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EFFECTS OF ALPHA-GPC AND HUPERZINE-A ON SHORT TERM MEMORY,
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CAFFEINE AND PLACEBO IN HEALTHY COLLEGE AGE STUDENTS

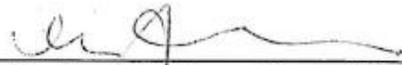
BY

JOHN P. ISAACS

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ANAEROBIS POWER OUTPUT, POST EXHAUSTION COMPARED TO
CAFFEINE AND PLACEBO IN HEALTHY COLLEGE AGE STUDENTS

BY

JOHN P. ISAACS

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

2019

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DEDICATION

This thesis is dedicated to someone that has always pushed me when it wasn't easy, kept my eyes on the prize, encouraged me to do more than I thought I was capable of, and supported me the entire time. He is the kind of mentor I hope everyone can find and a role model I strive to be more like every day. Thank you for all your help and support down this long road.

Thank you, Dr. Michael T. Lane.

ACKNOWLEDGEMENTS

I would like to acknowledge all the participants that made this study possible. Without you, none of this could have come to fruition and I applauded and am eternally grateful for the effort you gave. I would also like to thank Tyler Sutton, Conner Hutchinson, Ellen Reinhold, and BoDene Pinz for your help through this process. Lastly, thank you to the exercise and sports science department and the faculty for allowing me to use the space for my research.

ABSTRACT

Introduction: The use of multi-ingredient pre-workout supplements has been steadily on the rise in the fitness industries. Companies make claims about improvements in performance both physically and cognitively for users but seldom provide research to back up the claims made about the ingredients or dosages. **Purpose:** To examine the effects of Huperzine-A and Alpha-GPC on short term memory and anaerobic power output, post exhaustion compared to caffeine and placebo in healthy college age students.

Methods: The study was conducted as a double blind, placebo controlled, randomized design on 62 healthy adults (N=62 height 68.4 ± 3.5 in., weight 78.5 ± 15.1 kg.). The wash out period was a minimum of 48 hours after completion of the familiarization. Subjects reported to the exercise physiology lab thirty minutes before testing began and consumed either a caffeine, Alpha-GPC and Hup-A, or placebo solution. After the thirty-minute digestion period subjects performed one computer-based short-term memory test, and a thirty-second Wingate anaerobic power test. Subjects then performed an exhaustion protocol before repeating the memory and power test. Once all testing was completed subjects returned between 2 and 14 days after the last test and repeat the protocol. A power analysis was run using G* Power software 3.1.9.2 based from Zeigenfuss et al., (2008). The percent change between pre and post was compared across visits using ANOVA with repeated measures. Significance was found with an Alpha level $P \leq 0.05$ with Tukey Post Hoc analysis will be used to determine pairwise comparisons. All stats were run on IBM SPSS 23. **Results:** The ANOVA with repeated measures and Tukey Post Hoc analysis found there was no significant difference in performance pre to post,

between groups, or factoring the percent change pre to post. **Conclusion:** This result suggests there is no physical or mental benefit acutely dosing 600 mg. of Alpha-GPC and 200 mcg. of Huperzine-A in healthy recreationally active adults. This was the first study to look at the two in combination so, the finding is neither supported nor opposed to the current body of research. The finding does oppose the logic some supplement companies have been using to justify their sales tactics. Future research should investigate the effects of a loading period on physical and mental performance.

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Chapter 1

Introduction

Background

The use of supplements in fitness communities has been on the rise for nearly the last two decades (Dickinson, Blatman, El-Dash, & Franco, 2014). These supplements include but are not limited to Protein shakes, Meal replacement shakes, multi-vitamins, post- workout recovery shakes, nootropics (cognitive enhancers), and Pre-workouts. Many of these supplements include a long list of ingredients that claim to increase bioavailability of the substrate, cause a synergistic effect on the recovery process, increase the palatability upon ingestion, increase duration of workout and workload ability, or increase mental clarity and focus. Many of the claims about increased protein consumption post workout are well researched and backed by evidence, the same is not true for many pre-workout ingredient claims (Jeukendrup, & Gleeson, 2010)

Many different multi-ingredient pre-workout supplements make the same claim about being able to increase your strength, focus, workout intensity, and duration but very few have any empirical evidence to back up the claims made. One major issue with a large portion of these supplements is they may include some ingredients that do have some advantageous effects, but the amount is not enough to elicit the desired effect typically seen as “Proprietary Blends”. One common example is pre-workouts containing caffeine, this is a widely researched supplement that has been found to

increase aerobic performance. Supplement companies are highly aware of this claim and include 200 milligrams of caffeine and stake the claim “workout harder for longer”. This would be true if the person taking the supplement was 40 kg (88 pounds) because research shows that to increase aerobic performance with caffeine the ratio must be 5 milligrams per kilogram of body mass (Pasma, Van Baak, Jeukendrup, & De Haan, 1995). So, for the average sized adult, this dose would have little to no effect on performance. This is one of the most frequently consumed drugs in the world and is found in a wide variety of drinks and foods (Gilbert, 1984). This makes caffeine an excellent product to compare when examining the potential benefits of a new supplement. Caffeine in the correct dose has been shown to be effective in physical and mental performance (Ganio, Klau, Casa, Armstrong, & Maresh, 2009, Astorino, & Roberson, 2010), but the mechanisms in which caffeine affects performance is still unknown and highly speculated (Jeukendrup & Gleeson, 2010). The mechanism of action in caffeine is highly debated so, that may lead investigators to explore supplements that act on systems they understand, such as the cholinergic systems. The use of choline derived supplements has recently sparked the interests of performance researchers because of its role in acetylcholine synthesis, which plays an extremely important role in muscle contraction and the synaptic functions.

The manipulation of free choline in the body through ingestion of choline derivatives may prove to be advantageous in physical performance and cognitive capability. Alpha-glycerophosphocholine (Alpha-GPC) is a semi-synthetic derivative of lecithin that acts by releasing free choline, so it can be synthesized into acetylcholine (ACH) and phosphatidylcholine for biosynthesis in the brain (Gatti, Barzaghi, Acuto,

Abbiati, Fossati, & Perucca, 1992, Brownawell, 2010). Many studies have shown the efficacy of Alpha-GPC in decreasing the cognitive decline in patients with Alzheimer's disease and dementia (Traini, Bramanti, & Amenta, 2013), but few studies have investigated its ergogenic effects in exercise performance. Another supplement that has recently become a point of interest is Huperzine-A (Hup-A) an ancient Chinese herb from the herb *Huperzia Serrata* (Wang, Yan, & Tang, 2006).

Hup-A is a selective, reversible, and potent acetylcholinesterase inhibitor (Wang, et al., 2006). Acetylcholinesterase (ACHe) is the enzyme responsible for the break-down of ACH in the pre and post synaptic cleft of the neuromuscular and intraneuronal synaptic clefts. Once ACH is broken down by AChE it is returned to the presynaptic axon to be synthesized into ACH (Widmaier, Raff, Strang, 2004). Hup-A, like Alpha-GPC, was originally used in research on patients with neurodegenerative diseases and showed marked improvements on cognition in experimental trails (Wang, 2006). There is no current research on the effects of Hup-A in physical performance, but it has been shown to effectively inhibit AChE in healthy adults (Morasch, Aaron, Moon, & Gordon, 2015). AChE is the enzyme that breaks down ACH at the post-synaptic receptors, if this enzyme can be inhibited it may increase the firing rate of the synapse. The same principle may apply in the use of Hup-A to manipulate the process by which ACH is cycled in the neuromuscular junctions. The potential for increased cognitive functioning and physical performance when properly stacking Alpha-GPC and Hup-A may prove to be an effective replacement for caffeine in pre-workout supplements.

The current study proposed to examine the effectiveness of acute supplementation of Hup-A and Alpha-GPC, on cognitive performance and physical performance in healthy adults before and after an exhaustive protocol, compared to Caffeine and Placebo.

Limitations, Delimitations, Assumptions

The current limitations, delimitations, and assumptions are made from the pilot testing performed prior to the official data collection. As data collection and testing proceeds researchers may find some limiting factors in the true experimental design that cannot be controlled for. These limitations may include but are not limited to controlling for subject eating habits. Subjects will be asked to refrain from any heavy meals prior to testing because they may lead to potential issues with proper absorption of the supplements. Also, a heavy meal may reduce subject performance during the physical performance skewing test results. Problems with cramping, gut pain, or even vomiting may occur if over feeding occurs prior to the testing battery. Investigators will be sure to ask subjects when their last meal was and make sure they will not have any gastrointestinal issues during testing. If the subject feels they cannot perform their best, testing will be rescheduled. Consumption of caffeine prior to testing will also be discouraged in order to avoid a stacking effect or a potential overdose or synergistic effects from the combination of treatment and what subjects may potentially consumed on their own.

Subjects will be asked to refrain from any intense workouts 24 hours prior to testing as to avoid a premature fatiguing effect. Researchers will diligently question

subjects to make sure there was no previous intense exercise they may skew performance results.

Potential limitations may also include caffeine sensitivities in subjects that are non-users, or potential side effects from the dosing of Hup-A. Mild, occasional, side effects have been reported with oral supplementation of Hup-A (nausea, vomiting, diarrhea). These potential side effects will result in an immediate discontinuation of testing with that subject. This study will also be limited by the equipment technology and size of the lab hosting the testing protocol.

