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Effects of Pre-and Post-Exercise Intake of Performance Supplements on Body Composition, Muscle Isokinetic, Isometric, and Isotonic Strength and Power, and Mood in Trained Men Following 6 Weeks of Resistance Training

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EFFECTS OF PRE- AND POST-EXERCISE INTAKE OF PERFORMANCE SUPPLEMENTS
ON BODY COMPOSITION, MUSCLE ISOKINETIC, ISOMETRIC, AND ISOTONIC
STRENGTH AND POWER, AND MOOD IN TRAINED MEN FOLLOWING 6 WEEKS OF
RESISTANCE TRAINING.

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To my grandfather, William F. Mandler Sr.
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ABSTRACT

Performance supplements (PS) consumed in close proximity to resistance exercise (RE) are an increasingly common product, especially among young males\(^1\), \(^2\) and athletes\(^3\)\(^-\)\(^6\). The composition of PS vary widely, but the principle ingredients tend to include creatine monohydrate, caffeine, \(\beta\)-alanine (\(\beta\)A), the branched chain amino acids (BCAAs) leucine, isoleucine, and valine, as well as L-citrulline, and L-arginine. Manufacturer claims about effectiveness are often dubious and generally untested by peer-reviewed science. Of the small body of research specifically examining PS and RE training, none have used trained male subjects in conjunction with a pre- and post-exercise supplementation modality. This study sought to examine the effectiveness of two commercially available pre- and post-exercise supplements, No-Shotgun (SHOT) and NO-Synthesize (SYNTH) (Vital Pharmaceuticals, Davie, FL), respectively, at augmenting performance and muscle gains over the course of a 6-week training period. METHODS: Twenty-four, resistance trained men (age, 24.6 ± 4.9 years; height, 180.4 ± 5.5 cm; weight, 80.7 ± 8.8 kg) completed 6 weeks of periodized RE targeting muscles of the arms and shoulders, legs and core, and chest and back with three workouts per week. Resistance increased while repetitions decreased in two-week increments (week 1: 3x10, week 2: 3x6, and week 3: 3x4). Rest intervals of 60-90 seconds were constant between sets. Participants were assigned to one of two groups based upon maximal voluntary contraction of the quadriceps (Biodex) to lean mass ratio. Group 1 (n=6; Performance Supplement; PS) consumed one serving of NO-Shotgun\(^\text{®}\) before each workout and one serving of NO-Synthesize\(^\text{®}\) (Vital Pharmaceuticals, Inc., Davie, FL) immediately after each workout and on non-RT days. Group 2 (n= 3; Placebo; PLA) consumed a flavor-matched isocaloric maltodextrin placebo in the identical manner. Laboratory measurements included the following: body composition (dual-energy X-ray absorptiometry; DXA), circumferences of the shoulders, chest, waist, hip, and thigh, and maximal strength of the upper (chest press; CP) and lower body (leg press; LP) using one repetition maximum lifts (1RM), Anaerobic power using 30-second Wingate cycle ergometry tests, and isokinetic and isometric strength measurements. Statistical analysis was conducted using a 2x2 repeated measures analysis of variance. Significance was set at \(p<0.05\) and all values are reported as means ± standard deviation. RESULTS: Group x time interactions were observed for lean mass (LM, \(p = 0.017\)). The PS increased LM (+4.72%, \(p = 0.001\)), while the PLA group was
unchanged. Both groups demonstrated similar increases in leg press and bench press. Post-hoc analysis demonstrated that time effects were driven by the PS group in peak anaerobic power (+16.22%, p = 0.002), relative anaerobic power (+9.38%, p = 0.003), average anaerobic power (+9.94%, p = 0.015), and relative average anaerobic power (+8.21%, p = 0.028), while PLA remained unchanged. Neither group showed changes in fatigue index. The supplement had no effect on training volume for any week or exercise during the 6-week training period, but may have elevated feelings of vigor in PS (p = 0.019). **CONCLUSION:** Consumption of SHOT and SYNTH immediately before and after exercise during the course of a periodized exercise training program facilitated training-induced improvement in lean mass in trained males, whereas the consumption of isocaloric carbohydrate beverage did not. PS products most likely do not offer advantages in measures of muscle strength and power in this population. Sustained SHOT and SYNTH consumption has no negative effect on mood and may improve feelings of vigor, which has been shown to decrease with heavy training. Continued investigation of similar products is warranted in this and other populations.
CHAPTER ONE

INTRODUCTION

Resistance training is a widely popular pastime undertaken by many people worldwide in order to improve strength, power, athletic performance, body aesthetics, and health. Concomitantly, nutritional supplement manufacturers have enthusiastically developed and marketed products aimed at people engaged in resistance training. Performance supplements (PS) intended for consumption in close proximity to resistance exercise are extremely popular among young males\(^1,2\) and athletes\(^3\). This class of sports nutrition supplements accounted for approximately $2.5 billion dollars in sales in 2009, with projected 7.5% growth in sales for 2011\(^7\). The PS industry is lucrative and growing rapidly, with $2.8 billion in sales in 2008\(^8\). Manufacturers frequently claim that their PS increase energy, focus, and drive during fatiguing exercise. Supplement manufacturers also frequently tout the ability of their products to improve strength and muscle mass while decreasing fat mass with sustained use. The composition of PS vary widely, but the principle ingredients consistently include creatine monohydrate, caffeine, β-alanine (βA), the branched chain amino acids (BCAAs) leucine, isoleucine, and valine, as well as L-citrulline, and L-arginine. Most of these ingredients have been shown singularly\(^9-15\) and in combination\(^16-19\) to exert ergogenic effects during aerobic and anaerobic exercise or facilitate muscle hypertrophy over the course of a resistance training period.

