post biopsy

- leave the bandage(s) on for 24 hours (unless unexpected bleeding or pain occurs, which should be immediately reported to the lab)
- lightly clean around the bandage(s) if necessary
- report back to the lab within 24 hours to have the old bandage(s) removed, the incision inspected and new bandages applied
- refrain from vigorous physical activity with the leg during the first 24 hours post-biopsy

After the 24-hour follow-up, (for approximately 72 hours post biopsy)

- adequately cleanse the area surrounding the bandage with soap and water every 4-6 hours, and pat the area dry
- reapply a fresh adhesive bandage

At approximately 72 hours post biopsy

- allow the incision sight to be inspected, butterfly removed, and new bandages applied
- leave these bandages on for 24 hours (unless unexpected bleeding or pain occurs)
- return to normal hygiene practices unless complications arise

Possible pain & side effects

Soreness of the area comparable to that of a bruise will likely persist for 24 hours, and possibly even 36 hours, after the biopsy procedure. Following the procedures outlined above should significantly minimize pain and possible bleeding of the area. However, some participants experience no significant pain post biopsy.

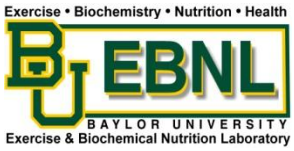
☺ If needed, the participant may take non-prescription analgesic medication such as Tylenol to relieve pain if needed.

☹ Medications such as aspirin, Advil, Bufferin, or Nuprin, are discouraged as they may lead to excess bruising at the biopsy site.

If any questions or complications arise please contact:

Lori Greenwood, Ph.D.	or	Adam Parker, BS
Room # 215 Marrs McLean Gym		Room # 122 Marrs McLean Gym
(254) 710-4026		(254) 733-3286

Lori_Greenwood@baylor.edu Adam_Parker1@baylor.edu



APPENDIX E

Medical History Questionnaire

BAYLOR UNIVERSITY
EXERCISE & SPORT NUTRITION LABORATORY

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name:_____ Age _____Date of Birth_____

Name and Address of Your Physician:_____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

____ Heart murmur, clicks, or other cardiac findings?
____ Frequent extra, skipped, or rapid heartbeats?
____ Chest Pain of Angina (with or without exertion)?
____ High cholesterol?
____ Diagnosed high blood pressure?
____ Heart attack or any cardiac surgery?
____ Leg cramps (during exercise)?
____ Chronic swollen ankles?
____ Varicose veins?
____ Frequent dizziness/fainting?
____ Muscle or joint problems?
____ High blood sugar/diabetes?
____ Thyroid Disease?
____ Low testosterone/hypogonadism?
____ Gluacoma?
____ Asthma/breathing difficulty?
____ Bronchitis/Chest Cold?
____ Cancer, Melanoma, or Suspected Skin Lesions?
____ Stroke or Blood Clots?
____ Emphysema/lung disease?
____ Epilepsy/seizures?
____ Rheumatic fever?
____ Scarlet fever?
____ Ulcers?
____ Pneumonia?
____ Anemias?
____ Liver or kidney disease?
____ Autoimmune disease?
____ Nerve disease?
____ Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?
_____
_____

Please provide any additional comments/explanations of your current or past medical history.
_____
_____

Please list any recent surgery (i.e., type, dates etc.).
_____
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.

What was the date of your last complete medical exam?

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study
(including strength and maximal exercise tests) ____ If yes, please explain:
_____
_____
_____

Recommendation for Participation

____ No exclusion criteria presented. Subject is ***cleared*** to participate in the study.
____ Exclusion criteria is/are present. Subject is ***not cleared*** to participate in the study.

Signed: _____ Date: _____

APPENDIX F

Personal Information Form

Baylor University
Exercise and Sport Nutrition Laboratory

Personal Information

Name: _____

Address: _____ City: _____

State: _____ Zip Code _____ SS# _____

Home Phone: (___) _____ Work Phone: (___) _____

Cellular (___) _____ email address: _____

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise & Supplement History/Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities

3. Describe any exercise training that you routinely participate.

4. How many days per week do you exercise/participate in these activities?

5. How many hours per week do you train?

6. How long (years/months) have you been consistently training?

APPENDIX G

Food Log Form

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ Date_____

INSTRUCTIONS

1. Record everything you eat for 4 days (including one weekend day). If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.
2. Record the Food, Amount, Brand Name, and Preparation Methods. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald's, Healthy Choice, or Frosted Flakes.
3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities.

