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Effects of Glycine-Arginine-Alpha-Ketoisocaproic Acid Calcium (Gakic) on Maximal Strength and Multiple Bouts of Resistance Exercise

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By

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Glycine-arginine-alpha-ketoisocaproic acid calcium (GAKIC) is an amino acid combination postulated to improve dynamic performance of skeletal muscle during acute, anaerobic exercise in healthy individuals. *Purpose:* The purpose of this study was to determine the ergogenic effects of GAKIC ingestion on resistance training performance in both trained male and female participants. *Methods:* Utilizing a double-blinded, crossover design, male participants completed a lower body leg press resistance exercise protocol and female participants completed a lower body leg extension resistance exercise protocol once using 10.2 g GAKIC and the other with a placebo. *Results:* A significant increase in TLV after GAKIC supplementation was observed in both male and female participants performing a lower body resistance exercise. No significant differences were found in lower body 1RM, HR, BLa, and Glucose between conditions in both groups. *Conclusion:* We concluded with the specific exercise protocols that were implemented, GAKIC increased TLV in the lower body exercises.

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. Success is not for the weak. With God, all things are possible.

ACKNOWLEDGEMENTS

I, Laura Hilton, would like to express my appreciation to my committee members for their outstanding work ethic and dedication towards the success of this project. Benjamin Wax, Heather Webb, Katherine Gilliland, and Andreas Kavazis have truly been a remarkable support throughout this entire process. Thank you to my family and loved ones from the bottom of my heart. Without these amazing people in my life, I would not be where I am today.

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CHAPTER I

INTRODUCTION

Dynamic all out high-intensity anaerobic exercise quickly leads to fatigue and a decrease in muscle force output. As a result, an athlete's performance output would, over time, decrease accordingly. In response, Stevens, Godfrey, Kaminski, and Braith (2000) developed a new supplement combining ketoacid/amino acid metabolic treatment that became known as Glycine-Arginine-Alpha-Ketoisocaproic Acid Calcium (GAKIC).

Specifically, GAKIC contains glycine, arginine, and ketoisocaproic acid. Glycine is a nonessential amino acid that is used for muscle biosynthesis (Chyun & Griminger, 1984). Furthermore, arginine is an essential amino acid that helps the body process both creatine and nitrogen. Creatine and nitrogen are two substances that are used during normal muscle metabolism (Camic, Housh, Zuniga, Hendrix, Mielke, Johnson, & Schmidt, 2010; Hambrecht, Hilbrich, Erbs, Gielen, Fiehn, Schoene, & Schuler, 2000; Zajac, Poprzecki, Zebrowska, Chalimoniuk, & Langfort, 2010). Lastly, ketoisocaproic acid functions as a precursor for the essential amino acid leucine (Anthony, J., Anthony, T, & Layman, 2010). Ketoisocaproic acid is an important precursor because leucine is used to stimulate protein synthesis in muscle and is closely related to the release of gluconeogenic precursors (Coburn, Housh, D, Housh, T, Malek, Beck, Cramer, Johnson, & Donlin, 2006; Mero, 1999; Mero, Leikas, Knuutinen, Hulmi, & Kovanen, 2009). This amino acid combination was postulated to improve dynamic performance of skeletal muscle during acute, anaerobic, exhaustive dynamic exercise in healthy individuals.

Stevens et al. (2000) reported that GAKIC ingestion increased muscle torque and work during intense acute anaerobic dynamic exercise. Specifically, GAKIC increased overall muscular performance by delaying muscle fatigue during the early stages of anaerobic dynamic exercise (Stevens et al, 2000). Several mechanisms are proposed to enhance performance including alterations in acidosis, clearance of ammonia and transamination waste products, and alterations in the enzymatic pathways concerning nitrogen and branched chain ketoacid metabolism (Buford, & Koch, 2004). Stevens et al. (1998) theorized the following: 1) GAKIC treatment may increase the ability to sustain athletic muscle force during intense anaerobic muscle exercise; 2) GAKIC treatment may also increase the ability to sustain athletic muscle work during intense anaerobic muscle exercise; and 3) GAKIC may further increase the overall muscle performance by decreasing absolute muscle fatigue while retarding the rate of muscle fatigue. However, to date, the exact mechanisms for GAKIC are still unknown.

According to Beis et al. (2011), the amino acids that make up GAKIC seem to be active in certain metabolic pathways. These metabolic pathways are associated with the biosynthesis of creatine, protein, and nitric oxide. GAKIC's effects may also be linked to stabilizing muscle pH or a reduction of ammonia concentration that is released from the purine nucleotide cycle. This possibly occurs through the alterations in the enzymatic pathways of nitrogen metabolism.

To date, there have only been three published studies investigating the effects of GAKIC supplementation on exercise performance (Stevens et al, 1998; Buford, & Koch, 2004; Beis, Mohammad, Easton, & Pitsiladis, 2011).The studies were all performed on either cycling or an isokinetic dynamometer and used only male participants. Stevens et

al. (2000) and Buford and Koch (2004) both used untrained males for their study. Both Stevens et al. and Buford and Koch reported positive effects for participants using GAKIC supplementation in their studies. In contrast, Beis et al. reported no significant differences in exercise performance for trained males on GAKIC supplementation. The conflicting results could be related to the different training status of the participants selected, the experimental design, and the methodology employed. None of the studies cited above used weight exercises or tested female participants. Therefore, the purpose of this study was to determine the ergogenic effects of GAKIC ingestion on resistance training performance in both trained male and female participants.

Statement of Problem

Little research has been done investigating the ability of GAKIC to enhance performance during a traditional resistance exercise protocol. While studies have demonstrated improvements in performance during high-intensity exercise, no research has examined whether an acute ingestion of GAKIC prior to resistance exercise can enhance the performance of trained male and female participants engaged in that exercise.

Hypotheses (Experiment 1 and 2)

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

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

HO_3: There is no significant difference in total load at 75% of 1RM for five sets between GAKIC and PL treatments in male participants.

HO_4: There is no significant difference in blood glucose between GAKIC and PL treatments in male participants.

HO_5: There is no significant difference in blood lactate (BLa) between GAKIC and placebo (PL) treatments in female participants.

HO_6: There is no significant difference in heart rate (HR) between GAKIC and PL treatments in female participants.

HO_7: There is no significant difference in 1RM between GAKIC and PL treatments in female participants.

HO_8: There is no significant difference in total load at 50% of 1RM for six sets between GAKIC and PL treatments in female participants.

Basic Assumptions

The following are basic assumptions for this study:

1. Measurements made on all equipment were accurately performed.
2. The participants did not consume any food for two hours prior to testing.
3. The participants understood the directions given to them.
4. The participants followed the directions given to them.
5. The participants abstained from intense exercise for 48 hours preceding each trial.
6. The participants did not consume any nonprescription medications or supplementation in the 48 hours prior to each trial.
7. The participants performed the exercise to failure.

8. The participants accurately recorded their diet and exercise recall for the 24 hours prior to testing.
9. The participants were non- anabolic steroid using individuals.

Limitations

The following are limitations of the study:

1. The investigators did not test the individuals for anabolic or exogenous steroid use.
2. Due to each participant monitoring his own food intake for 24 hours prior to testing, nutritional intake was neither exactly accurate nor duplicated.
3. Due to blood lactate being obtained from the finger tip, measurements may not accurately reflect what was occurring in the muscles.
4. The investigators did not analyze the supplement for purity of sample.

Delimitations

The following are delimitations of the study:

1. The participants were college students between the age of 18 and 40.
2. The participants were recreationally trained lifters (> six months of lifting experience).

Definitions

1. Failure: inability to complete a repetition without assistance.
2. Fatigue: a reduction in work capacity resulting from preceding work.

3. Maximal Strength: The maximal amount of weight an individual can lift one time.

Significance of the Study

Little research has been done investigating the effects of supplemental GAKIC ingestion on performance during a traditional resistance exercise protocol. While studies have demonstrated improvements in performance during high-intensity cycling exercise, no research has been conducted using traditional resistance exercise. Therefore, this study contributes information regarding GAKIC's effects during a traditional resistance protocol to the current body of scientific knowledge.

CHAPTER II

REVIEW OF LITERATURE

The following section will provide an overview of the basic energy systems, muscle contraction, fiber types, muscular strength, muscular endurance, and GAKIC. This will provide insight into the basic energy requirements needed for movement and lay the basic foundation, using information from the current literature, regarding GAKIC ingestion.

ATP-PC System

Muscular exercise requires a continuous supply of adenosine triphosphate (ATP) in order to provide enough energy to produce a contraction (Tullson & Terjung, 1991). ATP is an important compound because it couples the energy that is released from the breakdown of food into a form of energy that is usable and needed by all cells. Because muscle cells store only a limited amount of available ATP, metabolic pathways exist in the cells to supply additional energy. These pathways must have the ability to produce ATP at a rapid rate. Therefore, an individual's muscle cells have the capability to produce ATP by using one or a combination of three metabolic pathways. The three metabolic pathways include the following: 1) formation of ATP by phosphocreatine (PC) breakdown, 2) formation of ATP through the degradation of glucose or glycogen in a process known as glycolysis, and 3) oxidative formation of ATP. Because glycolysis and the PC pathway do not involve the use of oxygen, these methods of ATP formation are known as anaerobic pathways (Powers & Howley, 2007).