Delimitations of the study will be proper dose timing of 30 minutes which will begin once the subject has ingested the full treatment and time will be kept by the researchers on site. Physical performance will be collected via a single 30 second Wingate using a Monarch cycle ergometer, which has been shown to be a reliable and valid means to measure anaerobic power output. A standardized post exhaustive protocol recovery time of 5 minutes. Time will begin immediately once the subject finished the protocol completely. Subjects will be placed on a computer with noise canceling headphones/earplugs to ensure they are not distracted by outside influences when short term memory. Short term memory test will be simple and thoroughly explained by researchers and warm up rounds will be provided before each test to ensure instructions are understood.

Assumptions of this study are that subjects will come into each testing session well rested and will give best efforts in all trials (Investigators will record hours of sleep subjects had the previous night). It is also assumed that all subjects will be forth coming

with any injuries that may hinder performance during the Wingate, as well as any mental clarity issues that may impede cognitive functioning during the trials.

Problem statement

Supplement companies put arbitrary amounts of different ingredients and make claims about the effects their blend being advantageous for both physical and cognitive performance. Many supplements in these products have no research-based evidence that suggests they will cause an increase in performance and in some cases, these products will contain an ingredient that has been shown to have beneficial effects, but it is an insufficient dose. Alpha-GPC and Hup-A have been shown to improve cognitive performance and physical performance.

The purpose of this study is to test the effectiveness of Alpha-GPC and Hup-A in a stack supplement on improving cognitive and physical performance post exhaustion compared to placebo and caffeine healthy college aged adults. The approach investigators will use is an experimental double-blind placebo-controlled protocol.

Hypothesis

Hypothesis 1 – It was hypothesized that 600 mg of Alpha-GPC and 200 mcg of Hup-A would improve anaerobic power output and short-term memory pre and post an exhaustive protocol, in healthy college aged students compared to 5 mg/kg of caffeine and placebo.

Hypothesis 2 – It was hypothesized that 600 mg of Alpha-GPC and 200 mcg of Hup-A would improve anaerobic power output but not short-term memory pre and post

exhaustive protocol, in healthy college aged students compared to 5 mg/kg of caffeine and placebo.

Hypothesis 3 – It was hypothesized that 600 mg of Alpha-GPC and 200 mcg of Hup-A would improve short term memory but not anaerobic power output pre and post an exhaustive protocol, in healthy college aged students compared to 5 mg/kg of caffeine and placebo.

Chapter 2

Review of Literature

Background

Multi-ingredient pre-workouts have become popularized amongst fitness enthusiast and athletes at all levels in the health and wellness industry (Dickinson, Blatman, El-Dash, & Franco, 2014). Each specific supplement in the recipe having its own claim to how it increases performance, most of which make claims regarding increases performance, in strength, power, fatigue resistance, and better focus. In theory, these combinations of supplements may prove to be advantageous for users but, there is very little research as evidence to support the claims made. A. R. Jagim and associates published one of few studies analyzing the effects of Multi-Ingredient Pre-Workout Supplements (MIPS) against placebo (Jagim, Jones, Wright, Antoine, C. Kovacs, & Oliver, 2016). One issue with this method is it leaves much room for questions about which supplement or combination was responsible for the desired effects observed in the research, three main supplements used in the MIPS were caffeine, Alpha-Glycerolphosphocholine (Alpha-GPC), and Huperzine-A (Hup-A). Caffeine is a widely used substance that has been found to be efficacious for physical and mental performance tasks. Multiple systematic reviews on the effects of caffeine on sports performance have been published due to its wide use in athletics and ability to improve cognitive performance (Ganio, Klau, Casa, Armstrong, & Maresh, 2009, Astorino, & Roberson, 2010).

Alpha-GPC is a relatively new supplement in the performance world. There are very few studies done on the effectiveness of alpha-GPC in peak performance but author T. Ziegenfuss suggests it may be effective in increasing peak power, anaerobic power, and human growth hormone levels (Ziegenfuss, Landis, & Hofheins, 2008). Hup-A comes from the naturally occurring Chinese herb and is a reversible cholinesterase inhibitor and comes from *Huperzia serrata* (Rathee, Chaudhary, Rathee, & Rathee, 2008). This herb has been shown in some research to be an effective memory booster in monkeys (Ye, Cai, Wang, & Tang, 1999), suggesting the possibility of providing similar effects in humans. There has been little to no research of the effects of Hup-A in healthy adults on mental or physical performance. The purpose of this literary review is to gauge the effectiveness of Alpha-GPC and Hup-A against caffeine and their possible uses in mental and physical performance in healthy individuals.

Caffeine

Caffeine is arguably one of the most widely used drugs in the world according to author R.M. Gilbert, 1984. Caffeine is consumed primarily in the form of coffee 54%, and tea 43%, but is found in a wide variety of products. Other products with caffeine include chocolate, chocolate milk, candy bars, hot chocolate, sodas, and even decaffeinated coffee still contains some caffeine (Gilbert, 1984). In 1996, Author J. J. Barone reports from the available data that the average consumption in users in the United States is 4 milligrams per kilogram of bodyweight (mg/kg) (Barone, & Roberts, 1996). For a person of about 200 pounds that means they are taking approximately 365 mg of caffeine a day.

Physiological/psychological response to caffeine

Caffeine has been found to have a wide variety of uses in the average person's day to day life. Many of the uses for caffeine are beyond the scope of this review. While there is a plethora of benefits one may receive, some populations experience adverse health effects like an acute rise in blood pressure at rest (Robertson, Frölich, Carr, Watson, Hollifield, Shand, & Oates, 1978). Robertson, D. et al., (1978) reported a rise in mean blood pressure of 10/14 (systolic/diastolic) one hour after a 250 mg dose. If chronic use were to become chronically high blood pressure this could pose long term health problems. On the contrary, a meta-analysis on of coffee consumption showed that 4 cups a day can decrease chances of heart attack, but 10 cups a will return the risk back to baseline (Mostofsky, Rice, Levitan, & Mittleman, 2012). Evidence suggests that there are some potential benefits with caffeine consumption, and some slight complications, which both are dependent on dose and the tolerance of the individual. The effects on the physiology are easier to track because investigators can quantify the responses simply and uniformly, psychological responses are reasonably less straight forward. These responses may pose an issue in caffeine intolerant populations through increased levels of perceived anxiety and brain lactate levels, these increases were found after subjects were given a dose of 10mg/kg of caffeine (Dager, Layton, Strauss, Richards, Heide, Friedman, ... & Posse, 1999). Brain lactate levels were found not to be causal to increased anxiety levels by investigators. Hormonal response may be one of the factors leading to the perceived anxiety according to Lane J. D. et al., (1990). A robust increase in plasma epinephrine and cortisol at a dose of 3.5 mg/kg in both non and chronic users, as well as increases in blood pressure and heart rate (Lane, Adcock,

Williams, & Kuhn, 1990). This finding is hard to categorize as either psychological or physiological because it is sensationalized in both areas simultaneously. For a caffeine user with a relatively high tolerance, they may not experience any of the adverse effects of the high doses that come in some pre-workout supplements. But, for some individuals the side effects caused by high caffeine doses may out-weigh the benefits. For caffeine intolerant athletes and exercise enthusiasts use of cholinergic supplements may prove to be advantageous.

Aerobic physical performance

There are some clear disadvantages to acute and habitual use of caffeine but, there are some accompanying advantages to supplementation as well. One study provided evidence that caffeine can increase time to exhaustion in cyclists (Costill, Dalsky, & Fink, 1978). Researchers had experienced cyclists' bike until exhaustion on stationary bicycle ergometer at 80% of their VO₂ max. One trial was placebo controlled with decaffeinated coffee, but the other trial was performed with regular coffee containing 330mg of caffeine, results showed that the caffeine trial increased the average time to exhaustion to 90 minutes, compared to 75 minutes in placebo. Upon Further investigation of supplementation for performance, researchers have found the ergogenic effects of caffeine on endurance performance are dose dependent. In a review of caffeine by authors A. Jeukendrup and M. Gleeson they suggest that endurance performance response is dependent on dose relative to body weight. A study by researcher W. J. Pasman compared endurance performance time by giving subjects 0, 5, 9, and 13 mg/kg of caffeine and analyzing the difference in time to exhaustion. They found that each dose besides 0 mg/kg had a marked effect increasing performance but

no significant increases after 5 mg/kg (Pasma, Van Baak, Jeukendrup, & De Haan, 1995). The use of caffeine has shown to have an advantageous effect on aerobic performance, but there is little evidence suggesting the same for anaerobic performance.

Anaerobic physical performance

Once researchers established that the use of caffeine in aerobic performance attention turned toward its potential to enhance anaerobic performance, these studies specifically focused on trained vs untrained populations and a variety of sport-specific and non-specific movements. At a dose of 7 mg/kg trained athletes did not see an increase in performance during 3, 1-minute bouts on a cycle ergometer (Vanakoski, Kosunen, Meririnne, & Seppala, 1998). While this study's focus was to test the effects of creatine consumption on athletes, researchers did well in testing the effect of caffeine on anaerobic power as well. This study suggests caffeine has little effects on anaerobic power output during a 30 second Wingate on a cycle ergometer. Because the Wingate is a non-sport specific test, researchers thought to look at the effects of caffeine on a sport specific sprint. Researchers J.K. Davis and M. J. Green (2009) report in a review of caffeine on anaerobic power that caffeine had no marked effect on sprints for power or speed (J. Davis and M. Green, 2009). Similar results were found in a study testing division 1 football players during 6, 35-meter sprints. The caffeine taurine mixture administered found no improvements on power output compared to placebo (Gwacham & Wagner, 2012). Caffeine has shown marked effects in research on aerobic performance of athletes but, as previously stated there is little evidence to suggest that it has any advantageous effects on anaerobic power output in trained or untrained populations.

