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The Effects Of Phosphatidylserine On Reaction Time And Cognitive Function Following An Exercise Stress

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THE EFFECTS OF PHOSPHATIDYLSERINE ON REACTION TIME AND COGNITIVE FUNCTION FOLLOWING AN EXERCISE STRESS

by

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ABSTRACT

Phosphatidylserine (PS) is an endogenously occurring phospholipid that has been shown to have cognition and mood enhancing properties in humans, possibly through its role as an enzyme co-factor in cellular signal transduction. Specifically, PS has been identified as activator of classical isoforms of protein kinase C, an enzyme known to be involved in the growth and differentiation of neural cells, and is therefore thought to play a role in the protection of neurons.

The purpose of this study was to examine the effects of supplementation with PS and caffeine on measures of cognition, reaction time and mood prior to and following an exercise stress.

Twenty, healthy, resistance trained males (17) and females (3) (mean ± SD; age: 22.75 ± 3.27 yrs; height: 177.03 ± 8.44cm; weight: 78.98 ± 11.24kg; body fat%: 14.28 ± 6.6), volunteered to participate in this randomized, double-blind, placebo-controlled study. Participants were assigned to a PS group (400mg/day PS; 100mg/day caffeine, N=9) or PL (16g/day Carbs, N=11) delivered in the form of 4 candy chews identical in size, shape and color. Subjects performed an acute bout of full body resistance exercise, prior to (T1) and following 14 days of supplementation (T2). Measures of reaction time (Dynavision® D2 Visuomotor Training Device), cognition (Serial Subtraction Test, SST), and mood (Profile of Mood States, POMS) were assessed immediately before and following resistance exercise in both T1 and T2. Data was analyzed using two-way ANCOVA and repeated measures ANOVA.

Supplementation with 400mg PS and 100mg caffeine did not have a significant impact upon measures of reaction time or cognition between groups at baseline or following acute resistance exercise. However, there was a non-significant trend to the attenuation of fatigue

between groups, following acute resistance exercise ($p = 0.071$). Interestingly, our data suggests that acute resistance exercise alone may improve cognitive function.

Although more research is necessary regarding optimal dosage and supplementation duration, the current findings suggest that supplementation 400mg/day PS with 100mg/day caffeine may attenuate fatigue following acute resistance exercise. It is possible that the lack of significance may be the result of both an inhibition of the PS activated pathway and a withdrawal effect from caffeine.

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LIST OF ACRONYMS/ABBREVIATIONS

aPKC	Atypical Protein Kinase C
ATCA	Average Time per Correct Answer
BC-PS	Bovine Cortex Phosphatidylserine
BDNF	Brain Derived Neurotrophic Factor
cPKC	Classical/Conventional Protein Kinase C
DAG	Diacylglycerol
DHA	Decosehexenoic acid
EMG	Electromyography
ERGIC	Endoplasmic Reticulum Golgi Intermediate Compartment
ERK	Extracellular Signal-Regulated Kinase
HPA Axis	Hypothalamo-pituitary-adrenal Axis
HPL	Human Performance Lab
$ICC_{3.1}$	Interclass Correlation Coefficient (2-way fixed)
IP3	Inositol 1,4,5-triphosphate
LPS	Lysophosphatidylserine
MAM	Mitochondrial-Associated Membrane
MAPK	Mitogen-Activated Protein Kinase
NGF	Nerve Growth Factor
nPKC	Novel Protein kinase C
NSCA	National Strength & Conditioning Association
P75NTR	P75 Neurotrophin Receptor
PA	Phosphatidic Acid
PKC	Protein Kinase C
PC	Phosphatidylcholine

PDK-1	3-Phosphoinositide-Dependent Protein Kinase 1
PE	Phosphatidylethanolamine
PKCδ	Protein Kinase-C-Delta
PKCγ	Protein Kinase-C-Gamma
PKCξ	Protein Kinase-C-Zeta
PI3K	Phosphatidylinositol-3-Kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5-Triphosphate
PLCγ	Phospholipase C-Gamma
POMS	Profile of Mood States
PS	Phosphatidylserine
PSS1	Phosphatidylserine Synthase-1
PSS2	Phosphatidylserine Synthase-2
RM	Repetition Maximum
RT	Reaction Time
$SEM_{3.1}$	Standard Error of Measurement (2-way fixed)
SMS	Sensorimotor system
SST	Serial Subtraction Test
TGN	Trans-Golgi Network
TRK	Tropomyosin-Receptor- Kinase

CHAPTER 1: INTRODUCTION

Lipid bilayers form the core structure of the membranes that surround mammalian eukaryotic cells. These membranes serve to separate the interior of the cell from the outside environment. In addition, these membrane phospholipids play an important role in cell-to-cell communication and the transfer of biochemical messages into the cell (Alternative Medicine Review, 2008). All phospholipids are comprised of a glycerol molecule, two fatty acids and a phosphate group (Kingsley, 2006). Mammalian plasma membranes contain more than 1000 different types of phospholipid molecules (Kainu, Hermansson, & Somerharju, 2008), a diversity in part attributed to the vast variety of fatty acid chains that are bound to the sn-1 and sn-2 positions of the glycerol molecule of the phospholipid (Vance & Steenbergen, 2005) and in part to the phospholipid head at the sn-3 position (Lourenssen & Blennerhassett, 1998). The distinct lipid composition defines the thickness, permeability and fluidity of the membrane (van Meer, Voelker, & Feigenson, 2008), which in turn regulates the properties of the proteins embedded within it (Vance & Steenbergen, 2005). Therefore, different tissues and different cell types have distinct phospholipid compositions. The phospholipid phosphatidylserine (PS) has been shown to have cognition enhancing properties in humans, possibly through its role as an enzyme co-factor in cellular signal transduction. In addition, PS has been implicated in combating exercise-induced stress, improving reaction time and decision-making ability. This has tremendous implications in sporting and tactical arenas where reaction time and decision-making ability are key determinants of a successful outcome. PS has also been shown to improve cognitive function in individuals with age associated memory impairment. Further research into the efficacy of

supplemental PS in the maintenance and improvement of cognitive function is therefore warranted.

CHAPTER 2: LITERATURE REVIEW

Phosphatidylserine Synthesis & Hydrolysis

In humans, PS is an endogenously occurring phospholipid, synthesized by enzymes that are found in the endoplasmic reticulum of the cell, or in a sub-fraction of this called the mitochondria-associated membrane (MAM) (Vance & Steenbergen, 2005). Despite this, PS is a universal component of plasma membranes throughout the human body (Kidd, 2009) and is widely distributed throughout the membranes of other organelles that lack the capacity to produce it, suggesting that these phospholipids can be transported between organelles (Vance & Steenbergen, 2005). Quantitatively, PS is a relatively minor phospholipid, accounting for approximately 15% of the phospholipid pool, with the greatest concentration being found in the myelin of brain tissue (Jager, Purpura, & Kingsley, 2007). The total body PS pool is estimated to be approximately 60 grams, 30 grams of which is found in the brain (Jager et al., 2007). In animal cells, the fatty acid composition of PS varies from tissue to tissue, although its core structure remains the same. Like all phospholipids, it comprises of a phosphate group, a glycerol molecule and two fatty acids. However, unique to PS is the serine head linked at position sn-3 on the molecule. In contrast to choline and ethanolamine, which are cationic, serine is neutral making the PS molecule anionic (Leventis & Grinstein, 2010).

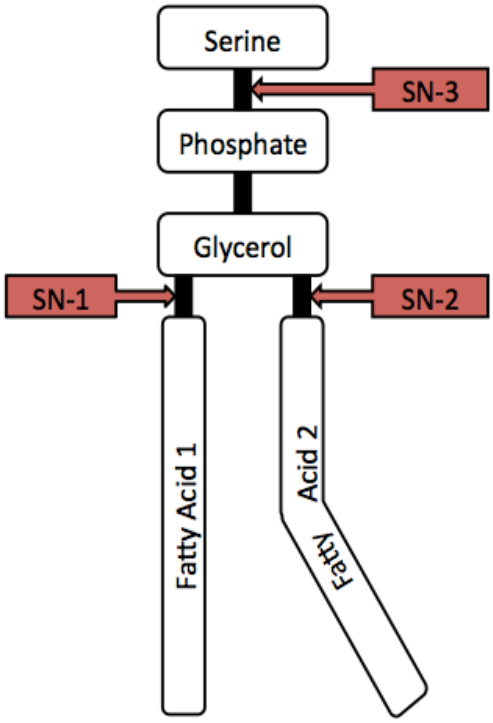

Figure 1: Phosphatidylserine Molecule

In mammalian cells, PS is endogenously synthesized via two pathways, both of which exchange the head group of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) for serine, generating PS in both instances (Vance, 2008). PS synthase-1 (PSS1) catalyzes the choline exchange reaction and PS synthase-2 (PSS2) catalyzes the ethanolamine exchange reaction.

Phosphatidylcholine + L-Serine $\xrightarrow{\text{PS Synthase 1}}$ Phosphatidylserine + Choline

Phosphatidylethanolamine + L-Serine $\xrightarrow{\text{PS Synthase 2}}$ Phosphatidylserine + Ethanolamine

Figure 2: Phosphatidylserine Synthesis

PS, both from endogenous synthesis and exogenous supplementation can be hydrolyzed via two pathways. This occurs via phospholipases, which are located in the plasmalemma

(Leventis & Grinstein, 2010). The sn-1acyl chain of PS is hydrolyzed via the action of PS-specific phospholipase A1, while the sn-2 acyl chain of PS is hydrolyzed via PS-specific phospholipase A2, with lysophosphatidylserine (LPS) being formed in both cases. Lysophosphatidylserine produced through the hydrolysis of the sn-2 acyl chain has been implicated in a number of biological processes including mast cell activation and neural differentiation (Hossono, Aoki, Nagai, Bandoh and Ishida (2001); Aoki, Nagai, Hosono, Inoue, and Arai (2002).

Efficacy of Soy-Derived Phosphatidylserine

Bovine cortex phosphatidylserine (BC-PS) has been shown to have cognition enhancing properties in rodents; however, because of the possible transfer of infectious diseases such as Mad Cow Disease, the use of bovine cortex PS is not considered safe in humans (Kingsley, 2006). Additionally, the yield of PS from bovine cortex is relatively low (Weihrauch & Son, 1983). Soybean PS is molecularly similar to bovine cortex PS with respect to the serine head group and the glycerol moiety; however the fatty acid composition is different. Bovine Cortex PS and egg PS are both rich in arachidonic acid and decosehexenoic acid (DHA) however; soybean PS is virtually devoid of these fatty acids (Blokland, Honig, Brouns, & Jolles, 1999). Since the lipid composition can in turn define the fluidity of the membrane and regulate the properties of the proteins embedded within it, it was questioned whether exogenously supplied soybean PS could have the same cognition enhancing properties of BC-PS. This was addressed in a study by Blokland et al. (1999), which compared the cognitive enhancing properties of subchronic PS supplied from bovine cortex, soybean and egg in middle-aged rats. Rats were

treated with 15mg•kg^{-1} of PS derived from each source or control vehicle and the effects were evaluated in three different behavioral tests. An open field test was utilized to examine the effects of treatment on psychomotor behavior, in addition to the Morris water escape task and two-way active avoidance tests, which assessed the effect of the treatment on cognitive performance. In the Morris water escape task, a rat or mouse is placed into a small pool of water, which contains an escape platform hidden a few millimeters below the water surface. Visual cues, such as colored shapes, are placed around the pool in plain sight of the animal. When released, the animal immediately begins to search for an exit. Time spent in each quadrant of the pool, the time taken to reach the platform and total distance traveled are recorded. The desire to escape from the water reinforces rapid location of the platform. Improvements in performance are thought to occur as a result of learning and memory with regards to platform location, relative to visual cues. Psychomotor and spatial discrimination performance were unaffected by all derivatives of PS. The performance of rats treated with egg PS did not deviate from that of the control group; however the cognition-enhancing effects BC-PS were also seen in rats treated with soy-PS, leading to the conclusion that soy PS has comparable effects to BC-PS with regards to cognition enhancement. These results were similar those posited by others (Drago, Canonico, and Scapagnini (1981); Sakai, Yamatoya, and Kudo (1996) and Zanotti, Valzelli, and Toffano (1989).

Effects of Phosphatidylserine Supplementation

The uptake biokinetics of PS are not well established. While much of exogenously supplied PS is hydrolyzed and degraded, small amounts of PS remain available (Pepeu, Pepeu, &

Amaducci, 1996) The addition of 80μm exogenous PS to cell cultures has been shown to result in the efficient incorporation of PS into Chinese hamster ovary (CHO) cells and utilized for membrane biogenesis (Nishijima, Kuge, & Akamatsu, 1986). While uptake kinetics have not been shown in humans, it does provide an indication that supplementation with higher doses of PS may lead to an increase in the overall PS pool. PS has been reported to be effective for combating exercise-induced stress (Monteleone, Maj, Beinat, Natale & Kemali, 1992). PS has also been shown to increase time to exhaustion in runners and cyclists (Kingsley, Wasdworth, Kilduff, Mceneny, and Benton (2005); Kingsley, Miller, Kilduff, Mceneny, and Benton (2006). Approximately 130 mg•day^{-1} of PS is ingested in the average diet (Jager et al., 2007) however; supplementation with higher levels of PS (300mg BC-PS) has been shown to improve cognitive function in tests related to learning and memory and tasks of daily living in subjects with age associated memory impairment (Crook, Tinklenberg, Yesavage, Petrie, Nunzi & Massari, 1991).

It has been theorized that PS may be beneficial for sports demanding high levels of concentration and coordination. Increased Beta-1 spectral power is an indicator of activation associated with cognitive task demands and higher neurophysiological function. The frontal and pre-frontal regions of the brain mediate executive processes such as attention, coordination and concentration and show a higher activation immediately following cognitive tasks (Sauseng, Klimesch, Schabus & Doppelmayr, 2005). Six weeks of supplementation with 200mg soy-derived PS has been shown to significantly decrease Beta-1 power in right hemispheric frontal brain regions before and after induced stress in healthy male subjects (Baumeister, Barthel, Geiss & Weiss, 2008). A decrease in Beta-1 power demonstrates a form of relaxation in subjects supplementing with PS. In contrast, subjects on placebo were unable to relax during performance of the cognitive task, as demonstrated by a higher Beta-1 power indicating a higher activation

state (Baumeister et al., 2008). Jager, Purpura, Geiss, Weib, Baumeister, Amatulli and Herwegem (2007) sought to investigate the effect of oral supplementation of 200mg of PS for 6 weeks on golf performance. The test population consisted of 20 healthy volunteers recruited from local golf courses with handicaps of 15-40. Subjects were assigned in a randomized fashion to either the PS group or a placebo. The 6-week supplementation period began immediately after pre-testing. Pre-testing consisted of a 10-minute golf specific warm-up followed by subjects teeing 20 consecutive tee shots in 15-second intervals toward a target 135 meters away. Ball flight quality was monitored by a professional golf trainer who adjudged shots as hit or missed based on a predetermined ball flight criterion. The time constraint and targeting task were designed to induce stress. Following the 20 tee shots, perceived stress was measured using a visual analogue scale. Subjects reported back after the six-week supplementation period and repeated the testing protocol in the same fashion. Golf club selection remained constant for each subject in the pre and post testing. PS supplementation was shown to significantly increase ball flight accuracy, whereas placebo had no effect on performance, and showed a trend towards improving perceived stress levels during tee-off. Stress levels remained unchanged under placebo. The proposed mechanism of action was the counteraction of stress-induced activation of the hypothalamo-pituitary-adrenal (HPA) axis. Although the results were not statistically significant, a 31% improvement in perceived stress levels was seen. Therefore, PS supplementation may have practical implications in the golfing arena.

It has been proposed that PS may have a beneficial effect on reaction time, alertness and focus during a state of fatigue. Hoffman, Ratamess, Gonzalez, Beller, Hoffman, Olsen, Purpura and Jager (2010) examined the effects of acute and prolonged (4-week) ingestion of a combination of 50 mg PS and PC (phosphatidylcholine). The supplement was tested in

combination with a high-intensity anaerobic exercise protocol designed to elicit fatigue. The test population consisted of nineteen recreationally active subjects randomly assigned to either a supplement or placebo group. The study was conducted in a double blind format. A crossover design was not utilized due to unknown washout periods for several of the supplement's ingredients. Subjects reported on two separate occasions for testing. For each testing session, subjects were provided with either supplement or placebo and instructed to complete a survey disclosing feelings relating to alertness, energy, fatigue, focus and well-being. Following this, reaction time was assessed using a Makoto testing device, whereby subjects had to respond to both auditory and visual stimuli. Upon completion of this test, subjects completed a 10-minute bout of exhaustive exercise consisting of a 30-second Wingate and maximal pushups and sit-ups within a one-minute period. Testing was repeated after 4 weeks. The results of this study indicated that acute ingestion of PS and PC can maintain reaction time to both visual and auditory stimuli following a high intensity bout of exhaustive exercise compared with placebo. Additionally, acute supplementation with 50mg PS and PC resulted in maintained focus and alertness following the exercise protocol, with focus being maintained after four weeks compared to placebo. The authors attributed the ability to maintain reaction performance following fatigue in part to the combined effects of PS, choline and the supplements energy matrix, citing that this combination may contribute to an enhanced neuro-protective effect. Prolonged supplementation did not produce the same effects as acute supplementation in this study with regards to alertness. The authors cited a possible habituation effect with prolonged supplementation, whereby the daily concentrations of ingredients may not have provided the same physiological affect after 4 weeks.

In a similar fashion, Parker, Gordon, Thornton, Byars, Lubker, Bartlett, Byrd and Kreider (2011) examined whether supplementation with 400mg of soy-derived PS for 14 days would improve cognitive performance prior to and/or following a stress induced bout of lower body resistance exercise. The test protocol consisted of subjects ingesting either the PS or placebo (rice flour) in a double blind, placebo-controlled, cross over design with no washout period. The test population consisted of 18 physically active college-aged males, free of any additional nutritional supplement for 30 days prior to the commencement of the study. The test protocol consisted of a pre-exercise serial subtraction test (SST) and profile of mood state (POMS), followed by an acute lower body resistance exercise, which included squat, leg press and leg extension. The exercise protocol was high volume, low intensity (5 sets of 10 repetitions, 90-second rest periods between sets and 180-second rest periods between exercises). 1RM and baseline measures for SST and POMS were established in a familiarization session. Following the familiarization session, subjects were randomly assigned PS or placebo for 14 days, after which they reported for the first testing session. SST and POMS were performed prior to exercise. Following the exercise session, SST and POMS were performed at 5 and 60 minutes post exercise. Upon completion of the first testing session, participants were given a 14-day supply of either placebo or PS, depending upon what they took for the first 14 days. After completing supplementation, subjects returned and completed a second testing session in the same fashion as the first testing session. PS supplementation significantly increased the speed of SST calculation by 20% ($p = 0.001$). In addition to this, the amount of mistakes made was decreased by 39% ($p = 0.53$) and the amount of correct calculations was increased by 13% ($p = 0.070$) prior to exercise when compared to placebo. Following exercise, there was no difference in SST scores between placebo and treatment groups. Therefore, it was concluded that PS

supplementation could significantly increase cognitive function prior to an acute bout of lower body exercise.

It is clear from the research that PS may maintain cognitive performance during fatigue. Additionally, research shows that PS may also improve baseline cognitive function. Decreased Beta-1 power in right hemispheric frontal brain regions indicates that fewer resources are required to perform cognitive tasks under PS supplementation and PS has been shown to result in maintained focus and alertness following fatigue. Additionally, research shows trends towards PS improving the perception of stress. The implications of this research regarding sports performance, degenerative cognitive disease and combat situations, warrants further research into the cognition enhancing properties of phosphatidylserine.

Possible Mechanisms Through Which PS May Enhance Cognition

Lysophosphatidylserine has been proposed to potentiate the effects of nerve growth factor (NGF) (Lourenssen & Blennerhassett, 1998) and in addition, PS in cooperation with DHA, acetyl-l-carnitine, α-lipoic acid and glycerophosphocholine has been suggested to contribute to an enhanced neuro-protective effect via a stronger defense on membrane integrity (Suchy, Chan, & Shea, 2009). In the field of neurobiology, a recurring motif is the role of neurotrophins. Neurotrophins are a set of molecules that support the proliferation, differentiation and survival of neurons and also act by preventing the associated neuron from initiating apoptosis, thus allowing the neurons to survive.

