THE ACUTE EFFECTS OF INTERMITTENT CREATINE AND CARBOHYDRATE SUPPLEMENTATION ON ANAEROBIC PERFORMANCE IN RECREATIONAL ATHLETES

A Thesis by

Joseph Lawrence Bach

Bachelor of General Studies, Wichita State University, 2014

Submitted to the Department of Human Performance Studies and the faculty of the Graduate School of Wichita State University in partial fulfillment of the requirements for the degree of Master of Education

May 2018
THE ACUTE EFFECTS OF INTERMITTENT CREATINE AND CARBOHYDRATE SUPPLEMENTATION ON ANAEROBIC PERFORMANCE IN RECREATIONAL ATHLETES

The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Education, with a major in Exercise Science.

______________________________
Michael Rogers, Committee Chair

______________________________
Heidi VanRavenhorst-Bell, Committee Member

______________________________
Kandatega Wimalasena, Committee Member
ABSTRACT

INTRODUCTION: Studies have shown that supplementing creatine can increase the power, strength, and increase the amount of energy stored in the muscle as glycogen. Creatine supplementation is often ingested with carbohydrates, which may enhance the absorption of creatine into the muscle. There have been few studies, however, that have viewed the intermittent effects of creatine supplementation within hours of anaerobic athletic performance. PURPOSE: The purpose of this study was to determine the effects of intermittent creatine and carbohydrate supplementation within two hours of a Wingate test. METHODS: 11 participants performed a Wingate test after intermittent supplementation of either a 1) control (CON), 2) 50 grams of dextrose (CHO), or 3) 50 grams of dextrose and 5 grams of creatine monohydrate (CHOCR). Each participant consumed one of the three supplements, in specific order, across three separate occasions. Each supplement was administered after a bout of submaximal exercise and prior to the Wingate test. Data were collected for analysis of the averaged peak power output (PP), amount of power produced in seconds 1-5 and 5-10 (AUC5 and AUC10), and the averaged end power (EP) RESULTS: There were significant differences (p<0.05) in PP between CHO and CHOCR, AUC5 between CON and CHOCR, AUC5 between CHO and CHOCR, and AUC10 between CON and CHOCR, respectively. CONCLUSION: The results of the study indicated that supplementation of creatine within two hours of anaerobic athletic performance may enhance athletic performance.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Purpose of the Study</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Research Hypothesis</td>
<td>2</td>
</tr>
<tr>
<td>1.4 Significance of Research</td>
<td>2</td>
</tr>
<tr>
<td>1.5 Variables</td>
<td>3</td>
</tr>
<tr>
<td>1.5.1 Independent variables: supplement dosages</td>
<td>3</td>
</tr>
<tr>
<td>1.5.2 Dependent variables: Wingate test performance</td>
<td>3</td>
</tr>
<tr>
<td>1.5.3 Controlled variables</td>
<td>3</td>
</tr>
<tr>
<td>1.6 Limitations</td>
<td>4</td>
</tr>
<tr>
<td>1.6.1 Standard addition protocol</td>
<td>4</td>
</tr>
<tr>
<td>1.6.2 Dietary intake</td>
<td>5</td>
</tr>
<tr>
<td>1.6.3 Vegetarian/vegans</td>
<td>5</td>
</tr>
<tr>
<td>1.6.4 Supplement dosages</td>
<td>5</td>
</tr>
<tr>
<td>1.6.5 No muscle biopsies</td>
<td>5</td>
</tr>
<tr>
<td>1.7 Delimitations</td>
<td>6</td>
</tr>
<tr>
<td>1.8 Assumptions</td>
<td>6</td>
</tr>
<tr>
<td>2. LITERATURE REVIEW</td>
<td>7</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>7</td>
</tr>
<tr>
<td>2.2 Adenosine Triphosphate</td>
<td>7</td>
</tr>
<tr>
<td>2.3 Creatine and Phosphocreatine</td>
<td>11</td>
</tr>
<tr>
<td>2.4 Carbohydrates, Glycogen, and Glycolysis</td>
<td>13</td>
</tr>
<tr>
<td>2.5 Anaerobic Exercise: An Integrated Approach for Cellular Bioenergetics</td>
<td>15</td>
</tr>
<tr>
<td>2.6 Creatine Supplementation and Ergogenic Benefits</td>
<td>16</td>
</tr>
<tr>
<td>2.7 Creatine Supplementation: Time Dependent or Dose Dependent</td>
<td>19</td>
</tr>
<tr>
<td>3. METHODOLOGY</td>
<td>21</td>
</tr>
<tr>
<td>3.1 Participants</td>
<td>21</td>
</tr>
<tr>
<td>3.2 Procedure</td>
<td>21</td>
</tr>
<tr>
<td>3.3 Data Analysis</td>
<td>23</td>
</tr>
<tr>
<td>4. RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>4.1 Descriptive Statistics</td>
<td>24</td>
</tr>
<tr>
<td>4.2 Averaged Peak Power (PP)</td>
<td>24</td>
</tr>
<tr>
<td>4.3 Power Produced: 1-5 s (AUC5)</td>
<td>25</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>4.4</td>
<td>26</td>
</tr>
<tr>
<td>4.5</td>
<td>27</td>
</tr>
<tr>
<td>5.</td>
<td>29</td>
</tr>
<tr>
<td>5.1</td>
<td>29</td>
</tr>
<tr>
<td>5.2</td>
<td>33</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>34</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>40</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>1.</td>
<td>Participant descriptives</td>
</tr>
<tr>
<td>2.</td>
<td>Averaged peak power</td>
</tr>
<tr>
<td>3.</td>
<td>Power produced: 1-5 s</td>
</tr>
<tr>
<td>4.</td>
<td>Power produced: 5-10 s</td>
</tr>
<tr>
<td>5.</td>
<td>Averaged end power</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine Monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>ATPase</td>
<td>Adenosine Triphosphatase</td>
</tr>
<tr>
<td>AUC</td>
<td>Power Produced</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CON</td>
<td>Control</td>
</tr>
<tr>
<td>CR</td>
<td>Creatine</td>
</tr>
<tr>
<td>CRK</td>
<td>Creatine Kinase</td>
</tr>
<tr>
<td>CRM</td>
<td>Creatine Monohydrate</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>IGF1</td>
<td>Insulin-like Growth Factor 1</td>
</tr>
<tr>
<td>ISSN</td>
<td>International Society of Sports Nutrition</td>
</tr>
<tr>
<td>K</td>
<td>Potassium</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide Dinucleotide</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>PCR</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PEGCR</td>
<td>Polyethylene Glycosylated Creatine</td>
</tr>
<tr>
<td>PP</td>
<td>Averaged Peak Power</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per Minute</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

1.1 Introduction

Creatine (CR) is a naturally occurring modified amino acid that functions in key roles in bioenergetics, the flow of energy in a biological system (Bessman and Carpenter, 1985). CR may be synthesized in the body through a three-enzymatic step process beginning in the kidneys and finishing in the liver (da Silva et al., 2009). CR may also be obtained through the ingestion and digestion of meats, dairy products, as well as through supplementation (Preen, 2003). CR and its energetically activated form, phosphocreatine (PCR), are involved in numerous biochemical processes regarding bioenergetics. Specifically, PCR provides sustenance for adenosine triphosphate (ATP) and other nucleoside analogs, which serve as an energy currency within cells, to remain largely in their energetically active form.

In general, when the energy state of the cell is in low need, CR may be converted into PCR and stored within the cytosol of the cell to be used at a later time. PCR is used to transfer a phosphate group, a process called phosphorylation, onto adenosine diphosphate (ADP) to make ATP. CR may then travel through the cytosol to sites of ATP production to be phosphorylated back into PCR, which then is able to repeat the process of ATP regeneration. The mechanism for CR and PCR is advantageous for many reasons. Regarding muscular contraction for physical exercise, PCR provides the means for continuing work rates at relatively high intensities for as long as 10 seconds before the work rate is limited by energy production (Hespel et al., 2001). Furthermore, it is well documented that the supplementation of CR contributes to greater athletic performance in anaerobic exercises by increasing power output (Kocak & Karli, 2003) and by extending the ability to work at higher intensities of physical exercise for longer duration (Miura
et al., 1999). There is, however, less evidence that PCR has any benefits to anaerobic exercises when supplemented within two hours of performance. Determining the effects of CR supplementation within two hours of anaerobic tests could benefit athletes whose performance relies on producing power efficiently. Therefore, the determination of any effects of CR supplementation on anaerobic tests within two hours of supplementation may warrant further research behind the claims.

1.2 Purpose of the Study

The purpose of this study is to determine the acute effects of intermittent CR supplementation prior to anaerobic performance. Specifically, the research will evaluate the effect of CR supplementation on the average peak power produced in the first second, the power generated during seconds 1-5 and 5-10, and the averaged end power production between seconds 25-26 during a 30-second sprint performed on a cycle ergometer.

1.3 Research Hypothesis

This research contains three separate hypotheses. The first hypothesis is that neither CR or carbohydrate (CHO) supplementation will affect increases in the averaged peak power output relative to the control. The second hypothesis is that there will be more power generated during the intervals of seconds 1-5 and 5-10 for CR supplementation compared to CHO. The third hypothesis is that the averaged end power production will be greater for CR supplementation compared to CHO.