Cognitive Effects

It is widely accepted that caffeine increases performance in an academic setting, but there is limited research for that specific area to support the claim. A large study (N=1604) compared the effect of caffeine on introverts versus extroverts. Researchers found large doses of caffeine increased intelligence performance tests in extroverts but not introverts (Gupta, 1988). Similar effects on working memory were found in extroverts at a dose of 200 mg (Smillie, & Gökçen, 2010). Caffeine's effect of individuals is largely found to be dose dependent and there is a threshold that can cause issues in memory with non-caffeine users. A dose of 450 mg of caffeine was found to decrease performance on memory tasks in non-users but, this high dosage increased performance in reaction time testing (Childs & De Wit, 2006). These results suggest that there is a bell curve effect on the use of caffeine in the ability to increase cognitive performance in individuals, optimal dosing varies based on previous exposure (tolerance) and the weight of the user.

Mechanism of action

The mechanism of action in which caffeine effects performance is highly debated but there are several theories on how it works. Authors Jeunkendrup and Gleeson provide several theories on these mechanisms of action. One, is due to caffeine's ability to stimulate fat oxidation (lipolysis) muscles can spare their glycogen stores more effectively, this in-turn provides longer lasting energy during endurance performance. Another theory is "Possibilities include the handling of ions, inhibition of phosphodiesterase leading to an increased concentration of 3',5 -cyclic adenosine monophosphate (cAMP)." This theory is based on the idea that enzyme manipulation by

caffeine changes the handling of ions that are used in exercise performance. One possible theory is the effect caffeine has on calcium, increasing calcium release from the sarcoplasmic reticulum has increases muscle cell excitability. Therefore, one might see an increase in ability to contract muscle as well as, increased time of ability to contract. Lastly, caffeine is a central nervous system stimulant that has a marked effect on "...perceptions of effort or affects the signal transduction from the brain to the neuromuscular junction." If caffeine can manipulate the ability to increase synaptic connections at the neuromuscular junctions then that of contractions would increase and possibly last longer (Jeukendrup and Gleeson, 2010 p. 269) (Table 1).

Table 1
Caffeine Doses and Effects

Use	Dosage	Effective	Source
Aerobic	330 mg	^ time to fatigue	Costill et al., 1978,
Anaerobic	5 mg/kg		Pasman et al., 1995
Cognitive	7 mg/kg	NO	Vanakoski et al., 1998
	200 mg	^ extrovert memory	Smillie, 2010
Physiological	250 mg- 3.5 mg/kg	Robust effect	Robertson et al., 1978, Lane et al., 1990
Psychological	10 mg/kg	Yes	Dager et al., 1999

Importance of Choline

The use of Acetylcholine (ACH) in cell function, muscle contraction and synaptic function, is well documented. Being the first neurotransmitter to be discovered in 1921 by German biologist Otto Loewi (Boeree, 2003) researchers have an in-depth understanding of its use in the body and where it is synthesized from. ACH is derived from the substrate choline, making choline essential to releasing action potentials all through the body, especially in muscle contraction. Authors Jeukendrup and Gleeson describe its connection very well “Acetylcholine transmits the electrical potential from neuron to muscle cell, leading to the calcium release from the sarcoplasmic reticulum and muscle contraction... The precursor of acetylcholine is choline, a normal component of the human diet” (Jeukendrup and Gleeson, 2010). The abundance of choline in one’s diet is important because of its lipotropic effects in the liver, choline helps prevent lipid accumulation on the hepatocytes (Best and Huntsmen, 1932). Until Best and Huntsman began their research initially looking at insulin but, discovered that choline inhibits fatty liver accumulation. Before this research, choline was underappreciated as a micronutrient essential to one’s health. Thanks to the wide variety of foods that contain choline mostly meats, legumes, and dairy products there is a low chance one would find themselves in a deficit while eating a well-balanced diet. Because of the crucial role ACH plays in the neuromuscular junction, this has led researchers to use concentrated forms of choline for supplementation. These concentrated forms of choline are broken down more easily into phosphorylcholine after ingestion than regular dietary choline. Phosphorylcholine is the metabolically active form of choline, it is used in the synaptic cleft through the entire central nervous

system (De Ferra, Hagerman, Purpura, Jaeger, Hagerman, & Zenoni, 2016). Author De Ferra elaborates well on the function of phosphorylcholine stating “Phosphorylcholine migrates to the synaptic nerve endings found throughout the entire central nervous system, and in turn increases ACH synthesis and release. ACH is a vastly important neurotransmitter present in both brain and muscle tissue. In the brain, ACH plays a key role in basically every cognitive function, while in muscle, it is vitally involved in muscle contraction, as it is the major neuro-transmitter involved in regulating physiological response to exercise.”

Some examples of choline concentrates are Citicoline (CDP-choline), this supplement has shown efficacy in preventing the cognitive decline associated with post stroke symptoms (Alvarez-Sabin, 2011). This form of choline concentrate demonstrates very important neuroprotective properties that may be useful in increasing cognitive performance in healthy individuals. These implications suggest that choline derived supplements are not only functional in physical performance, researchers may find use for increased cognitive performance as well. Another form of choline concentrate that is proving to be advantageous in mental, physical and physiological performance is Alpha-glycerophosphocholine.

Alpha-Glycerophosphocholine

Alpha-glycerophosphocholine (Alpha-GPC) is a natural derivative of the substrate choline, which is used in the body to synthesize ACH, a vastly important neurotransmitter. Alpha-GPC was developed to act by releasing free choline that in-turn will increase ACH and phosphatidylcholine biosynthesis in the brain (Gatti, Barzaghi, Acuto, Abbiati, Fossati, & Perucca, 1992). Brownawell et al. (2011) describes it as a “semi-synthetic derivative of lecithin. Following oral administration, it is converted to phosphorylcholine, a metabolically active form of choline able to reach cholinergic synaptic endings where it increases acetylcholine synthesis and release (Brownawell et al., 2011, Lopez et al., 1991, Trabucchi et al., 1986, Abbiati et al., 1991)”. This suggests that ingestion of Alpha-GPC could lead to an increase in plasma choline levels.

Effects on Physical Performance

Alpha- GPC has been hypothesized to increase physical performance in power, strength, and aerobic performance, one study found an interesting result on the post work-out growth hormone secretion and bench press in males. Researchers Tim Ziegenfuss, Jamie Landis, and Jennifer Hofheins tested the Alpha-GPC on seven resistance trained men to see its effect on explosive performance and growth hormone levels. Subjects were given 600 mg of Alpha-GPC or placebo 90 minutes before exercise, then performed an exhaustive squat protocol. After thirty minutes of rest they performed a bench protocol to assess peak power, serum samples to assess growth hormone secretion were taken immediately, fifteen minutes, thirty minutes, and every thirty minutes until the two-hour mark. Researchers found that peak growth hormone secretion increased 44-fold in the alpha-GPC trials, and bench press force increased by

fourteen percent. Increased bench press power was trending, as well as, lower post exercise respiratory exchange rate (Ziegenfuss, Landis, & Hofheins, 2008).

Investigators conclude that 600 Mg of Alpha-GPC increases bench press force and growth hormone secretion and suggest future research be directed toward its effects on binding proteins when coupled with resistance training, but the lowering of respiratory exchange ratio post exercise has interesting implications to future research as well.

From the result of this study there is reason to believe that Alpha-GPC may provide an ergogenic effect on endurance performance and muscle contractile time. Some

researchers have taken interest in loading Alpha-GPC and its unique effects on force production. Bellar, D. et al., (2015) investigated isometric strength after loading Alpha-

GPC compared to placebo. Research was performed using 13 healthy male college students, they were provided with either 600 mg of Alpha-GPC to take daily or a

placebo after baseline measurements were taken. After a 6-day loading phase of either Alpha-GPC or placebo subjects performed an isometric mid-thigh rack pull and

isometric pushup against force plates and force production was measured in both.

Researchers found significant increase in the mid-thigh pull strength and a trend toward

significant change in the push-up. Bellar reports “Magnitude based inferences suggest

that the A-GPC was 68.3% likely beneficial for increasing upper body isometric force and 86.5% likely beneficial for increasing lower body isometric force production.”

(Bellar, LeBlanc, & Campbell, 2015). Another study examined Alpha-GPC’s effects on

counter movement jump height, 40-yard dash time, and Wingate anaerobic power

output compared to placebo. Subjects were given 300 mg of Alpha-GPC administered

with a sports drink 1 hour before testing. Results found a significant improvement in the

counter movement jump compare to placebo but no improvement in the Wingate or 40-yard dash (Rickard, 2017). Research examining the effects of Alpha-GPC on physical performance are sparse and should be further pursued.

