

α -GLYCEROPHOSPHORYLCHOLINE AND THE
EFFECTS ON ANAEROBIC INDICES

by

Alex J. Rickard

An Abstract

of a thesis submitted in partial fulfillment
of the requirements for the degree of Master of Science
in the Department of Nutrition and Kinesiology
University of Central Missouri

May, 2017

ABSTRACT

by

Alex J. Rickard

The purpose of the investigation was to examine the acute effects of α -glycerophosphorylcholine (α -GPC) ingestion on anaerobic performance. This study was double-blind, placebo controlled, crossover design, with a one-week wash-out period—two testing sessions. One hour prior to the testing, subjects ingested a solution—placebo or α -GPC. Subjects ($N=17$) completed three assessments of anaerobic performance: the counter movement jump (CMJ), 40-yd dash, and 30-second Wingate anaerobic test (WAnT). The best of three attempts of the CMJ and 40-yd were used in analysis. They completed only one trial on the 30-second WAnT. Results showed a significant difference between placebo (68.5 ± 11.5 cm) and α -GPC (69.8 ± 11.5 cm) for the CMJ performance. A trend towards significance ($p=.069$) was found for the 30-second WAnT minimum power under the α -GPC condition. No other differences were noted. Alpha-GPC may be an ergogenic aid; however, more research is needed to fully elucidate its effectiveness as an ergogenic aid.

α -GLYCEROPHOSPHORYLCHOLINE AND THE
EFFECTS ON ANAEROBIC INDICES

by

Alex J. Rickard

A Thesis

submitted in partial fulfillment
of the requirements for the degree of Master of Science
in the Department of Nutrition and Kinesiology
University of Central Missouri

May, 2017

© 2017

Alex J. Rickard

ALL RIGHTS RESERVED

α -GLYCEROPHOSPHORYLCHOLINE AND THE
EFFECTS ON ANAEROBIC INDICES

by

Alex J. Rickard

May, 2017

APPROVED:

Thesis Chair: Dr. Matthew J. Garver

Thesis Committee Member: Dr. Steve Burns

Thesis Committee Member: Dr. Dennis Docheff

ACCEPTED:

Chair, Department of Nutrition & Kinesiology: Dr. Dennis Docheff

UNIVERSITY OF CENTRAL MISSOURI
WARRENSBURG, MISSOURI

ACKNOWLEDGMENTS

I would like to thank Dr. Garver, Dr. Burns, and Dr. Docheff for guiding me throughout the process of completing this thesis. I would also like to thank them for the time they have set aside in each of their busy schedules to give me their input and suggestions to better this thesis. These three are great mentors and made the process of completing this thesis as smooth as possible. A special thank you goes out to all faculty members in the Department of Nutrition & Kinesiology at UCM. They helped me gather my subjects. Without their help, I would not have been able to complete this research.

TABLE OF CONTENTS

	Page
LIST OF TABLES	ix
CHAPTER ONE: NATURE AND SCOPE OF THE STUDY	1
Significance of Study	6
Purpose of Study.....	6
Hypotheses	6
Delimitations	7
Limitations	7
Assumptions	7
Definition of Terms	8
CHAPTER TWO: REVIEW OF LITERATURE	9
Introduction	9
Acetylcholine	10
Acetylcholinesterase and Metabolism of ACh	11
Muscle Activation and Muscular Fatigue	13
Alpha-glycerophosphorylcholine	15
Free Plasma Choline: α -GPC and CDP Supplementation	15
Clinical Considerations	16
Alpha-glycerophosphorylcholine and Exercise	19
Summary	21
CHAPTER THREE: METHODOLOGY	22
Overview	22

Subjects	22
Procedures and Instrumentation	23
Data Analysis	25
CHAPTER FOUR: RESULTS	26
Demographics	26
Order Effect	26
Counter Movement Jump	27
Forty-Yard Dash	27
Wingate Anaerobic Test	28
CHAPTER FIVE: DISCUSSION	29
Future Directions	34
Conclusion	34
REFERENCES	35
APPENDICES	
A. Data Sheet	38
B. Human Subjects Approval	39
C. Consent Form	41

LIST OF TABLES

Table	Page
4.1 Demographics and Anthropometric Data for Subjects.....	26
4.2 Counter Movement Jump Means for the Placebo and α -GPC Conditions.....	27
4.3 40-yd Dash Mean Times for the Placebo and α -GPC Conditions.....	28
4.4 The 30s WAnT Results for the Placebo and α -GPC Conditions.....	28

CHAPTER ONE
NATURE AND SCOPE
OF THE STUDY

Athletes are constantly striving to perform to the best of their ability. It is no secret that fame is tied to participation in professional athletics. Participation may also be lucrative. For those and other reasons, some athletes are willing to do whatever it takes to win and/or perform at the professional level; sometimes this desire moves outside of the confines of the rules (Tokish, Kocher, & Hawkins, 2004). Some athletes utilize technology, modifications of equipment, new training modalities, supplements, and drugs to gain a competitive edge. Certain supplements and drugs pose health risks that may include pre-mature death (Tokish, Kocher, & Hawkins, 2004). Supplements and drugs are termed ergogenic aids if they improve some performance parameter, such as strength, speed, or focus.

Many companies produce and market supplements and drugs to athletes of all type looking for ergogenic effects. In fact, the supplement industry is a billion dollar enterprise (Cohen, 2012). Some ergogenic aids are drugs, like anabolic steroids, and are banned from many professional sport organizations and competitions (primary example, the International Olympic Committee). Other ergogenic aids, like caffeine, are allowed within certain limits and vary from organization to organization. Because of the lucrateness of both sport and industry, research is constantly being done on new supplements.

Choline, a water soluble vitamin, is necessary for both structural and functional roles and was declared an essential nutrient in 1998 (Deuster & Cooper, 2006). The daily requirement has been estimated at approximately 550 and 425 mg daily for males and females, respectively. Multiple lipids that preserve cell membrane integrity rely on choline as a component. Arguably, choline's most important function is acting as a metabolic precursor to the neurotransmitter

acetylcholine (ACh) (Deuster & Cooper, 2006). Although there are dietary and de novo pathways for obtaining choline, as might be surmised, companies know the important roles of choline in the body and therefore produce and market choline supplements. Ordered by effectiveness at increasing circulating choline levels, choline supplements include α -glycerophosphorylcholine (α -GPC), cytidine 5-diphosphocholine (CDP-choline), phosphatidylcholine, lecithin, and choline salts.

In order to better understand the clinical and potential ergogenic effects of α -GPC, it is necessary to think about the nature, mechanisms, and metabolic relationship between α -GPC, choline and ACh. First, α -GPC is precursor to choline. Second, choline is required for the synthesis of ACh. In effect, α -GPC is indirectly connected, through choline, to the synthesis of ACh. Acetylcholine is an important neurotransmitter for proper brain function and it is also required for muscle action. Therefore, the nootropic and ergogenic effects of α -GPC are appropriate to study.

Acetylcholine is released from the synaptic vesicles into the synaptic cleft to induce changes in the postsynaptic cell. Acetylcholinesterase, an enzyme present in the postsynaptic cleft, is responsible for the hydrolysis of ACh into choline and acetic acid. The majority of the choline is reabsorbed by the terminal branches of the motor neuron with the reuptake supporting the resynthesis of ACh (Zimmerman & Soreq, 2006). Muscle activation relies on nervous signaling from the motor cortex and the release of ACh at the synaptic cleft brings about changes that induce muscle contraction. This series of events is called excitation-contraction coupling mechanism.

Alpha-glycerophosphorylcholine is derived from lecithin phosphatidylcholine (Govoni, Lopez, Battaini, & Trabucchi, 1992; Ban, Panzarasa, Borra, Del Duchetto, & Fjetland, 1991).

Choline derived from α -GPC does not have a charge like dietary choline (Parnetti, Mignini, Tomassoni, Traini, & Amenta, 2007). Without a charge, the choline freely diffuses across the blood brain barrier, thus allowing it to increase choline levels in the brain—an important clinical consideration (Govoni et al., 1992; Parnetti et al., 2007). Gatti et al. (1992) has shown that α -GPC supplementation results in significant increases in plasma free choline levels. Moreover, an increased availability of choline has been positively correlated with increases in ACh synthesis (Gatti et al., 1992; Moreno, 2002; Govoni et al., 1992; Parnetti et al., 1993).

With this knowledge, several investigators have focused on the clinical outcomes associated with its usage. In one of the early trials, Ban et al. (1991) researched the effectiveness of α -GPC in patients suffering from dementia. All participants were given α -GPC in the form of 400mg gel capsules (three capsules a day for 180 days). The results of this clinical study suggested that α -GPC is an effective therapeutic agent in treating subjects with dementia stages 2 to 5 as supplementation improved patients in multiple dimensions measured.