In anaerobic ATP production, ATP production involves the donation of a phosphate group and its bond energy from a combination of PC to ADP to form ATP (Bessman & Carpender, 1985; Conley, 1994; DeZwaan & Thillard, 1985; Holloszy, 1982; Whipp & Mahler, 1980). However, the body's muscle cells do not store large amounts of PC. As a result, the amount of ATP formation through this reaction for energy use is limited. Therefore, the body uses a combination of stored ATP and PC known as the ATP-PC system (phosphagen system). The ATP-PC system provides the energy needed for muscular contraction at the start of exercise and during short-term, high-intensity exercise. The reformation of PC requires ATP (Cerretelli, Rennie, & Pendergast, 1980; di Prampero, Boutellier, & Pietsch, 1983). However, this only occurs during the recovery periods after exercise. The ATP-PC system is important for athletes who participate in short term, intense exercise because the exercises only require a few seconds to complete. In such activities, a rapid supply of ATP is required. As a result, the ATP-PC system provides a simple one-enzyme reaction to produce the ATP needed for these activities (Powers & Howley, 2007).

Anaerobic Glycolysis

Another method of anaerobic ATP production is known as glycolysis. In glycolytic reactions, two end products are created, pyruvic acid and hydrogen atoms combined with NAD^+ to form NADH and H^+. If either or both of these build up to excessive amounts, the glycolytic process is halted. Therefore, no further formation of ATP would take place. When this occurs, the two end products react with each other to form what is known as lactic acid (Guyton & Hall, 2006). As exercise intensity increases, a greater flow of excess hydrogen to pyruvate is produced causing the concentration of

lactate to quickly rise within the active muscle. During recovery, the excess hydrogen in the lactate oxidizes to result in the reformation of a pyruvate molecule (McArdle, Katch, & Katch, 2009). The enzyme, lactate dehydrogenase (LDH), catalyzes this reaction resulting in the formation of lactic acid and the reformation of nicotinamide adenine dinucleotide (NAD). Therefore, the formation of lactic acid is used for the reprocessing of NAD in order for glycolysis to continue (Armstrong, 1979; Johnson, 1987). Glycolysis is an important mean of releasing energy from the glucose molecule. It is the process of splitting the glucose molecule to form two molecules of pyruvic acid. Because it does not require oxygen in the chemical reaction for the breakdown of glucose into pyruvic acid, glycolysis is considered an anaerobic process (Guyton & Hall, 2006).

Intense exercise can possibly cause muscular performance to be weakened during both the acute and chronic phases of the exercise. Dynamic high-intensity anaerobic use of skeletal muscle can quickly lead to fatigue and a decrease in muscle force and work. As a result, an athlete's performance is reduced. Many ergogenic supplements have been used in an attempt to enhance muscle performance during acute and long-term exercise. However, most of these attempts have resulted in limited success.

Aerobic Glycolysis

Glycolysis is known as the anaerobic production of ATP. It is our body's pathway for producing ATP without oxygen. However, glycolysis has also been considered to be the first step in the aerobic degradation of carbohydrates, which in turn aids in Aerobic ATP Production. The Krebs cycle becomes the next stage in the process of the degradation of the glucose molecule to form ATP (Guyton & Hall, 2006). This process occurs in the mitochondria of the cell. The Krebs cycle is used to degrade the acetyl-CoA

substrate to carbon dioxide and hydrogen atoms. When the hydrogen atoms are oxidized during the electron transport-oxidative phosphorylation, the formation of ATP occurs. Oxygen is not directly used during the Krebs cycle reactions. However, within the pyruvate, the chemical energy transfers to ADP through the electron transport-oxidative phosphorylation. With adequate oxygen, enzymes, and substrate, NAD^+ and FAD regenerate allowing the Krebs cycle to continue (McArdle, Katch, F., & Katch, V., 2010). The process of aerobic ATP production is a three stage process. In stage 1, a vital two-carbon molecule is created known as acetyl-CoA. In stage 2, the oxidation of the molecule acetyl-CoA occurs in the Krebs cycle. In stage 3, the process of oxidative phosphorylation (ATP formation) in the electron transport chain occurs (Powers & Howley, 2007).

Muscle Contraction

Muscular contraction can be described as a complex process that involves a number of cellular proteins and energy production systems. Muscle contraction occurs through the sliding filament mechanism. The actin filaments being pulled completely inward with the myosin filaments results in their ends becoming overlapped to maximum extension. As a result, forces are generated by the interaction of the cross-bridges from the myosin filaments with the actin filaments (Guyton & Hall, 2006). The actin filaments are activated by calcium ions. Once activated, the heads of the cross-bridges from the myosin filaments become attracted to the actin filaments' active sites. The heads of the cross-bridges from the myosin filaments bend back and forth pulling the ends of the actin filaments toward the center of the myosin filament causing a contraction (Metzger, 1992; Rayment et al., 1992; Ruegg, 1992; Vale, 1994).

Muscle Fibers

Muscle fibers, irrespective of gender, are divided into two categories: slow twitch and fast twitch (Buchthal & Schmalbruch, 1970; Burke, 1986; Edgerton et al., 1983; Edgerton et al., 1986; MacIntosh, Gardiner, & McComas, 2005). Fiber type percentage found within the skeletal muscle can be influenced by many factors including: genetics, blood levels of hormones, and the exercise habits of the individual. Further, there are no noticeable differences in fiber type distribution with sex and age (Powers & Howley, 2007). Slow twitch fibers can also be called slow-oxidative or type I fibers. Type I fibers have four distinctive characteristics. They are the following: 1.) Low myosin ATPase activity, 2.) Slow calcium handling ability and shortening speed, 3.) Less well-developed glycolytic capacity than fast twitch fibers, and 4.) Large and numerous mitochondria. They produce energy for the resynthesis of ATP mostly through the aerobic system for the transfer of energy. The characteristics of Type I fibers cause them to be highly resistant to fatigue. Therefore, Type I muscle fibers are best used for prolonged aerobic exercises (McArdle, Katch, F., & Katch, V., 2010).

Fast twitch fibers can be subdivided into two categories: type IIx and type IIa. Type IIx can also be called fast-glycolytic fibers. They have a comparatively small number of mitochondria, less resistance to fatigue than slow twitch fibers, and a limited capacity for aerobic metabolism (Green, 1986; Pette & Spamer, 1986). On the other hand, type IIx fibers are high in glycolytic enzymes. These enzymes provide the type IIx fibers with a large anaerobic capacity (Pette, 1980). Also, type IIx fibers have higher myosin ATPase activity than any other fiber types. As a result, they have the highest Vmax. Because type IIx fibers have very high ATPase activity, it causes them to be less efficient and results in greater energy expenditure per unit of work performed (Powers &

Howley, 2007). Type IIa fast twitch muscle fibers show fast shortening speed and a moderately well-developed capacity to transfer energy from two sources, aerobic and anaerobic. Fast twitch muscle fibers are mostly utilized in anaerobic-type sprint activities and other forceful muscle actions. In other words, any actions that rely almost completely on anaerobic energy metabolism require a greater amount of fast twitch fibers (McArdle, Katch, F., & Katch, V., 2010).

Muscular Strength

Muscular strength is an essential component when assessing an individual's athletic performance. In order to develop strength, exercises of high-resistance, low-repetition using larger muscle masses to increase the maximal force production by a muscle or muscle group are necessary (Judge & Burke, 2010). An individual's ability to adapt to increasing training loads requires adequate knowledge and understanding of the volume and intensity of the different exercises. Also, another important aspect to consider is the appropriate amount of recovery time between training periods. If there is an inappropriate amount of recovery time, the postexercise fatigue could have an impact on maximal force production by a muscle. Therefore, postexercise fatigue could limit the effectiveness of the resistance training program (Judge & Burke, 2010).

When comparing absolute muscular strength in untrained men and women, it can be found that men are generally stronger. The greatest strength difference is most often found in the upper body, where men are nearly 50% stronger than women. In the lower body, men are only 30% stronger than women (Morrow & Hosler, 1981). However, this apparent strength difference between men and women is disregarded when force production is compared on the basis of the cross-sectional area of the muscle. Human

muscle, regardless of whether male or female, can produce 3 to 4 kg of force per cm² of muscle cross-section (Ikai & Fukunaga, 1968).