Effects on cognition and mood

There is evidence to support the claim that Alpha-GPC may also increase cognitive performance (Traini, Bramanti, & Amenta, 2013). Traini found that the use of cholinergic precursors and acetylcholinesterase inhibitors can improve memory and attention in patients with dementia. Due to the vast use of acetylcholine throughout the central nervous system and its importance in the neuromuscular junction use for Alpha-GPC to improve reaction time testing in healthy adults. Researchers found that “CRAM” a supplement containing Alpha-GPC (150 mg), choline bitartrate (125 mg), phosphatidylserine (50 mg), niacin (vitamin B3; 30 mg), pyridoxine HCl (vitamin B6; 30 mg), methylcobalamin (vitamin B12; 0.06 mg), folic acid (4 mg), L-tyrosine (500 mg), anhydrous caffeine (60 mg), acetyl-L-carnitine (500 mg), and naringin (20 mg), maintained reaction times pre and post exhaustive protocol (Hoffman, Ratamess, N. Gonzalez, Beller, Hoffman, Olson, & Jäger, 2010). As previously stated, phosphatidylcholine is the metabolically active form of choline which can be used in the neuromuscular junctions to synthesize acetylcholine, an important neurotransmitter for proper cell function and specifically for muscle contraction (De Ferra, 2016). In the research performed by Hoffman et al. (2010) subjects were given either placebo or CRAM supplement in the initial testing once given time to relax and digest they filled out a survey fatigue, focus, alertness and energy. Then, subjects performed a reaction time test and an exhaustive protocol followed by another survey and reaction test.

Hoffman, J. R. et al., (2010) reports that acute supplementation of CRAM has shown that subjects maintain feelings of focus and alertness but also maintain reaction times pre and post (Hoffman et al., 2010). These results may suggest the increased availability of phosphatidylcholine can lead to better performance in cognitive tasks. One issue with this study is that it was a multi ingredient supplement so there may be some compounding factors contributing the results found. Performance supplements such as caffeine are dose dependent (Pasman et al., 1995), dosing is typically measured as milligram per kilogram of body weight (mg/kg). Further research on the effects of Alpha-GPC relative to body size is needed, it is widely accepted that the consumption of Alpha-GPC at relatively high doses is safe for oral consumption (Bronawell, 2011).

Safety of ingestion

Researchers must be careful when testing relatively high doses in patients because of potential toxicity, Alpha-GPC has been found to be found and is widely considered to be safe for consumption in moderate to high doses. The oral ingestion toxicity rate has been found to be very high and doses in rats had to be up to 10,000 mg/kg to exhibit toxic effects or death (Brownawell, 2011). The research suggests that Alpha-GPC is safe for consumption at relatively high doses (Table 2).

Table 2
Alpha-GPC Dosing and Effects

Use	Dose	Timing	Effective	Source
Exercise performance	600 mg	90 minutes	↑ LE Peak, ↑ HGH	Ziegenfuss et al., 2008
	600 mg	6-day loading	↑ UE/LE isometric strength	Bellar et al., 2015
Cognitive performance	150 mg	20 minutes	↑ mood and attention?	Hoffman et al., 2010

Huperzine-A

Huperzine-A (Hup-A) is a novel alkaloid that comes from the Chinese herb *Huperzia serrata*, and is a selective, reversible and potent acetylcholinesterase inhibitor (Wang, Yan, & Tang, 2006). Acetylcholinesterase (AChE) plays an important role at the neuromuscular junction, where acetylcholine (ACh) is released in the synaptic cleft it binds to the postsynaptic ACh receptors. Once the action potential is released from the motor endplate the enzyme AChE breaks down the built up ACh into choline and acetate. After the breakdown, choline is returned to the presynaptic axon where it is reused for the synthesis of new acetylcholine (Widmaier, Raff, Strang, 2004). This well-established breakdown gives useful insight to how ACh and AChE are used in the neuromuscular junction, but as previously mentioned, these compounds are used throughout the entire nervous system. The series of events are similar but there are some slight variations in the intraneuronal synapses versus the neuromuscular junctions. In the nervous system the synapses are referred to as cholinergic, action potentials arrive at preganglionic axon releasing ACh into the “intra-axonal storage site” once the ACh crosses the cleft it causes the postsynaptic potential to travel down the ganglionic fiber, this is where AChE begins to break down ACh on the post synaptic membrane (Koelle, 1962).

Effects on cognition

There are many similarities between the two systems, both of which are affected by the manipulation of ACh and AChE. Hup-A has been found to improve cognitive deficits in the elderly population. It is used for treatments in patients with

“Alzheimer disease and vascular dementia, with minimal peripheral cholinergic side effects and no unexpected toxicity.” (Wang, 2006).

Animals

One study examined the effects of Hup-A on monkeys with scopolamine induced memory deficits (Ye, Cai, Wang, & Tang, 1999). In this study 8 monkeys, 4 young and 4 old, were tested using a delayed response task. They were shown two food bowls one which was filled, then they were separated from the bowls by a screen for increasingly longer periods of time. The monkeys were intravenously given doses of scopolamine to significantly decrease choice accuracy, and then given Hup-A or a placebo in the same manner, both were dosed according to body weight. They found that Hup-A increased delayed response accuracy in young monkeys given scopolamine with peak effect being a dose of .1 mg/kg with a decrease in effectiveness in .2 mg/kg. No negative side effects were reported at the administered doses and suggest optimal dosing for advantageous effects.

Humans

In one study on healthy adults, researchers tested the effect of 100 and 200 micrograms of Hup-A on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and neurobehavioral performance. Both doses of Hup-A significantly inhibited the release of AChE but not BChE, researchers speculate this is due to the selective property of Hup-A. No changes were found in the neurobehavioral performance (Morasch, Aaron, Moon, & Gordon, 2015). Morasch et al. (2015) states that that dose-response studies should be done to determine the neuroprotective properties of Hup-A and other cholinesterase inhibitors.

Safety

There have been few trails using Hup-A in healthy adults but there was little to no negative side effects associated in this group at dosages of 100- 200 mcg (Morasch et al., 2015). Most of the human clinical trials have been done in patient populations with Alzheimer's disease to enhance cognitive ability and alleviate symptoms associated with this neurodegenerative disease. In one meta-analysis Wang et al. (2009), focuses on the efficacy and safety of Hup-A specifically in patients with Alzheimer's disease. Authors examined 4 large clinical studies where primary outcomes were focused on mini-mental state examinations and activities of daily living (Wang, Wang, Wei, Song, Zhang, & Chen, 2009). The meta-analysis revealed there were no adverse effects on vital signs, blood work, or electrocardiogram readings. Effects were mild to moderate in the worst cases scenarios and typically diminished with time, side effects included nausea, vomiting, and diarrhea; investigators report it was more likely to happen with Hup-A treatment groups than placebo, but it was not statistically significant. The recommended doses were between 300-500 mcg a day in groups with Alzheimer's disease, western countries had a growing interest in the use of Hup-A as a cognitive enhancer in populations in the normal cognitive range and in the United States there were a few supplements that sold it in 100 mcg doses. The statistics from Wang et al. suggest that high doses of Hup-A are required to elicit positive effects in populations with neurodegenerative diseases.

Physical performance

Currently there is no research comparing the effects of Hup-A against placebo or any other supplement to examine its effects on performance or ability to recover from strenuous repeated bouts of exercise (Table 3).

Table 3
Hup-A Dosing and Effects

Use	Dose	Effective	Source
Healthy adults' cognitive performance	100-200 mcg	No	Morasch, K. C. et al., 2015
Adults with neurodegenerative disease	300-500 mcg	^ cog. Scores	Wang, B. S. et al., 2009
Animal trials	.1 mg/kg	∇ cog. Decline	Ye, J. W. et al., 1999
Physical performance	N/A	N/A	None

Chapter 3

Methods

Overview

The purpose of this study was to investigate the synergistic effectiveness of 600 mg of Alpha-GPC and 200 mcg of Hup-A against 5 mg/kg of caffeine and placebo, in increasing anaerobic power output and short-term memory pre and post an exhaustive protocol, in healthy college aged students. The study was conducted as a double blind, placebo controlled, randomized cross over design. The wash out period was a minimum of 48 hours after completion of the testing battery and up to 7 days. Subjects reported to the exercise physiology lab thirty minutes before testing began and consumed either the caffeine, Alpha-GPC and Hup-A, or placebo solution. After the thirty-minute digestion period subjects completed a computer based short term memory test and a thirty-second Wingate anaerobic power test. After 3-5 minutes of low intensity pedaling, subjects performed a high intensity training battery and then were given 5 minutes of full recovery before repeating the first testing battery. Once all testing was completed subjects returned between two and fourteen days after the last test and repeated the protocol.

Procedure and instruments

Subjects reported to the Exercise physiology lab for two testing sessions thirty-minutes prior to the beginning of the testing battery. During the first session subjects were required to fill out the proper IRB approved informed consent and Health history

questionnaire. Subjects were tested for body composition via bioimpedance spectroscopy (BIS) on the SOZO (ImpediMed limited Queensland, Australia) for body fat and lean mass percentage.

In the treatment trials, the subjects were given either solution of 5 mg/kg of caffeine (PURCAF caffeine by KAGED muscle, Boise, Idaho), 200 mcg of Hup-A (Huperzine-A by Double wood supplements, Philadelphia, Pennsylvania) and 600 mg of Alpha-gpc (AlphaSize by Chemi Nutra, Austin, Texas), or a placebo (KarbolyN Fuel by EFX Sports, San Jose, California) 30 minutes before the testing battery began. Randomization was based on rolling a dice by a third-party investigator. The dice roles were assigned to specific treatments by a third-party investigator was responsible for supplement dosage, but was not involved with data collection to ensure true double-blind protocol is followed. All treatments were administered with 8 ounces of water, mixed with 1 standard serving of “Blue Razz Watermelon” (KarbolyN Fuel by EFX Sports, San Jose, California) carbohydrate supplement to ensure subjects weren’t able to tell a difference in flavor across sessions. The Alpha-GPC, Hup-A and caffeine were administered in a white flavorless powder form mixed with the carb supplement for uniformity across visits.

