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entitled
The Effect of Creatine Supplementation on Exercise Performance following a Short-term
Low Carbohydrate Diet
by
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Submitted to the Graduate Faculty as partial fulfillment of the requirements for the
Doctor of Philosophy Degree in
Exercise Science

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Purpose: The purpose of this study was to determine if a creatine supplement prevents the decrease in performance while consuming a low carbohydrate (CHO) diet during high-intensity exercise. Compared to the placebo group where the low CHO diet was expected to result in reduced exercise tolerance, it was hypothesized that creatine supplementation would offset the adverse side effects of a low CHO diet, as demonstrated by a similar or improved exercise capacity. **Methods:** Twenty healthy subjects (8 males, 12 females; 27 ± 7 yrs, $\pm$ SD) were randomly assigned to a low CHO plus creatine monohydrate supplement (L-CHO+Cr), low CHO diet and placebo (L-CHO) group, or control (CON). Each subject performed 2 high intensity exercise tolerance tests (HIETT) to fatigue at 90% peak power output (PPO), separated by a 10 days where the subject followed a low CHO diet (<30% total consumption). Subjects completed a progressive exercise test to volitional fatigue to establish baseline fitness determined as peak O₂ uptake (VO₂peak) and to determine 90% of the peak work. The HEITT was on a 1:1 ratio of 30-seconds at 90% peak work rate followed by 30-seconds of no load active recovery. Heart rate was collected at baseline and every interval during

both exercise sessions. Subjects consumed a loading dose of creatine monohydrate (20 g/day) or placebo (5g/day), while adhering to a low-CHO diet of <30% CHO total daily consumption. Subjects kept detailed food and exercise logs for the duration of the study.

Results: The L-CHO+Cr group was of a similar fitness (36.1 ± 5.3 ml/kg/min) compared to the L-CHO group (36.9 ± 5.8 ml/kg/min) as determined by the progressive test to volitional fatigue. There was a statistically significant difference between the L-CHO+Cr group and the L-CHO HEITT performance pre- and post-dietary intervention ($p=0.001$). On average the L-CHO+Cr group demonstrated a 20% increase in the total number of bouts performed (pre/post), whereas the placebo group exhibited a 36% decrease. Both groups exhibited an average body fat loss of 1.0%, which was not statistically significant.

Conclusion: The results of the present study demonstrated that a creatine loading protocol attenuates the fatigue associated with consuming a diet low in carbohydrate content, and indeed, leads to an improvement in high-intensity interval exercise performance. While the specific mechanism(s) underlying the improvement in performance was not identified in the present study, these findings are consistent with the results of studied demonstrating a significant relationship between the rate of phosphocreatine utilization following a dietary intervention and exercise performance.

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Chapter 1

Introduction

In pursuit of a leaner, stronger, faster body, the idea of “less is more” can be particularly enticing to those populations who are trying to achieve a leaner body mass or improve their endurance capacity while at the same time, minimizing the amount of time spent on achieving these goals. Thus, it should not be surprising that a current trend in diet and exercise is coupling high intensity interval training (HIIT) with a low carbohydrate diet (L-CHO). HIIT appears to be a good method for promoting body fat loss in a shorter duration of time (Gillen & Gibala, 2014; Hunter, Weinsier, Bamman, & Larson, 1998; Tremblay, Simoneau, & Bouchard, 1994; Volek, Quann, & Forsythe, 2010). In addition, this method for training offers a variety of benefits physiologically, such as improved maximal aerobic capacity (i.e. maximal oxygen uptake, VO_{2max}) (Duffield, Edge, & Bishop, 2006; Esfarjani & Laursen, 2007), a shift in the lactate threshold to higher intensity exercise (Esfarjani & Laursen, 2007), as well as breaking up monotony and expediting training sessions (Gillen & Gibala, 2014). While consuming a L-CHO diet has been shown to expedite fat loss through greater lipid utilization, a number of studies have shown that performing intense exercise ranges from uncomfortable to nearly-impossible while on a L-CHO diet due to low glycogen stores in

the muscle and liver (Balsom, Gaitanos, Soderlund, & Ekblom, 1999; Coyle, 2004; Williams, 2008). Therefore, it would seem that these two approaches to losing body fat may circumvent each other, ultimately resulting in reduced exercise tolerance and greater susceptibility to fatigue (Helge, 2002), which may impair performance and fat loss efforts.

The high energy phosphate system (i.e. adenosine triphosphate – phosphocreatine, ATP-PCr) can sustain maximal efforts up to approximately 20 s and therefore, in order to maintain ATP levels, other energy pathways must contribute to ATP resynthesis (Greenhaff, Bodin, Soderlund, & Hultman, 1994). The glycolytic pathway, which relies upon a readily available source of glycogen, is able to maintain ATP resynthesis at a rate sufficient to sustain high intensity exercise for two to three minutes before fatigue ensues (Lima-Silva et al., 2013; Romijn et al., 1993). In order to maintain exercise for longer than two to three minutes, the exercise intensity must be reduced or maintained at submaximal intensities at which oxidative phosphorylation of glucose and fatty acids becomes the predominant energy pathway supplying the needed ATP (Lima-Silva et al., 2013; Romijn et al., 1993).

When performing high intensity exercise, while on a L-CHO diet, the contribution of the ATP-PCr system will diminish, the glycolytic system will decrease due to reduced glycogen stores, and oxidative phosphorylation will be unable to sustain physical activity without considerable fatigue (Lima-Silva et al., 2013; Sahlin, 2014). This typically results in reduced overall performance including both reduced intensity and duration of exercise, as well as a greater rating of perceived exertion (RPE). All of these factors may impact training volume and compliance with the training program, leading to a less than

optimal training adaptations (Helge, 2002). A possible solution to augment exercise performance in the face of such conditions is supplementation with creatine. Creatine supplementation has been shown to increase muscle power output and reduce the rate of fatigue thereby allowing the individual to work at a higher intensity for a greater duration (Grande & Graves, 2005; Izquierdo, Ibanez, Gonzalez-Badillo, & Gorostiaga, 2002; Tarnopolsky, 2010). As previously mentioned, the body has a finite amount of ATP and PCr stored; however, if the body's stores are increased through creatine loading, it is proposed that maximal efforts can be sustained for longer durations (Greenhaff, 1997; Herda et al., 2009; Izquierdo et al., 2002; Spriet, 2006; Tarnopolsky, 2010). The advantage of the PCr system is that energy can be produced quickly and it is self-buffering, as the proton is required to replace the phosphate group of the PCr and therefore, any change in acid-base status is considered minimal (Robergs, Ghiasvand, & Parker, 2004; Tarnopolsky, 2010). Utilizing creatine, and thereby maximizing the potential of the PCr energy system, may allow individuals to train at a higher intensity or sustain training duration, which can ultimately lead to greater increases in performance (Grande & Graves, 2005). It is not currently known if an individual performing high intensity interval exercise while on a L-CHO diet will be able to exercise at the same, or higher intensity with the use of creatine supplementation.

1.1 Objective and Hypothesis

The objective of this study is to determine if a creatine supplement prevents a decrease in exercise tolerance while consuming a L-CHO diet during an acute bout of high intensity interval exercise. Compared to placebo and control groups, it is hypothesized that creatine supplementation will offset the adverse effects of

consuming a L-CHO diet, as demonstrated by an improvement in exercise tolerance while performing high intensity interval exercise.

Chapter 2

Review of Literature

2.1 High Intensity Interval Training

2.1.1 Introduction

High intensity interval training (HIIT) is typically defined as repeated bouts of short to moderate duration exercise, ranging from 10 seconds to 5 minutes of activity, at an intensity that is greater than the lactate threshold (Gillen & Gibala, 2014; Laursen & Jenkins, 2002). Bouts of activity are separated by periods of low-intensity or complete rest that are shorter in duration than the bouts of intensity, which are intended to allow for partial but not full recovery (Gillen & Gibala, 2014; Laursen & Jenkins, 2002). The intention behind this methodology is to repeatedly stress the physiological systems relied upon for other endurance-type activity to a greater degree than is required during the aforementioned activity (Laursen & Jenkins, 2002).

2.1.2 Concepts and Variations

High intensity interval training (HIIT) is characterized by its level of work output, determined by VO_{2max} and/or rate of perceived exertion (RPE), and work to rest ratio (Perry, Heigenhauser, Bonen, & Spriet, 2008; Utter et al., 2007; Zuniga et al., 2011). These parameters can be adjusted in order to bring about desired physiological

adaptations to enhance performance (Perry et al., 2008; Utter et al., 2007; Zuniga et al., 2011).

For instance, Zuniga et al. (2011) demonstrated that for their sample of recreationally competitive triathletes, a 30 s-30 s protocol was more beneficial than a 3min-3min protocol when both were performed at 90% maximal power output and performed to exhaustion. The short intervals (30 s) yielded higher values for absolute VO_2 (L/min), total time (minutes) completed for each session, mean relative VO_2 (ml/kg/min), and heart rate (HR) but with lower values of blood lactate concentration (BLC) as compared to longer intervals of three 3 minutes (Zuniga et al., 2011). Additionally, the lower exercise intensity (90% mean power output (MPO)) produced higher values for absolute VO_2 (L/min) and total exercise time (minutes) completed for each protocol as compared to the higher exercise intensity of 100% MPO (Zuniga et al., 2011).

2.1.3 Physiological Adaptations

The training responses to HIIT vary depending on the initial fitness level of the individual. In untrained to moderately trained individuals, an improved capacity for oxidative metabolism, as seen by an increase in expression of type I fibers, capillarization and oxidative enzyme activity, is mostly commonly observed (Laursen & Jenkins, 2002). Additionally, a significant increase in $\text{VO}_{2\text{max}}$ is often observed (Duffield et al., 2006; Esfarjani & Laursen, 2007; Kohn, Essen-Gustavsson, & Myburgh, 2011). In well-trained athletes, training benefit more commonly presents in the form of increased peak treadmill speed rather than increased $\text{VO}_{2\text{max}}$, which has been considered more closely related to

racing performance (Kohn et al., 2011). Arguably, well-trained or elite athletes are already performing at or near their peak $\text{VO}_{2\text{max}}$, and it would therefore seem accurate that HIIT would serve to increase $\text{VO}_{2\text{max}}$ in untrained or moderately trained populations only (Kohn et al., 2011).

2.1.3.1 Metabolism Bioenergetics

Another training response to HIIT is exhibited in enzyme activity within the skeletal muscle. In well-trained subjects, 6 weeks of HIIT elicited an increase in LDH activity in skeletal muscle, particularly in type IIa fibers (Kohn et al., 2011). While the exact mechanism requires elucidation, the authors propose that the marked increase in LDH activity suggests an improvement in the activity of the lactate shuttle indicating a greater reliance on glycolytic pathways and oxidative metabolism (Kohn et al., 2011). Such training adaptations support the aforementioned increase in peak treadmill speed and overall performance evidenced in well-trained athletes. Further, Burgomaster et al. (2008) reported reductions in net muscle glycogenolysis and phosphocreatine (PCr) degradation following 6 weeks of spring interval training (SIT) as compared to lower-intensity endurance training (ET). Both training regimens produced the same metabolic adaptations, such as increased mitochondrial density and enzyme activity, despite a lesser overall training volume with the SIT group, indicating HIIT is capable of inducing equivocal training responses as ET with less total training time (Burgomaster et al., 2008).

2.1.3.2 Fat Adaptation

Fat adaptation is the state at which the body's oxidative capacity or ability to utilize lipids for energy increases to a greater rate and the body becomes potentially more energetically effective as a result of increases in intramuscular substrate stores, mitochondrial density, enzyme activity (i.e. PFK, PDK, CS, β -HAD), and the quantity of membrane transporters (i.e. GLUT4, MCT-1 & -4, FAT/CD36, FABP_{pm}) (Burgomaster et al., 2008; Gibala & McGee, 2008; Laursen & Jenkins, 2002; Perry et al., 2008). In addition, this metabolic adaptation is thought to spare muscle glycogen and improve PCr kinetics (Forbes, Slade, & Meyer, 2008; Stepto et al., 2002).

It is well-established that PCr resynthesis is reliant on oxidative metabolism, as it is mediated by mitochondrial membrane-bound creatine kinase (CK) (Forbes et al., 2008; Greenhaff et al., 1994). Therefore, improved oxidative capacity, such as that exhibited following HIIT, may also improve the recovery of the PCr system (Forbes et al., 2008). To test this, Forbes et al. (2008) had fourteen recreationally active subjects perform six sessions of HIIT, three days per week in a 1:8 work to rest ratio (30 seconds:4 minutes) at a typical Wingate-resistance protocol. The subjects demonstrated an improved rate of PCr recovery, in line with that of trained distance runners as compared to untrained subjects (Forbes et al., 2008).