Muscular Endurance

Muscular endurance is the body's way of efficiently postponing the onset of muscular fatigue. Endurance depends greatly on the nutritional support for the muscle. The main source of energy depends on the amount of glycogen that has been stored within the muscles before the start of any period of exercise (Guyton & Hall, 2006). Despite an abundant supply of oxygen to the muscles and an almost indefinite supply of energy to the muscles through the body's stored fat, fatigue occurs when the exercise continues past the limits of the liver and muscle glycogen stores (McArdle, Katch, F., & Katch, V., 2010). In order to comprehend muscular endurance, three metabolic systems are especially important in understanding the limits of physical activity. These three metabolic systems are (1) the phosphocreatine-creatine system, (2) the glycogen-lactic acid system, and (3) the aerobic system. However, it is imperative that it is understood that in order to make a contraction, the initial source of energy that is needed comes from adenosine triphosphate (ATP). For all training levels of individuals, the amount of ATP that is present in the muscles can only sustain about 3 seconds at maximal power output (McArdle, Katch, F., & Katch, V., 2010). The phosphocreatine-creatine system provides maximal power output for 8 to 10 seconds and is mainly used for maximal short bursts on muscular power. The glycogen-lactic acid system is also said to be anaerobic metabolism. The initial stage of this system is called glycolysis, which occurs without the use of oxygen. During glycolysis, glucose molecules are split into two pyruvic acid molecules. The energy that is released from the glucose molecules being split then forms ATP

molecules. In the second stage of the glycogen-lactic acid system, there is not a sufficient supply of oxygen for glucose metabolism to occur. Therefore, most of the pyruvic acid is converted into lactic acid. Because of this conversion to lactic acid, great amounts of ATP are formed completely without the utilization of oxygen. The glycogen-lactic acid system can provide 1.3 to 1.6 minutes of maximal muscle activity. The aerobic system is the oxidation of glucose, fatty acids, and amino acids from the foodstuffs in the mitochondria. The foodstuffs combine with oxygen to release great amounts of energy. This energy is used to convert AMP and ADP into ATP. The aerobic system provides unlimited amounts of muscular activity, as long as the nutrients stored in the body last (McArdle, Katch, F., & Katch, V., 2010).

GAKIC

Researchers, Stevens et al. (2000), created a new supplement combining glycine and L-arginine monohydrochloride salt of alphaketoisocaproic acid calcium. This ketoacid/amino acid combination metabolic treatment was hypothesized to improve dynamic performance of skeletal muscle during acute, anaerobic, exhaustive dynamic exercise in healthy adult males compared to the control isocaloric sucrose treatment. In their study, the effects of the treatments were assessed using the researchers' Fatigue Resistance Index (FRI). This supplement became known as GAKIC (Stevens, Godfrey, Kaminski, & Braith, 2000). The results showed that compared with isocaloric carbohydrate, the oral GAKIC treatment increased muscle torque and work continued during intense acute anaerobic dynamic exercise. Also, GAKIC increased overall muscular performance because it delayed the muscle fatigue during the early stages of anaerobic dynamic exercise (Stevens, Godfrey, Kaminski, & Braith, 2000).

In order to maintain muscular power during short-term and high-intensity exercise, dietary supplements have been used. Some such supplements include the following: creatine monohydrate and beta-hydroxy-beta-methylbutyrate (HMB). These supplements have been shown to provide the muscles with an ergogenic effect during high-intensity exercise (Buford & Koch, 2004). Researchers, Buford and Koch (2004), conducted a study using the supplement glycine-arginine-alpha-ketoisocaproic acid (GAKIC). The purpose of the study was to determine the different effects that GAKIC supplementation could have on an individual's repeated bouts of anaerobic cycling performance. When observing a significant treatment to time interaction, the study resulted in a change in mean power output over the repeated sprints between the GAKIC and placebo treatments (Buford & Koch, 2004). The researchers concluded that the data did support an ergogenic effect of GAKIC for reducing the decline in mean power during repeated bouts of supramaximal exercise (Buford & Koch, 2004). Further, speculation about the ergogenic effects of GAKIC are as follows: 1) increases the ability to sustain athletic muscle force during intense anaerobic muscle exercise; 2) increases the ability to sustain athletic muscle work during intense anaerobic muscle exercise; and 3) increases the overall muscle performance by decreasing muscle absolute fatigue while retarding the rate of muscle fatigue (Stevens, Godfrey, Kaminski, & Braith, 1998).

Researchers, Beis, Mohammad, Easton, and Pitsiladis (2011) also conducted a study using GAKIC supplementation. The purpose of their study was to investigate the effects of GAKIC on fatigue during a series of 10 sprints in trained cyclists. The researchers used an experimental design that was almost identical to the design used in Buford and Koch's study. It was hypothesized that the GAKIC supplementation would

enhance peak power output and reduce the decline in power output during repeated sprints in trained cyclists. When reviewing the two conditions, the study resulted in no significant difference between the GAKIC and PL conditions for any of the measured performance variables. The researchers concluded that the data from their study did not support the findings of previous studies that GAKIC will enhance high-intensity exercise performance. Further, with this study using well-trained participants, there appears to be very little evidence to support that GAKIC supplementation has any ergogenic effects during high-intensity exercise performance (Beis, Mohammad, Easton, & Pitsiladis, 2011).

CHAPTER III

METHODOLOGY

Participants

Seven apparently healthy, resistance trained adult males and nine healthy, resistance trained females ranging in age from 18 to 36 years were recruited for this investigation. To qualify, the participants were required to have a minimum of six months of continuous recreational resistance exercise training. Participants were free of any medical condition which might hinder their ability to perform the exercise protocol. Prior to acceptance into the investigation, participants were required to complete a medical history form (see Appendix A), to be screened for qualifying schedule conflicts, and to sign a statement of informed consent (see Appendix B). Participants were free of any anabolic steroid use for one year prior to participation in the investigation. The procedures and forms in this investigation were reviewed and approved by the Institutional Review Board at Mississippi State University (Appendix C).

Experimental Design

Treatment order (GAKIC or PL) was randomly assigned to participants. A double-blind crossover design was used, as neither the primary investigators nor the participants were aware of the treatment order. Sessions were separated by a minimum of 7 days but no longer than 14 days to minimize participant fatigue in each experimental protocol. Despite common misconceptions, this design was appropriate for females, as oral contraceptives (Nichols, Hetzler, Villanueva, Stickley, & Kimura, 2008) and

menstrual cycle conditions (Miskec, Potteiger, Nau, & Zebas, 1997) do not physiologically affect anaerobic performance.

Pretest Evaluation (Session 1)

During the initial session participants were given a thorough explanation of the informed consent and research protocol. The informed consent was signed prior to any evaluations of the participants. On this day all anthropometric measurements were determined. Participants' standing height, body mass, and percent body fat were initially assessed. Standing height and body mass were assessed using a height/weight 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 on the highest point. Participants were weighed in shorts without shirt or shoes. Body mass was measured in kilograms. Percent body fat was measured using the BOD POD (BOD POD, Life Measurement, Inc., Concord, CA). Participants were asked to wear compression shorts and compression shirts for females in order to minimize air resistance within the BOD POD. The participants' percent body fat was then analyzed by the BOD POD.

Experimental Protocol 1 (Session 2 & 3)

Each male participant performed the sessions a minimum of one week apart, in which the experimental treatments, GAKIC or a PL, were randomly assigned and administered in a double blind fashion. Upon arriving at the Sanderson Center weight room the participants were taken to a designated area for 5 minutes of quiet rest. Next, the participant's heart rate (HR) and resting blood lactate levels (BLa) were measured. HR was assessed using an automated machine (SunTech Medical Cycle). BLa was

assessed by taking a drop of blood (5 microL or approximately 2mm diameter drops) from the finger tip of the participant and immediately analyzed using the Lactate Pro Analyzer (LT 1710). Results were yielded in 60 seconds or less. Immediately following BLa assessment the participant was given GAKIC or PL, which was followed by a 40 minute rest period. Following this period, participants warmed up on a commercial upright stationary bike for 5 minutes. Participants then completed a warm-up set on the standard leg press. Participants used a weight of 200 pounds for 8-12 repetitions depending on strength level. Two minutes of rest was allotted between warm-up sets. One repetition maximum (1RM) was determined by increasing weight in 20 to 40 pound increments depending on the participant's strength level. One repetition maximum was obtained in 3-6 sets. One repetition maximum represents the maximum weight lifted once with proper form. Immediately following 1RM determination, the amount of 75% of 1RM was placed on the leg press, while participants rested for 3 minutes. Immediately following the recovery period, the participant completed 5 sets doing as many repetitions as possible until failure occurred. Failure was defined as the inability to complete a full repetition without assistance. Participants rested 3 minutes between each set. This procedure was repeated for a total of 5 sets. HR and BLa were assessed within 5 seconds of the final repetition during set 5. Total load volume (TLV) was calculated by multiplying the 75% of 1RM mass lifted by the sum of repetitions to failure. Each session was estimated to last approximately one hour and 30 minutes. Seven days later, the participants returned to the Sanderson Center weight room and completed the identical protocol using the other treatment.

