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THE ACUTE EFFECTS OF ALPHA-GPC ON HAND GRIP STRENGTH, JUMP HEIGHT, POWER
OUTPUT, MOOD, AND REACTION-TIME IN RECREATIONALLY TRAINED, COLLEGE-AGED
INDIVIDUALS

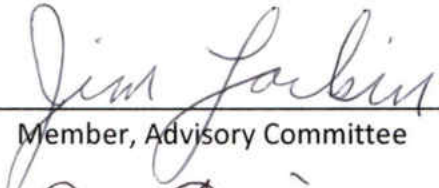
BY

JOSEY LUCAS CRUSE

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THE ACUTE EFFECTS OF ALPHA-GPC ON HAND GRIP STRENGTH, JUMP HEIGHT, RATE
OF FORCE DEVELOPMENT, MOOD, AND REACTION-TIME IN RECREATIONALLY
TRAINED, COLLEGE-AGED ATHLETES

BY

JOSEY LUCAS CRUSE

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2018

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DEDICATION

This document is dedicated to my parents Felix and Lisa Cruse, whom without them; I would not be the person I am today. I would like to thank them both for all their love and support in my decisions to pursue my education.

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I would like to thank my research committee who helped me with this research project. Dr. Lane, thank you for helping me every step along the way during this massive project. Dr. Sciascia, thank you for helping me run the stats. Dr. Adams-Blair (aka Dr. A), thank you for all your encouragement and help with all the grammatical issues in the study. Dr. Larkin, thank you for all your assistance and feedback. I would like to recognize all the investigators during this investigation. Lee Doernte, J.P. Isaacs, Pete Collopy, and April Spears, thank you all for everything you all did to help me during this journey.

God Bless.

ABSTRACT

Introduction: While alpha-GPC (A-GPC) has received recent praise as a mental and physical performance enhancement supplement, very limited research on this topic exist, and no current studies examine A-GPC in collegiate-aged trained individuals. This study hopes to bridge the gap in the current research literature on the topic of A-GPC ingestion in young healthy adults in the cognitive and anaerobic paradigms.

Purpose: To investigate and identify the acute effects of A-GPC ingestion on performance testing (hand-grip strength, jump height, power output/rate of force development, mood, & reaction time) of college-aged recreationally trained individuals.

Methods: This study utilized a random, double-blinded, cross over design in which 27 participants (m=15; f=12) ingested either the placebo (dextrose, Group 1) A-GPC (6 mg·kg⁻¹ body mass, Group 2). Baseline measurements were obtained on the initial visit. On supplemental visits, immediately following ingestion, subjects were required to sit in a rested state for 25-minutes. Subjects first completed the 4-item mood questionnaire to assess subjective feelings of energy, fatigue, alertness and focus for tasking, followed by the pre reaction-time (RT) test. Body composition was then measured via the Bod Pod, followed by a standardized questionnaire indicating age and training experience. Each test consisted of four stations assessing physical task performance. Subjects participated on three different occasions separated by 2-14 days.

Hypotheses: The author of this investigation predicted A-GPC would have a positive impact on the performance variables, improving power output and neurological stimulation. Furthermore, this study aimed to support preliminary evidence of the ergogenic properties in A-GPC

Results: Twenty-seven total subjects participated in this study (Age: mean 21.66 ±SD 1.88 years; Height: mean 68.4 ±SD 4.1 inches; Weight: mean 169.02 ±SD 32.2 lbs.; Body fat: mean 19.2 ±SD 8.8%; Heart rate: mean 67.96 ±SD 8.2 beats/minute; SBP: 120.59±SD12.39 mmHg; DBP 74.74±SD 9.12 mmHg). There were no significant differences between any of the variables pre to post testing. There were also no differences between males and females for any test variable. Repeated measures ANOVA: Multiple comparisons, LSD reveal statistical significances were found for the following the dependent variables (fatigue, plyometric push-up, mood change, and reaction-time test). Participants reported lower levels of fatigue following A-GPC ingestion, yielding a significance of (p=0.019) when compared to baseline. The A-GPC group showed increased levels of peak force production during the plyometric push-up when compared to placebo with significance of (p=0.014). Mood change increased with A-GPC ingestion compared to placebo with significance of (p=0.023); and subjects scored more accurate (less incorrect answers) during the reaction-time test post A-GPC ingestion compared to placebo (p=0.014).

Discussion: A-GPC supplementation showed a 12% increase in upper body power output, and 12% improvement in accuracy during the reaction-time test. This investigation supports A-GPC as an ergogenic aid for physical or cognitive stimulation.

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CHAPTER I

INTRODUCTION

Alpha-GPC

Alpha-glyceryl-phosphoryl-choline (A-GPC) supplementation has been shown to improve mental and physical performance (Parker et al., 2015), increasing mood and potentially power-output. While literature looks promising, only one pilot study exists examining the acute effects of power-output (Ziegenfuss et al., 2008), with the majority of literature on A-GPC being experimented on elderly persons and rodents. Therefore, more research in young healthy adults is needed to support A-GPC as an ergogenic aid (allowing the person to train with greater intensity for longer durations), possibly increasing force production.

A-GPC is a choline containing phospholipid (Tayebati et al., 2013), an intermediary of lecithin breakdown or simply lecithin molecule with two less fatty acids (Zeisel, 1981). The supplement A-GPC is found as a naturally occurring constituent of red meat products (Armah et al., 2008) and organ tissue (Schmidt et al., 1945). However, it can be challenging to consume effective amounts in naturally occurring sources (Zhang et al., 2012) therefore, it can be difficult to obtain enough A-GPC in an individual's diet to be beneficial, hence, supplementation may be necessary. A-GPC is organically created from egg (Brockerhoff & Yurkoski, 1965) or soy lecithin (Brownawell et al., 2011). When controlling the amount of choline in each supplement, A-GPC requires 46% the dose of CDP-Choline (also known as citicoline) (Tayebati et al.,

2013). A-GPC revealed to be more effective, when tested by potency on a weight basis, as a cholinergic, than both choline and CDP-choline when looking at serum values or benefits from clinical research (Parnetti et al., 2007; Di Perri et al., 1991).

Evidence supports citicoline and choline as positively impacting cognitive function, but little is known about either supplement's impact on physical function. The literature does contain some evidence that choline itself is vital to consider in respect to endurance performance (Conlay et al., 1986; Penry & Manore et al., 2008) revealing evidence of aerobic ergogenic properties in choline. One study reported that a multi-ingredient supplement containing A-GPC improved mean power during maximal effort sprint testing on a non-motorized treadmill (Jajim et al., 2015), illustrating anaerobic properties in A-GPC as an ergogenic aid. However, common performance tasks such as vertical jump and hand-grip strength (often used to assess physical function ability in both athletic and non-athletic individuals) have not been investigated.

Theoretically, with A-GPC being a more potent form of choline than citicoline, it would be logical to assume the supplement would serve as a more effective additive to physical performance efforts. Although A-GPC has been receiving recent praise as a performance enhancing supplement for some physical (Stoppani, 2010; Bellar et al., 2015; Ziegenfuss et al., 2008) and neurological aspects of human function (Scapicchio, 2013; Tomassoni et al., 2006), more research in human subjects on this topic is needed.