Caffeine, the active ingredient in coffee and tea\(^20\), acts as an antagonist to adenosine receptors, possibly improving motor function\(^21,22\) and decreasing the pain and fatigue\(^23-26\) associated with heavy resistance exercise (RE). One putative means by which PS are believed to improve the results of resistance training is delaying fatigue\(^27\) and reducing perceived exertion during exercise\(^28\), allowing the consumer to lift more weight for more repetitions\(^24,26\), resulting in an increased total training volume. Creatine may aid in improving substrate availability for muscle contractile activity\(^29,30\), as well as enhancing some muscle hypertrophic pathways\(^19,31-33\). BCAAs have also been shown to augment hypertrophy\(^34-37\) and decrease perceived exertion during exercise\(^38\). βA supplementation increases muscle carnosine concentration\(^39\), which in turn acts to dampen the decrease in pH associated with intense exercise\(^31,40\), potentially improving performance in activities limited by muscle acidosis\(^40-44\).
Claims about effectiveness and ergogenic enhancements provided by PS are often not supported by empirical data and worse, frequently reflect poor understanding or even a misappropriation of the underlying science. Accordingly, it is of importance to consumers and researchers that PS be evaluated in blinded, placebo-controlled trials. While there is a considerable body of research on the individual effects of creatine, caffeine, β-alanine and protein/AA consumption in proximity to exercise, there has been very little investigation of the combined effect of these ingredients on exercise performance, and even less with RE training. Thus far, the limited evidence available suggests that PS products of this general composition may offer an advantage for those wishing to increase muscle mass and strength.

Recently, researchers at the University of Oklahoma supplemented twenty-four moderately-trained recreational athletes with a pre-workout supplement (GT; Game Time®, Corr-Jensen Laboratories Inc., Aurora, CO), containing 18 g of a proprietary blend including whey protein, cordyceps sinensis, creatine monohydrate, citrulline, ginseng, and caffeine. Subjects in this study performed nine high intensity interval running training (HIIT) sessions over 3 weeks. Participants consumed GT or placebo 30 minutes prior to each training session. In contrast to the placebo group, the supplemented participants demonstrated a significantly higher training volume (distance to exhaustion per session) and tended to perform better in measures of anaerobic running capacity (p = 0.053). Training volume was 11.6% higher for the GT versus PL group (p = 0.041). Additionally, lean body mass increased from 54.2kg to 55.4kg (p = 0.0035) in the GT group and remained unchanged in the PL group (p = 0.69), while there were no significant changes in percent body fat.

Of the small body of work investigating workout supplement products and RE, a study by Shelmadine et al. (19) is the most relevant. This group examined the effects of 28 days of supplementation and concurrent resistance training. They used the commercially available pre-workout supplement NO-Shotgun® (SHOT), containing whey protein, caffeine, creatine, β-alanine, BCAAs, and L-arginine with 18 untrained males. One 27g serving of SHOT or maltodextrin placebo (PL) of identical taste and caloric content was consumed 30 minutes prior to each exercise session, and upon waking on non-exercising days. Subjects performed two upper body and two lower body routines each week, for a total of 16 sessions. Measurements of body composition, maximal strength in bench and leg press, serum hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), overall myofibrillar protein content, and myogenic
regulatory factors (MRFs) were measured pre and post training. One repetition maximum (1-RM) bench press strength improved significantly for both groups (p = 0.005), while there were no significant differences in improvements in 1-RM leg press strength. The SHOT group exhibited a significantly greater increase in bench press 1-RM than PL (p = 0.003). In terms of body composition, SHOT increased lean mass more than PL (p = 0.001), as well as myofibrillar protein (p = 0.014), and myof(p = 0.041). Increases in muscle mass were mirrored by augmented signs of satellite cell activation. Serum IGF-1 was not significantly greater for the SHOT group (p = 0.061), while serum HGF decreased for the PL and increased for the SHOT group (p = 0.02). Muscle c-met (CMET) was not different between groups (p = 0.067), while myofibrillar protein was greater for the SHOT group (p = 0.014).

While the findings of Shelmadine et al.\textsuperscript{19} are promising, especially in regards to significant increases in muscle mass and markers of satellite cell activation with supplementation, there are important limitations to be noted when drawing conclusions about populations other than untrained males. Performance supplements, particularly those of similar composition to SHOT and SYNTH are not intended for or marketed towards novice weightlifters. Rather, SHOT and similar PS are marketed towards athletes and those experienced with resistance training who are seeking to overcome training plateaus and gain a competitive edge. To date, there have been no investigations of the effectiveness of these products with trained male subjects over the course of an extended (6-week) RE program in a pre- post-exercise supplementation modality.