NGF, a member of the neurotrophin family of molecules, is a protein involved in a variety of signaling events leading to the maintenance, growth and survival of sympathetic and

sensory nerve cells (Sofroniew, Howe, & Mobley, 2001). It is secreted by tissues targeted by sympathetic and some sensory neurons and is mediated through its binding to two classes of receptors expressed on the surface of responsive cells (Levi-Montalcini, 1987). These receptors are TrkA (Tropomyosin-Receptor-Kinase A) and p75NTR (Low Affinity Nerve Growth Factor Receptor) (Kaplan & Miller, 1997). Trk is a gene family, which is comprised of related transmembrane neurotrophin specific receptors TrkA, TrkB and TrkC. TrkA is a single-pass transmembrane protein that serves as a receptor for NGF (Sofroniew et al., 2001); TrkB serves as the receptor for brain derived neurotrophic factor (BDNF), Neurotrophin-3 (NT3) and neurotrophin 4/5 (NT-4/5), while TrkC serves as a receptor for neurotrophin-3 (NT-3) (Kaplan & Miller, 1997). NGF binds to the TrkA receptor with a high affinity (Chao, 1992) creating a TrkA-NGF complex. This complex is thought to be essential for neurotrophic action (Loeb, Maragos, Martin-Zanca, Chao, Parada & Greene, 1991), serving as an important messenger that delivers the NGF signal from axon terminals to the cell bodies of neurons. Research also indicates that NGF also binds to a lower-affinity receptor called p75NTR, a transmembrane glycoprotein that binds all members of the neurotrophin family with equal affinity. It is thought that this low affinity receptor can cooperate with Trk receptors to increase signaling efficiency and the affinity of neurotrophin binding (Barker & Shooter, 1994). Activation of TrkA is followed by receptor dimerization and autophosphorylation of tyrosine residues on the TrkA receptor. These residues act as docking sites for signal transduction molecules. Activation of TrkA results in the rapid association of TrkA with phospholipase-C-gamma (PLCγ) and phosphatidylinositol-3-Kinase (PI3K). Tyrosine 785 is the tyrosine residue required for NGF-dependent recruitment of PLCγ to the tropomyosin-receptor-kinase (TrkA) and for the phosphorylation of and activation of PLCγ (Vetter, Martin-Zance, Parada, Bishop, & Kaplan,

1991). When tyrosine 785 binds to TrkA, PLCγ is activated and induced to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) (Sofroniew et al., 2001). This hydrolysis yields Inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), both of which function as intracellular secondary messengers. IP3 serves to interact with its specific receptor on the endoplasmic reticulum to induce the release of intracellular calcium, while DAG stimulates Protein Kinase-C-Delta (PKCδ), both of which play a role in activation of the PKC pathway. DAG is considered the key activator of classical isoforms of PKC (cPKC) and novel isoforms of PLC (nPKC) (Newton, 1997). PI3K also forms a complex with activated TrkA. PI3K converts PIP2 to PIP3 at the membrane. This in turn activates the protein kinase PDK-1, which activates atypical Protein Kinase-C-Zeta (aPKCζ). This leads to neural differentiation (Wooten, Zhou, Seibenhener & Coleman, 1994) and late-phase long term potentiation through Protein Kinase-M-Zeta (PKMζ), an atypical N-terminal truncated form of PKCζ (Serrano, Yao & Sacktor, 2005). Research ascribes a multiplicity of functions to PKC, specifically in the areas of cell growth regulation, learning and memory, and in regulating transcription (Newton, 1995), through its function in signal transduction pathways. However, PKC is maintained in an inactive state by its interaction with a pseudosubstrate (Newton, 1997). Activation requires the removal of the auto-inhibitory pseudosubstrate domain from the active site. In classical forms of PKC, this is achieved by highly specific binding of 1,2-sn-diacylglycerol and PS to the two membrane targeting domains, C1 and C2 of cPKC (Newton, 1997a). Both domains must be membrane bound for pseudosubstrate removal to occur and for maximal activation of PKC to be achieved (Newton, 1995). PS is the most effective phospholipid in the activation of classical isoforms of PKC (Takai, Kishimoto, Iwasa, Kawahara, Mori & Nishizuka, 1979). Calcium release via IP3 allosterically increases the C2 domains affinity for PS (Newton, 1997a). Additionally, the

presence of DAG induces high specificity for the phospholipid head-group L-Serine, with at least one order of magnitude higher affinity than other acidic lipids (Newton, 1997b). In the absence of DAG, as is the case with atypical isoforms, PKC exhibits no selectivity for phospholipid head group and will bind any phospholipid with a negative charge (Newton, 1997b). Therefore the cellular growth & transcription functions of classical isoforms of PKC may be dependent upon cellular levels of PS. Novel PKC Requires the presence of DAG but not calcium, while atypical PKC does not require DAG or calcium for its activation.

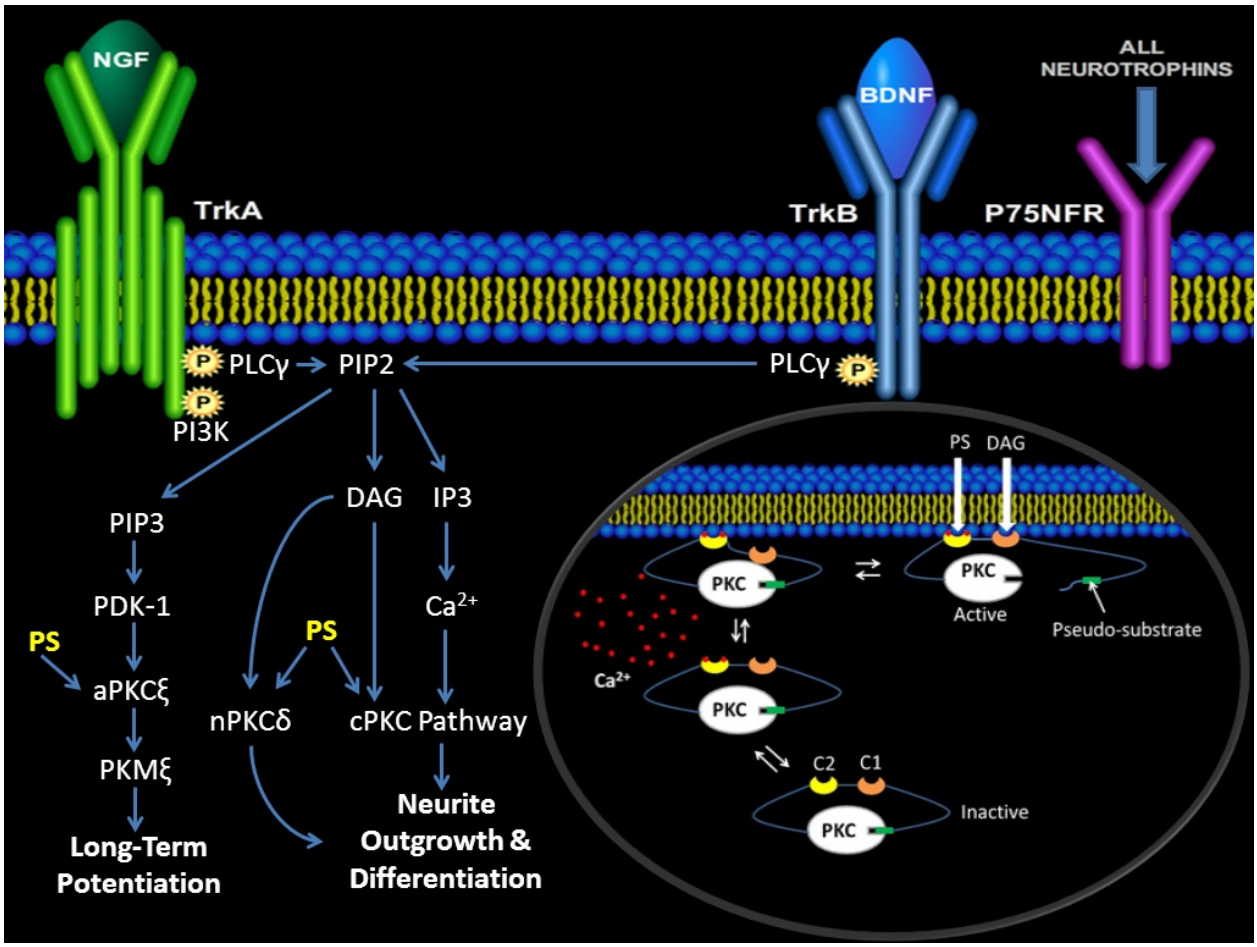

Figure 3: Phosphatidylserine Interaction with cPKC

It has been suggested that PKC activation may be involved in the neuro-protection of motor neurons (Timamatsu & Arakawa, 1993). Activation of PLCγ and PKC has been shown to occur in injured spinal motor neurons, with inhibition of the PLCγ/PKC pathway resulting in inhibited PKC phosphorylation and an increase in avulsion induced motor neuron loss (Zhao, Wang, Wang, Song, Li, Fu, Zheng, Wu & Zhou, 2012). However, motor neurons do not express the high affinity TrkA NGF receptor (Henderson, Camu, Mettling, Gouin, Poulsen, Karihaloo, Rullamas, Evans, McMahon, Armanini, Berkemeier, Phillips & Rosenthal, 1993); therefore NGF cannot exert its trophic effect upon motor neurons. Consequently, the activation of PLCγ within motor neurons must occur via a pathway other than that initiated by the TrkA-NGF complex. Subsequent research has indicated that the denervation of Schwann cells is followed by an up-regulation of BDNF, highlighting a role for this neurotrophin in motor neuron growth and repair (Meyer, Matsuoka, Wetmore, Olsen & Thoenen, 1992). Koliatsos, Clatterback, Winslow, Cayouette and Price (1993) reported that BDNF is indeed a trophic factor for motor neurons, citing trkB expression in α motor neurons of both neonatal and adult rats. Alpha motor neurons innervate extrafusal muscle fibers and are directly responsible for the initiation of muscle contraction. This notion is supported by Zirrgiebel, Ohga, Carter, Berninger, Inagaki, Thoenen and Lindholm (1995) who reported the expression of TrkB receptors in the developing cerebellar granule neurons of rats, the region of the brain responsible the coordination, precision and accurate timing of movement (Gordon, 2007). Veritably, PKC has been shown to be a cellular effector of PLCγ phosphorylation on TrkB. BDNF was reported to induce the autophosphorylation of TrkB receptors in these granule neurons, resulting in the subsequent phosphorylation and binding of PLCγ to the TrkB receptor for a survival promoting effect (Zirrgiebel et al., 1995; Ortega, Perez-Sen, Morente, Delicado and Miras-Portugal, 2010).

Therefore, BDNF signaling via the TrkB receptor may induce downstream activation of PKC, which in the presence of Phosphatidylserine may result in the outgrowth and differentiation of motor neurons.

Classical isoforms of PKC include PKC-alpha (PKCα), PKC-Beta1 (PKCβI), PKC-Beta2 (PKCβII) and PKC-Gamma (PKCγ). It is important to note that specific isoforms of PKC evoke their effect through a variety of pathways downstream of its activation. Cellular responses of PKC are regulated through its dynamic interactions with other substrates with which it acts on and thus, PKC action is dependent upon where and when it is activated (Nakashima, 2002). It is also important to note that each PKC isoform will exert positive as well as negative effects along the same signaling pathway. Additionally, different isoforms will sometimes play opposing roles in cellular function. It has been suggested that this contributes to the fine-tuning of signal transduction (Kawakami, Kawakami & Kitaura, 2002). Maintaining the balance between hypertrophic growth and apoptosis is critical for tissue homeostasis. Overexpression of PKC and unregulated cell growth can lead to various forms of tumor and pathological conditions, while an excessive or premature cell death can lead to conditions of impaired memory, cognition and motor functioning such as Alzheimer's, Parkinson's and Multiple Sclerosis.

PKCα is distributed throughout all tissues in the body and is activated by a number of stimuli, including signals binding to Trk receptors. It has been implicated in variety of cellular functions including proliferation and differentiation. PKCα functions as a potent activator of c-raf-1 and turns on the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) cascade (Kolch, Heidecker, Kochs, Hummel, Vahidi, Mischak, Finkenzeller, Marme & Rapp, 2002). These kinases are involved in cellular proliferation, differentiation, migration, survival and gene expression. It has been demonstrated that PKCα isoform specific

activation is involved in neurite outgrowth of hypothalamic neuronal cells via the ERK pathway (Choe, Lee & Kim, 2002).

PKCγ is expressed exclusively in the brain and spinal cord and its localization is restricted to neurons (Tanaka & Saito, 1992). PKCγ exhibits abundant expression in hippocampal pyramidal cells, cerebellar Purkinje cells and within the cerebral cortex. It has been implicated in both long term potentiation and long term depression through modulation of synaptic plasticity. A review of the function of PKCγ by Saito and Shirai (2002) suggests that PKCγ deficient mice exhibit mild deficits in spatial and contextual learning and impaired motor coordination. Thus it is possible that PKCγ may be involved in learning and memory, and neuronal development.

Evidence has shown that action of Rasagiline, a neuroprotective drug used in the treatment of Parkinson's disease, is mediated through PKC signaling (Weinreb, Bar-am, Amit, Chillag-Talmor & Youdim, 2004). Short-term treatment of PC12 cells with Rasagiline induces PKC phosphorylation in a dose-dependent manner, activating PKCα and novel PKCε and PKCδ isozymes, in association with Bcl-2 protein (B-cell lymphoma – apoptosis regulator protein). ERK/MAPK cascades have also been found to be upregulated by Rasagiline, leading to an inhibition of cell death. It is also reported that suppression of PKCα triggers apoptosis through down-regulation of Bcl-xL (B-cell lymphoma – extra-large anti-apoptotic protein).

PKCα, PKCβ and PKCγ have been shown to be present in both myleinated and unmyelinated axons. Within myelinated axons, all three isozymes have been shown to be widely spread throughout the subaxolemma peripheral zones and to a lesser extent, within the axoplasm, however not within the cytoskeletal domain. In contrast, within unmyelinated axons, PKCα, PKCβ and PKCγ are distributed widely in the axoplasm. Only Schwann cells of myelinated

nerve fibers exhibited PKC immunoreactivity. All three isozymes of classical PKC have been shown to be up-regulated and translocated into the growth cones of regenerating sciatic nerve axons in rats (Okajima, Mizoguchi, Tamai, Hirasawa & Ide, 1995). A rapid increase in axonal PKC levels has been shown to occur within hours of nerve injury, with cPKC co-stimulating axonal regeneration in the presence other factors. Therefore it is suggested that cPKC isozymes may play an important role in the regulation of growth cone activity in the peripheral nervous system (Okajima et al., 1995).

Lourenssen and Blennerhassett (1998) posited that lysophosphatidylserine (LPS) might potentiate the effects of NGF in inducing neural differentiation of PC12 cells. PC12 is a cell line from a neuroendocrine tumor of the rat adrenal medulla, expressing both trkA and p75NTR receptors (Greene & Tischler, 1976). These cells respond to NGF by growth arrest and differentiation into a neural phenotype with multiple neurites (Greene, Aletta, Rukenstein, & Green, 1986). The study compared three trials where by NGF, NGF+LPS and LPS were added to PC12 cells in a neural environment. LPS alone had no effect on cell morphology. The addition of NGF alone to PC12 cells resulted in a dose dependent increase in cells with neurites longer than one or three cell body diameters. This is suggestive of differentiation into a neural phenotype. NGF added concurrently with LPS had a dramatic effect on cell appearance characterized by the formation of a single very long neurite in lieu of the multiple neurites. The cell bodies displayed a more rounded appearance and were phase bright. In addition, the neurites were thinner and displayed well-defined growth cones. PC12 cells receiving NGF+LPS were more similar to primary neurons than cells receiving NGF alone. The number of PC12 cells responding to NGF and the magnitude of the response was strongly potentiated by the presence of LPS. Cells exposed to NGF + LPS tended to be unipolar or bipolar as opposed to the multi-polar appearance

observed with NGF alone. Notably, neither lysophosphatidylcholine nor lysophosphatidylinositol affected the morphology of the cells, indicating the absolute requirement for the serine group.

Effects of Exercise Induced Fatigue on CNS Function & Cognition

The participation in physical activity challenges a variety of physiological systems. It is possible that physical fatigue affects central nervous system function. The sensorimotor system (SMS) is responsible for the awareness, coordination and feedback in maintaining form, stability and mobility during athletic performance (Lephart, Riemann, & Fu, 2000). Muscle fatigue has been shown to affect the spatial-temporal aspects of fine motor control (Wojtys, Wylie, & Huston, 1996) such as that seen in the progressive decline of form and timing as one continuously repeats the same movement, for example a jump shot. Muscle fatigue has also been shown to affect upper extremity SMS function in both single motion isolated movements (Tripp, Boswell, Gansneder, & Shultz, 2004) and in multiplanar, multijoint movements (Tripp, Yochem, & Uhl, 2007) as a result of decreased SMS acuity.

Physical fatigue of the hamstrings and quadriceps muscle has been shown to impact CNS function. Wojtys et al. (1996) reported a significant decrease in both the reaction time and the number of responses from both the quadriceps and hamstrings muscle, as measured with EMG, following induced fatigue using an isokinetic dynamometer. Within the spinal reflex of the medial and lateral quadriceps, there was a significant delay in reaction time of 60-80 msec and 77-93 msec respectively. In addition to this, the percentage of muscle firing in both the medial and lateral quadriceps decreased by 40% after induced fatigue with concurrent decreases in the firing rates of the lateral and medial hamstring by 30% and 35%, respectively. There was also a

concurrent 45% decrease in the firing rate of the gastrocnemius despite this muscle not being exercised. The study utilized surface EMG at five muscle locations consisting of the medial and lateral quadriceps, medial lateral hamstring and gastrocnemius in 10 healthy subjects. Muscle fatigue was induced using an isokinetic dynamometer with fatigue being defined as decrease in work output greater than 50% in both the hamstrings and quadriceps muscle group. Muscle recruitment order remained unchanged in the hamstrings, quadriceps and gastrocnemius between baseline and induced fatigue. However this study did not evaluate whether this decrease in neural firing could impact cognitive function in the brain. If fatigue affects the function of the central nervous system, it is possible that fatiguing exercise will have a detrimental impact on cognitive function.

It has been hypothesized that short-term maximal aerobic exercise to exhaustion would affect simple reaction time, visual spatial memory, attention, short-term memory and working memory over time. However, in a study involving 26 healthy young females, Lo Bue-Estes, Willer, Burton, Leddy, and Wilding (2008), showed that simple reaction time was unaffected by maximal aerobic exercise to exhaustion, although active individuals had faster reaction times than sedentary individuals. Working memory performance was shown to be negatively affected during exercise at intensities up to 50% of VO2max, but then shown to rebound following recovery above that of baseline. Attention, short-term memory and delayed short-term memory were shown to be unaffected by short-term maximal aerobic exercise. The effects of resistance exercise were not considered in this study. The authors proposed that the decline in cognitive performance observed at low exercise intensities may be associated with competition for a limited amount of cerebral and peripheral resources during preparation for and participation in the early stages of exercise. It was suggested that the subsequent removal of competition for

these resources from the periphery and within competing areas of the brain, in addition to the physiological and metabolic changes occurring during exercise, may contribute to the increased performance seen following recovery. Hogervorst, Riedel, Jeukendrup, and Jolles (1996), hypothesized that simple task performance would be improved following endurance exercise and that complex cognitive performance would decline following endurance exercise. Fifteen healthy male triathletes and competitive cyclists performed a bicycle ergometer endurance test at 70% VO_2max. Simple reaction time, 3-choice reaction time, a finger tapping task, a stimulus-response incompatible reaction time test and the Stroop test were administered pre and post exercise. The results of the study supported the authors' first hypothesis. The exercise protocol was found to have a positive effect of performance speed in simple tasks. Simple reaction times were significantly lower than baseline after fatiguing exercise. The more complex stimulus response incompatible reaction time test results and color word interference in the Stroop test results also demonstrated an increase in speed of performance after endurance exercise relative to baseline. This invalidated the authors' second hypothesis, showing that endurance exercise in fact has a positive effect on complex cognitive functions.

Very few studies have examined the effects of an acute bout of resistance exercise on cognitive function. Pontifex, Hillman, Fernhall, and Thompson (2009) examined the influence of an acute bout of resistance exercise on executive control function relative to acute aerobic exercise and seated rest in 21 healthy undergraduate college students. The term executive control describes a subset of processes involved in the selection, scheduling and coordination of computational processes that underlie perception, memory, and action. Aerobic exercise consisted of 30 minutes of treadmill running at 60-70% VO2max. Resistance exercise consisted of a 30-minute session utilizing seven major muscle groups for 3 sets of 8-12 repetitions at 80%

1RM. Rest periods were set at 60 seconds between sets and 90 seconds between exercises. Working memory was assessed using task performance measures of reaction time and response accuracy during a modified Sternberg task administered prior to, immediately following and 30 minutes post exercise intervention and control. The authors postulated that shorter reaction time latency and increased response accuracy would be observed following both the aerobic and resistance exercise intervention, relative to seated rest. The results of this study showed that reaction time latency during a working memory task was improved immediately following and 30 minutes following an acute bout of aerobic exercise relative to the pre-test. These results were not seen after acute resistance exercise or seated rest. Shorter reaction time latency was observed for task conditions requiring increased working memory capacity after aerobic exercise relative to the pretest, indicating that changes in cognitive function after acute exercise are disproportionately larger for tasks requiring greater amounts of executive control. Kauranen, Siira and Vanharanta (1999) also found that 1 hour of resistance exercise had no effect on motor performance, reaction time and speed of movement in healthy females.

These results are supported by Tomporowski (2003), who conducted a review of studies that assessed the effects of acute bouts of physical activity on the cognitive performance of adults. The review spanned the effects of intense anaerobic exercise, short-duration anaerobic and aerobic exercise and steady-state aerobic exercise with measures of cognitive function being taken either during or immediately following the exercise protocol. However, the review did not consider any form of resistance exercise. Measures of cognitive function included coincidence timing, mathematical computation, letter detection, simple reaction time, choice reaction time, visual searches and decision-making tasks. The review was limited to exercise studies that involved the activation of the entire body to produce systematic changes in physiological

function. From the review of the literature, the author concluded that sub-maximal aerobic exercise performed for periods of up to 60 minutes facilitated specific aspects of information processing. In contrast, extended exercise that leads to dehydration was seen to compromise both information processing and memory function. In addition, intense exercise appeared to have a transient detrimental effect on processes that control response preparation. Exercise is said to have an inverted U effect on the performance of a cognitive task (Brisswalter, Collardeau, & Rene, 2002). An increase in arousal has been linked to increases in intensity with concurrent increases in heart rate and ratings of perceived exertion. Cue utilization theory suggests that moderate intensity exercise could improve cognitive performance while high intensity fatiguing exercise would lead to decrease in cognitive performance (Brisswalter et al., 2002). The ability to maintain normal cellular function during high intensity activity could be a determining factor in successful sports performance (Jager et al., 2007).