Of concern is that there is conflicting information in the literature regarding the effects of A-GPC. For example, previous literature found that 30 minutes prior to testing, A-GPC supplementation ($4 \text{ mg}\cdot\text{kg}^{-1}$) had no significant beneficial effect on mood, cognitive function, and physiological performance (Parker et al., 2015). Conversely, one study presented at the 2008 Annual Meeting Of The International Society Of Sports Nutrition reported that trained men taking ($6 \text{ mg}\cdot\text{kg}^{-1}$) A-GPC 90-minutes before workouts increased growth hormone (GH) levels by about 100% more than placebo (Stoppani, 2010). The lone pilot study on power output found evidence, that supplemental ingestion of A-GPC, given ($6 \text{ mg}\cdot\text{kg}^{-1}$) prior to a power test (bench throws), reported a 14% power output improvement relative to a placebo when taken 45-minutes prior to activity (Ziegenfuss et al., 2008). These findings revealed supplementation also increased post-exercise serum GH. In contrast, supplementation showed no significant beneficial effect on peak power, rate of force development, RMR, or cardiovascular hemodynamics, such as heart rate and blood pressure. However, one study revealed A-GPC supplementation may augment lower body force production. These findings reveal a 3% increase in lower body isometric strength, following 6 days of acute A-GPC supplementation (Bellar et al., 2015). This contrast in results supports the need for further investigations into A-GPC and its impact on physiological performance.

Summary and Objectives

Previous claims support the idea that A-GPC as a supplement can boost physical and mental performance; however, there is a gap in literature, noting only a hand full of research exist on this topic in human subjects. Therefore, this study aims to fill this gap in the literature, in regard to A-GPC as a power output and cognitive supplement in young healthy adults. The purpose of this investigation was to identify the acute effects of A-GPC in testing to placebo ingestion on jump height, hand-grip strength, rate of force development, power output, mood change, and reaction time in recreationally (moderately) trained, college-aged athletes. The author of this investigation predicts A-GPC ingestion will improve physical task performance on jump height, hand-grip strength, rate of force development, power output, mood, and reaction time in moderately trained young healthy adults. Primarily, the author of this study believed ergogenic properties in the supplement A-GPC exist, beneficially influencing power output and enhanced brain function.

Limitations

Subjects were subjectively screened using questionnaires only. It's possible a physical by an MD with blood work may have revealed differences within and between subjects possibly affecting the results. Not all performance tasks were utilized. It's possible different power tests and/or different reaction tests could elicit different results. It's possible different age groups and/or sub-groups (athletes, non-athletes, pediatrics, geriatrics, etc.) could have responded differently.

Delimitations

This investigation only examined participants (18-26y) because there is currently a large body of research in elderly persons and high number of college-aged individuals. Subjects received body composition opposed to underwater weighing because it is faster, and easier. The mood questionnaire assessed levels of energy, fatigue, alertness, and focus; used to determine an individual's mood because it was easy and self-reported on a scale. Reaction time was measured to determine accuracy and speed of response-time because it was a simple and easy for use. Hand-grip strength was used via the hand-grip dynamometer, a good overall indicator of strength, and easy to use. Standing vertical jump was measured to determine jump height and peak force production via the jump mat over the VerTec for its accuracy and simplicity. The plyometric push-up was tested to measure peak force production where only one preliminary piece of literature has supported upper body power output following A-GPC ingestion. Therefore, this investigation aims to bridge this gap on the current literature with supportive evidence pertaining to power output.

CHAPTER II

LITERATURE REVIEW

Background

Choline is a precursor required to produce acetylcholine (Parnetti et al., 2007). This is clinically valuable due to vast research suggesting learning, memory, intelligence, and mood are stimulated in part by acetylcholine metabolism in neural function (Hasselmo et al., 2006). L-alpha-glycerylphosphorylcholine (GPC) is a water-soluble, natural choline compound, deacylated phosphocreatine(PC) derivative, which evidence supports effectiveness against the loss of membrane function in central nervous system(CNS) injuries (Amenta et al. 1994).

Sangiorgi and colleagues (1994) noted, “GPC is a precursor molecule of the neurotransmitter acetylcholine and was recently tested as a centrally acting parasympathic precursor molecule of the neurotransmitter acetylcholine” (p. 253-69), and evidence support it as a centrally acting parasympathomimetic drug in dementia disorders and acute cardio vascular diseases (De Jesus Moreno Moreno 2003). GPC acts as a PC precursor and increases in uptake of membrane (Galazzini and Burg, 2009). This increased uptake of membrane-forming phospholipids, and PC exerted an anti-inflammatory stimulation in previous investigational research (Chao et al, 1995, El-Hariri et al. 1992). Parnetti and colleagues (2007) noted, “The deacylated, water soluble PC analogue GPC rapidly delivers choline to the brain across the blood-brain-barrier (BBB)” (264-69). Another study found that pre-treatment with GPC is protective

against CNS irradiation-induced peripheral effects (Tóké's et al., 2014). GPC is a choline donor compound where a recent experimental study revealed parasympathomimetic action (Gibellini & Smith, 2010). This parasympathomimetic action illustrates its activation of the “rest and digest” division of the nervous system.

A-GPC has been shown to increase the release of neurotransmitter of acetylcholine and facilitates learning and memory (Scapicchio, 2013). Another study found higher acetylcholine production allows A-GPC to boost GH response to weightlifting (Stoppani, 2010). One investigation observed the effects of A-GPC, caffeine or placebo on markers of mood, cognitive function, power, speed, and agility, where A-GPC prevented exercise-induced reductions in exhaustive levels (Parker et al., 2015). The only current evidence supporting A-GPC supplementation as effective for nootropic purposes in young healthy adults (18-35y) found A-GPC (Alpha BRAIN®) ingestion improved recent verbal memory when compared to controls (Solomon et al., 2016) as it appears to have cognitive improving properties. However, more research is needed because no current evidence in humans can be found in the literature. However, previous investigations supported A-GPC intake in rodents (Lopez et al., 1991; Amenta et al., 2001; Trabucchi et al., 1986; Abbiati et al., 1993). More evidence supports A-GPC supplementation on neuroprotective properties for ischemia attack, or stroke patients (Zhang et al., 2013; Kidd et al., 2009). Other findings reveal A-GPC may decrease the rate of cognitive decline when consuming (1200mg) of A-GPC daily (Scapicchio, 2013; Tóké's et al., 2015). Zhang and colleagues (2013) noted, “Plasma choline increased 30-130 minutes’ post-ingestion (spikes at 60 minutes)” (p. 96-103)

and returns to baseline at 4 hours (Kawamura et al., 2012) with A-GPC supplementation. A-GPC in Europe currently is being used as treatment of head trauma and other acute brain injuries in addition to stroke (Onishchenko et al., 2008).

Primary Studies

Preliminary evidence found A-GPC may be beneficial for power output, revealing a 14 % increase on bench throws in men with two years of training experience (Ziegenfuss et al., 2008), when consuming ($6 \text{ mg}\cdot\text{kg}^{-1}$) of A-GPC 45-minutes prior to exercise; this being the lone experimental study on power output following A-GPC consumption. Although the ergogenic properties in A-GPC look promising, more research is required to validate this evidence.

Stoppani (2010) found evidence that A-GPC consumption of ($6 \text{ mg}\cdot\text{kg}^{-1}$) 90-minutes before exercise increased growth hormone levels by about 100% more than placebo. Another study revealed evidence that A-GPC ingestion enhances strength, particularly in the lower body after six days of administration when taking ($6 \text{ mg}\cdot\text{kg}^{-1}$) of A-GPC 60-minutes prior to physical activity (Bellar et al., 2015). Other literature also found when taking ($6 \text{ mg}\cdot\text{kg}^{-1}$) of A-GPC prior to physical activity that acute supplementation was shown to augment bench press in a small sample of men with 2 years of training experience (Ziegenfuss et al., 2015). One study found ($4 \text{ mg}\cdot\text{kg}^{-1}$) of A-GPC 30-minutes before exercise prevents exercise induced reductions in choline levels, increases endurance performance and GH secretion (Parker et al., 2015). (See Table 1 for previous studies on acute supplementation of A-GPC).