The present study sought to target a population more likely to consume products like SHOT—regular weightlifters—by selecting only subjects who have been resistance training regularly (two or more sessions per week) for at least one year, with no significant breaks. With this specific population, any gains in strength should be almost entirely due to physiological and hypertrophic changes to the trained muscles, rather than improvements in neurological coordination. Furthermore, in order to provide a time course that would facilitate greater hypertrophy; the training took place over 6 weeks, three times a week, for a total of 18 sessions. Shelmadine et al. noted large increases in markers of satellite cell activation and hypertrophy, and modest increases in lean body mass (4.75%) for the supplement group after only 4 weeks. By increasing the time course and total volume of training, we aimed to augment the opportunity for muscle growth. In addition to ingesting SHOT before exercise, our subjects also consumed
one serving of NO-Synthesize® (SYNTH, Vital Pharmaceuticals, Davie FL) immediately post-exercise and on every non-training day. SYNTH is of similar composition and caloric value to SHOT, but does not contain the stimulants caffeine or phenylethylamine. This pre-post supplementation model provided a better environment for muscle hypertrophy and recovery than with only pre- or only post-supplementation. (For a detailed discussion of supplement timing and muscle hypertrophy, see literature review). In order to ensure compliance, pre- and post-exercise supplement consumption was monitored by our investigators. For the non-training day supplementation, subjects were given single serving baggies and asked to return them to the research team to confirm consumption. Whereas Shelmadine et al. allowed subjects to train independently, in this study all training sessions were monitored by experienced research staff who provided form corrections and spots for free-weight lifts.

To date, no other attempts have been made to investigate the effectiveness of PS products similar to SYNTH and SHOT over the course of an extended (greater than 4 weeks) RE training protocol with trained subjects using a PRE-POST and off-day supplementation model. Additionally, the emphasis placed on consistent lifting form in this study, coupled with researcher supervision, helped ensure full participant compliance with training, as well as reduce variability due to inter-subject differences or deficiencies in form.

The principal aim of this study was to determine the efficacy of supplementation with the SHOT and NO-Synthesize products on RE trained males over the course of a 6-week RE program. The variables examined were isokinetic strength, isometric strength, isotonic (1-RM) strength, and anaerobic power. Additionally, changes in mood pre- and post-training was evaluated using a Profile of Mood State (POMS) questionnaire.\textsuperscript{45}
Specific aims

1. To evaluate performance enhancements associated with pre- and post-exercise consumption of PS during the course of a 6-week resistance training program in trained males.
   a. Measures of isotonic, isokinetic, isometric strength, and power were be obtained using a Biodex™ dynamometer in the laboratory and 1-repetition maximum testing in the bench press and 45° leg press exercises.
   b. Anaerobic power was evaluated using a Wingate 30-sec cycle ergometry test.
2. To determine if PS consumption would increase training volume for each session. The caffeine contained in SHOT has been demonstrated to decrease feelings of fatigue and increase resistance training volume.
   a. Training volume (weight x repetitions x sets) were recorded for each exercise for each subject every week for 6 weeks.
   b. Mood state was evaluated pre- and post-training using a Profile of Mood State questionnaires.
3. To determine if the consumption of PS enhances body composition changes during the 6-week resistance training program in trained males. Increases in muscle diameter are associated with increases in strength and power.
   a. Body composition (body mass, lean mass, fat mass) were measured using dual x-ray absorptiometry (DXA).
   b. Circumference measurements were taken of the upper arm, chest, thigh, and gluteal regions.
Research hypotheses

1. Measures of 1-RM, isokinetic, and isometric strength would significantly increase for both groups following 6 weeks of resistance training, while the performance supplement group would experience gains greater than those of the placebo group.
2. The performance supplement group would perform a higher volume (weight x repetitions) than the placebo group over the course of the training period.
3. The performance supplement group would experience larger gains in muscle mass than the placebo despite higher level of training status exhibited by participants in this study.
4. Neither performance supplement or the placebo would significantly affect mood state.

Assumptions

The following assumptions were made in this study:

1. All laboratory equipment provided valid/reliable results.
2. Subjects followed instructions provided by investigators.
3. Subjects answered truthfully in the provided physical activity/health history questionnaires.
4. Subjects put forth a maximal effort during all testing and training sessions.
5. Each participant consumed his given dose of post-supplement on days when not directly supervised by research staff (i.e. non-training days).
Delimitations

Delimitations of the current study were as follows:

1. Individuals with uncontrolled hypertension (blood pressure $>140/90$), uncontrolled cholesterol/blood lipid levels or those that take cholesterol medication, diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, and any musculoskeletal complications that would impede one from exercising with weights were excluded.

2. Individuals who are currently regular smokers, take cholesterol medication, take nutritional supplements within the last 4 weeks (except for a multivitamin without known ergogenic enhancers), or have any allergies to milk products were excluded.

3. Participants must have been resistance training at least two times per week for the last year.

4. This study included only males aged 18-40 years.

Limitations

The major limitations of the study included:

1. The inability to control subject diet and daily activity level.

2. Subjects may not have honestly reported level of training status prior to the study.

3. Subjects may have performed additional exercise without the knowledge or consent of the researchers.

4. Some subjects might have initially exhibited a higher training status than others.

5. Training was conducted in a commercial gym, rather than in a distraction-free laboratory environment.

6. Subjects with prior experience with workout supplements may have been able to perceive the changes in performance brought about by stimulant consumption and determine their grouping, despite identical flavoring.