30 minutes after consumption, subjects began the short-term memory assessment. Investigators used a computer-based digit span task software to assess short term memory called a Digit Span memory test performed on PsychoPy by Python software created by Dr. Donald Varakin (Peirce, & MacAskill, 2018). The test took subjects roughly 11-13 minutes per session. Subjects were given a verbal explanation of

the testing procedure before the start and were allowed 1 trial test. The testing procedure is as follows;

One number at a time appeared in the center of the testing window for 1 second with a 1 second delay before the next number appears. Once each number has been displayed a blue enter bar appeared to cue the subject to enter their response. The goal was for the subject to enter all the numbers displayed in the order they appeared for each sequence. The test was divided into 8 parts of 3 number sequences, the first set of 3 had 2 digits, the second 3, the third 4, and increased until the last set contained 10 digits. Scoring was shown as the percent correct of each sequence from the different sequence lengths. The average percent was calculated from all sequence percentages as a cumulative score. Once subjects complete the full digit span task, they completed a Wingate anaerobic power test.

The 30 second Wingate anaerobic power test was performed on a Peak Bike cycle ergometer (Monark, Ergomedic 894E) where mean, peak, and minimal power output were recorded. Subjects were given between a 1 minute and 30 second to 5-minute warm-up requiring that they stay between 60 and 100 revolutions per-minute (rpm) before the onset of the test. Once the warm-up was over subjects were instructed to work up to a maximal sprint, at 120 rpm a weight of 7.5% of their weight in kilograms dropped and created a constant resistance until the end of the 30 seconds. Upon completion the investigator released the resistance and subjects were instructed to continue pedaling for 3 minutes as a cool down.

Peak power output was defined as the most mechanical power output during the test in watts, mean power was the average observed throughout the duration of the test,

minimal power was the least amount of mechanical power output during the test, all values were recorded in watts. Power outputs were calculated in the Monarch analysis software.

Upon completion of the Wingate anaerobic power test and cool down, subjects performed an exhaustive protocol. Three simple exercises were chosen to ensure each subject was able to complete them safely and until they are thoroughly fatigued; push-up, sit-up, and the body weight squat (to the best of their ability). ACSM standards for proper form in each movement to ensure all subjects are being tested uniformly (Proper form displayed in figures 1-3). Each exercise was performed for 1 minute as fast as possible, investigators were watching to ensure correct form is always being used. It is assumed that subjects gave their best effort to ensure a fatiguing effect. Protocol was chosen as an adaptation of the exhaustive protocol used by Hoffman et al., (2010). Once the subject had gone to failure in the exhaustive protocol, they were allowed a 5-7-minute break plus up to 6 ounces of water for rehydration (without the threat of stomach cramping). After the short break subjects will repeat all the testing measurements in the same order. Both pre-exhaustion and post-exhaustion scores were compared in all 3 supplement trials and compared to baseline (Figures 1 – 3).



Figure 1 Correct sit up form



Figure 2 Correct squat form



Figure 3 Correct push up form

Data Analysis

Descriptive statistics were reported for demographic variables with means and standard deviations provided for continuous variables and frequencies and percentages provided for categorical variables. Normality testing was performed on each variable of interest using the Shapiro Wilk test. For the cognitive and anaerobic power output performance comparisons, a sample size of 10-15 per-group was determined as an effective sample size. Based on the number of groups (3) total number of subjects to provide 80% power would be 30-45 to detect a difference between visits/groups. Based on the results of Ziegenfuss et al., (2008), with an average between groups difference being 875 watts (Group 1, 933 watts. Group two 818 watts), significant differences will

be found at an alpha level of $P \leq 0.05$. Power analysis was run using G* Power software 3.1.9.2 (Heinrich Heine, Universitat Dusseldorf). Data was analyzed in a two-step process. First, subject's performance was compared pre to post session to find the percent change in accuracy in the digit span test and peak power, average power, and minimum power output during the 30 second Wingate. These percentages were calculated for each of the subject visits familiarization, Placebo, Caffeine, and Hup-A/Alpha-GPC (treatment). Paired sample T-tests were ran to normalize data, using IBM SPSS 23 (Armonk, NY). Second, the changes were compared across groups using a repeated measures analysis of variance (ANOVA) using the same software as used for the T-test. All visits were compared across visits for each metric. Significance was set with an Alpha level of $P \leq 0.05$ with Tukey Post Hoc analysis used to determine pairwise comparisons significant differences.

Chapter 4

Results

Descriptive statistics

65 healthy adults volunteer to take place in the study, after three subjects were dropped from testing due to scheduling conflicts during the allotted time frame. 62 (N=62 height 1.74 ± 0.089 m., weight 78.5 ± 15.1 kg.) completed the study and were used to analyze results. There were two separate sample sizes used to calculate results from the two-testing metrics. Digit span memory test had a sample size of N=54 and the Wingate Anaerobic power test had a sample size of N=62. Differences in sample sizes were due to technical issues with the Lab computer, once the issue was resolved testing continued as usual. Each subject was randomly assigned to one of the following groups, Placebo (N=21), Caffeine (N=19), and Alpha-GPC/Huperzine-A (N=22) in Power output testing. Each group's results were normalized as a percent of change from pre to post among the following metrics of measure, Peak power visit 1 and 2 (PPv1 and PPv2), Average power visit 1 and 2 (APv1 and APv2), and Minimum power visit 1 and 2 (MPv1 and MPv2). Descriptive statistics for the power output percent change are shown in table 4.

Table 4
Percent power change descriptive statistics

Metric	Group	Mean \pm Std. Dev.	Std. Error
PP v1	Placebo	3.07% \pm 10.35%	2.26%
	Caffeine	8.36% \pm 11.90%	2.73%
	Treatment	4.15% \pm 10.63%	2.27%
PP v2	Placebo	-0.05% \pm 9.63%	2.10%
	Caffeine	1.98% \pm 12.65%	2.90%
	Treatment	-0.91% \pm 13.82%	2.95%
AP v1	Placebo	3.3% \pm 8.97%	1.96%
	Caffeine	8.49% \pm 8.55%	1.96%
	Treatment	3.86% \pm 9.46%	2.02%
AP v2	Placebo	0.44% \pm 10.69%	2.33%
	Caffeine	3.88% \pm 8.08%	1.85%
	Treatment	2.08% \pm 14.92%	3.18%
MP v1	Placebo	25.75% \pm 84.62%	18.47%
	Caffeine	-4.36% \pm 28.04%	6.43%
	Treatment	-11.82% \pm 50.05%	10.67%
MP v2	Placebo	-7.99% \pm 74.16%	16.18%
	Caffeine	-0.06% \pm 18.40%	4.22%
	Treatment	0.77% \pm 24.64%	5.25%

For the digit span memory test score were normalized for percent change in the same manner as power output and were coded as digit span cumulative 1 visit 1 and 2 (DSCv1 and DSCv2) and digit span total visit 1 and 2 (DSTv1 and DSTv2). For the digit span the group sizes were slightly different than the power output testing; placebo N=20, Caffeine N=16 and Treatment N=18. Descriptive statistics for the digit span memory testing percent change are shown in table 5.

Table 5
Digit span percent change descriptive statistics

Metric	Group	Mean \pm Std. Dev.	Std. Error	Min.	Max.
DSCv1	Placebo	3.72% \pm 15.00%	3.35%	-27.78%	21.73%
	Caffeine	4.08% \pm 18.53%	4.63%	-29.42%	36.85%
	Treatment	5.78% \pm 16.90%	3.98%	-30.76%	28.56%
DSCv2	Placebo	6.23% \pm 15.19%	3.40%	-16.67%	36.85%
	Caffeine	3.50% \pm 15.96%	3.99%	-30.76%	27.27%
	Treatment	1.09% \pm 20.29%	4.78%	-53.31%	29.42%
DSTv1	Placebo	0.57% \pm 4.18%	0.93%	-9.81%	10.95%
	Caffeine	0.85% \pm 4.36%	1.09%	-5.51%	11.92%
	Treatment	1.20% \pm 6.77%	1.60%	-6.80%	25.68%
DSTv2	Placebo	-0.07% \pm 3.97%	0.89%	-10.88%	5.56%
	Caffeine	-0.11% \pm 4.39%	1.10%	-9.28%	9.03%
	Treatment	1.88% \pm 3.99%	0.94%	-4.13%	10.70%

Analysis of Variance

An ANOVA was run to determine between group differences in the both digit span and power output percent changes. ANOVA found there was no significant differences between groups with no main effect in power output percent change as shown in table 6.

Table 6
ANOVA of power output percent changes

Metrics	F-Value	P-Value
PPv1	1.289	0.283
PPv2	0.297	0.744
APv1	1.966	0.149
APv2	0.428	0.654
MPv1	2.35	0.104
MPv2	0.255	0.799

The minimum power output percent change in visit 1 was trending toward significance and had the greatest effect size (F= 2.35, P= 0.104) but this was results from the familiarization and has no significant bearing on the findings of the study. ANOVA

found similar results for the digit span testing, no significance and no effect as shown in table 7.

