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Effects of Supplemental Citrulline Malate during a Resistance Training Protocol

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By

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Ergogenic L-citrulline and malate are amino acids used in specific combination to effect muscular endurance during athletic performances. *Purpose*: The study aimed to investigate the ergogenic properties of citrulline-malate (CM) during a resistance training protocol. *Methods*: Utilizing a randomized, counterbalanced, double blind study, fifteen trained males completed a resistance training protocol once using placebo (PL) and once with CM (8.0g). *Results*: CM supplementation increased repetitions in chin-ups, reverse chin-ups, push-ups, and total trial repetitions. Blood lactate was significantly increased post-exercise compared to pre-exercise, but was not significantly different between CM and placebo. Further, a significant interaction effect was revealed for systolic blood pressure, a significant condition effect for diastolic blood pressure, and a significant time effect for HR. Post-hoc analysis revealed that SBP responses were more elevated in the placebo condition during recovery. *Conclusion*: Collectively, these novel findings suggest CM increases muscular endurance during upper body resistance exercise.

DEDICATION

I would like to dedicate this body of work to my family and loved ones for their unfailing love and support in every endeavor of my life. I would also like to make a special dedication to my grandmother Earline who is no longer with me physically but still remains a constant source of encouragement.

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CHAPTER I

INTRODUCTION

For as long as there have been competitive sports, there has been a desire by the participants of their respective sports to utilize the most of their potential to give them any form of advantage over their counterparts. Logically the first step was to adopt the right training regimen to correlate with an increased performance in competition. The next step was to develop ways to get the most out of the adopted training regimen to further increase the performance benefits shown during competition. A number of methods have been tested ranging from simply modifying the individual's diet by increasing or decreasing certain nutrients to more novel and complex methods such as blood boosting (Leigh-Smith 2004) or gene mapping that determines which genes correlate with specific attributes which is still only being tested with animals (Huson et al. 2010). Within the last several decades of the 20th century and today's society use of nutritional ergogenic aids are receiving the most attention and have become a popular way to improve training and ultimately competitive performance among athletes (Thein,Thein, and Landry 1995, Applegate and Grivetti 1997).

The term "ergogenic aid" encompasses a large spectrum of techniques and methods used to enhance performance. Anything such as: a training method, specific diet, mechanical device, pharmacological substances, or psychological preparation that is used with the intention of improving performance in training and/or competition in sport

can be considered an ergogenic aid (Kreider et al. 2010). Nutritional and some pharmacological ergogenic aids can be considered as dietary supplements that include but are not limited to: multivitamins, creatine, and sports foods/drinks (Tian, Ong, Tan 2009). Use of nutritional/pharmacological ergogenic aids probably stemmed from simply from supplementing different aspects of an individual's normal diet with certain foods. These supplemented foods were used to add extra nutrients such as: carbohydrates, vitamins, minerals, amino acids/proteins, metabolites, or some form of combination of these nutrients that would fuel or improve performance in the activity or sport the individual participated in (Williams 2004). However, as technology improved over time the nutrients were manufactured into dietary supplements. These supplements claim to enhance performance, increase energy, maintain strength and health, and limit nutritional deficiencies by adding larger doses of desired nutrients to the diet in the form of tablets, powders, or liquids as needed by the individual to meet the demands of their specific activity/sport (Heikkinen et al. 2011). Some nutritional aids, especially those utilizing proteins/amino acids, are considered pharmacological aids because they mimic some of the effects of other pharmacological aids like anabolic steroids (Silver 2001).

In recent years, a new dietary supplement has begun to gain popularity among strength and power athletes ranging from professional bodybuilders to recreational strength athletes. Originally, citrulline-malate mixtures were developed as a treatment for asthenia, however in recent decades, its perceived benefits on performance has also led to its use as a pre-exercise dietary supplement (Bendahan et al. 2002, Pérez-Guisado and Jakeman 2010 . The term citrulline-malate comes from the components L-citrulline, which is a non-essential amino acid, and malate, which is a derivative of malic acid

(Schwedhelm et al. 2007, Zelle et al. 2008). This combination of citrulline and malate was developed to be the active ingredient of this supplement. Like creatine or the various protein/amino acid supplements on the market, citruline-malate is believed to enhance training by sustaining muscle force and work and delaying the effects of fatigue on the muscle (Pérez-Guisado and Jakeman 2010). Only a limited amount of research has been done on this supplement and the exact physiological mechanisms by which this supplement is believed to work are still in question. Some research even questions if this product works at all and if it does work is this merely due to a placebo effect. The purpose of this study was to test the effectiveness of citruline-malate on muscular performance and prevention of muscular fatigue and potentially observe the exact mechanisms by which citruline-malate improves these factors during exercise.

Statement of the Problem

Will muscular endurance be augmented as a result of ingestion of citrulline-malate prior to engaging in an acute bout of resistance exercise focusing on muscular endurance?

Hypotheses

HO_1: There is no significant difference in blood lactate (BLa) between citrulline-malate and placebo (PL) treatments in male participants

HO_2: There is no significant difference in heart rate (HR) between citrulline-malate and PL treatments in male participants.

HO_3: There is no significant difference in blood pressure (BP) between citrulline-malate and PL treatments in male participants.

HO_4: There is no significant difference in resistance training performance between citrulline-malate and PL treatments in male participants.

Basic Assumptions

1. Participants understood all directions given to them.
2. Participants followed the directions given to them.
3. Subjects did not participate in any strenuous exercise training 48 hours prior to testing.
4. Measurements were accurately performed on measured equipment.
5. Participants were non-supplement using participants.
6. Participants performed the given exercise to volitional failure.

Limitations

Potential limitations of this study:

1. Athletes were not tested for steroid use.
2. Nutrient intake may not have been repeated correctly due to each participant monitoring his food intake 48 hours prior to testing.

Delimitations

1. Most participants were in the traditional college age range of 18-22
2. Participants were recreationally trained lifters.
3. Participants selected had more than 6 months of consistent training at the time of testing

Definitions

1. Muscular fatigue: a decrease in the force generation of a working muscle across time.
2. Exhaustion or Failure: inability to complete one repetition due to muscular fatigue.
3. Anaerobic Muscular Endurance: maximum amount of repeated contraction repetitions of a muscle or muscle group sustained over a period time, yet does not utilize aerobic metabolic pathways as a primary source of energy.

Significance of the Study

In most competitive sports, especially in more elite levels, a matter of seconds or inches can be the difference between victory and defeat. For an athlete having enough muscular endurance to sustain the desired level of muscular performance in order to last longer or go further than their opponent is the true testament to their training. Many athletes seek to acquire the optimal benefits of their training by ingesting supplements, such as citrulline-malate, that will intensify the quality of their training. The examinations of this study will hopefully augment the modest body of literature in regards to the effects of citrulline-malate on muscular endurance.