1.4 Significance of the Research

The significance of this research is that, to the researcher’s knowledge, there has not been any studies that have determined if CR supplementation within two hours of exercise has any effects. This study may raise cause for further investigation into the supplementation of CR within
two hours of anaerobic testing protocols if the hypotheses are confirmed. Currently, the recommended dosage for CR supplementation is to ingest CR supplements at high loads for five to seven days. Specifically, the International Society of Sports Nutrition (ISSN) recommends consuming 0.3 g/kg/day for 5-7 days (Antonio et al., 2014). After the loading phase, the typical dose may be dropped to as little as 3-5 grams per day for maintenance. A common side effect from supplementing CR is water retention, which increases body weight. Athletes that strive for optimal power to weight ratios may not receive the known benefits associated with CR supplementation due to increases in body weight. Thus, this study provides the preliminary research that may elicit the need for further investigation to determine different protocols for supplementing CR.

1.5 Variables

1.5.1 Independent Variables: Supplement Dosages

1. Water flavoring
2. 50 grams of dextrose (CHO) + water flavoring
3. 5 grams of creatine monohydrate + 50 grams of dextrose + water flavoring

1.5.2 Dependent Variable: Wingate Test Performance

1. Averaged peak power production (0-1 second)
2. Averaged end power production (25-26 seconds)
3. Power produced during the interval 1-5 seconds
4. Power produced during the interval 5-10 seconds

1.5.3 Controlled Variables

1. Age of the participant
   The age was controlled to limit risks during the exercise procedures.
2. Physically capable to perform max effort exercises
The participants must be physically capable to perform maximum effort exercises in order to complete the study.

3. No prior history of glucose intolerance/metabolic disorders

Participants that have or have had prior history of glucose intolerances/metabolic disorders may experience adverse events during the supplementation of CHO and/or during the cycling tests.

4. No prior history of CR supplementation

Prior history of CR supplementation may hamper with the results and the integrity of the study.

5. Hydration

Hydration was controlled to help prevent adverse events that may be caused due to dehydration.

6. Resistance applied during warmups and tests on the cycle ergometer

The resistance applied during warmups and tests were controlled to demonstrate increases relative to the participant and not a standard.

7. No external influences that may positively or negatively affect the performances of the participants

The experiment was conducted early mornings before classes were conducted in adjoining and adjacent rooms to insure that there were no external influences that may positively or negatively affect the performances of the participants.

1.6 Limitations

1.6.1 Standard addition protocol for supplementation versus a double blind controlled study

The supplementation protocol was performed in a standard addition manner, in that each participant received the placebo, the CHO, then the CHO plus CR for each of the 3 trials. Ideally, the experiment would have been performed in a double-blind manner, where all of the participants
performed the baseline placebo test then they would have been partitioned into two groups where both the participants and the investigators are unaware of the supplement administered to the participants. The researcher took the standard addition approach due to limited participants.

1.6.2 Dietary Intake of Foods that Contain CR

It is known that many foods contain CR. This study did not address this concern for it is difficult to supersaturate CR stores in muscle cells from dietary intake alone. Furthermore, it is difficult to assess CR concentration within specific foods to determine new exclusion criteria.

1.6.3 Vegetarians/Vegans were not screened.

The effects of CR supplementation in vegetarians and vegans are relatively unknown regarding athletic performances. As they do not ingest CR from dietary sources, their bodies rely on CR synthesis and make use of relatively lower stores of Cr for bioenergetics (da Silva et al., 2009). If their bodies were supersaturated acutely (within two hours), it could confound the results.

1.6.4 Supplement dosages were standardized

Each participant received CHO and CR in 50 and 5-gram dosages, respectively. The standardization of the supplement dosages may affect the results of the study due to disproportionate nutrients delivered. Both CHO and CR are known to provide energy to sustain muscle contraction (Loike et al., 1988). By standardizing the dosages, a participant may be receiving excess or insufficient nutrients as the experiment progresses. In either case, this may affect the results of the study. Furthermore, the experiment should have been individualized to the participant based on body mass to increase the probability of eliciting similar responses from each participant.

1.6.5 The research did not conduct any muscle biopsies for biochemical analysis to determine causation
This experiment did not conduct any muscle biopsies to further clarify the causation of any increases or decreases for the testing parameters. This study just demonstrates the similarities shared with other studies and the investigator may speculate any causes that elicited specific responses.

1.7 Delimitations

Each of the following variables were accounted for in the research.

1. Recreational athletes
2. Between ages 18 and 24 years old
3. Cycling Sprint
4. Max effort exercises within 30-seconds of duration
5. Dose specific at 50 grams of CHO and 5 grams of CR

1.8 Assumptions

The following assumptions were made due to their subjective nature and were made based on the honesty of each participant.

1. Each participant completed the required forms truthfully and honestly in order to screen for potential health risks that could skew the data such as prior CR supplementation.
2. Each participant fasted for the 10 hours prior to testing.
3. Each participant abstained from physical exercise 24 hours prior to testing.
4. Each participant performed maximally and to the best of their capability when performing each of the Wingate tests.
Chapter 2

LITERATURE REVIEW

2.1 Introduction

Bioenergetics is the flow of energy in a biological system (Brooks et al., 2005). Primarily, bioenergetics is the metabolism of macronutrient substrates (carbohydrates, proteins, fats) into usable energy. The energy is then transferred to sites that consume energy to perform various biological functions such as muscular contraction and substrate metabolism. Much is known about the bioenergetics of the skeletal muscular system, although most literature is not conveniently congregated into an integrative view. Specifically, most texts do not integrate various energy pathways regarding the use of CR and PCR to transport energy from sites of energy production to sites that consume energy within the cell. The purpose of this review is to provide a basic overview of molecular cellular bioenergetics in respect to the skeletal muscular system, starting with the fundamental substrates used for energy production that drives muscle contraction and then discussing the integration of those substrates under anaerobic (without oxygen) conditions in order to sustain physical activity in a energy state of high need. To conclude the review, there will be a discussion regarding the ergogenic benefits of supplementing CR and research models that test the ergogenic benefits.

2.2 Adenosine Triphosphate

ATP is the main substrate used as energy within muscle cells. It is popular belief that the source of ATP’s energy is produced by the hydrolysis of the third phosphate group, where a water molecule breaks the covalent bond between the second and third phosphate groups of ATP. While hydrolysis of ATP is seen in the body, it is not the main method for which ATP provides energy. When ATP undergoes hydrolysis, the energy produced is not transferred between systems but is
released as heat to the environment. It is well known that increasing the temperature of a chemical reaction may increase the rate of product formation, but this is regarding increases of temperature to the reaction itself. In the human body, local temperatures may fluctuate in accordance to various hydrolysis reactions. The fluctuating temperature within cells is due to the release of energy in the form of heat but that heat-energy is quickly lost to the surrounding environment in compliance with the laws of thermodynamics. The human body will attempt to sustain an ideal temperature of 37 degrees Celsius (Jeukendrup and Gleeson, 2010). Therefore, the method that ATP provides energy more efficiently is through the transfer of the third phosphate group onto its target site, converting the ATP into ADP. This energy is in the form of potential energy by adding a highly electronegative chemical group onto the target molecule which in turn makes the molecule more reactive or creates a conformation change within the target molecule. The bound phosphate group will then undergo hydrolysis to return the target site and molecule back to its original form. One enzyme that assists in the ATP phosphate transfer onto specific target sites is adenosine triphosphatase (ATPase).

ATP and ATPases are located at numerous locations within the muscle cell that utilize the phosphate transfer to allow for biochemical processes to proceed. One example is the sodium (Na)/potassium (K) pump that uses the phosphate transfer mechanism of ATP within ATPase. As the phosphate is transferred onto the target site of the Na/K pump, there is a conformational change within the pump that makes the pump function to return the cellular concentrations of Na and K back to an unfavorable concentration gradient with higher K concentrations inside the cell and Na outside the cell (Mougios and Mougios, 2006). There are many other gradient pumps that function like the Na/K pump, in that they use ATPase for the phosphate transfer mechanism to provide the conformational changes needed to work against concentration gradients. As previously mentioned,
ATP is used to make a conformational change in the target site of the molecule by transferring a phosphate from the bound ATP to the target site. In the case of the Na/K pump ATPases, the phosphate is transferred to an asparagine within the protein complex of the Na/K ATPase and allows for the pumping of K and Na against the gradient (Mougios and Mougios, 2006).

ATPase may also be found within sites of energy production and consumption. The localization of ATPase within sites of energy production and consumption allows for greater attempts at energy balance through the maintenance of ATP and ADP concentrations, which are stored compartmentally within each ATPase (Sata, 1996). In general, this allows the body to maintain the availability of ATP needed for various biochemical reactions to proceed. The compartmentalization of ATP within the ATPase also serves to prevent the accumulation of ADP within the cytosol of the cell. ADP may be used in specific metabolic pathways, such as ATP synthesis through adenylate kinase, that produce byproducts that are known inhibitors to the energy-producing sites within the cell when concentrations rise above a certain level (Mougios and Mougios, 2006). The localization of ATP within ATPase contradicts most texts, in that it is either omitted, assumed, or told that ATP and ADP freely travel through the cell. While there are small concentrations of ATP and ADP traveling freely within the cytosol of cells, approximately 3-6 mM (Saks, 2007), it does not behoove the cell to have relatively large quantities of ATP and ADP unbound and traveling freely within the cell, especially when there are enzymes that need ATP for activation. This would also subsequently increase concentrations of ADP and the byproducts from the bioenergetic processes using ATP. One of the biggest competitors for ATP is found within the bioenergetic pathways needed for skeletal muscular contractions.