Table 7
ANOVA of digit span percent change

Metrics	F-Value	P-Value
DSCv1	0.08	0.923
DSCv2	0.422	0.658
DSTv1	0.069	0.933
DSCv2	1.372	0.263

Tukey Post Hoc Testing

Post Hoc testing was run using Tukey analysis to find significant differences between groups (Placebo, Caffeine, and Treatment). Because there were no significant differences in the ANOVA testing it was highly unlikely there would be a significant difference found in Post Hoc. All results for the power output percent change are reported in table 8.

Table 8
Tukey Post Hoc Testing Peak Power

Metric	Group	Group	Mean Difference \pm Std. Error	P-Value
PP v1	Placebo	Caffeine	-5.29% \pm 3.46%	0.285
		Treatment	-1.08% \pm 3.34%	0.944
	Caffeine	Placebo	5.29% \pm 3.46%	0.285
		Treatment	4.21% \pm 3.43%	0.441
	Treatment	Placebo	1.08% \pm 3.34%	0.944
		Caffeine	-4.21% \pm 3.43%	0.441
PP v2	Placebo	Caffeine	-2.03% \pm 3.85%	0.859
		Treatment	0.86% \pm 3.71%	0.971
	Caffeine	Placebo	2.03% \pm 3.85%	0.859
		Treatment	2.88% \pm 3.81%	0.731
	Treatment	Placebo	-0.86% \pm 3.71%	0.971
		Caffeine	-2.88% \pm 3.81%	0.731
AP v1	Placebo	Caffeine	-5.19% \pm 2.86%	0.173
		Treatment	-0.56% \pm 2.75%	0.977
	Caffeine	Placebo	5.19% \pm 2.86%	0.173
		Treatment	4.63% \pm 2.83%	0.238
	Treatment	Placebo	0.56% \pm 2.75%	0.977
		Caffeine	-4.63% \pm 2.83%	0.238
AP v2	Placebo	Caffeine	-3.44% \pm 3.72%	0.627
		Treatment	-1.64% \pm 3.58%	0.891
	Caffeine	Placebo	3.44% \pm 3.72%	0.627
		Treatment	1.80% \pm 3.68%	0.877
	Treatment	Placebo	1.64% \pm 3.58%	0.891
		Caffeine	-1.80% \pm 3.68%	0.877
MP v1	Placebo	Caffeine	30.11% \pm 18.89%	0.256
		Treatment	37.57% \pm 18.20%	0.106
	Caffeine	Placebo	-30.11% \pm 18.89%	0.256
		Treatment	7.46% \pm 18.68%	0.916
	Treatment	Placebo	-37.57% \pm 18.20%	0.106
		Caffeine	-7.46% \pm 18.68%	0.916
MP v2	Placebo	Caffeine	-7.93% \pm 14.80%	0.854
		Treatment	-8.76% \pm 14.26%	0.813
	Caffeine	Placebo	7.93% \pm 14.80%	0.854
		Treatment	-0.83% \pm 14.64%	0.998
	Treatment	Placebo	8.76% \pm 14.26%	0.813
		Caffeine	0.83% \pm 14.64%	0.998

For the digit span memory test similar results were found, as shown in table 9.

Table 9
Tuckey Post Hoc
Testing Digit Span

Metrics	Group	Group	Mean difference \pm Std. Error	P-Value
DSCv1	Placebo	Caffeine	-0.36% \pm 5.61%	0.998
		Treatment	-2.06% \pm 5.44%	0.924
	Caffeine	Placebo	0.36% \pm 5.61%	0.998
		Treatment	-1.71% \pm 5.75%	0.953
	Treatment	Placebo	2.06% \pm 5.44%	0.924
		Caffeine	1.71% \pm 5.75%	0.953
DSCv2	Placebo	Caffeine	2.73% \pm 5.79%	0.885
		Treatment	5.14% \pm 5.61%	0.633
	Caffeine	Placebo	-2.73% \pm 5.79%	0.885
		Treatment	2.41% \pm 5.93%	0.913
	Treatment	Placebo	-5.14% \pm 5.61%	0.633
		Caffeine	-2.41% \pm 5.93%	0.913
DSTv1	Placebo	Caffeine	-0.28% \pm 1.75%	0.986
		Treatment	-0.63% \pm 1.70%	0.927
	Caffeine	Placebo	0.28% \pm 1.75%	0.986
		Treatment	-0.35% \pm 1.80%	0.979
	Treatment	Placebo	0.63% \pm 1.70%	0.927
		Caffeine	0.35% \pm 1.80%	0.979
DSTv2	Placebo	Caffeine	0.04% \pm 1.38%	1
		Treatment	-1.95% \pm 1.33%	0.319
	Caffeine	Placebo	-0.04% \pm 1.38%	1
		Treatment	-1.98% \pm 1.41%	0.345
	Treatment	Placebo	1.95% \pm 1.33%	0.319
		Caffeine	1.98% \pm 1.41%	0.345

Gender differences in percent change

Results were analyzed dividing up the genders which were self-reported on the health history questionnaire. Sample size for men in power output was N=38 and N=36 for digit span (weight 85.97 \pm 12.65 kg. height 1.78 \pm 0.074 m.) and the sample size for women for power output was N=24 and N=18 for digit span (weight 66.54 \pm 10.06 kg. height 1.66 \pm 0.062 m.). Each metric (digit span and power output) was analyzed using ANOVA and reported in the following tables (Tables 10 – 13).

Table 10
ANOVA of male power output percent changes

Metrics	F-Value	P-Value
PPv1	2.053	0.144
PPv2	0.567	0.572
APv1	1.850	0.172
APv2	1.375	0.266
MPv1	0.789	0.462
MPv2	0.916	0.410

Table 11
ANOVA of female power output percent changes

Metrics	F-Value	P-Value
PPv1	0.079	0.924
PPv2	0.234	0.794
APv1	0.310	0.737
APv2	0.043	0.958
MPv1	1.626	0.221
MPv2	1.147	0.337

Table 12
ANOVA of male digit span percent change

Metrics	F-Value	P-Value
DSCv1	0.019	0.981
DSCv2	0.206	0.815
DSTv1	0.485	0.620
DSCv2	0.880	0.424

Table 13
ANOVA of female digit span percent change

Metrics	F-Value	P-Value
DSCv1	0.426	0.661
DSCv2	0.606	0.558
DSTv1	0.259	0.775
DSCv2	0.338	0.718

As shown in tables 10- 13 there was no significant differences within groups or between groups with genders separated.

Upon further investigation of the digit span memory test visit 1 pre-exhaustion scores and post-exhaustion were compared to the visit 2 pre and post using ANOVA with LSD and Tukey Post Hoc multiple comparisons. The scores were not based on percent change but what their percent of success was in DSC and DST. Descriptive statistics, ANOVA and Post Hoc multiple comparisons were reported in tables 14, 15 and 16.

Table 14
Post exhaustion digit span total score descriptive statistics

Metric	Group	N	Mean \pm Std. Dev.	Std. Error
V1 DST	Placebo	20	0.937 \pm 0.062	0.014
	Caffeine	17	0.938 \pm 0.044	0.010
	Treatment	19	0.937 \pm 0.067	0.015
V2 DST	Placebo	20	0.959 \pm 0.032	0.007
	Caffeine	17	0.948 \pm 0.041	0.009
	Treatment	19	0.933 \pm 0.045	0.010

Table 15
ANOVA Post exhaustion Digit Span total

Metric	F-Value	P-Value
V1 DST	0.001	0.999
V2 DST	2.047	0.139

Table 16

LSD multiple comparisons

Metric	Group	Group	Mean difference	Std. Error	P-Value
V1 DST	Placebo	Caffeine	-0.000	0.019	0.963
		Treatment	-0.000	0.019	0.982
	Caffeine	Placebo	0.000	0.019	0.963
		Treatment	0.000	0.019	0.981
	Treatment	Placebo	0.000	0.019	0.982
		Caffeine	-0.000	0.019	0.981
V2 DST	Placebo	Caffeine	0.011	0.013	0.424
		Treatment	.026*	0.012	0.049
	Caffeine	Placebo	-0.011	0.013	0.424
		Treatment	0.015	0.013	0.26
	Treatment	Placebo	-.026*	0.012	0.049
		Caffeine	-0.015	0.013	0.26

* The mean difference is significant at the 0.05 level

As seen in table 16, significance differences were found between groups at alpha level 0.049. The difference found was between the treatment and placebo groups based on the mean difference score of -0.026 subjects seemed to do better with the simple carb supplement in post exhaustion compared to the Supplement provided. Though found significant, it does not hold much bearing, the finding is likely a Type 1 Error (False Positive).

Chapter 5

Conclusion

The results of this study indicate that acute ingestion of 200 micrograms of Hup-A with 600 milligrams of Alpha-GPC had no effect on cognitive or physical performance in a healthy, recreationally active population. Further-more the results suggest that after exhaustion the combination of Hup-A and Alpha-GPC may decrease cognitive performance. This does not line up with the current body of knowledge about the combination of Hup-A and Alpha-GPC, because this is the first study of its kind to look at a combination at this dose in a controlled environment with healthy participants. As stated by Jay Hoffman, past investigators have suggested that cholinergic supplementation may be beneficial for exercise, particularly exhaustive exercise, based on the belief that exercise lowers levels of acetylcholine concentrations resulting in fatigue and decrease in performance (Hoffman et. al., 2010). Given that information, a logical assumption would be that increasing the amount of acetylcholine in the system would mitigate the effects of fatigue. But Spector and other investigators found that choline supplementation did not improve performance in fatiguing cycling (Spector, Jackman, Sabounjian, Sakkas, Landers, & Willis, 1995). These assumptions are widely accepted across supplement companies and the same logic is used in nutrition blogs or advertisements for pre-workout and nootropic supplements. Companies are making these claims based on untested assumption without support in the research. This is a

common practice in supplement companies to sell new lines of product to fitness enthusiasts (Jagim, et. al., 2016).