CHAPTER II

REVIEW OF LITERATURE

According Dale Ahrendt, M.D., in 1996 a study showed approximately 50% of the United States' general population reported some use of supplementation and other studies showed 76% of collegiate athletes and 100% of bodybuilders took supplements (Ahrendt 2001). Through the years numerous supplements claiming to utilize various ingredients to improve muscular performance have been used by professional and amateur athletes alike. As time goes by, some supplements are no longer used due to a belief they are not effective or because they may be too effective and have been deemed unfair and illegal in professional sports. No matter the reason, the fall of one supplement inevitably makes room for the rise of another. Currently products like GAKIC are gaining popularity for their perceived influence of enhancing muscular endurance and helping sustain overall muscular performance. Muscular endurance is generally described as the muscle's/muscles' ability to sustain repeated contractions over extended periods of time. To understand the importance of sustaining repeated contractions and their benefit to overall performance, it is important to have a thorough understanding of the mechanisms that interact to generate a single contraction. In addition it is necessary to understand the different aerobic and anaerobic metabolic processes that supply the energy needed to produce these muscular contractions. Both developers and consumers of the various ergogenic supplements should have a thorough understanding of these

pathways when making judgments on the validity of the effectiveness of these supplements on muscular contractions, muscular endurance, and overall muscular performance.

Utilization of Aerobic and Anaerobic Metabolism

The body produces energy for metabolic processes in a number of ways; however no matter which pathway is used each pathway can be described as being aerobic or anaerobic in nature. Whether aerobic or anaerobic, the energy for metabolic processes is supplied by the degradation and synthesis of the molecule adenosine triphosphate (ATP). ATP is considered the main currency of energy in all living organisms (Cloutier and Wellstead 2010). The differences between the two are the substrates used in the processes and whether or not oxygen is used to break these substrates down.

Aerobic Metabolism

Oxidation is the process by which oxygen (02) interacts with and removes an electron (e-) from another substance. Aerobic metabolism employs 02 to break down, or oxidize, fat (for fatty acids) and carbohydrates (for glucose) which are the primary substrates of aerobic ATP synthesis in skeletal muscle (Ergen et al. 2005). Inside skeletal muscle cells the double-membrane organelles known as mitochondria synthesize ATP by removing hydrogen molecules (H+) and e-‘s from pyruvate and fatty acids which ultimately are by-products from the breakdown of fats and carbohydrates (Scott 2005). The metabolic process responsible for the removal of H+ and e- from pyruvate and fatty acids is known at the Kreb’s Cycle. O2 inside the inner membrane of the mitochondria attracts e-‘s in what is known as an electron transport chain, and this chain is used to

pump H+ from higher concentrations to lower concentrations which creates an energy gradient (Scott 2005). The energy from this gradient produced in the mitochondria is used in the re-synthesis of ATP, and the overall process is referred to as oxidative phosphorylation. Endogenous triglycerols are a large fuel reserve of the body and are stored mostly in the adipose tissues (fat) of the body but small amounts are also found in skeletal muscle (Horowitz and Klein 2000). Fat is the primary fuel source during lower intensity exercise as well as the primary source of fatty acids, which makes aerobic metabolism more relevant to endurance athletes who perform at lower intensities for an extended period of time (Horowitz and Klein 2000, Scott 2005).

Anaerobic Metabolism

Compared to other metabolic processes of the body oxidation of fats is a slow process. Strength and power athletes perform at moderate to high intensities for short periods of time which means aerobic metabolism would only have a minimal influence on energy production for these individuals. Anaerobic metabolism requires minimal oxygen since the main two energy systems utilized by strength and power athletes are the phosphagen system and the glycolytic system which do not require oxygen to re-synthesize ATP (Baker, McCormick, and Robergs 2010). The energy supplied at the beginning of any form of exercise is the creatine-kinase system. This is the two-way system, which is part of the phosphagen system. H+ can be added to phosphocreatine (PCr) and adenosine diphosphate and then catalyzed by creatine-kinase to yield to ATP and a molecule of creatine (CR). The reverse can also occur where creatine-kinase can breakdown ATP and yield ADP and PCr. The creatine-kinase system is an important buffer of ATP when there is a change in energy demand such as going from rest to

exercise (Thompson et al. 1996). Within a few seconds of exercise the creatine-kinase system gives way to anaerobic glycolysis, which is the breakdown of glucose without the presence of 02. Glycolysis utilizes glucose in the blood and glycogen, the stored form of glucose in skeletal muscle, to re-synthesize ATP (Baker, McCormick, and Robergs 2010). During glycolysis glucose is broken down in a series of enzymatic reactions and a small amount ATP is resynthesized in a process that can be considered as a substrate level phosphorylation (Scott 1996). Depending on the rate H+ and e-‘s are released from glucose, glycolysis can result in the production of pyruvate or lactate. If H+ and e-‘s are released at a rate equivalent to the current rate of aerobic metabolism in the mitochondria, H+ enters the mitochondria of skeletal muscle cells and pyruvate is the byproduct of glycolysis; however if H+ and e-‘s are released at a rate that exceeds aerobic metabolism the H+ binds with pyruvate and ultimately lactate is the byproduct of glycolysis (Scott 1996). The end product of lactate is of concern because excessive buildup of lactate makes the pH of the muscle too acidic and results in fatigue and ultimately the inability of the muscle to continue to contract.

Muscle Contraction

Expression of skeletal muscles' mechanical capacity is demonstrated by the muscles' ability to exert force, shortening, and by combinations of force exertion and shortening (Rahe-Meyer et al. 2008). Skeletal muscles' ability to shorten and exert forces are vitally important to basic life functions and even more so if individuals intend to engage in physical activity/exercise and participate in competitive sports. Voluntary muscle contractions are initiated by electrical impulses that travel from the central nervous system (CNS) to the peripheral nervous system (PNS) and then the muscle.

Motor units, which are the neurons and the muscle fibers they innervate, interpret the intensity of impulses and regulation of these impulses determines the number of fibers to be recruited which correlates to the force of the contraction (Duchateau and Enoka 2008). Once the fibers are recruited, calcium ions (Ca2+) are released from the sarcoplasmic reticulum as a result of what is known as an action potential. The intracellular release of Ca2+ from the sarcoplasmic reticulum triggers the mechanical aspects of a muscular contraction (Kim et al. 2006). The Ca2+ released from the sarcoplasmic reticulum interacts with troponin, which is attached to the protein tropomyosin that is part of the thin myofilament actin. Binding with Ca2+ alters the position of tropomyosin and exposes the binding sites located on the actin filament (Vingradova et al. 2005). Once the binding sites are exposed, the sperm-like globular heads of the the thick myofilament, myosin, extend and bind to the now exposed binding sites on the actin filament (Hooper, Hobbs, and Thuma 2008). The binding of the globular heads of myosin to actin results in a discrete lever arm of rotation powered by ATP, which is believed to cause myosin to move the actin filament (Jackson and Baker 2009). This belief of a thick myofilament sliding along a thin filament was proposed within the last century and is known as the sliding-filament theory of muscle contractions (Vinogradova et al. 2005). Inability to sustain adequate repetitions of this process over time results in what is considered as muscular fatigue.

Muscular Fatigue

Muscular fatigue can be considered a transient decrease in the ability to perform physical actions resulting from a number of factors including: reduction in muscular force, exhaustion of contractile muscle function, acidosis of the muscle and even the

psychological decline of mental function (Enoka and Duchateau 2008). The physiological aspects of muscular fatigue could also be the result of the energy pathways of the body not producing enough adenosine triphosphate, or ATP, to supply the energy needed to continue muscular contractions and overall activity. This supports the notion if the energy supply does not meet the energy demands imposed on the working muscle during physical activity then muscular fatigue will be the inevitable outcome (Barclay, Arnold, and Gibbs 1995). It appears that muscle fatigue cannot simply be described as resulting from a single factor, but instead a combination of physiological and psychological factors.