7. Subjects with cycling experience or training may have had an advantage over inexperienced subjects in the Wingate test.
CHAPTER TWO

LITERATURE REVIEW

The concept of purposefully undertaking resistance exercise (RE) to improve athletic performance has been extant since at least the times of the ancient Greek Olympians, who exercised with types of stone or metal dumbbells known as *haltares*, which were also used as an ergogenic aid in the long jump\(^46\). Despite the resurgence in popularity in urban Europe of gymnasiums during the 19\(^{th}\) century, the modern scientific study of resistance training began only following the Second World War with the work of Delorme and Watkins\(^{47-49}\). Research volume has increased in parallel with the growing popular interest in weightlifting and bodybuilding, with a surge in the last twenty years.

An individual may undertake a RE program in order to fulfill any number of goals; prevention of age-related sarcopenia\(^{50,51}\), enhancement of body image and self esteem\(^52\), improvement in body composition and health\(^53\), or improvement in athletic performance\(^54\). The prerequisite factor underlying all of these larger goals is the capacity of resistance training to increase skeletal muscle mass, strength, and power.

The present review of literature will discuss the ergogenic effects of caffeine, creatine, β-alanine, and BCAAs supplementation alone and with training on anaerobic performance measures and mood. The performance measures of particular emphasis will be; 1 repetition maximum (1RM) weightlifting, isokinetic peak force, isometric maximal voluntary capacity, and anaerobic power as measured by Wingate sprint cycling tests. Furthermore, this review examines putative mechanisms through which the supplement ingredients may exert their effects.
Supplementation can enhance performance

Anaerobic capacity

The Wingate cycle ergometry test is a commonly used measure of anaerobic capacity, peak power output, and anaerobic fatigue resistance\(^{55}\). Briefly, the test consists of a 30-second cycling sprint against a resistance equal to 7-8% of bodyweight. American football players yielded no significant improvement in peak power during a 60-second Wingate test following 30 days of 4.5g/day β-alanine (βA) supplementation with off-season training. However, the rate of fatigue was reduced for the supplement group\(^{10}\). Ten-weeks of resistance training and supplementation with a creatine, amino acid, and protein containing drink yielded improvements in peak power and mean power during 3 30-sec Wingate anaerobic power tests, but no significant improvement in body composition, muscular strength or endurance\(^{18}\).

Fukuda et al. found that pre-workout ingestion of and caffeine, creatine, and amino acids induced a 10.8% increase in anaerobic running capacity, as well as significantly improving time to exhaustion at 110, 105, and 100% of anaerobic threshold\(^{16}\). The same group of researchers was unable to find a training effect. Three weeks of high intensity interval training did not significantly improve anaerobic running capacity vs. placebo, although VO\(_2\)max and total training volume were higher for the supplement group\(^{17}\).

Another common test of anaerobic performance is repetitions of a resistance exercise (commonly bench press) at a percentage of 1RM to failure. Recently, Duncan and Oxford demonstrated an increase in repetitions to failure and total weight lifted in trained males in the bench press exercise following ingestion of 200mg caffeine\(^{26}\). Their findings support those of similar caffeine studies using bench press\(^{56,57}\) and leg press\(^{28}\). Pre exercise ingestion of 250mL of Red Bull® energy drink, containing 80mg of caffeine, slightly improved total number of bench press repetitions (3 sets to failure at 70% 1-RM) but had no effect on Wingate peak or average power\(^{58}\). There is some evidence that βA supplementation may improve repetitions to failure and decrease feelings of fatigue\(^{10,32}\), although the data are equivocal\(^{59,60}\).
**Strength (1-RM)**

Shelmadine et al. reported that both bench press ($p = 0.005$) and leg press ($p = 0.001$) 1-RM significantly for both groups after 28 days of resistance training. The group supplemented with SHOT exhibited a significantly greater increase in bench press 1-RM than the placebo group. (18.40% vs. 8.82%, $p = 0.003$). In terms of body composition, NO increased lean mass more than the placebo (PL) group (4.75% vs. 1.69%, $p = 0.001$), as well as myofibrillar protein (70.39% vs. 26.34, $p = 0.014$), and total DNA (88.75% vs. 4.67%, $p = 0.041$).

Ten weeks of resistance training and supplementation with a post-workout beverage containing whey protein, amino acids, creatine, and carbohydrates resulted in no difference between the supplement and placebo group in 1-RM bench press or 45° leg press, despite larger gains in fat free mass for the supplement group. Furthermore, twenty-eight days of polyethylene glycosylated (PEG) creatine supplementation without resistance training increased muscle strength, anaerobic power, and work performed during exercise in trained14 and untrained61 men, while only seven days of 25g/day creatine monohydrate did not significantly improve upper body 1-RM strength in resistance trained females62.

To date, there has been relatively less research examining the effects of acute caffeine consumption and 1-RM strength, compared to aerobic performance measures. Acute caffeine ingestion yielded a small improvement in bench press 1-RM, but no improvement in leg extension in resistance trained males9. In a 10-week whole body resistance training study, Lockwood and colleagues found no significant improvement with a caffeine-containing low-calorie energy drink in 1-RM bench or leg press vs. placebo63.