One goal of this study was to put the supplement combination of Alpha-GPC and Hup-A to the test and see if there was an ergogenic effect based on the assumptions mentioned earlier. Based on the results from this study there was no effect on performance. The current body of research for these two supplements are done on an individual basis and in most cases are performed on subjects with Alzheimer's or Dementia (Wang, et. al., 2009, & Barbagallo, et. al., 1994). Barbagallo et. al. found that the use of Alpha-GPC over a period of 28 days in patients with recent stroke or transient ischemic attacks helped psychological recovery. Similarly, a meta-analysis by Wang et. al. on the effects and efficacy of Hup-A as a treatment for Alzheimer's disease. Wang found that after an 8-24-week period of ingesting 300-500 micrograms of Hup-A orally significantly improved the scores of patients with Alzheimer's disease. The tests they used were the Mini-mental state examination and the Activities of daily living scale. The Mini-mental state examination is brief test to screen for Dementia including questions on orientation, attention, recall and language (Galea & Woodard, 2005). As seen in these two studies each supplement has been shown to be safe and effective in increasing cognitive ability in those with neurodegenerative diseases. These studies demonstrate there is a use for these supplements in those specific populations but there is little other evidence to support the claims made by companies that they are beneficial for a healthy population. Because of this gap in literature we investigators felt it was necessary to see the potential in a healthy population. Both the mini-mental state exam and the activities of daily living scale are self-reported questionnaire. For this study the

investigators found it important that the treatment of Alpha-GPC and Hup-A be tested using quantifiable results to find if there is an actual change and not the perception of change. Another difference is the acute supplementation versus the chronic supplementation model. An acute model was chosen to more accurately represent how pre-workout supplements are taken by the recreationally trained population (30-60 minutes/upon arrival). The previous literature only shows the changes in ill populations and none of the testing in Hup-A is done using a demonstrable performance-based test, they are all self-reported tests based on self-perception which is not always indicative of reality.

The use of healthy recreationally trained subjects was purposeful because these are predominantly the ones being targeted by the supplement companies to buy their products. As previously mentioned, these companies are not required to show empirical evidence to support their claim that they work (Martinez, Campbell, Franek, Buchanan, & Colquhoun, 2016). Even without the proper support for their claim they shamelessly market their products. Take this article by Cellucor (a supplement company) for example;

“Studies have also suggested that Huperzine-A can boost your mental energy. Taken as a part of your pre-workout supplement, you may have more mental energy and a better ability to learn and remember new exercises.”

“As a pre-workout supplement, Alpha GPC can help to boost your mental energy.”

“It also helps with athletic performance. A study published in Journal of the International Society of Sports Nutrition demonstrated that Alpha GPC supplementation resulted in elevated levels of lower body strength output, and researchers confirmed it can be useful in promoting overall speed and power.”

“Finally, Alpha GPC has been said to pair extremely well with other nootropics, such as Huperzine A and caffeine, resulting in enhanced benefits.”

The only cited research specific to Alpha-GPC or Hup-A in this article references to the previously mentioned study by Bellar and colleagues stating it increases lower limb isometric strength by an average of 22.2 lbs. 6 days of loading at 600 mg. a day (Bellar, 2015). The supplement they sell using this article only contains 200 mg preserving. This is a classic example of supplement companies making false claims to sell a product.

Another comparison performed in the current study was comparing the effects of the Alpha-GPC/Hup-A to caffeine dosed at 5 milligrams per-kilogram of bodyweight. This dose was chosen because it was found to be efficacious in improving both physical and cognitive performance in healthy subjects (Pasma et. al., 1995, Davis and Green, 2009, Vanakoski et. al., 1998). Based on the results there was no significant difference between groups at any level. The results of this study found that neither Alpha-GPC/Hup-A or Caffeine influenced performance compared to placebo which is not supported by the current research. This has led the investigators to believe there are some design flaws in the method used to test the supplements.

The study used a randomized double-blind procedure to make sure there was no influence on the subjects from the testers. Optimally, it would not be three separate groups, but a randomized cross-over design where each subject was given all three treatments. This would control better for inter-subject variations because each subjects' results would be compared against themselves. Other issues with the design was the freedom given to subjects on the start time for their Wingate bike sprint. Subjects were given the option to start as early as 1 minute and 30 seconds or as late as 5 minutes after pedaling at 60-100 rpm. The variation in the warm-up time could have led to the consistency issues experienced between visits and between subjects. Another issue is

unfamiliarity with the equipment causing issues with executing the test properly even under instruction.

Gender specific comparisons were analyzed to determine if there was a possible gender specific effect. The result suggests there is no gender difference between groups implying one gender reacts more to the substances than another. Currently there is no published data looking at the differences between genders in these supplements. The cause of this may be due to what is known as survivorship bias in research (Brown & Goetzmann, 2018). This is based on the idea that in research many times only the studies that found that something worked are published. So, if there was a study that found that the supplement combination didn't work it may not have been published due to this bias.

Future Research

In the event future investigators try to recreate the current study they should take into consideration some of the issues that the primary investigators encountered. One issue with a two-visit test was the natural learning effect that subjects had when performing these new tasks. In many cases the subject would do better in the second Wingate sprint than in the first one. Based on the individuals experience and with performing Wingate's investigators believe that from pre to post the subjects had a better understanding of how to perform the testing. So, even after being put in a state of exhaustion, subjects would produce higher peak power. To control for this issue, if a subject comes in for the first test and scores higher in the post testing than the pre-testing, they should have to come in for another familiarization test. Another issue is proper warm-ups. Investigators should have used the standard 5 minutes at 60-100 rpm

warm up to control for variations between subject visits. Lastly, in several studies previously mentioned the supplements were given over a span of 28 days or 8 weeks. Given this information future investigators should consider examining the effects Alpha-GPC and Hup-A after a loading period.

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APPENDICES

Appendix A: Informed Consent

Consent to Participate in a Research Study

**Effects of Alpha-GPC and Huperzine-A on Short
Term
Memory, Anaerobic Power Output, Post Exhaustion
compared to Caffeine and placebo in Healthy
College Age Students**



Institutional Review Board
Protocol Number

2422

Approval Valid

6/11/19-5/1/ 20

Key Information

You are being invited to participate in a research study. This document includes important information you should know about the study. Before providing your consent to participate, please read this entire document and ask any questions you have.

Do I have to participate?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop at any time during the study and still keep the benefits and rights you had before volunteering. If you decide to participate, you will be one of about 60 people in the study.

What is the purpose of the study?

The purpose of the study is to determine if the two supplements (Alpha-GPC and Huperzine-A) will be more effective in improving mental and physical performance compared to a placebo or caffeine. Another purpose of this study is to determine if the two supplements will increase performance once you are tired after doing multiple exercises. You have been selected to participate because you are a healthy person between the ages of 18-40 years old and with the proper amount of training history (2 or more years), **and are not pregnant.**

Where is the study going to take place and how long will it last?

The research procedures will be conducted at the Moberly building on Eastern Kentucky University's campus. You will need to come to Moberly 223 a total of 2 times over the period of 2 weeks during the study. Each visit will take approximately 1 hour to 1 hour and 15 minutes. The total amount of time you will be asked to volunteer for this study is 2-3 hours over the next 14 days.

What will I be asked to do?

For the first visit, you will be required to complete a survey about your current caffeine usage and a health history questionnaire. If you are a female participant, you will be required to visit the student health center to take a pregnancy test to verify you are not currently pregnant. A note from the student health center must be provided to the research team prior to

participating in the testing procedures. Once the survey is completed, you will be asked to perform a simple body composition test to determine body fat and fat free mass. This will require you to stand still for one minute while standing on an electronic scale. Lastly, you will have your blood pressure and resting heart rate taken by an automatic blood pressure cuff. After all paper work and body composition testing is finished, you will be asked to perform a memory test. For the test, you will be required to watch a computer screen that will show a series of numbers to you. Once all the numbers in the sequence have been displayed, you will be asked to type the digits you saw in order, to the best of your ability. The sequences will increase from 2 to 10 digits.

After the memory test has been completed, you will then sit on a stationary bike and be asked to pedal for 1 minute at a medium pace. After 1 minute, you will sprint for 10 seconds against no resistance then return to a medium pace for 1 minute before working up to a 30 second all out sprint against a brake weight of 7.5% of your body weight. You will be allowed a period to pedal as a cool down for three minutes before moving on to the next step of testing which will include 3 different exercises. The exercises will include: 1 minute of body weight squats, 1 minute of push-ups (from toes or knees), and 1 minute of sit ups. You will be asked to perform as many repetitions of each exercise as you can during each minute. Once all 3 exercises are finished, you will rest quietly for 7-10 minutes and can drink water if needed. After the rest period, you will repeat the memory test and the bike test.