Although there is limited research on the effects of resistance exercise on cognition, research indicates that resistance exercise does not have a positive impact on cognitive function. Therefore, resistance exercise may serve as an excellent training modality in inducing physical fatigue. The combination of PS supplementation and resistance exercise induced fatigue, coupled with appropriate tests of cognitive function, may provide the means to further understand the efficacy of PS and its effects on cognitive function. The purpose of this study is to determine if supplementation with PS (400mg of soy-derived PS) and 100mg caffeine for 14 days, will improve reaction time, cognitive performance and mood state prior to and following a fatigue inducing bout of full body resistance exercise. It is hypothesized that supplementation with 400mg of PS and 100mg caffeine for 14 days will improve baseline reaction time and cognitive

performance against placebo, while maintaining baseline performance following an acute exercise stress.

CHAPTER 3: METHODOLOGY

Subjects

Twenty, healthy males (17) and females (3) (mean ± SD; age: 22.75 ± 3.27 yrs; height: 177.03 ± 8.44cm; weight: 78.98 ± 11.24kg; body fat%: 14.28 ± 6.6) volunteered to participate in this study. Subjects were required to be free of any physical limitations and have at least six months resistance training experience in the squat, bench press, deadlift, incline bench press and bent-over row exercises. Subjects were required to complete a medical history questionnaire in order to identify any exclusion criteria. Exclusion criteria included any allergy to soy, dairy, egg and wheat ingredients, peanuts, seeds and tree nuts. In addition, any chronic illness that required continuous medical care, the use of prescription or over-the-counter medicine, pregnancy, or a predisposition to insomnia served as exclusion criteria. Subjects were also required to cease all supplementation and be free of the influence of any performance enhancing substances. Participants in the study were required to read an informed consent prior to enrollment and made fully aware of the purpose of the study, as well as any potential risks and side effects from supplementation with PS and the resistance exercise workout. The study proposal and all procedures were approved by the University of Central Florida Institutional Review Board.

Table 1: PS Group Descriptive Data

	N	Min	Max	Mean	SD
Age	11	19.00	33.00	24.00	3.90
Height	11	170.50	190.00	178.64	7.10
Weight	11	69.30	106.70	82.17	10.74
Body Fat %	11	6.80	20.26	12.73	4.61

Table 2: PL Group Descriptive Data

	N	Min	Max	Mean	SD
Age	9	19.00	23.00	21.22	1.30
Height	9	154.00	185.50	175.06	9.91
Weight	9	61.10	95.20	75.07	11.16
Body Fat %	9	6.63	28.19	16.17	8.33

Study Protocol

This study investigated the effects of 2 weeks supplementation of phosphatidylserine or placebo on reaction time, cognitive function and mood, following an exercise stress. The study was conducted in a double-blind, randomized, placebo controlled fashion. Cross-over design was not utilized in this study, since the wash-out period for Phosphatidylserine was previously unestablished. Subjects reported to the University of Central Florida Human Performance Lab on three separate occasions. During the first visit, subjects completed a familiarization process with the instruments used to measure reaction time, cognitive function and mood. Following this, subjects were tested for 1RM strength in both the bench and squat exercises.

Visits 2 (T1) consisted of pre-supplementation testing. Visit 3 (T2) consisted of post supplementation testing. The protocol at T1 and T2 were identical. Subjects reported to the UCF HPL and underwent a standardized warm-up consisting of 10 minutes cycling (Monark Ergomedic 828 E cycle ergometer) at 80 rpm and a resistance of 1Kp, one warm-up set in the squat exercise and one warm-up set in the bench exercise. Immediately following this, subjects began tests of reaction time, cognition and mood. These tests consisted of 3 reaction time tests using a Dynavision D2 Visuomotor training device, a serial subtraction test and a POMS questionnaire. After completion of these tests, subjects then completed a resistance exercise workout. Immediately following the workout, subjects completed a second round of reaction

time and cognition tests, administered identically to the first round. T1 was followed by two weeks supplementation with either phosphatidylserine or placebo. Subjects then reported back to the UCF HPL for post testing (T2). Post testing was administered in a fashion identical to that in T1. Pre and post testing was administered at the same time of day to account for diurnal variation.

Familiarization & 1RM Testing

Subjects were asked to report to the University of Central Florida Human Performance Lab (HPL) for familiarization testing and 1RM testing in the bench and squat exercises. Subjects were instructed to perform a standardized warm up consisting of 10 minutes cycling (Monark Ergomedic 828 E cycle ergometer) at 80 rpm and a resistance of 1Kp and one warm up set in both the squat and bench press exercises. Subjects completed warm up sets in both the squat and bench exercises as they would prior to a normal workout. Following the warm-up, subjects performed familiarization trials in a Serial Subtraction test, three Dynavision D2 visuomotor training device tests and also the Profile of Mood States (POMS) Questionnaire. Familiarization consisted of 7 total trials for both the Dynavision D2 training device and the Serial Subtraction Test. The first trial was a practice test used to acquaint the subject with each test and provide instruction on how to complete each test. Following this, subjects completed 5 consecutive trials for each test in order to eliminate the possibility of a training effect within the results as much as possible. All subjects then completed each test in an identical configuration as would be performed during pre and post testing, to familiarize them with the complete process. Subjects were then familiarized with the POMS questionnaire, through completion of the questionnaire.

Following the familiarization process, subjects then completed 1RM testing in the squat and bench. The purpose of 1RM testing in this study was to identify target weights for the resistance workout. Target weights were also identified for each of the other resistance exercises. 1RM testing followed the National Strength and Conditioning Associations (NSCA) guidelines and was administered by a Certified Strength and Conditioning Specialist (CSCS).

Reaction Time, Cognitive Function & Mood Measurement

In order to measure reaction time, the Dynavision D2 Visuomotor training device was utilized. The Dynavision D2 is a light-training reaction device, developed to train sensory motor integration through the visual system. For the purposes of this study, the device provided 3 tests.

Dynavision Test 1

Test 1 measured simple reaction time. This was broken down into visual, motor and physical reaction time. This test was initiated when subjects placed and held their hand on an illuminated button. This remained lit for the course of the test and served as the "home" button. At this point, a second stimulus would appear on the Dynavision board and subjects were required leave the home button, strike the stimulus and then return to and hold the home button. This was repeated ten times per test. Visual reaction time ($ICC_{3.1}$: 0.83211; $SEM_{3.1}$ = 0.02181) was measured as the amount of time it took to identify the stimulus and initiate a reaction by leaving the home button. Motor response time ($ICC_{3.1}$: 0.43766; $SEM_{3.1}$: 0.06816) was measured as the amount of time (measured in 1/100's of a second) it took to physically strike the stimulus following the initial visual reaction and is measured as the amount of time between the hand

leaving the home button and striking the stimulus. Physical reaction time ($ICC_{3.1}$: 0.61405; $SEM_{3.1}$: 0.06853) was a measurement of the total elapsed time from the introduction of the target stimulus to the physical completion of the task (i.e. leaving the first home button, striking the stimulus, and returning to the home button). This is illustrated by the following equation:

PHYSICAL RESPONSE (PR) = VISUAL REACTION (VR) + MOTOR RESPONSE (MR)

PR = VR+MR

Dynavision Test 2

Test 2 was a measure of reactivity and lasted for 60 seconds. A stimulus presented on the Dynavision board in a random location and remained lit until it was extinguished through striking it. At this point, another stimulus illuminated in a random location on the board and again remained lit until it was extinguished. The cycle continued for the 60 seconds with the purpose being to extinguish as many stimuli as possible. The number of hits during the 60 seconds was scored for each subject ($ICC_{3.1}$: 0.80324; $SEM_{3.1}$: 5.58396). Additionally, the average time per hit, measured in 1/100's of a second, was recorded for each subject ($ICC_{3.1}$: 0.79978; $SEM_{3.1}$: 0.0442).

Dynavision Test 3

Test 3 was a measure of proactivity. In a similar fashion to test 2, a stimulus was presented in a random location on the Dynavision board. However in test 3, the stimulus only remained illuminated for 1 second before it changed to another random location on the board.

The purpose of this test was to extinguish the light before it moved to another location. Failure to do so counted as a miss, while successfully extinguishing the stimulus counted as a hit. The total number of hits ($ICC_{3.1}$: 0.81968; $SEM_{3.1}$: 6.82665) and misses ($ICC_{3.1}$: 0.66003; $SEM_{3.1}$: 4.75719) during the 60 seconds was recorded for each subject. In addition to this, throughout the test, a five digit number was periodically presented on the center screen of the Dynavision board and remained for one second. In addition to extinguishing the illuminated buttons, subjects were also required to verbally recite the five digit number. The inclusion of the digits served to place additional demands on the information processing resources of the subject.

Figure 4: Dynavision D2 Visuomotor Training Device

Serial Subtraction Test

The serial Subtraction test was utilized in order to analyze cognitive function. This test consisted of a two minute timed test in which subjects were required to subtract the number 7 from a randomly generated four digit number, in order to measure how quickly and accurately they can compute a simple mathematical problem. One hundred-four digit numbers were randomly generated using a computerized number generator. These numbers were then transcribed onto standard note cards as part of a subtraction calculation. Subjects were given a randomized stack of note cards and asked to complete as many calculations as possible in the two minute period. Subject and scorer sat opposite each other during testing. The answers to the calculations were written on the back of the note cards in pencil for the scorer to see. Subjects were unable to see the correct answer. The test was administered orally to the scorer who scored the answer as either correct or incorrect. Once the subject released the note card, their answer was considered "locked in" and unchangeable. The number of correct answers ($ICC_{3.1}$: 0.86331; $SEM_{3.1}$: 4.20812), average time per correct answer ($ICC_{3.1}$: 0.81383; $SEM_{3.1}$: 0.80543) and the number of incorrect answers ($ICC_{3.1}$: 0.21254; $SEM_{3.1}$: 2.55716) were recorded.

POMS Questionnaire

Analysis of mood was performed through the administration of the Profile of Mood States Questionnaire (POMS) (McNaire, Lorr, & Droppleman, 1971). This questionnaire is psychological rating scale, consisting of 58 adjectives that are rated by subjects on a 5-point scale (0 = Not at all; 1 = A little; 2 = Moderately; 3 Quite a bit; 4 = Extremely). It is used to measure transient mood states and measures six factors including tension, anger, depression,

confusion, fatigue and vigor. Scores for adjectives representing each factor were added together to give a total score for the factor. This score was then converted to a T-Score using a conversion table. Changes in the T-Score represented a change in mood state for each of the six factors. Additionally, a total mood score (TMS) was calculated by subtracting vigor from the sum of the T-Scores of the five other negative factors and adding 100 to avoid a negative result. TMS is representative of mood disturbance. An increase in TMS from baseline is indicative of an increase in mood disturbance and is considered a negative occurrence. In contrast, a decrease in TMS is indicative of a move to a more positive mood. The POMS was administered before and immediately after the resistance exercise protocol at T1 and T2. All questionnaires were performed under controlled conditions, in a quiet room with the investigator present and were representative of how the subject felt at the specific time the questionnaire was completed. In the present study, we did not perform internal test-retest reliability due to the transient nature of mood. Measures of consistency ranging between 0.85 and 0.95 and test-retest reliability estimates ranging between 0.65 and 0.74 have been previously reported for the POMS instrument (McNair et al., 1971). The lower coefficients of stability are thought to be a result of the transient and fluctuating characteristics of mood state.

Resistance Exercise Workout

The resistance exercise workout consisted of the squat, bench press, deadlift, incline bench press and bent-over row exercises. The exercise protocol required subjects to perform a high volume, low intensity workout, common in a hypertrophy phase of training. Subjects were required to complete 4 sets of 10-12 repetitions for each exercise, with 90 seconds of rest

between each set and 120 seconds of rest between exercises. The resistance workout was administered in the UCF HPL under the supervision of a CSCS. Workout intensity was set at 70% of 1RM in the core exercises (bench and squat). Selection of appropriate weight for the assistance exercises (deadlift, incline bench press & bent-over row) was based upon a 10 repetition maximum. During the exercise protocol at T1, weights were adjusted to maintain appropriate technique and the desired exercise volume (4 sets x 10reps). A workout log was recorded at T1. The workout log at T1 subsequently became the standard for the workout at T2. Sets, repetitions and loads were matched across exercise sessions from T1 to T2.

Supplementation Protocol

Subjects were randomly assigned to an experimental or placebo group. The supplement for both groups came in the form or a candy chew (Neutravail, LLC, VA), identical in taste and appearance and only distinguishable through the color of the wrapper. One color contained 100mg PS and 25mg caffeine per candy chew while the other did not. This was a double-blind study as both the subjects and the investigators were unaware which chew was active or placebo. Immediately following the pre-test (T1), subjects were given a 14 day supply of either PS or placebo. Subjects were required to consume 4 chews per day spread out evenly across the day. In the experimental group, this amounted to 400mg PS and 100mg caffeine per day. Supplementation commenced on the day of pre-testing (T1) and ceased the day before post-testing (T2). Subjects were required to return to the UCF HPL within 24 hours following the conclusion of supplementation for post-testing (T2). The resultant duration of time between last

supplement and post-testing ranged from 8-24 hours among subjects, depending upon the time of day testing was scheduled for.

Table 3: PS and PL Ingredients

PS – Per chew	**PL – Per chew**
Vitamin C 15 mg (25% DV)	N/A
Vitamin B1 0.38 mg (25% DV)	N/A
Niacin 7 mg (25% DV)	Niacin 7 mg (25% DV)
Vitamin B5 2.5 mg (25% DV)	N/A
Vitamin B6 0.5 mg (25% DV)	N/A
Vitamin E 3 IU (10% DV)	N/A
Calcium 25 mg (2.5% DV)	N/A
Magnesium 10mg (2.5% DV)	N/A
PS 100 mg	N/A
Caffeine 25 mg	N/A
Carbohydrates 4g	Carbohydrates 4g

Reliability of Tests

Interclass correlation coefficients ($ICC_{3.1}$) and standard error of measurements ($SEM_{3.1}$) between the final two trials of the familiarization process were calculated for each test to determine internal test re-test reliability, as recommended by Weir (2005). Since it is not possible to know the true score in our tests, true reliability cannot be calculated. Interclass correlation coefficients offer an estimate of reliability based upon the statistical concept of variance; expressed as the variability among scores within a sample. Interclass correlation coefficients range from 0.00 to 1.00. 0.00 indicates zero reliability, while 1.00 indicates perfect reliability. For the purposes of our study, minimum acceptable interclass correlation coefficients are based upon the recommendations of Portney & Watkins (2000). They suggest that an ICC above 0.75

represents good reliability. Consequently, an ICC below 0.50 represents poor reliability. An ICC from 0.50 to 0.75 represents moderate reliability.

Table 4: Internal Test-Retest Reliability

Test	$ICC_{3.1}$	$SEM_{3.1}$
Dynavision Test 1 - Visual RT (sec)	0.84211	0.02181
Dynavision Test 1 - Motor RT (sec)	0.43766	0.06816
Dynavision Test 1 - Physical RT (sec)	0.61405	0.06853
Dynavision Test 2 - Hits	0.80324	5.58396
Dynavision Test 2 - Avg. RT (sec)	0.79978	0.0442
Dynavision Test 3 - Hits	0.81968	6.82665
Dynavision Test 3 - Misses	0.66003	4.75719
SST - Correct Answers	0.86331	4.20812
SST - Incorrect Answers	0.21254	2.55716
SST - ATCA (sec)	0.81383	0.80543

RT = Reaction Time; SST = Serial Subtraction Test; ICC = Interclass Correlation Coefficient; SEM = Standard Error of Measurement; MD = Minimum difference; Avg. = Average

Statistical Analysis

Dependent T-Test was utilized to assess the effects of the exercise intervention. Two-way analysis of covariance (ANCOVA) was used to assess the changes in delta scores between T1 and T2, covarying for baseline values. Post-hoc Bonferroni correction was utilized to counteract the problem of multiple comparisons. Changes between pre-tests in T1 and T2 (Δ pre) and between post-tests in T1 and T2 (Δ post) were assessed using repeated measures ANOVA. A criterion alpha level of $p \leq 0.05$ was used to determine statistical significance. Data is represented as mean ± SD.

CHAPTER 4: FINDINGS

Dynavision Test 1

The resistance-exercise workout caused an increase in visual (0.0025 ± 0.0424), motor (0.0195 ± 0.0733) and physical reaction time (0.0225 ± 0.0919) among all subjects, although the increases were not significant (p = 0.795, p = 0.249 and p = 0.287 respectively).

Both groups decreased visual RT from pre to post workout at T2 (PL = -0.00901 ± 0.0234; PS = -0.0022 ± 0.0172), however the change was not significant in either group (p = 0.227 and p = 0.708 respectively). The change in delta scores for visual RT from T1 to T2 were not significant between groups (p = 0.476) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.852) or post workout measures (Δ post; p = 0.697).

Table 5: Dynavision Test 1 - Visual Reaction Time

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	0.3535 ± 0.0459 0.3560 ± 0.0458	0.0025 ± 0.0424	p = 0.795	N/A	N/A
PL	T1 PRE T1 POST	0.3664 ± 0.0423 0.3618 ± 0.0355	−0.0045 ± 0.0455	p = 0.747	N/A	P = 0.476
PS	T1 PRE T1 POST	0.3378 ± 0.0476 0.3489 ± 0.0575	0.0111 ± 0.0392	p = 0.420		
PL	T2 PRE T2 POST	0.3627 ± 0.0372 0.3536 ± 0.0427	−0.0091 ± 0.0234	p = 0.227	−0.009 ± 0.007[$]	
PS	T2 PRE T2 POST	0.3367 ± 0.0447 0.3344 ± 0.0336	−0.0022 ± 0.0172	p = 0.708	−0.002 ± 0.007[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[a] = Adjusted for multiple comparisons: Bonferroni; [$] = Evaluated at Δ T1 = 0.0025

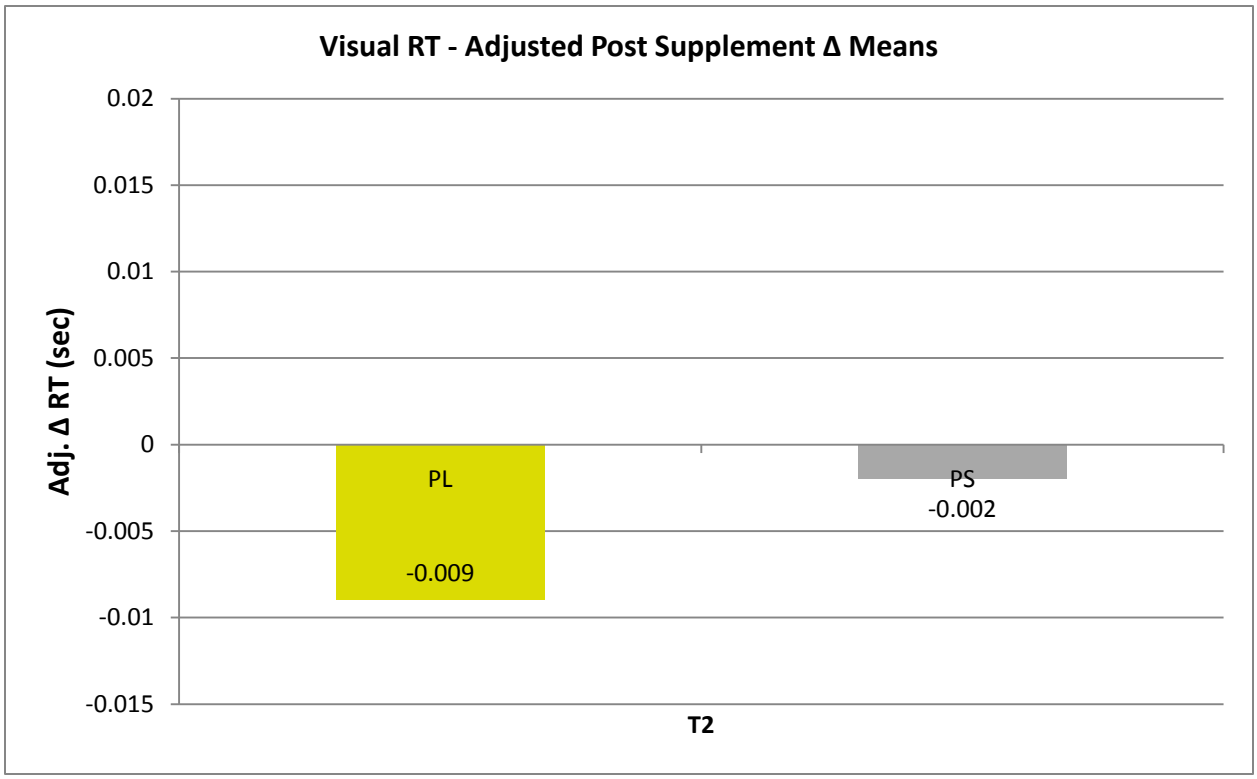

Figure 5: Dynavision Test 1 - Adjusted Δ Visual RT

The PL group saw a decrease in motor RT at T2 (-0.0018 ± 0.0407), whereas the PS group saw an increase in motor RT in T2 (0.0211 ± 0.0697), although these changes were not significant in either group ($p = 0.885$ and $p = 0.390$ respectively). The change in delta scores for motor RT from T1 to T2 were not significant between groups ($p = 0.460$) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; $p = 0.325$) or post workout measures (Δ post; $p = 0.337$).

