THE EFFECTS OF α-GPC SUPPLEMENTATION ON GROWTH HORMONE, FAT LOSS, AND BODY COMPOSITION IN OVERWEIGHT ADULTS

by

WILLIAM G. MALDONADO

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ABSTRACT OF THE THESIS

The Effects of α-GPC Supplementation on Growth Hormone, Fat Loss, and Body Composition in Overweight Adults

By WILLIAM GERARD MALDONADO

Thesis Director

Shawn M. Arent

In the United States, there is an increasing prevalence of obesity that is associated with health risks, and, as such, the need for effective weight loss methods is becoming increasingly more important. In the elderly, α-GPC has been shown to significantly increase growth hormone (GH) concentrations, a major stimulator of lipolysis and protein synthesis. However, very little work has been done in younger individuals. **PURPOSE**: to investigate if α-GPC, an acetylcholine precursor, could confer additional GH or weight loss benefits to active, overweight individuals while exercise and nutrition are maintained. **METHODS**: Participants were randomly assigned to either α-GPC (n=15, M_{age}=25.8±9.1y, $M_{BF\%}$=35.48±1.75%) or placebo (n=13 M_{age}=24.4±10.4y, $M_{BF\%}$=35.65±1.98%) after health/fitness screening. Both groups were instructed to consume two capsules of their respective supplement for a total of 1200 mg/day, one dose before their workout or on non-workout days with their midday meal, and the second dose before going to sleep, for eight weeks. Assessments were performed pre- and post-supplementation and included resting blood pressure and heart rate, activity level via Framingham Physical Activity Index (F-score), body composition via air-displacement

plethysmography (fat mass [FM], fat free mass [FFM[, body fat percentage [BF%], body mass [BM]), and girth measurements (waist, hips). Additionally, blood samples were obtained for analysis of growth hormone (GH). Throughout the duration of the study participants were instructed to maintain their current activity level and diet. During the weeks leading up to pre- and post-testing, daily caloric intake was reported using MyFitnesspal. RM-MANOVAs with univariate follow-ups were conducted to determine differences between groups over the course of the trial with significance set at $P<0.05$. **RESULTS**: There were no significant differences between groups for any body composition, girth measurements, GH, caloric intake, or F-score from pre- to post-intervention ($P>0.05$). There were significant main Time effects for decreases in BF%, FM, and waist measurements ($P<0.05$) as well as trends for decreased BM ($P=0.094$) and increased FFM ($P=0.064$). No main effects were observed for any other variable ($P>0.05$). Univariate follow-ups showed a significant Time-by-Group interaction for an increase in SBP in the α-GPC group ($P<0.05$). A negative trend was seen for total daily caloric intake among all subjects over time ($P=0.066$, ES=0.136). **CONCLUSIONS**: Overall, supplementation with α-GPC alone under the conditions of this study did not result in additional body mass loss, alterations in body composition, or changes in GH, when compared to a placebo. This study did show that the act of tracking diet may have been sufficient to alter behavior, however more research is required. As such a future direction should investigate if tracking diet in conjunction with maintained exercise is sufficient to produce significant body composition changes.

Acknowledgments and Dedications

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Chapter 1: α-GPC a history

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Introduction

Obesity in America has been on the rise over the last two decades, specifically in adults over the age of 20 where increases from 30.5% of the population to 39.6% have been noted from 1999 to 2016 (Hales et al., 2017). With this rise in obesity, the overall medical expenses related to obesity have also increased (Finkelstein et al., 2009). The most recently reported annual medical cost of obesity in the United States was $147 billion in 2008 (Finkelstein et al., 2009). Additionally, at this time, the average medical cost for an obese individual was $1,429 higher compared to a normal-weight individual (Finkelstein et al., 2009). In response to this increase in obesity rates, different methods of decreasing body fat and overall body mass have been sought after by the population as a whole to ameliorate this public health issue and other obesity-associated diseases. One well-known method of inducing weight loss is endurance-based exercise which has been shown to increase rates of fat oxidation up to 10-fold from rest. However, this option is often neglected (Achten & Jeukendrup, 2004). Significant weight loss results require long-term adherence, which is often not met. Therefore, weight-loss supplements appear to be an appealing alternative or adjunct to exercise, stemming from marketing claims of easy and fast ways to increase fat oxidation and improve weight loss (Jeukendrup & Randell, 2011). In 2002, it was shown that approximately 15% of adults in the U.S. had used weight-loss supplements (Blanck et al., 2007). In 2015, the sales of weight loss supplements pills reached $2.1 billion dollars, while weight-loss meal supplements produced $3.85 billion dollars in sales (Polito, 2016). Due to the escalating prevalence of obesity rates, research is needed to assess the efficacy and safety of various weight-loss supplements (Heinrich, 2002).

A common weight loss supplement that was also one of the first in the market is green tea extract (GTE), which has been shown to increase catecholamine levels, resulting in increased energy expenditure at rest (Dulloo et al., 1999). Early research observing respiratory quotient (RQ) data showed the increased energy expenditure was from fat; however, when individuals consumed additional caffeine along with the natural quantity found in GTE, the drop in RQ and increase in fat oxidation is not readily observed (Dulloo et al., 1999; Westerterp-Plantenga et al., 2005). This can be a major issue with this supplement because approximately 85% of Americans consume at least one cup of caffeinated beverage in a day, with a daily intake upwards of 380 mg caffeine/day (Mitchell et al., 2014). Another popular weight-loss supplement was ephedrine which showed promise in improving and maintaining weight loss over long periods of time through increasing norepinephrine release (Dulloo et al., 1991; Toubro et al., 1993). While ephedrine was effective in promoting weight loss, it also increased risk of psychiatric, autonomic, and gastrointestinal distress and heart palpitations and strokes, which lead to its eventual ban in 2004 due to several deaths (Food and Drug Administration, 2004; Morgenstern et al., 2003; Shekelle et al., 2003). Studies of efficacy and safety of these types of weight-loss supplements are crucial. Further, an alternative supplement that may be more applicable for weight-loss while mitigating these adverse effects is L-α-glycerylphosphorylcholine (α-GPC).