For the second visit, you will be required to drink one of the following: the combination of both AlphaGPC and Huperzine-A, caffeine, or placebo and then sit quietly for 30 minutes while the drink digests. Once the digestion period is over, you will be asked to complete the same testing process as the first visit. You have an equal chance of being assigned to each drink.

Are there reasons why I should not take part in this study?

You should not participate in this study if you are under the age of 18 or over 40 years old, have chronically high blood pressure (blood pressure greater than 140/90), a high resting heart rate (beats per minute greater than 100), a history of metabolic (diabetes), cardiovascular (heart disease), or respiratory disease (asthma). **If you are or could be pregnant.** Also, if you have orthopedic issues (joint, muscle, or ligament pain), or mobility limitations, you may not want to participate. Lastly, if you have allergies to the any of the supplements you may be taking in this study, you will want to refrain from participation.

What are the possible risks and discomforts?

Possible risks and discomforts that may occur are as follows.

- Bike test may cause muscle fatigue, heavy breathing, sweating, and mild nausea.
- Exercises may cause muscle fatigue, heavy breathing, sweating, muscle soreness, and mild nausea.
- Supplements may cause increased nervousness, anxiety, mild nausea, and a slim chance of diarrhea.

These discomforts may only last 1-2 minutes or as long as 15 minutes based on each individual. Muscle soreness after strenuous exercise is a common side effect but may not occur until 1-2 days after testing is complete. This discomfort (if any) will likely not last longer than 3 days. If you have any further questions/concerns, please contact the research team, contact information is located on page 3.

You may, however, experience a previously unknown risk or side effect.

What are the benefits of taking part in this study?

You are not likely to get any personal benefit from taking part in this study. Your participation is expected to provide benefits to others by finding if the combination of the previously mentioned supplements are as effective or more effective than caffeine.

If I don't take part in this study, are there other choices?

If you do not want to be in the study, there are no other choices except to not take part in the study.

Now that you have some key information about the study, please continue reading if you are interested in participating. Other important details about the study are provided below.

Other Important Details

Who is doing the study?

The person in charge of this study is John Isaacs, a graduate assistant in the Exercise and Sports Science Department at Eastern Kentucky University. Dr. Michael Lane, Dr. Aaron Sciascia, and Dr. Donald Varakin will be advising him. There may be other people on the research team assisting at different times during the study.

What will it cost me to participate?

There are no costs associated with taking part in this study.

Will I receive any payment or rewards for taking part in the study?

You will not receive any payment or reward for taking part in this study.

Who will see the information I give?

Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers, we will write about this combined information. You will not be identified in these written materials.

All data will be kept confidential and you will not be identifiable. All records will be kept under lock and key. Only those researchers listed above will have access to identifiable information and data results.

Can my taking part in the study end early?

If you decide to take part in the study, you still have the right to decide at any time that you no longer want to participate. You will not be treated differently if you decide to stop taking part in the study.

The individuals conducting the study may need to end your participation in the study. They may do this if you are not able to follow the directions they give you, if they find that your being in the study is more risk than benefit to you, or if the University or agency funding the study decides to stop the study early for a variety of reasons.

What happens if I get hurt or sick during the study?

If you believe you are hurt or get sick because of something that is done during the study, you should call John Isaacs at 502-320-3371 or Dr. Michael Lane at 859-622-1890 immediately. It is important for you to understand that Eastern Kentucky University will not pay for the cost of

any care or treatment that might be necessary because you get hurt or sick while taking part in this study. Also, Eastern Kentucky University will not pay for any wages you may lose if you are harmed by this study. These costs will be your responsibility.

Usually, medical costs that result from research-related harm cannot be included as regular medical costs. Therefore, the costs related to your care and treatment because of something that is done during the study will be your responsibility. You should ask your insurer if you have any questions about your insurer's willingness to pay under these circumstances.

What else do I need to know?

You will be told if any new information is learned which may affect your condition or influence your willingness to continue taking part in this study.

We will give you a copy of this consent form to take with you.

Consent

Before you decide whether to accept this invitation to take part in the study, please ask any questions that come to mind now. Later, if you have questions about the study, you can contact the investigator, John Isaacs at 502-320-3371 John_isaacs38@mymail.eku.edu or Dr. Michael Lane at Michael.lane@eku.edu (859-622-1890). If you have any questions about your rights as a research volunteer, you can contact the staff in the Division of Sponsored Programs at Eastern Kentucky University at 859-622-3636.

If you would like to participate, please read the statement below, sign, and print your name.

I am at least 18 years of age, have thoroughly read this document, understand its contents, have been given an opportunity to have my questions answered, and voluntarily agree to participate in this research study.

Signature of person agreeing to take part in the study

Date

Printed name of person taking part in the study

_____ Name of person providing information to subject

Appendix B: Caffeine Survey

Effects of Alpha-GPC and Huperzine-A on Short Term Memory, Anaerobic Power Output, Post Exhaustion compared to Caffeine and placebo in Healthy College Age Students
Caffeine Consumption survey

Name _____ Age _____ Weight _____
Date _____

Scale of 1 – 10 (1=not at all, 10=can't be without). How much do you depend on caffeine on a daily basis to function properly? _____

What form of caffeine do you most often consume?

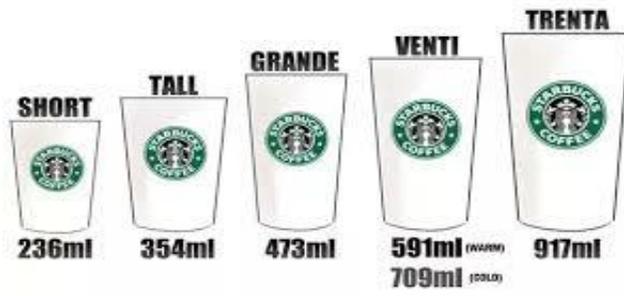
Give a rough estimate of how many milligrams (Mg) of caffeine you consume daily (Example 1 cup of coffee = roughly 90-100 mg of caffeine) _____

Weekly _____

Intake guidelines chart

Drinks/Foods	Volume	Caffeine content mean & range
Filter coffee	125 ml (4 Fl. Oz. cup)	85 (65-135)
Espresso	30 ml (1 shot)	60 (35-100)
Soluble Instant Coffee	125 ml (4 Fl. Oz. cup)	65 (35-105)
Decaffeinated	125 ml (4 Fl. Oz. cup)	3 (1-5)
Tea	150 ml (5 Fl. Oz. cup)	32 (20-45)
Iced Tea	330 ml (11 Fl. Oz. cup)	20 (10-50)
Hot chocolate	150 ml (5 Fl. Oz. cup)	4 (2-7)
Caffeinated soft drinks	330 ml (11 Fl. Oz. cup)	39 (30-48)
Sugar-free Caffeinated soft drinks	330 ml (11 Fl. Oz. cup)	41 (26-57)
Energy Drinks	330 ml (11 Fl. Oz. cup)	80 (70-120)
Pre-workout	1 powder scoop	300 (200-400)
Chocolate bar	30 g (1 Oz.)	20 (5-36)
Milk Chocolate	30 g (1 Oz.)	6 (1-15)
Dark Chocolate	30 g (1 Oz.)	60 (20-120)

Source (<https://www.coffeeandhealth.org/topic-overview/sources-of-caffeine/>)



Appendix C: Recruitment Script

Recruitment script.

Thank you for allowing me to come speak to your class about the current research I will be conducting in the exercise physiology lab here at Eastern Kentucky University.

The current study is “Effects of Alpha-GPC and Huperzine-A on Short Term Memory, Anaerobic Power Output, Post Exhaustion compared to Caffeine and placebo in Healthy College Age Students”. In this study you will be asked to come to the exercise physiology lab for 2, 1-hour long visits. In each visit you will be asked to perform a digit span memory test, Wingate anaerobic power test, exhaustive protocol, and then repeat the digit span and Wingate tests.

In the first visit you will be divided randomly into either a caffeine group, placebo group, or treatment group. The group you are assigned to will be blinded to both the researcher conducting the tests and you. Each supplement will be in specific doses to provide a specific ergogenic effect (caffeine 5mg/kg, Alpha-GPC 600mg and Huperzine-A 200mcg). All doses have been tested for safety and been shown to be safe for use in acute dosing.

All subjects must have a least 2 years’ experience in some form of physical training and be between 18-40 years of age. Sign ups are with me and you can contact me at John_isaacs38@mymail.eku.edu if you are interested.

Thank you,

John Isaacs

Appendix D: Data Collection

Effects of Alpha-GPC and Huperzine-A on Short Term Memory, Anaerobic Power Output, Post Exhaustion compared to Caffeine and placebo in Healthy College Age Students

Primary investigator: John Isaacs. Sub-Investigators: Michael Lane

Familiarization

Subject Name _____(optional)

Date _____

Subject number _____ Height _____ Weight _____ Body

Comp _____

Hours of sleep last night _____

Digit span score Pre-test _____

Wingate Pre-test-Peak _____ Average _____

Minimum _____

of sit ups _____ # of push up _____ # of squats _____

Digit span score Post-Test _____

Wingate Post-Test- Peak _____ Average _____

Minimum _____

Visit 2

Date _____

Digit span score Pre-test _____

Wingate Pre-test-Peak_____

Average_____

Minimum_____

of sit ups_____

of push up_____

of squats _____

Digit span score Post-Test_____

Wingate Post-Test- Peak_____

Average_____

Minimum_____