Physiological Fatigue

A study conducted by David Jones, published in The *Journal of Physiology* in 2010, observed the relationship between force and velocity in fatigued muscle. His study supported the belief a cause of fatigue in working skeletal muscle is the loss of the muscle's ability to produce force. Jones compared the contraction of a fresh quadriceps muscle to a fatigued quadriceps muscle and noticed a significant decrease in power. The decrease in power was a result of a combination of a 20-30% decrease in force as well as velocity in the fatigued muscle. In order to apply this to anaerobic activity, Jones described this decrease in power as the reason runners struggle to maintain their speed in the last 100 meters of a 400m sprint or individual's rapid reduction in power output at the end of a Wingate Test. Acidosis of the muscle, which is a commonly believed to cause fatigue, was alluded to in this study. It was stated in this study that acidosis plays a minimal effect on fatigue because a previously observed patient with McArdle's disease, who did not produce the byproducts that lead to acidosis, still showed the characteristic

loss of force associated with fatigue. From this study, changes in contractile properties and issues with the cross bridge mechanisms of the myofilaments seem to correlate more with loss of force and fatigue rather than acidosis (Jones 2010).

Exhaustion of contractile properties of muscle is also a viable presumption in when explaining the cause of fatigue. As stated by Jones, de Ruiter, and de Haan in their study published in 2006, two contractile properties could be contributors to muscular fatigue. They recognized the slowed process of removing Ca2+ from muscle, or the reduction in the speed in which myosin cross bridges detach from actin filaments could lead to inadequate muscle function and decrease in muscular performance (Jones, de Ruiter, and de Haan 2006). Though it was unclear as to what extent rate of Ca2+ removal played in fatigue, a primary finding of this study was that the slowed relaxation of fatigued muscle correlated with changes in cross-bridge function (Jones, de Ruiter, and Haan 2006).

Psychological Fatigue

More novel approaches not only acknowledge the physiological factors of fatigue, but are now starting to recognize the potential psychological and neurological factors as well. Studies like the study published by Noakes, St. Clair Gibson, and Lambert in 2005 examines these novel approaches to fatigue that incorporate these factors. Agreeing with Jones' 2010 study, Noakes, St. Clair Gibson, and Lambert also put little emphasis on acidosis playing a major role during fatigue in muscle. However, where Jones' 2010 study uses physiological evidence to support their statement, Noakes, St. Clair Gibson, and Lambert state acidosis instead leads to an increase in perception of discomfort. This perception of discomfort supports psychological factors' influence on fatigue. The

authors also take a novel approach to the effect of ATP on fatigue. It is widely accepted that depletion of ATP is responsible for the decline in contractions over time; however, the authors state that even during the most intense exercise ATP stores never drop to less than 50% of their resting values. This interpretation of ATP presumes that, like acidosis, ATP may play a smaller role in fatigue than previously believed. As stated in this study, older beliefs look at physiological factors or "peripheral events" as the cause of fatigue and minimize psychological and neurological influences. Yet the reverse may be true and peripheral events may play a lesser role in comparison to these psychological and neurological influences. The central governor model states that exercise performance is centrally regulated and that influence of the central nervous system(CNS) changes the perspective of fatigue from physiological to more of an emotion or feeling (Noakes, St. Clair Gibson, and Lambert 2005). This article supports the notion that defining fatigue cannot be limited to only physiological factors.

Muscle Fiber Type

Just as there different energy pathways in the body that are utilized during different exercise intensities and durations, there are different muscle fibers in the body whose usage depends on the same conditions of exercise. Human skeletal muscle can be classified into two primary types: fatigue-resistant slow twitch fibers known as type-I fibers or fatigue-sensitive fast twitch fibers known as type-II fibers (Baguet et al. 2011). Type-II fibers can be further divided into subgroups: type-IIa, type-IIx, and type-IIb (Casas et al. 2008).

Type-I Fibers

Type-I muscle fibers have been characterized as "slow-twitch" because the twitch force they generate takes approximately four times longer to generate than type-II fibers (Macdonald and Stephenson). These muscle fibers are of great significance to physical activity/exercise that lasts for longer duration and rely on more aerobic pathways for production of ATP. It has been shown that muscles composed primarily of the highly oxidative type-I fibers contain a high mitochondrial density and are utilized more during prolonged repetitive activity (Grifone et al. 2004, Casas et al. 2008). Skeletal muscles of athletes participating in exercise/sports that are more aerobic in nature, such as marathons and triathlons, usually are comprised of more type I fibers.

Type-II and Intermediate Fibers

Type-II fibers are characterized as fast-twitch fibers in the body. As stated earlier, they are able to produce a twitch force much faster than their type-I counterparts. Athletes, whose physical activity involves more intermittent bursts over shorter periods of time, utilize muscles that have a lower mitochondrial density and contain more glycolytic type-II fibers (Grifone 2004, Casas et al. 2008). It was also stated earlier in this section that type-II fibers could be further classified into smaller and more specified phenotypes: type-IIa, type-IIx, and type-IIb. Type-IIb fibers characteristically have significantly less mitochondria and rely primarily on anaerobic energy pathways in comparison to type-I fibers; whereas type-IIa and type-IIx fibers have oxidative capacities valued somewhere between those of type-I and type-IIb fibers (Casas et al. 2008). Type-IIb fibers can be considered opposite in nature to type-I fibers, and types-IIa/IIx can be considered as intermediates between the two. Skeletal muscles of athletes

participating in exercises that are anaerobic or more anaerobically dominant in nature, such as power-lifters, 100m sprinters, or soccer players, tend to be comprised more of the type-II muscle fiber phenotypes.

Metabolic Dynamics

Onset of exercise causes the body to shift from a state of storing energy to a state of utilizing energy. This shift is the result of nueruoendocrine changes, contraction of working muscles, and redistribution of blood flow primarily to the working muscles (Younk et al. 2011). Nueroendocrine changes triggered by the autonomic aspect of the peripheral nervous system influence the delivery of muscle substrates and change the normal distribution of the blood (Helge et al. 2007). Once these neuroendocrine changes are initiated changes in heart rate, utilization of glucose, and production of lactate can occur.

Heart Rate

Heart rate is the amount of time the heart beats per min and is a factor cardiac output, which is the amount of blood pumped out by the heart per minute. As an individual goes from rest or light exercise to more intense physical activity, both heart rate and cardiac output increase resulting from circulation of norepinephrine and epinephrine released by the neuroendocrine system (Snyder, Johnson, and Joyner 2008). Increases in heart rate allows for a higher cardiac output. The increased cardiac output supplies more blood to the working muscle since blood flow is directed away from areas with lesser need for oxygen and directed to working muscles, which require more oxygen (Snyder, Johnson, and Joyner 2008). At the time of this increase in heart rate and cardiac

output, glucose is released from the liver and is transported by the blood to the working muscles to be broken down for energy (Goodwin 2010).

Glucose

As stated in the aerobic and anaerobic metabolism section of the current review, break down of glucose is the primary fuel source for early and more intense physical activity/exercise. The initial source of fuel for working muscle is its own stores of glucose in the form of glycogen (Younk et al. 2011). However, these glucose stores deplete quickly and other fuel sources are required to continue the physical activity/exercise. By the time the muscles' glycogen stores have been depleted blood glucose becomes the primary fuel source for muscles, through anaerobic glycolysis, until more aerobic pathways take over. Glucagon, an enzyme produced by pancreatic cells, initiates glucongenolysis and gluconeogenesis in the blood to provide a new supply of glucose to working muscle (Younk et al. 2011).