α-GPC (chemical structure shown in Figure 1) is a semi-synthetic derivative of lecithin which, *in vivo*, is an intermediate in the biosynthetic pathway of phosphatidylcholine (Brownawell et al., 2011). This essential step results in the synthesis of choline, which serves an important role in cell membranes, particularly in neurons, as

well as a component to the neurotransmitter acetylcholine (ACh) (Dross & Kewitz, 1972; Spanner & Ansell, 1982). α-GPC has also been shown to cross the blood-brain barrier and increase choline availability for ACh synthesis in cholinergic neurons, leading to an increase in the production of ACh (Abbiati et al., 1993; Trabucchi et al., 1986). Following ingestion of α-GPC, it is hydrolyzed in the gut mucosa by phosphodiesterases, and, when administered intravenously, it is oxidized by glycerophosphorylcholine phosphodiesterase (GPCP) in the brain to produce choline and glycerophosphate, (Abbiati et al., 1991; Lopez et al., 1991; Trabucchi et al., 1986). Acetylcholinesterase (AChE, or ChAT) in cholinergic neurons then forms ACh through the combination of choline and acetyl-CoA (summarized in Figure 2) (Oda, 1999). In neuronal tissues in the central and peripheral nervous system, ACh is a common neurotransmitter supplied mainly from dietary intake of choline (Halbach & Dermietzel, 2002; Webster, 2001). At the level of the neuron, only approximately 50% of choline from ACh hydrolysis is recovered, and, as a result, neurons require additional supply for ACh synthesis (Schwartz, 1991).

Figure 1: Chemical structure of α-GPC (adapted from Brownawell et al., 2011)

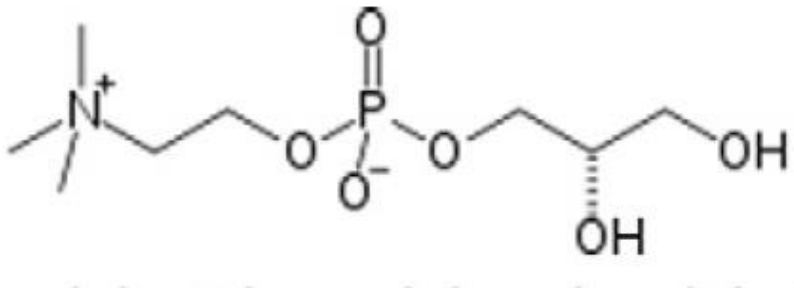
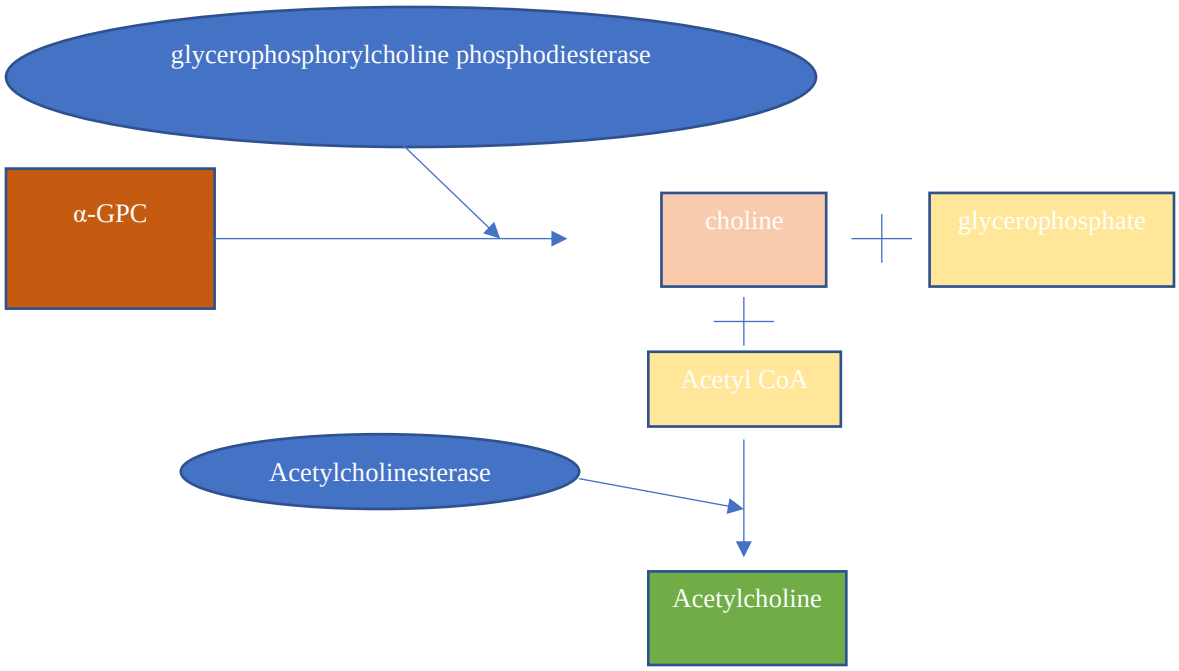