The most recent study evaluating the effects of A-GPC on physical and psychomotor performance found A-GPC (2.5 mg·kg⁻¹) consumption prior to exercise improved maximal velocity and maximum mechanical power on counter movement jump (Marcus et al., 2017). Based on previous literature regarding A-GPC, it seems logical for the supplement to be at least considered as an emerging ergogenic aid.

Table 1: Previous Studies on Alpha-GPC

Investigator	Nutrient Timing	Findings
Stoppani, 2010	(6 mg·kg ⁻¹) 90-min	Increased GH levels 100% more than placebo
Ziegenfuss et al, 2008	(6 mg·kg ⁻¹) 90-min	Improved p-output 14% relative to placebo
Bellar et al., 2015	(6 mg·kg ⁻¹) 60-min	Enhanced strength, particularly in lower body after 6-days of administration
Parker et al., 2015	(4 mg·kg ⁻¹) 30-min	Prevents exercise induced reductions in choline levels, increases endurance performance, and growth hormone secretion
Marcus et al., 2017	(2.5 mg·kg ⁻¹) 120-min	Improved maximal velocity and mechanical power on counter movement jump
Hoffman et al., 2010	(1.5 mg·kg ⁻¹) 10-min	Prevent exercise-induced decline in subjective feelings of focus and alertness following exhaustive exercise

Reaction time was maintained post-exhaustive exercise (Parker et al., 2015). A study by Parker and colleagues (2015) found “acute supplementation with A-GPC had no statistically significant beneficial effect on measures of mood, cognitive function, or physiological performance, in part due to large individual variability between subjects”

(p. 41). However, A-GPC seemed to improve physical and mental performance tasks.
(See Figure 1 for a recent study on A-GPC and Caffeine).

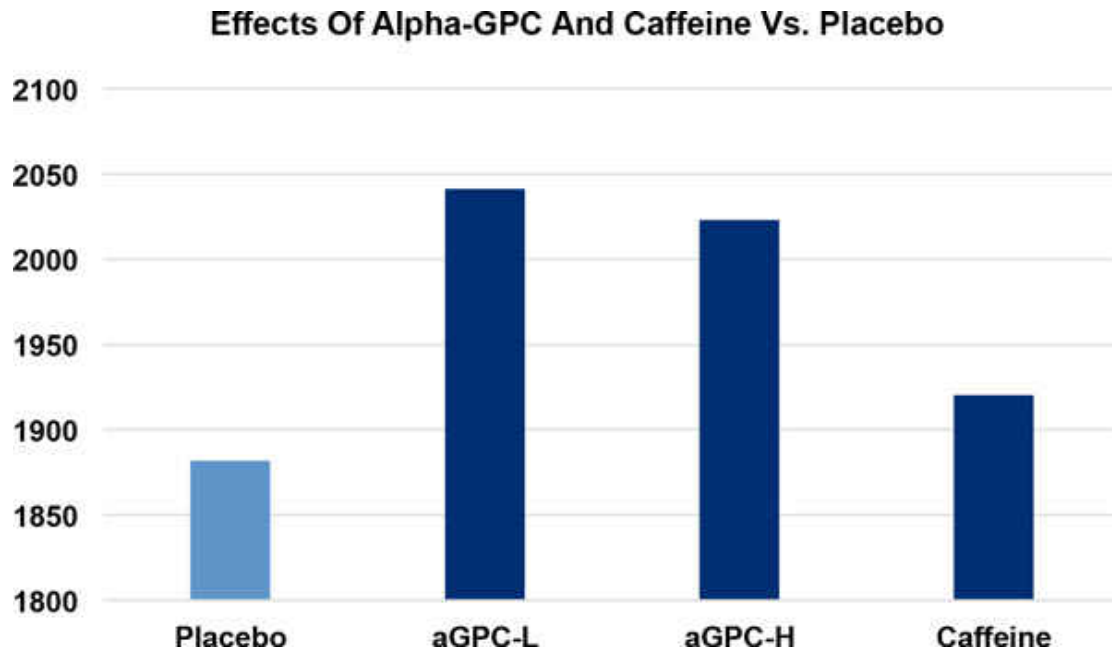


Figure 1: The effects of alpha-GPC-L (+8.5%), alpha-GPC-H (7.5%), and caffeine (+2.0%), in comparison to placebo, on vertical jump peak power (in watts) (Parker et al., 2015)

Growth Hormone Response to A-GPC

Ceda and company (1992) noted, “Growth hormone secretion in response to stimulation (via GHRH) is amplified with administration of A-GPC, and although it can be effective in both young and elderly it appears that elderly persons require a greater relative increase, due to having less output initially” (p. 119-21). A-GPC’s primary target is to augment the growth-hormone response to exercise, which can subsequently induce the release of the anabolic hormone insulin-like growth factor-1, which alone is enough to make A-GPC a valuable supplement (Barr, 2010). A-GPC

ingestion ($3-6 \text{ mg}\cdot\text{kg}^{-1}$) of may be beneficial for power output and act as an ergogenic aid (Ziegenfuss et al., 2008).

Previous literature found A-GPC was able to maintain reaction time in humans following exhaustive exercise (Hoffman et al., 2010). One study illustrated A-GPC provided evidence for power output improvements following ingestion for upper body force production during bench throws (Ziegenfuss et al., 2008). Another study found increases in lower body strength but failed to find evidence for improved upper-body strength during isometric testing. A-GPC seems promising however; more evidence is required to validate the ergogenic effects of acute supplementation. A-GPC has shown to augment acetylcholine levels in neurons in rat CNS (Traini et al., 2013). More research needed to determine the neurological and physical properties of A-GPC in human subjects. No studies look at chronic hormone spikes from A-GPC supplementation. One study revealed A-GPC supplementation may augment lower body force production. These findings reveal a 3% increase in lower body isometric strength, following 6 days of acute A-GPC supplementation (Bellar et al., 2015). Therefore, athletic trainers and sport professionals may consider adding A-GPC to the diet of athletes to improve muscle performance. This investigation hopes to shed new light on these contradictive findings for upper body power output during the plyometric push-up.

Nutrient Interactions

Previously literature claim A-GPC may have a synergistic effect with caffeine (Bruce et al., 2014). Uridine may be effective in supplemental form to increase brain GPC concentrations (Agarwal et al., 2018), but contradictive findings reveal this may not be the case (Silveri et al., 2008). The current literature gives mixed evidence on the influence A-GPC has on interacting with iron. One study found A-GPC supplementation improved absorption of nonheme iron from food products comparable to that of vitamin C when given at 2:1 ratio (Armah et la., 2008). However, another study failed to find evidence supporting this theory when doubling the ratio to 4:1 (Troesch et al., 2009).

Performance Testing

Mood Questionnaire. The 4-item mood questionnaire was created to assess subjective feelings of energy, fatigue, alertness and focus for tasking (Hoffman et al., 2010) consisting of a 10-point rating scale by verbal selection according to previously used methods. Specifically, subjects were asked to rate their response using verbal cues on a scale of a 1-10 which corresponded from 1= very low to 10 = very high. One previous study found acute ingestion of an A-GPC containing supplement can prevent the exercise-induced decline in subjective feelings of focus, and alertness in healthy college students following exhaustive exercise (Hoffman et al., 2010). Although cognitive influencing properties of A-GPC seem promising, more research is required to clarify these nootropic benefits.

Hand Grip Strength

The purpose of this test was to measure the maximum isometric strength of the hand and forearm muscles and is a good overall indicator of strength. A handgrip dynamometer needed to perform this assessment. Currently there has been research examining the acute effects A-GPC supplementation has on hand-grip strength and this investigation is the first to look at these variables. (See Appendix A for figure).