Food (include brand)	Method of Preparation	Quantity (cups, oz., no.)
BREAKFAST:		
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
LUNCH:		
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
DINNER:		
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
SNACKS:		
_____	_____	_____
_____	_____	_____
_____	_____	_____

APPENDIX H

Adverse Reaction Questionnaire

Weekly Follow-up Assessment

Effects of Supplementation on Training Adaptations

Subject Name: _____ Subject #: _____ Date: _____

Week	1	2	3	4	Please use this column to provide any additional comments
Are you training on schedule?	Yes / No	Yes / No	Yes / No	Yes / No	
Below, please rate the ***frequency*** of the following symptoms according to the scale where: 0 = none 1 = minimal (1-2 per/wk) 2 = slight (3-4 per/wk) 3 = occasional (5-6 per/wk) 4 = frequent (7-8 per/wk) 5 = severe (9 or more per/wk)					
Dizziness?					
Headache?					
Fast or racing heart rate?					
Heart skipping or palpitations?					
Shortness of breath?					
Nervousness?					
Blurred Vision?					
Any other unusual or adverse effects?					
Below, please rate the ***severity*** of the following symptoms according to the scale where: 0 = none 1 = minimal 2 = slight 3 = moderate 4 = severe 5 = very severe					
Dizziness?					
Headache?					
Fast or racing heart rate?					
Heart skipping or palpitations?					
Shortness of breath?					
Nervousness?					
Blurred Vision?					
Any other unusual or adverse effects?					

PLEASE REMEMBER TO REPORT ANY SIDE EFFECTS IMMEDIATELY. REMEMBER TO ALSO REPORT WEEKLY TO THE RESEARCH NURSE IN THE ESNL.

Directions: If necessary, please contact either Maureen Head, BS or Mike Greenwood 254-710-7687. You may also e-mail either at Maureen_Head@baylor.edu or Mike_Greenwood@baylor.edu.

Thanks for your participation!

Baylor University

APPENDIX I

Data Collection Sheet

Data Collection Sheet

Determining related work-to-rest ratios following a maximal effort isokinetic leg extension bout in trained and untrained males: A double-blind creatine/placebo controlled study

Subject Name:_____ Group:_____
Testing Session:_____ Date: _____
Dominant Leg: L / R

Food Log _____ Informed Consent _____ Supplement log _____

Ht. _____ Biodex _____
Wt. _____ Leg Cr. _____cm
BIA _____
Blood _____ (2 tiger, 1 purple)
HR _____
BP _____
SF _____, _____ (chest) _____ average
_____, _____ (tricep) _____ average
_____, _____ (mid-axilla) _____ average
_____, _____ (sub-scapula) _____ average
_____, _____ (iliac) _____ average
_____, _____ (abdomen) _____ average
_____, _____ (thigh) _____ average _____ Σ7
_____ %BF

Leg Press
_____x_____; _____x_____; _____x_____; _____x_____;_____x_____;
_____x_____;_____x_____
Max _____

T4

Blood _____ (pre) _____ (post) _____ (3 min) _____ (5 min)

Biopsy _____ (pre) _____ (post) _____ (3 min) _____ (5 min)

BIBLIOGRAPHY

Aaserud, R., Gramvik, P., Olsen, S. R., & Jensen, J. (1998). Creatine supplementation delays onset of fatigue during repeated bouts of sprint running. *Scandinavian Journal of Medicine & Science in Sports, 8*(5 Pt 1), 247-251.

Abernethy, P. J., Jurimae, J., Logan, P. A., Taylor, A. W., & Thayer, R. E. (1994). Acute and chronic response of skeletal muscle to resistance exercise. *Sports Medicine (Auckland, N.Z.), 17*(1), 22-38.

Alvestrand, A., Furst, P., & Bergstrom, J. (1983). Intracellular amino acids in uremia. *Kidney International.Supplement, 16*, S9-16.