Figure 2: α-GPC Breakdown and Utilization



When the exogenous supply of choline is insufficient, membrane phospholipids can be hydrolyzed in order to provide choline for neurotransmitter synthesis. This degradation has been implicated as a mechanism of action in Alzheimer's disease (AD) patients, where a dysregulation of the cholinergic system occurs, resulting in cell apoptosis in an attempt to provide enough choline from the membrane phospholipids (Bartus et al., 1982; Blusztajn et al., 1990). Preliminary research of α-GPC focused mainly on rodents to demonstrate the potential use in human subjects with AD. Early mouse studies utilized cholinolytic scopolamine in order to simulate the cholinergic dysregulation seen in AD patients. α-GPC was able to increase endogenous ACh release by 2.5-fold with effects lasting up to 30 hours in mice (Lopez et al., 1991). Further, rat studies providing a diet designed to induce cognitive decline, α-GPC supplementation was able to prevent neurodegeneration (Suchy et al., 2009). When scopolamine was used in humans, α-GPC was shown to mitigate the induced cognitive decline (Canal et al., 1991). These findings provide evidence that α-GPC can serve to increase compromised

ACh levels, resulting in a partial rescue phenotype, the ability to restore back to a normal phenotype, even under the presence of a disease such as Alzheimer's.

As a result of this mechanism of action, animal research, and potential efficacy for preventing neurodegenerative disease, a majority of the research on α-GPC has been done in the elderly population for the potential effects on cognition as an ACh precursor. Within two weeks of supplementation in elderly adults, α-GPC improved memory, attention, and visual learning, as well as the ability to perform activities of daily living (Richter et al., 2011). Following an additional ten weeks of supplementation, the same population saw further improvements in performance of activities of daily living (Richter et al., 2011). More recent work in a college-aged population showed that with acute supplementation of α-GPC, subjects were able to maintain reaction time, as well as subjective feelings of focus and alertness following exhaustive exercise (Hoffman et al., 2010).

While α-GPCs does have impact on ACh levels resulting in improved cognitive measures, ACh also has involvement in the endocrine system. ACh binds to cholinergic receptors on the hypothalamus inhibiting the release of somatostatin. This prevents somatostatin's inhibition of GH secretion from the anterior pituitary (Casanueva et al., 1986; Locatelli et al., 1986; Maier et al., 2004; Mazza et al., 1994; Richardson et al., 1980; Torsello et al., 1988; Wehrenberg et al., 1992). Furthermore, ACh stimulation of cholinergic receptors in the GI tract results in the release of ghrelin, which also acts to stimulate GH secretion (Casanueva et al., 1986; Locatelli et al., 1986; Maier et al., 2004; Mazza et al., 1994; Richardson et al., 1980; Torsello et al., 1988; Wehrenberg et al., 1992). The main secretagogue of GH is growth hormone releasing hormone (GHRH),

which is released from the hypothalamus into hypothalamic-hypophyseal circulation to stimulate GH secretion from the anterior pituitary (Mayo et al., 2000). GH plays a critical role in lipid metabolism by decreasing fatty acid synthesis in adipose tissue and increasing plasma FFA concentrations through increasing the activity of hormone sensitive lipase (HSL) in adipocytes through post-transcriptional mechanisms (Fain & Wilhelmi, 1962; Goodman, 1963a, 1963b; Raben, 1962; Raben & Hollenberg, 1959; Slavin et al., 1994; Zimmermann et al., 2003). HSL acts to hydrolyze triacylglycerols, diacylglycerols, cholesterol esters, and retinal esters, which result in increased FFA release from adipose tissue (Zimmermann et al., 2003). In addition to its role of increasing amino acid uptake in skeletal muscle, GH also increases synthesis of insulin-like growth factor 1 (IGF-1) predominantly from the liver and has been shown to be synthesized and secreted in a *de novo* fashion (Francis & Hill, 1975; Kostyo et al., 1959; Kostyo & Schmidt, 1961; Mayo et al., 2000; Mcconaghey & Sledge, 1970; Moses et al., 1980; Noall et al., 1957; Phillips et al., 1976; Riggs & Walker, 1960; Rosenfeld et al., 1989; Schwander et al., 1983; Scott et al., 1985). IGF-1 functions as part of a negative feedback loop through binding to receptors on somatotrophs to inhibit further GH synthesis. Like ACh, IGF-1 also acts to stimulate hypothalamic somatostatin which inhibits GH secretion. GH secretion is also inhibited via a negative feedback loop mediated by GH receptors in the hypothalamus (Mayo et al., 2000; Rosenfeld et al., 1989). Due to its role in mediating cholinergic dysregulation through increasing choline levels, α-GPC may also augment growth hormone (GH) levels. With aging, there is a decreased pituitary response to GHRH, and ACh precursors, like α-GPC, have been previously shown to stimulate both basal GH levels and exogenous GHRH-mediated GH

release four-fold and two-fold, respectively, in this population (Ceda et al., 1989; Ceda et al., 1991).