BA supplementation appears to have no acute impact on 1RM strength10,32,60, although it may influence 1-RM indirectly by increase training volume to a small degree32 over the course of a RE program.

Timing of supplement dosing may also be crucial for maximizing gains. Ingestion of creatine/protein/CHO immediately pre and post RE sessions improved 1RM strength and type II muscle fiber cross-sectional area more than when the supplement was consumed upon waking and in the evening64.
Isokinetic peak force

Bond and colleagues investigated the role of caffeine and isokinetic force. Peak force of knee extension and flexion at 30, 150, and 300 degrees per second was examined using an electronic dynamometer. Twenty male athletes ingested a 7mg/kg dose of caffeine. The researchers found significantly greater peak torque for knee extensors at 30 and 300°sec⁻¹ for flexors at all velocities. Jacobson et al. conducted a series of studies with acute caffeine supplementation using trained⁶⁵ and untrained⁶⁶ subjects. They found no significant difference in performance with the untrained subjects, however the trained subjects significantly increased peak torque during unilateral knee extension at 30° and 300°sec⁻¹, and at all velocities (30°, 150°, and 300°sec⁻¹) for knee flexion. Acute ingestion of caffeine significantly (p = 0.05) enhanced peak knee flexion torque, knee extension/flexion total work, and knee extension/flexion power in the first of two bouts of 40 repetitions of maximal knee extension/flexion²⁴.

Seven days of 25g/day creatine monohydrate supplementation did not significantly improve peak concentric or eccentric isokinetic peak torque in resistance trained females⁶², it did however improve total work performed. Five days of 25g/day creatine supplementation yielded similar results with untrained males⁶⁷. Six days of 9g/day creatine supplementation with trained competitive power lifters yielded a significant increase in knee extension isokinetic peak torque as well as average power and total work during bouts of maximal deadlift⁶⁸.

Kendrick et al. found no improvement in isokinetic leg extension/flexion following 10 weeks of 6.4g/day βA supplementation⁵⁹. Four weeks of 4.8g/day βA supplementation did not improve peak isokinetic strength, although it did slightly attenuate fatigue repeated bouts of exhaustive dynamic contractions in sprint trained athletes, despite muscle carnosine increases (47-37%).

Endocrine response

Growth hormone (GH) increases 15-30 minutes following RE⁶⁹. Some ingredients in workout supplements are included with the purpose of augmenting this increase to take advantage of the anabolic properties of GH and IGF-1. Wu et al. found that ingestion of 6mg/kg of caffeine attenuates GH response following exercise⁷⁰, in contrast with early in vitro findings⁷¹. They also reported significantly higher plasma FFA following RE in the caffeine group, possibly due to
caffeine-induced elevation of epinephrine levels. No significant difference was evident in insulin, testosterone, or cortisol.

SHOT (27g/day) supplementation during 28 days of resistance training resulted in a significant (p<0.05) increase in HGF (+47.42%), while the placebo group decreased 8.71%. The authors postulated that the presence of arginine in the supplement also increased endogenous NO production, a modulator of HGF release. There was no significant difference between groups in changes in serum IGF-1 (p = 0.061). Muscle c-met, the gene that encodes HGF-receptor, was not different between groups (p = 0.067), while myofibrillar protein was greater for the NO group (p = 0.014).

Using a pre-workout supplement sold as “Amino Shooters” (Champion Nutrition Inc., Concord, C) consisting of 19 g of a powder containing essential BCAAs (3000 mg L-leucine, 1100 mg L-isoleucine, and 1100 mg valine), essential amino acids (1100 mg L-lysine, 300 mg L-methionine, 1100 mg/mL phenylalanine, 700 mg histidine, and 1100 mg L-threonine), 5000 mg creatine monohydrate, 1500 mg L-taurine, 350 mg glucuronolactone, and 110 mg of caffeine Hoffman et al. demonstrated increases in GH, insulin, and cortisol immediately following a bout of 6 sets of no more than 10 reps of back squat at 75% of 1RM. The supplement group was able to perform slightly more reps during the 5th set, resulting in a higher work volume, which may account for the elevated cortisol. The higher insulin concentration vs. isocaloric maltodextrin placebo was possibly due to the glycogen-sparing effect of BCAA consumption. The higher GH levels were most likely due to increased training volume of the supplement group. Willoughby et al. found that supplementing 20g of protein (14 g whey and casein protein, 6 g free amino acids), 1hr before and after training sessions (40g/session) during 10 weeks of resistance training augmented serum IGF-1, as well as myosin heavy chain (MHC) I and IIa and total myofibrillar protein content in untrained males.

In contrast, Hoffman et al. reported that 30 days of 4.8g/day of βA supplementation did not significantly alter the endocrine response to an acute bout of exercise consisting of 6 sets of 12 repetitions of the squat exercise at 70% of 1-RM with 1.5 minutes of rest between sets. These despite increased mean power and total work performed by the supplement group.
Protein synthesis and supplement timing

With regard to RE, protein balance can be described as the net ratio of muscle protein synthesis over degradation. The balance of protein synthesis/degradation is also referred to as the fractional synthesis rate (FSR). In a fasted state, a bout of RE enhances muscle protein synthesis immediately following exercise, but also stimulates protein breakdown, leading to a diminished or even net negative FSR\textsuperscript{74-76}. Furthermore, the post-exercise increase in protein synthesis appears to decline as training status increases\textsuperscript{77}. With this in mind, for those interested in increasing muscle FSR and hypertrophy, information pertaining to optimal nutrition before and after exercise is of elevated consequence. The most investigated strategies have been feedings protein/amino acids and CHO a short time before or immediately after a bout of exercise, or some combination of the two.