Baechle, T. R., Earle, R. W., & National Strength & Conditioning Association. (2008). *Essentials of strength training and conditioning* (3rd ed.). Champaign, IL: Human Kinetics.

Baldwin, J., Snow, R. J., Carey, M. F., & Febbraio, M. A. (1999). Muscle IMP accumulation during fatiguing submaximal exercise in endurance trained and untrained men. *The American Journal of Physiology, 277*(1 Pt 2), R295-300.

Baldwin, J., Snow, R. J., Gibala, M. J., Garnham, A., Howarth, K., & Febbraio, M. A. (2003). Glycogen availability does not affect the TCA cycle or TAN pools during prolonged, fatiguing exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 94*(6), 2181-2187.

Balsom, P. D., Soderlund, K., Sjodin, B., & Ekblom, B. (1995). Skeletal muscle metabolism during short duration high-intensity exercise: Influence of creatine supplementation. *Acta Physiologica Scandinavica, 154*(3), 303-310.

Bergstrom, J., Furst, P., Holmstrom, B. U., Vinnars, E., Askanasi, J., Elwyn, D. H., et al. (1981). Influence of injury and nutrition on muscle water electrolytes: Effect of elective operation. *Annals of Surgery, 193*(6), 810-816.

Bevegard, S., Castenfors, J., Danielson, M., & Bergstrom, J. (1977). Effect of saluretic therapy on muscle content of water and electrolytes in relation to hemodynamic variables. *Acta Medica Scandinavica, 202*(5), 379-384.

Bogdanis, G. C., Nevill, M. E., Boobis, L. H., & Lakomy, H. K. (1996). Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 80*(3), 876-884.

Bogdanis, G. C., Nevill, M. E., Boobis, L. H., Lakomy, H. K., & Nevill, A. M. (1995). Recovery of power output and muscle metabolites following 30 s of maximal sprint cycling in man. *The Journal of Physiology, 482 (Pt 2)*(Pt 2), 467-480.

Bompa, T. O., & Carrera, M. (2005). *Periodization training for sports* (2nd ed.). Champaign, IL: Human Kinetics.

Borsheim, E., & Bahr, R. (2003). Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. *Sports Medicine (Auckland, N.Z.), 33*(14), 1037-1060.

Broberg, S., & Sahlin, K. (1989). Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 67*(1), 116-122.

Brockett, C. L., Morgan, D. L., & Proske, U. (2001). Human hamstring muscles adapt to eccentric exercise by changing optimum length. *Medicine and Science in Sports and Exercise, 33*(5), 783-790.

Brooks, G. A. (2007). Lactate: Link between glycolytic and oxidative metabolism. *Sports Medicine (Auckland, N.Z.), 37*(4-5), 341-343.

Buresh, R., Berg, K., & French, J. (2009). The effect of resistive exercise rest interval on hormonal response, strength, and hypertrophy with training. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 23*(1), 62-71.

Casey, A., & Greenhaff, P. L. (2000). Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance? *The American Journal of Clinical Nutrition, 72*(2 Suppl), 607S-17S.

Cottrell, G. T., Coast, J. R., & Herb, R. A. (2002). Effect of recovery interval on multiple-bout sprint cycling performance after acute creatine supplementation. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 16*(1), 109-116.

Drouin, J. M., Valovich-mcLeod, T. C., Shultz, S. J., Gansneder, B. M., & Perrin, D. H. (2004). Reliability and validity of the biodex system 3 pro isokinetic dynamometer velocity, torque and position measurements. *European Journal of Applied Physiology, 91*(1), 22-29.

Febbraio, M. A., & Dancey, J. (1999a). Skeletal muscle energy metabolism during prolonged, fatiguing exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 87*(6), 2341-2347.

Febbraio, M. A., & Dancey, J. (1999b). Skeletal muscle energy metabolism during prolonged, fatiguing exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 87*(6), 2341-2347.

Finn, J. P., Ebert, T. R., Withers, R. T., Carey, M. F., Mackay, M., Phillips, J. W., et al. (2001). Effect of creatine supplementation on metabolism and performance in humans during intermittent sprint cycling. *European Journal of Applied Physiology, 84*(3), 238-243.