In 1993, Parnetti et al. (1993) investigated α -GPC and acetyl-L-carnitine on subjects with probable senile dementia. The study involved 126 subjects with clinical diagnoses of dementia. Behavioral and neuropsychological testing was completed at each test point (baseline, four months, and after six months). While both treatment groups experienced improvements, the α -GPC treatment group experienced greater outcomes. Authors concluded that α -GPC is a more effective treatment than acetyl-L-carnitine for treating these types of patients (Parnetti et al., 1993).

More recently, Moreno (2002) examined the effects of α -GPC supplementation on mild to moderate Alzheimer's dementia in a multicenter, double-blind, randomized, placebo controlled trial. In patients with Alzheimer's, the cerebrocortical cholinergic system's capacity

diminishes and this reduction is linked to decreased ACh synthesis, release, and uptake. The purpose of the investigation was to determine the efficacy and tolerability of α -GPC ingestion for treating patients with mild to moderate Alzheimer's disease. Subjects included 105 women and 27 men (mean age of 72.2 ± 7.5 yrs). Volunteers were randomized into a placebo (capsules that looked identical to the treatment group) or treatment group (α -GPC—3, 400mg capsules per day). Efficacy of the treatment was assessed at 90 and 180 days—primary efficacy point being slowing of cognitive decline which was measured by the Alzheimer's Disease Assessment Scale-Cognitive Subscale. Moreno's investigation showed that treatment with α -GPC significantly improved cognition and global function. The author concluded that the improvements were likely caused by the improvement of neurotransmission as well as α -GPC's anabolic effect on neuronal cell loss (Moreno, 2002).

The nootropic benefits described above have enlivened researchers to consider the potential beneficial effects of α -GPC during exercise. In one of the earliest exercise studies, Ziegenfuss, Landis, and Hofheins (2008) investigated the effects of AlphaDopa, a supplement containing 100mg of α -GPC, on growth hormone, explosive performance, and post-exercise substrate utilization in eight healthy adults with at least five years of resistance training experience. The study was randomized, double-blind, placebo controlled, crossover fashion. Subjects ingested the placebo or AlphaDopa capsules 90 minutes prior to completing six sets of squats at 70% 1RM. Thirty minutes after the exercise, subjects completed three sets of bench press throws at 50% 1RM. Subjects returned a week later to complete the same exercise protocol under the opposite condition. The authors found that AlphaDopa improved peak force post-exercise (Ziegenfuss et al., 2008). These results may be a result of the α -GPC in AlphaDopa.

Similar to the previous researchers, Hoffman et al. (2010) examined the effect of a supplement containing α -GPC, called CRAM. The CRAM capsule contains 150mg α -GPC. The authors examined the acute and prolonged effect of this supplement on reaction time, mental focus, and exercise ability. Randomly assigned groups were given their treatment, placebo or CRAM, and they rested for ten minutes before completing a focus related questionnaire and a reaction time test. Thereafter, they completed an exercise protocol which included the 30-second Wingate Anaerobic Test (WAnT). After the exercise was completed, subjects were retested on the questionnaire and reaction time. The authors found no acute effect for the CRAM supplement; however, after four weeks of CRAM supplementation, reaction time was better maintained after exercise. No other effects were found (Hoffman et al., 2010).

The most recent study conducted by Bellar, LeBlanc, and Campbell (2015) investigated the effect of pure α -GPC on isometric strength in thirteen college-aged males. The study was double-blind, placebo controlled, crossover fashion, with a one-week wash-out period. Baseline performance was assessed followed by their initial treatment—placebo or 600mg of α -GPC. One hour after ingestion, subjects performed isometric exercises. For the following six days, subjects ingested their treatment before returning and repeating the same assessments. One week later, subjects switched groups and repeated the other protocol. No acute effect was found with α -GPC supplementation contrary to the findings of Ziegenfuss et al. (2008), but after 6 days of α -GPC supplementation, lower body strength did improve (Bellar et al., 2015).

Alpha-glycerophosphorylcholine has demonstrated its nootropic effects in clinical investigations with consistency; however, α -GPC's potential ergogenic effects during exercise are not well established. This research increased the current body of literature on α -GPC's effect on acute anaerobic exercise performance.

Significance of Study

Anaerobic power is an important consideration for activities across the sports spectrum. While a plethora of research exists on anaerobic power and ergogenic aids, the effect of α -GPC on anaerobic performance has not been elucidated. The results from this investigation provided evidence supporting α -GPC as an acute ergogenic aid for anaerobic exercise performance, specifically the counter movement jump (CMJ).

Purpose of Study

The purpose of this investigation was to examine the acute effects of α -GPC ingestion on acute anaerobic performance as measured by the CMJ, 40-yard dash (40-yd), and 30-second WAnT.

Hypotheses

H₁: It was hypothesized that ingestion of α -GPC would increase power, thereby improving vertical jump height during a CMJ test.

H₂: It was hypothesized that ingestion of α -GPC would improve a 40-yd dash as reflected in a decreased time to complete the task

H₃: It was hypothesized that ingestion of α -GPC would increase peak power and mean power during a 30-second WAnT.

H₀: It was hypothesized that ingestion of α -GPC would not improve indices of anaerobic performance as measured by the respective apparatus.

Delimitations

The investigation was delimited to:

1. Students attending University of Central Missouri;
2. Subjects with some exercise experience;
3. Two ninety-minute sessions to assess anaerobic indices under both the placebo and experimental conditions;
4. Experimental supplementation of 300mg of α -GPC dissolved in 0.25L of orange sports drink and placebo supplementation of 0.25L of orange sports drink, a single dose, respectively.

Limitations

The investigation was limited by:

1. Velotron ergometer technology accurately measuring each 30-second WAnT;
2. Subjects reporting to the assessment without consuming any choline-containing food for 12 hours prior to the assessment;
3. Subjects reporting to the assessment without consuming any caffeine or alcohol for 24 hours prior to the assessment; and
4. Subjects reporting to the assessment without undergoing any exercise/practice for 24 hours prior to the testing session.

Assumptions

It was assumed that:

1. Subjects performed the CMJ test to their maximum ability;
2. Subjects performed the 40-yd dash to their maximum ability;
3. Subjects performed the 30-second WAnT to their maximum ability;

4. The software utilized to collect 30-second WAnT trial data provided the most accurate measure for each trial; and
5. Subjects did not know which treatment they were receiving at the time of testing.

Definition of Terms

1. Ergogenic aid: a substance that improves performance.
2. Supplement: substance that contains some dietary ingredient; allowable in certain doses and concentrations by sport organizations.
3. Drugs: substances banned from sport.
4. De novo synthesis: synthesis of a complex molecule from simple molecules.
5. Gorge: a narrow valley with steep walls.
6. Motor endplate: the synapse between a branch of the motor axon and a muscle fiber; each endplate contains a cluster of synaptic terminals (Åstrand, Rodahl, Dahl, & Strømme, 2003).
7. Peak Power: the highest mechanical power elicited during the test.
8. Mean Power: the average power sustained.
9. Minimum Power: the lowest mechanical power elicited during the test.
10. Fatigue Index: the degree of power drop off.
11. Quanta: a quantity or amount.

CHAPTER TWO REVIEW OF LITERATURE

Introduction

To better understand the clinical and potential ergogenic effects of α -glycerophosphorylcholine (α -GPC), it is necessary to understand the nature, mechanisms, and metabolic relationship between α -GPC, choline, and acetylcholine (ACh). To begin, α -GPC is a precursor to choline and is required for the synthesis of ACh. In effect, α -GPC is indirectly connected, through choline, to the synthesis of ACh. Acetylcholine is an important neurotransmitter for proper brain function and it is also required for muscle action. Therefore, the nootropic and ergogenic effects of α -GPC are appropriate to study.

In *Sports Nutrition: Vitamins and Trace Elements*, Deuster and Cooper (2006) discuss choline, including its role and functions in the body. Choline, declared an essential nutrient in 1998, is a quaternary amine that exists in most animals and plants. Nervous tissue typically has greater amounts of choline when compared to other tissues and, as a testament to that fact, the highest concentration of choline in the human body is found in the brain. Choline is necessary for both structural and functional roles. Multiple lipids that preserve cell membrane integrity rely on choline as a component. As an example, phosphatidylcholine is an integral part of the phospholipid bilayer of the cell. Choline can also serve in the synthesis of creatine. Creatine is used to form phosphocreatine, which is an important high-energy compound. Choline is also a precursor for compounds, such as platelet-activating factor. Arguably, choline's most important function is acting as a metabolic precursor to the neurotransmitter acetylcholine (ACh) (Deuster & Cooper, 2006).

Adult males and females need approximately 550 and 425 mg of choline daily, respectively, and it is primarily acquired from exogenous sources, which include meats, poultry, and fish. When ingested, choline is absorbed by the small intestine and is delivered to the liver via portal circulation. In addition, there is de novo synthesis of choline. Two de novo pathways exist and both pathways require methylation reactions. The first pathway requires the decarboxylation of serine, an amino acid, to ethanolamine as the first step. Ethanolamine is then methylated to form choline. The second pathway involves three methylation reactions to convert phosphatidylethanolamine to phosphatidylcholine (Deuster & Cooper, 2006). As might be surmised, companies also produce and market choline supplements. Ordered by effectiveness at increasing circulating choline, choline supplements include α -GPC, cytidine 5-diphosphocholine (CDP-choline), phosphatidylcholine, lecithin, and choline salts (Deuster & Cooper, 2006).