Table 6: Dynavision Test 1 - Motor Reaction Time

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	0.2225 ± 0.0440 0.2420 ± 0.0635	0.0195 ± 0.0733	p = 0.249	N/A	N/A
PL	T1 PRE T1 POST	0.2155 ± 0.0373 0.2264 ± 0.0590	0.0109 ± 0.0511	p = 0.495	N/A	p = 0.460
PS	T1 PRE T1 POST	0.2311 ± 0.0521 0.2611 ± 0.0223	0.0300 ± 0.0963	p = 0.377		
PL	T2 PRE T2 POST	0.1927 ± 0.0366 0.1909 ± 0.0386	−0.0018 ± 0.0407	p = 0.885	−0.0085 ± 0.17[$]	
PS	T2 PRE T2 POST	0.2322 ± 0.0550 0.2533 ± 0.0631	0.0211 ± 0.0697	p = 0.390	0.019 ± 0.018[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 0.0195

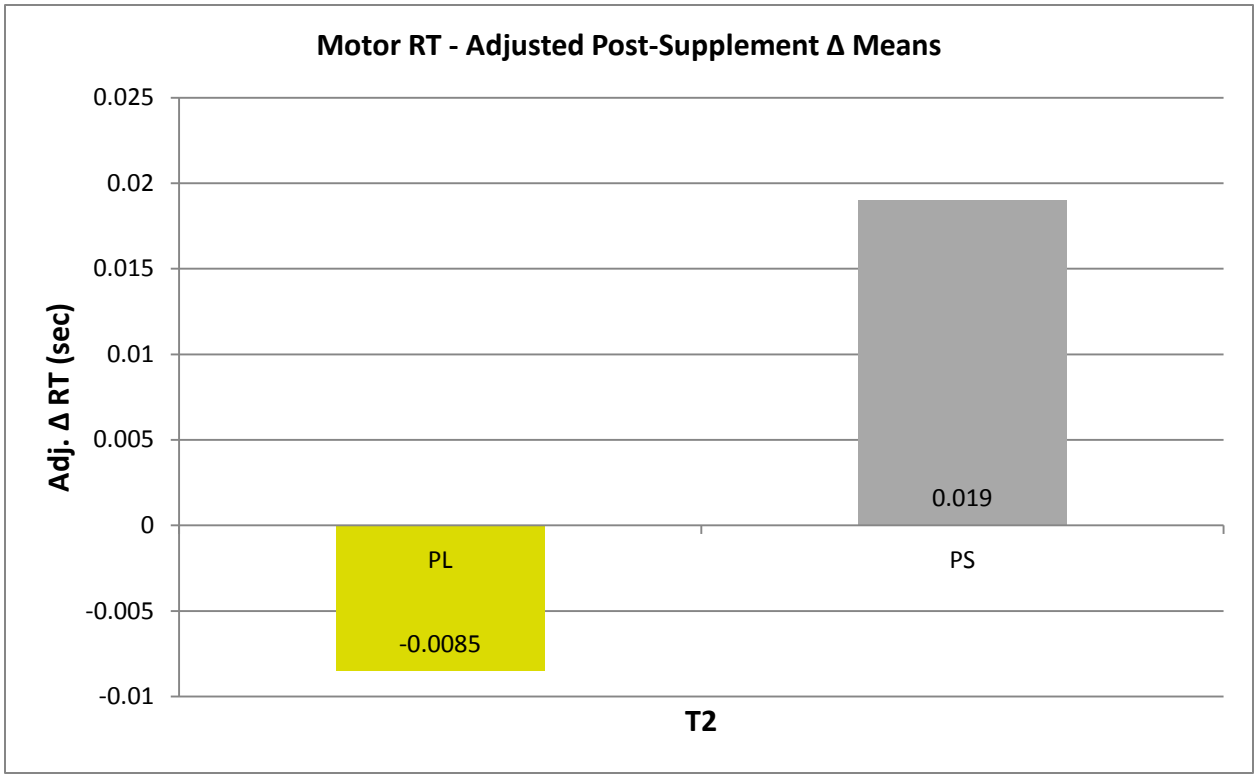

Figure 6: Dynavision Test 1 - Adjusted Δ Motor RT

The PL group saw a decrease in Physical RT at T2 (-0.0118 ± 0.0431) whereas the PS group saw an increase in physical reaction time (0.0222 ± 0.0778). These changes were not significant in either group (p = 0.384 and p = 0.416 respectively). The change in delta scores for physical RT from T1 to T2 was not significant between groups (p = 0.312) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.422) or post workout measures (Δ post; p = 0.487).

Table 7: Dynavision Test 1 - Physical Reaction Time

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	0.5780 ± 0.0529 0.6005 ± 0.0873	0.0225 ± 0.0919	p = 0.287	N/A	N/A
PL	T1 PRE T1 POST	0.5827 ± 0.0420 0.5918 ± 0.0662	0.0091 ± 0.0691	p = 0.672	N/A	p = 0.312
PS	T1 PRE T1 POST	0.5722 ± 0.0661 0.6111 ± 0.1113	0.0389 ± 0.1165	p = 0.346		
PL	T2 PRE T2 POST	0.5564 ± 0.0482 0.5445 ± 0.0537	−0.0118 ± 0.0431	p = 0.384	−0.009 ± 0.018[$]	
PS	T2 PRE T2 POST	0.5667 ± 0.0841 0.5889 ± 0.0609	0.0222 ± 0.0778	p = 0.416	0.019 ± 0.020[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 0.0225

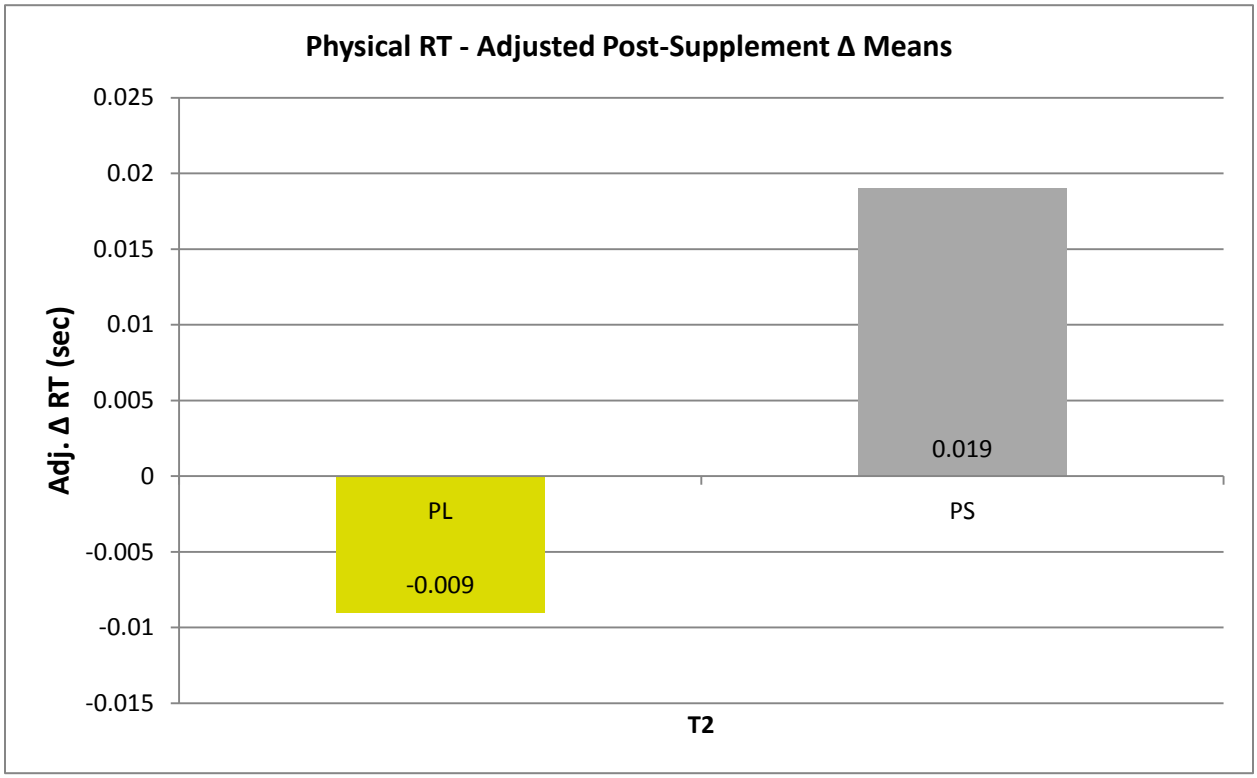

Figure 7: Dynavision Test 1 - Adjusted Δ Physical RT

Dynavision Test 2

The resistance-exercise workout caused a significant decrease in the number of hits (-4.60 ± 9.00) and a significant increase in average RT time per hit (0.0405 ± 0.0836) from pre to post workout at T1 among all subjects ($p = 0.034$ and $p = 0.043$ respectively).

Both groups saw a decrease in the number of hits from pre to post workout at T2 (PL = -3.09 ± 8.51; PS = -1.00 ± 5.66), however neither was significant ($p = 0.256$ and $p = 0.610$ respectively). The change in delta scores for the number of hits from T1 to T2 was not significant between groups ($p = 0.722$) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; $p = 0.552$) or post workout measures (Δ post; $p = 0.519$).

Table 8: Dynavision Test 2 - Hits

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	90.55 ± 9.57 85.95 ± 11.67	−4.60 ± 9.00	p = 0.034	N/A	N/A
PL	T1 PRE T1 POST	89.45 ± 10.72 83.64 ± 12.24	−5.82 ± 10.21	p = 0.088	N/A	p = 0.722
PS	T1 PRE T1 POST	91.89 ± 8.39 88.78 ± 10.95	−3.11 ± 7.50	p = 0.254		
PL	T2 PRE T2 POST	92.64 ± 7.78 89.55 ± 12.83	−3.09 ± 8.51	p = 0.256	−2.653 ± 2.062[$]	
PS	T2 PRE T2 POST	93.33 ± 9.50 92.33 ± 8.54	−1.00 ± 5.66	p = 0.610	−1.535 ± 2.283[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
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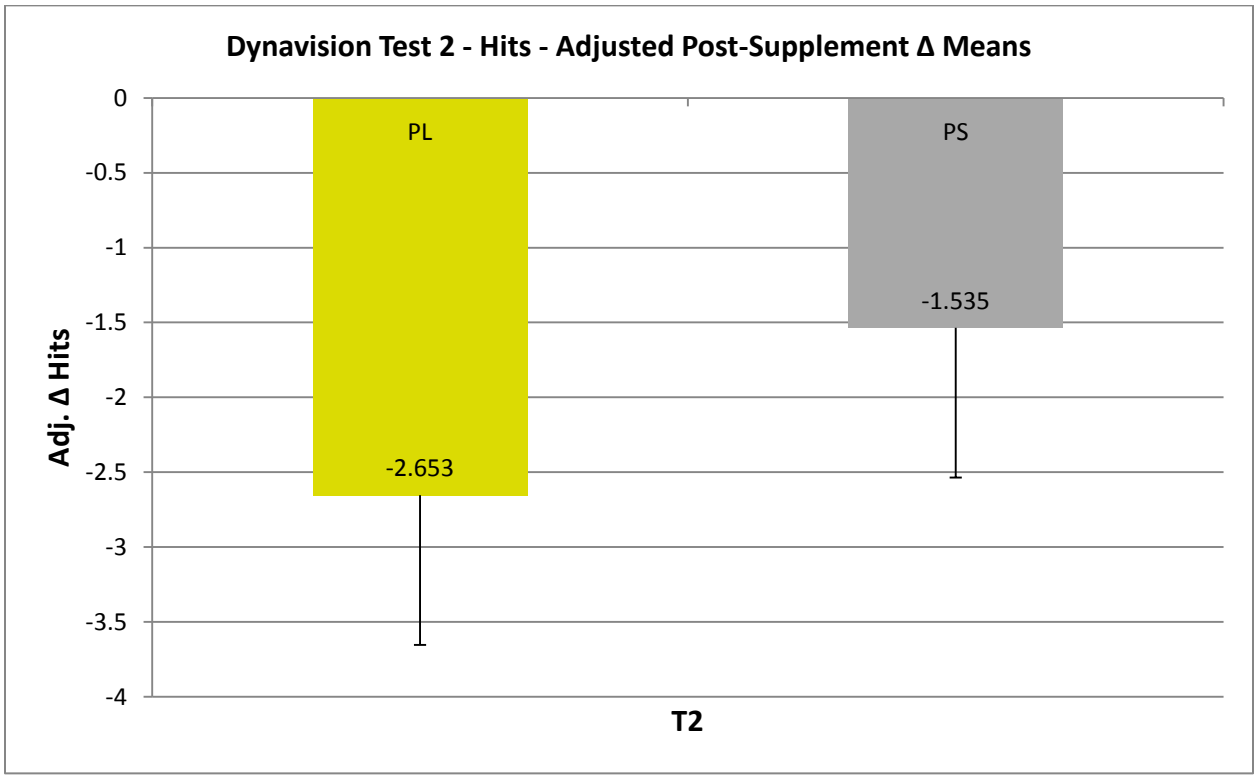

Figure 8: Dynavision Test 2 - Adjusted Δ Hits

Both groups saw an increase in average RT per hit at T2 (PL = 0.0400 ± 0.1149; PS = 0.0067 ± 0.0430), however neither was significant (p = 0.275 and p = 0.654 respectively). The change in delta scores for average RT per hit was not significant between groups from T1 to T2 (p = 0.537) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.309) or post workout measures (Δ post; p = 0.736).

Table 9: Dynavision Test 2 - Average RT

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	0.6655 ± 0.0719 0.7060 ± 0.1073	0.0405 ± 0.0836	p = 0.043	N/A	N/A
PL	T1 PRE T1 POST	0.6755 ± 0.0819 0.7245 ± 0.1234	0.0490 ± 0.102	p = 0.142	N/A	p = 0.537
PS	T1 PRE T1 POST	0.6533 ± 0.0600 0.6833 ± 0.0852	0.0300 ± 0.0581	p = 0.160		
PL	T2 PRE T2 POST	0.6418 ± 0.0458 0.6818 ± 0.1214	0.0400 ± 0.1149	p = 0.275	0.035 ± 0.023[$]	
PS	T2 PRE T2 POST	0.6444 ± 0.0684 0.6511 ± 0.0601	0.0067 ± 0.0430	p = 0.654	0.013 ± 0.025[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 0.0405

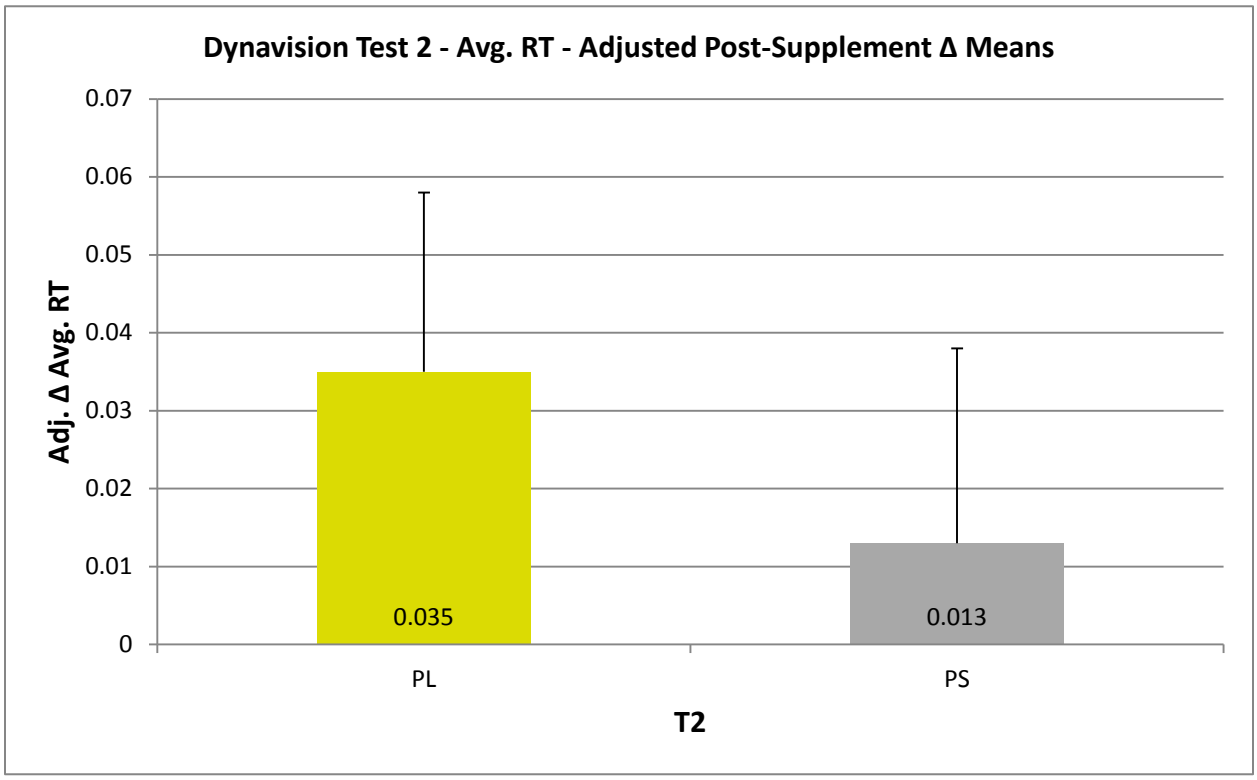

Figure 9: Dynavision Test 2 - Adjusted Δ Avg. RT

Dynavision Test 3

The resistance-exercise workout caused a decrease in hits (−2.80 ± 10.81) and an increase in the number of misses (0.50 ± 5.03) from pre to post workout at T1 among all subjects, however these changes were not significant (p = 0.261 & p = 0.662 respectively).

The PL group saw a decrease in the number of hits (-1.64 ± 13.89) from pre to post workout at T2 (p = 0.704), whereas the PS group saw an increase in the number of hits (2.22 ± 3.35) from pre to post workout at T2 (p = 0.081). The change in delta scores for the number of hits from T1 to T2 was not significant between groups (p = 0.580) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.489) or post workout measures (Δ post; p = 0.680).

Table 10: Dynavision Test 3 - Hits

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	76.50 ± 13.84 73.70 ± 13.14	−2.80 ± 10.81	p = 0.261	N/A	N/A
PL	T1 PRE T1 POST	75.09 ± 14.82 70.91 ± 12.75	−4.18 ± 9.61	p = 0.180	N/A	p = 0.580
PS	T1 PRE T1 POST	78.22 ± 13.19 77.11 ± 13.52	−1.11 ± 12.50	p = 0.797		
PL	T2 PRE T2 POST	80.55 ± 9.66 78.91 ± 16.87	−1.64 ± 13.89	p = 0.704	−8.296 ± 2.873[$]	
PS	T2 PRE T2 POST	81.11 ± 9.21 83.33 ± 9.55	2.22 ± 3.35	p = 0.081	−5.861 ± 3.181[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = -4.30

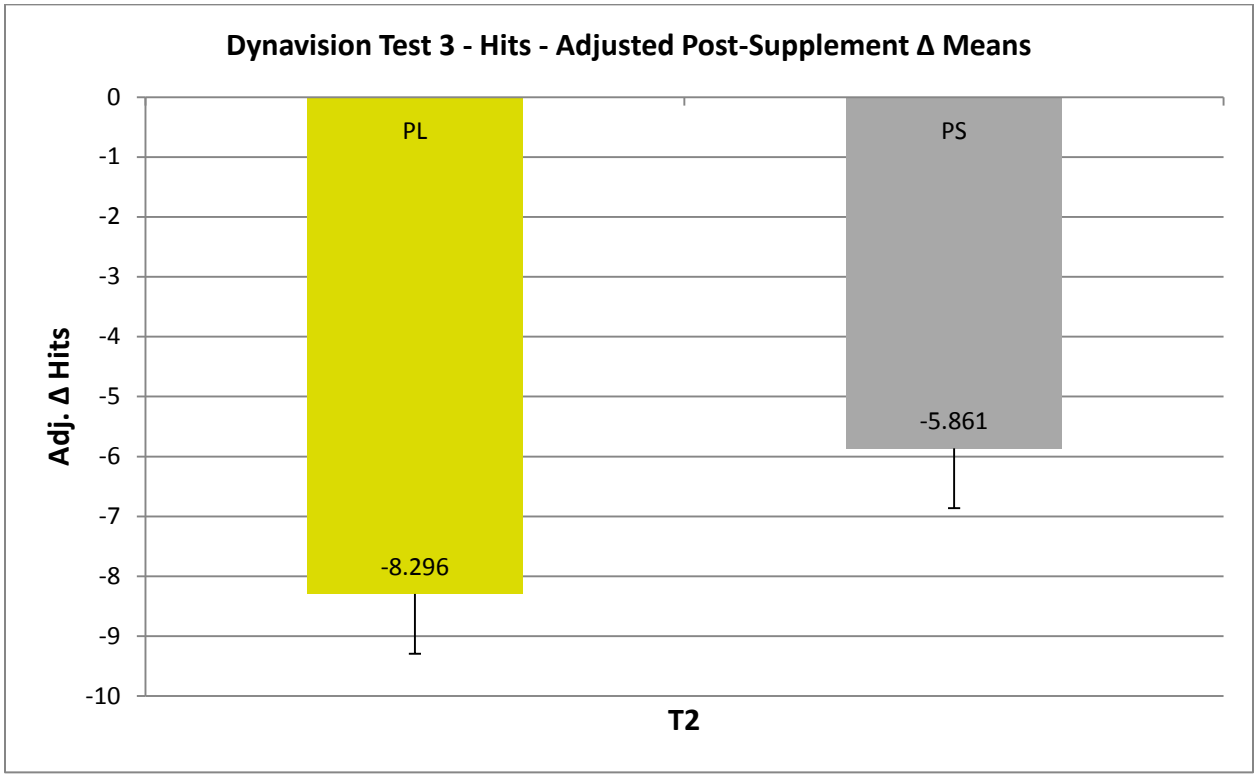

Figure 10: Dynavision Test 3 - Adjusted Δ Hits

The PL group saw an increase in the number of misses (0.64 ± 7.65) at T2, whereas there was a significant decrease in the number of misses (-1.89 ± 2.15) from pre to post workout in the PS group at T2 ($p = 0.030$) The change in delta scores for the number of misses from T1 to T2 was not significant between groups ($p = 0.403$) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; $p = 0.939$) or post workout measures (Δ post; $p = 0.686$).