Forsberg, A. M., Nilsson, E., Werneman, J., Bergstrom, J., & Hultman, E. (1991). Muscle composition in relation to age and sex. *Clinical Science (London, England : 1979), 81*(2), 249-256.

Glaister, M. (2005). Multiple sprint work : Physiological responses, mechanisms of fatigue and the influence of aerobic fitness. *Sports Medicine (Auckland, N.Z.), 35*(9), 757-777.

Glaister, M., Stone, M. H., Stewart, A. M., Hughes, M., & Moir, G. L. (2005). The influence of recovery duration on multiple sprint cycling performance. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 19*(4), 831-837.

Gordon, A. M., Homsher, E., & Regnier, M. (2000). Regulation of contraction in striated muscle. *Physiological Reviews, 80*(2), 853-924.

Green, H. J., Jones, S., Ball-Burnett, M., Farrance, B., & Ranney, D. (1995). Adaptations in muscle metabolism to prolonged voluntary exercise and training. *Journal of Applied Physiology (Bethesda, Md.: 1985), 78*(1), 138-145.

Green, H. J., Thomson, J. A., Daub, B. D., & Ranney, D. A. (1980). Biochemical and histochemical alterations in skeletal muscle in man during a period of reduced activity. *Canadian Journal of Physiology and Pharmacology, 58*(11), 1311-1316.

Greenhaff, P. L. (1996). Creatine supplementation: Recent developments. *British Journal of Sports Medicine, 30*(4), 276-277.

Greenhaff, P. L., Bodin, K., Soderlund, K., & Hultman, E. (1994). Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. *The American Journal of Physiology, 266*(5 Pt 1), E725-30.

Greenhaff, P. L., Casey, A., Short, A. H., Harris, R., Soderlund, K., & Hultman, E. (1993). Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. *Clinical Science (London, England : 1979), 84*(5), 565-571.

Greenwood, M., Kreider, R. B., Melton, C., Rasmussen, C., Lancaster, S., Cantler, E., et al. (2003). Creatine supplementation during college football training does not increase the incidence of cramping or injury. *Molecular and Cellular Biochemistry, 244*(1-2), 83-88.

Grindstaff, P. D., Kreider, R., Bishop, R., Wilson, M., Wood, L., Alexander, C., et al. (1997). Effects of creatine supplementation on repetitive sprint performance and body composition in competitive swimmers. *International Journal of Sport Nutrition, 7*(4), 330-346.

Hargreaves, M., McKenna, M. J., Jenkins, D. G., Warmington, S. A., Li, J. L., Snow, R. J., et al. (1998). Muscle metabolites and performance during high-intensity, intermittent exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 84*(5), 1687-1691.

Harris, R. C., Soderlund, K., & Hultman, E. (1992). Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clinical Science (London, England : 1979), 83*(3), 367-374.

Haseler, L. J., Hogan, M. C., & Richardson, R. S. (1999). Skeletal muscle phosphocreatine recovery in exercise-trained humans is dependent on O2 availability. *Journal of Applied Physiology (Bethesda, Md.: 1985), 86*(6), 2013-2018.

Hasko, G., Sitkovsky, M. V., & Szabo, C. (2004). Immunomodulatory and neuroprotective effects of inosine. *Trends in Pharmacological Sciences, 25*(3), 152-157.

Hill-Haas, S., Bishop, D., Dawson, B., Goodman, C., & Edge, J. (2007). Effects of rest interval during high-repetition resistance training on strength, aerobic fitness, and repeated-sprint ability. *Journal of Sports Sciences, 25*(6), 619-628.

Houston, M. E. (2006). *Biochemistry primer for exercise science* (3rd ed.). Champaign, IL: Human Kinetics.

Hultman, E., Soderlund, K., Timmons, J. A., Cederblad, G., & Greenhaff, P. L. (1996). Muscle creatine loading in men. *Journal of Applied Physiology (Bethesda, Md.: 1985), 81*(1), 232-237.

Huxley, A. F. (1975). The origin of force in skeletal muscle. *Ciba Foundation Symposium, (31)*(31), 271-290.