For muscle contraction to occur according to the sliding filament theory, there must be sufficient ATP for myofilaments actin and myosin to bind and unbind. Specifically, ATP binds to
myosin which in turn releases actin from the myosin binding site (Lehninger et al., 2013). The hydrolysis of the ATP then changes the conformation of myosin in such a manner that allows for the myosin binding site to rebind to actin after the sequential release of the free phosphate and then the ADP from myosin. When there is a high need for muscular contraction such as during physical exercise, ADP begins to accumulate as ATP is used. To prevent the accumulation of ADP, the body may metabolize two ADP molecules through the enzyme adenylate kinase to produce one ATP and one adenosine monophosphate (AMP). Adenylate kinase works similar to ATPase, in that adenylate kinase transfers a phosphate group from one ADP onto the second ADP. The ATP generated may then be used as described above while AMP may be used in other biochemical processes such as signal transduction pathways (Jentjens, 2003). Thus, adenylate kinase functions as an alternative method for ATP production. ATP production through adenylate kinase requires no intermediates, contrasting ATP production from sugars and fats. Adenylate kinase also prevents the rise of ADP concentrations. It is not, however, the primary method for ATP synthesis. This is primarily due to the energy that is needed to convert AMP back into ATP is so high that AMP will likely continue down other metabolic pathways and create a net loss of ATP.

ATP is accepted as the body’s universal source of energy, and the maintenance of ATP concentrations are tightly regulated. It has been found that ATP concentrations do not fluctuate greatly in accordance to energy demands of the cell (Earnest, 1995). Fluctuation of ATP within the cell would correlate to increases in byproducts of ATP metabolism, such as ADP and hydrogen molecules (H). The gradual accumulation of ADP and H within the cell would eventually cause the cell to fail to function properly. This is in part due to the inhibition of many metabolic processes as the acidity of the cell, or H concentrations increase. Another reason for the lack of proper cell function is in accordance to adenylate kinase activity. As long as the energy state of the cell is in
high need, adenylate kinase will remain active in an attempt to sustain the energy needed for cellular function to the point that the concentrations of ADP, and subsequently ATP, dwindle to inhibitory status. To accommodate for inhibitory factors, in conjunction with ATP and ADP being stored compartmentally, there must be a transfer mechanism that allows for the sustenance of adequate ATP concentrations to perform necessary cellular functions. This transfer mechanism is accomplished through CR and its energetically active form, PCR.

2.3 Creatine and Phosphocreatine

When paired with ATP, PCR composes the second half of the phosphagen energy system (Brooks et al. 2005). The phosphagen energy system is named after the energy produced through the metabolic processes involving the transfer of phosphates between ATP, ADP, PCR, and CR. PCR is able to transfer its phosphate group to ADP through the enzyme creatine kinase (CRK) to generate an ATP and is converted to a CR molecule (Walsh, 2001), similar to adenylate kinase’s mechanism. The metabolic process through CRK is reversible, in that ATP can transfer its phosphate group to CR, converting it into PCR and generate an ADP molecule. Whereas ATP is termed as the body’s universal source of energy, PCR has been coined the universal phosphate donor.

CRK has been found functionally coupled with ATPases as dimers that help maintain consistent ATP concentrations (Sata et al., 1996). Dimers are two enzymes that are functionally coupled to work synergistically to allow for the enzymatic functions to occur more readily. For CRK, the functional coupling with ATPase allows for the exchange of phosphate groups between ATP and CR, as well as PCR and ADP, in such a manner that sustains consistent ATP concentrations. One study found that the strength of contractions in frog hearts decreased with the decrease in PCR availability, even though ATP stores remained around 75% of its max capacity.
Other knockout models that inhibited PCR function also found that at
the time of muscle failure, ATP stores were at or above 75% maximum capacity (Wallimann et al., 1992). Thus, PCR’s function benefits the cell not only to ensure that it has the necessary energy needed to perform essential functions to sustain life, but it also suggests that without PCR and CR the cell cannot function properly. It has been found that many bioenergetic systems utilize PCR and CR and possess unique isoforms of CRK that are specific to the cell type (Wallimann et al., 1992). These bounded isoforms of CRK include mitochondrial, myofilament, cytosolic, brain, and myocardial. The specificity of these isoforms is due to genetic factors that account for the specific cell type according to its location on the chromosome. Regardless of the specificity of the isoforms, all CRK function in the same manner: phosphate transfer. Therefore, what is discovered for one isoform of CRK generally applies to the others. In the research of ATP concentrations after PCR decrements, isoforms of cardiac muscle and skeletal muscle confirm the similarities while possessing minute genetic differences (Echegaray and Rivera, 2001). To further the previous claims, Sata et al. (1996) not only found that PCR and myocardial CRK serve to maintain functional concentrations of ATP cardiac muscular contractions, but they found that it also assisted in the availability of ATP for the actin/myosin crossbridge. The research previously mentioned indicates that while ATP is pertinent for bodily function, the transportation of energy through PCR is just as important for sustaining life.

The use of CR/PCR through CRK is also referred to as the Phosphocreatine Shuttle System (Bessman et al., 1985). The Phosphocreatine Shuttle System behooves cellular bioenergetics because the use of CR and PCR for biochemical reactions is limited to sites that contain CRK. While ATP and ADP are mostly bound compartmentally within ATPase, CR and PCR may travel freely and relatively unperturbed within the cell to the metabolic sites that produce or use ATP for
the maintenance of ATP concentrations. When the energy state of the cell is in low need, PCR may be synthesized and stored in the cytosol. In skeletal muscle, the stored PCR can help accommodate high levels of energy need. It should be noted that the Phosphocreatine Shuttle System is integrated with all sources of ATP production that use ATPase. What limits the quantity or duration of relative maximum effort in physical activity is the amount of PCR available to phosphorylate ADP back to ATP as it is used. The CR produced will then travel through the cytosol to sites of ATP synthesis to be phosphorylated back into PCR, which will then travel through the cytosol to be used where it is needed. While there are many means to synthesize ATP in anaerobic modes of exercise, which is characterized by less than two minutes of maximal effort physical exercise, most of the ATP synthesized is from the metabolism of carbohydrates and glycogen in a process called glycolysis.

2.4 Carbohydrates, Glycogen, and Glycolysis

Carbohydrates are one type of the macronutrient substrates used for energy production that fuel cells such as red blood cells and muscle cells (Richter, 2013). There are a variety of carbohydrates that may be consumed such as lactose and fructose, but the body eventually digests and metabolizes most carbohydrates into glucose. Glucose is the primary form of carbohydrate that is used in cellular bioenergetics. In physical exercise, skeletal muscle cells may use glucose for ATP production, called glycolysis (Bogardus, 1983), and indirectly through the Citric Acid Cycle and Electron Transport Chain (Goodyear, 1990). Much like CR and PCR, glucose may be stored in the body when the energy state of the cell is in low need.

When there is an abundance of blood glucose and the demand for energy is low, glucose will either be stored for later use or excreted, although storage is the more likely fate. When glucose is stored in the body, it is converted into either triacylglycerol (triglyceride) and stored within fat
cells and skeletal muscle (Jeukendrup and Gleeson, 2010) or it can be converted to glycogen which is stored in muscle cells and the liver (Ivy, 1988). As with glucose, glycogen’s main purpose is to produce ATP. Glycogen may undergo glycogenolysis, the process of liberating glycogen from its storage site to be converted into an intermediate that may be used for ATP production (Casey et al., 1995). Glycogen stored in the liver may also be used for ATP production but it may also be used for gluconeogenesis, the synthesis of glucose from non-carbohydrate sources (Ivy, 2004).

[Glucose and glycogen are two substrates utilized in glycolysis (Grewe, 1999). While glucose may enter glycolysis once transported into the cell, glycogen must be converted into an intermediate of glycolysis prior to entry. As these substrates undergo glycolysis, their fate is determined by the energy state of the cell and body. When the energy state of the cell is in high need, every glucose molecule that enters glycolysis will produce four ATP while consuming two ATP in the process to yield two pyruvates, two nicotinamide adenine dinucleotides (NADH), two ATP (Lehninger et al., 2013). The pyruvates and NADHs may be sent onto other metabolic pathways for ATP production while the ATP synthesized is used to phosphorylate CR into PCR per the PCR Shuttle System or, to a lesser extent, be released into the cytosol.] When the energy needs of the cell are low, glucose molecules that enter glycolysis may undergo an alternate reaction that stores a glucose metabolite, glucose 6-phosphate, as glycogen. [Since glycogen is stored within the cell, in conjunction with the location at which glycogenolysis occurs for reentry into glycolysis, glycogen only requires one ATP in the metabolic process that produces four ATP in glycolysis. Thus, ATP production through the metabolism of glycogen yields three ATP, one more than glucose metabolism that yields only two ATP. With the location of glycogen storage already in the cell and the higher yield of ATP, glycogen provides a more efficient method for ATP production. It is for that reason that glycogen provides the ATP production when glucose
availability is low. As glucose transport into the cell increases and subsequently enters glycolysis, glycogen metabolism will decrease to spare its utilization to better accommodate future needs. To prolong these biochemical processes as glucose stores diminish, the body has developed mechanisms to replenish blood glucose levels accordingly through gluconeogenesis.