2.1.3.3 Fat Loss

HIIT has been associated with greater body fat loss over time with a lesser total training volume as compared to moderate to low intensity training regimes, with total training volume referring to both individual exercise bouts (minutes) and overall program

duration (weeks) (Esfandiari, Sasson, & Goodman, 2014; Gillen & Gibala, 2014; Hunter et al., 1998; Tremblay et al., 1994). A study by Tremblay et al. (1994) demonstrated significantly greater subcutaneous fat loss following 15 weeks of HIIT as compared to 20 weeks of ET, despite the total energy cost of the ET protocol being more than twice that of HIIT. The subcutaneous adiposity lost was nine-fold greater following the HIIT protocol as compared to the ET. These results are thought to be, in part, a result of a post-exercise training negative energy balance equivalent to a lipid deficit (Tremblay et al., 1994). There was also an observed increase in a citrate synthase, a citric acid cycle enzyme marker, which is thought to be supportive of the concept that oxidative phosphorylation is essential for creatine phosphate resynthesis in muscle (Tremblay et al., 1994). Additionally, this study resulted in a substantial increase in β -HAD enzyme activity following HIIT but not ET, which suggests HIIT may lead to better lipid utilization in the post-exercise state, and may lead to greater energy expenditure and lipid deficit (Tremblay et al., 1994). Lastly, it is suggested that vigorous exercise may result in a reduced post-exercise energy intake as compared to low to moderate intensity training assisting overall weight loss (Schuenke, Mikat, & McBride, 2002; Tremblay et al., 1994; Tucker, Angadi, & Gaesser, 2016).

2.1.3.4 VO₂ and Caloric Expenditure

In order to improve VO_{2max} it may be necessary to train near or at VO_{2max} (Esfarjani & Laursen, 2007). It is thought that a HIIT training program may induce adaptations that would increase an individual's VO_{2max} and overall fitness (Esfarjani & Laursen, 2007; Sartor et al., 2010). For instance, in a study by Sartor et al. (2010), HIIT was effective in increasing VO_{2peak} by 16% (on average) in two weeks of training. An

improvement in overall fitness and $\text{VO}_{2\text{max}}$ may increase the basal metabolic rate of the individual, leading to greater caloric expenditure during exercise, as much as a proposed 33% (Esfarjani & Laursen, 2007; Hunter et al., 1998; Perry et al., 2008; Sartor et al., 2010).

2.1.3.5 Excess Post-Exercise Oxygen Consumption (EPOC)

Dubbed “excess post-oxygen consumption” in 1984 by Gaesser and Brooks, EPOC refers to an elevated VO_2 following exercise for a time (LaForgia, Withers, & Gore, 2006). It was originally thought that EPOC was in direct correlation to the oxygen debt of exercise, but it is currently thought that EPOC is a result of general metabolic perturbations, and only partially a result of oxygen debt (LaForgia et al., 2006). EPOC that continues for greater than one hour following exercise is thought to be associated with triacylglycerol/fatty acid cycling, with a causal hypothesis relating to catecholamine concentrations, and exercise-induced increases in lipolytic responsiveness of adipocytes to catecholamines (LaForgia et al., 2006). Alternatively, Tremblay et al. (1994) suggested that EPOC is potentially explained by an enhancing effect of HIIT on post-exercise lipid utilization. There have been considerable research efforts to more clearly identify the magnitude of EPOC and its impact on the thermic effect of activity following exercise (LaForgia et al., 2006).

Post-exercise, oxygen is used for lactate clearance and rephosphorylation of creatine and ADP (Schuenke et al., 2002). In addition, the body is also recovering homeostatic imbalances of hormones and/or protein degradation (Schuenke et al., 2002). To what extent EPOC is responsible for these mechanisms is currently unknown, but it is thought to be attributed to such (Schuenke et al., 2002). Regardless, EPOC and its

duration, are identified to be in direct correlation to the length of the exercise bout and intensity (Drummond, Vehrs, Schaalje, & Parcell, 2005; Kaminsky, Padjen, & LaHam-Seager, 1990; LaForgia et al., 2006; Mukaimoto & Ohno, 2012; Schuenke et al., 2002; Thornton, Rossi, & McMillan, 2011; Tremblay et al., 1994).

Research suggests that the duration of EPOC depends on type of training (aerobic vs. anaerobic), the order in which combination training is performed, and meal timing following exercise (Drummond et al., 2005; LaForgia et al., 2006; Schuenke et al., 2002). EPOC appears to continue for a longer duration in a fasted post-exercise state, and meal consumption following exercise results in the culmination of exhibited EPOC effects (LaForgia et al., 2006). Additionally, EPOC appears to continue for a longer duration following resistance or anaerobic training as compared to aerobic training, and increases exponentially in relation to exercise intensity (LaForgia et al., 2006; Schuenke et al., 2002). Lastly, in consideration of combining aerobic training with anaerobic training such as running and resistance training, the order in which these are performed can also impact EPOC duration (Drummond et al., 2005). It has been observed that EPOC was elevated to a greater degree and for longer duration following a Run-Resistance protocol as compared to a Resistance-Run protocol (Drummond et al., 2005). It would seem that aerobic exercise offers a measure of physiological recovery following resistance training that minimizes or negates the exhibited EPOC (Drummond et al., 2005).

2.1.3.6 Lactate Threshold and Exercise Tolerance

HIIT has been reported to reduce blood lactate concentration levels during exercise, elevate the lactate threshold, and be more tolerable (as reported via rate of perceived exertion (RPE)) than steady state exercise (Duffield et al., 2006; Esfarjani &

Laursen, 2007; Utter et al., 2007; Zuniga et al., 2011). Esfarjani and Laursen (2007) state that blood lactate levels are linked to muscle oxidative capacity, which is a physiological adaptation often linked to HIIT. It is also suggested that, through these physiological adaptations and the repetitive stimulus of HIIT, running economy, defined as the steady-state relative VO_2 , also improves (Esfarjani & Laursen, 2007; Perry et al., 2008).

Improvement in running economy and an increased lactate threshold assist to improve exercise tolerance (Esfarjani & Laursen, 2007; Utter et al., 2007). Additionally, Utter et al. (2007) argue that HIIT, by design, may be more tolerable due to the shorter bouts of intensity separated by rest periods, as defined by RPE.

2.1.4 Summary

It has been suggested that the very intense nature of HIIT is what stimulates rapid physiological changes, while more traditional moderate intensity training yields adaptations much more slowly (Gillen & Gibala, 2014; Perry et al., 2008). With “lack of time” being a commonly cited barrier to regular exercise, it would present as a viable solution for many people (Gillen & Gibala, 2014). Additionally, it can be more tolerable due to the variability of the exercise protocol, which may prove to be more mentally stimulating as compared to continuous exercise (Hunter et al., 1998). It appears that HIIT is an equally successful exercise protocol as compared to continuous moderate-intensity training for inducing rapid training adaptations, while requiring less total work (Esfandiari et al., 2014).

2.2 Carbohydrates and Dietary Interventions for Performance

2.2.1 Role of Carbohydrates in Exercise

Glycogen availability is often considered a limiting factor in long-term exercise performance (Balsom et al., 1999; Sherman, Doyle, Lamb, & Strauss, 1993); particularly during high intensity exercise where there is a rapid breakdown of muscle glycogen (Balsom et al., 1999). Often, athletes will utilize dietary interventions in the form of macronutrient ratios in conjunction with specialized training to achieve optimal performance results (Stephens et al., 2002). Carbohydrates, in particular, are often increased or decreased in an attempt to yield greater performance, due, in part, to their role as the preferred fuel during intense exercise, and reasoned to be the amount of energy derived per unit of oxygen consumed being greater with carbohydrates as compared to fat (Stephens et al., 2002).

2.2.2 Dietary Interventions: High Carbohydrate

A diet is considered high in carbohydrates when the total caloric energy intake derived from carbohydrate is 50% or higher (Balsom et al., 1999; Green, Ball-Burnett, Jones, & Farrance, 2007). This is often balanced by the intake of fat and protein, with the greater intake typically fat (Balsom et al., 1999; Green et al., 2007). In regards to exercise performance, a high carbohydrate diet yields greater performance output and a lesser time to fatigue, as compared to a low carbohydrate diet (Balsom et al., 1999; Green et al., 2007; Utter et al., 2007).

A study conducted by Green et al (2007), compared the effects of high carbohydrate dietary manipulation with that of low carbohydrate on the exercise performance of eight untrained male subjects. The high carbohydrate diet was based on a

3000 kcal/day intake, and an approximate macronutrient split of 50/40/10 of carbohydrate/fat/protein (Green et al., 2007). The low carbohydrate diet was also based on 3000 kcal/day, and an approximate macronutrient split of 20/60/20 of carbohydrate/fat/protein (Green et al., 2007). The subjects were asked to cycle at 68% of $\text{VO}_{2\text{peak}}$ for 90-120 min, stopping to rest at 30 min intervals (Green et al., 2007). Ultimately, the high carbohydrate protocol resulted in greater performance as measured by time to fatigue and spared phosphocreatine (Green et al., 2007).

Another example in support of high carbohydrate consumption is an experiment performed by Balsom et al. (1999), in which they examined high-intensity exercise and muscle glycogen availability in humans. In this study, there was a drastic difference in the carbohydrate manipulation with the low carbohydrate macronutrient percentage split as 4/64/32 of carbohydrate/fat/protein, and the high carbohydrate macronutrient percentage split as 67/20/13 of carbohydrate/fat/protein (Balsom et al., 1999). The seven males subjects were asked to participate in two exercise protocols at differing levels of intensity, while consuming either the high or low carbohydrate macronutrient split, selected at random (Balsom et al., 1999). Overall, there was a 265% increase in total work output and overall performance time with the subjects consuming the high carbohydrate diet as compared to subjects consuming the low carbohydrate diet (Balsom et al., 1999).

2.2.3 Dietary Interventions: Low Carbohydrate

Consuming less than 30% carbohydrate of total daily intake, regardless of other macronutrient contributions, is considered low carbohydrate (Coyle, 2004; Westman, Mavropoulos, Yancy, & Volek, 2003). As many tissues in the body are glucose

metabolizing, particularly the central nervous system (CNS), other energy fuel sources are particularly important when consuming a low carbohydrate diet (Westman et al., 2003). Although, research suggests that a low carbohydrate diet practiced for up to 12 weeks does not obviously impact systemic glucose levels is not detrimental to the CNS and other glucose metabolizing tissues (Bowden et al., 2007).

2.2.3.1 Benefits to Fat Loss

Research suggests that consuming a low carbohydrate/high fat diet can yield greater quantities of free fatty acids circulating in the blood, indicating more adipose tissue being broken down and utilized for energy as compared to individuals consuming moderate to high carbohydrate diets (Balsom et al., 1999; Sartor et al., 2010; Westman et al., 2003; Yeo et al., 2008). Balsom et al (1999) demonstrated that, during and following exercise, subjects consuming a low carbohydrate/high fat diet exhibited a greater contribution to total energy from fat combustion as compared to subjects consuming a high carbohydrate diet. Additionally, Yeo et al. (2008) offer similar findings with subjects consuming a low carbohydrate/high fat diet and exercising at high-volume intensity. In this particular case, the experiment utilized the low carbohydrate/high fat diet for 5 days, followed by a single day of rest and carbohydrate restoration, resulting in greater rates of whole body fat oxidation and decreased rates of muscle glycogenolysis during submaximal training (Yeo et al., 2008).

In considering muscle glycogen availability in conjunction with high intensity exercise, Balsom et al. (1999) utilized a L-CHO dietary intervention consisting of 4% CHO, 64% fat, and 32% protein based on a 3000 kcal total intake. Subjects performed HIIT to exhaustion. The results demonstrated elevated levels of pre-exercise plasma free

fatty acid concentrations among low carbohydrate/high fat diet subjects, and evidence of a greater contribution of total energy expenditure from fat combustion overall (Balsom et al., 1999).

In another study, Lima-Silva, et al. (2013) examined the effects of CHO consumption on time to exhaustion (TTE) during high intensity exercise. L-CHO subjects were instructed to consume 25% CHO, 45% fat, and 30% protein, maintaining an isocaloric diet based on a 24-hr dietary recall (Lima-Silva et al., 2013). The primary findings support the body of research that consumption of a L-CHO diet impairs high intensity exercise performance (Lima-Silva et al., 2013).