Standing Vertical Jump Test

The purpose of this test was to assess average jump height and peak jump performance. Vertical jump was recorded using the Just Jump via force plates to examine rate of force development and peak force production. Previous literature found improvement during the counter movement jump following A-GPC ingestion (Marcus et al., 2017). (See Appendix A for illustration)

Plyometric Push-up

The purpose of the plyometric push-up was to assess impulse and rate of force development. This test determines how much upper extremity force one can produce and how fast the individual can produce it. While only a preliminary piece of literature exists, giving evidence on improved power output (Ziegenfuss et al., 2008) following A-GPC ingestion. Another study (Bellar et al., 2015) found no evidence to support upper body power output post A-GPC consumption. Therefore, this investigation hopes to

shed new light on these contradictory findings following A-GPC ingestion on upper body power output. (See Appendix A for execution during the plyometric push-up).

Reaction-time Test

Reaction-time test using PsychoPy-Psychology software for Python (V1.82) determined reaction time in participants. This is a computer-based reaction time test that allows for custom programming. It also has the ability to program multiple scenarios, preventing errors from participants memorizing patterns of other reaction-based tests (Peirce, 2007). The purpose of this test, originally written by Peirce as a proof of concept – is that a high-level scripting language could generate experimental stimuli in real time. Previous literature indicated acute ingestion of a supplement containing A-GPC can prevent induced-decline of reaction time in healthy college student's following exhaustive exercise (Hoffman et al., 2010). However, limited studies exist examining reaction time following A-GPC ingestion and more research is needed to support the current evidence. (See Appendix A for figures of the reaction-test).

Summary

This examination explores the influence of A-GPC in various anaerobic paradigms with particular attention given to the impact on performance variables. While there are lots of promise regarding A-GPC as a supplement to boost physical and mental performance, there is currently not enough literature examining A-GPC to

make this conclusion. Therefore, this study aims to fill the gap in the literature, in regard to A-GPC as a power output and cognitive supplement for college-aged individuals. The purpose of this investigation was to identify the acute effects of A-GPC in testing to placebo ingestion on jump height, hang-grip strength, power output, mood change, and reaction time in recreationally (moderately) trained, college-aged athletes. The author of this investigation predicts A-GPC ingestion will improve physical task performance on the aforementioned variables, benefiting cognitive function and power output. Currently only one piece of literature examines the acute effects A-GPC ingestion has on power output, where the population was men ($30.1 \pm 7y$) with two years of training experience (Ziegenfuss et al., 2008). Therefore, this research investigation was designed in efforts to bridge the gap in the current dearth of literature, in hopes to shed new light on A-GPC as a power output supplement one can use as an ergogenic aid to enhance strength via anaerobic paradigms, as well as stimulate cognitive facilitation, improving mood and reaction time in young healthy moderately trained adults.

CHAPTER III

METHODOLOGY

Inclusion/Exclusion

The following inclusion criteria was established: College aged (18-26), recreationally (moderately) trained, healthy volunteer (resting BP under 140/90 mmHg, resting HR < 100 bpm). According to the American College of Sports Medicine (Thompson et al., 2010), moderate training includes aerobic activity performed for a minimum of 30-min five times a week. Health history was assessed using a standardized university form to ensure that all applicants met the current standards and regulations of being a healthy participant in order to participate in the trials. Subjects were excluded from the study if the individual was untrained in recreational related activities (did not meet the aforementioned requirements of being recreationally trained); unhealthy (did not meet aforementioned requirements of being a healthy volunteer); or had a resting BP over 140/90 mmHg and/or a resting HR < 100 bpm.

This research project took place in a university exercise physiology lab, where 35 subjects were recruited to participate in the study. Twenty-seven subjects completed all three visits looking at the acute effects of A-GPC on performance enhancement.

Prospective participants were recruited via recruitment letters and emails sent by the primary investigator. The cover letter and email briefly explained information

about how the participant was identified to receive the letter; who was responsible for conducting the study and why; the study procedures; overview of any risks or potential benefits; contact information; and proper notification about the confidentiality issues associated with email communication.

Research Procedures

Study Design. The study utilized a random, double-blinded, cross over design where subjects reported to the laboratory on three different occasions with each visit separated by 2-14 days. All subjects read and signed the informed consent application prior the initial familiarization trial, which included instructions on how to complete a 24-hour food log, height and weight measurements, body plethysmography via BOD POD (Cosmed, USA), mood questionnaire, reaction time test, hand-grip strength, standing vertical jump, and the plyometric push-ups. Upon arrival to the testing facility and completion of informed consent procedures, subjects performed a brief standardized dynamic warm-up in preparation for physical activity.

Following the dynamic warm-up, subjects were directed through the performance testing protocol where stations were structured in the following order; mood questionnaire, reaction-time test, hand-grip strength, standing vertical jump, and plyometric push-up (See Table 2 for procedure outline, Figure 2 for schematic of stations and Appendix 1 for descriptions of each maneuver).

Table 2: Procedure Outline

Baseline Visit	Placebo Visit	A-GPC Visit
Height	Ingestion	Ingestion
Mood Questionnaire/ Reaction-Time Test-PRE	Mood Questionnaire-PRE	Mood Questionnaire-PRE
Blood Pressure/ Heart Rate/ Body Composition	Reaction-Time Test-PRE	Reaction-Time Test-PRE
Reaction-Time Test-PRE	Body Composition/ Heart Rate/ Blood Pressure	Body Composition/ Heart Rate/ Blood Pressure
Hand-Grip Strength	Hand-Grip Strength	Hand-Grip Strength
Warm-Up	Warm-Up	Warm-Up
Standing Vertical Jump	Standing Vertical Jump	Standing Vertical Jump
Plyometric Push-Ups	Plyometric Push-Ups	Plyometric Push-Ups
Mood Questionnaire-POST	Mood Questionnaire-POST	Mood Questionnaire-POST
Reaction-Time Test-POST	Reaction-Time Test-POST	Reaction-Time Test-POST

The subjects and research assistants who supervised the stations of the test were blind to the treatment. Subsequent testing sessions included the same procedures; the only difference was subject randomization into one of two groups prior to completing the testing stations. Group 1 (placebo trial) was required to consume (Dextrose); Group 2 consumed 6 mg·kg⁻¹ (A-GPC group). All test subjects were instructed to refrain from consuming food; caloric beverage and A-GPC substances at least 8 hours prior to each trial; and instructed to come in on a fasted state during testing days. Understanding nutrient-nutrient interaction between A-GPC combined with either uridine, iron, or caffeine may potentially skew the results (Troesch et al., 2009; Armah et al., 2008; Agarwal et al., 2018; Silveri et al., 2008). Therefore, the participants were asked to come in on a fasted state before treatment trials (visits 2 &3). Coming in on a fasted state requires the participants not consume anything eight hours prior to supplement ingestion on the treatment trials, preventing any possible unknown health-related risk or nutrient interaction that may exist.

Subjects were asked to continue current training throughout the duration of the study. However, subjects were instructed to abstain from hard training (exerting maximal effort to exhaustion) for 24 hours prior to each visit. Similar to baseline testing, the subjects completed blood pressure measurements, heart rate, mood questionnaire, and reaction-time testing each visit. At the end of the third and final session, data analysis compared the effects of the nutrient on physical task performance. Each trial was administrated, conducted, and separated by 2-14 days.

Testing Procedure

Baseline/Familiarization (Pre-Treatment). Subjects underwent a baseline trial during their first visit to the laboratory where subjects submitted a completed 24-hour food log that listed all foods and beverages the subject consumed in the previous 24-hours. This was used to replicate caloric and macronutrient intake for the 24-hours prior to future trials. Subjects then completed baseline assessments for height, weight, blood pressure, and heart rate, followed by guidance through a standardized warm-up and completion of the aforementioned performance testing protocol. Subjects were asked to come to the treatment visits in a fasted state and to abstain from hard training for 24-hours prior to performance testing.