Gluconeogenesis is the process of chemically converting non-glucose substrates into glucose (Hickner et al., 1997). As the body enters a high-energy need state, it will liberate all substrates that can enter gluconeogenesis in an attempt to sustain adequate blood glucose levels to assist in meeting the energy needs of the body. Gluconeogenesis is mostly completed in the liver but may also occur in the kidneys to a lesser extent (Bogardus, 1983). The substrates that may enter gluconeogenesis include lactate, glycerol, alanine, and glycogen. While glycogen is the primary substrate used in gluconeogenesis, there are more amino acids that can undergo gluconeogenesis, with alanine being most common due to its simplicity. Once glucose enters the bloodstream, it is then shuttled throughout the body and transported into cells that have a need for glucose.

2.5 Anaerobic Exercise: An Integrated Approach for Cellular Bioenergetics

The previous sections encapsulate cellular bioenergetics regarding anaerobic exercise. Anaerobic exercise is defined as a mode of skeletal muscle contraction that does not involve energy that primarily comes from oxygen metabolism (Kavanagh, 1988). In anaerobic exercise, there are two distinct phases of energy production that sustain muscular contractions. The first phase consists of the phosphagen energy system that allows for relatively high levels of power output. As previously stated, muscle contraction involves the linkage of myofilaments actin and myosin through ATP and ATP is replenished through the phosphorylation of ADP by PCR. Initially, the ATP that is stored compartmentally within the ATPase in the actin/myosin crossbridge is utilized
first. Stored ATP can generally provide for the energy needed for less than one second (Esbjornsson-Liljedahl et al., 1999). The generated ADP signals for PCR to phosphorylate ADP back to ATP at sites of ATP consumption. As the rate of ATP consumption increases, the amount of stored PCR decreases. Stored PCR can sustain high-energy needs for approximately three to five seconds before it is diminished and relies on the phosphate transfer from sites of ATP synthesis (Sata et al., 1996). In anaerobic exercise, the phosphorylation of CR to PCR occurs within the ATP producing enzymes of glycolysis and through adenylate kinase, both of which comprise the second phase of energy production. Although PCR and CR can freely travel through the cell to sites of ATP synthesis and consumption to provide sustenance for the cell’s energy needs, the rate limiting factor for the ability to sustain muscular contractions is the ability to produce ATP in adequate quantities. These mechanisms are often exploited through supplementation of CR and the ergogenic benefits have been extensively researched.

2.6 Creatine Supplementation and Ergogenic Benefits

One popular method to enhance capabilities in anaerobic exercise is to supplement CR. The premise behind CR supplementation is to increase the quantity of CR that the body possesses and eventually stores as PCR (Harris, 1992). While there are many forms of CR available to supplement, creatine monohydrate (CRM) is the form that has the most research supporting its potency, bioavailability, as well as the actual quantity of CR per gram when compared to other forms of CR (Jager, 2011). A common method for supplementing CR is to consume CR supplements at high volumes for five to seven days followed by maintenance dosages for as long as desired. Specifically, the ISSN suggests supplementing Cr at 0.3 grams per kilogram per day for five to seven days for the loading phase (Antonio et al., 2014). While one can calculate the exact quantity of CR to supplement, many research designs implement supplementing CR at high
volumes of 20 grams per day or greater for the loading phase at intervals of five-gram doses ingested throughout the day. After the loading phase, then the maintenance dosages may be dropped to as little as three to five grams per day.

There is a plethora of research on CR supplementation regarding its ergogenic benefits to exercise. Traditionally, most CR supplementation research on humans implement a baseline test for some measurable parameter then the participants consume either a placebo or CR supplement in the loading phase manner previously mentioned. Upon completion of the loading phase, the testing protocol is performed again and measured for deviations from baseline tests. For example, a study conducted by Birch et al. (1994) examined the effects of creatine supplementation on a repeated cycling test. Fourteen college aged males each performed three bouts of maximal effort exercise on a cycle ergometer on two separate occasions. Participants performed their maximum effort cycling bouts with four minutes of active rest between each 30 second cycling test. The participants were separated into two groups of seven in a double-blind study. Each group either consumed creatine or a placebo of an unsweetened glucose polymer during their loading phase, supplementing five gram dosages four times a day, every day, for five consecutive days that totaled 20 grams of CR supplemented per day. After the supplementation period, participants repeated another set of three 30 seconds cycling bouts. The experiment tested for differences in peak power output, mean power output, and total work output for each of the three bouts of cycling for both groups. While the placebo group demonstrated no changes in performance, the CR group demonstrated a significant difference after supplementation in the first bout of cycling at approximately 8% increase in power production. The CR group demonstrated significant differences within the first and second bouts of exercise for both mean power output and total power output, where each measurement demonstrated an approximate 6% increase. Although no
muscle biopsies were taken to determine if CR/PCR was the true cause of the power increases, the supplementation of CR coincides with CR’s role in bioenergetics. Thus, these findings indicate that CR supplementation can increase peak power production as well as the power production in the first 30 seconds of maximal effort cycling.

Another example may be seen in the research of Kocak and Karli. Their research studied the effects of CR supplementation on anaerobic capacity in 20 elite wrestlers of the Turkish national team (Kocak and Karli, 2003). Participants performed a 30 second Wingate test for baseline measurements in conjunction with measuring body mass. Following the baseline testing, the participants were separated into two supplementation groups consuming either CRM or milk powder for five days at four, five-gram dosages per day throughout the day for a total of 20 grams of CR per day. After the fifth day of supplementation, follow up testing revealed that that the CR group had increase average and peak power by approximately 12% and 17%, respectively, whereas the placebo did not demonstrate any significant increases. The CR group also demonstrated an average weight gain of 1.02 kg, whereas the placebo group measured an average loss of 0.2 kg. This study further fortifies the claims that CR supplementation can increase peak power and the amount of power produced within the first 30 seconds of maximal effort exercise.

Studies conducted by Aaserud et al. (1998), Zuniga et al. (2012), Smith et al. (1998), and Earnest et al. (1995) all fortify the notions made by Kocak and Karli (2003) and Birch et al. (1994), in that CR supplementation may benefit performance on maximum effort cycling tests. Not only has CR supplementation been shown to provide ergogenic benefits to cycling, but CR supplementation has been shown to benefit other modes of exercise. In a study conducted by Herda et al. (2009), the investigators viewed the effects of supplementing CRM or a CR isoform, polyethylene glycosylated creatine (PEGCR), on muscular strength, endurance, and power output.
Specifically, the testing parameters for the placebo controlled experiment included a countermovement vertical jump, two consecutive 30 second Wingate tests that tested for peak and mean power production, a one repetition maximum strength test for barbell bench press and incline leg press, a multiple rep maximum for both the barbell bench press and incline leg press at a resistance of 80% of the one repetition maximum, as well as body mass assessment. College-aged males (n=58) were recruited for the research. The experiment was a double-blind study where participants performed two tests separated by 30 days. After the first series of tests, the participants were separated into one of four groups that either consumed 3.6 grams of the placebo of microcrystalline cellulose, five grams of CRM, 1.8 grams of PEGCR, or 3.6 grams of PEGCR per day during the 30-day separation between testing days. The results indicated that both CRM and PEGCR, at both dosages, significantly increased the performance of the countermovement vertical jump, anaerobic peak power output from cycling, the 1 repetition maximum for bench press and leg press, and the multiple repetition maximum for both the bench press and the leg press. The CRM group also demonstrated significant increases in body mass and anaerobic mean power production in cycling.