2.2.3.2 Negative Effects

According to Westman et al. (2003), potential negative side effects of a low carbohydrate diet, although unsubstantiated through monitored clinical trials, include kidney stones, electrolyte deficiencies, elevated fatty acids, and gout if too much protein is consumed. Extremely rare side effects are optic neuropathy, acute variegate porphyria, and arrhythmia (Westman et al., 2003), although it is important to note that these conditions were not necessarily caused by a low carbohydrate/high fat diet, and also that the particular macronutrient ratios differed and in some cases were extreme. With regards to exercise, consuming a low carbohydrate diet often results in lethargy, increased fatigue, and reduced performance/capacity (Sherman et al., 1993; Stepto et al., 2002).

2.2.3.3 Fat Adaptation

It has been speculated that, as even the leanest of individuals is in possession of adipose stores that would be adequate fuel for exercise lasting several days, if we could

use more fat for fuel for exercise we could drastically improve endurance performance (Burke & Hawley, 2002). Therefore, in an effort to delay fatigue, many studies have sought to improve endurance performance by increasing the availability and/or the muscle's ability to oxidize fat, while concurrently sparing muscle glycogen stores (Burke & Hawley, 2002; Yeo, Carey, Burke, Spriet, & Hawley, 2011). One path of investigation into this realm has been through dietary interventions, particularly that of “fat adaptation.”

Consuming a L-CHO diet for three days or more is associated with alterations in skeletal muscle gene expression, substrate stores, metabolic flux, and fuel oxidation (Coyle & Hodgkinson, 2001; Yeo et al., 2008). There are several mechanisms that may explain these skeletal muscle fat adaptations (Yeo et al., 2011). L-CHO diets facilitate an increase in rates of whole-body lipolysis, with a corresponding increase in circulating free fatty acids (FFA), thought to be due, in part, to higher circulating catecholamine concentrations (Hawley, 2011). The increase in FFA circulation is likely to increase transporter protein (FAT/CD36) content, as well as concentrations of enzymes β -hydroxyacyl-CoA-dehydrogenase (β -HAD), carnitine palmitoyl-transferase I (CPT I), and hormone sensitive lipase (HSL) (Hargreaves & Cameron-Smith, 2002; Hawley, 2011; Spriet, 2014; Yeo et al., 2011). Additionally, fat adaptation may result in a decrease in key CHO-metabolizing enzymes such as glycogen phosphorylase (PHOS), phosphofructokinase (PFK), and pyruvate dehydrogenase (PDH) (Yeo et al., 2011). These shifts in metabolic flux are thought to be triggered by the increase in circulating FFAs, as the presence of one macronutrient in abundance automatically lowers the

energetic contribution of the others (Achten & Jeukendrup, 2004; Zderic, Davidson, Schenk, Byerley, & Coyle, 2004).

Of particular interest to researchers, are the effects of a low carbohydrate diet and its effect on the pyruvate dehydrogenase complex (PDHc). This enzyme complex is considered to be the primary regulator of substrate utilization, in that it is responsible for the conversion of pyruvate to acetyl-CoA, or simply the shift from substrate level phosphorylation to that of oxidative phosphorylation (Putman et al., 1993). It has been demonstrated that a low carbohydrate diet rapidly down-regulates the amount of PDH-active (PDHa) at rest, with a corresponding increase in PDH kinase (PDK) (Putman et al., 1993; Yeo et al., 2011). This shift in enzymatic activity is representative of a decrease in CHO oxidation at rest and over a wide range of exercise intensities (Yeo et al., 2011). This shift causes a decrease in PHOS activity, thereby suppressing glycogenolysis (Yeo et al., 2011). Interestingly, restoration of glycogen levels will not immediately alter these adaptations (Burke et al., 2002; Stellingwerff et al., 2006; Yeo et al., 2011). The exact mechanism is unknown, but it is speculated that the lasting effects of the adaptation has to do with the combination of intense training and a L-CHO diet (Burke et al., 2002).

Depending on the duration, high-intensity exercise ($>70\% \text{VO}_{2\text{peak}}$), while on a low carbohydrate diet ranges from uncomfortable and difficult to nearly impossible (Coyle, 2004, 2011; Fleming et al., 2003; Hawley, 2011; Lima-Silva et al., 2013; Stepto et al., 2002). Indeed, a low carbohydrate diet can impair submaximal performance when compared to a high carbohydrate diet (Fleming et al., 2003). At supramaximal intensities, a low carbohydrate diet reduced time to exhaustion (TTE), but a high carbohydrate diet did not enhance performance above baseline measures (Lima-Silva et

al., 2013). According to Fleming et al (2003), a L-CHO diet not only fails to improve exercise performance, it may impair maximal aerobic capacity, inhibit endurance capacity, and reduce peak power output.

The exact mechanisms explaining the decrease in performance while on a low carbohydrate diet are unknown. It is postulated that reduced work power output during endurance training, even following glycogen restoration, indicates that some mechanism beyond glycogen content is responsible for the decreased performance (Fleming et al., 2003). It is further suggested that changes in membrane composition, buffering capacity, perceived exertion, reduced body mass, or negative energy balance may be responsible (Fleming et al., 2003; Greenhaff, Gleeson, & Maughan, 1988; Greenhaff, Gleeson, Whiting, & Maughan, 1987).

2.3 Fatigue

2.3.1 Introduction

Skeletal muscle fatigue can be generally defined as the decrease in force or power production in response to contractile activity (Kent-Braun, Fitts, & Christie, 2012).

Fatigue is discussed as central and peripheral parts, so as to more specifically examine the potential mechanisms that cause skeletal muscle fatigue (Davis & Walsh, 2010).

However, limits to the energetic processes (bioenergetics) must first be discussed.

2.3.2 Metabolism

During exercise, stored energy, such as carbohydrates, triglycerides, and proteins, is converted to high-energy phosphates through various energetic processes, which is an intermediate step between substrate catabolism and energy utilization (Sahlin, 2014).

Nearly all processes require energy, and during exercise ATP turnover must happen rapidly in order to maintain power output (Sahlin, Tonkonogi, & Söderlund, 1998). Metabolic limitations set the upper margin for energy production and exercise capacity (Sahlin, 2006; Sahlin et al., 1998).

2.3.3 Phosphocreatine

The energy source that can maintain the highest rate of ATP generation is from the degradation of phosphocreatine (PCr) (Sahlin, 2006; Sahlin et al., 1998). Catalyzed by creatine kinase (CK), this near-equilibrium reaction is activated by an increase in free ADP, and the flux is dependent on the extent to which the reaction is displaced from equilibrium (Sahlin, 2006; Westerblad, Bruton, & Katz, 2010).

Flux of the CK reaction is also dependent on the PCr to Cr ratio, thus PCr availability may be a limiting factor in energy production before muscle stores of PCr deplete (Sahlin, 2006, 2014; Sahlin et al., 1998). Therefore, the total capacity of the ATP-PCr system is limited by the amount of stored PCr (Sahlin, 2006, 2014; Sahlin et al., 1998).

2.3.4 Glycolysis

During high rates of glycolysis, glycogen is the primary substrate and lactate is the primary end-product (Sahlin, 2006, 2014). Historically, an accumulation of lactate in the muscle was considered a major cause of fatigue (Kent-Braun et al., 2012; Spriet, Howlett, & Heigenhauser, 2000). However, research suggests that it is more likely the fall in pH as a result of accumulated hydrogen ions, as opposed to that of lactate, that

inhibits force production (Kent-Braun et al., 2012; Sahlin, 2006; Sahlin et al., 1998). It is thought that this decrease in pH (acidosis) may inhibit phosphofructokinase (PFK) and glycogen phosphorylase (PHOS), which are the two main enzymes of glycolysis (Sahlin, 2006).

Prolonged exercise performed between 65% and 90% maximal oxygen uptake can result in hypoglycemia and muscle glycogen depletion, which can contribute to fatigue (Kent-Braun et al., 2012). Although there is no current explanation for the ostensibly requisite oxidation of glucose, one hypothesis is that a certain rate of oxidation is necessary to provide intermediates for the citric acid cycle (CAC) and electron transport chain (ETC) (Kent-Braun et al., 2012). Another consideration in terms of fatigue is that, with the depletion of glycogen and subsequent increase in fat oxidation, the uptake and transport of free fatty acids (FFA) may be a rate limiting step in energy production depending on intensity of exercise (Kent-Braun et al., 2012).

2.3.5 Oxidative Phosphorylation

For exercise lasting more than 1-2 minutes, oxidative phosphorylation is the predominating process for ATP generation (Sahlin, 2006; Sahlin et al., 1998). It is generally accepted that oxidation of fatty acids cannot process at the same rate as that of carbohydrate, and therefore has a reduced energy contribution at exercise intensities exceeding 65% $\text{VO}_{2\text{max}}$ (Sahlin, 2006; Spriet, 2011). Fat is stored in copious amounts in the body, and its energetic potential is vast (Sahlin, 2006; Sahlin et al., 1998). For exercise performed at low intensities, where fat can be the sole fuel source, metabolic factors do not limit performance (Sahlin, 2006). It is carbohydrate that is store in limited

quantities, and therefore potentially limits exercise performance through metabolic pathways (Sahlin, 2006). Particularly, depletion of muscle glycogen can result in fatigue through a number of mechanisms (Sahlin, 2006).

2.3.6 Whole-body Energy Production

Energy production appears to be limited by enzyme activity, particularly that of phosphofructokinase (PFK), creatine kinase (CK), and myosin ATPase (Sahlin, 2006; Spriet, 2014). Conversely, aerobic power appears to be limited by oxygen delivery rather than inherent muscle parameters (Sahlin, 2006). Both anaerobic and aerobic pathways depend, and are thereby limited, by substrate availability (ie. glycogen) and the capacity to distribute interfering metabolic end products (Sahlin, 2006; Sahlin et al., 1998).

2.3.7 Central Fatigue

Skeletal muscle contraction is controlled by a series of events, and fatigue can be a result of impairment to any particular event along the series (Westerblad et al., 2010). The series starts in the central nervous system (CNS) with the motor cortex, which is primarily responsible for individual motivation, and thereby results in a higher neural drive (Sahlin, 2006; Westerblad et al., 2010). Large fast-twitch motor neurons have a higher activation threshold, and thereby require a higher neural drive as a result of the potential difficulty and discomfort of the exercise (typical of high-intensity exercise) (Sahlin, 2006).

2.3.7.1 Motor Cortex

A reduction in motivation/drive may be a result of supraspinal effects of group III and IV muscle afferents, which react to changes in core temperature, as well as the chemical/mechanical environment within the muscle (Sahlin, 2006). A rise in core temperature could occur during strenuous exercise as a result of the atmospheric environment, as evident exercise in extreme heat and/or humidity (Sahlin, 2006). Extreme heat may result in an increased cardiovascular demand that results from blood being redistributed away from the skin (Sahlin, 2006). Metabolic perturbations could occur as a result of substrate availability, or lack thereof, such as glycogen depletion (Sahlin, 2006). Muscle glycogen stores can become depleted following >2 hours of prolonged exercise, without carbohydrate supplementation (Sahlin, 2006). Glucose is the primary substrate for neural tissue, thus hypoglycemia may impair CNS function and exercise performance (Sahlin, 2006).

2.3.7.2 Spinal Cord

Activation of the motor cortex results in the activation of alpha motor neurons in the spinal cord, where fatigue is expressed as an altered or failed firing frequency of motor neuron signal transmission (Davis & Walsh, 2010; Sahlin, 2006; Westerblad et al., 2010). Efferent and afferent signs are collected in the somas of the motor neurons, both excitatory and inhibitory, and will temper the signal output to the muscle (Kent-Braun et al., 2012). Therefore, as muscle fatigue manifests, changes in the signal output from the motor cortex, in conjunction with changes in several sensory receptors that relay information to the spinal cord, background excitability and total signal output are

changed in the motor neuron may result in impaired firing frequency (Kent-Braun et al., 2012).

Several factors play into the role of central fatigue vs. peripheral fatigue, such as the motivation and training status of the subject; the duration, intensity and type of exercise; and the type of muscles involved (Sahlin, 2006). It is generally accepted that central fatigue plays a larger role in prolonged lower intensity exercise, where metabolic changes are of a lesser extent, while peripheral fatigue predominates short-term high-intensity exercise (Sahlin, 2006; Westerblad et al., 2010)

2.3.8 Peripheral Fatigue

Peripheral fatigue relates to intramuscular factors that cause impaired contractile functioning during exercise (Westerblad et al., 2010). Experimental techniques used to study peripheral fatigue are more established, and central fatigue a more complex phenomenon, thus the majority of the literature of skeletal muscle fatigue focuses on peripheral fatigue (Sahlin, 2006). Additionally, peripheral fatigue can be examined utilizing both animals and single muscle fibers, while central fatigue in animals does not particularly correlate to human subjects (Sahlin, 2006).