Experimental Protocol 2 (Session 2 & 3)

Each female participant performed the sessions a minimum of one week apart, in which the experimental treatments, GAKIC or a PL, were randomly assigned and administered in a double blind fashion. Upon arriving at the Exercise Physiology Laboratory, the participants were taken to a designated area for 5 minutes of quiet rest. Next, the participant's heart rate (HR) and resting blood lactate levels (BLa) were measured. HR was assessed using an automated machine (SunTech Medical Cycle). BLa was assessed by taking a drop of blood (5 microL or approximately 2mm diameter drops) from the finger tip of the participant and immediately analyzed using the Lactate Pro Analyzer (LT 1710). Results were yielded in 60 seconds or less. Immediately following BLa assessment the participant was given GAKIC or PL, which was followed by a 40 minute rest period. Next, participants warmed up on an upright stationary bike for five minutes. Following this general warm-up, participants performed fifteen repetitions of two sets on the leg extensions with a mass of 11 kg. Then, in order to determine each participant's 1RM, a trained technician determined a beginning resistance for each participant to perform their session. The 1RM was then determined by increasing mass in 4.5 to 9 kg increments relative to the participant's ability to lift the first mass. The 1RM was obtained in three to six sets. Importantly, the accepted 1RM was defined as the ability of the participant to complete a full repetition without assistance. Following a five minute rest period, 50% of 1RM was placed on the leg extension machine and each participant completed as many repetitions as possible until failure occurred. Failure was defined as the inability to complete a full repetition without assistance. This was followed by a three minute rest, and then participants performed another set on the leg extension machine by using the same mass. This procedure was repeated for a total of six sets.

Total load volume was calculated by multiplying the 50% of 1RM mass lifted by the sum of repetitions to failure. Seven days later, the participants returned to the Exercise Physiology Laboratory and completed the identical protocol using the other treatment.

Dietary and Supplement Intake

Participants completed a 24 hour diet and exercise recall during the 40 minute inactive period at each experimental session. They were also restricted from taking any medication or supplementation during the 48 hours prior to each session. Participants were also asked to abstain from intense exercise in the 48 hours preceding each session. Participants who failed to meet this standard, which was determined approximately 8 to 10 hours prior to reporting for their session (questioned during the courtesy call the night before), were rescheduled. Rescheduling was dependent on washout period and testing schedule. No dietary analysis was performed.

Treatment Ingestion

Over-the-counter GAKIC (manufactured by Muscle Technology; see Appendix D) or a cellulose based PL (Apotheca Company Inc., Woodbine, IA) were provided to participants 40 minutes pre-exercise. GAKIC ingestion was 10.2 grams taken in pill form. Prior studies have used a similar dose in powder form (Stevens, Godfrey, Kaminski, & Braith, 2000). A minimum of seven days later, participants ingested the other treatment, and repeated the identical exercise protocol. There have been no reported side effects of GAKIC when taken in amounts recommended by the manufacturer or cited in prior literature.

Statistical Analysis

All statistical analyses were performed by using the GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Data for total load volume between GAKIC and PL were analyzed using repeated measures t-test. Data for blood lactic acid, blood glucose, and heart rate were analyzed by using a 2 (condition; GAKIC or PL) x 2 (time; pre or post) repeated measures analysis of variance (ANOVA). When the main effects of the 2 x 2 ANOVA were found to be significant ($p < 0.05$), a repeated measures t-test was performed to identify statistical differences between groups. Significance was established at $p < 0.05$. Data are reported as mean ± standard deviation.

CHAPTER IV

RESULTS

Experimental Protocol 1

Participants

This study consisted of seven apparently healthy, resistance trained male participants. A sample size analysis was performed and showed that participants were required to achieve a power of 0.80. The intra-class correlations were $\geq$ 0.80.

Table 1 Male Participant Characteristics (mean $\pm$ SD)

Age (y)	Height (m)	Body mass (kg)	Body fat (%)
20.7 ± 1.3	1.78 ± 0.07	89.8 ± 12.5	19.7 ± 7.9

Total Load Volume

Total load volume was calculated by multiplying the 75% of the 1RM mass by the total number (i.e. sum of five sets) of repetitions to failure. Our data showed a significant ($p < 0.05$) increase in TLV after GAKIC supplementation. Specifically, GAKIC's TLV was 31564 ± 9132 kg, whereas the PL TLV was 25763 ± 6595 kg. **(Figure 1)**

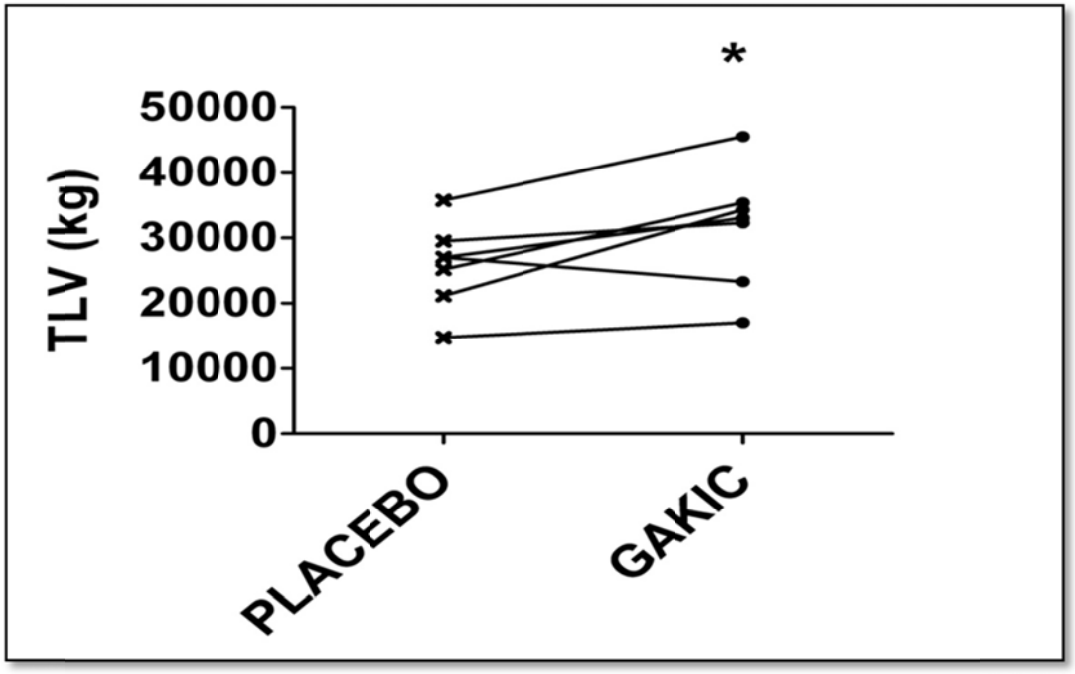

Figure 1 Total Load Volume (1)

Lower body muscular endurance was determined by calculating the total load volume (TLV = 75% of one repetition maximum mass lifted X the sum of repetitions to failure). The data points for each participant are shown in the graphs. * indicates $p < 0.05$ between GAKIC and PL.

Heart Rate

Heart rate was measured as an indicator of exercise intensity and to document that participants in both groups exerted similar effort following GAKIC and PL supplementation. At rest (pre), there were no differences for heart rate between GAKIC (64.6 ± 7.6 beats per minute (bpm)) and PL (63.2 ± 6.6 bpm) ($p > 0.05$). Following the exercise protocol, heart rate increased significantly in both groups (GAKIC = 142.4 ± 15.5 bpm; PL = 140.7 ± 9.5 bpm; $p < 0.05$ compared to pre) but it was not different ($p > 0.05$) between groups. **(Figure 2)**