Lactate

Glycogenolysis, breakdown of glycogen, and glycolysis, breakdown of glucose yield the byproduct lactate (Service 1991, Scott 1996). Higher intensity exercises, which use anaerobic glycolysis as a primary fuel source, cannot be sustained over extended periods of time due to the higher concentrations of lactate they produce (Lewis et al. 2010). As mentioned earlier in the aerobic and anaerobic metabolism section of the current review, lactate, a byproduct of glycolysis, makes the environment of the working muscle more acidic. In the muscle fatigue section of the current review it was referenced that this lactate-induced acidosis correlates physiologically by a highly acidic

environment and psychologically by perceptions of discomfort. Both aspects of fatigue lead to a decrease in muscular endurance and overall performance.

Performance Enhancing Substances

There is a vast array of performance enhancing substances (PES's) that claim to improve athletic performance by increasing strength gains, reducing the effects of muscular fatigue, promoting faster recovery from injury, improving body mass index, and increasing level of focus during exercise (Buckman et al. 2009). In a 1997 study it was documented that Americans spent approximately $11.8 billion on supplements and it was predicted that this amount would continue to increase in the following years (Ahrendt 2001). Many PES's have been vilified and their use has become illicit in more elite levels of sports; however use of these substances are still common among athletes of various skill levels (Buckman et al. 2009). With an abundant number of substances, many whose effects have been minimally researched, the line between which substances' usage should be considered licit or illicit is very thin.

Illicit PES's

When it comes to illicit PES's, substances that are synthesized versions of hormones that occur naturally in the human body are the most recognized with anabolic-androgenic steroids (AAS's) being the most popularly discussed (Hoffman 2002). These substances can be administered orally or injected directly into the blood stream (Thein, Thein, and Landry 1995). The term anabolic refers to "tissue building" and androgenic refers to "masculinizing" (Thein, Thein, and Landry 1995). AAS's are synthetic versions of testosterone and have been shown anabolically to provide large increases in muscle

mass and further promote protein synthesis (Thein, Thein, and Landry 1995, Hoffman 2002, Kanayama, Hudson and Pope 2010). As appealing as these physiological effects may be to athletes, evidence supporting adverse effects on the body such as sterility in males, increased stress to the cardiovascular system, increased aggression, and development of mood disorders has increased the infamy of AAS usage (Thein, Thein, and Landry 1995, Hoffman 2002, Kanayama, Hudson and Pope 2010). Due to the extent of their effectiveness many deem their usage as cheating and offensive to the true "spirit of competition" (Savulescu, Foddy, and Clayton 2004). A combination of dangerous adverse effects and being associated with cheating are the reason AAS's are the most infamous illicit PES's.

Licit PES's

Dietary supplements, some that are anabolic in nature, have also been manufactured to enhance performance. Popular dietary supplements contain active ingredients such as: creatine, various vitamins, stimulants, nitrous oxide and protein/amino acids (Silver 2001, Kersick and Leutholtz 2005). Creatine has been associated with increases in muscular performance by enhancing intracellular ATP and is used to enhance anaerobic athletes' training (Silver 2001, Tokish, Kocher, and Hawkins 2004). Nitrous oxide is naturally present in the body and is shown to aid in vasodilation. Nitrous oxide supplement manufacturers have claimed their products enhance performance by increasing blood flow to working muscles by increasing the amount of nitrous oxide in the body (Bloomer et al. 2010). Proteins, which are composed of amino acids, are the building blocks of muscles and there are numerous protein/amino acid supplements on the market. According to studies analyzing the effectiveness of protein

supplements, there is strong evidence that supports administering protein/amino acid supplements during periods of rest and post-exercise aid in increasing protein balance and synthesis in skeletal muscle (Kersick and Leutholtz 2005). Dietary may or may not have as the same adverse side effects as AAS's. Due to the numerous available dietary supplements and steady introduction of new supplements, these ergogenic aids are deemed safe or licit until adverse effects are reported in scientific/medical literature (Kreider et al. 2010).

Citrulline-Malate

Active Ingredients

As mentioned earlier in this paper, citrulline-malate is a combination of the non-essential amino acid L-citrulline and malate. In humans L-citrulline is converted to L-argininosuccinate and by ensuing metabolic processes it becomes L-arginine (Schwedhelm et al. 2007). L-arginine is used in the synthesis of nitric oxide, which promotes vasodilation of the smooth muscle found in blood vessels (Bloomer 2010). The importance of malate in the citrulline-malate mixture is in its use as an intermediate in the Kreb's cycle (Pérez-Guisado and Jakeman 2010). The Kreb's cycle utilizes malate-asparate and malate-citrate shuttle pathways to transfer electrons. The malate-asparate shuttle facilitates transfer of electrons from the cytoplasm of the cell to the mitochondrial matrix, which helps regulate glycolysis and lactate oxidation (Lu et al. 2008). However, once the number of electrons transported by this shuttle becomes too low, lactate is no longer oxidized and begins to accumulate.

Functions

It has already been mentioned that citrulline-malate has been utilized as a treatment for asthenia, which is a medical term generally used to describe energy loss or weakness. As a treatment for asthenia, citrulline-malate has been shown to improve muscle performance in subjects suffering from weakness and fatigue associated with this condition (Bendahan et al. 2002). The improvements in muscle performance seen in these individuals in relation to use of citrulline-malate has led to interest in its potential ergogenic effectiveness among a healthy and more athletic population. As an ergogenic aid, citrulline-malate may potentially improve muscle performance by: facilitating clearance of ammonium which is a factor in fatigue, limiting lactic acid accumulation, and increasing nitric oxide production (Pérez-Guisado and Jakeman 2010). Ingestion of citrulline-malate, in regards to its use as an ergogenic aid, should be conducted orally prior to exercise. As a pre-exercise supplement, it is recommended to ingest citrulline-malate in 4-10 g doses 1 hour prior to exercise/competition (Pérez-Guisado and Jakeman 2010). Oral ingestion at the proper dosages is preferred because it has been observed to increase plasma concentrations of both L-arginine and L-citrulline, which facilitates a correlating enhancement in nitric oxide-dependent signaling (Schwedhelm et al. 2007). The enhancements in nitric oxide signaling will lead to increases in nitric oxide and its function as a vasodilator of blood vessels. Theoretically, in regards to ergogenic increases in nitric oxide, further increases in vasodilation will facilitate optimal blood flow and superior delivery of oxygen and nutrients to working muscle (Bloomer 2010). Optimal oxygen and nutrient delivery in combination with the attrition of fatigue

resulting in enhanced performance potentially makes citrulline-malate an effective pre-workout supplement.

CHAPTER III

METHODOLOGY

Participants

Male participants, preferably within the traditional college age range of 18 to 22, were recruited for this study. To qualify, potential participants were required to have a minimum of six months of continuous strength training. Participants were free of any medical conditions, which may hinder their ability to perform the exercise protocol. Prior to participating in the study, participants were required to complete health history questionnaires and sign a statement of informed consent. Patients who reported having not used anabolic steroids for at least one year prior to participation in the investigation were qualified for this experiment. Potential participants not meeting these criteria were eliminated from this investigation. The procedures outlined were reviewed and approved by the Institutional Review Board at Mississippi State University.