24-hour Food Log/Alpha-GPC Questionnaire/Health History

Subjects were provided with a list of foods and beverages that contained A-GPC (See Appendix 3 for nutritional sources on A-GPC), and subjects were asked to refrain

from consuming these foods for 24-h before exercise testing. Subjects were provided with a 24-h food log before the first testing session, so each subject can replicate their diet before each of the following testing sessions. Subjects reported regular consumption of A-GPC via a questionnaire.

Equipment

Upon arriving to the laboratory, subjects first obtained baseline measurements for blood pressure and heart rate using a digital blood pressure cuff (Omron, USA), height was assessed using a tape measure from the base of the floor to the peak height of the individual (i.e. “wall height”), weight and body fat percentage was measured using body plethysmography via BOD POD (Cosmed, USA) following ingestion. The BOD POD is highly accurate and can detect even small changes in body fat and body mass (National Institute for Fitness & Sport, 2017). A digital blood pressure cuff was used to determine BP and HR, and PsychoPy-Psychology software (Python, V1.82) was used to determine reaction time. The Just Jump Mat (Probotics, INC: Huntsville, AL) was used to assess force kinetics and jump height, the digital hand-grip dynamometer (Takei Scientific Instruments, Japan) assessed overall strength while the force plates (Summit, INC) were used to determined peak force output and RFD during the vertical jump and plyometric push-ups. The force plate data was interfaced with a NI DAQ (national instruments) with custom written LabVIEW software (national instruments).

Treatment Protocol

Supplementation Administration (Dosage). Prior to testing, on the second and third visits, subjects were randomly provided with a moderate dose of either A-GPC (6 mg·kg⁻¹) or the same dosage of placebo (dextrose). The samples were weighed and placed in a cup and mixed with 10 ounces of water, assigned in a randomized fashion. Test subjects consumed a different substance on each occasion, outside of the baseline trial where no substance was ingested. Subjects were instructed to sit in a rested state for 25-minutes following ingestion of either the supplement or placebo. The assigned supplement was prepared and delivered to the laboratory by a member of the research team outside of the testing facility. Each sample was labeled with the subject's name on a shaker bottle. These procedures were followed in order to maintain the double-blind procedure. During placebo visits, subjects ingested a dose of dextrose that was administered and calculated to increase plasma glucose concentrations by <0.3 mmol. L⁻¹. These amounts have been utilized in previous literature and therefore were adopted for this current study (Anselme et al., 1992; Schneiker et al., 2006; Bell et al., 2001; Stuart et al., 2005; Astorino et al, 2010; Paton et al., 2010; Bellar et al., 2015; Stoppani, 2010; Ziegenfuss et al., 2008). The placebo consisted of dextrose, which was designed to mimic the actual supplement in terms of consistency for taste, texture, and color.

Warm-up Protocol

Following the treatment protocol, prior to the testing battery, subjects were directed through standardized dynamic warm-up station, consisting of 10 body weight squats, and 10 push-ups, in preparation for physical activity. Subjects were assessed on hand-grip strength, jump height, rate of force development, power output, mood, and reaction-time.

Testing Protocol

Following a 25-minute passive rest period following ingestion of the supplement or placebo, subjects performed a mood questionnaire. The mood questionnaire was completed post-ingestion, directly before each reaction-time test. The same researcher administered the questionnaires each time. Body composition, blood pressure and heart rate measurements were then obtained. Subjects then completed the warm-up protocol and participated in the performance test. The test took place in the exercise physiology laboratory, designed with four stations assessing physical task performance, specifically measuring hand-grip strength, jump height, power output, mood, and reaction-time. (See Figure 2 for schematics of stations).

Schematics of Stations

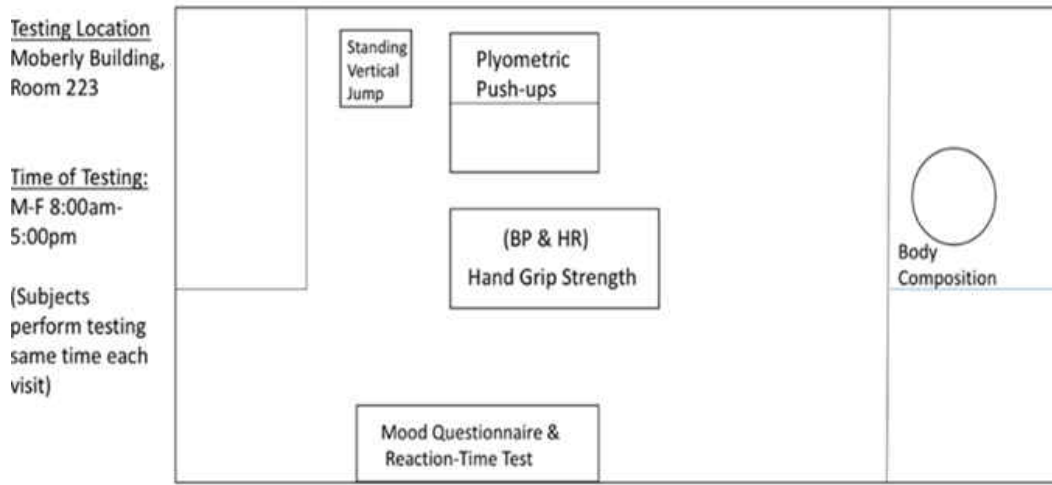


Figure 2: Schematics of Stations

The primary investigator and research team inspected the testing area prior and post each visit to ensure participant safety. Participants who noticed any unordinary discomfort during the duration of the performance testing were terminated from the study in order to limit any potential minimal risk. To help limit muscle soreness and body fatigue, participants were asked to refrain from high intensity exercise 24-hours leading up to each visit. Maximum efforts were taken to ensure participant safety, limiting any potential health-related risk.

Reaction Time coding

When analyzing the reaction time test, the column titles indicate the different combinations of manipulated variables. The first three letters indicate the flanker type. Flankers are the shapes that appear on the left and the right of the target participants

respond to. Con – congruent, which means the target is flanked by arrows that point the same direction, like this <<<<< and this >>>>>. Inc – incongruent, which means the target is flanked by arrows that point in the opposite direction, like this <<><< and like this >><>>. The fourth through sixth letters indicate the cue type. The cue is what appears right before the target and flankers. Val – Valid Cue, which means that the cue appears at the same location as the target (above or below the central fixation cross). Ori – Orienting Cue, which means that the cue appears at the center of the screen, but does not indicate the location of the target. It just alerts the participant that the target is about to appear. Non – No cue appears. The last letters indicate the dependent variable. RT – response time, which is reported in seconds, and only includes trials with accurate responses. Acc – proportion accurate. For example: ConValRT – congruent flankers, valid cue, Response time; ConOriRT – congruent flankers, orienting cue, Response time; ConNonRT – congruent flankers, no cue, response time; incValRT - incongruent flankers, valid cue, Response time; incOriRT - incongruent flankers, orienting cue, Response time; incNonRT - incongruent flankers, no cue, response time and so forth.

Statistical Analysis

A statistical power analysis was performed prior to testing to determine the adequate number of subjects needed to find a 10% difference in task performance between the placebo and A-GPC ingestion. Using a beta level of 0.80 was used to determine the probability of accepting the null hypothesis when it is false and an alpha

level of 0.05 determined the probability of rejecting the null hypothesis when it is true, and sample size was determined to be 11 subjects per group. Inter-rater reliability was established for the assessment of HR, BP, and hand-grip strength. Utilizing a 2-way mixed effect model and a consistency of agreement design (3, k), (ICC) intra-class correlations coefficients were determined to be between .80-.98 for the metrics.