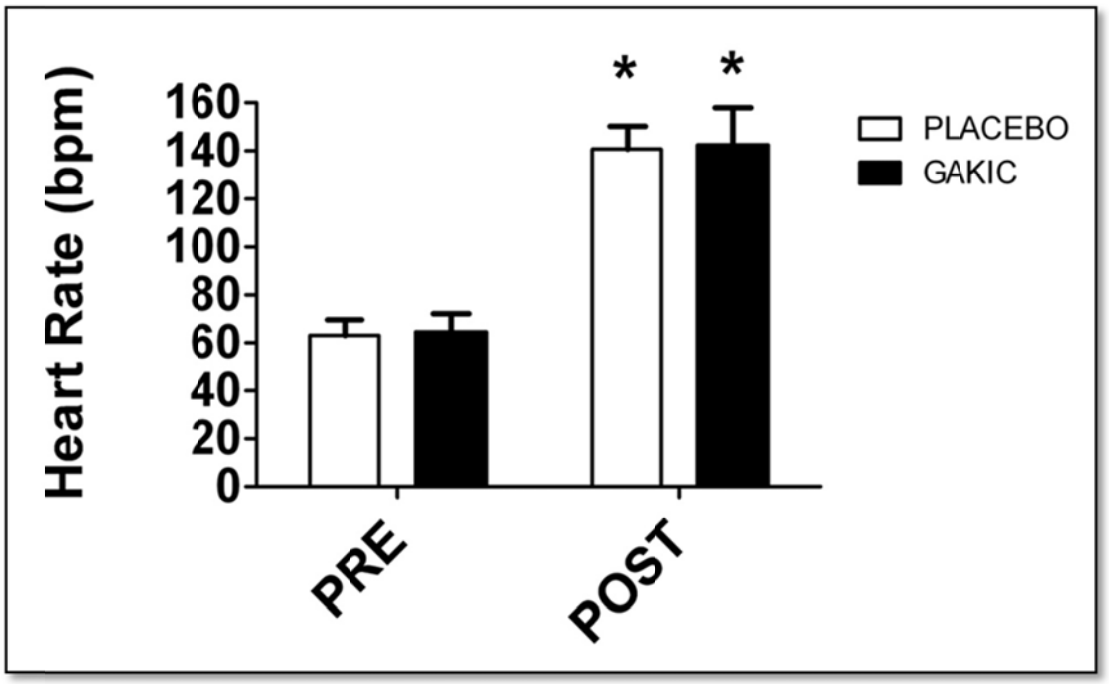

Figure 2 Heart Rate (1)

Heart rate (beats per minute; bpm) at PRE (i.e. rest) and POST (i.e. following the exercise protocol). * indicates $p < 0.05$ between PRE and POST exercise.

Blood Lactic Acid

Blood lactic acid was also measured as an indicator of exercise intensity. At rest (pre), there were no differences ($p > 0.05$) between GAKIC (1.5 ± 0.7 mmol/l) and PL (1.6 ± 0.7 mmol/l). Following the exercise protocol, BLa increased significantly in both groups (GAKIC = 12.8 ± 3.0 mmol/l; PL = 10.7 ± 2.2 mmol/l; $p < 0.05$ compared to pre) but it was not different ($p > 0.05$) between groups. **(Figure 3)**

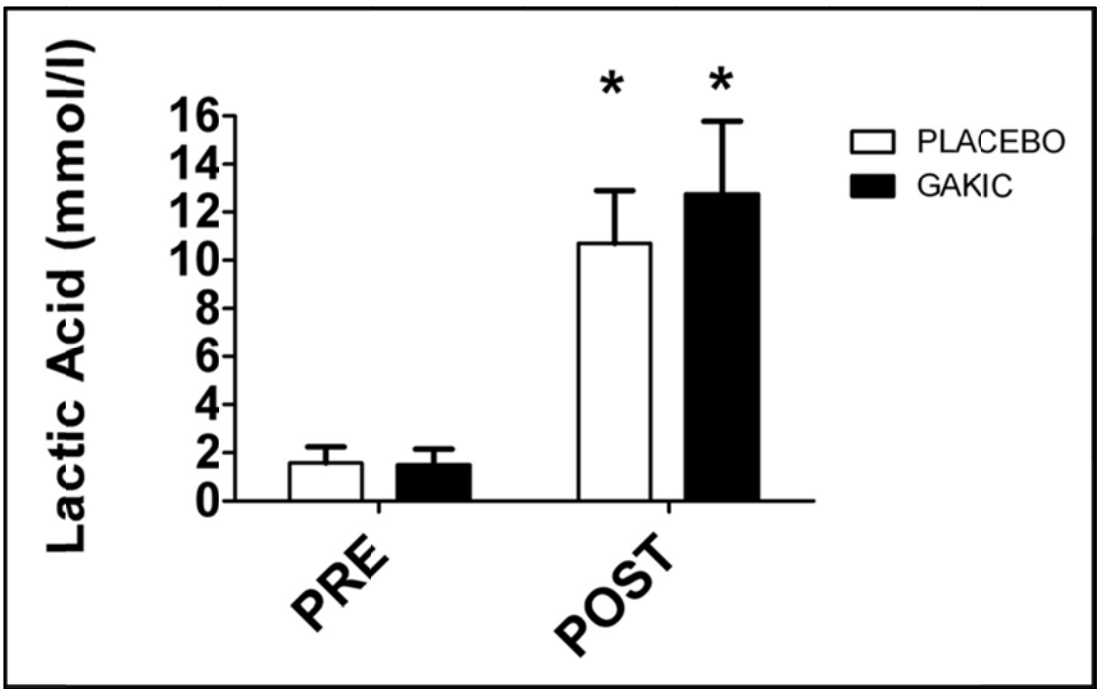

Figure 3 Blood Lactic Acid (1)

Blood lactic acid (mmol/l) at PRE (i.e. rest) and POST (i.e. following the exercise protocol). * indicates $p < 0.05$ between PRE and POST exercise.

GAKIC vs. Placebo

There were no differences between GAKIC and PL in pre-exercise blood glucose concentration (GAKIC = 99.4 ± 16.4 mg/dl; PL = 90.0 ± 7.8 mg/dl; $p > 0.05$). Unlike BLa concentration, blood glucose levels were not significantly different ($p > 0.05$) between pre and post-exercise. Also, there were no significant differences in blood glucose levels between GAKIC and PL following the completion of the exercise protocol (GAKIC= 87.6 ± 7.3 mg/dl; PL = 82.6 ± 8.7 mg/dl; $p > 0.05$). **(Figure 4)**

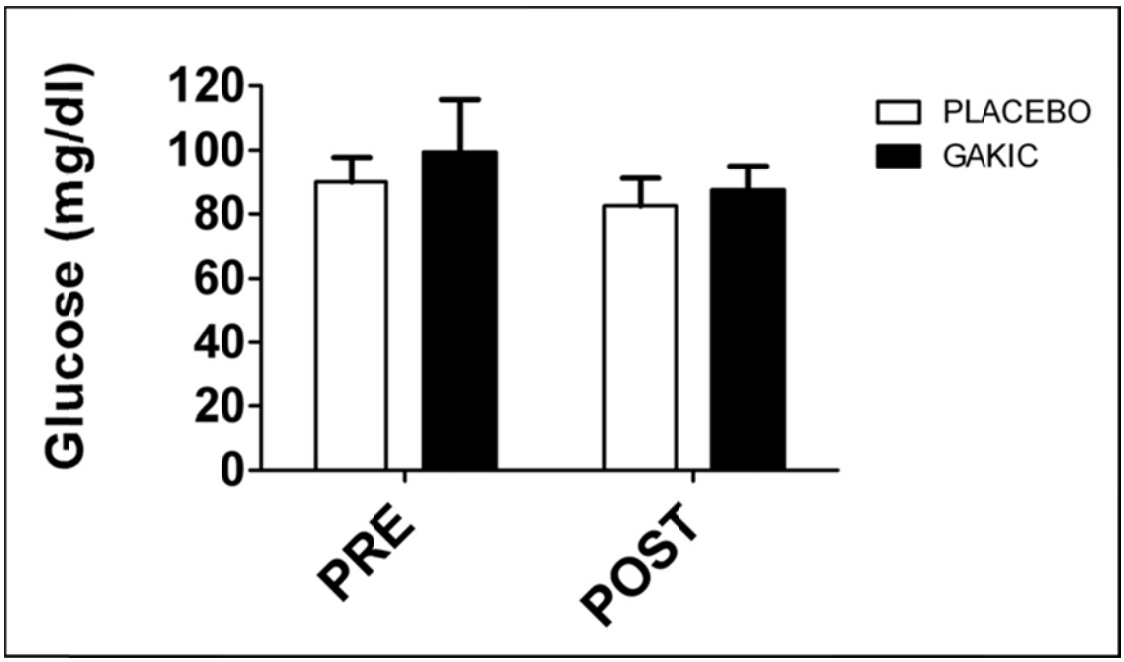

Figure 4 Blood Glucose (1)

Blood glucose (mg/dl) at PRE (i.e. rest) and POST (i.e. following the exercise protocol). No significant differences were detected ($p > 0.05$) between groups.

Experimental Protocol 2

Participants

This study consisted of nine healthy, resistance trained female participants. A sample size analysis was performed and showed that participants were required to achieve a power of 0.80. The intra-class correlations were ≥ 0.80.

Table 2 Female Participants Characteristics (mean ± SD)

Age (y)	Height (m)	Body mass (kg)	Body fat (%)
21.8 ± 1.0	1.62 ± 0.07	64.1 ± 14.4	22.4 ± 9.9

1 RM and Total Load Volume

Data indicate that the 1RM was not significantly different ($p > 0.05$) between GAKIC and PL. Specifically, 1RM of GAKIC was 34.1 ± 9.1 kg and 1RM of PL was 32.3 ± 8.5 kg ($p < 0.05$) (**Figure 5A**). Total load volume was calculated by multiplying the 50% of one repetition maximum mass lifted by the sum of repetitions (i.e. six sets) to failure. Our data showed a significant ($p < 0.05$) increase in TLV after GAKIC supplementation. Specifically, GAKIC's TLV was 1721.7 ± 479.9 kg, whereas the PL TLV was 1479.1 ± 396.8 kg. **Figure 5B** illustrates the individual response of each participant after GAKIC and PL supplementation. Importantly the TLV increased in 8 of the 9 participants following GAKIC ingestion.