2.3.8.1 Neuromuscular Junction

Neuromuscular propagation refers to the process of converting an axonal action potential (AP) into a sarcolemmal AP (Enoka, 1994). Prolonged activity can impair some of these processes, resulting in the reduced force production associated with fatigue (Enoka, 1994). Sites of potential impairment include: failure of the axonal AP to invade all branches of the axon, depletion of neurotransmitters, reduction in exocytosis, and/or

decreased sensitivity of the postsynaptic membrane (Enoka, 1994). Muscle fiber excitability can be tested using electromyography (EMG) via the m-wave (Kent-Braun et al., 2012; Sahlin, 2006). The m-wave is a compound AP recorded with surface EMG in response to an electrically-induced stimulation of the motor neuron (Kent-Braun et al., 2012; Sahlin, 2006). The size of the m-wave reflects electrical transmission at the neuromuscular junction and excitability of the muscle fiber membranes (Kent-Braun et al., 2012). Failure of excitation-contraction coupling (ECC) is demonstrated by a change in twitch force without a change in the m-wave (Kent-Braun et al., 2012).

2.3.8.2 Excitation-Contraction Coupling

There are seven series processes involved in the conversion of an AP into muscle force: (1) propagation of the AP along the sarcolemma; (2) propagation of the AP down the transverse tubule; (3) the change in calcium (Ca^{2+}) conductance of the sarcoplasmic reticulum (SR); (4) movement of Ca^{2+} down its concentration gradient into the sarcoplasm; (5) reuptake of Ca^{2+} by the SR; (6) binding of Ca^{2+} to troponin; (7) the interaction of actin and myosin, and the work of the crossbridge cycling (Enoka, 1994). Impairment of any event along the series can result in impaired force production.

Although causes of impairment of the propagation of the AP along the surface of the sarcolemma (process 1) is not attributed to be a cause of fatigue, it is considered to be a result of repeated activation (Allen, Lamb, & Westerblad, 2008). Repeated activation can slow the propagation of the AP, reduce its size, and increase its duration due to fiber depolarization (Allen et al., 2008). The changes in the sarcolemmal properties can also lead to intermittent failure in a progression of APs, but the following slowing of fusion

frequency indicates that such AP failure does not evidently contribute to decreased force (Allen et al., 2008).

Disturbances to the propagation of the AP down the transverse tubule (processes 2 & 3) are attributed to the high chloride (Cl^-) conductance within the T-tubular system, as the Cl^- channels are responsible for potassium (K^+) clearance or reducing the rate of K^+ accumulation (Allen et al., 2008). A build-up of K^+ within the T-tubule system, as a result of high frequency of APs, results in the depolarization of the fiber as a whole causing sarcolemma AP failure (Allen et al., 2008).

Changes to the intracellular environment, as a result of metabolic disturbances, can reduce the amount of Ca^{2+} released and/or returned to the SR (processes 3 & 4) (Enoka, 1994; Sahlin, 2006). The release of Ca^{2+} from the SR Ca^{2+} pumps is thought to be impaired by depleted stores of Ca^{2+} and/or inactivation of the release channels (Enoka, 1994). It is also potentially due to the change in AP size along the surface of the sarcolemma (process 1), which results in reduced activation of the SR Ca^{2+} pumps (Allen et al., 2008). Reuptake inhibition (process 5) is likely due to a reduced concentration gradient, and the resulting change in the flux of Ca^{2+} across the SR membrane (Enoka, 1994).

Furthermore, glycogen stores have an integral role beyond being an energy source, as it has been implicated in serving to maintain cell organelle integrity and function (Sahlin, 2006). Prevention of glycogen resynthesis in single mouse fibers resulted in incomplete force recovery, decreased Ca^{2+} release, and decreased Ca^{2+} sensitivity (Sahlin, 2006). The same impairment, as a result of diminished glycogen

stores, have been evidenced in humans; thus, muscle glycogen seems to be important for maintaining fiber excitability, possibly due to a structural role of glycogen in association with the SR (Gejl et al., 2014; Sahlin, 2006).

2.3.8.4 Metabolic Changes

Increases in hydrogen (H^+), inorganic phosphate (P_i), and ADP change the intracellular environment, such as decreased pH, which in turn inhibits Ca^{2+} binding and cross-bridge cycling (processes 6 & 7) (Kent-Braun et al., 2012). Accumulation of P_i occurs as a result of PCr breakdown, a process that is particularly active during high-intensity efforts (Allen et al., 2008). Additionally, H^+ and ADP are also by-products of metabolism and ATP turnover.

The hypothesis that fatigue is a result of energetic processes failing to meet energy demands is archetypal, and many studies have shown that the onset of fatigue can be delayed through improved ATP generating processes (Sahlin, 2006). It is well-established that the enhancement of substrate availability can improve exercise tolerance and performance (Sahlin, 2006). However, as cellular ATP homeostasis is well-maintained during exercise, rarely decreasing by more than 30%, the hypothesis remains in question, and leaves room for further experimentation and research (Sahlin, 2006).

2.4 Creatine

2.4.1 Role in the Body and Exercise

Creatine is a naturally created compound that is synthesized from the amino acids arginine and glycine in the liver and kidneys (Tarnopolsky, 2010). In the absence of dietary supply, the body will make 1-2 g/day (Grande & Graves, 2005). Creatine can be

found in many food sources, such as cod, tuna, beef, salmon, pork, and herring; with trace amounts available through shrimp, cranberries, and milk (Grande & Graves, 2005). Over 90% of total body creatine is stored in skeletal muscle, with approximately 60% of that in the form of phosphocreatine (PCr) (Flanagan, 2007; Greenhaff, 1997; Tarnopolsky, 2010). Short bursts of energy, approximately the first 10-20s of work, are fueled by the ATP-PCr system (Flanagan, 2007; Grande & Graves, 2005; Tarnopolsky, 2010). Approximately half of the exercising muscle's PCr content can be restored within one minute of recovery, but it can take up to six minutes for the PCr pool to replenish completely (Flanagan, 2007; Greenhaff et al., 1994).

2.4.2 Creatine and Exercise Performance

The ability of creatine supplementation to enhance high intensity exercise with repeated bout performance is well established (Graef et al., 2009; Herda et al., 2009; Izquierdo et al., 2002; Kendall et al., 2009; Prevost, Nelson, & Morris, 1997). It is hypothesized that the increase in phosphocreatine (PCr), as achieved through supplementation, acts to postpone skeletal muscle fatigue by delaying the accumulation of ADP and inorganic phosphate (P_i), which are activators of glycolysis (Kendall et al., 2009). By reducing the reliance on glycolysis, it is thought that exercise efficiency and aerobic performance may also be improved (Kendall et al., 2009).

The mechanisms by which creatine supplementation is thought to improve high intensity exercise with repeated bout performance, or HIIT, are thought to be a result of increased phosphogen stores, metabolic shift, and an improved Ca^{2+} sensitivity (Bemben & Lamont, 2005; Greenhaff, 1997; Greenhaff et al., 1994; Prevost et al., 1997). High-

energy phosphate systems fuel short duration (<20s) exercise, characteristic of HIIT, and therefore an increase in intracellular content may thereby extend performance (Prevost et al., 1997). Additionally, a greater reliance on the phosphogen system may result in a reduced reliance on anaerobic glycolysis, resulting in a reduction in lactate accumulation and the resulting drop in pH (Greenhaff et al., 1994; Prevost et al., 1997). Lastly, it is suggested that, by virtue of the reduced ionic strength that occurs when water follows creatine osmotically into the muscle fiber, there may be a subsequent improvement in Ca^{2+} sensitivity (Graef et al., 2009). The ability to produce more force for a given intracellular concentration of Ca^{2+} may enhance performance and alleviate force loss resulting from reduced sarcoplasmic reticulum release of Ca^{2+} during the fatiguing state (Graef et al., 2009).

2.4.3 Loading

It has been demonstrated that creatine stores can be increased through supplemental loading, up to a 20% increase in muscle concentration (Greenhaff, 1997). The ability of the subject to increase their creatine stores varies depending on a variety of characteristics, such as s fitness level, dietary habits, and individuality – meaning that there are responders and non-responders to creatine (Greenhaff, 1997; Rawson, Stec, Frederickson, & Miles, 2011)

Creatine has a delayed onset of action, and is best loaded into the system over time (Rawson et al., 2011). Consuming 20 grams (g) per day, divided into 4-5 doses, over the course of 5-7 days is a well-established protocol (Greenhaff, 1997). However, it has been suggested that it would be more accurate to individualize the dosing based on body mass as creatine is stored within the skeletal muscle (Rawson et al., 2011). This

customized protocol is typically 0.03g/kg/dose, consumed 4-5 times per day for 5-7 days (Rawson et al., 2011)

In an attempt to study the effects of creatine supplementation on muscle power, endurance, and sprint performance, Izquierdo et al. (2002) used a short-term loading protocol of 5g of creatine monohydrate, 4 times per day, for 5 days. The subjects were then allowed 4-5 single attempts to determine 1RM with a 2 minute rest between attempts (Izquierdo et al., 2002). Creatine appeared to enhance 1RM performance in the half-squat, but not in the bench press, suggesting performance improvement in the lower extremity large muscles only (Izquierdo et al., 2002). Additionally, supplementation significantly improved performance during the first 5 meters of repeated bouts of 15m sprints (Izquierdo et al., 2002). Overall, increased urinary creatinine excretion suggests creatine supplementation was effective at raising creatine stores (Izquierdo et al., 2002).

Another experiment, by Rawson et al. (2011), utilized a slower loading protocol of approximately 2.3g of creatine per day for 6 weeks, utilizing the 0.3g of creatine per kilogram of body weight method. Maximal strength testing was performed using a 3RM leg extension, and maximal fatigue was tested also using a leg extension of 30 peak torques per set (of 5 sets) with 60s rest between sets (Rawson et al., 2011). No improvement was found in strength performance, but creatine supplementation enhanced resistance to fatigue (Rawson et al., 2011). It is speculated, in this study, that creatine is shed through creatinine in urine at a rate of 2g/day, and that loading doses must exceed this rate in order to enhance performance (Rawson et al., 2011), suggesting that the low-dose, slow loading was not sufficient to elevated creatine levels sufficiently to enhance muscular performance.

Two similar studies utilized the same creatine loading protocol of 20g/day for 30 days, divided into two 10g doses to be consumed pre- and post-training, respectively (Graef et al., 2009; Kendall et al., 2009). They examined the effects of a creatine supplement in conjunction with HIIT, utilizing the same HIIT protocol with a 2:1 work:rest ratio, with the work set at a supramaximal intensity (Graef et al., 2009; Kendall et al., 2009). However, they examined different indicators of performance, with Graef, et al. looking at ventilatory threshold (VT), VO_{2peak} , and total work done (TWD), and Kendall, et al. looking at critical power (CP) and anaerobic work capacity (AWC) (Graef et al., 2009; Kendall et al., 2009).

Graef, et al. (2009) demonstrated improvement in VT, with no concurrent improvements to VO_{2peak} or TWD. Considered to be an indicator of endurance performance, VT has been suggested to be a gauge for the ability of the cardiovascular system to adequately supply oxygen to the working muscles (Graef et al., 2009). Therefore, an improvement in VT may correspond to an increased time to exhaustion (TTE) and greater resistance to fatigue (Graef et al., 2009).

Kendall, et al. (2009) demonstrated an improvement to CP, without an improvement to AWC. CP indicates the highest sustainable output that can be maintained for an extended period of time, whereas AWC reflects the total metabolic work capacity independent of oxygen use (Kendall et al., 2009). An increase in CP lends to the hypothesis that PCr recovery relies on aerobic metabolism (Graef et al., 2009).