This investigation ran statistics on the continuous variables to determine mean and standard deviation and frequencies and percentages for categorical variables. Normality was examined using a Shapiro-Wilk test, which indicated the study variables were not normally distributed ($p \leq 0.05$). Non-parametric Mann-Whitney-U rank sum tests were performed to determine if a difference existed between pre and post testing for the following variables (mood change, reaction-time test, hand-grip strength, and jump height, upper and lower body rate of force development and peak force production). Finally, in order to assess differences between the two supplementation options and the study variables, a repeated measures analysis of variance (RMANOVA) was performed. Multiple comparisons were assessed in a post-hoc fashion using a least squared difference (LSD) procedure. Analyses were performed using a using SPSS software (Version 24, IBM, Armonk, New York). The level of significance was set at $p < 0.05$ for all comparisons. Data was normalized to baseline results, change scores represented the difference between the supplementation trial in comparison to the baseline.

CHAPTER IV

RESULTS

Descriptive Statistics

Twenty-seven total subjects (m=15; f=12) participated in this study (Age: mean 21.66 ±SD 1.88 years; Height: mean 68.4 ±SD 4.1 inches; Weight: mean 169.02 ±SD 32.2 lbs.; Body fat: mean 19.2 ±SD 8.8%; Heart rate: mean 67.96 ±SD 8.2 beats/minute; SBP: 120.59±SD12.39 mmHg; DBP 74.74±SD 9.12 mmHg). Frequency and percentages found 56% (m=15) of the participants in the study were males, and 44% (f=12) of the subjects who completed the study were females. (See Table 3 for mean difference and standard deviation results for demographics of the study).

Table 3: Descriptive Statistics

	Age	HT	BM	Bfperc	SBP	DBP
Mean	21.667	68.380	169.022	19.233	120.593	74.741
SD	1.881	4.106	32.226	8.796	12.395	9.121
Range	8	15	109.6	32.8	48	35
Minimum	19	60	109.8	7.9	92	55
Maximum	27	75	219	40.7	140	90
Count	27	27	27	27	27	27

There were no statistically significant differences between any of the variables pre to post testing. There were also no significant differences between males and females for any test variable.

Repeated measures ANOVA: Multiple comparisons, LSD

Results revealed statistical differences for the following dependent variables of this study: fatigue, plyometric push-up peak force production, mood change, and accuracy during the reaction-time test. When examining fatigue during the mood questionnaire, the placebo and A-GPC groups reported lower levels of fatigue compared to baseline (0.719 points, $p \leq 0.019$). Peak force production during the plyometric push-up was significantly greater (12% $p \leq 0.014$) for A-GPC versus placebo (Table 4). Subjects reported being in a better mood during the A-GPC group compared to the placebo with a mean difference of 0.667 points ($p \leq 0.023$) When looking at accuracy on the reaction-time test, subjects scored with less incorrect answers with A-GPC ingestion when compared to placebo (12%, $p \leq 0.014$).

Table 4: Dependent Variables

Dependent Variable	A-GPC Treatment Compared To:	Mean Difference	Significance (P Value)	95% Confidence Interval Level (Lower Bound)	95% Confidence Interval Level (Upper Bound)
Fatigue	Baseline	-0.719 points	0.019	-1.314	-0.123
PP Peak	Placebo	-12%	0.014	-22%	-2.5%
Mood Change	Placebo	0.667 points	0.023	0.092	1.241
RT-Test Accuracy	Placebo	-12%	0.014	-22%	-2.5%

CHAPTER V

DISCUSSION

Summary

The author of this investigation hypothesized that A-GPC would have positive acute effects on the performance variables of this study, improving physical task performance in college-aged recreationally trained athletes. When focusing on A-GPC consumption, subjects reported lower levels of fatigue and positive change in mood on the mood questionnaire post ingestion; increased force production during the plyometric push-up and participants had less incorrect answers when receiving the A-GPC supplement. These findings illustrate young healthy adults may benefit from A-GPC ingestion if one is feeling fatigued, or before exercise (mentally or physically).

A-GPC may influence fatigue in athletes, by preventing exercise-induced reductions in choline levels, increases endurance performance and growth hormone secretion (Parker et al., 2015). One study reported a multi-ingredient supplement that contained A-GPC during a maximal effort sprint test on a non-motorized treadmill but did not find any changes in counter-movement performance peak or mean power (Jagim et al., 2016). The current study demonstrated less fatigue occurred with A-GPC ingestion, which is in line with these previous investigations. However, the fatigue was reported by the subject in a qualitative manner and was not physiologically measured. Therefore, this finding should be interpreted with caution until physiologic verification

of less fatigue can be investigated. This evidence is in agreement with previous literature (Hoffman et al., 2010), but more physiological evidence is needed.

While participants in this investigation reported feeling in a better mood following A-GPC ingestion, one previous study reported no changes in mood after A-GPC supplementation (Parker et al., 2015). It should be noted that although a significant difference was found between supplement ingestion and mood, the difference was less than 1 point on the Likert scale suggesting the mood improvement may not be clinically meaningful.

A-GPC has been identified as a nutrient for the brain, otherwise known as a nootropic. Previous literature supports this supplement for cognitive function and mood enhancement (Hoffman et al., 2010), which would partially explain the improvement in accuracy on the reaction-time test (12% increase in accuracy) and the change in mood when subjects ingested A-GPC compared to the baseline in this study. One previous investigation supports the results of this study indicating acute ingestion of A-GPC can maintain reaction time, feelings of focus, alertness to both visual and auditory stimuli following high-intensity bouts of exercise (Hoffman et al., 2010).

A-GPC ingestion yielded greater power output, creating more force production (12% increase) during the plyometric push-up movement compared to baseline, providing evidence that A-GPC may have ergogenic effects during upper body power output. However, no lower body benefits were found during the vertical jump or hand-grip strength test when compared to placebo or baseline trials. Previous literature supports this evidence where one study found supplementation of A-GPC increased

upper body power output by 14% (Ziegenfuss et al., 2008). While another investigation supports lower body force production following acute A-GPC supplementation, however, the same study found no improvements in upper body force production (Bellar et al., 2015). These contradictive findings could be due to inter-individual variability between differences in sample, responder's vs non-responders etc. However, more research is needed to determine the physical and cognitive benefits of A-GPC

Future research in this area

Although this study supports A-GPC as an ergogenic aid, providing additional evidence to contradictive literature on force production, more research is needed. This investigation was the first to examine the acute effects A-GPC ingestion has on cognitive facilitation (reaction-time test and mood questionnaire) and power output (Hand-grip strength, standing vertical jump, and plyometric push-up) via anaerobic paradigms in young healthy adults. A future study may focus on examining a collegiate athletic program and the athletes by position to limit individual variability between subjects. Although previous studies support the evidence found in this investigation, further research is needed on this topic to clarify the benefits of A-GPC ingestion on physical and mental performance in young healthy adults. Ziegenfuss and colleagues (2008), noted, "Future work should examine how resistance exercise + A-GPC affect the growth hormone-insulin growth factor axis and their associated family of binding proteins" (p. 15). There is recent literature claiming citicoline and caffeine have a

theoretical synergistic effect, although there is no evidence currently (Bruce et al, 2014), with A-GPC being a more potent form of choline, future research may want to examine this synergist effect that could potentially exist between A-GPC and caffeine. More research is needed to determine interactions between other nutrients such as uridine (Agarwal et al., 2018; Silveri et al., 2008) and iron (Armah et al., 2008; Troesch et al., 2009).