Pre-Testing Evaluation

During the initial session, participants were provided with an explanation of the informed consent and research protocol (Day 1). Informed consent was signed prior to any evaluations on the participant. On day one, all anthropometric measurements were determined. Participants' standing height, body weight, and body fat percent was assessed. Standing height and body weight was assessed using a Doctor's scale

(Healthometer 402EXP). Participants removed their shoes and stood erect with their hands down at their side in an erect position. Heels were placed together. The measurement stick was placed at the center of the participants' head at the highest point. Participants were weighed in shorts without shoes. Body weights were measured in kilograms. Body fat percentages were determined using the 3-site skinfold method described by Pollack and Wilmore (1990).

Experimental Protocol (Session 2 & 3)

Each participant performed the trials a minimum of one week apart, in which the experimental treatments, citrulline-malate (CM) or placebo (PL), were randomly assigned and administered in a double-blind fashion. Upon arriving to the Sanderson Center weight room on the campus of Mississippi State University, the participants were taken to a designated area and given the treatment designated prior to their arrival. Fifty minutes after ingesting the treatment, the participant's heart rate (HR) and resting blood lactate levels (BLa) were measured. Heart rate and blood pressure (BP) were assessed using an automated blood pressure monitor (SunTech Medical Cycle). Blood lactate (BLa) was assessed by taking blood samples from the finger sticks from the participants and samples were immediately analyzed using the Lactate Pro Analyzer.

After these measurements were obtained, the participants remained seated until the total time proceeding ingestion of the treatment was 60 minutes. The time from ingestion to the beginning of the workout, including pre-workout measurements, was exactly 60 minutes. This period allowed adequate time for the supplement to be absorbed by the body's system.

Participants completed three sets each of chin-ups (hands pronated), reverse chin-ups (hands supinated), and push-ups to failure with 3 minutes of rest between each set. The order of the exercises were the same for each session and all participants. Participants waited on a "go" command at the initial hanging position beginning at full elbow extension for each chin-up and reverse chin-up repetition. The repetition was counted if the chin reached a parallel level with the bar. A tester held their arm in front of the participant to prevent excessive swinging of the participant's body. Markings were placed on the apparatus to ensure the same hand position is maintained during each trial and set. The participant's push-up repetitions were counted if the participant's chest made contact with the tester's fist, which rested on the ground beneath the participant's nipple line. Participant's elbows were fully extended after the concentric portion of the repetition. Failure for either exercise was defined as the inability to complete a full repetition without assistance. This protocol was used as a measure of muscular endurance. BLa was immediately assessed within 5 seconds of the final push-up repetition. HR and BP were measured at five minutes and ten minutes following the last push-up repetition.

Experimental Design

Treatment order was randomly assigned to participants. A double-blind crossover design was used, as neither the principal investigators nor the participants were aware of the treatment order. The treatments were placed in small plastic bags marked with either a red or black dot. Experimental days were separated by a minimum of 1 week but no more than 2 weeks to minimize participant fatigue.

Dietary and Supplement Intake

Participants completed a 24-hour diet and exercise recall during the 60 min pre-exercise period of each experimental trial. Participants were also restricted from taking any medications or supplements 48 hours prior to each trial, and participants were also asked to abstain from intense exercise within the 48 hours preceding each trial. Those individuals who failed to acknowledge meeting these criteria during the phone call preceding the test were rescheduled. Rescheduling was dependent on testing availability and washout period.

Treatment Ingestion

Over-the-counter CM or a PL were provided to participants 60 minutes pre-exercise. The CM or PL will be administered to the participants mixed in 300 mL of chilled crystal light lemonade juice. This drink was administered in one full dose 60 minutes prior to exercise. The amount of CM ingested by all participants was approximately 8 grams in accordance with recommended dosages being between 4-10 grams (Pérez-Guisado and Jakeman 2010). Seven days later, participants ingested the alternative treatment, and repeated the identical exercise protocol. There have been no reported side effects of CM when taken in recommended amounts by the manufacturer or cited in prior literature.

Statistical Analysis

Performance differences for resistance exercises were analyzed using 2 (trial) x 3 (time) repeated measures analyses of variance (RMANOVAs). Paired t-tests were used to determine significant differences for total trial repetitions and total repetitions per

exercise (set 1 + set 2 + set 3). One way ANOVA and paired t-tests were used to determine differences between conditions as appropriate. Statistical analyses were performed using commercially available software (SPSS Inc., Chicago, IL, USA) and acceptance for statistical significance was $p \leq 0.05$.

CHAPTER IV

RESULTS

Participants

This study consisted of fifteen apparently healthy, resistance trained male participants (see Table 1).

Table 1 Demographic data for subjects

Variable	Mean ± SD
Age (yrs.)	23.73 ± 2.28
Height (cm)	178.91 ± 6.73
Weight (kg)	86.91 ± 9.37
Body fat %	15.73 ± 4.95

Resistance Performance

Analyses of performance on the resistance exercises (pull-ups, reverse pull-ups, and push-ups) demonstrated similar responses across all three exercises. No significant interaction effect was seen for the resistance exercises, although all three exercises demonstrated a significant main effect for condition (pull-ups: $F_{1, 14} = 13.49$, $p = .003$; reverse pull-ups: $F_{1, 14} = 9.64$, $p = .008$; and push-ups: $F_{1, 14} = 27.04$, $p = .001$), with the CM condition resulting in greater number of total repetitions performed in each exercise between the two groups (Figure 1). The total number of repetitions for each

exercise and complete session was calculated by adding the repetition sum of all individual exercises and the trial collectively (Figure 2).

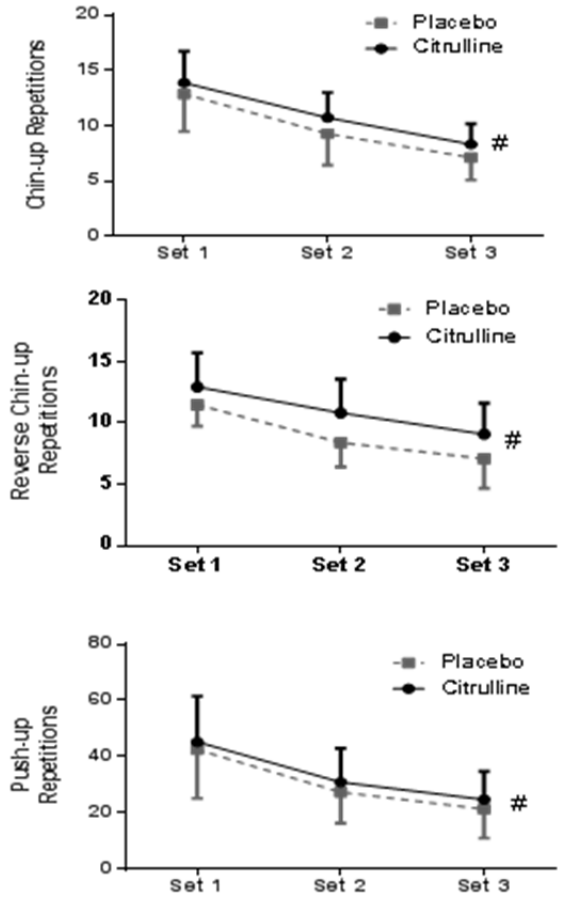

Figure 1 Chin-up, reverse chin-up, and push-up repetitions (group) performed collectively were significantly greater in the CM than PL group

(# indicates $p < 0.05$).