2.4.4 Potential Negative Effects of Creatine Supplementation

The proposed observed safe limit (OSL) for creatine supplementation is 5g/day (Herda et al., 2009). A number of negative side effects have been proposed in

conjunction with creatine supplementation: muscle cramping, liver dysfunctions, muscle fiber rupture, kidney impairments, and gastrointestinal distress (Grande & Graves, 2005; Kim, Kim, Carpentier, & Poortmans, 2011). However, it is suggested that muscle cramping might be caused by the intensity of the exercise rather than the supplementation of creatine, and maintaining a proper hydration status may reduce this risk (Kim et al., 2011). To date, liver and tissue damage has only been reported in mice, and no other species, suggesting that these results may be species-specific and would not translate to humans (Kim et al., 2011). The elevated PCK levels associated with muscle fiber rupturing are more likely attributed to heavy training as opposed to any creatine supplementation (Kim et al., 2011). Lastly, there is insufficient evidence to support the impairment of kidney function or gastrointestinal distress being caused by the ingestion of creatine (Kim et al., 2011).

Further, Kim et al. (2011) argues that there are no real incidents of any of these side effects associated with creatine supplementation. It is advised, however, that great care is taken to ensure the creatine supplement is pure, and should not be used by anyone with a pre-existing renal disease or predisposition for renal dysfunction (Kim et al., 2011). Lastly, it is suggested that a low-dose, slow protocol for loading may be easier on the body, and better tolerated, as compared to a short-term, high dose protocol (Kim et al., 2011).

2.4.5 Conclusion

Creatine, as an ergogenic aid, has been investigated thorough since 1992 (Tarnopolsky, 2010). The logical hypothesis was that if creatine stores could be increased, there could be an extension of the anaerobic energy systems in brief, intense

exercise efforts (Tarnopolsky, 2010). The advantage of the PCr system is that energy can be produced quickly and, in contrast to anaerobic glycolysis where lactate is produced, the proton is buffered from this reaction (Tarnopolsky, 2010). Utilizing creatine, and thereby maximizing the potential of the PCr energy system, may allow athletes to train more intensely, which can ultimately lead to greater increases in performance (Grande & Graves, 2005).

Chapter 3

Methods

3.1 Subjects and Subject Demographics

Seventeen healthy men (n=8) and women (n=12) volunteered to participate in this study, which was approved by the University of Toledo Institutional Review Board for Biomedical Research, and conducted in accordance with the Declaration of Helsinki. All subjects were fully informed of the experimental procedures and possible risks before providing their written consent to participate.

The present study was limited to healthy male and female subjects between the ages of 18 and 50 years old. Subjects were excluded from the study if they: had been diagnosed with a metabolic, pulmonary, and/or cardiovascular disorder, including diabetes, hypertension, currently smoke or recently quit smoking in the last twelve months, had any orthopedic-related injuries effecting their ability to perform cycling exercise, and/or currently pregnant or nursing, as determined by a medical history questionnaire.

Standard physiological measurements were recorded before and after the dietary intervention including: age (years), height (meters, m), weight (kilogram, kg), waist and hip circumference (centimeters, cm) to calculate waist to hip ratios, resting heart rate

(beats per minute, bpm), resting blood pressure (millimeters of mercury, mmHg), and body composition (percent body fat, %fat). Resting heart rate and blood pressure were measured using an automated blood pressure cuff (Omron Digital Blood Pressure Monitor HEM-907XL, Omron Healthcare, Lake Forest, IL). Body composition was assessed using an air displacement plethysmography technique (BodPod, COSMED, Chicago, IL). The air displacement plethysmography technique is similar to hydrostatic weighing, with the exception that body volume is measured using changes in pressure (P_1 and P_2) and a known volume (V_1) to solve for the unknown body volume (V_2) using the equation for Boyle's Law, (i.e. $P_1V_1 = P_2V_2$). Whole body density is calculated using the subjects body mass, which in turn is used to calculate body composition using the two compartment model to estimate fat mass and lean body mass (Siri Equation). This approach has been shown to agree very well with widely accepted hydrostatic weighing technique (Dempster & Aitkens, 1995; McCrory, Gomez, Bernauer, & Mole, 1995).

3.2 Experimental Design

This study utilized three groups in a single-blind, randomly distributed experimental design. Subjects were randomly assigned to one of three groups, control (CON), low carbohydrate diet and placebo (L-CHO) or low carbohydrate diet and creatine supplementation (L-CHO+Cr). Subjects reported to the laboratory on four separate occasions over the course of two weeks. Before the dietary intervention, subjects underwent a progressive incremental ramp exercise test to volitional fatigue on an electronically braked cycle ergometer (Lode, Groningen, the Netherlands) with pulmonary gas exchange measured (VMax Carefusion, BD Worldwide, USA) continuously throughout the exercise. The maximal test protocol is outlined below under

Experimental Procedures. The maximal exercise test was used to measure peak oxygen uptake ($\text{VO}_{2\text{peak}}$) and peak power output (PPO). This value (PPO) was then used to determine the subject's work rate (90% of PPO) for the high intensity exercise tolerance test (HIETT), which was performed before and after the 10-day dietary intervention (see Experimental Procedures).

3.3 Dietary Intervention and Supplementation

Subjects that were randomized to either of the low carbohydrate intervention groups (L-CHO+Cr or L-CHO) were required to adhere to a restricted diet for 10 days. Subjects were instructed to log their daily food intake using the myfitnesspal app on their phone or computer. They were directed to consume <30% of their total calories from carbohydrate. Subjects were not controlled for total caloric intake, nor were they directed to meet any particular protein or fat percentage intake. A list of foods low in carbohydrate content and foods to avoid was provided to each of the subjects, in addition to dietary guide offering suggestions on how best to adhere to the dietary intervention, to include upper limit definitions for food labels (i.e. <10g CHO per food item).

Subjects were provided with supplement (creatine monohydrate, NOW® Foods or taurine, NOW® Foods) that was divided into premeasured individual serving vials, each containing a total of 1 g (taurine for the L-CHO group) or 4 g of (creatine monohydrate for the L-CHO+Cr group). Subjects were instructed to dissolve each premeasured portion with 8 oz of tepid water and to consume a total of 5 servings each day with approximately 2 hours between servings. The subjects were provided with written instructions and directions for use were thoroughly explained during the familiarization session. The subjects were all contacted a minimum of six times over the course of the

10-day intervention period by the investigator to offer support and answer any questions the subject may have had.

3.5 Experimental Procedures

All data collection for the present study was conducted in the Cardiopulmonary and Metabolism Research Laboratory at the University of Toledo (Toledo, OH). Each subject reported to the laboratory on four separate occasions within a two-week timeframe to complete three physical assessment sessions (2 pre-diet and 1 post-diet). Subjects were also required to visit the laboratory during a familiarization session where the investigator provided specific instructions on the low carbohydrate diet, how to keep an accurate food and exercise log, and instructions on how to consume the nutritional or placebo supplement. Each subject was instructed to refrain from any strenuous physical activity 24 hour prior to each exercise session, and directed to maintain their current exercise habits.

3.5.1 Resting Respiratory Exchange Ratio (RER)

Resting respiratory data was collected by measuring the amount of oxygen breathed in and the amount of carbon dioxide breathed out using a canopy hood and analyzed using a commercially available metabolic measurement system (VMax Carefusion, BD Worldwide, Franklin Lakes, NJ). The metabolic measurement system was calibrated prior to each exercise test according to the manufacturer's instructions. The O₂ and CO₂ analyzers were calibrated using gases of known concentrations. The volume signal was calibrated using a syringe of known volume (3.0 L). Corrections for variations in ambient temperature and water vapor were made for conditions measured

near the mouth. Subjects were asked to lie in a supine position on a comfortable bed for 40 minutes. Data was collected and analyzed prior to the start of the intervention to determine the primary substrate utilized at rest, used to affirm that the subject was not already adapted to a low carbohydrate diet. In a subset population (n=4), data was collected and analyzed both before and after dietary intervention to determine if the low carbohydrate diet did alter the primary substrate utilized at rest.

3.5.2 Determination of $\text{VO}_{2\text{peak}}$ and Peak Power Output (PPO)

Preliminary exercise testing of each subject was performed to both familiarize the subjects with the exercise protocol and for the determination of peak cardiorespiratory fitness (expressed as $\text{VO}_{2\text{peak}}$) and peak power output (PPO). Subjects performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, the Netherlands), with pulmonary gases collected and analyzed using a commercially available metabolic measurement system (VMax Carefusion, BD Worldwide, Franklin Lakes, NJ). Seat height and handlebar position were adjusted on the cycle ergometer prior to the first cycling exercise test for each subject and returned to the same position for all subsequent exercise tests. The ramp exercise test consisted of 4 min of very low intensity cycling (25 W) followed by a progressive increase in exercise intensity where the work rate increased as a ramp function at 25 W/min until the subjects reached volitional fatigue. The highest VO_2 averaged over a 20 s interval was taken as $\text{VO}_{2\text{peak}}$. Subjects were instructed to maintain a pedaling cadence of ≥ 80 revolutions per minute (rpm) during the test; volitional fatigue was determined when the subject could no longer maintain a pedaling cadence of 40 rpm. Subjects were required to breathe through a mouthpiece while wearing a nose clip for the measurement of oxygen uptake (VO_2),

carbon dioxide output (VCO_2) and minute ventilation (VE). The measurement system was calibrated prior to each exercise test according to the manufacturer's instructions. The O_2 and CO_2 analyzers were calibrated using gases of known concentrations. The volume signal was calibrated using a syringe of known volume (3.0 L). Corrections for variations in ambient temperature and water vapor were made for conditions measured near the mouth.

3.5.3 High-Intensity Exercise Tolerance Test (HIETT)

Pre- and post-intervention, subjects performed a high intensity interval exercise session on an electronically braked cycle ergometer (Lode, Groningen, the Netherlands). Work rate and pedal cadence were monitored and recorded using Lab Chart 7 software (Powerlab/16sp, ADInstruments, South San Francisco, CA). Subjects completed a 4 minute warm-up, followed by a 30 s high intensity interval (set at 90% of their PPO achieved during the maximal exercise test), and followed by 30 s of loadless cycling as an active recovery interval. The interval duration was established to adequately diminish the high energy phosphate systems during exercise, and to prevent the full recovery of these energy stores during the recovery period. Each subject was instructed to perform as many intervals as possible, maintaining a pedal cadence of 80-100 rpm. The end-point of the HIETT (i.e. exercise tolerance) was defined as the point when they were no longer able to complete an entire exercise interval from onset to end at >80 rpm. Heart rate data was monitored and recorded for the duration of the exercise session using an electrocardiograph (Schiller AT-10, Schiller, Switzerland) with the leads placed in a 4 lead configuration.

3.6 Dietary Analysis

Subjects were instructed to keep a food log for three days prior to the dietary intervention which was to be considered baseline. Subjects also kept a food log during the 10-day dietary intervention. Dietary intake data, as recorded by the subject via the myfitnesspal app, was analyzed for average caloric and macronutrient intake using Nutritionist Pro (Axxya Systems, San Bruno, CA). The dietary data was entered in conjunction with subject gender, height, weight, and year of birth. If the brand of food recorded in the food log was not listed in Nutritionist Pro, an internet search was performed to find the weight, calories, and macronutrient content. After all dietary intake data was entered, the specified time frame (i.e. pre-dietary and dietary intervention periods) were analyzed for macronutrient content (%CHO/%Fat/%Protein) presenting an overall average of consumption. Subjects were instructed to focus on keeping their CHO intake <30% for each day of the 10-day intervention period. They were not to be concerned with overall caloric ingestion, or any other macronutrient, but were allowed to eat to satiety and choose fats or proteins to replace the restricted CHOs from their diet.

3.7 Other Variables

The following data was collected pre- and post-dietary intervention for comparison: weight, body composition, dietary data, total number of bouts completed during HIIT sessions. Other variables of interest include age, height, weight, heart rate (resting and exercising), resting blood pressure, and resting and exercising respiratory exchange ratio.

3.8 Statistical Analysis

Exercise tolerance, body composition, weight, and all dietary data were analyzed using a two-way analysis of variance (ANOVA) with one repeated measure. All mean %change (Δ) data, as well as baseline variables, were analyzed using a one-way ANOVA. Significant differences were further analyzed using a Student-Newman-Keuls post hoc analysis. Significance was set at a priori at $p \leq 0.05$. All statistical analyses were conducted using SigmaPlot 13.0 (Systat Software, San Jose, CA). Unless specified otherwise, all data are presented as the mean $\pm$ standard deviation (SD).

3.9 Study Timeline

Day 1 Pre-Diet Assessment/ Data Collection	Minimum 4 Days	Day 2 Pre-Diet Assessment/ Nutritional Review	Minimum 3 Days	Day 3 Group Assignment/ Supplement Distribution	Day 4-13 Diet & Supplement Compliance	Day 14 Post-Diet Assessment/ Nutritional Review
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Figure 3-1. Outline of the study timeline.