Conclusion

This study provides support, similar to previous literature, that A-GPC maybe beneficial as a cognitive and power output supplement. Significance of the study reveals a 12% increase in power output during the plyometric push-up and accuracy during the reaction-time test. This study sheds new light on the potential benefits A-GPC may have on young healthy adults and the ergogenic effects of A-GPC. Specifically, A-GPC may improve trained college-aged individuals' level of fatigue, mood, force production, and reaction time. For college students, the amount of time spent working and attending school may create challenges with expending and managing energy requirements. A-GPC could potentially serve as a reasonable supplement for young healthy adults attempting to deal with the stressors of everyday life or for the student looking for an advantage academically or with physical activity. Although future research is needed to give merit to these findings, A-GPC looks promising in supplementation as an ergogenic aid or to help facilitate cognitive function, promoting learning via enhanced memory recall.

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APPENDICES

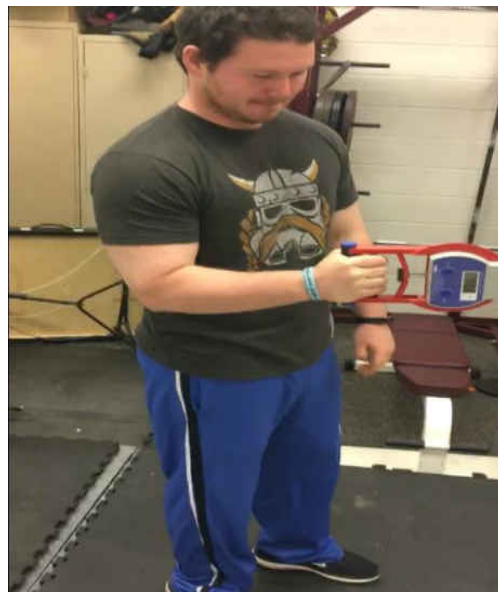
APPENDIX A:

Description of Stations

DESCRIPTION OF STATIONS

1. Hand Grip Strength

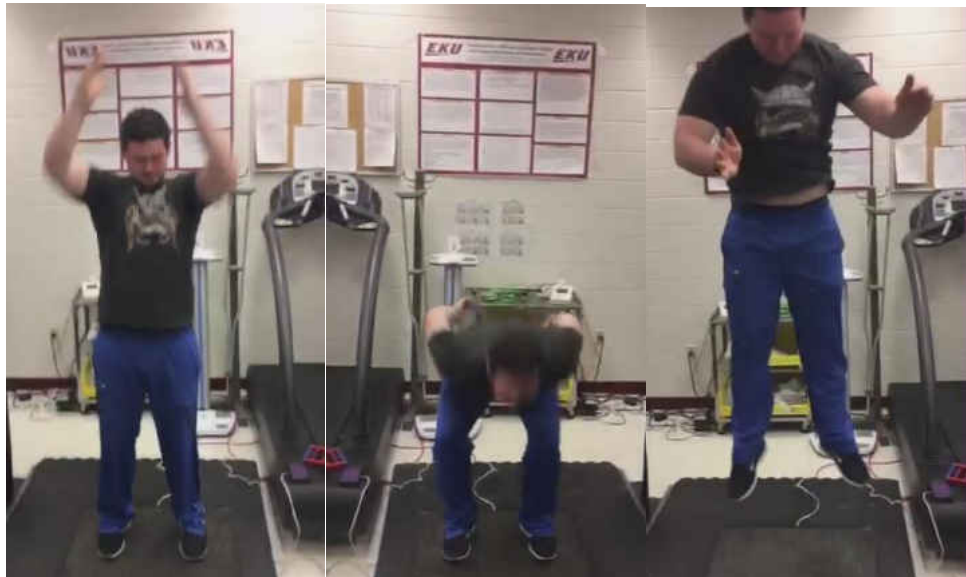
The purpose of this test is to measure the maximum isometric strength of the hand and forearm muscles. Handgrip strength is important for any sport in which the hands are used for catching, throwing or lifting. Also, as a general rule people with strong hands tend to be strong elsewhere, so this test is often used as a general test of strength. A handgrip dynamometer is needed to perform this assessment. Following procedure: The subject holds the dynamometer in the hand to be tested, with the arm at right angles and the elbow by the side of the body. The handle of the dynamometer is adjusted if required - the base should rest on first metacarpal (heel of palm), while the handle should rest on middle of four fingers. When ready the subject squeezes the dynamometer with maximum isometric effort, which is maintained for about 5 seconds. No other body movement is allowed. The subject should be strongly encouraged to give a maximum effort. The position of the arm and hand can vary in different grip strength protocols. Various positions include the elbow being held at right angles as per the above procedure, the arm hanging by the side, and the extended arm being swung from above the head to by the side during the squeezing motion. The Eurofit Test Manual recommends squeezing for 3 seconds. The procedure for the Groningen Elderly Tests has the subject hang their hand by their side, one practice trial, best of three attempts with 30 seconds rest between. Scoring, the best result from several trials for each hand is recorded, with at least 15 seconds recovery between each effort.



2. Standing Vertical Jump Test

(To assess average force on jumps and peak jump performance)

Vertical jump will be recorded using the Just Jump or Run mat. Participants were instructed to jump and reach their maximum height. Just Jump or Run mat is measured in units of 0.1 inches. Participants will not be allowed to have a moving start. Each participant was given three attempts at the Just Jump or Run mat. The purpose of this test is to measure the leg muscle power. Equipment needed will be the jump mat. Procedure: Vertical jump height can be measured using a jump mat, which measures the displacement of the hips. To be accurate, you must ensure the feet land back on the mat with legs nearly fully extended. The vertical jump is usually performed with a countermovement, where there is bending of the knees immediately prior to the jump. Scoring: the jump height will be recorded as a distance score (possible conversions for jump height into a power or work score may be calculated).



3. Plyometric Push-up Test

(To assess impulse and rate of force development)

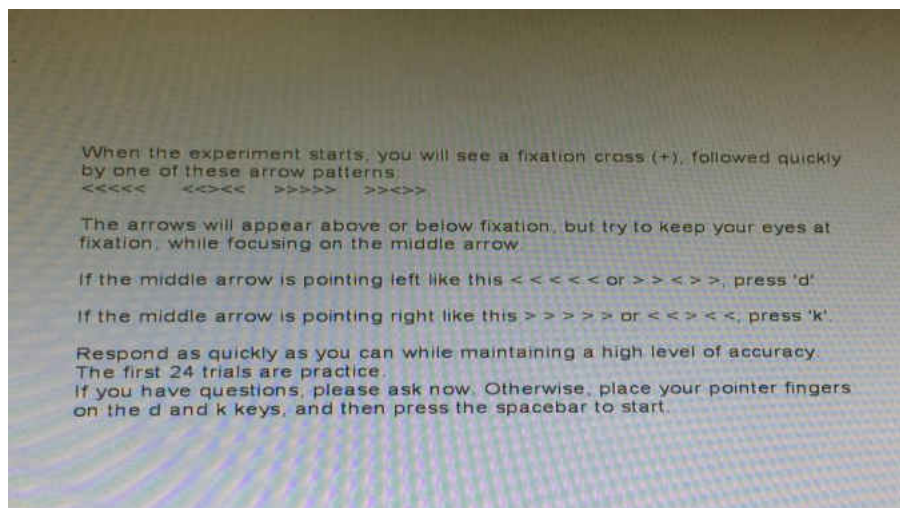
During the plyometric push-ups (otherwise known as clap push-ups), the participants will lower the chest, down toward the force plates, while maintaining a straight line with their body. After this phase, the subject would forcefully push up and while in the air clap their hands together (if possible) before landing back onto the force plates. One full repetition was defined as successfully completing both phases of the exercise and returning to the starting position after landing back onto the force plates (Koch et al., 2012). The subjects will be instructed to perform each repetition consecutively with maximal explosiveness and no pause (rest) between repetitions. After the completion of the 4 repetitions of each set. The