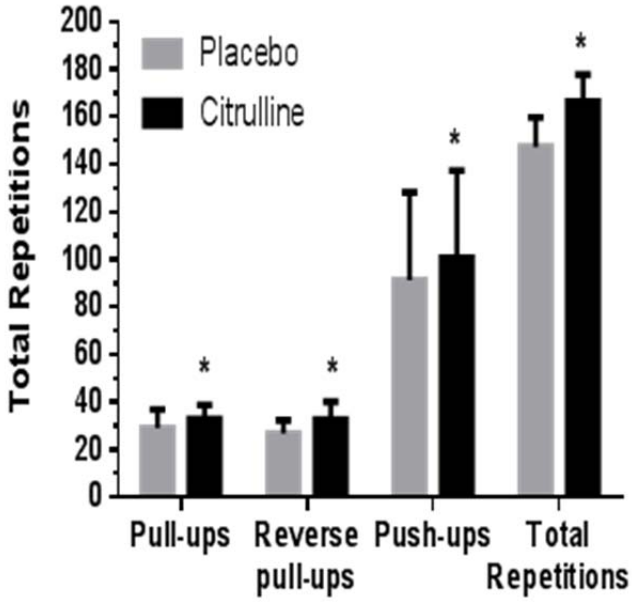

Figure 2 Mean (± SD) total repetitions for exercise and complete trial

*Significantly different ($p < 0.05$) between trials.

Blood Lactate

Blood lactate values demonstrated a significant increase from pre- to post-exercise in both conditions (treatment $t14 = 27.14$, $p > .001$; control $t14 = 15.44$, $p > .001$). No differences were shown between conditions either prior to or after exercise, thus demonstrating no significant differences in lactate responses as a result of supplementation (Figure 3).

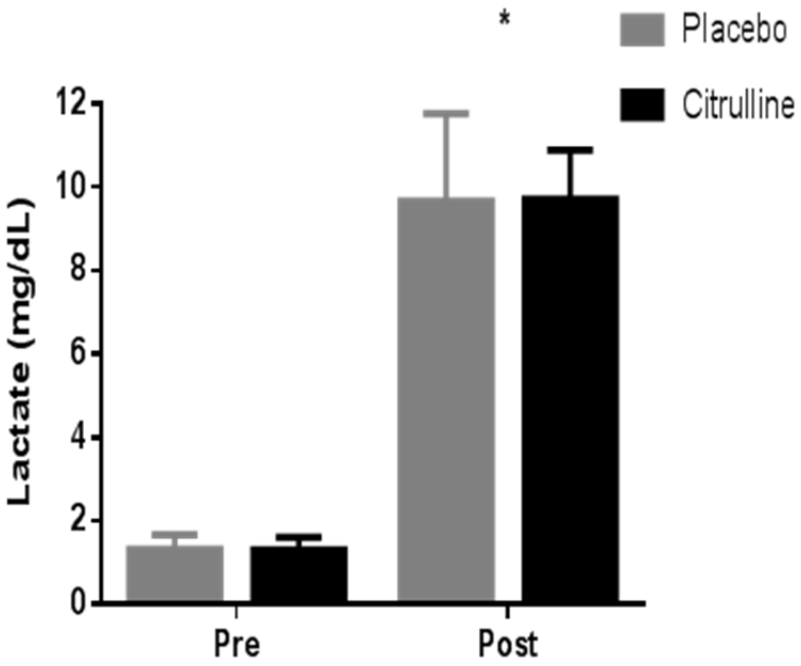

Figure 3 Blood lactate (mmol/l) at PRE and POST (i.e. following the exercise protocol).

No differences between trials were found.

Cardiovascular Variables

Repeated measures analysis of cardiovascular variables (HR, SBP, and DBP) demonstrated a significant interaction effect for SBP ($F_{2, 28} = 4.70$, $p = .02$), a significant main effect for condition for DBP ($F_{1, 14} = 13.15$, $p = .003$), and a significant time main effect for HR ($F_{2, 27} = 100.55$, $p < .001$). Post-hoc analysis revealed that SBP responses were more elevated in the placebo condition at R5 ($t_{14} = 3.28$, $p = .005$) and R10 ($t_{14} = 3.74$, $p = .002$). These results suggest that the CM supplement did have an impact on cardiovascular responses during the resistance exercise, although the mechanisms by which these responses occurred needs to be further investigated (Figure 4).

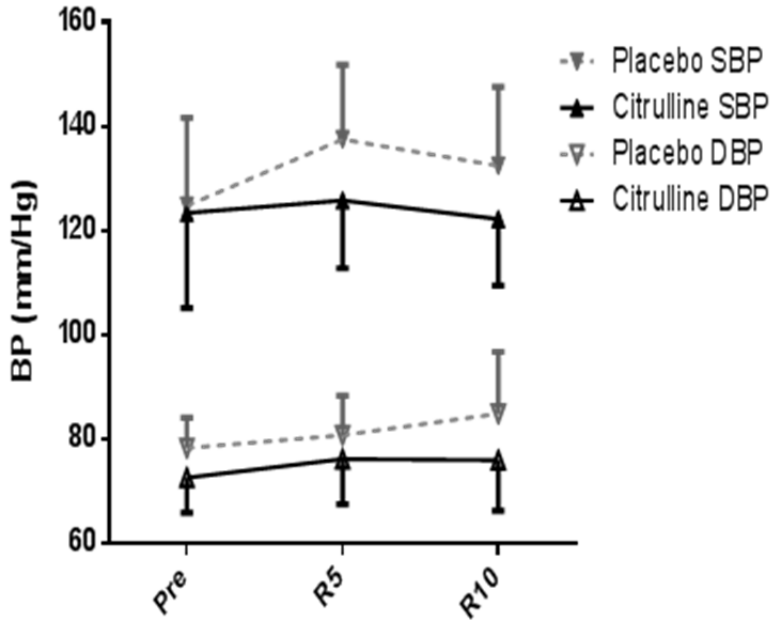

Figure 4 SBP and DBP (group) for Pre-Exercise and Recovery 5 and 10 minutes post-exercise.

*Indicates $p < 0.05$ between various time points.

CHAPTER V

DISCUSSION

The purpose of this investigation was to investigate the effects of citrulline-malate (CM) on resistance exercise performance (i.e. number of repetitions performed), blood lactate, blood pressure, and heart rate during repeated bouts of upper body exercises in resistance trained college-age male subjects. In this study, we hypothesized that CM supplementation would not effect the number of repetitions performed by trained males during a nine set upper body exercise session.

Implications

The results in our investigation are in agreement with a prior study (Perez-Guisado and Jakeman 2010) that investigated the effects of CM supplementation on resistance exercise performance. Specifically, Perez-Guisado and Jakeman reported that a single dose of CM (8 g) increased performance by an average of 19%, measured as the number of repetitions performed until exhaustion occurred. Additionally, the authors reported a significant decrease of 40% in muscle soreness by subjects during their CM trial 24 and 48 hours following their resistance training session.