Table 11: Dynavision Test 3 - Misses

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	10.65 ± 6.65 11.15 ± 5.98	0.50 ± 5.03	p = 0.662	N/A	N/A
PL	T1 PRE T1 POST	11.18 ± 7.49 12.36 ± 6.53	1.18 ± 3.54	p = 0.295	N/A	p = 0.403
PS	T1 PRE T1 POST	10.00 ± 5.83 9.67 ± 5.22	−0.33 ± 6.56	p = 0.883		
PL	T2 PRE T2 POST	9.45 ± 4.48 10.09 ± 8.63	0.64 ± 7.65	p = 0.788	0.555 ± 1.823[$]	
PS	T2 PRE T2 POST	8.44 ± 3.57 6.56 ± 3.28	−1.89 ± 2.15	p = 0.030	−1.789 ± 2.018[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 0.50

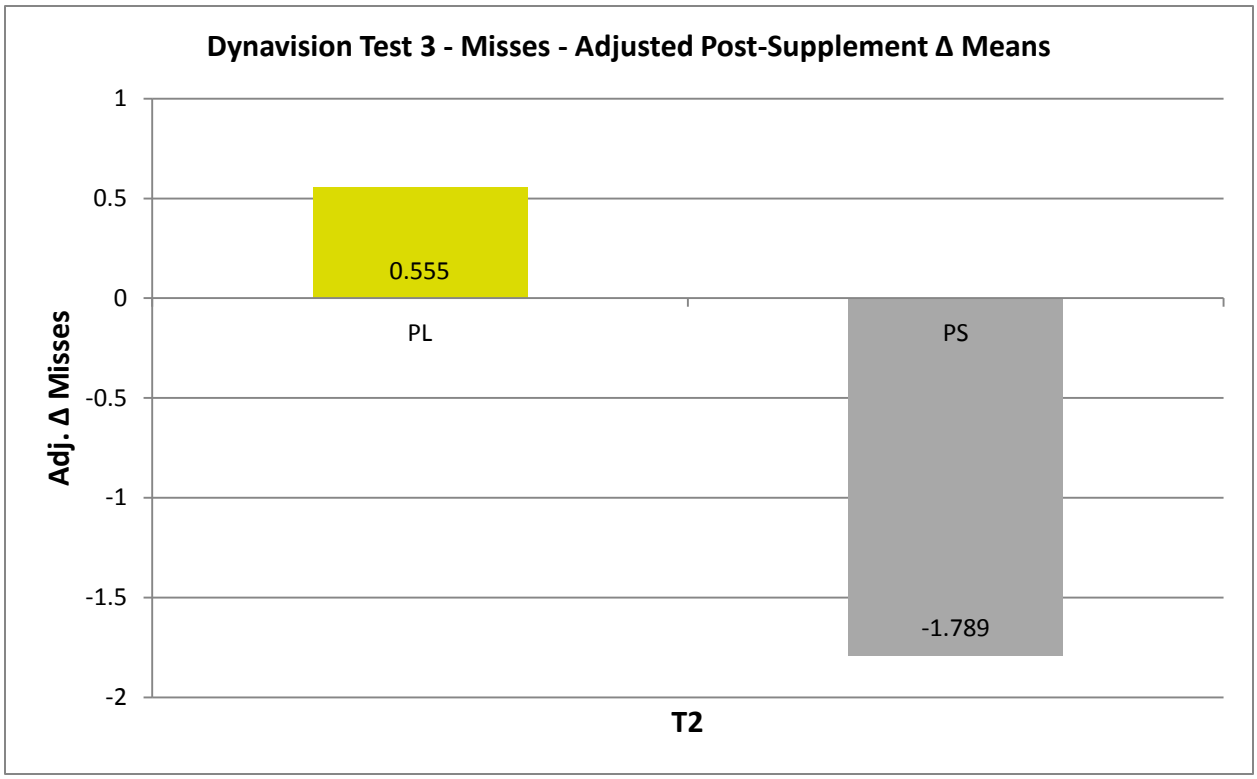

Figure 11: Dynavision Test 3 - Adjusted Δ Misses

Serial Subtraction Test (SST)

The resistance-exercise workout caused a significant increase in the number of correct answers (2.50 ± 3.80) and significant decrease in average time per correct answer (ATCA) (0.30 ± 0.52) from pre to post workout at T1 among all subjects ($p = 0.008$ and $p = 0.018$ respectively). Additionally, the workout caused an increase in incorrect answers (0.30 ± 1.13) at T1, however the increase was not significant ($p = 0.249$).

There was an increase in the number of correct answers within both groups at T2 (PL = 2.45 ± 3.30; PS = 2.00 ± 3.46). This was significant within the PL group ($p = 0.033$), but not significant in the PS group ($p = 0.122$). The change in delta scores for the number of correct answers from T1 to T2 was not significant between groups ($p = 0.869$) after adjustment for

baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.727) or post workout measures (Δ post; p = 0.401).

Table 12: SST - Correct Answers

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	32.20 ± 9.13 34.70 ± 9.50	2.50 ± 3.80	p = 0.008	N/A	N/A
PL	T1 PRE T1 POST	34.18 ± 8.84 36.27 ± 3.18	2.09 ± 3.14	p = 0.052	N/A	p = 0.869
PS	T1 PRE T1 POST	29.78 ± 9.39 32.78 ± 8.25	3.00 ± 4.64	p = 0.088		
PL	T2 PRE T2 POST	36.82 ± 12.06 39.27 ± 11.58	2.45 ± 3.30	p = 0.033	2.365 ± 1.016[$]	
PS	T2 PRE T2 POST	31.56 ± 9.29 33.56 ± 9.26	2.00 ± 3.46	p = 0.122	2.110 ± 1.124[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
SST = Serial Subtraction Test; [$] = Evaluated at Δ T1 = 2.50

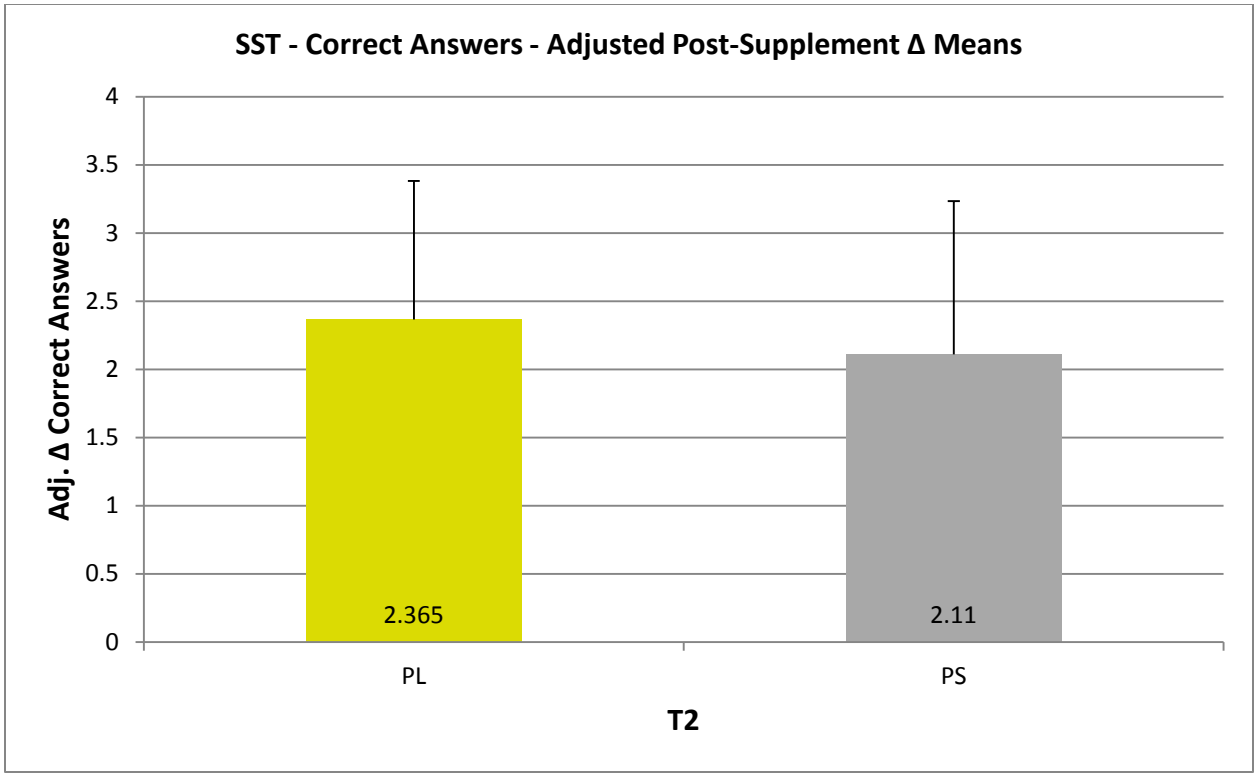

Figure 12: SST - Adjusted Δ Correct Answers

There was a decrease in ATCA within both groups at T2 (PL = 0.32 ± 0.47sec; PS = 0.24 ± 0.57sec). This was significant within the PL group (p = 0.048), but not significant in the PS group (p = 0.245). The change in delta scores for ATCA from T1 to T2 was not significant between groups (p = 0.984) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.509) or post workout measures (Δ post; p = 0.854).

Table 13: SST - Average Time per Correct Answer (ACTA)

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	4.03 ± 1.20 3.73 ± 1.11	0.30 ± 0.52	p = 0.018	N/A	N/A
PL	T1 PRE T1 POST	3.75 ± 1.07 3.59 ± 1.14	0.16 ± 0.38	p = 0.196	N/A	p = 0.984
PS	T1 PRE T1 POST	4.38 ± 1.33 3.90 ± 1.11	0.48 ± 0.63	p = 0.053		
PL	T2 PRE T2 POST	3.77 ± 1.86 3.44 ± 1.53	0.32 ± 0.47	p = 0.048	−0.281 ± 0.158[$]	
PS	T2 PRE T2 POST	4.05 ± 0.99 3.81 ± 1.04	0.24 ± 0.57	p = 0.245	−0.286 ± 0.176[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
SST = Serial Subtraction Test; [$] = Evaluated at Δ T1 = -0.3019

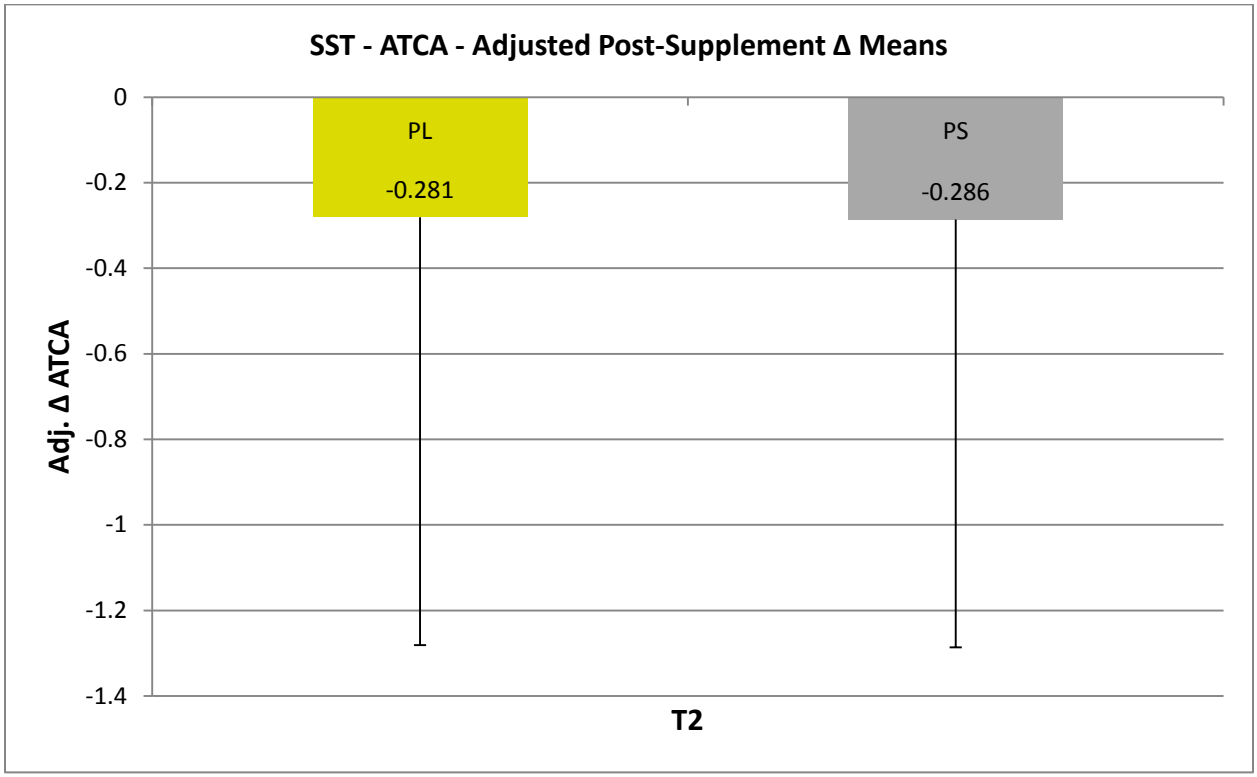

Figure 13: Adjusted Δ Average Time per Correct Answer (ATCA)

There was a decrease in the number of incorrect answers at T2 for the PS group (-0.33 ± 2.69), whereas the PL group showed an increase (0.64 ± 1.21) in incorrect answers, however neither were significant (p = 0.720 and p = 0.111 respectively). The change in delta scores for the number of incorrect answers from T1 to T2 was not significant between groups (p = 0.305) after adjustment for baseline scores. There was no significant interaction between groups for changes in baseline measures (Δ pre; p = 0.352) or post workout measures (Δ post; p = 0.854).

Table 14: SST - Incorrect Answers

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	2.45 ± 2.48 2.75 ± 2.69	0.30 ± 1.13	p = 0.249	N/A	N/A
PL	T1 PRE T1 POST	2.55 ± 2.16 3.09 ± 2.55	0.55 ± 1.21	p = 0.167	N/A	p = 0.305
PS	T1 PRE T1 POST	2.33 ± 2.96 2.33 ± 2.96	0.00 ± 1.00	p = 1.000		
PL	T2 PRE T2 POST	1.82 ± 1.17 2.45 ± 1.51	0.64 ± 1.21	p = 0.111	0.656 ± 0.631[$]	
PS	T2 PRE T2 POST	2.78 ± 2.64 2.44 ± 2.92	0.33 ± 2.69	p = 0.720	−0.357 ± 0.700[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
SST = Serial Subtraction Test; [$] = Evaluated at Δ T1 = -0.3000

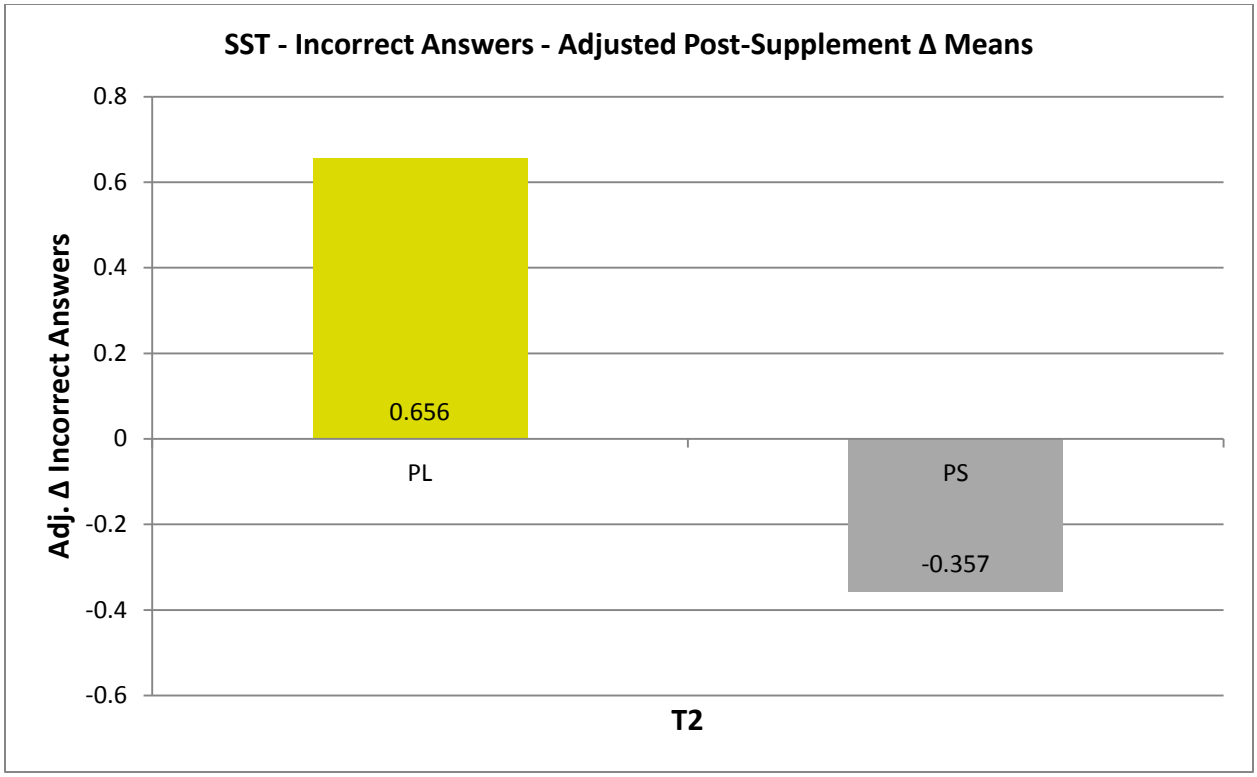

Figure 14: Adjusted Δ Incorrect Answers

Profile of Mood States (POMS)

The resistance-exercise workout caused a significant increase in TMS (23.65 ± 21.50) from pre to post workout at T1, among all subjects (p = 0.000). Fatigue T-Score (14.25 ± 11.84) tension T-Score (3.20 ± 5.74) and depression T-Score (0.20 ± 0.41) were also significantly increased (p = 0.000; p = 0.022; p = 0.042 respectively). Vigor T-Score (-6.60 ± 11.85) was significantly decreased from pre to post workout at T1 among all subjects (p = 0.022). T-Scores for anger (-1.00 ± 1.25; p = 0.725) and confusion (−0.50 ± 2.89; p = 0.449) were not significantly changed by the resistance exercise protocol.

An increase in TMS was seen within both groups at T2 (PL = 26.91 ± 22.95; PS = 8.00 ± 16.07). The increase was significant within the PL group (p = 0.003), however the increase

within the PS group was not significant (p = 0.174). The change in delta scores for TMS from T1 to T2 were not significant between groups (p = 0.112) after adjustment for baseline scores.