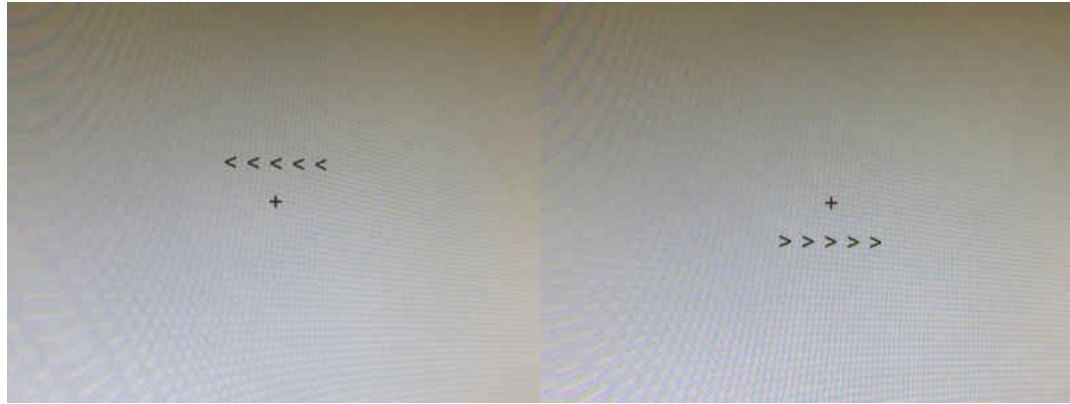
standard modified push-up will be an alternative for subjects who cannot get full extension and separation from the force plates.



4. Reaction-Time Test

Reaction-time test will be conducted using PsychoPy-Psychology software for Python V1.82. This is a computer based reaction time test that allows for custom programming. It also has the ability to program multiple scenarios, preventing errors from participants memorizing patterns of other reaction based tests (Peirce, 2007). In 2003, PsychoPy was originally written by Peirce as a proof of concept - that a high-level scripting language could generate experimental stimuli in real time. Reaction-time will be assessed prior ingestion and post-test, directly before each mood test. Each subject will have a letter on the keyboard designated for left and right arrows which will appear on the computer screen, with the index finger on each hand pressing the key that represents the arrow as soon as the subject specifies the direction of the arrow. Reaction-time test will be employed following the mood questionnaire, post-ingestion, pre and post-test.





APPENDIX B:

Food Sources and Content of A-GPC

FOOD SOURCES OF A-GPC

- Toasted wheat germ (33.78mg/100g), wheat crackers (10.94mg/100g), and wheat bread (4.93mg/100g)
- Oat bran (33.25mg/100g)
- 2% Milk (9.98mg/100g) and skim milk (9.70mg/100g)
- Cheese (2.30mg/100g), Cottage cheese (8.39mg/100g), Cream cheese (9.26mg/100g)
- Yogurt (7.79-9.10mg/100g)
- Eggs (0.60mg/100g)
- Chicken breast (1.12-1.20mg/100g) and liver (8.80mg/100g)
- Pork sausage (7.41mg/100g), cooked pork loin (22.51mg/100g)
- Beef liver (77.93mg/100g)
- Atlantic cod (30.04mg/100g), Salmon (5.89mg/100g)
- Bananas (5.60mg/100g), blueberries (0.61mg/100g), avocado (0.73mg/100g), grapefruit (1.16mg/100g), cantaloupe (0.71mg/100g), oranges (1.10mg/100g), and strawberries (0.86mg/100g)
- Broccoli (1.32mg/100g), cabbage (3.47mg/100g), cucumber (0.48mg/100g), spinach (0.21mg/100g), onions (0.57mg/100g), sauerkraut (0.94mg/100g), sweet potatoes (1.97mg/100g), Russel sprouts (3.18mg/100g), and raw mushrooms (5.11mg/100g)
- Beer (light at 2.98mg/100g, regular at 5.06mg/100g) and coffee (0.67mg/100g)

Steven H. Zeisel², Mei-Heng Mar, Juliette C. Howe*, and Joanne M. Holden (2003) The American Society for Nutritional Sciences Concentrations of Choline Containing Compounds and Betaine in Common Foods.

APPENDIX C:

Recruitment Letter



Recruitment Letter

The Acute Effects of Alpha-GPC on Hand Grip Strength, Jump Height, Rate of Force Development, and Reaction-Time in Recreationally Trained, College-Aged Athletes

Researcher: Josey Lucas Cruse

College of Health Sciences

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859-302-4443

Dear Potential Participant,

You are being invited to participate in a research study to examine the ergogenic effect of strength and power training supplement, A-GPC, during physical task performance. A-GPC is a supplement that has previously been researched and proven to increase levels of acetylcholine in the blood and growth hormone secretion. Increased levels of acetylcholine in the body have been shown to enhance strength and explosive ability (power). Elevated levels of GH in the body have also been shown to increase strength and explosive ability. Few studies have examined the effect of A-GPC on sport performance. Therefore, your participation in this investigation hopes to further the research literature on this topic, aiming to identify the acute effects of A-GPC ingestion on Hand Grip Strength, Jump Height, Rate of Force Development, and Reaction-Time in Recreationally Trained, College-Aged Athletes

For this study you will be asked to report to the exercise physiology lab in the Moberly facility, on 3 separate occasions, separated by 2-7 days. You will be asked to come at the same time for each testing. You will be provided with a list of foods and beverages that contain A-GPC and asked to refrain from these foods. You will be asked to abstain from hard training for at least 24 hours prior to each visit. Before and after each visit you will be asked to perform a reaction-time test conducted using PsychoPy-Psychology software (Python V1. 82.). Next, measurements for blood pressure, heart rate, height and weight will be assessed, and body fat percentage will be determined using the BOD POD.

After that, you will be asked to perform a series of physical performance tests (4 stations, consisting of the hand grip strength, standing vertical jump, plyometric push-ups, and reaction-time). First, you will perform the reaction-time test before physical performance. Next you will be tested on hand grip strength using a dynamometer, followed by the standing vertical jump test in which you will be asked to jump as high as you can to test your lower body explosive ability will be assessed using the

jump mat. Next, you will perform the plyometric push-ups on the force plate to assess impulse and rate of force development.

Prior to testing, on the second and third visit, you will randomly be provided with a moderate dose of either alpha-GPC (6 mg·kg⁻¹) or the same dosage of placebo (dextrose), which will be weighed and placed in a cup and mixed with 10 ounces of water and assigned in a randomized fashion. You will consume a different substance on visit two and three, outside the baseline trial, where no substance will be ingested. Subjects will be instructed to sit in a rested state for 45 minutes following ingestion of either the supplement or placebo. The assigned supplement was prepared by an outside member of the research staff and delivered to the laboratory with the subject's name on a shaker bottle in order to maintain a double-blind procedure.

If you chose to start this study, you should be aware that you will receive no benefits such as extra credit or course credit in any College of Health Sciences course. You may choose to stop the study at any time and will face no penalty. Your participation is completely voluntary. The study will take approximately 5 hours of your time over the course of 15 days, and you may experience some muscle soreness as a result of the exercise.

The intention is to analyze the data after the study is complete and make a report and publish in a scientific journal. Your name will not be part of any reporting of any information from this study. The files from the study will be stored in an encrypted file, on a password protected computer accessible only to Dr. Lane. If you have any questions about this study, please contact either Josey Cruse at josey_cruse10@mymail.eku.edu or Dr. Michael Lane at Michael.lane@eku.edu.
Respectfully,

Josey Cruse