Acetylcholine

Acetylcholine has vital neurotransmitter roles in the central nervous system (CNS) and the peripheral nervous system (PNS), but its mechanisms of action differ by location (Åstrand, Rodahl, Dahl, & Strømme, 2003). The action of ACh is distinctly different in the CNS and PNS because of the receptors to which it binds. In the CNS, the cholinergic receptor is muscarinic, while in the PNS, the receptor is nicotinic. The muscarinic receptor works indirectly, while the nicotinic receptor works directly as an excitatory receptor (Åstrand, Rodahl, Dahl, & Strømme, 2003). The parasympathetic and sympathetic preganglionic neurons and the parasympathetic postganglionic neurons use ACh as a neurotransmitter. In the CNS, interplay of excitatory and inhibitory impulses arriving at the particular neuron determines whether threshold potential is reached and if an action potential will occur (Åstrand, Rodahl, Dahl, & Strømme, 2003). In other

words, the sum of the electrochemical activity in a given time frame determines the result at any given neuron.

In the PNS, ACh is most widely known for its role in muscle action. The role of ACh in muscle action occurs in the motor endplate, the synapse between the terminal branches of the motor neuron and the skeletal muscle fiber. The action potential that has been initiated somewhere in the CNS travels down the length of the motor neuron's axon to the terminal branches. Calcium ions flow into the axon terminal causing the synaptic vesicles to release 100 to 200 quanta of ACh into the motor endplate. ACh quickly binds to cholinergic receptors, specifically nicotinic receptors on the sarcolemma of the associated muscle fibers (Åstrand, Rodahl, Dahl, & Strømme, 2003). The binding of ACh causes an influx of sodium ions into the fiber. One synaptic terminal does not cause enough depolarization to reach threshold potential; however, spatial summation is able to take place because of all synaptic terminals releasing ACh simultaneously, which reaches well above threshold potential (Åstrand, Rodahl, Dahl, & Strømme, 2003). This depolarization causes a subsequent muscle fiber contraction.

Acetylcholinesterase and Metabolism of ACh. ACh is released from the synaptic vesicles into the synaptic cleft to induce changes in the postsynaptic cell. The enzyme acetylcholinesterase (AChE) is present in the postsynaptic cleft and is responsible for the hydrolysis of ACh into choline and acetic acid. The majority of the choline is reabsorbed by the terminal branches of the motor neuron with the reuptake supporting the resynthesis of ACh. The substrate turnover rate of AChE is known to be incredibly high (Zimmerman & Soreq, 2006). In humans, AChE, is comprised of an invariable 534 amino acid sequence, a variable C-terminus of 14, 26, or 40 amino acids as well as an N-terminus of 60 or 66 amino acids (Zimmerman & Soreq, 2006). These variances allow for 6 possible AChE subunits. The catalytic domain of

AChE is composed of a serine-histidine-glutamate triad and located at the bottom of a gorge dubbed the active site gorge. Within the active site gorge are 14 aromatic residues. Outside the entrance of the active site gorge are a group of 5 residues collectively called the peripheral anionic site. The positively charged substrate, ACh, is attracted to the peripheral anionic site and transiently bonds to it. It is assumed that ACh structurally changes after bonding to the peripheral anionic site in order to continue movement into the active site gorge (Zimmerman & Soreq, 2006). The aromatic groups guide ACh to the bottom of the gorge where the enzyme's active site resides. At this site, serine displaces choline forming an acetyl-enzyme intermediate. Subsequently hydrolysis occurs cleaving the acetate group from the acetyl-enzyme intermediate.

A single synaptic vesicle releases enough ACh to saturate all ACh receptors and cholinesterases within a 0.5 μm diameter (Zimmerman & Soreq, 2006). ACh is able to bind to the ACh receptors more quickly than AChE can hydrolyze it. The amount of ACh that binds to cholinergic receptors is expressed as a ratio of receptors to esterase—typically 4:1 receptors to esterase, respectively. However, because hydrolysis of ACh occurs more rapidly than ACh is able to bind and unbind from the receptors, AChE is readily available to hydrolyze ACh. This means two things: 1) ACh concentration in the synaptic cleft is kept relatively low at all times; and 2) it is not conceivable that a single ACh would be able to activate numerous receptors (Zimmerman & Soreq, 2006).

Choline acetyltransferase (ChAT) is responsible for catalyzing the synthesis of ACh. In the case of the motor endplate, ChAT is located in the terminal branch of the cholinergic neurons. ACh is synthesized from choline and acetyl-CoA. How acetyl-CoA, which is located in the inner membrane of the mitochondria, reaches the cytoplasm, where the ChAT is found, is unclear. Choline, which exists in the blood plasma, is taken up by either the high affinity choline

uptake (HACU) or the low affinity choline uptake (LACU) system (Deuster & Cooper, 2006; Taylor & Brown, 1999). The HACU system is temperature, energy, and sodium dependent and is the primary transport system of choline into the cholinergic neuron (Deuster & Cooper, 2006). The HACU system should be able to maintain ACh levels in the terminal branch of the neuron even during high demands; however, when the choline demand is exceptionally high, the LACU system is activated to support the uptake of plasma choline for synthesis of ACh (Deuster & Cooper, 2006; Taylor & Brown, 1999). More than half of the choline used for ACh synthesis is recycled from the hydrolysis reaction catalyzed by AChE in the synaptic cleft. Choline is not stored in the cells, but instead, is immediately used for synthesis of ACh (Deuster & Cooper, 2006). Unlike choline, ACh is stored in two distinct pools: the depot pool and the stationary pool. The depot pool is readily available and is utilized by the neuron as needed. The stationary pool supplies the depot pool with ACh and if the amount supplied is not adequate for the imposed demands, ACh release can be limited (Taylor & Brown, 1999).

Muscle Activation and Muscular Fatigue. Muscle activation relies on nervous signaling from the motor cortex. Release of neurotransmitter at the synaptic cleft induces muscle contraction. The series of events is called excitation-contraction coupling mechanism. Fatigue can be defined as “any exercise-induced reduction in the maximal capacity to generate force or power output” (Åstrand, Rodahl, Dahl, & Strømme, 2003). Fatigue cannot be tied to one single causative agent, but instead, is the result of multiple factors which vary situationally. Fatigue can be broken down into central and peripheral factors. Central factors, labeled central fatigue, result from insufficient neural activation of the muscle. Peripheral fatigue, resultant to muscle factors, is due to an inability of muscle to respond maximally to adequate stimulation. With respect to fatigue at the motor end plate, there are two localized areas to consider: presynaptic and

postsynaptic (Åstrand, Rodahl, Dahl, & Strømme, 2003; Ament & Verkerke, 2009; Kirkendall, 1990). Presynaptic considerations are focused on issues with the motor neuron, whereas postsynaptic considerations are focused on the innervated cell.

One factor that might cause muscular fatigue is the release of a limiting quantity of ACh. What might cause a decreased release of ACh into the synaptic cleft is not completely clear, but it may relate to decreased terminal depolarization, which is a result of a decrease in calcium ion entry caused by decreased calcium sensitivity. Because one motor neuron can innervate hundreds of muscle fibers, it is possible that fewer terminal branches are depolarizing, which leads to lower force and power output (Åstrand, Rodahl, Dahl, & Strømme, 2003; Ament & Verkerke, 2009).

Another factor that may cause muscular fatigue may be decreased ACh release due to fewer vesicles releasing ACh per action potential and decreased quantal size (Åstrand, Rodahl, Dahl, & Strømme, 2003; Kirkendall, 1990). The decrease in number of vesicles may be attributable to altered vesicle recycling, i.e., the reuptake of the vesicle membrane is diminished (Åstrand, Rodahl, Dahl, & Strømme, 2003). There is some evidence that ACh quantal size decrements may be related to decreased choline availability leading to a decrease in ACh synthesis (Wurtman & Lewis, 1991; Deuster & Cooper, 2006). Also, long-term exposure to ACh, or ACh accumulation, has been shown to decrease sensitivity of the ACh receptors to ACh. This desensitization logically leads to a decrease in depolarization of the sarcolemma or, in other words, a decrease in sarcolemmal excitability. Decreased sarcolemmal excitability then causes a decrease in muscle contraction-excitation coupling (Åstrand, Rodahl, Dahl, & Strømme, 2003; Deuster & Cooper, 2006).