Kerksick, C., Taylor, L.,4th, Harvey, A., & Willoughby, D. (2008). Gender-related differences in muscle injury, oxidative stress, and apoptosis. *Medicine and Science in Sports and Exercise, 40*(10), 1772-1780.

Kilduff, L. P., Vidakovic, P., Cooney, G., Twycross-Lewis, R., Amuna, P., Parker, M., et al. (2002). Effects of creatine on isometric bench-press performance in resistance-trained humans. *Medicine and Science in Sports and Exercise, 34*(7), 1176-1183.

Korzeniewski, B. (2006). AMP deamination delays muscle acidification during heavy exercise and hypoxia. *The Journal of Biological Chemistry, 281*(6), 3057-3066.

Kraemer, W. J., Adams, K., Cafarelli, E., Dudley, G. A., Dooly, C., Feigenbaum, M. S., et al. (2002). American college of sports medicine position stand. progression models in resistance training for healthy adults. *Medicine and Science in Sports and Exercise, 34*(2), 364-380.

Kreider, R. B. (2003). Effects of creatine supplementation on performance and training adaptations. *Molecular and Cellular Biochemistry, 244*(1-2), 89-94.

Kreider, R. B., Ferreira, M., Wilson, M., Grindstaff, P., Plisk, S., Reinardy, J., et al. (1998). Effects of creatine supplementation on body composition, strength, and sprint performance. *Medicine and Science in Sports and Exercise, 30*(1), 73-82.

Kreider, R. B., Melton, C., Rasmussen, C. J., Greenwood, M., Lancaster, S., Cantler, E. C., et al. (2003). Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. *Molecular and Cellular Biochemistry, 244*(1-2), 95-104.

Larsson, J., & Bergstrom, J. (1978). Electrolyte changes in muscle tissue and plasma in tourniquet-ischemia. *Acta Chirurgica Scandinavica, 144*(2), 67-73.

Lowry, O. H., & Passonneau, J. V. (1972). *A flexible system of enzymatic analysis*. New York: Academic Press.

MacDougall, J. D., Sale, D. G., Ward, G. R., & and Sutton, J. R. (1977). Biochemical adaptation of human skeletal muscle to heavy resistance training and immobilization. *Journal of Applied Physiology, 58*, 700-703.

MacDougall, J. D., Ward, G. R., Sale, D. G., & Sutton, J. R. (1977). Biochemical adaptation of human skeletal muscle to heavy resistance training and immobilization. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 43*(4), 700-703.

Madapallimattam, A. G., Cross, A., Nishio, M. L., & Jeejeebhoy, K. N. (1994). Stability of high-energy substrates in fast- and slow-twitch muscle: Comparison of enzymatic assay of biopsy with in vivo 31P nuclear magnetic resonance spectroscopy. *Analytical Biochemistry, 217*(1), 103-109.

McMahon, S., & Jenkins, D. (2002). Factors affecting the rate of phosphocreatine resynthesis following intense exercise. *Sports Medicine (Auckland, N.Z.), 32*(12), 761-784.

McNeil, C. J., Allman, B. L., Symons, T. B., Vandervoort, A. A., & Rice, C. L. (2004). Torque loss induced by repetitive maximal eccentric contractions is marginally influenced by work-to-rest ratio. *European Journal of Applied Physiology, 91*(5-6), 579-585.

Metzger, J. M., & Moss, R. L. (1987). Greater hydrogen ion-induced depression of tension and velocity in skinned single fibres of rat fast than slow muscles. *The Journal of Physiology, 393*, 727-742.

Meyer, R. A., & Terjung, R. L. (1979). Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. *The American Journal of Physiology, 237*(3), C111-8.

Meyer, R. A., & Terjung, R. L. (1980). AMP deamination and IMP reamination in working skeletal muscle. *The American Journal of Physiology, 239*(1), C32-8.

Nevill, A. M., Jones, D. A., McIntyre, D., Bogdanis, G. C., & Nevill, M. E. (1997). A model for phosphocreatine resynthesis. *Journal of Applied Physiology (Bethesda, Md.: 1985), 82*(1), 329-335.