2.7 Creatine Supplementation: Time Dependent or Dose Dependent?

The performance studies provided are just a few that demonstrate the ergogenic benefits from CR supplementation. As previously mentioned, the ISSN recommends to supplement CR at 0.3 g/kg/day for 5-7 days then follow with maintenance dosages at three to five grams per day for as long as desired (Antonio et al., 2014). Although many CR study designs supplement 20 grams per day for five days, Herda et al. (2009) revealed that low quantities of CR supplementation, 1.8 grams per day, can elicit ergogenic benefits after 30 days of continuous supplementation and Ziegenfuss et al. (2002) demonstrated ergogenic benefits after 3 days of supplementation at 20
grams per day. The findings by Herda et al. (2009) and Ziegenfuss et al. (2002) demonstrate different supplementation methods of CR that increase anaerobic performances, specifically in max effort cycling tests. It is known that once CR is ingested and enters the blood stream, it may then cross the cell membrane through co-transportation with sodium through specific sodium/chloride transport proteins (Miller et al., 2013). Furthermore, it has been shown that the CR transport across a cell membrane is amplified by the hormones epinephrine, insulin, and insulin-like growth factor 1 (IGF1) (Odoom et al., 1996). These are common hormones produced in the human body in response to specific stimuli: epinephrine from the adrenal cortex in response to stress, insulin from the pancreas in response to an increase in blood glucose levels, and IGF1 in various stages of sleep. Considering the mechanisms for CR transport in conjunction with the results from Herda et al. (2009) and Ziegenfuss et al. (2002), one may ask whether the ergogenic benefits of CR supplementation is a time dependent or dose dependent response. Specifically, does the body need the known five-day loading phase or may CR enter the cell and demonstrate the associated power increases in a shorter amount of time. Current research provides mixed results with some research suggesting that ergogenic benefits may be obtained in as little as three days with high volume (>20 grams per day) CR supplementation (Ziegenfuss et al., 2002). However, to the researchers’ knowledge there is no research that has viewed the effects of CR supplementation at high volumes on anaerobic modes of exercise within two hours of the testing parameters. Determining if there are ergogenic benefits to supplementing CR within two hours of the testing parameters may help guide future research by the implementation of new supplementation schemes that could enhance our understanding of bioenergetics.
CHAPTER 3
METHODOLOGY

3.1 Participants

Male (n=6) and female (n=5) participants between the ages of 18 and 30 years who were capable of performing maximum effort exercise were recruited from classes at Wichita State University. Participants were informed about the design of the study and all provided written informed consent prior to participation. Participants could not have glucose intolerance, dyslipidemia, chronic high blood pressure, previous and/or current medical conditions that may cause adverse events during exercise, or have previously used creatine as a supplement. These exclusion criteria were assessed during the first lab visit using the American College of Sport Medicine's (ACSM) medical history questionnaire (Pescatello, 2014) as well as a sport supplement questionnaire that determined prior use of caffeine, beta-alanine, and creatine. If a participant met any exclusion criterion, they were asked to obtain a physician’s note of clearance to participate in the study. The study was approved by the Institutional Review Board at Wichita State University and was in accordance with the Declaration of Helsinki.

3.2 Procedure

Each participant was asked to visit the lab on three separate occasions and each visit must be at least 48 hours apart. Participants were tested following a 24-hour abstinence from vigorous exercise and a 10-hour fast. Although they were asked to refrain from eating, they were strongly encouraged to drink plenty of water to ensure that they were properly hydrated. Hydration status was measured using a specific gravity refractometer (RHC-300ATC Specific Gravity Refractometer, Ade Advanced Optics) before testing began each day. Each participant urinated approximately 100 mL into a plastic cup. A pipet was used to transfer a small quantity of urine
onto the glass pane of the refractometer and specific gravity was measured via light diffraction. To meet the hydration requirement, the specific gravity for each participant’s urine sample had to be between 1.000 and 1.020 (Su et al., 2006). If this criterion was not met, then they were asked to drink water until adequate hydration was achieved or they were asked to reschedule their visit. Throughout each lab visit, participants drank water ad libitum in addition to what was administered in the supplement protocol (approximately 500 mL per supplement drink).

The supplement administration and exercise testing procedures were conducted in sequential order during each visit: hydration test, consumption of the first supplement drink, 30 minutes rest, seven-minute warm-up on a cycle ergometer at a resistance of 3.75% of their body weight at a speed of 70 revolutions per minute (RPM) or greater, consumption of the second supplement drink, 30 minutes of rest, seven-minute warm-up on the cycle ergometer at a resistance of 3.75% of their body weight at a speed of 70 RPM or greater, consumption of the third supplement drink, 30 minutes of rest, and the 30-second Wingate test. The Wingate test was performed by warming-up at no resistance for three minutes at 70 RPM or greater followed by 30 seconds of cycling without resistance or set RPM, a 10 second ramp-up period without resistance where the participant attempted to achieve maximum RPM, and then 30 seconds of cycling with a resistance of 7.5% of their body weight where they attempted to maintain maximum RPM. Upon completion of the Wingate test, participants continued cycling without resistance or walked on a treadmill for five minutes for a cool-down. An electromagnetically-braked cycle ergometer (Velotron RacerMate®, RacerMate, Inc., Seattle WA) was used for all cycling procedures and the seat height was adjusted to the height of the participant’s greater trochanter.

Each lab visit differed by the supplement administered. Each participant consumed, in a set order, one of three different supplement drinks during each visit, eventually consuming all three
supplements accordingly. The order of supplement drinks consumed per visit consisted of either flavored water, 50 grams of dextrose (carbohydrates) + flavoring, or 50 grams of dextrose + five grams of creatine monohydrate (Creapure®, AlzChem AG, CHEMIEPARK TROSTBERG, Germany) + flavoring. Each supplement drink was consumed with approximately 500 mL of room temperature (20°C) water. Each supplement drink was consumed within five minutes. The 30 minute rest period began after the completion of each supplement drink.

3.3 Data Analysis

The data was analyzed through SPSSv.23 by conducting descriptive analysis and a repeated measures ANOVA test for each testing parameter: averaged peak power (PP), power produced for seconds one through five and one through ten (AUC5 and AUC10, respectively), and averaged end power (EP). A linear regression was used to determine the area under the curve for both AUC5 and AUC10, from which the data points collected and used were at every one tenth (0.1) second increments for the respective timeframes. First, a Mauchly’s Test of Sphericity was calculated to determine if there was no significance in the variances from the performances within each of the three supplement trials (p>0.05). If sphericity was met, then no adjusted values were used to determine the F-value and the p-value for Within-Subjects effects between the three supplement trials. Then, a Partial ETA Squared ($\eta_p^2$) and observed power was calculated to determine the effect size (ES) for the Within-Subjects tests. Lastly, a pairwise-comparisons test was calculated to determine any significant differences ($p=0.05$) between supplement groups.
CHAPTER 4

RESULTS

4.1 Descriptive Statistics

Descriptive statistics are presented in Table 1. Although the population consisted of males (n=6) and females (n=5), all of the participants were combined for analysis (N=11).

<table>
<thead>
<tr>
<th>PARTICIPANT DESCRIPTIVES</th>
<th>Participants (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year) ± SD</td>
<td>21.2±1.7</td>
</tr>
<tr>
<td>Height (cm) ± SD</td>
<td>179.5±6.2</td>
</tr>
<tr>
<td>Body Mass (kg) ± SD</td>
<td>71.9±8.2</td>
</tr>
</tbody>
</table>

4.2 Averaged Peak Power (PP)

PP was analyzed using a repeated measures ANOVA. A Mauchly’s Test of Sphericity determined that each of the variances from the three performance tests were not significantly different (p=0.053). With Sphericity assumed, the Within-Subjects was found to be statistically significant, $F(2,10) = 4.497$, $p = 0.024$, $\eta^2_p = 0.31$, observed power = 0.701. A pairwise comparisons test was then implemented to determine if there were any significant differences ($p<0.05$) between trials (Table 2). The pairwise comparisons determined that there were no significant differences between the control (CON) and CHO groups ($p = 1.0$) or between CON and CHOCR groups ($p=0.055$). There was a significant difference between CHO and CHOCR ($p=0.012$), demonstrating a higher averaged power output of 36.1 watts in CHOCR over CHO.
The difference was a 3.93% increase in power produced. The Paired Samples Test calculated significance ($p<0.05$) between the CON and CHOCR ($p=0.018$) and between CHO and CHOCR ($p=0.004$).

**TABLE 2**

**AVERAGED PEAK POWER**

<table>
<thead>
<tr>
<th></th>
<th>Mean (watts) ± SD</th>
<th>Pairwise Comparison (p value)</th>
<th>Paired Samples Test: t value</th>
<th>Paired Samples Test: Significance (2-Tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>vs CON</td>
<td>vs CHO</td>
<td>vs CHOCR</td>
</tr>
<tr>
<td>CON</td>
<td>915.5±197.3</td>
<td>N/A</td>
<td>1.0</td>
<td>0.055</td>
</tr>
<tr>
<td>CHO</td>
<td>918.3±190.6</td>
<td>1.0</td>
<td>N/A</td>
<td>0.012</td>
</tr>
<tr>
<td>CHOCR</td>
<td>954.4±195.1</td>
<td>0.055</td>
<td>0.012</td>
<td>N/A</td>
</tr>
</tbody>
</table>

4.3 Power Produced: 1-5 s (AUC5)

AUC5 was first calculated using linear regression to determine the area under the curve for seconds 1-5 with data points collected at every tenth (0.1) second. The value calculated was then analyzed using a repeated measures ANOVA. A Mauchly’s Test of Sphericity determined that each of the variances from the three performance tests were not significantly different ($p=0.429$). With sphericity assumed, the Within-Subjects was found to be statistically significant, $F (2,10) = 16.011, p = 0.000$, $\eta_p^2 = 0.616$, observed power = 0.998. A pairwise comparison test was then implemented to determine if there were any significant differences ($p<0.05$) between trials (Table 3). The pairwise comparisons test determined that there was no significant difference between CON and CHO ($p=1.0$). There were significant differences between CON and CHOCR ($p=0.001$) and between CHO and CHOCR ($p=0.001$), demonstrating a 275.4 and a 294.9 watt increase, respectively. The difference between CON and CHOCR indicates an 8.75% increase in power produced and 9.42% between CHO and CHOCR. The Paired Samples Test demonstrated
significance \((p<0.05)\) between CON and CHOCR \((p=0.000)\) and between CHO and CHOCR \((p=0.000)\).