Alpha-glycerophosphorylcholine

Alpha-glycerophosphorylcholine, also known as choline alfoscerate, is a semi-synthetic derivative of lecithin phosphatidylcholine that, after oral ingestion, acts as a precursor to choline (Moreno, 2002; Parnetti et al., 1993). Following oral ingestion, α -GPC is converted into glycerophosphate and free choline by glycerolphosphorylchoine diesterase (Gatti et al., 1992). Choline derived from α -GPC does not have a charge like regular choline found in natural food sources (Parnetti, Mignini, Tomassoni, Traini, & Amenta, 2007). This allows the choline to cross the blood brain barrier thereby increasing choline levels in the brain—an important clinical consideration (Govoni et al., 1992; Parnetti et al., 2007). Without a charge, the choline freely diffuses across the blood brain barrier allowing it to be utilized as needed. Choline, as discussed earlier, is necessary for the synthesis of ACh (Deuster & Cooper, 2006). An increased availability of choline has been positively correlated with increases in ACh synthesis (Gatti et al., 1992; Moreno, 2002; Govoni et al., 1992; Parnetti et al., 1993). Beyond just serving as a precursor to choline, and by extension ACh, α -GPC also serves as a precursor to the synthesis of phospholipids and glycerolipids (Gatti et al., 1992; Govoni et al., 1992; Parnetti et al., 1993; Parnetti et al., 2007), which are important components for neuronal integrity and which have known connection to pathologies including Alzheimer's disease (Gatti et al., 1992; Parnetti et al., 1993)

Free Plasma Choline: α -GPC and CDP Supplementation. Noted earlier, a variety of choline supplements exist. The two most effective choline supplements are α -GPC and CDP-choline. Gatti et al. (1992) investigated the differences between α -GPC and CDP choline's supplementation on free plasma choline levels. The randomized, crossover design employed healthy, young adult males (age 26 ± 2) as subjects. A one-week wash-out period was utilized.

Subjects were administered either α -GPC or CDP choline in 1000mg doses intramuscularly. Blood samples were collected before injection and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, and 6 hours post. Samples were analyzed by using a high performance liquid chromatography method with a post-column enzymatic reactor and electrochemical detector. Supplementation resulted in significant increases in plasma free choline levels; however, α -GPC increased choline levels beyond that achieved by CDP choline at all points between 0.25 hours and 2 hours. Speculatively, the reason for this difference was attributed to the choline moiety in α -GPC (405mg in 1000 mg) and CDP choline (213mg in 1000mg) treatments. This investigation is just one example of α -GPC's ability to increase plasma choline levels (Gatti et al., 1992).

Clinical Considerations. Ban et al. (1991) conducted a clinical study on the effectiveness of α -GPC in patients suffering from dementia. Subjects were included if diagnosed with primary degenerative dementia of the Alzheimer's type, multi-infarct dementia or mixed dementia at a stage of 2 to 5 on the Global Deterioration Scale and a score less than 23 on the Mini-Mental State Exam (MMSE). All participants were given α -GPC in the form of 400mg gel capsules. Subjects were instructed to take three capsules a day for 180 days. All subjects were assessed at baseline, 30 days, 90 days, and 180 days. The results of this clinical study suggested that α -GPC is an effective therapeutic agent in treating subjects with dementia stages 2 to 5. The α -GPC treatment improved patients in multiple dimensional measures—MMSE, GDS—and also improved performance measures and social behavior based on the Sandoz Clinical Assessment-Geriatric (SCAG). The treatment was also well-tolerated (Ban et al., 1991).

Abbati, Rondi, Rosola, Vavassori, and Bosio (1991) investigated the effects of two different treatments for cerebral aging: oxiracetam and α -GPC. Subjects were male, and between the ages of 55 and 65, and diagnosed with medium severity senile organic brain syndrome

(according to *Revised Diagnostic and Statistical Manual (DSM)*). To remove the confounding influence of medications, a two-week, medication-free wash-out period was utilized. The forty subjects were randomly assigned to the oxiracetam or the α -GPC groups. Both groups had 1g doses of treatments administered intramuscularly at 8:00 a.m. for 12 weeks. The study assessed cognitive function, relational condition, and biohumoral parameters. Oxiracetam resulted in earlier overall improvement in the twelve-week period, but α -GPC had a more pronounced and significant improvement longer term (in the follow-up weeks). The safety and efficacy of the nootropic effects are well-documented by these findings (Abbati et al., 1991).

A multicenter, randomized, controlled study designed to compare the efficacy of α -GPC and acetyl-*l*-carnitine on subjects with probable senile dementia was investigated by Parnetti et al. (1993). The study involved 126 subjects with clinical diagnoses of dementia based on criteria established in *DSM-III*. Subjects were excluded if they had any concomitant diseases or other systemic diseases. Groups were randomly generated using a balanced-block list, with 65 being in the α -GPC treatment group and 61 being in the acetyl-*l*-carnitine treatment group. After a two-week wash-out period, patients received their treatment: 800mg at 8 a.m. and 400 mg at 4 p.m. of α -GPC or 1000mg at 8 a.m. and 500mg at 4 p.m. of acetyl-*l*-carnitine. Behavioral and neuropsychological testing was completed at baseline, after four months, and after six months. Both groups scored significantly better on the MMSE at four months and six months. Neither group performed better at four months on Rey's 15-word test; however, at six months, the α -GPC group scored significantly better on immediate and delayed recall, while acetyl-*l*-carnitine only scored significantly better on delayed recall. While both treatment groups experienced improvements, the α -GPC treatment group experienced greater outcomes. Parnetti et al. (1993) concluded that α -GPC is a more effective treatment than acetyl-*l*-carnitine for this demographic

and attributed the change to an ability of α -GPC to increase choline and subsequently ACh synthesis. The authors noted the outcome may also be due to α -GPC's ability to improve anabolic processes in neuronal membranes, i.e., phospholipid synthesis/maintenance (Parnetti et al., 1993).

Moreno (2002) examined the effects of α -GPC supplementation on mild to moderate Alzheimer's dementia in a multicenter, double-blind, randomized, placebo controlled trial. Alzheimer's is a cognitive disease that is characterized by impaired motor function, memory, and learning. Cerebrocortical neuronal loss and brain atrophy are common pathological characteristics of Alzheimer's disease. In patients with Alzheimer's, the cerebrocortical cholinergic system's capacity diminishes and this reduction is linked to decreased ACh synthesis, release, and uptake, as well as diminished enzymatic activities i.e. ChAT and AChE. The purpose of the investigation was to determine the efficacy and tolerability of α -GPC ingestion for treating patients with mild to moderate Alzheimer's disease. Subjects included 105 women and 27 men, with the mean age of 72.2 ± 7.5 . All subjects displayed progressive clinical impairment with probable Alzheimer's according to the criteria in the *DSM-IV*. Subjects were excluded if they had other concomitant pathologies—depression—or other systemic diseases such as cancer or congestive heart failure. Volunteers were randomized into a placebo or treatment group. The treatment group received α -GPC—(3-400mg capsules per day)—and the placebo group received capsules that looked identical to the treatment group. Subjects were given one capsule orally three times a day—at breakfast, lunch, and dinner. Efficacy of the treatment was assessed at 90 and 180 days—the primary metric being slowing of cognitive decline as measured by the Alzheimer's Disease Assessment Scale- Cognitive Subscale. The α -GPC supplement was shown to significantly improve cognition and global function. Moreno

(2002) concluded that the improvements are likely caused by the improvement of neurotransmission as well as α -GPC's anabolic neuronal effect, which may offset neuronal cell loss.

Alpha-glycerophosphorylcholine and Exercise. In one of the earliest exercise studies using α -GPC, Ziegenfuss et al. (2008) investigated the effects of AlphaDopa on growth hormone (GH), explosive performance, and post-exercise substrate utilization. The crossover, clinical study was conducted in randomized, placebo controlled, double-blind, crossover fashion. AlphaDopa contains 100 mg α -GPC, 666.6 mg L-Dopa, 50 mg Bacopa Monniera extract. Eight healthy individuals with a minimum of five years of resistance training experience, who were able to squat 1.5 times their body weight, were enrolled. Anthropometric measures, a health history, clinical blood chemistry, and RMR were gathered on all subjects. Subjects consumed either AlphaDopa, or the placebo, 90 minutes prior to completing six sets of squats at 70% 1RM. Thirty minutes post-exercise, RMR was assessed followed by three sets of bench press throws at 50% 1RM. Blood was collected prior to exercise and 5, 15, 30, 60, 90, and 120 minutes post-exercise. Subjects returned for the second trial approximately one week after the first trial. Results indicated that AlphaDopa ingestion resulted in a 100% increase in growth hormone response during the first thirty minutes post-exercise and an increased potential to generate peak force. A potentially meaningful increase in fat utilization post-exercise was also observed, but the difference did not reach statistical significance. This early study showed that AlphaDopa containing 100 mg of α -GPC may be enough to increase the response of GH secretion and peak force during acute resistance training. This study demonstrates the role that AlphaDopa may serve as an ergogenic aid (Ziegenfuss et al., 2008).