Table 15: Profile of Mood State (POMS) - Total Mood Score (TMS)

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	231.80 ± 14.37 255.45 ± 16.10	23.65 ± 21.50	p = 0.000	N/A	N/A
PL	T1 PRE T1 POST	231.73 ± 18.47 260.55 ± 16.57	28.82 ± 26.37	p = 0.005	N/A	p = 0.112
PS	T1 PRE T1 POST	231.89 ± 7.98 249.22 ± 13.89	17.33 ± 12.10	p = 0.003		
PL	T2 PRE T2 POST	227.27 ± 12.77 254.18 ± 20.58	26.91 ± 22.95	p = 0.003	25.342 ± 6.040[$]	
PS	T2 PRE T2 POST	233.00 ± 9.59 241.00 ± 14.30	8.00 ± 16.07	p = 0.174	9.915 ± 6.703[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 23.6500

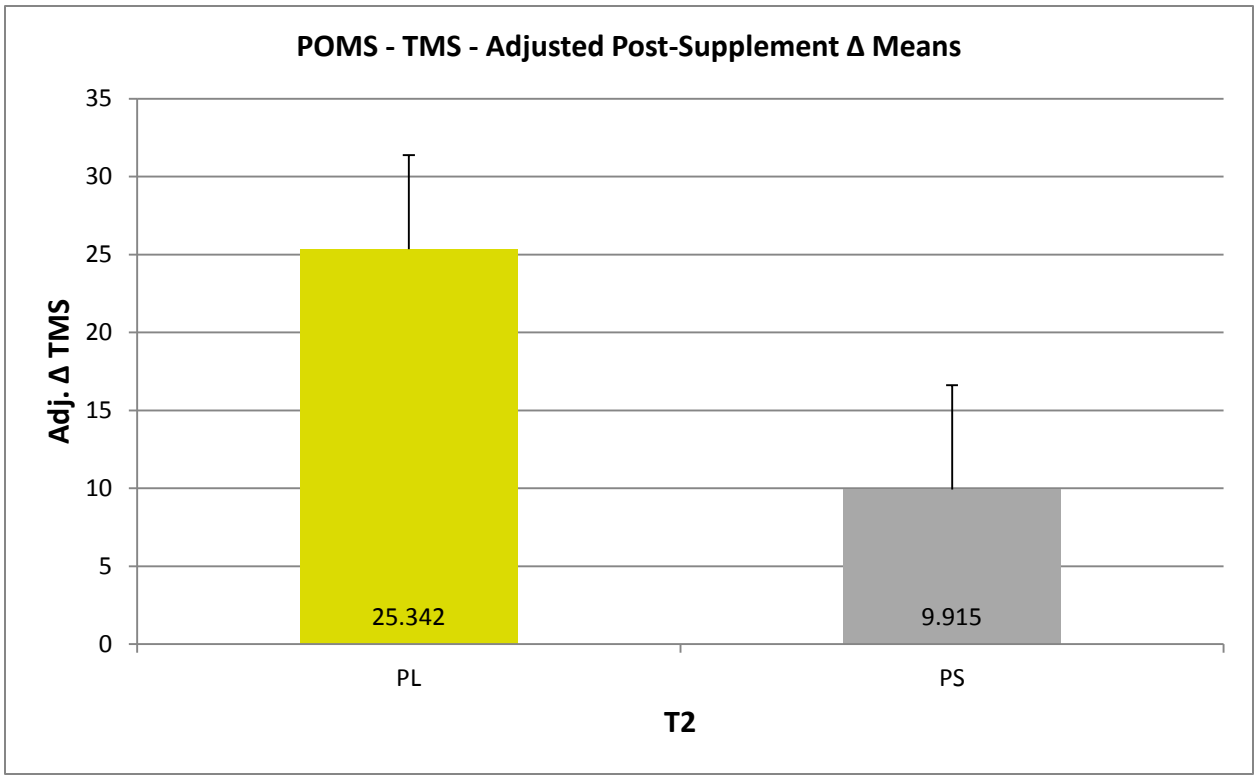

Figure 15: POMS - Adjusted Δ TMS

Fatigue T-Score increased significantly in both groups (PL = 15.27 ± 8.42; PS = 8.00 ± 7.50) from pre to post workout at T2 ($p = 0.000$ and $p = 0.013$ respectively). The change in delta scores for fatigue T-score from T1 to T2 were not significant between groups ($p = 0.071$) after adjustment for baseline scores.

Table 16: Profile of Mood State (POMS) - Fatigue T-Score

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	37.50 ± 5.75 51.75 ± 9.34	14.25 ± 11.84	p = 0.000	N/A	N/A
PL	T1 PRE T1 POST	38.91 ± 6.91 54.18 ± 9.72	15.27 ± 14.60	p = 0.006	N/A	p = 0.071
PS	T1 PRE T1 POST	35.78 ± 3.60 48.78 ± 8.41	13.00 ± 7.97	p = 0.001		
PL	T2 PRE T2 POST	36.45 ± 5.56 51.73 ± 9.52	15.27 ± 8.42	p = 0.000	15.057 ± 2.364[$]	
PS	T2 PRE T2 POST	38.00 ± 5.22 46.00 ± 7.48	8.00 ± 7.50	p = 0.013	8.264 ± 2.614[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 14.2500

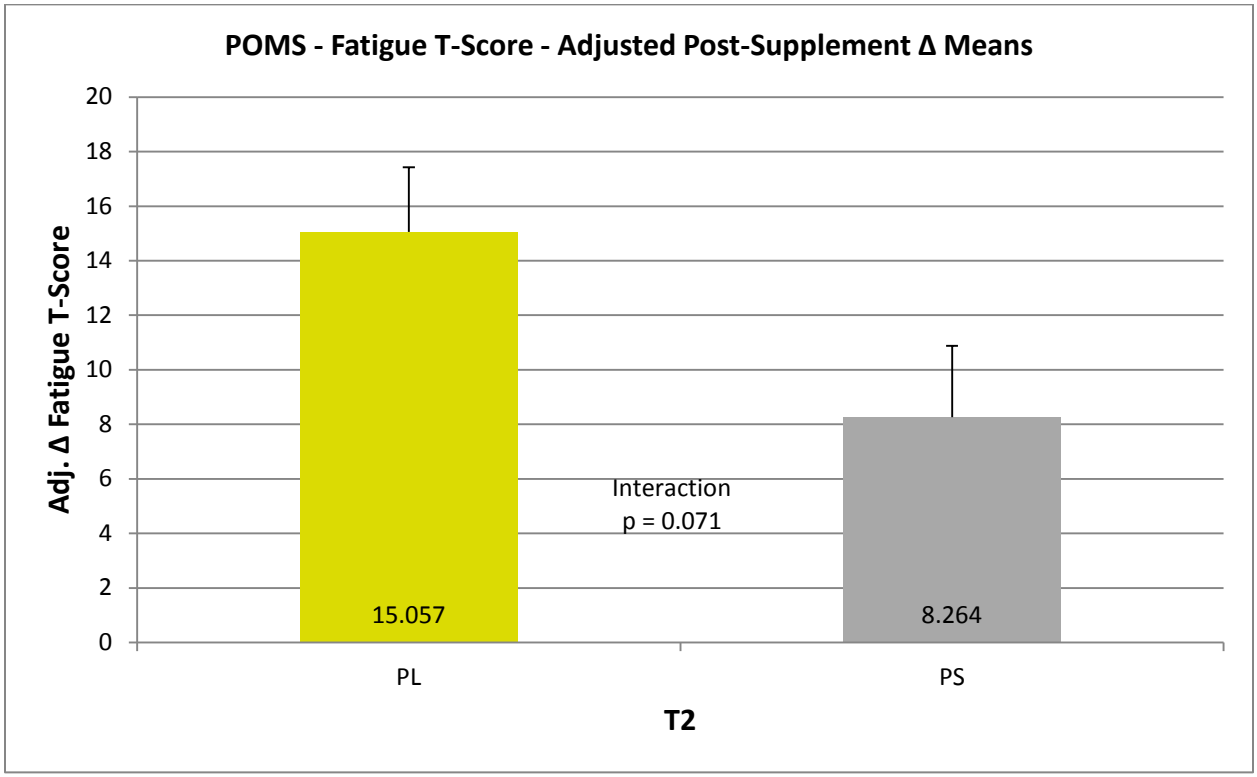

Figure 16: POMS - Adjusted Δ Fatigue T-Score

Tension T-Score increased within the PL group (3.73 ± 7.58) at T2, whereas a decrease in tension T-score was seen in the PS group (-0.67 ± 3.00), however the change was not significant in either group (p = 0.134 and p = 0.524 respectively). The changes in delta scores for Tension T-Score from T1 to T2 were not significant between groups (p = 0.124) after adjustment for baseline scores.

Table 17: Profile of Mood State (POMS) - Tension T-Score

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	39.30 ± 4.96 42.50 ± 7.24	3.20 ± 5.74	p = 0.022	N/A	N/A
PL	T1 PRE T1 POST	39.00 ± 3.82 41.45 ± 6.11	2.45 ± 3.98	p = 0.068	N/A	p = 0.124
PS	T1 PRE T1 POST	39.67 ± 6.32 43.78 ± 8.64	4.11 ± 7.52	p = 0.140		
PL	T2 PRE T2 POST	35.55± 4.13 39.27 ± 7.72	3.73 ± 7.58	p = 0.134	3.785 ± 1.863[$]	
PS	T2 PRE T2 POST	39.11 ± 5.35 38.44 ± 4.10	0.67 ± 3.00	p = 0.524	−0.737 ± 2.062[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = 3.2000

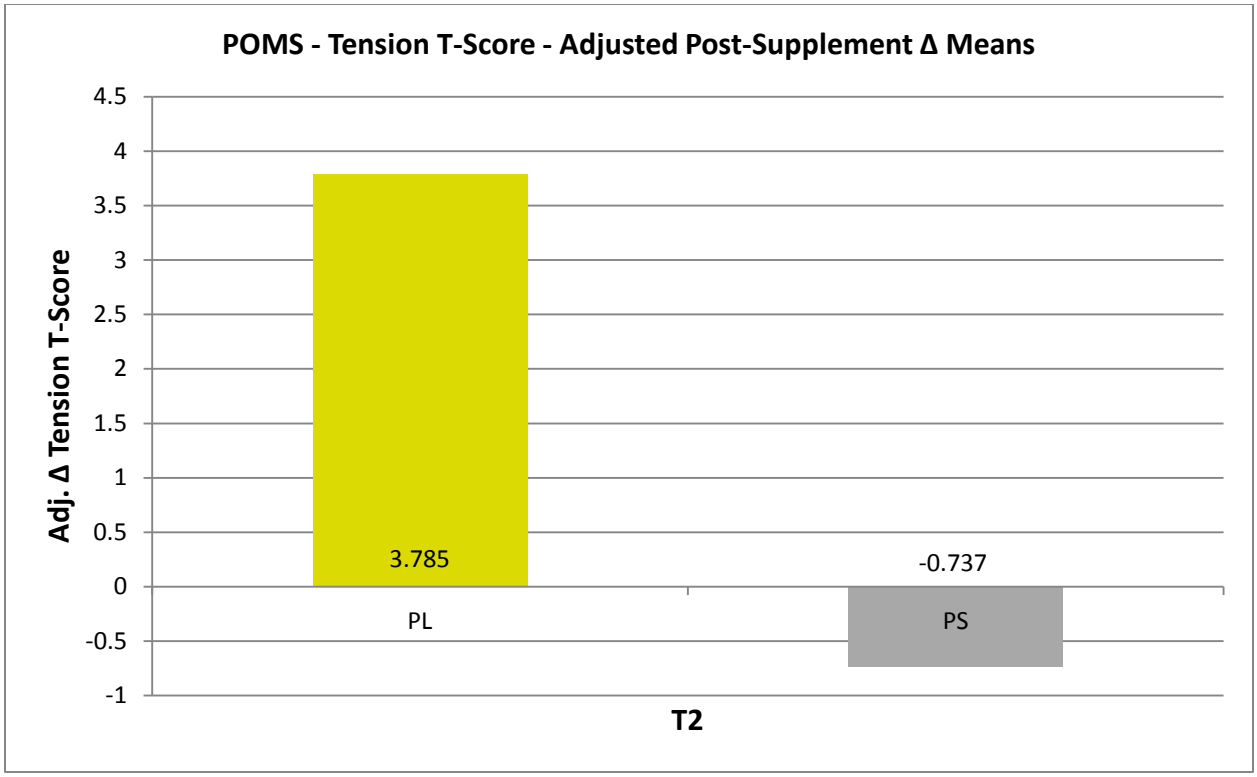

Figure 17: POMS - Adjusted Δ Tension T-Score

Vigor T-Score decreased within both groups (PL = -8.45 ± 14.60; PS = -2.33 ± 9.30) from pre to post workout at T2, although this decrease was not significant in either group ($p = 0.084$ and $p = 0.473$ respectively). The changes in delta scores for vigor T-Score between groups from T1 to T2 were not significant ($p = 0.767$) after adjustment for baseline scores.

Table 18: Profile of Mood State (POMS) - Vigor T-Score

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	55.90 ± 12.45 49.30 ± 14.18	−6.60 ± 11.85	p = 0.022	N/A	N/A
PL	T1 PRE T1 POST	56.36 ± 13.71 45.18 ± 9.92	−11.18 ± 11.08	p = 0.007	N/A	p = 0.767
PS	T1 PRE T1 POST	55.33 ± 11.51 54.33 ± 17.40	−1.00 ± 10.77	p = 0.788		
PL	T2 PRE T2 POST	52.82 ± 14.52 44.36 ± 12.79	−8.45 ± 14.60	p = 0.084	−6.510 ± 3.796[$]	
PS	T2 PRE T2 POST	55.00 ± 10.05 52.67 ± 13.23	−2.33 ± 9.30	p = 0.473	−4.711 ± 4.242[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
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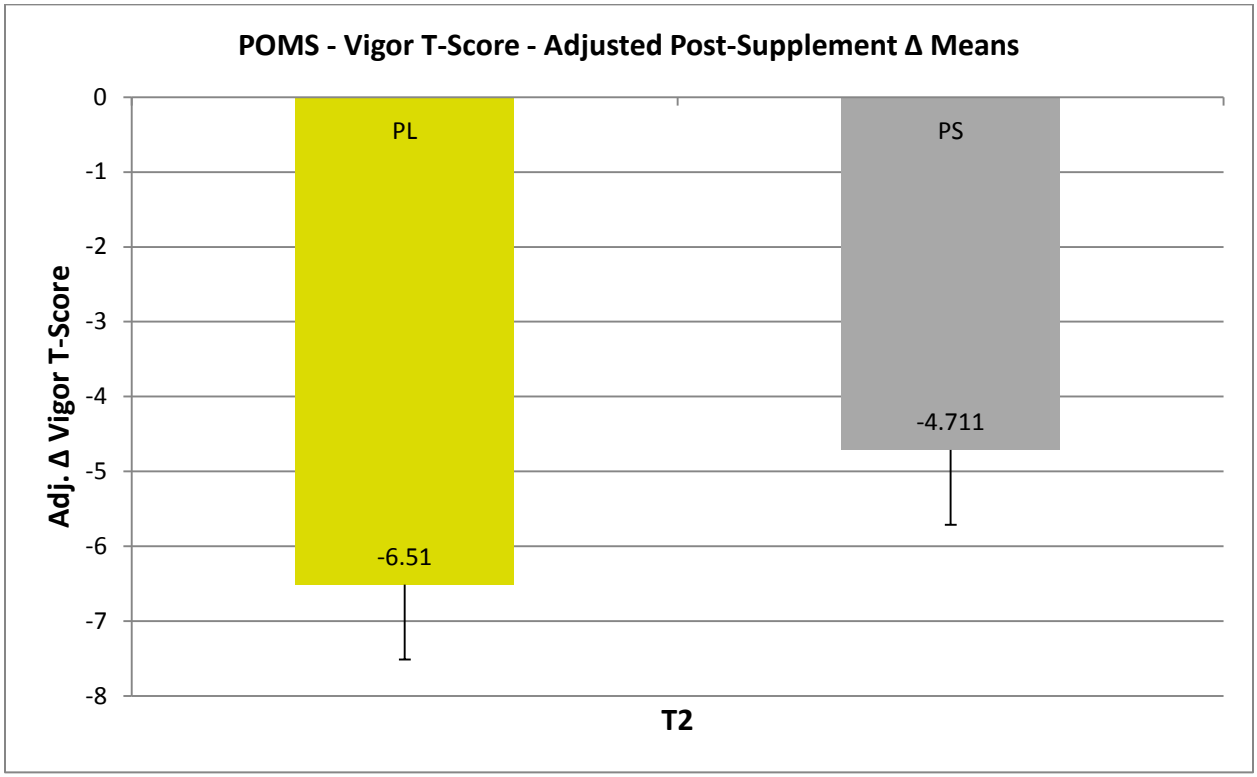

Figure 18: POMS - Adjusted Δ Vigor T-Score

Confusion T-Score decreased within both groups (PL = -0.64 ± 2.34; PS = -1.78 ± 2.17) from pre to post workout at T2. The change was significant in the PS group (p = 0.039) but not in the PL group (p = 0.387). The changes in delta scores for confusion T-Score between groups from T1 to T2 were not significant (p = 0.389) after adjustment for baseline scores.

Table 19: Profile of Mood State (POMS) - Confusion T-Score

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	35.60 ± 2.52 35.10 ± 3.11	−0.50 ± 2.89	p = 0.449	N/A	N/A
PL	T1 PRE T1 POST	35.18 ± 1.40 35.09 ± 2.70	−0.09 ± 2.39	p = 0.902	N/A	p = 0.389
PS	T1 PRE T1 POST	36.11 ± 3.48 35.11 ± 3.72	−1.00 ± 3.50	p = 0.416		
PL	T2 PRE T2 POST	33.64 ± 2.42 33.00 ± 1.79	−0.64 ± 2.34	p = 0.387	−0.764 ± 0.645[$]	
PS	T2 PRE T2 POST	35.56 ± 2.40 33.78 ± 3.42	−1.78 ± 2.17	p = 0.039	−1.622 ± 0.715[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = -0.5000

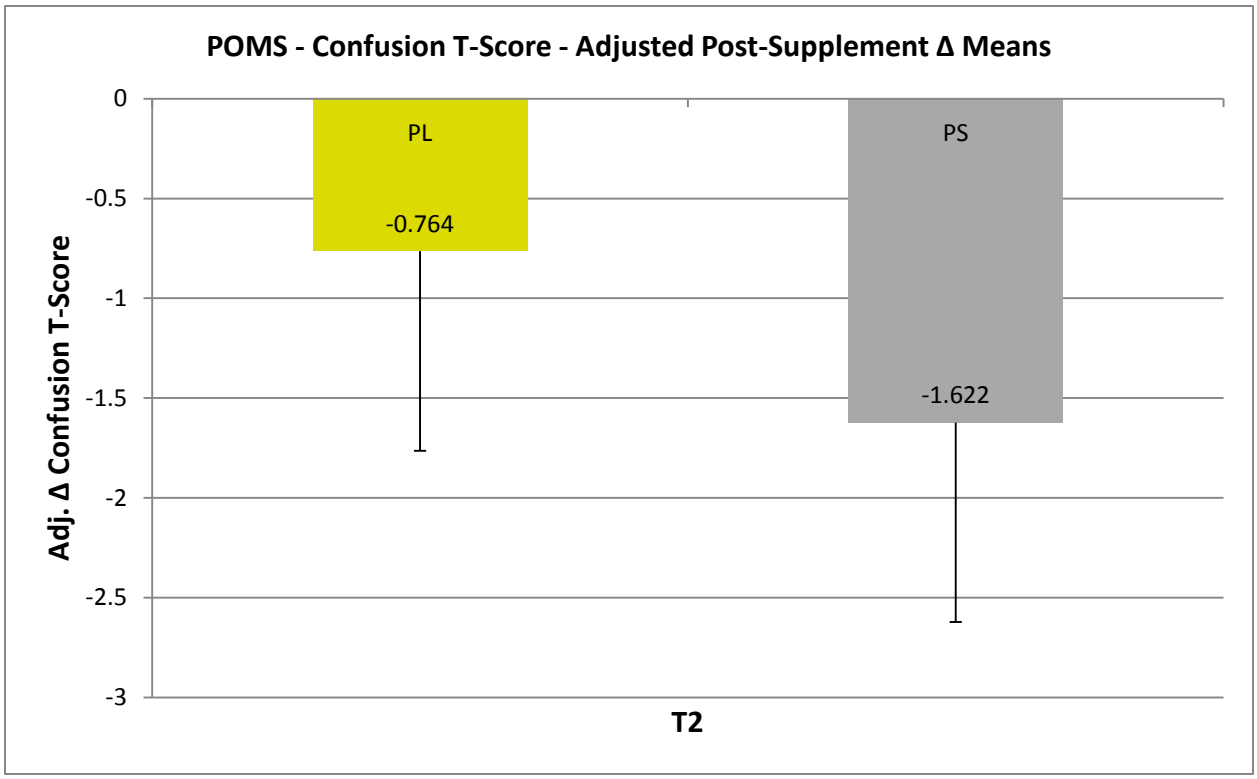

Figure 19: POMS - Adjusted Δ Confusion T-Score

Depression T-Score increased at T2 for the PL group only (0.18 ± 0.40), however the change was not significant ($p = 0.167$). Depression T-Score remained unchanged in the PS group. The changes in delta scores for confusion T-Score between groups from T1 to T2 were not significant ($p = 0.345$) after adjustment for baseline scores.

Table 20: Profile of Mood State (POMS) - Depression T-Score

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	37.00 ± 0.00 37.20 ± 0.41	0.20 ± 0.41	p = 0.042	N/A	N/A
PL	T1 PRE T1 POST	37.00 ± 0.00 37.27 ± 0.47	0.27 ± 0.47	p = 0.082	N/A	p = 0.345
PS	T1 PRE T1 POST	37.00 ± 0.00 37.11 ± 0.33	0.11 ± 0.33	p = 0.347		
PL	T2 PRE T2 POST	37.00 ± 0.00 37.18± 0.40	0.18 ± 0.40	p = 0.167	0.147 ± 0.072[$]	
PS	T2 PRE T2 POST	37.00 ± 0.00 37.00 ± 0.00	N/A*	N/A	0.042 ± 0.080[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement

*Cannot be computed because the standard error of the difference is zero.