### TABLE 3

**POWER PRODUCED: 1-5 S**

<table>
<thead>
<tr>
<th></th>
<th>Mean (watts) ± SD</th>
<th>vs CON</th>
<th>vs CHO</th>
<th>vs CHOCR</th>
<th>Paired Samples Test: t value</th>
<th>Paired Samples Test: Significance (2-Tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>3147.4±679.9</td>
<td>N/A</td>
<td>1.0</td>
<td>0.001</td>
<td>N/A</td>
<td>0.001</td>
</tr>
<tr>
<td>CHO</td>
<td>3127.9±628.2</td>
<td>1.0</td>
<td>N/A</td>
<td>0.001</td>
<td>-0.288</td>
<td>0.780</td>
</tr>
<tr>
<td>CHOCR</td>
<td>3422.8±725.4</td>
<td>0.001</td>
<td>0.001</td>
<td>N/A</td>
<td>5.908</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**4.4 Power Produced: 5-10 s (AUC10)**

AUC10 was first calculated using linear regression to determine the area under the curve for seconds 5-10 with data points collected at every tenth (0.1) second. The value calculated was then analyzed using a repeated measures ANOVA. A Mauchly’s Test of Sphericity determined that each of the variances from the three performance tests were not significantly different \((p=0.317)\). With sphericity assumed, the Within-Subjects was found to be statistically significant, \(F (2,10) = 4.589, p = 0.023, \eta_p^2 = 0.315\), observed power = 0.710. A pairwise comparison test was then implemented to determine if there were any significant differences \((p<0.05)\) between trials (Table 4). The pairwise comparisons test determined that there were no significant differences between CON and CHO \((p=1.0)\) or between CHO and CHOCR \((p=0.051)\). There was a significant difference between CON and CHOCR \((p=0.032)\), demonstrating a 263.9 watt increase. The difference between CON and CHOCR indicates a 3.93% increase in power produced. The Paired Samples Test demonstrated significance \((p<0.05)\) between CON and CHOCR \((p=0.011)\) and between CHO and CHOCR \((p=0.017)\).
TABLE 4
POWER PRODUCED: 5-10 S

<table>
<thead>
<tr>
<th></th>
<th>Mean (watts) ± SD</th>
<th>Pairwise Comparison (p value)</th>
<th>Paired Samples Test: t value</th>
<th>Paired Samples Test: Significance (2-Tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vs CON</td>
<td>vs CHO</td>
<td>vs CHOCR</td>
<td>vs CON</td>
</tr>
<tr>
<td>CON</td>
<td>6719.2±1448.8</td>
<td>N/A</td>
<td>1.0</td>
<td>0.032</td>
</tr>
<tr>
<td>CHO</td>
<td>6744.9±1345.7</td>
<td>1.0</td>
<td>N/A</td>
<td>0.051</td>
</tr>
<tr>
<td>CHOCR</td>
<td>6983.1±1446.9</td>
<td>0.032</td>
<td>0.051</td>
<td>3.124</td>
</tr>
</tbody>
</table>

4.5 Averaged End Power (EP)

EP was analyzed using a repeated measures ANOVA. A Mauchly’s Test of Sphericity determined that each of the variances from the three performance tests were not significantly different (p=0.83). With sphericity assumed, the Within-Subjects was found to be statistically significant, \( F (2,10) = 0.505, \ p = 0.611, \ \eta_p^2 = 0.048, \) observed power = 0.121. A pairwise comparisons test was then implemented to determine if there were any significant differences (\( p<0.05 \)) between trials (Table 2). Pairwise comparisons determined that there was no significant difference between CON and CHO groups (\( p=1.0 \)), CON and CHOCR groups (\( p=1.0 \)), or CHO and CHOCR (\( p=1.0 \)). The Paired Samples Test demonstrated no significance (\( p<0.05 \)) between CON and CHO groups (\( p=0.376 \)), CON and CHOCR groups (\( p=0.566 \)), or CHO and CHOCR (\( p=0.652 \)).
### TABLE 5

**AVERAGED END POWER**

<table>
<thead>
<tr>
<th></th>
<th>Mean (watts) ± SD</th>
<th>Pairwise Comparison (p value)</th>
<th>Paired Samples Test: t value</th>
<th>Paired Samples Test: Significance (2-Tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>vs CON vs CHO vs CHOCR</td>
<td>vs CON vs CHO vs CHOCR</td>
<td>vs CON vs CHO vs CHOCR</td>
</tr>
<tr>
<td><strong>CON</strong></td>
<td>443.1±92.3</td>
<td>N/A 1.0 1.0</td>
<td>N/A -0.927 -0.593</td>
<td>N/A 0.376 0.566</td>
</tr>
<tr>
<td><strong>CHO</strong></td>
<td>453.6±93.5</td>
<td>1.0 N/A 1.0</td>
<td>0.927 N/A 0.464</td>
<td>0.376 N/A 0.652</td>
</tr>
<tr>
<td><strong>CHO CR</strong></td>
<td>448.7±107.4</td>
<td>1.0 1.0 N/A</td>
<td>0.593 -0.464 N/A</td>
<td>0.566 0.652 N/A</td>
</tr>
</tbody>
</table>
CHAPTER 5

DISCUSSION

5.1 Discussion

The results of this study indicate that more power may be generated during a Wingate test within two hours of supplementing CR compared to a similar protocol of CHO supplementation or a CON. Participants exhibited a significant difference in power output after CR supplementation for the averaged peak power output (CHO vs CHOCR) and the power produced during seconds 1-5 (CON vs CHOCR and CHO vs CHOCR) as well as 5-10 (CON vs CHOCR) in comparison to the CON and CHO supplementation protocols. While the experiment demonstrated ergogenic benefits with CR supplementation, only one of the three hypotheses made by the investigator was supported.

The first hypothesis, that neither CR nor CHO supplementation will affect increases in the averaged peak power output relative to the control, was not supported. A significant difference in peak power production was observed between CHO and CHOCR tests \((p=0.012)\), while the difference between CON and CHOCR was approaching significance \((p=0.055)\). In the CHO vs. CHOCR comparison, there was an increase in peak power produced after CR supplementation calculated at 3.93%. In theory, the number of muscle fibers capable of contracting to generate the peak power produced should not differ significantly based on the amount of energy available in the form of PCR. One possible explanation could be that while a neural stimulus is needed for muscle contractions, the force of the contraction is limited to the amount of PCR available to phosphorylate ADP back into ATP in accordance to the sliding filament theory. As described in Chapter 2, ATP is used to create a conformational change in the myosin myofilament that allows for it to bind to actin and then allows for the contraction of muscles. If there is a greater
concentration of PCR in the muscle cell, then the increased concentration could increase the amount of myosin myofilaments capable of binding to actin through the increased rate at which ADP may be phosphorylated. This increased phosphorylation would subsequently increase the force of contractions through greater quantities of actin and myosin interactions.

The second hypothesis, that there will be more power generated during the intervals of seconds 1-5 and 5-10 when viewing CR supplementation but not CHO, was confirmed. In the study, there was more power produced from the Cr supplementation trials than the CON and CHO trials. There were significant differences between CHOCR and CON ($p=0.001$) and between CHOCR and CHO ($p=0.001$) for the power produced during seconds 1-5 as well as between CHOCR and CON ($p=0.032$) during seconds 5-10. This provided for a calculated 8.75% increase between the CON and CHOCR trials and 9.42% between CHO and CHOCR trials. Although there was no significant difference between CHOCR and CHO during seconds 5-10, it was approaching significance $p=0.051$. The significant differences in the amount of power produced was likely due to the increase of PCR and CR in the muscle cells. As stated in Chapter 2, it has been established that supplementing CR will increase power production associated with anaerobic endurance (Jager et al., 2011). The results from the current study suggest that by supplementing CR and CHO in a fasted state versus a placebo or CHO, it is possible to receive similar ergogenic benefits as seen in Zuniga et al. (2012) where their participants obtained a mean increase of 5.4% in mean power produced during a Wingate test.

The third hypothesis, that the averaged end power production will be greater in the CR supplementation but not in the CHO, was disproved after observing no significant differences in the averaged end power production between all supplementation groups. In theory, the increased concentration of CR within the muscle cell would increase the transportation rate from sites of
ATP production to sites of ATP consumption. The reason why there were no significant differences in the observed effect between supplement groups is likely due to the mode of energy production. While the mitochondria produce most of the body’s ATP at rest, it is known that the production of ATP in the mitochondria is grossly slower than the ATP produced through glycolysis in the cytosol when energy needs are high (Richter, 2013). The Wingate test is an anaerobic capacity test that determines the efficiency at which a person’s glycolytic enzymes can produce the ATP needed to sustain maximum effort cycling after initial stores of ATP and PCR have been depleted. If intracellular concentrations of CR were elevated, then the rate-limiting step in bioenergetics would be the production of ATP and/or the phosphorylation of CR into PCR due to the increased concentration of CR available for phosphorylation. Therefore, any ergogenic benefits associated with CR supplementation during a Wingate test would not be a good representation of CR’s effects on mitochondrial ATP production. The third hypothesis would be better applied to an aerobic test such as a VO2max test or a timed 5 Km run where more of the energy produced is coming from mitochondrial sources rather than through glycolytic means.