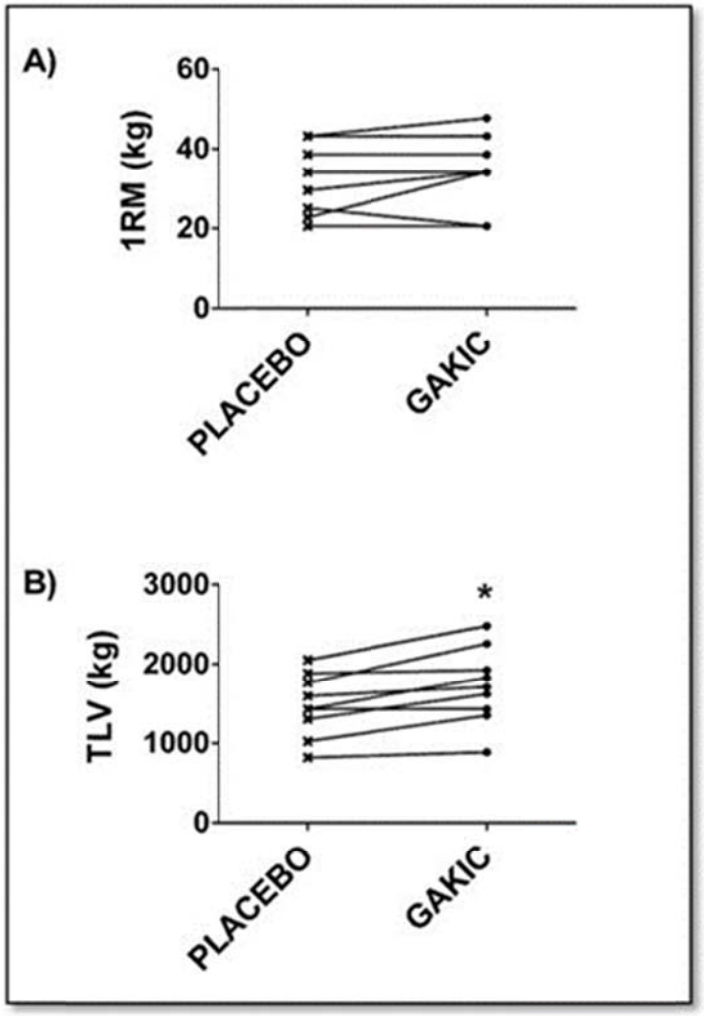

Figure 5 1RM and Total Load Volume (2)

A) One repetition maximum (1RM) following either glycine-arginine-α-ketoisocaproic acid (GAKIC) or PL ingestion. No significant difference ($p > 0.05$) was observed between GAKIC and PL. **B)** Total load volume (TLV) following either GAKIC or PL ingestion. The TLV was calculated by multiplying the 50% of 1RM mass by the sum of repetitions performed to failure in six sets. * indicates $p < 0.05$ between GAKIC and PL. The data points for each participant are shown in both graphs.

Heart Rate

Heart rate was measured as an indicator of exercise intensity and to document that participants in both groups exerted similar effort following GAKIC and PL supplementation. At rest (pre), there were no differences for heart rate between GAKIC

and PL ($p > 0.05$). Following the exercise protocol, heart rate increased significantly in both groups ($p < 0.05$) but it was not different ($p > 0.05$) between groups (**Figure 6**).

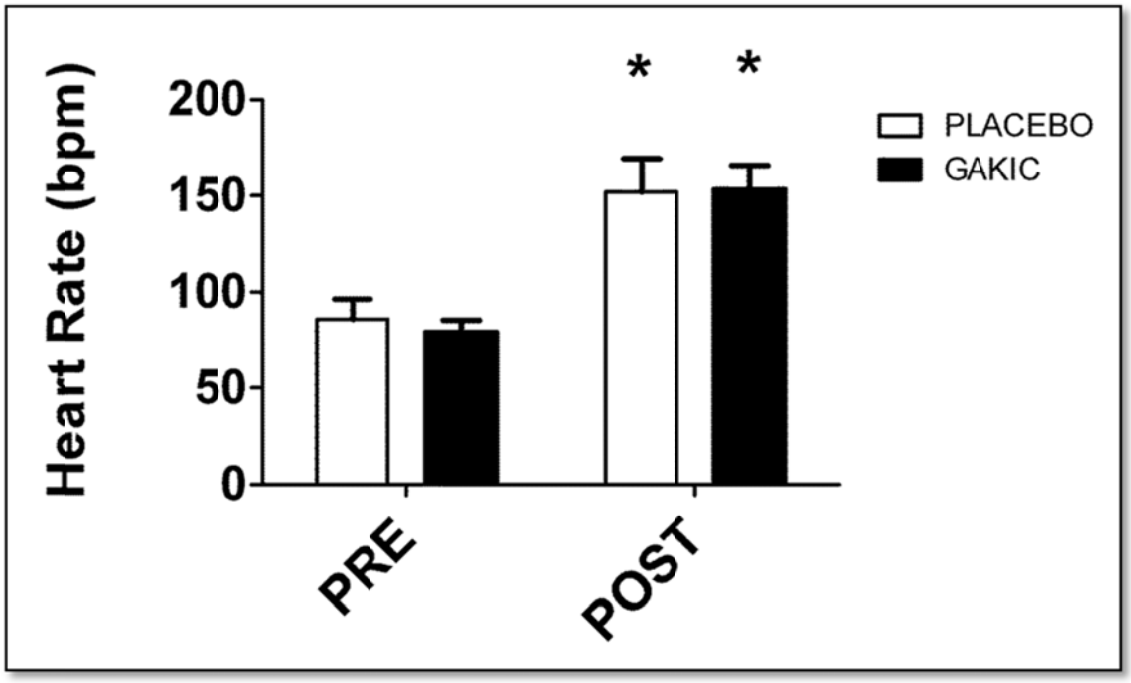

Figure 6 Heart Rate (2)

Heart rate (beats per minute; bpm) at PRE (i.e. rest) and POST (i.e. following the exercise protocol). * indicates $p < 0.05$ between PRE and POST.

Blood Lactic Acid

Blood lactic acid was also measured as an indicator of exercise intensity. At rest (pre), there were no differences ($p > 0.05$) between GAKIC and PL. Following the exercise protocol, blood lactic acid increased significantly in both groups ($p < 0.05$) but it was not different ($p > 0.05$) between groups (**Figure 7**).

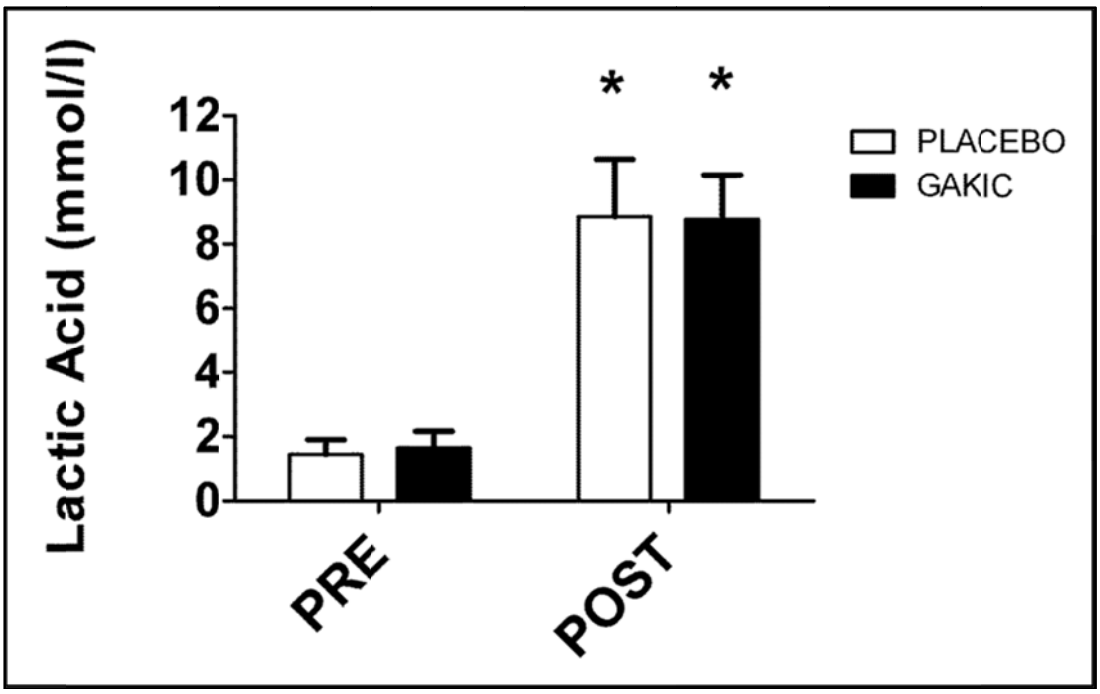

Figure 7 Blood Lactic Acid (2)

Blood lactic acid (mmol/l) at PRE (i.e. rest) and POST (i.e. following the exercise protocol). * indicates $p < 0.05$ between PRE and POST.