[$] = Evaluated at Δ T1 = 0.2000

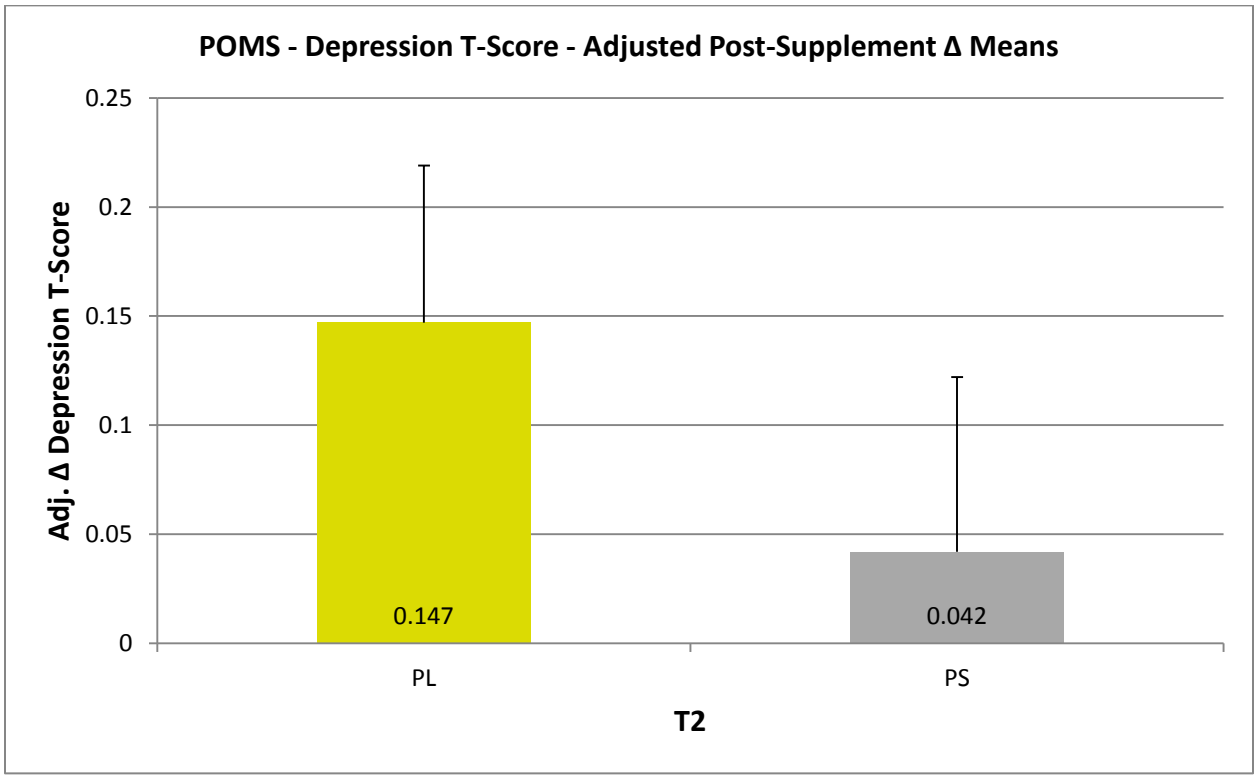

Figure 20: POMS - Adjusted Δ Depression T-Score

Anger T-Score increased at T2 for the PL group (-0.18 ± 1.40), whereas a decrease in anger T-Score was seen in the PS group (-0.11 ± 0.78), however the change was not significant in either group (p = 0.676 and p = 0.681 respectively). The change in delta scores for anger T-Score between groups from T1 to T2 were not significant (p = 0.519) after adjustment for baseline scores.

Table 21: Profile of Mood State (POMS) - Anger T-Score

Supp	Trial	Mean ± SD	Δ Mean ± SD	Sig.[a]	Adjusted Δ Mean ± SE	Sig. Between Groups
All Subjects	T1 PRE T1 POST	38.30 ± 2.00 38.20 ± 2.09	−1.00 ± 1.25	p = 0.725	N/A	N/A
PL	T1 PRE T1 POST	38.00 ± 1.61 37.73 ± 1.27	−0.27 ± 1.27	p = 0.493	N/A	p = 0.519
PS	T1 PRE T1 POST	38.67 ± 2.45 38.78 ± 2.77	0.11 ± 1.27	p = 0.799		
PL	T2 PRE T2 POST	37.36 ± 0.81 37.55 ± 1.29	0.18 ± 1.40	p = 0.676	−0.209 ± 0.359[$]	
PS	T2 PRE T2 POST	38.44 ± 2.51 38.33 ± 2.06	−0.11 ± 0.78	p = 0.681	0.145 ± 0.397[$]	

PL = Placebo; PS = Phosphatidylserine; T1 = PRE Supplement; T2 = POST Supplement
[$] = Evaluated at Δ T1 = -0.1000

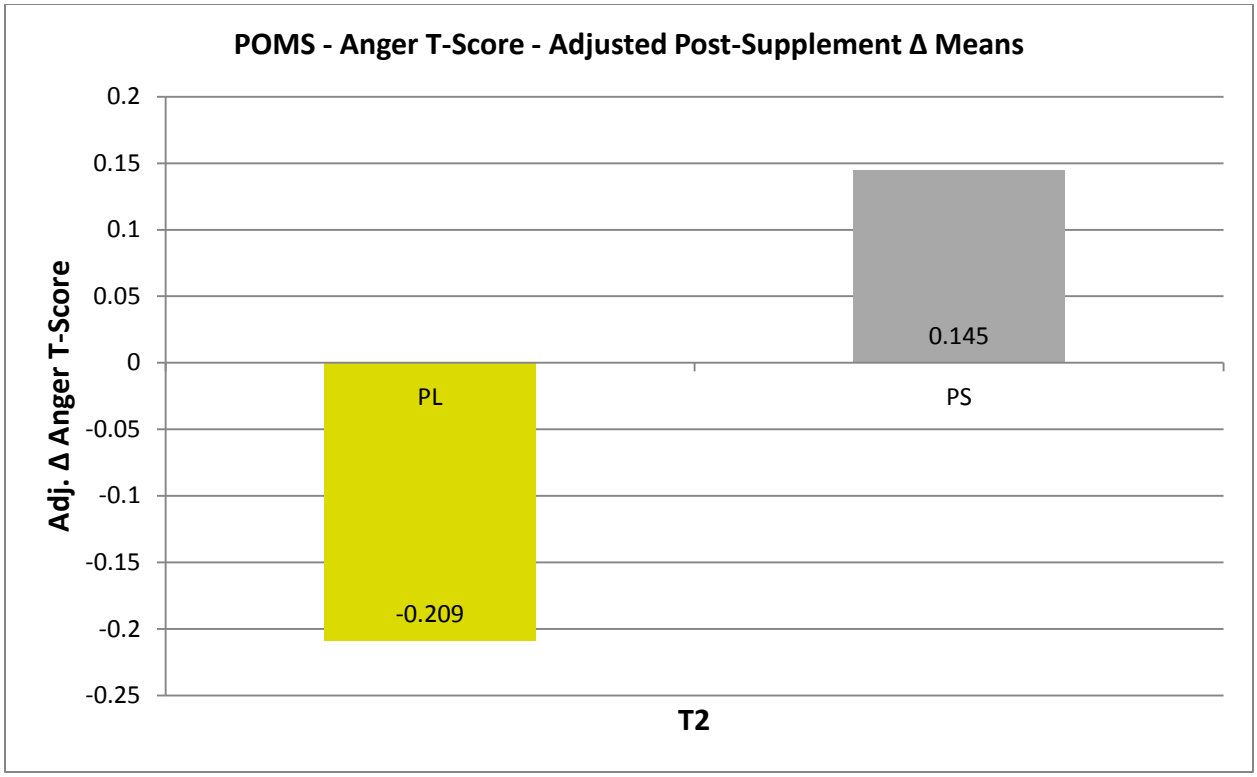

Figure 21: POMS - Adjusted Δ Anger T-Score

CHAPTER 5: DISCUSSION

Cue utilization theory suggests that moderate intensity exercise could improve cognitive performance while high intensity fatiguing exercise may lead to a decrease in cognitive performance. The ability to maintain normal cellular function during high intensity activity could therefore be a determining factor in successful sports performance and have further implications in other areas, such as the prevention of neurodegenerative disease. The findings in the present study suggest that 400mg PS taken with 100mg caffeine per day for two weeks in healthy males and females does not have a significant positive effect on measures of cognition or reaction time following an exercise stress. However, there is a trend in the data indicating that PS way attenuate fatigue.

When interpreting the results of this study, it is necessary to first ascertain the appropriateness of the exercise protocol in inducing fatigue. A significant increase in TMS was observed between pre and post workout testing at T1. This occurred through the interaction of a significant increase in fatigue, tension and depression, coupled with a significant decrease in vigor among all subjects. Additionally, reaction time increased from pre to post workout at baseline for all Dynavision tests and increased significantly in Dynavision test 2. These results suggest that the resistance protocol was adequate in inducing the desired fatigue. Interestingly, there was a significant increase in the number of correct answers in the SST and a significant decrease in ATCA between pre and post workout at in T1. Limited research on the effects of resistance exercise on cognition suggests that resistance exercise (30mins) does not induce an acute increase in cognitive performance or a decrease in reaction time (Pontifex et al., 2009). Kramer, Erickson and Colcombe (2012) report both acute increases in cognitive performance

with exercise in addition to a neuroprotective effect on later life cognition. However, their review of the literature does not make the distinction as to the effects of an acute bout of resistance exercise. It is possible that the increase in SST scores between pre and post testing at baseline may be reflective of inadequate familiarization with the test. However, pairwise comparison of the six familiarization trials indicates that familiarization had occurred after the fifth trial (data not shown). Consequently, our data may suggest that an acute bout of resistance exercise results in an improvement in acute cognitive functioning.

Analysis of the data indicates that supplementation with PS does not have a significant effect on reaction time. In Dynavision test 1, supplementation with PS failed to significantly impact visual, motor or physical reaction time (Tables 5, 6 & 7). Additionally, supplementation with PS failed to have a significant effect upon reaction time in Dynavision test 2 (Table 9) or performance in Dynavision test 3 (Tables 9 & 11). In Dynavision test 3, the PS group had a statistically significant decrease in misses at T2 (Table 11). However, there was no interaction between groups following adjustment for baseline scores ($p = 0.403$). One of the limitations of the Dynavision Visuomotor training device is the inability to control the spread of the stimuli on the board in tests 2 and 3. The Dynavision board is arranged into five concentric circles beginning at the center of the board. Additionally, it can be divided into four quadrants (Figure. 4). The spread refers to the distribution of the stimuli across each concentric circle and where they fall within each quadrant. Stimuli that are principally presented on the inner circles and that are confined to one quadrant may result in quicker reaction times, whereas stimuli that are principally presented across multiple quadrants and the outer circles may result in slower reaction times. The inability to control for the distribution means that the spread of the board

may not be uniform across all subjects or across all trials. Therefore a change in scores may be the result of an unfavorable or favorable spread.

Previous research has shown evidence that cognitive function may be improved through PS supplementation. Parker et al. (2011) concluded that supplementation with 400mg of soy derived PS for 14 days significantly reduced ATCA by 20% ($p = 0.001$), improved correct answers by 13% ($p = 0.070$) and reduced errors by 39% ($p = 0.053$) at baseline (Δ pre) in the serial subtraction test. This is in direct contrast to the present study where we found that PS did not increase SST performance at baseline (Δ pre; $p = 0.727$). A possible reason behind these contrasting results is an inadequate test familiarization process on the part of Parket et al., (2011). Parker et al., (2011) indicate that only one familiarization trial was performed for the serial subtraction test. In our study, pairwise comparison of 6 familiarization trials indicated statistically significant increases in performance between familiarization trial 2 and 3 and also familiarization trials 4 and 5 of the SST. Therefore, the performance gains at baseline seen by Parker et al., (2011) may not be a result of PS supplementation, but a result of subjects becoming more familiarized with the test protocol. Our results did indicate a significant increase in correct answers within the PL group from pre to post workout in T2 ($p = 0.033$). This effect was not seen in T1 for the PS group or at T1 or T2 for the PL group. However, the interaction between PS and PL groups was not significant ($p = 0.869$) after adjusting for T1 Δ scores. ATCA decreased significantly within the PL group, however the interaction between PS and PL groups was not significant ($p = 0.984$). The number of incorrect answers was not significantly changed in either group. Parker et al., (2011) did not report internal test retest reliability for their serial subtraction test. Additionally, they did not indicate the testing methodology used in administration of the SST. The use of different testing modalities between independent studies

can confound the interpretation of results. However, because the test protocol of Parker et al., (2011) is unknown, we are unable to make any inferences as to the effect of the test methodology.

Our findings did not show a significant impact upon mood. Total mood score (TMS) was significantly increased from pre to post workout in T1 and T2 within the PL group. However, although TMS increased in T2 for the PS group, the change was not significant. Additionally, there is a trend ($p = 0.071$) for an interaction between groups for fatigue T-Score, following adjustment for pre-supplement Δ scores. Previous research suggests that supplementation with PS may counteract stress induced activation of the hypothalamo-pituitary-adrenal axis. The HPA axis is a major part of the neuroendocrine system that controls the body's reaction to stress and regulates a number of body processes including mood and emotion (Monteleone et al., 1992). Our data indicates that PS may attenuate fatigue during an exercise stress. This may contribute to a decrease in mood disturbance. Confusion T-Scores decreased in both groups following resistance exercise at both T1 and T2, providing support for the exercise induced increase in cognitive performance seen in the SST.

The fact that the PS supplement contained caffeine while the PL supplement did not needs to be addressed. This offers a compelling avenue as to why our results showed a trend in the attenuation of fatigue, but no significance. Supplementation of four chews per day equated to an intake of 100mg of caffeine per day for fourteen days, in addition to any caffeine that the subjects may have already been consuming. In the present study, supplementation commenced on the day of pre testing and ceased the day before post testing. Therefore, there was no acute effect of caffeine on the selected measures within our study. However, it is possible that cessation of supplementation may have resulted in caffeine withdrawal. Research indicates that a

significant caffeine withdrawal can occur after abstinence from a dose as low as 100mg/day (Evans & Griffiths, 1999). The subsequent time course of caffeine withdrawal has been shown to typically emerge 12-24 hours after caffeine abstinence (Schuh & Griffiths, 1997), a time course consistent with the short half-life of caffeine (4-6hrs). A critical review of caffeine withdrawal symptoms was conducted by Juilano and Griffiths (2004). According to the authors, caffeine withdrawal can result in tiredness and fatigue, decreased energy, decreased alertness, drowsiness, depressed mood, irritability and fogginess in the head. In another study, Griffiths, Evans, Heishman, Preston, Sannerud, Wolf and Woodson (1990) reported the effects of withdrawal from 100mg/day of caffeine using the POMS questionnaire. Their study protocol consisted of two phases. Phase one consisted of an initial exposure to 100mg of caffeine for 9 to 14 days, followed by substitution with placebo for 12 days. Following this, a re-exposure to 100mg caffeine for 7 to 12 days was initiated. Phase two consisted of a 6-week period whereby placebo would be substituted for caffeine on five days. Placebo days were separated by a mean of 9.3 days of 100mg caffeine supplementation. They authors reported that compared to days when subjects received caffeine, intermittent placebo was associated with a significant increase in fatigue, confusion, bewilderment and total mood disturbance (total mood score) and a significant decrease in vigor. This study closely simulates both the caffeine intake and supplementation time frame seen in the present study. It is therefore possible that the effects of caffeine contained within the supplement in the present study, may counteract any beneficial effect that the PS may offer. This becomes even more apparent when the effects of caffeine on the signaling pathways involved in the activation of PKC are analyzed. In order for classical isozymes of PKC to be activated, PS, DAG and calcium must all be present. Research indicates that caffeine may inhibit the release of calcium from IP3 downstream of PLCγ (Bezprozvanny, Bezprozvannaya &

Ehrlick, 1995; Kang, Han, Ku, Lee, Hong, Shin, Almonte, Woo, Brat, Hwang, Yoo, Chung, Park, Paek, Roh, Lee, Park, Traynelis & Lee, 2010), possibly by inhibiting its receptor on the endoplasmic reticulum (Sei, Gallagher & Daly, 2001). Calcium release via IP3, allosterically increases the C2 domain of cPKC's affinity for PS. Research also indicates that calcium may function as a bridge between PKC and membrane phospholipids (Bazzi & Nelsestuen, 1990). Therefore, inhibition of the IP3 receptor may have a detrimental effect on the function of PS as an enzyme co-factor. It has been suggested that mobilization of intracellular calcium stores will induce an influx of extracellular calcium until the intracellular calcium pool is refilled, as part of a compensatory mechanism to maintain intracellular calcium levels (Dolor, Hurwitz, Mirza, Strauss & Whorton, 1992: Hoth & Penner, 1992). However, an inhibition of calcium release would not deplete the intracellular calcium store; it would merely render it inaccessible. It is currently unclear whether compensatory mechanisms exist to initiate extracellular calcium influx during IP3 receptor inhibition. Research does suggests that additional calcium pools may exist within the cell that are not released via the action of IP3 (Bian, Ghosh, Wang & Gill, 1991), although it is unclear whether these pools are mobilized during IP3 receptor inhibition.

It remains questionable whether exogenous PS is in fact incorporated into cellular membranes. It has been shown that the addition of exogenous PS to cultured fibroblasts results in the transportation of PS to the Golgi apparatus (Kobayashi & Arakawa, 1991), possibly through the use of flippases. Flippases are aminophospholipid translocases that transport specific phospholipids from the extracellular leaflet to the cytosolic leaflet of the plasma membrane. They also transport phospholipids from the luminal leaflet of intracellular organelles to the cytosolic leaflet (Laventis et al., 2010). A possible pathway for the incorporation of endogenously synthesized PS into the cell membrane has been outlined by Laventis et al.,

(2010). According to Laveltis et al. (2010), endogenous PS is synthesized on the cytoplasmic leaflet of the endoplasmic reticulum and mitochondrial associated membrane. It is then moved, via vesicular trafficking, from the endoplasmic reticulum to the endoplasmic reticulum Golgi intermediate compartment (ERGIC) and finally to the Golgi apparatus. Within the trans-Golgi network (TGN), PS is enriched in budding secretory vesicles. Flippases in the TGN and secretory vesicles then translocate PS to the cytoplasmic face. PS is then transported to the plasma membrane where flippases maintain PS exclusively on the cytosolic leaflet. PS is then delivered to sorting endosomes via endocytosis. At the early endosome, PS enriched vesicles are recycled to the plasma membrane via vesicular traffic. Since exogenous PS has been shown to enter the Golgi apparatus, it is possible that its incorporation into the membrane follows the same pathway downstream of the Golgi apparatus. Nishijima et al., (1986) suggest that an exogenous supply of PS may result in three-five fold suppression of endogenous PS biosynthesis with no change in PS turnover. This indicates that exogenous PS can be successfully integrated into cellular membranes despite the suppression of endogenous synthesis. Therefore it is conceivable that supplementation with PS in amounts that exceed the decline in endogenous synthesis may provide the ability to enhance the PS pool. However, incorporation of PS into the Golgi apparatus has been shown to result in the subsequent metabolism of PS to PE (Kobayashi & Arakawa, 1991). The successful incorporation of exogenous PS into the cell membrane therefore remains equivocal, despite positive results from practical studies supplementing PS. It may be possible that supplementation may benefit individuals with a deficit in the PS pool.

The appropriate dosage and duration of PS supplementation needs to be ascertained. Research indicates that supplementation levels as high as 600mg/day are well tolerated in elderly populations (Jorissen, Brouns, Boxtel & Riedel, 2002). Hellhammer, Fries, Buss, Engert, Tuch,

Rutenberg and Hellhammer (2004) examined the effects of three weeks supplementation on pituitary adrenal reactivity and the psychological response to a mental and emotional stressor. Dosages of soy-derived PS within a complex of PS and Phosphatidic acid (PA) equated to 400mg, 600mg and 800mg respectively. Results indicated that a dosage of 400mg/day resulted in profoundly blunted adrenocorticotropic hormone and cortisol response to the Trier Social Stress Test (TSST), as well as an attenuated salivary cortisol response and a positive emotional response. These results were not seen in individuals supplementing with 600mg and 800mg, or in the placebo group. 300mg/day of BC-PS for 12 weeks has been shown to result in improved performance with regards to memory tasks of daily life, learning and attention in subjects with age-associated memory impairment (Crook et al., 1991). Additionally, 300mg/day supplementation of BC-PS for 6 months has been shown to positively affect behavioral and cognitive parameters in geriatric patients (Cenacchi, Bertoldin, Farina, Fiori & Crepaldi, 1993). Thus, it seems that a dosage of approximately 300-400mg per day may be adequate to elicit significant results. In the present study, supplementation with 400mg/day PS for 14 days showed a trend for the attenuation of fatigue. Much of the research regarding the supplementation of PS published to date has been focused on elderly, memory impaired populations, and those in cognitive decline. With the exception of Parker et al., (2011), to our knowledge only two additional studies have focused on the cognitive efficacy of phosphatidylserine within a young, healthy population. Benton, Donohoe, Sillance and Nabb (2001) reported that supplementation with 300mg PS for 1 month was associated with feeling less stressed and having a better mood in young adults with above normal neuroticism scores. Baumeister et al., (2008) reported no significant difference between 42 days supplementation with 200mg/day of soy derived PS and PL in cognitive task performance. Supplementation duration may therefore need to be

substantially longer to illicit discernible results in a young, healthy population. Conversely, it is possible that within a young healthy population, the potential efficacy of PS may not manifest due to normal cellular functioning in terms of cell homeostasis. Indeed, the opposing roles of various isoforms of PKC have been elucidated. It is therefore conceivable that growth expression via one isoform of PKC may be offset by the signaling of apoptosis through another in an attempt to maintain cell homeostasis and regulate growth. In contrast, a significant effect may be seen in an impaired population as a result of the body attempting to bring a cell back to homeostasis. Therefore, further research into the dosing and duration of supplementation is necessary.

The results of research within populations experiencing cognitive decline however, remain equivocal. Some research indicates a positive effect (Cenacchi et al., 1993; Crook, 1998), while others found no effect (Jorissen, Brouns, Van Boxtel, Ponds, Verhey, Jolles & Riedel, 2000). Interestingly, it has been asserted that soy-derived phosphatidylserine species may not hold the most promise as a brain nutrient. Chen and Li (2008) compared molecular species of various transphosphatidylated PS with that of BC-PS, using mass spectrometry. They determined that a combination of fish liver PS and squid skin PS could serve as potential alternatives to soy-derived PS, solely based upon the lack of DHA species present in soy-derived PS. Early optimistic research involving phosphatidylserine was undertaken with the use of bovine-derived PS, a species containing about 10% DHA within its fatty acid chain. Therefore it may be possible that these results were representative of an interaction between PS and DHA. A study by Richter, Herzog, Cohen and Steinhart (2010), utilized a study protocol that called for supplementation with 300mg/day PS enriched with 37.5mg of Eicosapentaenoic acid plus DHA, for six weeks in elderly subjects with subjective memory complaints. They found that the combination of

supplements resulted in both a significant decrease in the decay in memory loss from immediate to delayed recall, and a significant improvement in delayed recall capability. It is therefore possible that the action of PS is combination with DHA may manifest at lower relative dosages. Consequently, future research should consider PS species rich in DHA as an alternative to soy-derived PS.