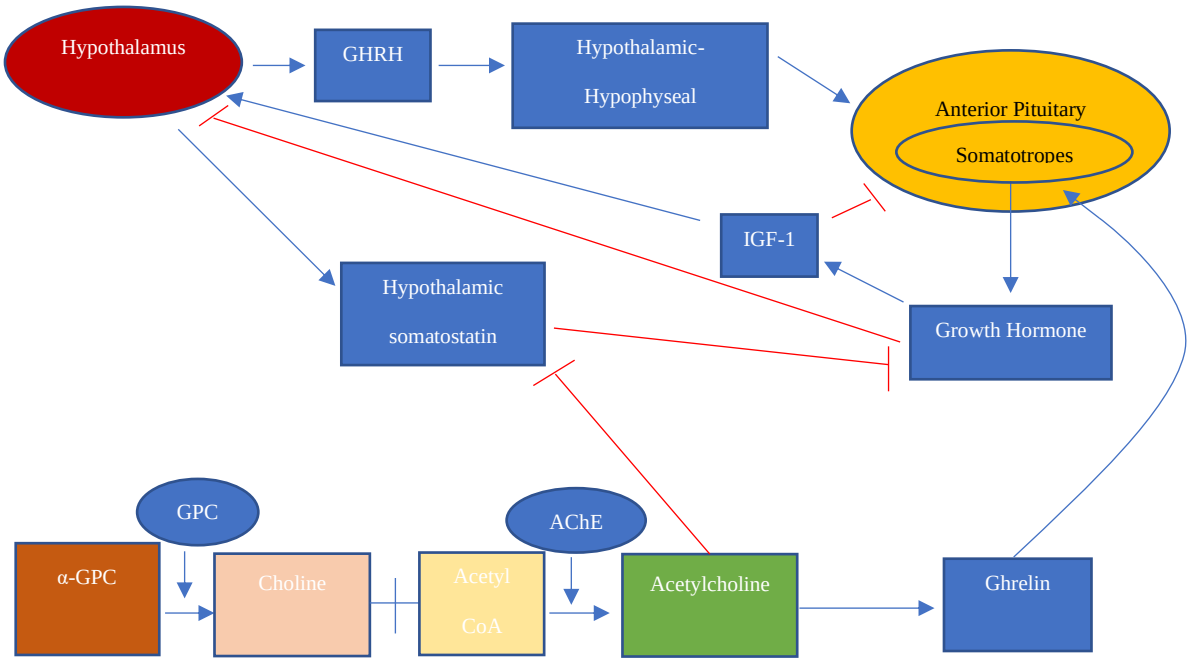
The effect of α-GPC on GH stimulation was further investigated in an elderly population with subjects receiving either an injection of 1 μg/kg GHRH or 1μg/kg GHRH plus an additional 2 grams of α-GPC in saline infusion over 15 minutes (Ceda et al., 1992a). Subjects who received GHRH plus α-GPC had a significantly higher GH response than those who received GHRH only. These results show that in elderly individuals with a diminished GH response to GHRH, α-GPC can serve to potentiate GH release (Ceda et al., 1989; Ceda et al., 1992a; Mayo et al., 2000). A second protocol within the same study involved subjects receiving two injections of 1 μg/kg GHRH separated by 120 minutes. Half of the subjects then received an additional intravenous (IV) injections of 2 grams α-GPC 15 minutes prior to the second injection of GHRH. Subjects who did not receive α-GPC had increased GH secretion after the first dose, but suppressed secretion after the second. However, subjects who received α-GPC showed a further increase in GH following the second injection of GHRH (Ceda et al., 1992a; Ceda et al., 1992b).

Following this research in the elderly, a single-dose oral administration of 1 g of α-GPC was shown to result in an acute increase in GH secretion in healthy young males, which coincided with increases in plasma GH and serum free fatty acid (FFA) levels at 60- and 120-minutes post-ingestion compared to a placebo group (Kawamura et al., 2012). Additional research in men between the ages of 30 and 40 years with at least two-years resistance training (RT) experience supported these findings, as 600 mg α-GPC 90-minutes prior to resistance training led to a 44-fold increase in peak GH levels after the

bout compared to a 2.6-fold in the placebo group (Ziegenfuss et al., 2008). While training history was not controlled between groups and may have contributed to the large difference observed between groups, this study indicated that α-GPC may have efficacy outside of an elderly population.

More recent work has investigated the potential ergogenic effects of the ACh precursor in college-aged individuals. One study showed that subjects who received a supplement containing 600 mg α-GPC displayed a significant increase in peak bench press force when compared with a placebo group (Ziegenfuss et al., 2008). Additionally, when only 300 mg of α-GPC was provided in a multi-ingredient supplement 30-min prior to testing, a statistically significant 3% increase in vertical jump power was observed in a crossover design with caffeine and a placebo supplement (Shields et al., 2014). Similarly, when given daily for a week, 250 mg α-GPC was shown to significantly improve max velocity and max mechanical power during a countermovement jump (Marcus et al., 2017). Despite these findings, the role of α-GPC in augmenting body composition remains to be examined.

Figure 3: Proposed Mechanism of α-GPC on GH



Given that α-GPC may influence GH levels, it could be postulated that this supplement may cause favorable body compositional changes, as GH has been shown to increase FFM and decrease FM (Fain & Wilhelmi, 1962; Goodman, 1963a, 1963b; Kawamura et al., 2012; Kostyo et al., 1959; Kostyo & Schmidt, 1961; Mayo et al., 2000; Noall et al., 1957; Raben, 1962; Raben & Hollenberg, 1959; Riggs & Walker, 1960; Rosenfeld et al., 1989; Slavin et al., 1994; Ziegenfuss et al., 2008; Zimmermann et al., 2003). Thus, the purpose of the current study was to investigate the effects of daily supplementation with 1200 mg of α-GPC on weight loss, body composition changes, and GH levels in overweight adults who maintain current exercise and dietary habits. Given the previous literature showing that α-GPC may increase GH levels, which can alter body composition, it was hypothesized that α-GPC would induce increased loss of FM. Additionally, we hypothesize an increase in GH levels and FFM and a decrease in body mass.