CHAPTER V

DISCUSSION

The purpose of this study was to investigate the effects of GAKIC on TLV, BLa, and heart rate during repeated bouts through a lower body resistance exercise protocol. It was hypothesized that GAKIC supplementation would increase the total mass lifted during repeated bouts of submaximal leg exercises in male and female participants. The major finding of this study is that GAKIC supplementation increases lower body TLV in both male and female groups. Following are the variables and their data points resulting from experimental protocol 1 and experimental protocol 2.

Experimental Protocol 1

Total Load Volume

Muscular strength and endurance were measured on the lower body using the leg press. Muscular strength was measured by using a 1RM on the leg press. Muscular endurance was measured by using TLV, which was calculated by multiplying the 75% of the 1RM mass by the total number of repetitions to failure. The total number of repetitions to failure was the sum of five sets. In order to make an initial muscular contraction, the energy source needed is adenosine triphosphate (ATP). However, ATP only sustains about 3 seconds of maximal muscle power. For a single, maximum short bout of muscle power, ATP is combined with cell phosphocreatine to form the phosphagen energy system (ATP-PCr) (Guyton & Hall, 2006). In order to maintain

maximum strength and to maintain endurance, the phosphagen energy system must recover and resynthesize ATP quickly and efficiently. After prolonged duration of a workout, the muscles' phosphocreatine stores are depleted. This depletion causes the body to switch to energy systems involving aerobic pathways (McArdle, Katch, F., & Katch, V., 2010).

With experimental protocol 1, the major finding during our leg press exercise protocol was that with GAKIC supplementation an increase in TLV for the lower body was revealed. This result could be due a decrease of ammonia and the system utilized. GAKIC supplementation may show improvements of the anaerobic system, which was the primary energy system used for this exercise protocol.

Heart Rate

Heart rate increases linearly in direct relation to exercise intensity (Maciel, Gallo, Marin Neto, Lima Filho, & Martins, 1986; Myers & Gullestad, 1998; Rotto & Kaufman, 1988). The heart rate measurements were used as a guide for exercise intensity for participants in both conditions. This allowed for observing similarities in effort following both of the treatment conditions (GAKIC and PL).

During exercise, work output, oxygen consumption, and cardiac output are directly correlated to exercise intensity. To illustrate, muscular work output increases oxygen consumption. The increased oxygen consumption dilates the muscle blood vessels, which therefore increase venous return and cardiac output (Guyton & Hall, 2006). Exercise intensity is calculated by a percentage of the participant's achieved heart rate maximum (Lamberts & Lambert, 2009; McArdle, Katch, F., & Katch, V., 2007; Rowell, 1988). With the participants' mean age being 20 years of age, the calculated

average maximum heart rate was 200 bpm. Therefore, the intensity of the TLV of the lower body exercise protocol was moderate (71% for the GAKIC and 70% for the PL) for both groups.

Blood Lactic Acid

Blood lactic acid was also used to indicate exercise intensity for both conditions. Lactate continually forms during rest and moderate exercise in all individuals (Brooks, 2009; Donovan & Brooks, 1983; Hashimoto & Brooks, 2008). Lactate formation is catalyzed by lactate dehydrogenase in a reversible reaction from pyruvate. Great accumulations of blood lactate occur rapidly during maximal exercises lasting between 60 and 180 seconds. However, BLa accumulations do not occur at all levels of exercise. Blood lactate production equals lactate removal and oxygen consuming reactions to sufficiently meet the energy demands of the exercise during light to moderate exercise (<50% of aerobic capacity). As exercise intensity increases, lactate production and lactate accumulation increases. For aerobic metabolism, blood lactate begins to store and rise at about 50 to 55% of the maximal capacity for healthy and untrained individuals (McArdle, Katch, F., & Katch, V., 2010). Therefore, it is expected that as exercise intensity and duration increases, the BLa levels should also be increased. Because there were no significant differences in BLa levels post exercise between the GAKIC supplementation and the PL, it can be hypothesized that GAKIC was not a lactic acid buffer.

Blood Glucose

Glucose is often referred to as "blood sugar." During exercise, muscle cells break down glycogen into glucose in a process called glycogenolysis. The glucose is then used as an energy source for muscular contraction (Powers & Howley, 2007). There were no

significant differences in blood glucose levels between pre and post-exercise, which could be due to the large quantities of glucose that are accumulated in the glycogen-storing cells, liver and muscle cells. The exercise protocol chosen for this study made it unlikely that there would be any significant decreases in glucose due to the duration of the exercise bouts. Therefore, the results suggest that the GAKIC supplementation is not a precursor for simple sugar or glucose.

Experimental Protocol 2

Total Load Volume

Muscular strength and endurance were measured on the lower body using the leg extension. Muscular strength was measured by using a 1RM on the leg press. Muscular endurance was measured by using TLV, which was calculated by multiplying the 50% of the 1RM mass by the total number of repetitions to failure. The total number of repetitions to failure was the sum of six sets.

Experimental protocol 2 major finding is that supplemental GAKIC ingestion increases TLV during leg extension exercise. This result could be due to the increase in phosphocreatine utilization, resulting from ammonia and clearance of substances that decrease muscle pH levels.

Heart Rate

Heart rate increases linearly in direct relation to exercise intensity (Maciel, Gallo, Marin Neto, Lima Filho, & Martins, 1986; Myers & Gullestad, 1998; Rotto & Kaufman, 1988). The heart rate measurements were used as a guide for exercise intensity for participants in both conditions. This allowed for observing similarities in effort following both conditions (GAKIC and PL).

Exercise intensity is calculated by a percentage of the participant's achieved heart rate maximum (Lamberts & Lambert, 2009; McArdle, Katch, F., & Katch, V., 2007; Rowell, 1988). With the participants' mean age being 21 years of age, the calculated average maximum heart rate was 199 bpm. Therefore, the intensity of the TLV of the lower body exercise protocol was moderate (≈75% for both the GAKIC and the placebo) for both groups.

Blood Lactic Acid

Blood lactic acid was also used to indicate exercise intensity for both conditions. Lactate continually forms during rest and moderate exercise in all individuals (Brooks, 2009; Donovan & Brooks, 1983; Hashimoto & Brooks, 2008). Great accumulations of BLa occur rapidly during maximal exercises lasting between 60 and 180 seconds. However, BLa accumulations do not occur at all levels of exercise. BLa production equals lactate removal and oxygen consuming reactions to sufficiently meet the energy demands of the exercise during light to moderate exercise (<50% of aerobic capacity). Therefore, it is expected that as exercise intensity and duration increases, the blood lactate levels should also be increased. Because there were no significant differences in BLa levels post exercise between the GAKIC supplementation and the PL, it can be hypothesized that GAKIC was not a lactic acid buffer.

Conclusion

In conclusion, with the specific exercise protocol that was implemented in order to demonstrate the effects of GAKIC treatment as compared to a PL treatment, it was revealed an improvement in maximum strength in the leg press exercise and the leg extension exercise with lower body TLV. When considering body mass and dosage of

supplement, it was concluded in previous studies that GAKIC improvements were closely related. With both protocols only having two testing sessions per participant separated by one week, it gave limited observation and data collection. Further research on GAKIC's effects could be beneficial when conducting research over a longer period of time allowing GAKIC to remain in the body.

Summary of Results (Experimental Protocol 1 & 2)

1) No significant difference was observed in blood lactate between GAKIC and placebo treatments in male participants.
2) No significant difference was observed in heart rate between GAKIC and placebo treatments in male participants.
3) A significant difference was observed in total load at 75% of IRM for five sets between GAKIC and placebo treatments in male participants.
4) No significant difference was observed in blood glucose between GAKIC and placebo treatments in male participants.
5) No significant difference was observed in blood lactate between GAKIC and placebo treatments in female participants.
6) No significant difference was observed in heart rate between GAKIC and placebo treatments in female participants.
7) No significant difference was observed in 1RM between GAKIC and placebo treatments in female participants.
8) A significant difference was observed in total load at 50% of 1RM for six sets between GAKIC and placebo treatments in female participants.

Recommendations for Further Research

1) Studies should compare how supplemental GAKIC ingestion effects total load volume during multiple exercises.
2) Studies should compare the effects of GAKIC supplementation over an 8-12 week period.
3) A similar study should be performed comparing supplemental GAKIC ingestion effects on trained vs. untrained participants.
4) A similar study should be performed comparing supplemental GAKIC ingestion effects on trained participants over a longer duration of supplementation.