Conclusions

Although more research is necessary regarding optimal dosage and supplementation duration, the current findings suggest that supplementation 400mg/day PS with 100mg/day caffeine may attenuate fatigue following acute resistance exercise. It is possible that the lack of significance may be the result of both an inhibition of the PS activated pathway and a withdrawal effect from caffeine.

Recommendations for Future Research

Future research should control for caffeine intake among subjects for the duration of supplementation to fully elucidate the role of PS in cognition and mood and reaction time. Additionally, future research should consider the use of DHA rich species of PS. The elucidation of appropriate dosing and duration is also warranted, particularly in young, healthy subjects where positive effects may take substantially longer to manifest. The possibility of a supplement loading period should also be explored. Since exercise resulting in dehydration has been shown to result in a deficit in cognitive function, it is important for future studies to control workout

hydration. Additionally, it is recommended that sleeping patterns be recorded, with regards to the effect upon training volumes, mood and fatigue.

LIST OF REFERENCES

Alternative Medicine Review (2008). Phosphatidylserine. *Alternative Medicine Review, 13*(3), 245-247.

Aoki, J., Nagai, Y., Hosono, H., Inoue, K., & Arai, H. (2002). Structure and function of phosphatidylserine-specific phospholipase A1. *Biochimica et Biophysica Acta, 1582*, 26-32.

Barker, P. A., & Shooter, E. M. (1994). Disruption of NGF binding to low affinity neurotrophin receptor p75LNTR reduces NGF binding to trkA on PC12 cells. *Neuron, 13*, 203-215.

Baumeister, J., Barthel, T., Geiss, K. R., & Weiss, M. (2008). Influence of phosphatidylserine on cognitive performance and cortical activity after induced stress. *Nutritional Neuroscience, 11*(3), 103-110.

Bazzi, M. D., & Nelsestuen, G. L. (1990). Protein kinase C interaction with calcium: A phospholipid-dependent process. *Biochemistry, 29*(33), 7624-7630.

Benton, D., Donohoe, RT., Sillance, B., & Nabb, S. (2001). The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. *Nutritional Neuroscience, 4*(3), 169-178.

Bezprozvanny, I., Bezprovvannaya, S., & Ehrlich, B. E. (1994). Caffeine-induced inhibition of inositol(1,4,5)-triphosphate-gated calcium channels from cerebellum. *Molecular Biology of the Cell, 5*(1), 97-103.

Bian, J., Ghosh, T. K., Wang, J., & Gill, D., L. (1991). Identification of intracellular calcium pools: Selective modification of thapsigargin. *Journal of Biological Chemistry 266*(14),

8801-8806.

Blokland, A., Honig, W., Brouns, F., & Jolles, J. (1999). Cognition-enhancing properties of subchronic phosphatidylserine (PS) treatment in middle-aged rats: Comparison of bovine cortex PS with egg PS and Soybean PS. *Nutrition, 15*(10), 778-783.

Brisswalter, J., Collardeau, M., & Rene, A. (2002). Effects of acute physical exercise characteristics on cognitive performance. *Sports Medicine, 32*(9), 555-566.

Cenacchi, T., Bertoldin, T., Farina, C., Fiori, M. G., & Crepaldi, G. (1993). Cognitive decline in the elderly: A double-blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. *Aging (Milano), 5*, 123-133.

Chao, M. V. (1992). Neurotrophin receptors: a window into neuronal differentiation. *Neuron, 9*, 583-593.

Chen, S., Li, K. W. (2008). Comparison of molecular species of various transphosphatidylated phosphatidylserine (PS) with bovine cortex PS by mass spectrometry. *Chemistry and Physics of Lipids, 152*, 46-56.

Choe, Y., Lee, B. J., & Kim, K. (2002). Participation of protein kinase C alpha isoform and extracellular signal-regulated kinase in neurite outgrowth of GT1 hypothalamic neurons. *Journal of Neurochemistry, 83*(6), 1412-1422.

Crook, T. H., Tinklenberg, J., Yesavage, J., Petrie, W., Nunzi, M. G., & Massari, D. C. (1991). Effects of phosphatidylserine in age-associated memory impairment. *Neurology, 41*(5), 644-649.

Crook, T. H. (1998). Treatment of age-related cognitive decline: Effects of phosphatidylserine. In: R. M. Klatz (Ed.), *Anti-aging Medical Therapeutics* (pp. 20-29). Chicago, IL: Health

Quest Publications.

Dolor, R. J., Hurwitz, L. M., Mirza, Z., Strauss, H. C., & Whorton, A. R. (1992). Regulation of extracellular calcium entry in endothelial cells: Role of intracellular calcium pool. *American Journal of Cell Physiology, 262*(1), C171-C181.

Drago, F., Canonico, P. L., & Scapagnini, U. (1981). Behavioral effects of phosphatidylserine in aged rats. *Neurobiology of Aging, 2,* 209-213.

Evans, S. M., & Griffiths, R. R. (1999). Caffeine withdrawal: A parametric analysis of caffeine dosing conditions. *Journal of Pharmacology & Experimental Therapeutics. 289,* 285-294.

Gordon, N. (2007). The cerebellum and cognition. *European Journal of Paediatric Neurology, 11*(4), 232-234.

Greene, L. A., Aletta, J. M., Rukenstein, A., & Green, S. H. (1986). PC12 pheochromocytoma cells: Culture, nerve growth factor treatment and experimental exploitation. *Methods in Enzymology, 147,* 207-216.

Greene, L. A., & Tischler, A. S. (1976). Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *PNAS, 73*(7), 2424-2428.

Griffiths, R. R., Evans, S. M., Heishman, S. J., Preston, K. L., Sannerud, C. A., Wolf, B., & Woodson, P. P. (1990). Low-dose caffeine physical dependence in humans. *Journal of Pharmacology & Experimental Therapeutics, 255*(3), 1123-1132.

Hellhammer, J., Fries, E., Buss, C., Engert, V., Tuch, A., Rutenberg, D., Hellhammer, D. (2004). Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the

endocrine and psychological responses to mental stress. *Stress, 7*(2), 119-126.

Henderson, C. E., Camu, W., Mettling, C., Gouin, A., Poulsen, K., Karihaloo, M.,...Rosenthal, A. (1993). Neurotrophins promote motor neuron survival and are present in embryonic limb bud. *Nature, 363*, 226-270.

Hoffman, J. R., Ratamess, N. A., Gonzalez, A., Beller, N. A., Hoffman, M., Olsen, M.,...Jager, R. (2010). The effects of acute and prolonged CRAM supplementation on reaction time and subjective measures of focus and alertness in healthy college students. *Journal of the International Society of Sports Nutrition, 15*(7), 39-47.

Hogervorst, E., Riedel, W., Jeukendrup, A., & Jolles, J. (1996). Cognitive performance after strenuous physical exercise. *Perceptual and Motor Skills, 83*, 479-488.

Hossono, H., Akoi, J., Nagai, Y., Bandoh, K., Ishida, M., Taguchi, R.,...Inoue, K. (2001). Phosphatidylserine-specific phospholipase A1 stimulates histamine release from rat peritoneal mast cells through production of 2-acyl-1-lysophosphatidylserine. *Journal of Biological Chemistry, 276*(32), 29664-29670.

Hoth, M., & Penner, R. (1992). Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature 355*, 353-356.

Jager, R., Purpura, M., Geiss, K. R., Weib, M., Baumeister, J., Amatulli, F., & Herwegen, H. (2007). The effect of phosphatidylserine on golf performance. *Journal of the International Society of Sports Nutrition, 4*(1), 23-28.

Jager, R., Purpura, M., & Kingsley, M. (2007). Phospholipids and sports performance. *Journal of the International Society of Sports Nutrition, 4*, 5, 1-8.

Jorissen, B. L., Brouns, F., Van Boxtel, M. J., Ponds, R. M., Verhey, F. J., Jolles, J., & Riedel,

W. J. (2001). The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. *Nutritional Neuroscience, 4*, 121-134.

Jorissen, B. L., Brouns, F., Van Boxtel, M. J., & Riedel, W. J. (2002). Safety of soy-derived phosphatidylserine in elderly people. *Nutritional Neuroscience, 5*(5), 337-343.

Juliano, L. M., & Griffiths, R. R. (2004). A critical review of caffeine withdrawal: Empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology, 176*, 1-29

Kainu, V., Hermansson, M., & Somerharju, P. (2008). Electrospray ionization mass spectrometry and exogenous heavy isotope-labeled lipid species provide detailed information on aminophospholipid acyl chain remodeling. *Journal of Biological Chemistry, 283*(6), 3676-3687.

Kang, S. S., Han, K. K., Ku, B. M., Lee, Y. K., Hong, J., Shin, H. Y.,...Lee, C. J. (2010). Caffeine-mediated inhibition of calcium release channel inositol 1,4,5-triphosphate receptor subtype 3 blocks glioblastoma invasion and extends survival. *Cancer Research, 70*(3), 1173-1183.

Kaplan, D. R., & Miller, F. D. (1997). Signal transduction by the neutrophin receptors. *Current Opinion in Cell Biology, 9*, 213-221.

Kauranen, K., Siira, P., & Vanharanta, H. (1999). Strength training for 1h in humans: Effect on the motor performance of normal upper extremities. *European Journal of Applied Physiology, 79*, 383-390.

Kawakami, T., Kawakami, Y., & Kitaura, J. (2002). Protein kinase C beta (PKC beta): Normal functions and diseases. *Journal of Biochemistry, 132*, 677-682.

Kidd, P. M. (2009). *Phosphatidylserine: The nutrient building block that accelerates all brain functions and counters Alzheimer's disease.* New Canaan, CT: Keats Publishing, Inc.

Kingsley, M. (2006). Effects of Phosphatidylserine supplementation on exercising humans. *Sports Medicine, 36*(8), 657-669.

Kingsley, M. I., Miller, M., Kilduff, L. P., Mceneny, J., & Benton, D. (2006). Effects of phosphatidylserine on exercise capacity during cycling in active males. *Medicine & Science in Sports & Exercise, 38*(1), 64-71.

Kingsley, M. I., Wasdworth, D., Kilduff, L. P., Mceneny, J., & Benton, D. (2005). Effects of phosphatidylserine on oxidative stress following intermittent running. *Medicine & Science in Sports & Exercise, 37*(8), 1300-1306.

Kohler, G., Hering, U., Zschornig, O., & Arnold, K. (1997). Annexin V interaction with phosphatidylserine-containing vesicles at low and neutral pH. *Biochemistry, 36*, 8189-8194.

Kolch, W., Heidecker, G., Kochs, G., Hummel, R., Vahidi, H., Mischak, H.,...Rapp, U. R. (1993). Protein kinase C-alpha activates Raf-1 by direct phosphorylation. *Nature, 364*, 249-252.

Koliatsos, V. E., Clatterbuck, R. E., Winslow, J. W., Cayouette, M. H., & Price, D. L. (1993). Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. *Neuron, 10*(3), 359-367.

Kraemer, A. F., Erickson, K, I., & Colcombe, S. J. (2012). Exercise, cognition, and the aging brain. *Journal of Applied Physiology, 101*, 1237-1242.

Lephart, S. M., Riemann, B. L., & Fu, F. H. (2000). *Proprioception and neuromuscular control*

in joint stability. Champaign, IL: Human Kinetics.

Leventis, P. A., & Grinstein, S. (2010). The distribution and function of phosphatidylserine in cellular membranes. *Annual Review of Biophysics, 39,* 407-427.

Levi-Montalcini, R. (1987). The nerve growth factor: Thirty-five years later. *EMBO Journal, 6,* 1145-1154.

Lo Bue-Estes, C., Willer, B., Burton, H., Leddy, J. J., & Wilding, G. R. (2008). Short-term exercise to exhaustion and its effects on cognitive function in young women. *Perceptual and Motor Skills, 107,* 933-945.

Loeb, D. M., Maragos, J., Martin-Zanca, D., Chao, M. V., Parada, L. F., & Greene, L. A. (1991). The trk proto-oncogene rescues NGF responsiveness in mutant NGF non-responsive PC12 cell lines. *Cell, 66,* 961-966.

Lourenssen, S., & Blennerhassett, M. G. (1998). Lysophosphatidylserine potentiates nerve growth factor-induced differentiation of PC12 cells. *Neuroscience Letters, 248,* 77-80.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1971). *Profile of mood states manual.* San Diego, CA: Educational and Industrial Testing Service.

Meyer, M., Matsuoka, I., Wetmore, C., Olson, L., & Thoenen, H. (1992). Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *Journal of Cell Biology, 119*(1), 45-54.

Monteleone, P., Maj, M., Beinat, L., Natale, M., & Kemali, D. (1992). Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. *European Journal of Clinical Pharmacology,*

42(4), 385-388.

Nakashima, S. (2002). Protein kinase C alpha (PKC alpha): Regulation and biological function. *Journal of Biochemistry, 132,* 669-675.

Newton, A. C. (1995). Protein Kinase C: Structure, function and regulation. *Journal of Biological Chemistry, 270*(48), 28495-28498.

Newton, A. C. (1993). Interaction of proteins with lipid headgroups: Lessons from protein kinase C. *Annual Review of Biophysics and Biomolecular Structure, 22,* 1-25.

Newton, A. C. (1997a). Regulation of protein kinase C. *Current Opinion in Cell Biology, 9,* 161-167.

Newton, A. C. (1997b). Cofactor regulation of protein kinase C. In: L. V. Dekker & P. J. Parker (Eds.), *Protein kinase C* (pp. 25-44). Austin, TX: RG Landes.

Nishijima, M., Kuge, O., & Akamatsu, Y. (1986). Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. I. Inhibition of de novo phosphatidylserine biosynthesis by exogenous phosphatidylserine and its efficient incorporation. *Journal of Biological Chemistry, 261,* 5784-5789.

Okajima, S., Mizoguchi, A., Tamai, K., Hirasawa, Y., & Ide, C. (1995). Distribution of protein kinase C (alpha, beta, gamma subtypes) in normal nerve fibers and in regenerating growth cones of the rat peripheral nervous system. *Neuroscience, 66*(3), 645-654.

Ortega, F., Perez-Sen, R., Morente, V., Delicado, E. G., & Miras-Portugal, M. T. (2010). P2X7, NMDA and BDNF receptors converge on GSK3 phosphorylation and cooperate to promote survival in cerebellar granule neurons. *Cellular and Molecular Life Sciences, 67*(10), 1723-1733.

Parker, A. G., Gordon, J., Thornton, A., Byars, A., Lubker, J., Bartlett, M.,...Kreider, R. (2011). The effects of IQPLUS focus on cognitive function, mood and endocrine response before and following acute exercise. *Journal of the International Society of Sports Nutrition, 21*(8), 16-22.

Pepeu, G., Pepeu, I. M., & Amaducci, L. (1996). A review of phospahtidylserine pharmacology and clinical effects. Is phosphatidylserine a drug for the ageing brain? *Pharmacology Research, 33*, 73-80.

Pontifex, M. B., Hillman, C. H., Fernhall, B., & Thompson, K. M. (2009). The effects of acute aerobic and resistance exercise on working memory. *Medicine & Science in Sports & Exercise, 41*(4), 927-934.

Portney, L. G., & Watkins, M. P. (2000). *Foundations of clinical research: Applications to practice.* Upper Saddle River, NJ: Prentice-Hall, Inc.

Richter, Y., Herzog, Y., Cohen, T., & Steinhart, Y. (2010). The effect of phosphatidylserine-containing omega-3 fatty acids on memory abilities in subjects with subjective memory complaints: A pilot study. *Journal of Clinical Interventions in Aging, 2*(5), 313-316.

Saito, N., & Shirai, Y. (2002). Protein kinase C gamma (PKC gamma): Function of neuron specific isotype. *Journal of Biochemistry, 132*, 683-687.

Sakai, M., Yamatoya, H., & Kudo, S. (1996). Pharmacological effects of phosphatidylserine enzymatically synthesized from soybean lecethin on brain functions in rodents. *Journal of Nutritional Science and Vitaminology, 42*, 47-54.

Sauseng, P., Klimesch, W., Schabus, M., Doppelmayr, M. (2005). Fronto, parietal EEG coherence in theta and upper alpha reflect central executive functions of working

memory. *International Journal of Psychophysiology, 52*(2), 97-103.

Schuh, K. J., & Griffiths, R. R. (1997). Caffeine reinforcement: The role of withdrawal. *Psychopharmacology, 130*, 320-326.

Sei, Y., Gallagher, K. L., & Daly, J. W. (2001). Multiple effects of caffeine on calcium release and influx in human B lymphocytes. *Cell Calcium, 29*(3), 149-160.

Serrano, P., Yao, Y., & Sacktor, C. (2005). Persistent phosphorylation by protein kinase M-Zeta maintains late-phase long-term potentiation. *The Journal of Neuroscience, 25*(8), 1979-1984.

Sofroniew, M. V., Howe, C. L., & Mobley, W. C. (2001). Nerve growth factor signaling, neuroprotection and neural repair. *Annual Review of Neuroscience, 24*, 1217-1281.

Suchy, J., Chan, A., & Shea, T. B. (2009). Dietary supplementation with a combination of Éø-lipoic acid, acetyl-l-carnatine, glycerophosphocholine, decosahexaenoic acid and phosphatidylserine reduces oxidative damage to murine brain and improves cognitive function. *Nutrition Research, 2*9, 70-74.

Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., & Nishizuka, Y. (1979). Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *Journal of Biological Chemistry, 25*4, 3692-3695.

Tanaka, C., & Saito, N. (1992). Localization of subspecies of protein kinase C in the mammalian central nervous system. *Neurochemistry International, 2*1, 499-512.

Tomimatsu, N., & Arakawa, Y. (2008). Protein kinase C-mediated protection of motoneurons from excitotoxicity. *Neuroscience Letters, 43*9(2), 143-146.

Tomporowski, P. D. (2003). Effects of acute bouts of exercise on cognition. *Acta Psychologica,*

*11*2, 297-324.

Tripp, B. L., Boswell, L., Gansneder, B. M., & Shultz, S. J. (2004). Functional fatigue decreases 3-dimensional multijoint position reproduction acuity in the overhead-throwing athlete. *Journal of Athletic Training, 3*9, 316-320.

Tripp, B. L., Yochem, E. M., & Uhl, T. L. (2007). Functional fatigue and upper extremity sensorimotor system acuity in baseball athletes. *Journal of Athletic Training, 4*2(1), 90-98.

Vance, J. E. (2008). Phosphatidylserine and phosphatidylethanolamine in mammalian cells: Two metabolically related aminophospholipids. *Journal of Lipid Research, 4*9, 1377-1387.

Vance, J. E., & Steenbergen, R. (2005). Metabolism and functions of phosphatidylserine. *Progress in Lipid Research, 4*4, 207-234.

Vetter, M. L., Martin-Zance, D., Parada, L. F., Bishop, J. M., & Kaplan, D. R. (1991). Nerve growth factor rapidly stimulates tyrosine phosphorylation of phospholipase C-gamma 1 by kinase activity associated with the product of the trk protooncogene. *Proceedings of the National Academy of Sciences,* 88, 5650-5654.

Weihrauch, J. L., & Son, Y. S. (1983). The phospholipids content of foods. *Journal of the American Oil Chemists Society, 6*0, 1971-1978.

Weinreb, O., Bar-Am, O., Amit, T., Chillag-Talmor, O., & Youdim, M. B. (2004). Neuroprotection via pro-survival protein kinase C isoforms associated with Bcl-2 family members. *FASEB Journal, 1*8, 1471-1473.

Weir, J. P. (2005). Quantifying test-retest reliability using the interclass correlation coefficient and the SEM. *Journal of Strength and Conditioning Research, 1*9(1), 231-240.

Wojtys, E. M., Wylie, B. B., & Huston, L. J. (1996). The effect of muscle fatigue on neuromuscular function and anterior tibial translation in healthy knees. *American Journal of Sports Medicine, 2*4(5), 615-621.

Wooten, M. W., Zhou, G., Seibenhener, M. L., & Coleman, E. S. (1994). A role for zeta-protein kinase C in nerve growth factor-induced differentiation of PC12 cells. *Cell Growth & Differentiation,* 5, 395-403.

Zanotti, A., Valzelli, L., & Toffano, G. (1989). Chronic phosphatidylserine treatment improves spatial memory and passive avoidance in aged rats. *Psychopharmacology, 9*9, 316-321.

Zhao, X. C., Wang, L. L., Wang, Y. Q., Song, F. H., Li, Y. Q., Fu, R.,...Zhou, L. H. (2012). Activation of phospholipase-C gamma and protein kinase C signal pathways helps the survival of spinal motoneurons injured by root avulsion. *Journal of Neurochemistry, 12*1, 362-372.

Zirrgiebel, U., Ohga, Y., Carter, B., Berninger, B., Inagaki, N., Thoenen, H., & Lindholm, D. (1995). Characterization of TrkB receptor-mediated signaling pathways in rat cerebellar granule neurons: Involvement of protein kinase C in neuronal survival. *Journal of Neurochemistry, 6*5(5), 2241-2250.

van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: where are they and how they behave? *Nature Reviews Molecular Cell Biology,* 9, 112-124.