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Chapter 2: The Effects of α-GPC Supplementation on Growth Hormone, Fat Loss, and Body Composition in Overweight, Active Adults

By

WILLIAM G. MALDONADO

Introduction

Obesity in America has been increasing steadily over the past two decades, increasing by nearly ten percent in adults age 20 or older (Hales et al., 2017). This rise has also brought a sharp rise in medical expenses, with an estimated cost of $147 billion in 2008 (Finkelstein et al., 2009). Exercise has been an often overlooked means of combating this epidemic by increasing fat loss, with weight loss supplement usage being the far more popular option (Achten & Jeukendrup, 2004; Jeukendrup & Randell, 2011). The weight loss supplement industry recently reported sales of $2.1 billion in 2015, however more research is needed to validate the claims of these products (Heinrich, 2002; Polito, 2016). One popular weight loss supplement, green tea extract (GTE), has been shown to increase energy expenditure and fat oxidation, however efficacy of the supplement is lost with regular caffeine consumption (Dulloo et al., 1999; Mitchell et al., 2014; Westerterp-Plantenga et al., 2005). A formerly popular supplement, ephedrine, was shown to promote body mass loss. However, due to several documented health complications and deaths, this supplement was banned (Dulloo et al., 1991; Morgenstern et al., 2003; Shekelle et al., 2003; Toubro et al., 1993). With these issues in mind, more possible solutions are needed with proper evidence, with once such solution potentially being α-GPC.

α-GPC is a semisynthetic derivative of phosphatidylcholine which works to increase synthesis of the critical component of both the neuronal phospholipid bilayer and the neurotransmitter acetylcholine, choline (Brownawell et al., 2011; Dross & Kewitz, 1972; Spanner & Ansell, 1982). With the ability to increase choline, this supplement was first intended for use in individuals with Alzheimer's disease (AD) where it was

theorized that α-GPC could supply choline, thus neurons would not need to undergo apoptosis to replenish a diminished choline supply (Bartus et al., 1982; Blusztajn et al., 1990; Schwartz, 1991). Early work in mouse models showed that in a simulated AD state induced by scopalamine, α-GPC attenuated neurodegradation as well as increased acetylcholine (ACh) levels (Lopez et al., 1991; Suchy et al., 2009). When scopolamine was utilized in young adults, α-GPC was shown to mitigate the cognitive impairment induced by scopolamine (Canal et al., 1991) as well as prevent declines in reaction time following exhausting exercises in college-aged students (Hoffman et al., 2010). In elderly adults, α-GPC was shown to improve memory and performance of activities of daily living (Richter et al., 2011). These studies showed that α-GPC can confer a partial rescue phenotype, the ability to restore individuals back to a normal phenotype, in the AD population, as ACh levels and cognition are improved with supplementation. Given that α-GPC can cause increases in ACh, studies assessed whether this increase was enough to elicit increases in GH, as previous work has shown that ACh precursors can stimulate GH by inhibiting somatostatin release and increasing ghrelin release (Casanueva et al., 1986; Ceda et al., 1991; Locatelli et al., 1986; Maier et al., 2004; Mazza et al., 1994; Richardson et al., 1980; Torsello et al., 1988; Wehrenberg et al., 1992; Wren et al., 2000). From this research, α-GPC was shown to significantly stimulate GH release (Ceda et al., 1989; Ceda et al., 1992a; Ceda et al., 1992b; Kawamura et al., 2012), particularly when given before exercise. GH increases of 44-fold were found in one study compared to a modest 2.6-fold increase in the placebo group (Ziegenfuss et al., 2008).

GH, which is released from the anterior pituitary, has been shown to favorably improve body composition through increasing fat free mass (FFM) and decreasing fat

mass (FM) (Fain & Wilhelmi, 1962; Goodman, 1963a, 1963b; Kawamura et al., 2012; Kostyo et al., 1959; Kostyo & Schmidt, 1961; Mayo et al., 2000; Noall et al., 1957; Raben, 1962; Raben & Hollenberg, 1959; Riggs & Walker, 1960; Rosenfeld et al., 1989; Slavin et al., 1994; Ziegenfuss et al., 2008; Zimmermann et al., 2003). GH initiates these changes by increasing the uptake of amino acids into skeletal muscle, as well as stimulating hormone sensitive lipase (HSL) in adipose tissue which causes an increase in lipolysis (Fain & Wilhelmi, 1962; Goodman, 1963a, 1963b; Kostyo et al., 1959; Kostyo & Schmidt, 1961; Mayo et al., 2000; Noall et al., 1957; Raben, 1962; Raben & Hollenberg, 1959; Riggs & Walker, 1960; Rosenfeld et al., 1989; Slavin et al., 1994; Zimmermann et al., 2003). α-GPC is theorized to stimulate ACh, which in turn inhibits hypothalamic somatostatin, and stimulates ghrelin, resulting in an increased GH secretion (Casanueva et al., 1986; Locatelli et al., 1986; Maier et al., 2004; Mazza et al., 1994; Richardson et al., 1980; Torsello et al., 1988; Wehrenberg et al., 1992; Wren et al., 2000).

Previous α-GPC work in a younger population has focused on performance outcomes. However, given the evidence to suggest that α-GPC may increase GH levels, alterations in body composition may also occur. Considering thatGH increases FFM while decreasing FM, and acetylcholine precursors can stimulate GH, analysis of body composition changes with α-GPC supplementation is warranted (Ceda et al., 1991; Fain & Wilhelmi, 1962; Goodman, 1963a, 1963b; Lee & Schaffer, 1934; Raben, 1962; Raben & Hollenberg, 1959; Slavin et al., 1994; Zimmermann et al., 2003). The purpose of the current study was to investigate the effects of daily supplementation with 1200 mg of α-GPC on weight loss, body composition, and GH levels in moderately active overweight adults who maintain current exercise and dietary habits. We hypothesized that

supplementation will result in greater improvements in body composition while also increasing GH.