Unlike a double-blind study, this experiment followed a standard addition approach. The standard addition approach was derived from a method used in chemical analysis. To perform the chemical analysis, a control sample is prepared and then the control sample is titrated with known quantities of a substrate being investigated in an unknown sample. Each sample that was prepared would have been analyzed to form a best-fit line from which interpolation of the unknown sample could determine the concentration of the substrate in the unknown sample. In the current study, each participant performed the same procedure three separate times under the control, CHO, and then CHOCR supplements. This method was implemented for many reasons. First, the standard addition approach accounts for physiological variances in the participants’ physical capabilities.
The capability to perform maximum effort tests like the Wingate test vary from participant to participant, where some may perform relatively better than others. In a double-blind study, the participants are randomly distributed into treatment groups and this creates a probability that participants may potentially be distributed unevenly and could skew the results. [The odds of uneven distribution of participants are less likely with greater sample sizes, but this research only recruited 11 participants which increases the risks of uneven distribution.] The researcher can limit this probability of error by specifying the inclusion criteria to only pertain to certain participants, but the researcher cannot account for each participant’s body type and the subsequent responses to each supplement. The standard addition approach accounts for the discrepancy by having each participant perform each supplement protocol. This also would allow the researcher to expand their inclusion criterion to maximize the participants without hampering the results due to physical performance discrepancies. The standard addition approach was used for that reason: maximizing sample size. The number of participants available for the research was limited. With only 11 participants enrolled in the study, the use of a double-blind study became impractical. The use of the standard addition approach allowed for each supplement group, CHO and CHOCR, to be tested 11 times versus five or six, depending on the distribution. The biggest reason for the use of the standard addition approach was for time. In an ideal study, not only would the researchers conduct a double-blind study but they would also perform a crossover. To properly perform a crossover study regarding CR supplementation, there must be a washout period to ensure any CR potentially consumed in the first trial does not hamper the performance in the second. Other studies that have done a crossover implement a six to eight week washout period (Preen, 2003). During the washout protocol, various stressors, physical and/or emotional, could enter the participants’ lives that could elicit a physiological adaptation and subsequently affect the second trial. The standard addition
approach does not require a washout period for CR supplementation, for it is always the last supplement to be tested in this research design. The standard addition approach allowed for each participant to finish their participation in as little as six days, dramatically reducing potential effectors and stressors. One pitfall to this research design is bias due to the researcher knowing what the participant is consuming. In that regard, it is up to the investigator to conduct the experiment honestly and accept that any findings from their research need to be verified through unbiased methods such as a double blind study.

5.2 Conclusion

In conclusion, CR supplementation within two hours of maximal effort testing was shown to increase anaerobic performance in recreational athletes. Specifically, CR supplementation demonstrated significant differences in the averaged peak power output between CHO and CHOCR, the power produced for seconds 1-5 between the control and CHOCR as well as between CHO and CHOCR, and in the power produced for seconds 5-10 between the control and CHOCR. The study also suggests that CR may be absorbed quicker than previously thought. This indicates that there may be alternative methods to supplementing CR and further research is warranted to verify the finding of this study.
BIBLIOGRAPHY


APPENDICES
Medical History Questionnaire

Participant ID:

Section A
1. When was the last time you had a physical examination?
2. If you are allergic to any medications, foods, or other substances, please name them.
3. If you have been told that you have any chronic or serious illnesses, please list them.
4. Give the following information pertaining to the last 3 times you have been hospitalized. 
   *Note:* Women, do not list normal pregnancies.

<table>
<thead>
<tr>
<th>Hospitalization 1</th>
<th>Hospitalization 2</th>
<th>Hospitalization 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reason for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hospitalization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month and year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of hospitalization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>City and state</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Section B
During the past 12 months
1. Has a physician prescribed any form of medication for you? ☐ Yes ☐ No
2. Has your weight fluctuated more than a few pounds? ☐ Yes ☐ No
3. Did you attempt to bring about this weight change through diet or exercise? ☐ Yes ☐ No
4. Have you experienced any faintness, light-headedness, or blackouts? ☐ Yes ☐ No
5. Have you occasionally had trouble sleeping? ☐ Yes ☐ No
6. Have you experienced any blurred vision? ☐ Yes ☐ No
7. Have you had any severe headaches? ☐ Yes ☐ No
8. Have you experienced chronic morning cough? ☐ Yes ☐ No
9. Have you experienced any temporary change in your speech pattern, such as slurring or loss of speech? ☐ Yes ☐ No
10. Have you felt unusually nervous or anxious for no apparent reason? ☐ Yes ☐ No
11. Have you experienced unusual heartbeats such as skipped beats or palpitations? ☐ Yes ☐ No
12. Have you experienced periods in which your heart felt as though it were racing for no apparent reason? ☐ Yes ☐ No
At present

1. Do you experience shortness or loss of breath while walking with others your own age?  □ Yes  □ No
2. Do you experience sudden tingling, numbness, or loss of feeling in your arms, hands, legs, feet, or face?  □ Yes  □ No
3. Have you ever noticed that your hands or feet sometimes feel cooler than other parts of your body?  □ Yes  □ No
4. Do you experience swelling of your feet and ankles?  □ Yes  □ No
5. Do you get pains or cramps in your legs?  □ Yes  □ No
6. Do you experience any pain or discomfort in your chest?  □ Yes  □ No
7. Do you experience any pressure or heaviness in your chest?  □ Yes  □ No
8. Have you ever been told that your blood pressure was abnormal?  □ Yes  □ No
9. Have you ever been told that your serum cholesterol or triglyceride level was high?  □ Yes  □ No
10. Do you have diabetes?
    If yes, how is it controlled?
    □ Dietary means  □ Insulin injection
    □ Oral medication  □ Uncontrolled
11. How often would you characterize your stress level as being high?
    □ Occasionally  □ Frequently  □ Constantly
12. Have you ever been told that you have any of the following illnesses?  □ Yes  □ No
    □ Myocardial infarction  □ Arteriosclerosis  □ Heart disease  □ Thyroid disease
    □ Coronary thrombosis  □ Rheumatic heart  □ Heart attack  □ Heart valve disease
    □ Coronary occlusion  □ Heart failure  □ Heart murmer
    □ Heart block  □ Aneurysm  □ Angina
13. Have you ever had any of the following medical procedures?  □ Yes  □ No
    □ Heart surgery  □ Pacemaker implant
    □ Cardiac catheterization  □ Defibrilator
    □ Coronary angioplasty  □ Heart transplantation

Section C

Has any member of your immediate family been treated for or suspected to have had any of these conditions? Please identify their relationship to you (father, mother, sister, brother, etc.).

A. Diabetes

B. Heart disease

C. Stroke

D. High blood pressure

APPENDIX A.2
# Checklist for Signs and Symptoms of Disease

*Instructions:* Ask your clients if they have any of the following conditions and risk factors. If so, refer them to their physicians to obtain a signed medical clearance prior to any exercise testing or participation. See the glossary on p. 411 for definitions of terms.

Client’s name ____________________________ Date ________________

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart murmurs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction (heart attack)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fainting/dizziness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Claudication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tachycardia (rhythm disturbances)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankle edema</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pulmonary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturnal dyspnea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coughing up blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise-induced asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breathlessness during or after mild exertion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>Yes</td>
<td>No</td>
<td>Comments</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----</td>
<td>----</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Metabolic (continued)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose intolerance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McArdie’s syndrome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Musculoskeletal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low back pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscular atrophy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swollen joints</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthopedic pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artificial joints</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male older than 45 yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female older than 55 yr, or had hysterectomy, or are postmenopausal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking or quit smoking within previous 6 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure &gt; 140/90 mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t know blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking blood pressure medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood cholesterol &gt; 200 mg · dL⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not know cholesterol level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have close relative who had heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physically inactive (&lt;30 min of physical activity more than 4 days/wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight by more than 20 lb (9 kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*If you have two or more risk factors, you should consult your physician before engaging in exercise.*
Lifestyle Evaluation

Smoking habits
1. Have you ever smoked cigarettes, cigars, or a pipe?  □ Yes  □ No
2. Do you smoke presently?  □ Yes  □ No
   Cigarettes ____________ a day
   Cigars ____________ a day
   Pipefuls ____________ a day
3. At what age did you start smoking? ____________ years
4. If you have quit smoking, when did you quit? ____________

Drinking habits
1. During the past month, how many days did you drink alcoholic beverages? ____________
2. During the past month, how many times did you have 5 or more drinks per occasion? ____________
3. On average, how many glasses of beer, wine, or highballs do you consume a week?
   Beer ____________ glasses or cans
   Wine ____________ glasses
   Highballs ____________ glasses
   Other ____________ glasses

Exercise habits
1. Do you exercise vigorously on a regular basis?  □ Yes  □ No
2. What activities do you engage in on a regular basis?