Hoffman et al. (2010) examined acute and prolonged effects of the supplement CRAM on reaction time, measures of focus, and exercise ability. The CRAM supplement contained 150 mg α -GPC and the placebo had similar visual characteristics. Subjects were randomly assigned to either the treatment group or the placebo group. Subjects ingested their given treatment, CRAM or placebo, and rested for ten minutes. The subjects then filled out a questionnaire related to feelings on the ability to focus. A reaction time test was then completed. Following the reaction time test, subjects engaged in an exhaustive exercise protocol, which consisted of a 30-second Wingate Anaerobic Test (WAnT), a 1-minute push-up test, and a 1-minute sit up test. Following exercise, subjects filled out the same questionnaire and repeated the same reaction time test. After four weeks of daily supplementation—one dose a day—the subjects returned and followed the same protocol. Hoffman et al. (2010) found that the CRAM supplementation allowed subjects to maintain a similar reaction time after exhaustive exercise as compared with before exercise. This finding was not evidenced in the placebo trial. Contrary to hypotheses, there were no statistically significant differences between groups for measures of focus, 30-second WAnT performance, or number of push-ups or sit-ups completed. Speculatively, the timing of testing may have influenced outcomes. Hoffman et al. (2010) suggested further research to add to the limited literature.

Bellar, LeBlanc, and Campbell (2015) examined the effect of α -GPC on isometric strength in thirteen college-aged males. The study was conducted in double-blind, placebo controlled crossover fashion, with a one-week wash-out period. Baseline performance was assessed—*isometric mid-thigh pull test and upper body isometric test*—and subjects were then given their initial treatment. The α -GPC treatment was provided as a gel capsule containing 600 mg of α -GPC. The placebo looked identical to the α -GPC treatment. Subjects continued to ingest

their given treatment for six days, after which they returned to repeat the assessments. A one-week wash-out period was utilized, after which subjects repeated trial assessment only with the opposite treatment. Results were mixed. Contrary to Ziegenfuss et al. (2008), there were no effects of α -GPC on upper body strength ($p=0.127$) (Bellar et al., 2015). Chronic effects showed a statistically significant increase in isometric lower body strength after 6 days of supplementation. Bellar et al. (2015) suggest that the improvements that were evidenced may be due to increased bioavailability of choline for the synthesis of ACh.

Summary

While it is clear that α -GPC is an effective clinical treatment option for neural pathologies, it has not been well established what potential ergogenic effect(s) it may offer during exercise. There is value in additional research evidence to elucidate if there is any efficacy of α -GPC supplementation's acute effect on anaerobic exercise. The prevailing theory is α -GPC's effect on free plasma choline may increase ACh availability during anaerobic activity; however, there is limited evidence to support α -GPC as an ergogenic aid. At least two studies have shown an ergogenic benefit for α -GPC (one acutely (Ziegenfuss et al., 2008) and the other chronically (Bellar et al., 2015)). By examining α -GPC's effect on counter movement jump, 40-yard dash, and 30-second WAnT performance, this researcher will be able to add additional evidence about the potential ergogenic effects of α -GPC to the existing literature.

CHAPTER THREE METHODOLOGY

Overview

The aim of this study was to investigate the acute effectiveness of 300 mg of α -GPC as an ergogenic aid with respect to anaerobic indices in young recreationally-trained females. This study was double-blind, placebo controlled, crossover design, with a one-week wash-out period. Subjects reported to the Human Performance Lab for two testing sessions. One hour prior to the testing, subjects ingested a solution—placebo or α -GPC. A structured warm-up, consisting of an 800m run and dynamic stretching was completed. After the warm-up, subjects completed 3 assessments of anaerobic performance: the CMJ, 40-yd dash, and 30-second Wingate anaerobic test (WAnT). Subjects completed three trials of the CMJ and 40-yd dash, with two minutes rest between attempts. They completed only one trial on the 30-second WAnT. The 30-second WAnT was conducted last in the session due to the strenuous physical output required and the obvious influence it would have on maximal efforts during the other assessments. Subjects returned one week later, ingested the other treatment, and completed the same testing protocol.

Subjects

Recruiting for the investigation commenced with the approval of the Institutional Review Board at the University of Central Missouri, Warrensburg, Missouri. The twenty recreationally-active females were recruited from the University of Central Missouri. Recreationally-active, for the purpose of this investigation, was defined as engagement in approximately 90 to 150 minutes of moderate intensity activity a week. An informed consent, approved by the University Human Subjects Committee, was read and signed by each subject before testing was initiated.

Procedures and Instrumentation

Subjects reported to the Human Performance Lab for two sessions. During the first session, subjects signed the informed consent form and had the anthropometric measures of height and weight recorded. Height was assessed using a Seca[®] stadiometer (Seca[®], Chino, CA) and recorded in centimeters. Weight was assessed using a Befour[®] digital scale (Befour[®], Saukville, WI) and recorded in kilograms.

Subjects were randomly administered one of two treatments: placebo or α -GPC. Randomization was based upon an online random numbers generator (www.random.org)—a website that offers true random numbers based on “atmospheric noise” (True Random Number Service, Feb. 5, 2017). A member of the research team who did not participate in data collection utilized the randomization sequence to prepare the test solutions in a double-blind fashion. Neither the research-staff nor the subject knew which solution was being ingested. The randomization sequence was not revealed until data collection for the full study was completed. The placebo treatment was .25 L of orange sports drink. The α -GPC treatment was 300 mg of α -GPC dissolved in .25 L of orange sports drink. The sports drink was used to mask the treatment even though α -GPC is odorless, tasteless, and clear in solution. Essentially, the orange flavor was an additional layer of protection to mitigate perceptions on the part of the subjects—which could lead them to apply or withhold some measure of effort.

One hour after supplement ingestion, the subjects completed a structured, researcher-led warm-up, consisting of an 800m run and dynamic stretching. The dynamic stretching routine was comprised of high knees, lunges, butt-kicks, and walking leg rises. After completing the warm-up, subjects completed the CMJ, 40-yd dash, and 30-second WAnT.

For the CMJ, subjects were instructed to complete a single movement down before exploding up maximally, reaching up as high as possible. The overhead reach and vertical jump height were assessed by the Vertec (Sport Imports, Hilliard, OH) and recorded in cm. The researchers demonstrated the CMJ prior to having the subject jump. The overhead reach was recorded prior to any subject jump performances. Subjects were provided three attempts, each separated by 2 minutes of rest.

The 40-yd dash was completed on an indoor track and was recorded in seconds. All subjects used a standing start without blocks and ran through two laser gates: one at 20 yards the other at 40 yards. Subjects were instructed to run at maximal effort from start to finish before volitionally slowing down. The Brower Timing System® (Brower Timing, Draper, UT) assessed 20-yd split and 40-yd dash times. Subjects were provided three attempts, each separated by 2 minutes of rest.

For the 30-second WAnT, subjects completed a 5-minute warm-up on the Velotron®—used for all warm-up and testing—at 60 RPM. During the warm-up, subjects completed 3 short maximal effort sprints lasting approximately five seconds at the 2, 3, and 4-minute time points. Following the warm-up, subjects began the 30-second WAnT, pedaling against a constant resistance (torque factor of 0.0750 per kilogram) for 30 seconds. Verbal encouragement was given throughout the duration of the test. Subjects cooled down by pedaling against a light resistance for 5 minutes. The Velotron® Wingate 2008, version 1.0.2, (Velotron®, Seattle, WA) was used to assess peak power, mean power, and fatigue index. Peak power was defined as the highest mechanical power elicited during the test. Mean power was defined as the average power sustained over 30 seconds. The minimum power was the lowest power output of the test. The

fatigue index was defined as the degree of power drop off during the test [mathematically: (Peak power – minimum power)/peak power*100].

One week after the initial testing, subjects returned for their final testing session. Subjects were administered the other treatment, and then completed the same protocol. They were not provided information about their initial performance. Subjects were solely encouraged to give maximal effort on each test.

Data Analysis

The placebo control, crossover design established one between-subjects factor (solution: placebo or α -GPC) and one within-subjects factor (session: pre and post). Subjects were either in sequence AB (placebo/ α -GPC) or sequence BA (α -GPC/placebo), with each person serving as their own control. For analysis of CMJ and 40-yd dash, only the best attempt during session 1 and session 2 was used for statistical analysis. It was hypothesized that ingestion of α -GPC would increase power, thereby improving vertical jump height during a CMJ test. It was also hypothesized that ingestion of α -GPC would improve the 40-yd dash as reflected in a decreased time to complete the 40-yd task. Finally, it was hypothesized that ingestion of α -GPC would increase peak power and mean power during the 30-second WAnT.

To assess each dependent variable (CMJ, 40-yd dash, selected measures during the 30-second WAnT), separate dependent t-tests were utilized. Data were analyzed with IBM SPSS Statistics 23 (Armonk, NY). A criterion alpha level of $p \leq 0.05$ was used. Data are reported as mean \pm SD.