Norman, B., Sollevi, A., Kaijser, L., & Jansson, E. (1987). ATP breakdown products in human skeletal muscle during prolonged exercise to exhaustion. *Clinical Physiology (Oxford, England), 7*(6), 503-510.

Parcell, A. C., Sawyer, R. D., Tricoli, V. A., & Chinevere, T. D. (2002). Minimum rest period for strength recovery during a common isokinetic testing protocol. *Medicine and Science in Sports and Exercise, 34*(6), 1018-1022.

Pincivero, D. M., & Campy, R. M. (2004). The effects of rest interval length and training on quadriceps femoris muscle. part I: Knee extensor torque and muscle fatigue. *The Journal of Sports Medicine and Physical Fitness, 44*(2), 111-118.

Pincivero, D. M., Lephart, S. M., & Karunakara, R. G. (1997). Effects of rest interval on isokinetic strength and functional performance after short-term high intensity training. *British Journal of Sports Medicine, 31*(3), 229-234.

Preen, D., Dawson, B., Goodman, C., Lawrence, S., Beilby, J., & Ching, S. (2001). Effect of creatine loading on long-term sprint exercise performance and metabolism. *Medicine and Science in Sports and Exercise, 33*(5), 814-821.

Richmond, S. R., & Godard, M. P. (2004). The effects of varied rest periods between sets to failure using the bench press in recreationally trained men. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 18*(4), 846-849.

Robinson, J. M., Stone, M. H., Johnson, R. L., Penland, C. M., Warren, B. J., & Lewis, R. D. (1995). Effects of different weight training exercise/rest intervals on strength, power, and high intensity exercise endurance. *Journal of Strength and Conditioning Research, 9,* 216.

Roussel, M., Bendahan, D., Mattei, J. P., Le Fur, Y., & Cozzone, P. J. (2000). 31P magnetic resonance spectroscopy study of phosphocreatine recovery kinetics in skeletal muscle: The issue of intersubject variability. *Biochimica Et Biophysica Acta, 1457*(1-2), 18-26.

Sahlin, K., Gorski, J., & Edstrom, L. (1990). Influence of ATP turnover and metabolite changes on IMP formation and glycolysis in rat skeletal muscle. *The American Journal of Physiology, 259*(3 Pt 1), C409-12.

Sahlin, K., Katz, A., & Broberg, S. (1990). Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *The American Journal of Physiology, 259*(5 Pt 1), C834-41.

Sahlin, K., Palmskog, G., & Hultman, E. (1978). Adenine nucleotide and IMP contents of the quadriceps muscle in man after exercise. *Pflugers Archiv : European Journal of Physiology, 374*(2), 193-198.

Sahlin, K., & Ren, J. M. (1989). Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. *Journal of Applied Physiology (Bethesda, Md.: 1985), 67*(2), 648-654.

Snow, R. J., McKenna, M. J., Selig, S. E., Kemp, J., Stathis, C. G., & Zhao, S. (1998). Effect of creatine supplementation on sprint exercise performance and muscle metabolism. *Journal of Applied Physiology (Bethesda, Md.: 1985), 84*(5), 1667-1673.

Soderlund, K., & Hultman, E. (1991). ATP and phosphocreatine changes in single human muscle fibers after intense electrical stimulation. *The American Journal of Physiology, 261*(6 Pt 1), E737-41.

Sora, I., Richman, J., Santoro, G., Wei, H., Wang, Y., Vanderah, T., et al. (1994). The cloning and expression of a human creatine transporter. *Biochemical and Biophysical Research Communications, 204*(1), 419-427.

Spencer, M. K., Yan, Z., & Katz, A. (1991). Carbohydrate supplementation attenuates IMP accumulation in human muscle during prolonged exercise. *The American Journal of Physiology, 261*(1 Pt 1), C71-6.

Spriet, L. L., Howlett, R. A., & Heigenhauser, G. J. (2000). An enzymatic approach to lactate production in human skeletal muscle during exercise. *Medicine and Science in Sports and Exercise, 32*(4), 756-763.