Materials and Methods

Study Design

A randomized, placebo-controlled, double-blind, parallel-group, clinical trial was used. Participants were randomly assigned into the supplement group (α-GPC) which received two daily doses of 600 mg of α-GPC to total 1,200 mg per day or placebo group (PLA) which received equal doses of rice flour for a total of eight weeks. Both α-GPC and PLA were supplied by North West Pharmanaturals (Farese, 2008) with purity certifications included through batch testing. Procedures were reviewed and approved by the Institutional Review Board at Rutgers University, and all participants provided written informed consent.

Participants

Thirty-two participants were recruited for this study. Of the 32, 28 completed the eight-week protocol (N=28; 10 male and 18 female). At the time of inclusion, subjects were between the ages of 18 and 45, had a body mass index (BMI) >23, and body fat percentage (BF%) >20% for men and >25% for women. Additional inclusion criteria included being in good health as determined by medical history questionnaire, following a regular structured exercise program for at least six months, and within the status of low-medium activity levels according to the Framingham Physical Activity Index (F-Score) (Kiely et al., 1994). Exclusion criteria included individuals who were pregnant or lactating, highly-trained, possessed major metabolic disorders or inborn errors of

metabolism, migraines, any history of hepatorenal, musculoskeletal, autoimmune, or neurological disease, or had any personal history of heart disease or hypertension. Additionally, individuals who had any self-reported intake of other weight loss supplements or drugs, recent gain or loss of greater than 13.6 kg within the past 30 days, regular consumption of more than three cups of coffee per day, smoking or had quit smoking within the past six months, had any known allergies to the supplement or placebo, or were participating in other weight loss or exercise-related research studies were excluded. Signed informed consents were obtained for all participants prior to enrollment, and the Rutgers University Institutional Review Board approved all aspects of the study. Subject characteristics at the beginning of the study are listed in Table 1.

Table 1: Subject demographics

	α-GPC (n=15)	Placebo (n=13)
M_{age} (years)	25.8 ± 9.1	24.4 ± 10.4
M_{BM}(kg)	82.0 ± 15.1	86.0 ± 15.2
M_{height} (cm)	163.9 ± 9.6	169.5 ± 10.5
$M_{BF\%}$ (%)	35.5 ± 1.8	35.7 ± 2.0

Protocol

Subjects reported to the Rutgers University Center for Health and Human Performance (CHHP) for pre- and post-intervention assessments. For the initial assessment, subjects reported following an overnight fast. Procedures were explained to participants including the potential risks and benefits. Upon arrival, participants completed a medical history questionnaire and the Framingham Physical Activity Questionnaire (Kiely et al., 1994). Resting heart rate was measured via radial pulse and blood pressure was measured by a member of the research staff using a sphygmomanometer in conjunction with a stethoscope to obtain systolic (SBP) and diastolic (DBP) values (American Diagnostic Corporation, Hauppauge, NY, USA) (Welch Allyn, Skaneateles Falls, NY, USA). Height was obtained using a mechanical stadiometer (Detecto, Webb City, MO, USA), and body composition was measured via air displacement plethysmography (BODPOD, COSMED, Concord, CA, USA) according to manufacturer guidelines. The Siri formula was used to determine BF%, which was then subsequently used to calculate fat free mass (FFM) and fat mass (FM) (Wells & Fuller, 2001). In addition, body mass (BM) was measured using the calibrated BODPOD scale. If subjects were within the inclusion criteria of greater than 20% body fat for males, and 25% for females, girth measurements at the waist (WC) and hips (HC) were collected. During the treatment period, subjects were instructed to not alter their activity level or diet. Subjects were instructed to record caloric intake via MyFitnessPal (Under Armor, Baltimore, MD, USA) and entries were monitored by researchers for compliance.

Experimental Period

Each subject was instructed to consume two doses of 600 mg/day of their assigned supplement. The first dose was taken either before a workout on workout days or midday on non-workout days, and the second dose was consumed immediately prior to sleep. The timing of the first dose was employed as this dosage has previously been shown to significantly increase peak growth hormone concentrations following a resistance exercise bout (Ziegenfuss et al., 2008). The second dosing time was chosen to correspond to the heightened GHRH response with sleep, which has been shown to be potentiated with α-GPC (Ceda et al., 1992a).

Sample Collection

Blood samples were collected from subjects at the beginning of the study and within one week following the supplementation protocol. Subjects were supplied with enough supplement to ensure that their last dose occurred the night prior to post supplementation assessment. Subjects reported to the CHHP after an overnight fast in a euhydrated state having refrained from exercise for at least 48 hours. Blood samples were collected via antecubital venipuncture with collection into a vacutainer serum separator tube (Becton Dickinson, Franklin Lakes, NJ, USA). Approximately 10 mL of whole blood was collected pre and post intervention, for a total of 20 mL over the duration of the study. Samples were centrifuged at 3500 x g for 15 minutes at 4° and then stored at -80°C until analysis.

Growth Hormone Analysis

GH was analyzed in duplicate in a single run across two plates using a commercially-available ELISA kit (DRG International Inc., Springfield, NJ, USA). The Lyphochek® Immunoassay Plus Control (Bio Rad, Irvine, CA, USA) levels 1, 2, and 3 were utilized to account for the plate's selective binding of HGH at varying concentrations. Controls and standard stock solutions were repeated on each plate. Determination of the concentrations of HGH were carried out using the CLARIOstar Plus microplate reader (BMG Labtech, Ortenberg, Germany). The %CV intra-assay was 8.38, while for the inter assay the %CV was 6.74. The software used to analyze the samples was CLARIOstar software version 5.21 R2, and MARS Data analysis Software version 3.20 R2 (BMG Labtech, Ortenberg, Germany).