Pre-exercise supplementation

Before and during exercise, elevated plasma amino acid (AA) and glucose may help enhance muscle FSR and hypertrophy. Tipton et al. have conducted a substantial amount of research investigating nutrient timing in relation to RE. In a study published in 2001, this group set out to determine if ingestion of an essential amino acid-carbohydrate (EAA-CHO, 6g EAA + 36g CHO) supplement resulted in greater muscle phenylalanine uptake if consumed immediately before or immediately following a bout of heavy RE. The authors regarded phenylalanine uptake as a marker of muscle protein synthesis. Six healthy males participated in two randomly ordered trials, PRE and POST with the supplement consumed before or after exercise, respectively. In both conditions blood and muscle phenylalanine concentrations were increased by \(~130\%\) and amino acid delivery to the leg was increased during exercise and remained elevated for the 2 h after exercise in both trials. Total net phenylalanine uptake across the leg was greater ($P = 0.0002$) during PRE (209 ± 42 mg) than during POST (81 ± 19), as measured by arteriovenous difference. Phenylalanine uptake rate increased after EAA-CHO consumption in both trials. The authors concluded that net muscle protein synthesis is greater in response to pre-exercise supplementation, possibly due to increased muscle delivery of amino acids ([amino acid] * blood flow), which was significantly greater in PRE than in POST during the exercise bout and in the 1st h after exercise ($P = 0.05$).
Post-exercise supplementation

Following exercise, proper nutrition is essential for adequate muscle recovery. Tipton et al. randomly assigned participants to one of three groups\(^78\). Each group consumed either placebo (PL; \(N = 7\)), 20 g of casein (CS; \(N = 7\)), or whey protein (WH; \(N = 9\)). Supplements were consumed 1 hour after the conclusion of a leg extension exercise bout. Leucine and phenylalanine concentrations were measured in femoral arteriovenous samples to determine balance across the leg. The researchers found that arterial amino acid concentrations were elevated by protein ingestion. Net amino acid balance switched from negative to positive after ingestion of both proteins. Peak leucine net balance over time was greater for WH (347 ± 50 nmol·min\(^{-1}\)·100 mL\(^{-1}\) leg) than CS (133 ± 45 nmol·min\(^{-1}\)·100 mL\(^{-1}\) leg), but peak phenylalanine balance was not different for CS and WH. Ingestion of both CS and WH stimulated a significantly larger net phenylalanine uptake after RE, compared with the PL (PL -5 ± 15 mg, CS 84 ± 10 mg, WH 62 ± 18 mg). The authors conclude that WH or CS supplementation post-exercise result in different patterns of blood amino acid responses, however, net muscle protein FSR was similar. Borsheim\(^79\) et al set out to examine the importance of essential amino acid (EAA) consumption following RE. Subjects consumed 3g EAA 1 and 2 h after RE. Subjects received a primed constant infusion of radiolabeled phenylalanine and leucine. Samples from femoral artery and vein and biopsies from vastus lateralis were obtained. Net muscle protein balance (NB) increased proportionally more than arterial AA concentrations in response to supplementation. Area under the curve for net phenylalanine uptake above basal value was similar for the first hour after each drink (67 ± 17 vs. 77 ± 20 mg/leg, respectively). Because the increase in NB was roughly double in this study than in a previous investigation of 3g of EAA and 3g of NEAA consumption following a similar bout of exercise, the authors conclude that NEAA are not required for post-exercise promotion of muscle protein synthesis.

In addition to protein and AA, other nutrients may have a pro-anabolic effect when consumed post exercise. Cribb et al.\(^80\) placed resistance-trained males into one of three strength-matched supplementation groups: protein (PRO), PRO-CHO, or the same PRO-CHO supplement (1.5 g·kg\(^{-1}\) body weight·d\(^{-1}\)) with added creatine monohydrate (CrM) (Cr-PRO-CHO) (0.1 g·kg\(^{-1}\) body weight·d\(^{-1}\)). Subjects completed 10 weeks of a supervised RE program, with post-exercise supplementation. At the conclusion of the training period, Cr-PRO-CHO provided greater improvements in 1RM strength in barbell bench press, squat, and cable pull-down. Cr-PRO-
CHO also resulted in greater increases in LBM, fiber CSA, and contractile protein compared with PRO and PRO-CHO

**Pre- and Post-exercise supplementation**

Pre and post-exercise consumption of 42g of protein significantly reduced creatine kinase (CK) levels and increased the number of repetitions performed in follow-up sessions following a bout of 10 repetitions of 80% 1-RM of the squat, dead-lift, and barbell lunge exercises in 15 male strength/power athletes. Consumption of protein, creatine, and glucose immediately before and after RE (PRE-POST) resulted in greater (P < 0.05) increase in lean body mass and 1RM strength after 10 weeks of structured, supervised training than consumption of the same nutrients several hours before and after exercise. The changes in body composition were supported by a greater (P < 0.05) increase in CSA of the type II fibers and contractile protein content. PRE-POST supplementation also resulted in higher muscle Cr and glycogen values after the training program (P < 0.05).