3. If you walk, run, or jog, what is the average number of miles you cover each workout? ____________ miles
4. How many minutes on the average is each of your exercise workouts? ____________ minutes
5. How many workouts a week do you participate in on average? ____________ workouts
6. Is your occupation?
   ____________ Inactive (e.g., desk job)
   ____________ Light work (e.g., housework, light carpentry)
   ____________ Heavy work (e.g., heavy carpentry, lifting)
7. Check those activities that you would prefer in a regular exercise program for yourself:

- Walking, running, or jogging
- Stationary running
- Jumping rope
- Bicycling
- Stationary cycling
- Step aerobics
- Handball, racquetball, or squash
- Basketball
- Swimming
- Tennis
- Aerobic dance
- Stair-climbing
- Other (specify)

Dietary habits

1. What is your current weight? _____ lb _____ kg height? _____ in. _____ cm
2. What would you like to weigh? _____ lb _____ kg
3. What is the most you ever weighed as an adult? _____ lb _____ kg
4. What is the least you ever weighed as an adult? _____ lb _____ kg
5. What weight-loss methods have you tried?

6. Which do you eat regularly?
   - Breakfast
   - Midafternoon snack
   - Midmorning snack
   - Dinner
   - Lunch
   - After-dinner snack

7. How often do you eat out each week? ____________ times

8. What size portions do you normally have?
   - Small
   - Moderate
   - Large
   - Extra large
   - Uncertain

9. How often do you eat more than one serving?
   - Always
   - Usually
   - Sometimes
   - Never

10. How long does it usually take you to eat a meal? ____________ minutes

11. Do you eat while doing other activities (e.g., watching TV, reading, working)? ____________

12. When you snack, how many times a week do you eat the following?
   - Cookies, cake, pie _____
   - Candy _____
   - Diet soda _____
   - Soft drinks _____
   - Doughnuts _____
   - Fruit _____
   - Milk or milk beverage _____
   - Potato chips, pretzels, etc. _____
   - Peanuts or other nuts _____
   - Ice cream _____
   - Cheese and crackers _____
   - Other ______________________

13. How often do you eat dessert? ____________ times a day ____________ times a week

14. What dessert do you eat most often?

15. How often do you eat fried foods? ____________ times a week

16. Do you salt your food at the table?  □ Yes  □ No
   - Before tasting it
   - After tasting it

APPENDIX A.5

The Acute Effects of Intermittent Carbohydrate and Creatine Supplementation on Anaerobic Performance in Recreational Athletes

Informed Consent Form

PURPOSE: You are being invited to participate in a research study that will measure the effects of creatine and carbohydrate supplementation on exercise performance.

PARTICIPANT SELECTION: You were selected as a possible participant in this study because you are between 18 and 30 years of age, able to perform maximal exercise, do not have cardiovascular disease, and are not glucose intolerant. Approximately 100 participants will be recruited. You will be asked to complete a medical history questionnaire and a sport supplement questionnaire to ensure your eligibility.

PROCEDURES: If you decide to participate, you will visit the Human Performance Lab (210 Heskett, WSU) on 3 days with at least 48 hours between visits. You will be asked to not consume any food overnight for 10 hours prior to each lab visit. On each visit, your hydration status will be measured. We will ask you to urinate approximately 100 mL into a plastic cup and from that sample a specific gravity refractometer will be used to determine hydration status. All samples will be discarded once specific gravity is measured. If you are not sufficiently hydrated, you will be asked to consume water until adequate hydration is met or you will be asked to participate another day. After documents are completed on the 1st lab visit and hydration status is met, each lab visit will proceed with the consumption of your 1st supplement drink, a 30 minute rest period, a 7 minute warm-up, consumption of your 2nd supplement drink, a 30 minute rest period, a 7 minute warm-up, consumption of your 3rd supplement drink, a 30 minute rest period, and a standard wingate test.

On the first day and after you consume your first supplement drink, your height and weight will be measured during the 1st 30 minute rest period. Your body composition (i.e., muscle, fat, bone) will be assessed with a full body DXA scan. The DXA emits a low radiation dose that presents minimal exposure to you - about one-tenth of what you would get in a chest x-ray. If you have had an x-ray exam or nuclear medicine isotope study within the last 7 days, notify the investigators and the DXA assessment will be postponed until 7 days since the exam has passed. Limb lengths (thigh, leg, foot, big toe) will also be measured using a large set of calipers.

The 7 minute warm-ups will be performed at a resistance set at 3.75% of your weight previously measured via magnetic brake and you will be asked to maintain a speed of 70 revolutions per minute (RPM) or greater. The standard wingate test is performed by warming up at 0% resistance for 3 minutes at 70 RPM or greater followed by cycling to a computer program that proceeds with cycling for 30 seconds at 0% resistance at no set RPM, a 10 second ramp-up period at 0% resistance where you will attempt to achieve the highest RPM as possible, then you will be asked to maintain that RPM for 30 seconds after a weight of 7.5% of your body weight previously measured is dropped via magnetic brake. Upon completion of the standard wingate test, you will be asked to either continue cycling at 0% resistance or walk on a treadmill for 5 mins for a cooldown, whichever feels most comfortable performing on.

The 1st lab visit should take approximately 2.25 hours to complete (2 hours, 15 mins), and the 2nd and 3rd visits should take approximately 2 hours to complete. You will be consuming 1 of 3 different
supplement drinks during each visit, with the remaining supplement drinks being consumed during the subsequent lab visits. The 3 supplement drinks will consist of either flavoring, 50 grams of dextrose (carbohydrates) + flavoring, or 50 grams of dextrose + 5 grams of creatine monohydrate + flavoring.

DISCOMFORT/RISKS: Some nausea, dizziness, muscle fatigue, and shortness of breathe may occur from the anaerobic tests, which is why proper warm-up and cool-down steps will be taken to help prevent any discomforts. You will also be encouraged to stay hydrated throughout the study. There is a very small risk of cardiovascular or cerebrovascular incident during exercise. There is an extremely small risk of sudden death during vigorous physical activity. However, a risk of cardiovascular incident, stroke and sudden death during exercise has only been demonstrated for individuals with established coronary artery disease and risk factors, cardiac rhythm disturbances or other serious medical conditions. Furthermore, the risk of serious medical complications are most persistently associated with very high intensity exertion in high risk subjects with infrequent exercise habits. In the unlikely event of a medical emergency, staff will be trained to respond appropriately. Procedures for the notification of Emergency Medical Services will be formalized in writing and the program personnel will understand these procedures.

BENEFITS: Your participation will aid in future research regarding sports supplementation. As part of your participation, you will be given a free DXA scan that measures the amount of lean muscle mass, amount of body fat, and bone mineral density upon completion of the study.

CONFIDENTIALITY: Every effort will be made to keep your study-related information confidential. However, in order to make sure the study is done properly and safely there may be circumstances where this information must be released. By signing this form, you are giving the research team permission to share information about you with the following groups:

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- The Wichita State University Institutional Review Board;
- The sponsor or agency supporting the study.

The researchers may publish the results of the study. If they do, they will only discuss group results. Your name will not be used in any publication or presentation about the study.

COMPENSATION OR TREATMENT FOR RESEARCH RELATED INJURY: Wichita State University does not provide medical treatment or other forms of reimbursement to persons injured as a result of or in connection with participation in research activities conducted by Wichita State University or its faculty, staff, or students. If you believe that you have been injured as a result of participating in the research covered by this consent form, you can contact the Office of Research and Technology Transfer, Wichita State University, Wichita, KS 67260-0007, telephone (316) 978-3285.

REFUSAL/WITHDRAWAL: Participation in this study is entirely voluntary. Your decision whether or not to participate will not affect your future relations with Wichita State University. If you agree to participate in this study, you are free to withdraw from the study at any time without penalty.

CONTACT: If you have any questions about this research, you can contact: Dr. Michael Rogers, office #106, Heskett Center, telephone (316) 978-5959 or Joseph Bach at jlbach@shockers.wichita.edu If you have questions pertaining to your rights as a research subject, or about research-related injury, you can contact the Office of Research and Technology Transfer at Wichita State University, 1845 Fairmount Street, Wichita, KS 67260-0007, telephone (316) 978-3285

You are under no obligation to participate in this study. Your signature below indicates that:

- You have read (or someone has read to you) the information provided above,
You are aware that this is a research study,
You have had the opportunity to ask questions and have had them answered to your satisfaction,
and
You have voluntarily decided to participate.

You are not giving up any legal rights by signing this form. You will be given a copy of this consent form to keep.

____________________________________________________
Printed Name of Subject

____________________________________________________
Signature of Subject                                      Date

____________________________________________________
Witness Signature                                         Date
Sport Supplementation Questionnaire

The following questions are regarding sport supplementation. Sport supplementation can potentially have an adverse reaction when taken before maximal exercise. With this in mind, please answer each question to the best of your ability.

1. Do you currently take caffeinated supplements, including pre-workout powders and coffee? If you do, please answer the following questions to the best of your knowledge.
   a. How often do you take caffeinated supplements?
      __________________________________________
   b. How much do you usually take in 1 day?
      __________________________________________

2. If you do take pre-workout supplements, please answer the following.
   a. What brand of pre-workout do you take?
      __________________________________________
   b. How often do you take pre-workout supplements?
      __________________________________________
   c. When was the last time you took a pre-workout supplement?
      __________________________________________

3. Have you consumed creatine or supplements that promote creatine synthesis such as glycine? If you answered yes, please answer the following questions to the best of your knowledge.
   a. When was the last time you consumed these
supplements? ________________________________

b. How much creatine do you consume in 1 day? ________________________________

____________________________________________________

Printed Name of Subject

____________________________________________________

Signature of Subject __________________________ Date

____________________________________________________

Witness Signature __________________________ Date