Statistical Analysis

Statistical analyses were conducted using SPSS version 24 (IBM, Armonk, NY, USA). RM-MANOVAs with univariate follow-ups were conducted to determine differences between groups over the course of the trial. Statistical significance was set at $P<0.05$, with trends defined as $P<0.1$. Sample sizes between measures varied due to subject noncompliance and/or experimental error (reported in Tables 2, 3, and 4). Effect sizes (ES) were calculated to determine magnitude of change pre- to post-intervention within each group using Hedges' g formula. Additionally, ES were calculated to compare change scores between α-GPC and PLA.

Results

Findings revealed a significant Time main effect for body composition measures ($P=0.047$). Univariate analyses revealed significant reductions in FM ($P=0.02$), BF%

(P=0.001), and WC (P=0.03) and trends for a reduction in BM (P=0.094), and an increase in FFM (P=0.064) across both groups over time. However, there were no significant differences between groups for any body composition measures, girth measurements, GH, F-score, resting heart rate, or caloric intake from pre- to post-intervention (P>0.05). Additionally, a significant Time-by-Group interaction was shown for an increase in SBP in the α-GPC group (P=0.03). Moreover, a trend for decreased caloric intake (P=0.066, ES=0.136) was reported from week one to week eight of supplementation for all participants. For all variables, differences within groups from pre- to post-intervention as well as ES for within groups and for change scores between groups are displayed in Tables 2, 3, and 4.

Table 2: Body Composition and Anthropometric Data

	Group	Pre	Post	ES (within groups)	ES (change scores between groups)
Body Mass (kg)	α-GPC (n=15)	82.21±3.90	81.29±3.70	-0.05	0.38
	PLA (n=13)	85.99±4.20	84.94±3.99	-0.07	
Fat Free Mass (kg)	α-GPC (n=15)	52.69±2.56	53.39±2.62	0.07	0.11
	PLA (n=13)	55.07±2.77	55.58±2.84	0.05	
Fat Mass (kg)	α-GPC (n=15)	29.35±2.21	27.8±2.10*	-0.18	-0.04
	PLA (n=13)	30.93±2.18	29.50±2.37#	-0.15	
Body Fat Percentage (%)	α-GPC (n=15)	35.45±1.75	34.05±1.80*	-0.21	0.14
	PLA (n=13)	35.65±1.96	34.52± 1.81#	-0.16	
Waist Circumference (cm)	α-GPC (n=14)	89.11±2.88	88.14±2.65	-0.23	0.25
	PLA (n=11)	89.39±3.25	87.27±2.99#	-0.08	
Hips Circumference (cm)	α-GPC (n=14)	108.27±2.62	107.75±2.26	-0.05	0.37
	PLA (n=11)	110.71±1.90	109.82±1.84	-0.05	
BMI (kg/m^2)	α-GPC (n=15)	30.79± 1.58	30.54 ± 1.55	-0.04	0.10
	PLA (n=13)	29.79± 1.02	29.4615± 1.04	-0.09	

*Significantly different from Pre (P<0.05), #Trend for difference from Pre (P<0.10)

Table 3: Caloric, Cardiac, and Activity Measures

	Group	Pre	Post	ES (within groups)	ES (change score between groups)
SBP (mmHg)	α-GPC (n=15)	114.33±2.46	118.93±3.242*	0.48	0.38
	PLA (n=12)	123.58±1.57	119.83±2.99	-0.69	
DBP (mmHg)	α-GPC (n=15)	74.53±1.70	77.67±2.94	0.48	0.35
	PLA (n=12)	79.467±1.38	55.5±10.3	-0.14	
Caloric Intake (kcal)	α-GPC (n=10)	1534.23±191.10	1368.28±183.76#	-0.28	0.14
	PLA (n=7)	1672.46±144.80	1544.00±148.74	-0.34	
Resting Heart Rate (bpm)	α-GPC (n=15)	70.00±2.63	72.60±2.96	0.26	0.36
	PLA (n=12)	73.58± 1.55	72.33± 2.94	0.26	

*Significantly different from Pre ($P<0.05$), #Trend for difference from Pre ($P<0.10$)

Table 4: Growth Hormone

	Group	Pre	Post	ES (within groups)	ES (change scores between groups)
GH (ng/mL)	α-GPC (n=13)	2.4±0.9	1.7±0.6	-0.33	-0.41
	PLA (n=11)	1.8±0.8	1.7±0.9	-0.041	

*Significantly different from Pre (P<0.05), #Trend for difference from Pre (P<0.10)

F-Score

There were no significant differences for F-Score between groups and over time (P>0.843). F score for α-GPC (n=11) (31.1±1.2 points vs.30.7±1.2 points) or for Placebo (n=10) (29.51± 0.67 points vs. 30.01± 0.77 points) (P>0.05).

Discussion

The purpose of the current study was to investigate daily supplementation of 1200 mg α-GPC compared to a placebo in an overweight, moderately active population. We hypothesized that α-GPC supplementation would result in greater loss of FM, increased FFM, decreased BM, and increased GH levels when dietary and exercise habits were maintained during this eight-week intervention. There was a significant Time effect for body composition measures, however no significant differences were seen between groups for any variables over the intervention. These findings suggest α-GPC does not confer any additional benefits to weight loss, body composition, and GH level beyond placebo in this population.