**Profile of mood state**

Mood can be defined as shifting affective states that reflect how an individual feels in a specific instance or time period. The Profile of Mood State (POMS) is a common measure of changes in mood associated with exercise. The present study uses the classic POMS developed by McNair in 1974, consisting of 65 feelings or adjectives that can describe mood. Examples include; “happy,” “confused,” “energetic,” and “gloomy.” Subjects are asked to rank on a scale of 0-4 how much each word describes how they have felt during a recent period of time, with zero representing “Not at all,” and 4 “extremely.” Items can be categorized into six separate subscales: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. Subscale scores are summated and form a general measure titled total mood disturbance (TMD). TMD can be a useful tool in examining changes brought about by exercise or supplementation.

Acute bouts of aerobic exercise have generally shown to improve mood, based on post exercise POMS scores, an effect that may persist for as long as 12hrs. Moderate intensity aerobic training for 4-8weeks has been shown to generally improve mood state scores, with decreases seen in the overall TMD and especially in the tension-anxiety category, depression-
dejection, and anger-hostility subscores. High intensity aerobic training, especially when associated with overtraining syndrome, tends to increase TMD, especially in the fatigue-inertia and vigor-activity categories.

Less investigation has been conducted on the effects of RE and mood. Acute bouts of 70% of 1-RM lower body RE improved vigor-activity ratings compared with non-exercisers in untrained females. Twelve weeks of an off-season strength and conditioning program produced no significant changes in POMS categories or TMD in Female NCAA Division-III soccer (n = 28), field hockey (n = 28), and softball (n = 19) athletes.

Several ingredients contained in the SHOT product are marketed as energy and focus enhancers. These ingredients, which combined in 376mg per serving of a proprietary blend labeled as “Meltdown™,” include caffeine anhydrous, β-phenylethylamine HCL, barley (Hordeum vulgare) bud, R-β-methyphenylethylamine HCL, and L-tyrosine. Ingestion of 140mL solution (230mg caffeine) of only the Meltdown™ formula produced no change in POMS in healthy, physically active women, but increased tension and confusion in a mixed group of men (n=5) and women (n=5). Ingestion of 200mg of orally administered caffeine increased vigor and reduced fatigue and total mood disturbance POMS scores, as well as decreasing reaction time and increasing visual acuity, compared with placebo. When caffeine was consumed habitually (5.25mg·kg⁻¹·day⁻¹) for four weeks, it had no significant enhancing effects of mood or performance. Phenylethylamine and its derivatives have been associated with the antidepressant effects of exercise. A combination of β-phenylethylamine and caffeine ingested pre-workout produced no change in mood at three hours following consumption. Mood was unaffected by pre-exercise creatine supplementation. L-tyrosine supplementation produced no effect in ratings of perceived exertion during a 90-minute moderate intensity cycling bout.

Mechanisms of performance enhancement

**Hypertrophy pathway enhancement**

Skeletal muscle is highly plastic and responsive to external and internal stimuli, including mechanical stress associated with resistance training and nutritional status. The ability of muscle to respond to these stimuli by changing size and protein content is governed by a number of complex and interrelated molecular pathways. Some of the ingredients in SHOT and SYNTH may influence these pathways.
IGF-1/mTOR

Classically, the endocrine hormone insulin-like growth factor 1 (IGF-1) is synthesized in the liver in response to changes in circulating concentrations of growth hormone released by the anterior pituitary. This systemic isoform of IGF-1 acts to promote cell growth and development in almost every tissue. It is now apparent that several other isoforms of IGF-1 may be synthesized in extrahepatic tissues in response to a variety of stimuli.

Three isoforms of IGF-1 are expressed by skeletal muscle through a process of alternative gene splicing; IGF-1Ea, IGF-1Eb, and IGF-1Ec. Of these isoforms IGF-1Ea and IGF-1Ec, also known as mechano-growth factor (MGF), are responsive to mechanical activity. The release of IGF-1 by skeletal muscle is up regulated by mechanical overload or stretch, and is closely associated with skeletal muscle hypertrophy.

The receptor for IGF-1 phosphorylates insulin receptor substrate 1 (IRS-1), which activates the phosphatidylinositol 3-kinase (PI3K), increasing levels of phosphatidylinositol 4,5-bisphosphate (PIP2), leading to Akt activation. Akt1, a member of the serine/threonine-specific protein kinase family, is activated during muscle hypertrophy, although this is debated by some. At the center of this pathway is the mammalian target of rapamycin (mTOR). mTOR, when associated with the scaffolding protein RAPTOR, activates the 70-kDa S6 protein kinase, which phosphorylates the ribosomal protein S6, inducing ribosomal biogenesis, and eventual hypertrophy. Concurrently, mTOR deactivates Eukaryotic translation initiation factor 4E (eIF4E), an inhibitor of protein synthesis.