CHAPTER FOUR RESULTS

The methods of this study were designed to test the hypotheses that α -glycerophosphorylcholine supplementation would improve CMJ performance, 40-yd dash performance, and selected measures during the 30-second WAnT. Data were collected in two, 90-minute sessions. Paired *t*-tests were utilized to determine differences between the best of the aforementioned measures when the subjects supplemented with the α -GPC and placebo solutions. The results will be discussed as follows: demographics, order effect, CMJ, 40-yd dash, and 30-second WAnT.

Demographics

Twenty female subjects completed the study; however, three subjects ($n=3$) were outliers due to excessive body weight and removed for data analysis. Performance analyzed relative to weight, and overall interpretation of the data, was significantly altered by inclusion of these individuals. Subjects' mean age, height, and weight during placebo and α -glycerophosphorylcholine (α -GPC) trials are reported in Table 4.1.

Table 4.1
Demographics and Anthropometric Data for Subjects

	Mean	Std. Deviation	Mean of Subjects Removed
Age, yrs	21.18	1.47	22.00
Height, cm	165.58	5.99	173.13
<i>Weights</i>			
Placebo Weight, kg	62.10	6.46	94.45
α -GPC Weight, kg	62.28	6.49	95.35

Notes: yrs=years; cm=centimeters, kg=kilograms, and α -GPC=alpha-glycerophosphorylcholine

Order Effect

To mitigate the confounding influence of any potential learning effect, subjects were randomly assigned to the placebo and α -GPC solution trials. A familiarization trial was not

utilized for this study. To confirm the adequacy of this design, order effect was analyzed by using dependent *t*-tests to compare results from session 1 and session 2 for the dependent variables. There were no order effects found for CMJ, 40-yd dash, 30-second WAnT peak power or fatigue index ($p > 0.05$).

Counter Movement Jump

Before any jumping occurred, an overhead reach was determined for each subject. Under the influence of both solutions, subjects had three attempts at achieving a maximal jump height. Only the best jump of each session was used for data analysis. Jump height was calculated by subtracting overhead reach height from the maximal jump height. For the placebo trial, the mean maximal jump height was 68.5 ± 11.5 cm. For the α -GPC trial, the mean maximal jump height was 69.8 ± 11.5 cm. All jumps in the α -GPC solution trial were 1.3 cm greater than those completed in the placebo trial. A paired samples *t*-test revealed α -GPC maximal jump heights were statistically greater than placebo maximal jump heights [$t(16) = -851.00$, $p < 0.001$]. Raw data for the CMJ can be found in Table 4.2.

Table 4.2

Counter Movement Jump Means for the Placebo and α -GPC Conditions (N=17)

Variable	Mean	Std. Deviation
<i>Standing Reach</i>		
Over Head Reach, cm	213.4	11.5
<i>Maximal Jump Height</i>		
Placebo, cm	68.5	11.5
α -GPC, cm	*69.8	11.5

Notes: * = denotes significance compared to Placebo, $t(16) = -851.00$, $p < 0.001$
cm=centimeters, and α -GPC=alpha-glycerophosphorylcholine

Forty-Yard Dash

Under the influence of both solutions, subjects had three attempts at completing the 40-yd dash. Laser-timed 20-yd split, 20 to 40-yd split, and 40-yd cumulative times were recorded. The

best performance of each segment was compared. Separate paired samples *t*-tests showed no significant differences between the placebo or α -GPC treatments for 20-yd split, 20 to 40-yard split, or 40-yd cumulative times. Forty-yard dash results can be found below in Table 4.3. The results of one subject were not used due to inability to complete the test.

Table 4.3
40-yd Dash Mean Times for the Placebo and α -GPC Conditions (N=16)

	Placebo	α -GPC	<i>p</i> value
20-yd split, sec	3.16 \pm .20	3.18 \pm .24	.221
20-40-yd split, sec	2.78 \pm .25	2.80 \pm .27	.344
40-yd time, sec	5.95 \pm .44	5.99 \pm .48	.174

Notes: yd=yards, and sec=seconds

Wingate Anaerobic Test

Each subject completed two 30-second WAnT trials: one under the placebo condition and the other under the α -GPC condition. Recorded measures include peak power (PP), mean power (MeP), minimum power (Min. P), fatigue index (FI), and anaerobic capacity (AnC). Paired samples *t*-tests showed no significant differences between the placebo or α -GPC treatments for any of the variables described (N=13). Due to the loss of subject number (inability to complete the test, *n*=4) there was not adequate power to detect a difference. Results of the 30-second WAnT indices are shown below in Table 4.4. The corresponding *p* values are also included.

Table 4.4.
The 30s WAnT Results for the Placebo and α -GPC Conditions (N=13)

	Placebo	α -GPC	<i>p</i> value
PP (W)	628.0 \pm 108.1	626.0 \pm 91.6	.896
MeP (W)	403.8 \pm 41.7	413.2 \pm 39.8	.198
Min. P (W)	263.9 \pm 43.6	279.1 \pm 41.1	.069
FI (W/s)	12.2 \pm 3.1	11.8 \pm 2.8	.392
AnC (W/kg)	6.6 \pm .60	6.8 \pm .48	.185

Notes: W=watts, s=seconds, kg=kilograms, PP=peak power, MeP=mean power, Min. P=minimum power, FI=fatigue index, and AnC=anaerobic capacity
 α -GPC=alpha-glycerophosphorylcholine

CHAPTER FIVE
DISCUSSION

The primary purpose of this study was to examine the acute effects of α -GPC ingestion on acute anaerobic performance as measured by the CMJ, 40-yd dash, and 30-second WAnT. It was hypothesized that ingestion of α -GPC would improve CMJ height, 40-yd dash times, as well as peak and mean power during the 30-second WAnT. These hypotheses were developed around the claim that α -GPC has ergogenic potential (Ziegenfuss et al., 2008; Hoffman et al. 2010; Bellar et al., 2015). The primary findings were that α -GPC supplementation significantly improved CMJ height, while neither the 40-yd dash times nor the peak or mean power during the 30-second WAnT improved. Secondary findings show minimum power during the 30-second WAnT were trending towards significance, but failed to reach significance.

The principal finding of this study was an increase in CMJ height following ingestion of α -GPC supporting hypothesis one. Strangely, all subjects ($N=17$) improved by exactly 1.3 cm (~0.5 in.) when offered the exact same verbal directions and demonstration under both conditions. Data analysis was completed on seventeen ($N=17$) subjects for the CMJ because all subjects were able to complete the CMJ under both conditions. The CMJ, commonly referred to as a vertical jump, is a functional test for lower extremity power output. After determining CMJ height, the vertical distance covered can be placed into a power estimation equation. In any equation used to calculate power from CMJ height, the higher the jump, the greater the power output. The increase in CMJ height observed in this study demonstrated α -GPC's ability to increase power output as measured by CMJ. This improvement in power output could lead to increased sport performance after ingesting α -GPC and supports the claim that α -GPC has potential to serve as an ergogenic aid.

Other research is equivocal with regards to the results of this study. Ziegenfuss et al. (2008) found acute supplementation with α -GPC increases peak bench press force, but not peak bench press power. Both the present study and Ziegenfuss et al. (2008) observed ergogenic effects as a result of acute α -GPC supplementation; however, they differ in at least one significant way. The primary difference is the parameter that improved: peak force in Ziegenfuss et al. (2008) and power in the present study. Force is measured irrespective of velocity while power output is contingent on velocity and force (power=force x velocity). Peak force generated will not generate the most power because velocity decreases as force increases (Zivkovic Zivkovic, Djuric, Cuk, Suzovic, & Jaric, 2017). These same authors demonstrated in a recent study examining muscle force-velocity relationships in different functional tests—one of which being a CMJ—that the force-velocity relationship may be more linear than previously thought, that is, as force increases velocity decreases and vice versa.

Bellar et al. (2015) also studied α -GPC's ergogenic effect on upper and lower body isometric strength. They found α -GPC supplementation increased lower body isometric strength using a mid-thigh pull, but not upper body isometric strength after 6 days of α -GPC supplementation. While both Bellar et al. (2015) and the present study found significant improvements in lower body indices (CMJ height and mid-thigh pull), Bellar et al. (2015) used chronic supplementation, and the present study used acute supplementation. It is impossible to determine whether or not the subjects participating in Bellar et al. (2015) would have experienced an acute effect since acute data was not collected. This author speculates that α -GPC works by increasing the bio-availability of ACh at the neuromuscular junction, which is the same conclusion as Bellar et al. (2015). Alpha-GPC may be related to muscle mass activated, but that is purely speculative because this was not a mechanistic study.

With the present subject number and data, hypothesis two ($N=16$ after attrition) and hypothesis three ($N=13$ after attrition) were not supported by the data of this study. There were no improvements in 40-yd dash time nor peak or mean power during the 30-second WAnT. The 40-yd dash splits (0 to 20-yd and 20-yd to 40-yd) were analyzed and also failed to reveal any significant differences between α -GPC and placebo trials. Pauole, Madole, Garhammer, Lacourse, and Rozenek (2000) showed a low correlation between CMJ and 40-yd dash times explaining that a 40-yd dash is more a measure of leg speed and less of a measure of leg power. That may explain why in this study CMJ height improved, but 40-yd dash times did not. Unfortunately, four subjects suffered upper anterior thigh muscle issues sustained during the first trial of the 40-yd dash; however, only one subject did not complete their first trial in an adequate time (\leq one standard deviation different than their average time) leaving sixteen subjects ($N=16$) for data analysis.