Chapter 4

Results

Subject anthropometric characteristics are presented in Table 4.1. A one-way ANOVA indicates there was no difference in baseline fitness levels, as defined by $\text{VO}_{2\text{peak}}$ among groups.

4.1 HIETT: Number of Bouts Completed

The results of the HIETT are presented in Figure 4-1 and are expressed as the % change in improvement compared to the first HIETT (%change). There was significant difference ($p < 0.001$) in the total number of bouts performed, comparing pre- and post-dietary exercise performance, between the L-CHO and both the L-CHO+Cr and CON groups. The L-CHO group demonstrated a -35.8 ± 15.0 % decrease in performance from baseline to post-diet intervention. The L-CHO+Cr demonstrated a 20.7 ± 27.6 % increase in performance from baseline to post-diet intervention. The CON group demonstrated a 11.9 ± 9.4 % increase in performance from baseline to post-diet intervention. There was no difference ($p = 0.42$) in the number of exercise bouts completed between the CON and L-CHO-Cr groups.

4.2 Body Composition

The results of both weight and percent body fat (BF%) are presented in Table 4.2. Although the changes were small, there was a significant difference ($p=0.027$) between the dietary intervention groups (L-CHO+Cr and L-CHO) and the CON group in weight lost, as well as a significant difference ($p=0.009$) in %BF lost. The L-CHO+Cr group demonstrated a mean weight loss of 2.0 ± 1.2 kg, with a corresponding 1.4 ± 1.5 % loss in body fat. The L-CHO group demonstrated a mean weight loss of $2.0 \text{ kg} \pm 0.8$ kg, with a corresponding 1.3 ± 0.8 % loss in body fat. The CON group demonstrated a mean weight loss of $0.2 \text{ kg} \pm 1.1$ SD, with a mean gain of 0.3 ± 0.5 % change in BF%; this was not significantly different ($p=0.733$).

4.3 Dietary Compliance

The overall dietary responses during baseline and the dietary intervention are presented in Table 4.3 and in Figures 4.2-4.5. There was a significant difference ($p=0.013$) in total energy (Kcals) intake from baseline compared to during the dietary intervention within the L-CHO+Cr group only (Figure 4.2 and Figure 4.3). The L-CHO+Cr group reported a decrease in total energy intake of 313.9 ± 343.3 Kcals. However, there was no significant difference within conditions in either the L-CHO or CON group, nor was there a significant difference in total energy intake across the L-CHO and CON groups (Figure 4.2 and Figure 4.3).

There was a significant difference ($p<0.001$) within both the L-CHO+Cr and L-CHO groups for macronutrient consumption when expressed as percent change from their respective baseline consumption (i.e. pre-dietary intervention) (Figure 4.4 and

Figure 4.5). The L-CHO+Cr group consumed 50.8 ± 17.3 % fewer CHOs from baseline compared to during the dietary intervention. Similarly, the L-CHO group consumed 58.8 ± 8.9 % fewer CHOs from baseline compared to during the dietary intervention. There was no significant difference in the decrease in CHOs consumed between the L-CHO+Cr and L-CHO groups. The L-CHO+Cr group consumed 71.2 ± 51.1 % more protein from baseline compared to the 10-day dietary intervention whereas the L-CHO group consumed 99.5 ± 69.1 % more protein from baseline versus the 10-day dietary intervention; this difference was significantly higher for L-CHO versus the L-CHO+Cr group. The L-CHO+Cr group consumed 31.0 ± 18.0 % more fat from baseline to during intervention. The L-CHO group consumed 31.0 ± 28.9 % more fat from baseline to during intervention. There was no significant difference in the amount of fat consumed during the dietary intervention between the L-CHO and L-CHO+Cr groups when expressed relative to their baseline values. As shown in Figure 4-4, the CON group did not significantly change their dietary habits over the period they were enrolled in the study.

n=20 (M=8;F=12)	CON n=6 (M=3;F=3)	L-CHO n=7 (M=3;F=4)	L-CHO + Cr n=7 (M=2;F=5)
Age, yr	33 ± 9	25 ± 6	26 ± 5
Height, cm	169.2 ± 7.8	171.4 ± 8.1	164.6 ± 5.8
Weight, kg	73.2 ± 21.0	68.4 ± 9.0	70.5 ± 18.8
Body Fat, % (female subjects)	28.7 ± 1.7	32.0 ± 6.6	32.4 ± 9.0
Body Fat, % (male subjects)	25.9 ± 1.3	16.0 ± 9.5	20.7 ± 6.4
Systolic Blood Pressure, mmHg	117 ± 15	115 ± 9	120 ± 18
Diastolic Blood Pressure, mmHg	74 ± 6	74 ± 7	74 ± 15
Resting Heart Rate, bpm	62 ± 10	65 ± 9	63 ± 9
Resting Respiratory Exchange Ratio (RRER)	0.76 ± 0.03	0.78 ± 0.03	0.79 ± 0.03
Exercising Respiratory Exchange Ratio (ExRER)	1.02 ± 0.06	1.03 ± 0.04	1.03 ± 0.05
VO _{2peak} , ml/kg/min	35.4 ± 3.2	36.9 ± 5.8	36.1 ± 5.3
Heart rate achieved at VO _{2peak} , bpm	180 ± 9	188 ± 16	182 ± 6

Table 4.1. Subject characteristics at baseline. All values are Mean ± SD for N=20. VO_{2peak}, peak oxygen consumption; ml/kg/min, relative peak oxygen consumption. CON, control; L-CHO, low carbohydrate + placebo; L-CHO+Cr, low carbohydrate + creatine.

Figure 4-1. Number of total completed bouts, expressed as a % change, group mean and standard deviation. Each bout lasted 30 seconds, interspersed with 30 seconds of loadless recovery. Resistance was established as 90% peak power output as determined by a 25W ramp test performed prior to dietary intervention. *, significant difference between dietary intervention groups ($p < 0.05$). †, significant difference between L-CHO+Cr and L-CHO groups.

Group	Weight – Pre (kg)	Weight – Post (kg)	Weight Change (%)	BF – Pre (%)	BF – Post (%)	BF Change (%)
CON						
Females	56.9	56.9	0.5	28.7	29.3	2.3
Males	89.5	89.2	0.0	25.9	25.9	0.0
± SD						
Females	5.2	5.2	0.6	1.7	1.8	1.7
Males	16.6	16.0	0.4	1.3	1.6	1.3
L-CHO						
Females	62.5	60.7	-3.0	32.0	30.4	-2.8
Males	76.3	74.3	-2.7	16.0	15.2	-3.6
± SD						
Females	6.5	5.8	0.9	6.6	7.1	1.2
Males	4.0	3.1	1.3	9.5	9.6	3.1
L-CHO+Cr						
Females	66.1	64.3	-2.5	32.2	30.9	-3.2
Males	81.7	79.0	-3.1	20.7	19.0	-7.9
± SD						
Females	19.2	18.1	1.0	7.8	6.3	4.2
Males	17.3	15.8	1.1	6.4	5.4	2.3

Table 4.2. Body composition pre- and post-dietary intervention. All values Mean ± SD for N=20.

Group	Kcals – Pre	Kcals – Post	Kcals Change	CHO – Pre (%)	CHO – Post (%)	CHO Change (%)	Fat – Pre (%)	Fat – Post (%)	Fat Change (%)	Pro – Pre (%)	Pro – Post (%)	Pro Change (%)
CON (n=6)	1456.86 ± 401.34	1557.96 ± 622.62	101.10 ± 340.54	50.7 ± 5.8	49.3 ±5.5	-1.4 ± 7.3	33.4 ± 5.7	35.5 ± 4.7	2.1 ± 6.6	15.9 ± 2.9	14.3 ± 1.5	-1.7 ± 2.6
L-CHO (n=7)	1386.76 ± 371.83	1436.75 ± 342.75	49.99 ± 192.62	42.5 ± 6.4	17.5 ± 4.1	-25.0 ± 5.7	37.3 ± 7.7	47.3 ± 6.3	10.0 ± 7.5	17.7 ± 4.9	32.9 ± 4.0	15.2 ± 6.3
L- CHO+Cr (n=7)	1648.41 ± 379.23	1334.48 ± 389.25	-313.92 ± 343.30	42.8 ± 6.6	21.0 ± 8.8	-21.8 ± 9.2	37.3 ± 4.7	48.7 ± 7.9	11.4 ± 6.2	18.2 ± 3.3	29.9 ± 4.4	11.7 ± 6.4
Subset (n=4)	1604.71 ± 305.93	1451.05 ± 213.39	-153.66 ± 245.96	44.6 ± 6.9	17.5 ± 3.6	-27.1 ± 10.1	38.0 ± 3.7	59.7 ± 4.3	21.7 ± 4.5	15.6 ± 3.4	24.9 ± 3.9	9.3 ± 6.6

Table 4.3. Total energy intake pre- and post-dietary intervention. CHO, carbohydrate; Fat; Pro, protein. All values are the mean ± SD for N=20.

Figure 4-2. Group mean and standard deviation for total energy (kcal) intake averaged 3 days before dietary intervention (baseline) and 6 days during the dietary intervention (10 days). *, significant difference between baseline and dietary intervention within the L-CHO+Cr group, $P<0.05$.

Figure 4-3. Group mean and standard deviation for the total energy (kcal) intake, represented as percent (%) change from baseline to during intervention. No significant difference between dietary intervention groups.

Figure 4-4. Group mean and standard deviation for the contribution of macronutrients to total energy intake, expressed as percent (%) change from baseline dietary logs compared to the dietary logs recorded during the intervention period. *, significant difference between the change in macronutrient intake compared to baseline intake within each of the study groups, $P < 0.05$.

Figure 4-5. Group mean and standard deviation for macronutrient contribution (%) to total energy intake at baseline and during the dietary intervention. (A) carbohydrate, (B) fat and (C) protein. *, significant difference within study group between baseline intake and the dietary intervention, $P < 0.05$.

Chapter 5

Discussion

The aim of the present study was to determine if a creatine monohydrate supplement would attenuate the adverse effects of consuming a low carbohydrate diet on exercise performance. The results of the present study indicate that consuming a standard loading dose (20g/day) of creatine monohydrate while on a low carbohydrate (<30% CHO) diet will moderate the fatiguing effects of the possible metabolic shift experienced with a reduction in CHO consumption. It was hypothesized that consuming a creatine monohydrate supplement while on a L-CHO diet would result in equal to, or greater exercise performance as demonstrated by a high-intensity repeated bout exercise tolerance test as compared to a control group. The results of the present study support the original hypothesis with the L-CHO+Cr group outperforming the CON group by approximately 10% and the L-CHO group by more than 50%.

It is generally accepted that peripheral fatigue during high-intensity exercise occurs as a result of the accumulation of metabolic by-products and/or the depletion of high energy phosphate stores (Kent-Braun et al., 2012; Larson, Hesslink, Hrovat, Fishman, & Systrom, 1994; Sahlin et al., 1998). However, there is evidence to suggest that the accumulation of P_i and the resulting fall in PCr/ P_i ratio plays a significant role in

determining the time to fatigue during high intensity exercise (Larson, Hesslink, Hrovat, Fishman, & Systrom, 1994). The improvement in exercise performance observed in the present study may be associated with the beneficial effects of creating loading on the intracellular phosphorylation potential. Consistent with our findings, Larson and colleagues (1994) demonstrated a significant correlation between exercise performance and the slope of PCr/P_i ratio using ³¹P-MRS techniques following consumption of either a high fat or high carbohydrate diet. Although muscle PCr content was not measure in the present study, it could be reasonably argued that the creatine loading protocol used in this study resulted in appreciable increases in muscle PCr content which may have lead to a lower PCr/P_i slope during exercise and improved exercise tolerance, similar to the findings reported by Larson et al. (1994).

The mechanisms behind this are two-fold, including the effect of P_i accumulation on muscle fatigue, and the role that PCr has in determining the time to exhaustion. The accumulation of P_i may impair force production directly by inhibition of cross-bridge function (Kent-Braun et al., 2012; Westerblad et al., 2010). It may also impair for production indirectly by causing a reduction in myofibrillar Ca²⁺ sensitivity and sarcoplasmic reticular Ca²⁺ handling (Kent-Braun et al., 2012; Westerblad et al., 2010). Therefore, any change in the intracellular phosphorylation potential that would attenuate or slow the accumulation of P_i in the muscle might be expected to delay the onset of muscle fatigue. Conversely, an increase in intracellular PCr stores may alter the magnitude of the oxygen deficit, the reduction of which may increase time to exhaustion during high intensity exercise (Demarle et al., 2001). In the present study, the L-CHO+Cr group performed 24 bouts, or 24 minutes of total exercise at baseline. The L-

CHO and control groups performed 37 and 33 bouts, respectively, at baseline. Following intervention, the groups performed 32, 22, and 37 bouts, respectively, resulting in significant improvements in the time to exhaustion in the L-CHO+Cr group.