During the course of the study, the lack of significant changes in F-score indicate that activity levels remained consistent throughout the intervention period. The trending decrease in energy intake could be partially due to subject under-reporting portion sizes, as is a common issue that can range upwards of 51% error (Guthrie, 1984). Based on the average caloric values obtained in this study, underreporting was suspected. However, if the underreporting was consistent across the intervention, the trend for decreased energy intake from pre to post across subjects may be an additional factor contributing to the Time effect on body composition variables, as it is possible subjects reduced caloric intake during the intervention (Flechtner-Mors et al., 2000; Frost et al., 2007). Considering that participants may have decreased or underrecorded dietary intakes, which in conjunction with the maintained exercise habits, suggests the subjects may have inadvertently added a confounding variable to the study (Goris et al., 2000; Livingstone et al., 1990). However, it is an interesting finding and suggests the act of tracking energy

intake may have been enough to raise awareness with the subjects and cause them to modify diet in a way that facilitated positive body composition changes even when activity was reportedly maintained.

Additionally, there was a trend for a significant increase of FFM seen in both groups across the intervention. This, however, may be a function of the regular exercise the subjects underwent and probably lends some credence to the notion that energy intake was underreported overall. Based on this study's findings, α-GPC did not appear to elicit greater changes in body composition or antropometric measures than the placebo alone. There were small ES seen in these measures; the largest calculated effect size between groups was in BM at 0.38. However, there was no significant changes in BM over time in either group, which likely explains the lack of change in BMI.

Contrary to our hypothesis, there were no changes in GH levels with α-GPC after the intervention. An important note for the GH measures is that subjects refrained from exercise prior to blood draws for 48 hours. This is an important factor as exercise can alter GH levels as a response closely following an acute bout of resistance training and even through much of the day (Kraemer et al., 1991; Nindl et al., 2001). However, the purpose of the current study was not to examine the GH systemic gain following a stressor such as exercise. While previous literature has shown a 44-fold increase in peak GH acutely following exercise with α-GPC supplementation (Ziegenfuss et al., 2008), the current study demonstrates basal GH is unchanged in this population after eight weeks of supplementation. Furthermore, while researchers attempted to obtain blood samples at the same time of day due to the pulsatile nature of GH, it is possible that some samples were obtained at different points of the pulsatile GH pattern than others (Edén, 1979; Frohman

et al., 1990; Hartman et al., 1991; Jaffe et al., 1993; Plotsky & Vale, 1985; Sato et al., 1988; Saunders et al., 1976; Tannenbaum & Martin, 1976). Additional studies showed other ACh precusors can significantly increase GH levels with chronic supplementation in the elderly, although this effect may be explained by the ACh precursor serving a rescue effect for GH (Ceda et al., 1991). Furthermore, it is likely that the younger population does not display a need for increased choline availability while the elderly population does (Bartus et al., 1982; Blusztajn et al., 1990). Additionally, in the elderly population, the anterior pituitary becomes less sensitive to the actions of GHRH, therefore it is possible that with a diminished GHRH repsonse, αGPC could serve to increase GH to a more physiologically normal range (Ceda et al., 1989; Mayo et al., 2000). Given this phenomenon in the elderly, future research should investigate if α-GPC as such can confer any additional body composition changes in this population.

One aspect of α-GPC that needs further investiagtion is it's asorption when ingested. Previous literature in the elderly showed significant impact of α-GPC on growth hormone, especially in potentiating the action of GHRH,when administered through invtravenous infusion (Ceda et al., 1992a; Ceda et al., 1992b). Additionally in a younger population, oral adminsitration of α-GPC has shown similar significant GH rises in an acute fashion (Kawamura et al., 2012). The tissue absorption of α-GPC through intravenous and oral administration has been studied in the rat model, however further work is needed in humans in order to assess the different absorption rates of varying tissues (Abbiati et al., 1993). In this model, radioactivly labeled α-GPC was shown to be metabolized faster after intravenous administration than oral dosing, showing different

metabolism for the two routes (Abbiati et al., 1993). Further research is needed in order to ascertain the degree to which metabolism of α-GPC plays a role in its efficacy.

The only significant group difference over time was seen for SBP, which increased more with αGPC compared to PLA. ACh has been shown to act as an endothelium-dependent vasodilator in smooth muscle tissue through muscarinic receptors, and in subjects with hypertension, this vasodilation effect is reduced (Furchgott, 1955; Furchgott & Bhadrakom, 1953; Furchgott & Zawadzki, 1980; Taddei et al., 1993). Considering that αGPC has been shown to elicit its main action through increasing ACh production, the results of this study are contradictory to what has been previously established (Abbiati et al., 1993; Trabucchi et al., 1986). Additionally, DBP showed a non-significant time-based decrease in the PLA group. However, these results along with the SBP results may be impacted by inter-tester variability using the sphygmomanometer and stethoscope. Finally, given that the SBP values for α-GPC were still in the "healthy" or "normal" range, this may be an example of statistical significance but not clinical importance.

A inherent limitation of the study was the use of self-report measures for dietary intake despite efforts by the researchers to provide instruction and reminders. A strength of the study was that this intervention was done in a free-living environment, so while some issues arose, these results are more externally valid for supplementation as a result.

The overall implications of this study indicate that α-GPC does not confer any additional weight loss benefits or changes in growth hormone in a younger population of overweight, moderately active individuals. While literature in an elderly population demonstrates α-GPC can increase GH levels, that same effect was not seen in the current

population. Additionally, we did not see any significant changes in body composition. Given that α-GPC has already shown efficacy in the elderly population for increasing GH serum levels, future work should assess whether the effects of an exercise intervention coupled with α-GPC supplementation would confer any superior body composition or GH changes over time in this population. Elderly individuals would be of particular interest as this could be a method of combating sarcopenia through combining the benefits of resistance trainig as well as the prevously seen GH increases. Another future direction could involve utilizing a young adult population in order to see if supplementing with α-GPC immediately prior to a resistance training bout could confer any additional benefits on body composition. Considering GH levels are highest immediately following resistance exercise, α-GPC could potentially bolster this acute response.

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