The precise mechanism by which CM leads to an increase in resistance exercise performance remains elusive, however, several have been proposed: 1) citrulline detoxifies ammonia , 2) alters the acid base balance, in particular lactic acid formation, 3)

increases plasma L-arginine concentrations (Hickner et al. 2006), while 3) malate acts as a metabolic shuttle between the cytoplasm and mitochondria by mitigating lactic acid accumulation and enabling continued pyruvate genesis, synergistically 4) increasing the availability of ATP (Bendahan et al. 2002) and nitric oxide via NO synthases catalytic effects on L-arginine and oxygen (Weissman and Gross 2001).

Nitric oxide (NO) is a gaseous molecule that plays an important role in many functions in the body including vasodilatation regulation, blood flow, and mitochondrical respiration. Additionally, NO regulates glucose uptake and oxidation, mitochondriogenesis, and other contractile functions in skeletal muscle (Petrovic et al. 2008). Availability of plasma arginine to endothelial cells is a limiting factor for NO synthesis (Nussler 1994). Citrulline supplementation increases levels of plasma L-arginine (Hickner et al. 2006); thereby, augmenting plasma arginine concentrations following exercise (Goodwin, Solomonson, and Eichler 2004, Mori 2007). Finally, malate affects oxidative ATP production through anaplerotic reations (Gibala, Young, and Taegtmeyer 2000), which mitigates ammonia's blockade of the oxidative pathway, therefore continuing pyruvate genesis (latic acid ↔ pyruvate).

In prior studies, (Bescos et al. 2012) authors speculated that CM would impact metabolic pathways. Specifically, lactic acid is quickly dissociated (loses a hydrogen) into a molecule called lactate, which is constantly being produced in humans at both rest and during exercise (Brooks 2009, Donovan and Brooks 1983, Hashimoto and Brooks 2008). Blood lactate concentrations can range from a resting value of 1 mmol/kg of muscle to more than 25 mmol/kg during intense exercise (Kilding and Jones 2005, Kon et al. 2010). In our study, blood lactate increased following both trials; however, there was

no significant difference in blood lactate between the CM and placebo treatments. This suggests that CM does not have a positive or negative influence lactate production in the glycolytic system.

An acute bout of resistance exercise can significantly increase heart rate and blood pressure, especially if the individual uses the Valsalva maneuver. Peak heart rate of 170 beats per minute and blood pressures of 320/250 mmHg have been reported during high intensity lower body exercise (MacDougall et al. 1985). The present study confirmed a rise in heart rate, DBP, and SBP between pre and post-exercise for both treatments. There were no differences between treatments in heart rate; however, SBP and DBP were significantly lowered during the CM treatment. This may have been attributed to a vasodilatory effect mediated by NO due to the activity of nitric oxide synthase (Orozco-Gutierrez et al. 2010).

In closing, the current study supports previous literature and agrees the use of citrulline-malate (CM) as a pre-workout supplement would be advantageous to performance. The exact mechanisms by which CM would improve performance are still not clear. However, from the results of the current study, evidence of improved cardiovascular conditions seen during the CM treatment is a likely mechanism. The current study focused on the use of CM in a more anaerobic setting. Future research following a more aerobic protocol may provide further support and novel findings to explain improvements in performance after ingestion of CM.

Recommendations for Future Research

Future research of citrulline-malate (CM) could test alternative variables such as:

1. Use of a more aerobic protocol.

2. Administration of CM in pill form versus powder
3. Testing ingestion of CM in multiple dosages over the course of days or "loading" versus an acute ingestion prior to exercise.
4. Monitoring additional biomarkers such as blood glucose.
5. Testing highly trained and elite athletes.
6. Testing and older age group.

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APPENDIX A

CONSENT TO PARTICIPATE

Mississippi State University

Department of Kinesiology
(662) 325-2963
(662) 325-4525 (fax)

Consent to Participate in an Experimental Study
Effects of Citrulline on Resistance Exercise

Investigators:

Ben Wax, Ph.D.
Mississippi State University
Department of Kinesiology
124 McCarthy Gymnasium
Mississippi State, Mississippi 39762
(662) 325-6800

Description: You are being asked to voluntarily participate in a research project designed to investigate the effects of the non-essential amino acid, Citrulline, on repeated bouts of muscular endurance resistance exercise using your bodyweight. Citrulline is an over-the-counter supplement that may delay muscle fatigue. Your participation will include coming to the Applied Physiology Lab (Room 131, McCarthy Gymnasium) for one-testing session and to the Sanderson Center weight room for three testing sessions. Below is an explanation of what you will be asked to do during those four sessions.

During the first session you will be introduced to the research team, complete a medical history questionnaire and informed consent. Also, you will be asked to participate in an assessment of your body-fat percent, body weight, height, and perform 8 chin-ups (pronated or supinated). The introductory information should take approximately 15 minutes, and the assessment should take no more than 15 minutes. Thus, the total time for this first session should be approximately 30 minutes.

If you qualify for the study, upon arriving to the Sanderson Center weight room you will be escorted to a designated area (conference room) where you will rest quietly for 5 minutes. Following 5 minutes of quiet rest, your heart rate (HR), and blood pressure (BP) level will be measured. Immediately following, you will be given Citrulline (3 grams) or placebo (60 mg of saccharine) mixed in chilled crystal light lemonade juice (200 ml). The mixture will be given to you for consumption, served 45 minutes before exercise. The beverages will be served in identical containers and you will be instructed to pinch your nose as you drink in an effort to disguise differences between Citrulline and placebo and maintain the double blind experiment. Following 45 minutes of rest, another measurement of HR and BP will be taken. You will then complete three sets each of chin-ups (hands pronated), reverse chin-ups (hands supinated), and push-ups to failure with 3 minutes of rest between each set. You will wait on a "go" command at the initial hanging position with full elbow extension for each chin-up or reverse chin-up repetition. The repetition will be counted if your chin reaches a parallel level with the bar. A tester will hold his arm in front of you to prevent excessive swinging of the body. Markings will be on the apparatus to ensure you maintain the same hand position during each trial and set. Push-up repetitions will be counted if your chest makes contact with the tester's fist resting on

MSU IRB
Approved: 1/5/12
Expires: 11/15/12

the ground, and your elbows should fully extend after the concentric portion of the repetition. The order of the exercises will be the same for each session and all participants.

You should know that we are looking for specific characteristics in our subjects. These include; a) having no history of chronic illness, b) no sickle cell anemia or sickle cell trait, c) musculoskeletal problems in the previous 6 months, d) not taking any prescribed medications (excluding contraceptive medication), supplements (vitamins and minerals permitted), or tobacco products, e) consuming an average of less than 10 alcoholic beverages per week, and f) be able to perform 8 chin-ups (pronated or supinated) without assistance. This information will be assessed during the initial testing session, as well as during the confirmation phone call the night before sessions two and three. Any subject who starts a prohibited substance will be excused from the research study.

Risks: Your participation in this study may involve some risks. The most common are the possibility of feeling general fatigue following one or both of the testing procedures. If you experience any of the following, please contact Dr. Wax or seek medical attention: dark urine, excessive muscle fatigue, excessive muscle pain, and/or swollen or hard muscles. Other potential risks will be the same as you would normally have during a weight training session. In a study performed by Gordon et. Al. (1995) in conjunction with the Cooper Institute confirmed that maximal strength testing is relatively safe procedure for healthy individuals (no comprehensive data is available on the cardiovascular safety of maximal strength testing to the researchers' knowledge). There have been no reported side effects of Citrulline when taken in recommended amounts by the manufacturer or reported in prior literature; however, Citrulline may present a risk of allergic reaction. Individuals with a history of drug allergies or allergies to dyes should review the ingredients of Citrulline before participating in the study. Every effort will be made to minimize any risks by constant supervision by the Principal Investigator while you are participating in this research project. Citrulline, the supplement being investigated in this research project, has not been evaluated by the Food and Drug Administration. Citrulline is not intended to diagnose, treat, cure or prevent any disease.