The 30-second WAnT is an established anaerobic power test (Inbar, Bar-Or, & Skinner, 1996). The CMJ and 30-second WAnT for peak power are moderately correlated with r values of approximately .70, but the 30-second WAnT and 40-yd dash are more strongly correlated with r values of approximately .84 (Inbar, Bar-Or, & Skinner, 1996). This demonstrates and perhaps explains to some degree why there was no significant differences seen between placebo and α -GPC conditions for both the 40-yd dash and the 30-second WAnT, but was seen for the CMJ. The subjects who hurt themselves were also unable to begin the 30-second WAnT, lowering the subject pool down to thirteen ($N=13$) for the 30-second WAnT. Unfortunately, this study does not have statistical power for data based on the 30-second WAnT.

This study did not utilize a familiarization trial in its methodology. Without a familiarization trial, it was important to establish that there is not an order effect before

determining statistical differences between placebo and experimental (α -GPC) conditions. Along these lines, data from session 1 and session 2 were compared using a paired samples *t*-test. There was no order effect found for CMJ, 40-yd dash, or peak power during the 30-second WAnT ($p > 0.05$). This lends credence to the adequacy of the research design. A familiarization trial may have been difficult to utilize for this study, even if an order effect had been found. Anecdotally, subjects expressed an unwillingness to complete three 30-second WAnT over the course of three weeks, that is, subject drop-out would have been expected to be greater. Unfortunately, there was enough subject attrition ($n = 7$; 35% of initial recruitment) to lose statistical significance for the 30-second WAnT.

Some of the limitations of the present study are obvious. The two primary limitations are training status of the subjects and subject drop-out. The subjects of this study were recreationally trained, defined as participation in 90 to 150 minutes of moderate physical activity a week. Subjects were determined to be recreationally active by verbal confirmation only. All subjects replied in the affirmative. Anecdotally, it became obvious that some of the subjects fell outside of the parameters of recreationally active. Authors speculate that the vigorous nature of the testing led to muscle damage, particularly in the subjects who were not adequately training to meet the recreationally-trained definition. This may explain the reports of quadriceps and hamstring tightness and the dropout rate at session number two ($n = 4$ total; 3 for control and 1 for GPC).

Subjects that fell below the set parameters could be considered untrained, and subjects that are above the parameters could be considered more than recreationally trained. Pauole et al. (2000) examined differences in performance for four functional tests across three training statuses—matching those just described—in college-aged women. They utilized the 40-yd dash

and vertical jump as two of the function assessments. They found that untrained women were significantly slower than both recreationally trained and trained women at the 40-yd dash, and recreationally trained women were significantly slower than trained females (Pauole et al., 2000). Vertical jump performance was significantly different between recreationally trained women and trained women, but there was no significant difference between untrained and recreationally trained women (Pauole et al., 2000). This demonstrated that if some of the participating subjects were untrained or trained, the data could be skewed.

Another important consideration for maximal exertion tests like the ones utilized by the present study is ratings of perceived exertion (RPE). While this study did not use any RPE scale, it is an important perspective in regards to training status of the subjects. It is well established that untrained individuals feel like they are working harder at submaximal and maximal intensities (Demello, Cureton, Boineau, & Singh, 1987). This means that untrained subjects may not have been truly giving maximal effort during the 40-yd dash and 30-second WAnT because of their perception that they were working harder than they actually were.

Skill is the last consideration for subject training status. Pauole et al. (2000) suggested part of the reason trained, recreationally trained, and untrained females all performed differently in the 40-yd dash was skill. While the authors did not delve into more detail, the differences are likely tied to the amount training using those ranges of motion and velocities used in the 40-yd dash, that is, untrained subjects were not used to performing that activity. It is possible the same information could be delineated from the present study. While Pauole et al. (2000) did not use the 30-second WAnT in their methodology, it can be logically deduced that some skill would also play a role in 30-second WAnT performance.

Future Directions

The current research did not have a homogenous subject pool. It would be advisable that future research use a sports team in which all athletes receive comparable training intensity and duration. Ideally, similar relative fitness among subjects could assist in maximizing the likelihood to detect a difference, if α -GPC does truly offer ergogenic benefit. These considerations would eliminate the primary limitation of this study: training status. It is also plausible that a dose-response relationship exists. Future research may aim to determine the optimal amount of α -GPC to elicit ergogenic effects for a given sport, required athletic activity, or body weight. Perhaps, the dose is dependent on weight. It may be related to muscle mass and bioavailability of ACh at the neuromuscular junction. If α -GPC is found in future studies to be ergogenic in nature, looking at the mechanisms of action would be a necessary step in understanding α -GPC. No study has looked at both acute and chronic effects of α -GPC, which could aid in determining if there is a difference between the acute and chronic effect of α -GPC.

Conclusion

The current study indicated acute ingestion of α -GPC improves CMJ performance, while having no effect on 40-yd dash or 30-second WAnT performance. Seventeen recreationally trained females completed the study. The data collected by this methodology supported the first research hypothesis, suggesting that α -GPC may be an ergogenic aid; however, the data did not support the second and third research hypothesis, which challenges the aforementioned statement. Unfortunately much like previous research, the results were equivocal. More than anything, this research and previous research have opened the door for future research to determine the true ergogenic nature of α -GPC and begin to unfold its mechanisms of action.

REFERENCES

- Ament, W., & Verkerke, G. J. (2009). Exercise and fatigue. *Sports Medicine*, 39(5), 389-422.
- Åstrand, P., Rodahl, K., Dahl, H. A., & Strømme, S. B. (2003). *Textbook of work physiology: Physiological bases of exercise* (4th ed.). Champaign, IL: Human Kinetics.
- Ban, T. A. (1991). Choline alfoscerate in elderly patients with cognitive decline due to dementing illness. *New Trends Clinical Neuropharmacology*, 5, 87.
- Bellar, D., LeBlanc, N. R., & Campbell, B. (2015). The effect of 6 days of alpha glycerylphosphorylcholine on isometric strength. *Journal of the International Society of Sports Nutrition*, 12(1), 42.
- Cohen, P. A. (2012). Assessing supplement safety—the FDA's controversial proposal. *New England Journal of Medicine*, 366(5), 389-391.
- Demello, J. J., Cureton, K. J., Boineau, R. E., & Singh, M. M. (1987). Ratings of perceived exertion at the lactate threshold in trained and untrained men and women. *Medicine and Science in Sports and Exercise*, 19(4), 354-362.
- Deuster, P.A., & Cooper, J.A. (2005). Choline In J. A. Driskell & I. Wolinsky (Eds.) *Sports Nutrition: Vitamins and Trace Elements* (pp. 139-161). Boca Raton, FL: Taylor and Francis.
- Gatti, G., Barzaghi, N., Acuto, G., Abbiati, G., Fossati, T., & Perucca, E. (1992). A comparative study of free plasma choline levels following intramuscular administration of L-alpha-glycerylphosphorylcholine and citicoline in normal volunteers. *International Journal of Clinical Pharmacology, Therapy, and Toxicology*, 30(9), 331-335.
- Govoni, S., Lopez, C. M., Battaini, F., & Trabucchi, M. (1992). Chronic treatment with an acetylcholine synthesis precursor, alpha-glycerylphosphorylcholine, alters brain

- parameters linked to cholinergic transmission and passive avoidance behavior. *Drug Development Research*, 26(4), 439-447.
- Hoffman, J. R., Ratamess, N. A., Gonzalez, A., Beller, N. A., Hoffman, M. W., Olson, M., ... & Jäger, R. (2010). The effects of acute and prolonged CRAM supplementation on reaction time and subjective measures of focus and alertness in healthy college students. *Journal of the International Society of Sports Nutrition*, 7(1), 39.
- Inbar, O., Bar-Or, O., & Skinner, J. S. (1996). *The Wingate anaerobic test*. Champaign, IL: Human Kinetics.
- Kirkendall, D. T. (1990). Mechanisms of peripheral fatigue. *Medicine and Science in Sports and Exercise*, 22(4), 444-449.
- Moreno, M. D. J. M. (2003). Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial. *Clinical Therapeutics*, 25(1), 178-193.
- Parnetti, L., Abate, G., Bartorelli, L., Cucinotta, D., Cuzzupoli, M., Maggioni, M., ... & Senin, U. (1993). Multicentre study of l- α -glyceryl-phosphorylcholine vs st200 among patients with probable senile dementia of alzheimer's type. *Drugs & Aging*, 3(2), 159-164.
- Parnetti, L., Mignini, F., Tomassoni, D., Traini, E., & Amenta, F. (2007). Cholinergic precursors in the treatment of cognitive impairment of vascular origin: ineffective approaches or need for re-evaluation? *Journal of the Neurological Sciences*, 257(1), 264-269.
- Pauole, K., Madole, K., Garhammer, J., Lacourse, M., & Rozenek, R. (2000). Reliability and validity of the T-test as a measure of agility, leg power, and leg speed in college-aged men and women. *The Journal of Strength & Conditioning Research*, 14(4), 443-450.