Spriet, L. L., Lindinger, M. I., McKelvie, R. S., Heigenhauser, G. J., & Jones, N. L. (1989). Muscle glycogenolysis and H+ concentration during maximal intermittent cycling. *Journal of Applied Physiology (Bethesda, Md.: 1985), 66*(1), 8-13.

Stathis, C. G., Febbraio, M. A., Carey, M. F., & Snow, R. J. (1994). Influence of sprint training on human skeletal muscle purine nucleotide metabolism. *Journal of Applied Physiology (Bethesda, Md.: 1985), 76*(4), 1802-1809.

Syrotuik, D. G., & Bell, G. J. (2004a). Acute creatine monohydrate supplementation: A descriptive physiological profile of responders vs. nonresponders. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 18*(3), 610-617.

Syrotuik, D. G., & Bell, G. J. (2004b). Acute creatine monohydrate supplementation: A descriptive physiological profile of responders vs. nonresponders. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 18*(3), 610-617.

Terjung, R. L., Clarkson, P., Eichner, E. R., Greenhaff, P. L., Hespel, P. J., Israel, R. G., et al. (2000). American college of sports medicine roundtable. the physiological and health effects of oral creatine supplementation. *Medicine and Science in Sports and Exercise, 32*(3), 706-717.

Tomlin, D. L., & Wenger, H. A. (2001). The relationship between aerobic fitness and recovery from high intensity intermittent exercise. *Sports Medicine (Auckland, N.Z.), 31*(1), 1-11.

Trappe, S. W., Costill, D. L., Goodpaster, B. H., & Pearson, D. R. (1996). Calf muscle strength in former elite distance runners. *Scandinavian Journal of Medicine & Science in Sports, 6*(4), 205-210.

Tullson, P. C., Bangsbo, J., Hellsten, Y., & Richter, E. A. (1995). IMP metabolism in human skeletal muscle after exhaustive exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 78*(1), 146-152.

van Loon, L. J., Oosterlaar, A. M., Hartgens, F., Hesselink, M. K., Snow, R. J., & Wagenmakers, A. J. (2003). Effects of creatine loading and prolonged creatine supplementation on body composition, fuel selection, sprint and endurance performance in humans. *Clinical Science (London, England : 1979), 104*(2), 153-162.

Verdijk, L. B., van Loon, L., Meijer, K., & Savelberg, H. H. (2009). One-repetition maximum strength test represents a valid means to assess leg strength in vivo in humans. *Journal of Sports Sciences, 27*(1), 59-68.

Walter, G., Vandenborne, K., McCully, K. K., & Leigh, J. S. (1997). Noninvasive measurement of phosphocreatine recovery kinetics in single human muscles. *The American Journal of Physiology, 272*(2 Pt 1), C525-34.

Willardson, J. M. (2006). A brief review: Factors affecting the length of the rest interval between resistance exercise sets. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 20*(4), 978-984.

Willardson, J. M., & Burkett, L. N. (2006a). The effect of rest interval length on bench press performance with heavy vs. light loads. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 20*(2), 396-399.

Willardson, J. M., & Burkett, L. N. (2006b). The effect of rest interval length on the sustainability of squat and bench press repetitions. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association, 20*(2), 400-403.

Wynants, J., & Van Belle, H. (1985). Single-run high-performance liquid chromatography of nucleotides, nucleosides, and major purine bases and its application to different tissue extracts. *Analytical Biochemistry, 144*(1), 258-266.

Yao, S., Shen, X., Terada, S., Nagamune, T., & Suzuki, E. (1999). A new method of ATP regeneration rate measurement using a pH meter. *Journal of Bioscience and Bioengineering, 87*(2), 238-240.

Yquel, R. J., Arsac, L. M., Thiaudiere, E., Canioni, P., & Manier, G. (2002). Effect of creatine supplementation on phosphocreatine resynthesis, inorganic phosphate accumulation and pH during intermittent maximal exercise. *Journal of Sports Sciences, 20*(5), 427-437.

Zhao, S., Snow, R. J., Stathis, C. G., Febbraio, M. A., & Carey, M. F. (2000). Muscle adenine nucleotide metabolism during and in recovery from maximal exercise in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985), 88*(5), 1513-1519.