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APPENDIX A

INFORMED CONSENT

Mississippi State University

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

Consent to Participate in an Experimental Study
Effects of GAKIC on Multiple-bout Resistance Exercise

Investigators:

Ben Wax, Ph.D. and Heather Webb, Ph.D., ATC, LAT
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 amino acid, Glycine-Arginine-Alpha-Ketoisocaproic Acid (GAKIC) on repeated bouts of muscular endurance (75% of 1-RM) resistance exercise. GAKIC 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 two testing sessions. Below is an explanation of what you will be asked to do during those three 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 and height. 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), blood pressure (BP), and blood lactic acid level will be measured. A small sample of blood will be taken from the tip of your finger (fingerstick method) to measure your blood lactate level. Immediately following, you will be given 8 caplets (10.2 grams) of GAKIC or placebo 40 min before resistance exercise. Following 30 minutes of rest, another measurement of HR and BP will be taken. Immediately following you will warm up on a commercial upright stationary bike for 5 minutes. A third measurement of HR and BP will be recorded 4 minutes into the warm-up. You will then complete a warm-up set on the standard barbell bench press at a load of 135 pounds for males or 45 pounds for females. Two minutes of rest will be allotted between sets, and 1-repetition maximum (1-RM) will be determined in 3-6 sets. 1-RM represents the maximum weight lifted once (maximum effort) with proper form "as explained and determined by the investigator". Immediately following 1-RM determination, 75% of 1-RM will be placed on the bar, and you will complete as many repetitions as possible until failure occurs. You will repeat this process for 4 additional sets. This will be used as a measure of muscular endurance during repeated bouts of upper body resistance exercise. HR, BP, and rating of perceived exertion (RPE) will be recorded within 5

seconds of the final repetition on set five. RPE will be measured by verbally asking you to rate your perceived effort of the exercise bout on a scale of 0-10 (0 - nothing at all to 10 – very very hard). Also, a second measure of blood lactic acid level will be taken. Approximately 5 minutes later, you will warm-up on the leg press exercise at a load of 200 pounds for males or 90 pounds for females. Two minutes of rest will be allotted between sets, and 1-RM will be determined in 3-6 sets. Immediately following 1-RM determination, 75% of 1-RM will be placed on the leg press, and you will complete as many repetitions as possible until failure occurs. You will repeat this process for 4 additional sets. This will be used as a measure of muscular endurance during repeated bouts of lower body resistance exercise. HR, BP, and rating of perceived exertion (RPE) will be recorded within 5 seconds of the final repetition on set five. RPE, again will be measured by verbally asking you to rate your perceived effort of the exercise bout on a scale of 0-10 (0 - nothing at all to 10 – very very hard). Also, a third measure of blood lactic acid level will be taken. There will be a total of three finger sticks for session two. You will repeat the identical protocol one week later after ingestion of the other treatment. After session three you will be asked whether or not you could identify the GAKIC trial. Each session is estimated to last approximately one hour and 45 minutes. There will be a total of six finger sticks to measure the blood lactic acid levels for the entire research project.

You should know that we are looking for specific characteristics in our subjects. These include; a) having no history of chronic illness, b) musculoskeletal problems in the previous 6 months, c) not taking any prescribed medications (excluding contraceptive medication), supplements (vitamins and minerals permitted), or tobacco products, and d) consuming an average of less than 10 alcoholic beverages per week. 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. 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 GAKIC when taken in recommended amounts by the manufacturer or reported in prior literature; however, GAKIC may present a risk of allergic reaction. Individuals with a history of drug allergies or allergies to dyes should review the ingredients of GAKIC 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. GAKIC, the supplement being investigated in this research project, has not been evaluated by the Food and Drug Administration. GAKIC is not intended to diagnose, treat, cure or prevent any disease.

Benefits: The benefits of this study to the general population include gaining in knowledge pertaining to the effects of GAKIC 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, maximal muscular strength (1RM) 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 GAKIC on Maximal Strength and Muscular Endurance*",

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:** _____

MSU IRB
Approved: 2/22/11
Expires: / /

APPENDIX B

HEALTH HISTORY

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 _____ Work/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 GAKIC, prior to the introduction to this research project? _____ YES _____NO

If YES, have you ever taken the supplement GAKIC? _____ 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	___NOS drinks/pills
___Glycerol	___Caffeine	___Sodium Bicarbinate
___Ephedrine/Ephedra	___wt. loss products	___natural testosterone enhancers (pro-hormones)

Other (please list)_____

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

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

APPENDIX C

IRB APPROVAL

MISSISSIPPI STATE UNIVERSITY

Compliance Division
Administrative Offices
Animal Care and Use (IACUC)
Human Research Protection Program (IRB)
1207 Hwy 182 West, Suite C
Starkville, MS 39759
(662) 325-3496 - fax

Safety Division
Biosafety (IBC)
Radiation Safety
Hazardous Waste
Chemical & Lab Safety
Fire & Life Safety
70 Morgan Avenue
Mississippi State, MS 39762
(662) 325-8776 - fax

http://www.orc.msstate.edu
compliance@research.msstate.edu
(662) 325-3294

November 23, 2010

Ben Wax
Kinesiology
Mail Stop 9575

RE: IRB Study #10-294: Effect of GAKIC on Maximal Strength and Muscular Endurance

Dear Dr. Wax:

On 11/10/2010, the Mississippi State University Institutional Review Board for the Protection of Human Subjects in Research voted to approve your application titled "Effect of GAKIC on Maximal Strength and Muscular Endurance" with contingencies.

The contingencies for approval are as follows:

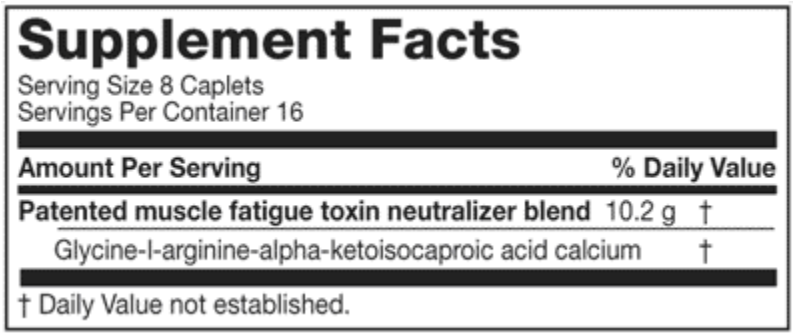
1. Revise the IRB application (and attachments if applicable) to indicate that individuals will not be asked to obtain a physician clearance to participate in the study, but will instead be excluded from participation if there are contraindications to study procedures as was discussed during the IRB meeting (item #6, page 9 of 13).
2. Please revise the personnel table on page 3 of 13 of the IRB application to remove Heather Webb as advisor and also not list Justin Knight and Heather Webb on the same line. A form for additional investigators is available at http://orc.msstate.edu/quicklinks/docs/addl_invs.doc if there are more investigators than will fit in the table provided on the form (a link to this form is also provided at the top of the personnel table within the application form itself).
3. In the first paragraph of the consent form, revise the statement "...an over-the-counter supplement designed to delay..." to read "...an over-the-counter supplement that may delay..."
4. Add a statement to the risks section of the consent form that GAKIC may present a risk of allergic reaction, and that individuals with a history of drug allergies or allergies to dyes should review the ingredients of GAKIC before participating in the study.
5. Add a statement such as the following to the risks section of the consent form: "GAKIC, the supplement being investigated in this research, has not been evaluated by the Food and Drug Administration. GAKIC is not intended to diagnose, treat, cure or prevent any disease."

These contingencies must be reviewed and approved by the IRB before you can begin your project.

needed to complete the project, you will need to su
ew Request form 30 days prior to the date of expirati
modification to the project must be reviewed and ap
mplementation. Any failure to adhere to the approve
ispension or termination of your project. Please note

Dwight Hare, Ph.D.
IRB Chair

Nutritional Information



Supplement Facts

Serving Size 8 Caplets
Servings Per Container 16

Amount Per Serving		% Daily Value
Patented muscle fatigue toxin neutralizer blend	10.2 g	†
Glycine-l-arginine-alpha-ketoisocaproic acid calcium		†

† Daily Value not established.

OTHER INGREDIENTS: MICROCRYSTALLINE CELLULOSE, HYDROXYPROPYL CELLULOSE, COATING (POLYVINYL ALCOHOL, POLYETHYLENE GLYCOL, HYDROXYPROPYL CELLULOSE, TITANIUM DIOXIDE, TALC, SOY LECITHIN, POLYSORBATE 80, FD&C RED NO. 40, FD&C YELLOW NO. 6), CROSCARMELLOSE SODIUM, STEARIC ACID, MAGNESIUM STEARATE, SILICA, ACESULFAME-POTASSIUM.