- Taylor, P., & Brown, J. H. (1999). Synthesis, storage and release of acetylcholine In G. J. Siegel, B. W. Agranoff, & R. W. Albers (Eds). *Basic Neurochemistry: Molecular, Cellular and Medical Aspects* (6th ed.). Philadelphia, PA: Lippincott-Raven.
- Tokish, J. M., Kocher, M. S., & Hawkins, R. J. (2004). Ergogenic aids: a review of basic science, performance, side effects, and status in sports. *The American Journal of Sports Medicine*, 32(6), 1543-1553.
- Wurtman, R. J., & Lewis, M. C. (1991). Exercise, plasma composition, and neurotransmission. In *Advances in Nutrition and Top Sport* (Vol. 32, pp. 94-109). Basel, Switzerland: Karger Publishers.
- Ziegenfuss, T., Landis, J., & Hofheins, J. (2008). Acute supplementation with alpha-glycerylphosphorylcholine augments growth hormone response to, and peak force production during, resistance exercise. *Journal of the International Society of Sports Nutrition*, 5(1), P15.
- Zimmerman, G., & Soreq, H. (2006). Termination and beyond: acetylcholinesterase as a modulator of synaptic transmission. *Cell and Tissue Research*, 326(2), 655-669.
- Zivkovic, M. Z., Djuric, S., Cuk, I., Suzovic, D., & Jaric, S. (2017). Muscle force-velocity relationships observed in four different functional tests. *Journal of Human Kinetics*, 56(1), 39-49.

APPENDIX A
DATA SHEET

Alpha-Glycerophosphorylcholine and Its Effect On Anaerobic Exercise in Recreationally-Trained Females

Descriptive Information and Eligibility

Subject #: _____ Age: _____ Height: _____
Informed Consent Completed: _____ Par-Q Completed: _____
Collected By: _____ Eligible? Yes/No Enrolled? Yes/No

Session 1: α-GPC or Placebo (Circle one)

Date: ____/____/____ Time of Consumption: _____ Test Time: _____					
Overhead Reach: _____ in. Weight: _____ lbs.					
	Trial 1	Rest	Trial 2	Rest	Trial 3
Vertical Jump (in.)		2 min		2 min	
20 split/40 Yard Dash (sec.)	/	2 min	/	2 min	/
	Peak Power	Mean Power	Min. Power	Fatigue Index	Anaerobic Capacity
Wingate					

Session 2: α-GPC or Placebo (Circle one)

Date: ____/____/____ Time of Consumption: _____ Test Time: _____					
Overhead Reach (copied from above): _____ in. Weight: _____ lbs.					
	Trial 1	Rest	Trial 2	Rest	Trial 3
Vertical Jump (in.)		2 min		2 min	
20 split/40 Yard Dash (sec.)	/	2 min	/	2 min	/
	Peak Power	Mean Power	Min. Power	Fatigue Index	Anaerobic Capacity
Wingate					

APPENDIX B
HUMAN SUBJECTS APPROVAL

Full Review
12/20/2016
Protocol Number: 636

Dear Alex Rickard:

Your research project, ' α -Glycerophosphorylcholine and the Effects on Anaerobic Indices', was approved by the University of Central Missouri Human Subjects Review Committee on 12/19/2016. You may collect data for this project until **12/19/2017**.

If an adverse event (such as harm to a research participant) occurs during your project, you must IMMEDIATELY stop the research unless stopping the research would cause more harm to the participant. If an adverse event occurs during your project, notify the committee IMMEDIATELY at researchreview@ucmo.edu.

The following will help to guide you. Please refer to this letter often during your project.

- If you wish to make changes to your study, submit an “Amendment” through Blackboard under the “Amendment and Renewals” tab. **You may not implement changes to your study without prior approval of the UCM Human Subjects Review Committee.**
- If the nature or status of the risks of participating in this research project change, submit an “Amendment” through Blackboard under the “Amendment and Renewals” tab. **You may not implement changes to your study without prior approval of the UCM Human Subjects Review Committee.**
- If you are nearing the expiration date for collecting data for this project (12/19/2017) and you have not finished collecting data:
 1. submit your project application via Blackboard under the “Amendment and Renewals” tab (include any revisions and/or amendments approved since you submitted your application initially)

AND

 2. submit a “Renewal Report” through Blackboard under the “Final/Renewal Report” tab.
- **When you have completed your collection of data, please submit the “Final Report” found on Blackboard under the “Final/Renewal Report” tab.**

If you have any questions, please feel free to contact me at researchreview@ucmo.edu.

Sincerely,



Kathy Schnakenberg
Program Administrator/Research Compliance Officer
Office of Sponsored Programs and Research Integrity
University of Central Missouri

cc: garver@ucmo.edu

APPENDIX C
CONSENT FORM

α -Glycerophosphorylcholine and the Effects on Anaerobic Indices

Name: _____ **Date:** _____ / _____ / _____

Identification of Researchers: This research is being conducted by Alex Rickard (graduate student) and co-investigators Taylor Dinyer (graduate student) and Dr. Matthew Garver (professor). We are current graduate students at the University of Central Missouri.

Purpose of the Study: The purpose of this investigation is to examine the effects of α -glycerophosphorylcholine (α -GPC) ingestion on acute anaerobic performance as measured by the counter movement jump, 40 yard sprint, and 30 second Wingate anaerobic test performance.

Request for Participation: We are inviting you to participate in a study on the supplement α -GPC and its effects on anaerobic exercise performance. It is up to you whether you would like to participate. If you decide not to participate, you will not be penalized in any way. You can also decide to stop at any time without penalty. You may withdraw your data at any time. Results will be provided to you, at your request, after the completion of the second session.

Exclusions: You must be at least 18-24 years of age to participate in this study, female, non-pregnant, without lower body musculoskeletal injury, and not diagnosed with any CVD, metabolic disease, and/or pulmonary disease. You must pass the PAR-Q.

Description of Research Method: This study involves two sessions and ingesting two treatments (α -GPC and placebo). The first session you will ingest one of the two treatments. One hour after ingestion, you will be asked to complete a structured, researcher led warm-up. After the warm-up, you will complete 3 anaerobic tests: the vertical jump (VJ), 40 yard sprint, and the Wingate 30 second high intensity anaerobic cycling test. The first test will be the VJ. You will be instructed to complete a single movement downward before exploding up maximally, reaching up as high as possible. The second test will be the 40 yard dash. You will be instructed to run at maximal effort from start to finish. You will complete the VJ and 40 yard sprint 3 times. The final test will be the Wingate anaerobic cycling test. You will warm up for 5 minutes on the cycle ergometer. After warming up, you will pedal against a constant resistance for 30 seconds. Immediately following this test, you will cool down for 5 minutes. One week after your first session, you will return and follow the same testing protocol. At the second visit you will ingest the second treatment. The order of ingestion is randomized and double blinded. Neither you nor the investigators will know which one you ingest.

Privacy: Your information will be kept confidential. All information will be kept in a locked file cabinet and data sheets will be shredded at the appropriate time-point. For data analysis, all information and data will be de-identified so your personal information will not be linked to your results. Your personal results will be associated with a number. Your results will be stored on a password protected computer.

Explanation of Risks: The risks associated with participating in this study are similar to the risks involved with vigorous intensity exercise. As with any exercise there exists the possibility of certain changes occurring during the exercise. Risks include; delayed muscle soreness, an abnormal response of blood pressure, fainting, irregular fast or slow heart rhythm, and in rare instances, heart attack, stroke, or death. You will also be monitored by a research member at all times. Remember, you are allowed to stop testing at any point for any reason. If you require medical treatment or emergency service, any associated costs will be your responsibility. I understand the risk associated with testing. I attest I have had my questions at this time answered. INITIAL HERE_____

Explanation of Benefits: You will benefit from participating in this study by gaining firsthand experience in physiological research and receiving information about your anaerobic fitness. You may also enjoy completing the anaerobic exercise tests.

Questions: If you have any questions about this study, please contact the primary investigator, Dr. Matthew Garver (garver@ucmo.edu; 1(660)543-4629). If you have any questions about your rights as a research participant, please contact the UCM Research Compliance Officer at (660) 543-8562.

I understand this study is approved by the UCM Institutional Review Board. I acknowledge that I have read this document in its entirety. I consent to the rendition of all procedures as explained herein by all research personnel. My signature below attests to my full understanding, cooperation, and agreement.

Signature_____ Date:_____

Witness Signature:_____ Date:_____