The ability of creatine supplementation to enhance high intensity exercise with repeated bout performance is well established (Graef et al., 2009; Herda et al., 2009; Izquierdo et al., 2002; Kendall et al., 2009; Prevost et al., 1997). It is hypothesized that the increase in phosphocreatine (PCr), as achieved through supplementation, acts to postpone skeletal muscle fatigue by delaying the accumulation of ADP and inorganic phosphate (Pi), which are activators of glycolysis (Kendall et al., 2009). By reducing the reliance on glycolysis, it is thought that exercise efficiency and aerobic performance may also be improved (Kendall et al., 2009).

The hypothesis is that if creatine stores could be increased, there could be an extension of the high phosphate energy systems in brief, intense exercise efforts (Tarnopolsky, 2010). The advantage of the PCr system is that energy can be produced quickly and, in contrast to anaerobic glycolysis where lactate is produced, the proton is buffered from this reaction (Tarnopolsky, 2010). Utilizing creatine, and thereby maximizing the potential of the PCr energy system, may allow athletes to train more intensely, which can ultimately lead to greater increases in performance (Grande & Graves, 2005).

Consumption of a L-CHO diet for >3 days is associated with metabolic and cellular adaptations that lend towards increased lipid utilization both at rest and during exercise (Coyle & Hodgkinson, 2001; Hawley, 2011; McCartney et al., 1986; Spriet,

2011; Yeo et al., 2011). This metabolic shift, or fat adaptation, results in greater body fat loss within the first six months of a reduced calorie diet, as compared to an isocaloric low-fat diet (Volek et al., 2010). It has also been long-speculated that greater lipid utilization during exercise may prolong submaximal endurance training (Burke & Hawley, 2002; Lima-Silva et al., 2013). However, it has been suggested that a L-CHO diet, and subsequent fat adaptation, may increase the lipid contribution to intense exercise efforts ($>60\%$ $\text{VO}_{2\text{max}}$) as well (Lambert, Hawley, Goedecke, Noakes, & Dennis, 1997; McCartney et al., 1986; Romijn et al., 1993)

The impairment of exercise tolerance and performance while on a L-CHO diet has been well established (Balsom et al., 1999; Fleming et al., 2003; Lima-Silva et al., 2013; Stepto et al., 2002). Depending on the intensity and duration of the exercise, performance ranges from uncomfortable and difficult to nearly-impossible. Even in the case of elite cyclists, while the exercise program may well be accomplished with intensity and duration maintained, the exercise session is poorly tolerated (Stepto et al., 2002). As HIIT can result in training adaptations equivocal to that of endurance training in a fraction of the time, it is generally accepted that high intensity exercise is integral to both body fat loss and improvements in markers of exercise performance (i.e. $\text{VO}_{2\text{max}}$, lactate threshold) (Duffield et al., 2006; Esfarjani & Laursen, 2007; Gillen & Gibala, 2014; Hunter et al., 1998; Tremblay et al., 1994). Thus, it is paramount for optimal training adaptations to be realized by those individuals who engage in HIIT, to be able to maintain exercise intensity and duration while consuming a low carbohydrate diet. The addition of a nutritional supplement containing creatine monohydrate may provide these individuals

with a way to cope with the adverse effects of consuming a L-CHO diet thereby allowing them to maintain a training volume that promotes optimal physiological adaptations.

5.1 Dietary Considerations

One particular finding of interest from the results of the present study resides within the dietary analysis. Although both the L-CHO and L-CHO+Cr groups participated in the dietary intervention (L-CHO and L-CHO+Cr) and adhered to the CHO restriction, both groups consumed what could be considered a high-fat diet (>45% fat) since the greatest macronutrient increase was in protein. Both groups drastically increased their protein consumption from the pre-dietary intervention baseline, with the L-CHO+Cr group demonstrating a 71.2% increase and the L-CHO group increasing protein intake by 99.5%.

Subjects were not specifically directed to increase fat or protein more than the other, but were allowed to choose their foods liberally, so long as the consumption of CHO was kept to <30% of the total macronutrient intake. However, during the familiarization session, and during follow-up conversations throughout the 10-day intervention period, subjects were advised to build their plate in a particular order for each meal: protein, non-starch vegetable, and cook/season the food with fat (such as olive oil, coconut oil, cheese, nut butters, etc). This technique was imitative of the Lean Eating behavior modification model established by Precision Nutrition (Berardi & Andrews, 2010). It is possible that the increase in dietary protein consumption aided in the maintenance of lean body mass, allowing for the fat loss exhibited in this study, there

is little reason to suspect that the alteration in the dietary intake of protein was associated with the improved performance observed in the L-CHO+Cr group.

5.2 Potential Limitations

Although subjects consumed a typical loading dose of creatine monohydrate (20g/day for 10 days), they were not examined for increased muscle phosphocreatine content. However, previous studies have demonstrated, using muscle biopsy, that a loading dose of 20g/day for 5 days is sufficient to increase intramuscular stores of PCr (Greenhaff et al., 1994; Harris, Soderlund, & Hultman, 1992). Additionally, as performance was increased in the L-CHO+Cr and CON groups, but was decreased by a mean 36% in the L-CHO group, the results suggest that creatine loading was effective in increasing muscle stores.

Improvement in exercise performance could be attributed to familiarization with the equipment and protocol, as the study did not offer familiarization session. However, again, performance was increased in only L-CHO+Cr and CON groups, and decreased in the L-CHO group. This would suggest that while familiarization may have improved performance by a small margin in the groups that had adequate energy for the exercise, it did not impact the L-CHO group. Therefore, it would not have impacted the overarching results of this study.

Both groups that underwent the dietary intervention (L-CHO+Cr and L-CHO) exhibited changes in body composition. In particular, both groups lost weight and body fat. While the subjects were not directed to control their caloric intake, they were directed to maintain their current exercise habits. While the dietary data cannot

ultimately account for the body composition changes, as caloric intake and expenditure was not controlled for, group means report caloric deficits. Nevertheless, the demonstrated decrease in exercise performance exhibited by the L-CHO group would suggest subject adherence to the dietary intervention.

In conclusion, the results of the present study support the hypothesis that creatine monohydrate, consumed in a typical loading protocol (20g/day for 10 days) off-sets the negative effects on high-intensity exercise performance following a L-CHO diet. While the results of the present study does not provide any direct evidence, the findings suggests that an increase in intracellular PCr stores may have a significant effect on longer duration repeated-bout intense exercise. This conclusion is supported by the higher number of bouts completed and improved exercise tolerance in those subjects that underwent the creatine monohydrate loading protocol while also on L-CHO diet.

5.3 Future Research

Based upon the present findings, with the demonstrated improvement to exercise performance with creatine supplementation while consuming a L-CHO diet, a logical next step would be to incorporate a HIIT training component. It has been demonstrated that HIIT promotes similar cellular and metabolic adaptations to that of a L-CHO but without the negative side effects typically associated with consuming a L-CHO diet (Burgomaster et al., 2008; Perry et al., 2008; Tremblay et al., 1994). It has also been demonstrated that these adaptations persist following training, despite CHO restoration (Burke & Hawley, 2002; Burke et al., 2002). Creatine loading does not appear to inhibit body fat loss, and has hereby been demonstrated in the present study to delay the early

onset of fatigue associated with an acute bout of high intensity interval exercise while on a L-CHO diet. To this end, incorporating a high intensity training component to the present research model may enable researchers to further explore the physiological benefits to exercise performance following fat adaption. In addition, the issue of reduced compliance to an exercise training program, particularly while consuming a L-CHO diet, could also be addressed using the same research model. As mentioned in the introduction, the negative side effects associated with consuming a L-CHO diet, often leads to individuals avoiding regular exercise. The addition of a nutritional supplement containing creatine monohydrate while consuming a diet low in carbohydrate may prevent the feelings of fatigue thereby improving exercise program adherence and promote a healthy lifestyle.

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Appendix A

Informed Consent Form

IRB # 201542

ICF Version Date: 08/22/2016

Department of Kinesiology
2801 W. Bancroft St. MS 119
Toledo, Ohio 43606
Phone: 419.530.2741
Fax: 419.530.2477

ADULT RESEARCH SUBJECT INFORMATION AND CONSENT FORM

CAN CREATINE SUPPLEMENTATION PREVENT THE DECREASE IN PERFORMANCE ASSOCIATED WITH A LOW-CARBOHYDRATE DIET?

Principal Investigator: Barry W. Scheuermann, Ph.D
Other Staff (identified by role): Stephanie Born, M.S. (co-investigator)
Contact Phone number(s): (419) 530-2757 Office
(419) 530-2058 Lab

What you should know about this research study:

- We give you this consent/authorization form so that you may read about the purpose, risks, and benefits of this research study. All information in this form will be communicated to you verbally by the research staff as well.
- Routine clinical care is based upon the best-known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.
- We cannot promise that this research will benefit you. Just like routine care, this research can have side effects that can be serious or minor.
- You have the right to refuse to take part in this research, or agree to take part now and change your mind later.
- If you decide to take part in this research or not, or if you decide to take part now but change your mind later, your decision will not affect your routine care.
- Please review this form carefully. Ask any questions before you make a decision about whether or not you want to take part in this research. If you decide to take part in this research, you may ask any additional questions at any time.
- Your participation in this research is voluntary.

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UNIVERSITY OF TOLEDO IRB
APPROVAL DATE: 09/09/2016
EXPIRATION DATE: 08/17/2017

PURPOSE (WHY THIS RESEARCH IS BEING DONE)

You are being asked to take part in a research study to determine if the ingestion of a nutritional supplement containing creatine will either prevent or attenuate the feeling of fatigue and the fall in exercise performance that typically accompanies a low-carbohydrate diet. Therefore, the purpose of the study is to compare exercise performance and exercise tolerance before and after a short-term low carbohydrate diet (2-weeks) with or without creatine supplementation.

You were selected as someone who may want to take part in this study because you indicated an interest in this study by contacting Dr. Barry Scheuermann or Stephanie Born, and you meet the criteria outlined below. This study will include 45 subjects recruited from the University of Toledo Community.

In order to participate in this study, you must be between the ages of 18-40 years and be free of any known cardiovascular, pulmonary, and/or metabolic diseases as determined by a medical history questionnaire and an activity level questionnaire. If you do not meet these criteria, we greatly appreciate your willingness to volunteer but unfortunately, you will not be able to participate in this study.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this study, you will be asked to report to the Cardiopulmonary and Metabolism Research Laboratory (room 1407, Health Science Human Service Building, University of Toledo) on 4 separate occasions within a 3 week period. Each session will last approximately 1-2 hours in duration. You will complete 3 physical assessment sessions (2 pre-diet and 1 post-diet), and have 1 familiarization session to learn about the diet, how to keep an accurate food and exercise log and/or use the free phone app (myfitnesspal), and how/when to consume the nutritional supplement.

All exercise performed during the study will be performed on a stationary bike. You will be asked to not participate in any exercise 24 hours prior to each session. Over the course of the 3 weeks, you may be contacted (via phone, text, and/or email) up to 6 times for compliance and maintenance check-in, where we will discuss your progress and any particular challenges you may be having complying with the diet.

Day 1: Pre-diet Assessment Session

During the first pre-diet assessment session, the research procedures will be explained to you by the investigator(s). You will be encouraged to ask any questions regarding the study to the research investigator(s). Also please remember that you can take as much time as you need to make an informed decision regarding your participation in the study. You are solely volunteering to participate in the research study and you can stop your participation in the research study at any time, for any reason, without any consequence. Once you feel comfortable with the research procedures, if you would still like to participate within the research study, you will have to provide written informed consent (by signing this document). Next you will be asked to complete a medical history questionnaire and an activity level questionnaire that will help to determine if you meet the criteria defined above for participation within the research study.

Following the completion of the questionnaires, you will be given a brief tutorial on how to keep an accurate food log. You will be asked to keep a detailed food and exercise log of all food and drink consumed for a minimum of 9 days over the course of the entire study, with 3 pre-diet days and 6 during-diet days.