Benefits: The benefits of this study to the participants are information regarding their blood pressure, body fat percentage and strength levels, while the general population benefits include gaining knowledge regarding the effects of Citrulline on resistance types of exercise.

Incentives: You will not receive any financial compensation for the initial session, but you will be provided with the results of your body composition and muscular endurance test and you may request information on how to improve your muscular strength and muscular endurance strength levels.

Confidentiality: The results of the tests and all the associated records will be kept strictly confidential, and only members of the investigative team will have access to these documents*. If your individual test results are reported at a scientific meeting or published in a scientific journal, only your assigned participant number, rather than full name, will be used.

On occasion, we may take photos or videotape you during your participation in the study for presentations at conferences or in manuscripts. We will make every attempt to keep you from being recognized in the video and/or photos. We will inform you if we wish to photograph or

record you. You may choose to participate in this research, while opting out of being photographed or recorded by initialing your preference below.
*Please note that these records will be held by a State entity and therefore are subject to disclosure if required by law. Also, all records of this research project may be inspected by the Food and Drug Administration (FDA).

If you agree to allow photographic and videographic recording of your participation in the study entitled, "*Effects of Citrulline on Resistance Exercise*",

Please initial "Yes" _____**YES** _____ **NO**

Research Questions: If you should have any questions about this research project, please feel free to contact Dr. Ben Wax at 662-325-6800. This study has been reviewed by the Mississippi State University Institutional Review Board (IRB). The IRB has determined that this study meets the ethical obligations required by federal law and University Policies. If you have any questions, concerns or reports regarding this study, please contact the MSU Regulatory Compliance Office at 662-325-3994.

Compensation for Illness or Injury: You are not waiving any legal rights or releasing the institution or their agents from liability from negligence. In addition to reporting an injury to Dr. Ben Wax (662-325-6800) and to the Regulatory Compliance Office (662-325-3994), you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the University Police Department at *MSU UNIVERSITY POLICE DEPARTMENT, Williams Building, Mississippi State, MS 39762, (662) 325-2121.*

Right to Withdraw: Your participation is totally voluntary and you can withdraw at any time for any reason. If you start the study and decide that you want to withdraw, you need only to inform Dr. Ben Wax or anyone else on the research team. You can do this in person, by letter, or by telephone at the Department of Kinesiology, Mississippi State University, MS 39762, or 325-6800. Whether or not you choose to participate will not affect your standing within the Department of Kinesiology, or with the University, and will not cause you to lose any benefits to which you are entitled. For additional information regarding your rights as a research subject, please feel free to contact the MSU Regulatory Compliance Office at 662-325-3994.

Statement of Consent: Please take all the time you need to read through this document and decide whether you would like to participate in this research study.

If you agree to participate in this research study, please sign below. You will be given a copy of this form for your records.

Signature of Participant: _____ **Date:** _____

Signature of Investigator: _____ **Date:** _____

APPENDIX B

PARTICIPATION AND HEALTH HISTORY QUESTIONNAIRE

PARTICIPATION AND HEALTH HISTORY QUESTIONNAIRE

Complete each question accurately. All information provided is strictly confidential.

Part I: Participant Information

Name (Print)	Home Phone #
Current Mailing Address	Cell Phone #
Personal Physician	Email Address
Emergency Contact (relationship)	Emergency Contact Phone #
Gender: _____ Female _____ Male	Date of Birth:_____
Height _____ Weight _____	Age _____

Part II. Health History

List any physical injuries or limitations that you have at this time: _____

_____

Have you ever been diagnosed as having any cardiovascular abnormalities?_____ Yes _____ No

If yes, what was diagnosed and when was the diagnosis conducted?

_____

_____

Please circle any of the following for which you have been diagnosed or treated by a physician, health care professional, or which you have experienced personally:

Heart Attack	Bypass Surgery	Sickle-Cell Anemia
Heart Palpitations	Arrhythmia	Chest Pain
Shortness of Breath	Stroke	Anemia
Heart Valve Problems	Seizure	Hypoxemia
Heart Murmur	Heart Rhythm Abnormalities	
Fainting		

Do you have or have had any form of respiratory (breathing) ailments in the previous three months? Please circle those that apply.

Asthma	COPD	Common cold
Emphysema	Bronchitis	H1N1 flu

Have you been diagnosed with any of the following? If yes, please circle the appropriate ailment.

Rheumatic Fever	High Blood Pressure	Obesity
Kidney/Liver Disease	High Cholesterol	Diabetes
Hemophilia	Multiple Sclerosis	

Other_____

Does anyone in your family have any of the conditions listed above? If yes, please list relation to family member and problem: _____

_____

Is your mother living? _____ Yes _____ No (age at death _____; cause_____)

Is your father living? _____ Yes _____ No (age at death _____; cause_____)

Do you have any allergies (latex, food, drug, etc.)? _____ Yes _____ No

If yes, please list: _____

_____

Have you ever performed a one repetition muscular strength tests? _____ Yes _____ No

****If yes, when and what were the latest results (bench press and/or leg press)? _____

_____

Have you ever experienced any adverse responses during or after exercise (i.e. dizziness, difficulty breathing, racing heart beat, fainting)? _____ Yes _____ No

If yes, what were the symptoms? _____

_____

Part III. Health Related Behavior

Do you smoke? _____ YES _____ No

If yes, indicate number of cigarettes per day?
_____ Less than ½ a pack _____ 1 pack ____ Greater than 1 pack

Do you drink alcohol? _____ YES _____ No

IF YES, INDICATE NUMBER OF ALCOHOLIC BEVERAGES PER WEEK?
_____ Less than 10 _____ 10 ____ GREATER THAN 10

Do you exercise regularly (30 minutes, 3 times per week)? _____ Yes _____ No

If so, what exercises do you participate in regularly? _____

_____

Are you taking any medications (prescription/nonprescription) or supplements?

_____ Yes _____ No

If yes, please list: _____

_____

Have you ever heard of the supplement Citrulline Malate, prior to the introduction to this research project? _____ YES _____NO

If **YES**,
Where?_____

If **YES**, have you ever taken the supplement Citrulline Malate? _____ YES _____NO

If **YES**, please indicate time usage of the supplement:

____ week (7 days or less)	____ 9 months
____ month (4 weeks or less)	____ 12- 18 months
____ 3 months	____ 2 years
____ 6 months	____ 3 years

Other (please explain-_____

What supplement(s) (ergogenic aid for weightlifting) have you cycled on (6 weeks or longer) in your past?

Check all that apply:

___GAKIC	___Protein Powder	___Creatine
___NO explode	___Citrulline Malate	___NOS drinks/pills
___Glycerol	___Caffeine	___Sodium Bicarbinate
___Ephedrine/Ephedra enhancers (pro-hormones)	___Wt. loss products	___Natural testosterone

Other (please list)_____
_____

Are you taking Citrulline Malate or any other supplement at the moment time? ____ YES ____NO

If YES, are you willing to discontinue usage during this experimental protocol? ___Yes ___NO

Thank You