Next standard physiological baseline measurements will be recorded including: age (years), height (meters, m), weight (kilogram, kg), waist and hip circumference (centimeters, cm), resting heart rate (beats per minute, bpm), resting blood pressure (millimeters of mercury, mmHg), and body composition (percent body fat, %fat). Resting heart rate and blood pressure will be measured using an automated

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blood pressure cuff. Body composition will be measured using a BodPod; an Air Displacement Plethysmograph (ADP) that uses whole body densitometry to determine body composition (fat vs. lean).

At this time we will also collect resting respiratory exchange ratio data by measuring the amount of oxygen you breathe in and the amount of carbon dioxide you breathe out using a metabolic cart. For this, you will be asked to lie in a supine position on a comfortable bed for 40 minutes. We will collect breathing samples; you will be asked to breathe through a canopy hood to collect data for the amount of oxygen inhaled and the amount of carbon dioxide exhaled at rest. Using the metabolic cart for analysis we will be able to determine your body's primary source of energy at rest (ie. carbohydrate vs. fat).

You will then be asked to perform a maximal exercise test that will measure the amount of oxygen you breathe in and the amount of carbon dioxide you breathe out. Prior to the start of the test, you will be prepped for the measurement of heart rate. We will shave (if needed) and clean 4 small areas on your chest. We will then place 4 small adhesive pads will be placed on the skin (2 pads close to your shoulders 2 pad just below your ribs) to measure the heart rate response to exercise.

The test will begin on a stationary bike where you will warm-up for 4 minutes at a low intensity (20 watts). Next, you will perform continuous exercise at increasing levels of resistance, similar to cycling uphill, at a pedaling cadence of ≥ 80 revolutions per minute (rpm). The test will be considered complete when you reach fatigue, determined by an inability to pedal at a rate of 40 revolutions per minute (rpm) consistently. For the duration of the test, you will be asked to breathe through a rubber mouthpiece to collect data for the amount of oxygen inhaled and the amount of carbon dioxide exhaled during the exercise. This test will tell us how "fit" you are.

Day 2: Pre-diet Assessment Session and Nutritional Review
(a minimum of 4 calendar days following Day 1)

During the second pre-diet assessment you will be asked to perform a high intensity interval exercise session. You will complete a 4 minute warm up set, followed by a 1 minute high intensity interval (set at 90% of your maximal work rate), and followed by 30 seconds of no-load cycling as a recovery interval. You will be asked to perform as many intervals as you are able to complete. The test will be considered finished when you are no longer able to complete an entire interval start to finish.

Following this session, we will also review your pre-diet food and exercise log for accuracy, and collect it for data analysis.

Day 3: Group Assignment and Supplement Distribution
(approximately 3 calendar days following Day 2)

For this meeting, you will be given your dietary guidelines and carbohydrate exclusion list, as well as your nutritional supplement containing creatine portions to be consumed over the next 2 weeks. You will be asked to adhere to the dietary guidelines as closely as possible, and consume approximately 20g of a nutritional supplement containing creatine per day, divided into 5 servings. Your supplement portions will be pre-measured and divided for you into individual serving vials, to be consumed 5 times per day. You will be given 50 vials for the entirety of the study. Supplement directions will be thoroughly explained and provided in written form with the vials. Please note that any and all benefits associated with the consumption of the nutritional supplement containing creatine have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

There will be 3 groups in this study: a diet-creatine group, a diet-placebo group, and a control (no intervention) group. These group assignments will be kept confidential to the participants. Group

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assignment will be determined using baseline data collected from the pre-diet exercise tests as well as the preliminary food and exercise log.

Group assignment will be based on:

- Baseline fitness as determined by the maximal exercise test performed on Day 1, with an attempt to evenly distribute fitness levels between groups to avoid bias.
- Baseline dietary date, with any subject consuming less than 30% carbohydrate daily being automatically designated to the control group.
- Information collected from the Medical History Questionnaire, with any subject with Bipolar Disorder or taking nephrotoxic medications being automatically designated to the control group.

Days 4-13: Diet and Supplement Compliance

During this time, you will simply eat according to the dietary guidelines and take the nutritional supplement as outlined in the Supplement Directions. You are free to follow your normal exercise habits, but we ask that you do not change normal exercise patterns during the study. For the duration of this time period, you will be contacted up to 6 times (via phone, text, and/or email) for a maintenance and compliance check-in, and to address any particular concerns or issues you may be having. You will be asked to keep a food and exercise log of a minimum of 6 days during this time period.

Day 14: Post-diet Assessment

You will be asked to report to the lab to repeat your high intensity interval exercise session, which will be the same as performed on Day 2. At this time, we will also repeat your physiological baseline measures, as collected on Day 1. Lastly, we will review your food and exercise log for accuracy, and collect it for data analysis.

RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH

Immediate risks to cycling exercise may include muscle cramping, strain, or soreness, especially following the maximal fatigue exercise test.

You may also experience gas, diarrhea, and/or constipation during the oral ingestion of the tested nutritional supplement containing creatine used for this research study. Creatine causes muscles to draw water from the rest of your body. Be sure to drink extra water to make up for this. Also, if you are taking creatine, don't exercise in the heat. It might cause you to become dehydrated.

Presently there is not sufficient information regarding the consumption of creatine to pregnant or nursing women, and you must therefore stop the study immediately should you become pregnant as the risks to unborn children are at this time unknown.

All subject data and information is kept in a secured storage area to minimize the risk of a breach of confidentiality.

POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH

We cannot and do not guarantee or promise that you will receive any benefits from this research study. The benefit of participating in this study is to help further research regarding the effects of a low carbohydrate diet and creatine supplementation on high intensity exercise.

COST TO YOU FOR TAKING PART IN THIS STUDY

You are not directly responsible for making any type of payment to take part in this research study. However, you are responsible for providing your own means of transportation to and from the Cardiopulmonary and Metabolism Research Laboratory at The University of Toledo's main campus in the

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Health Science and Human Services Building (room 1407). You will not be compensated for gas, travel, or any other expenses to participate in this research study.

PAYMENT OR OTHER COMPENSATION TO THE RESEARCH SITE

The University of Toledo is not receiving money or other benefits from the sponsor of this research as reimbursement for conducting the research.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH

Since there are no therapeutic treatments or interventions involved in this study, there are no alternatives to taking part in this research study. Exclusion from the study, however, will not affect the quality of care you may receive at the sports medicine/physical therapy facility, doctor's office, and/or other medical facilities.

CONFIDENTIALITY - (USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION)

By agreeing to take part in this research study, you give to The University of Toledo (UT), the Principal Investigator and all personnel associated with this research study your permission to collect information we obtain in connection with this study. We will use this information solely for the purpose of conducting the present research study as described in the research consent/authorization form.

The information that we will use includes *all the data collected during the 4 visits. In summary, we are collecting information regarding your health history, medications that you are currently taking, your family medical history (including risk factors related to cardiovascular disease), your eating habits, anthropometrics, and fitness level.* Under some circumstances, the Institutional Review Board, or the Research and Sponsored Programs of the University of Toledo may review your information for compliance audits.

IN THE EVENT OF A RESEARCH-RELATED INJURY

In the event of injury resulting from your taking part in this study, treatment can be obtained at a health care facility of your choice. You should understand that the costs of such treatment will be your responsibility. Financial compensation is not available through The University of Toledo or The University of Toledo Medical Center.

In the event of an injury, contact Barry Scheuermann, PhD at (419) 258-0283.

VOLUNTARY PARTICIPATION

Taking part in this study is voluntary. You may refuse to participate or discontinue participation at any time without penalty or a loss of benefits to which you are otherwise entitled. If you decide not to participate or to discontinue participation, your decision will not affect your future relations with the University of Toledo or The University of Toledo Medical Center.

NEW FINDINGS

You will be notified of new information that might change your decision to be in this study if any becomes available.

OTHER IMPORTANT INFORMATION

It is important that you are the only one that takes the nutritional supplement or placebo that you are given as part of this research. It is very important that you keep it (these) out of the reach of children and persons who may not be able to read or understand the label.

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IRB # 201542

ICF Version Date: 08/22/2016

OFFER TO ANSWER QUESTIONS

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over. If you have questions regarding the research at any time before, during or after the study, you may contact Barry Scheuermann, PhD at (419) 530-2692, or Stephanie Born, MS at (419) 283-6965.

If you have questions beyond those answered by the research team or your rights as a research subject or research-related injuries, please feel free to contact the Chairperson of the University of Toledo Biomedical Institutional Review Board at 419-383-6796.

SIGNATURE SECTION (Please read carefully)

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ THE INFORMATION PROVIDED ABOVE, YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND YOU HAVE DECIDED TO TAKE PART IN THIS RESEARCH.

BY SIGNING THIS DOCUMENT YOU AUTHORIZE US TO USE OR DISCLOSE YOUR PROTECTED HEALTH INFORMATION AS DESCRIBED IN THIS FORM.

The date you sign this document to enroll in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to the bottom of each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study. Each page of this Consent/Authorization Form is stamped to indicate the form's validity as approved by the UT Biomedical Institutional Review Board (IRB).

_____ Name of Subject (please print)	_____ Signature of Subject or Person Authorized to Consent	_____ Date
_____ Relationship to the Subject (Healthcare Power of Attorney authority or Legal Guardian)	_____ Time	_____ a.m. p.m.
_____ Name of Person Obtaining Consent (please print)	_____ Signature of Person Obtaining Consent	_____ Date
_____ Name of Witness to Consent Process (when required by ICH Guidelines) (please print)	_____ Signature of Witness to Consent Process (when required by ICH Guidelines)	_____ Date

YOU WILL BE GIVEN A SIGNED COPY OF THIS FORM TO KEEP.

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**UNIVERSITY OF TOLEDO IRB
APPROVAL DATE: 09/09/2016
EXPIRATION DATE: 08/17/2017**

Appendix A – Medical History Questionnaire

APPROVED BY
UNIVERSITY OF TOLEDO IRB

Age: _____ Gender: M/F Subject ID #: _____

Weight (lbs): _____ (kg): _____ Height (in): _____ (cm): _____

Do you have any of the following health conditions?*	Yes	No
Family history of heart disease? i.e. Heart attack, bypass, stroke, or sudden death before age 55 in 1st degree male relative (father, brother, son) or before age 65 in 1st degree female relative (mother, sister, daughter)		
Smoking habit? i.e. Current cigarette smoker <i>or</i> one who has quit within the previous 6 months		
High blood pressure? i.e. $\geq 140/90$ on two separate occasions <i>or</i> currently on antihypertensive medication		
Abnormal cholesterol levels? i.e. Total Cholesterol >200 mg/dL, <i>or</i> LDL >130 mg/dL, <i>or</i> HDL <35 mg/dL, <i>or</i> currently on lipid lowering medication		
High fasting glucose? i.e. Fasting blood glucose ≥ 110 on two separate occasions		
Are you inactive? i.e. Accumulate <30 minutes of moderate physical activity on most days of the week		
If you can answer yes to 2 or more above please obtain medical clearance for exercise from your personal physician .		

Do you current have any of the following?*	Yes	No
Pain in the chest, neck, jaw, or arms?		
Shortness of breath at rest or with mild exertion?		
Dizziness or fainting?		
Awakened by a shortness of breath?		
Swelling in your ankles?		
Rapid heart rate while at rest?		
Leg pain or cramping while walking; stops with rest?		
Heart murmur?		
Unusual fatigue or shortness of breath with usual activities?		
If you can answer yes to any of the above please obtain medical clearance for exercise from your personal physician .		

Do you have a history of the following?*	Yes	No
Heart attack or stroke?		
Heart surgery (CABG, angioplasty, other)?		
Metabolic disorder (diabetes, kidney, thyroid)?		
Respiratory problems (asthma, COPD)?		
Hospitalization or surgery within the last 6 months?		
If you can answer yes to any of the above please obtain medical clearance for exercise from your personal physician .		

Contact Information
Street:
City:
Zip:
Home Phone:
Work Phone:
Cell Phone:
E-mail:
Preferred contact method:
Emergency Contact Information
Name:
Relationship:
Home Phone:
Work Phone:
Cell Phone:

Other Health Related Questions:
Current Medications (ie. Prescription, Over The Counter, Herbals, Vitamins, and Dietary Supplements): _____
Do you currently consume caffeine or caffeinated beverages? If so, approximately how much? _____
Allergies: _____
Do you have any orthopedic conditions/arthritis that may limit your activities? _____
Are you pregnant? If yes, how many weeks? _____
Do you have any other problems or medical conditions not addressed on this form including any disorders that might affect the ability of your blood to clot normally? _____
How long have you had your medical condition(s)? _____

Signature: _____ Date: _____

*Adapted from ACSM's Guidelines for Exercise Testing and Prescription, 6th Edition.