Myogenic regulatory factors

Discovered by Mauro in the early 1960’s, satellite cells are mononucleated precursor cells which occupy spaces between the basal lamina and sarcolemma of adult myofibers. In response to increased workload and myofiber damage, the normally quiescent satellite cells are activated, divide, and donate nuclei to the growing muscle cell host. Rather than existing as a homogeneous population, satellite cells may exhibit more than one phenotype, with one group more committed to retaining its progenitor role and the other “daughter” nucleus entering the sarcoplasm to initiate myogenic activities. Concomitantly, satellite cells activate expression of
several myogenic regulatory factors (MRF)\textsuperscript{126}; Myf5 and MyoD, followed by Myogenin and MRF4\textsuperscript{127}. MRFs bind to promoter regions of genes for several important sarcomeric proteins, increasing biogenesis of new contractile units and eventual hypertrophy\textsuperscript{128}. Satellite cell activation and proliferation appear to be necessary for hypertrophy and proper recovery following exercise. Phelan and Gonyea demonstrated that gamma irradiation of synergist-ablated soleus muscle completely prevented the hypertrophy seen in the non-irradiated controls, despite increases in muscle IGF-1 and hepatocyte growth factor (HGF)\textsuperscript{129}. Lovering and colleagues found that satellite cell inactivation through irradiation increased sarcolemma permeability in rats for several days following high intensity eccentric contractions, as well as reduced the MyoD and myogenin production\textsuperscript{130}.

**Satellite cell activation**

There are a number of important triggers of satellite cell activation. Early work involved examination of IGF-1\textsuperscript{131} and fibroblast growth factors (FGFs) as potential mediators of satellite cell activation, but it became evident that they exerted their differentiation promoting effects post-activation\textsuperscript{132}. Attention subsequently turned to Hepatocyte Growth Factor (HGF). Allen and colleagues successfully activated quiescent rat satellite cells \textit{in vitro} with HGF\textsuperscript{133}, and later observed satellite cell activation in living rat tibialis anterior muscle following HGF injection\textsuperscript{72}. HGF appears to activate satellite cells through binding with its receptor, c-met\textsuperscript{72,127}. HGF is released by muscle cells in response to mechanical overload\textsuperscript{134,135}, crushing injury\textsuperscript{72}, and RE \textit{in vivo}\textsuperscript{136}. Muscle-derived HGF quickly associates with quiescent satellite cell c-met, initiating activation\textsuperscript{137}. Interestingly, activated satellite cells may be capable of producing HGF in a positive feedback autocrine manner\textsuperscript{137}.

Another modulator of satellite cell activation is nitric oxide (NO). Long known as a potent vasodilator produced by the vascular endothelium\textsuperscript{138-140}, NO may also be produced in skeletal muscle by neuronal nitric oxide synthase-I\textmu (NOS-I\textmu). NO production occurs as a byproduct of the conversion of the substrate L-arginine to citrulline\textsuperscript{141}. Muscle HGF production appears to be dependent on proper NO signaling. The early introduction of L-arginine methyl ester (L-NAME) \textit{in vivo}, an inhibitor of NOS function, following crush injury prevents HGF release and subsequent satellite cell activation\textsuperscript{142}. Ten days of intense aerobic interval training produced significant increases in muscle NOS-I\textmu in all fiber types\textsuperscript{143}. In the acute sense, 45
minutes of exhaustive treadmill running enhanced NOS activity 37% compared with N-G-monomethyl-L-arginine (L-NMMA) NOS blockaded rats. Amplified NO production during exercise may also serve to increase regional limb blood flow via its vasodilatory effects on the vascular endothelium\textsuperscript{144, 145}, one effect of which may be to enhance glucose uptake\textsuperscript{146}.

In summation, there are several complex pathways for exercise generated hypertrophy. These pathways are interrelated and not necessarily linear, but in general begin with the increase in activity of muscle NOS-I\textsubscript{μ} in response to muscle damage potentially generated by heavy contractile activity. NO produced by NOS-I\textsubscript{μ} enhances muscle HGF production, as well as increases local blood flow to enhance delivery of substrates necessary for hypertrophy. IGF-1 and HGF act as primary activators of quiescent satellite cells, which begin to proliferate and migrate into the cytoplasm of the damaged host fiber where they release myogenic regulatory factors. The MRFs enhance muscle protein synthesis to form new contractile and structural proteins necessary for hypertrophy. IGF-1, produced locally and systemically in response to exercise, enhances the myogenic activity of already activated satellite cell nuclei, as well as signaling for the hypertrophic mTOR pathway. With so many important molecular factors in play, there are a myriad of targets for enhancement with dietary supplements and indeed, manufacturers have marketed products aimed at just that.

**Supplementation and training augment hypertrophic pathways**

Creatine, BCAAs, and L-arginine are ingredients in SHOT/Synthesize are included at least in part to stimulate hypertrophic pathways. *In vitro*, methionine incorporation into sarcoplasmic and myofibrillar proteins was increased by the addition of creatine to myogenic cell cultures. Creatine also increased the number of nuclei incorporated within myotubes\textsuperscript{33}. Myosin heavy chain type II was increased 1,300%, while troponin T was increased by 65%, and titin by 40%. Similar effects were not present with the addition of taurine or beta-alanine. Introduction of rapamycin, completely inhibited the effect, which suggests that creatine is involved in upregulation of the mTOR pathway\textsuperscript{33}, potentially through augmenting muscle IGF-1 production\textsuperscript{31}. Caffeine has been shown to inhibit mTOR activity by approximately 80%, but did not decrease in association with raptor\textsuperscript{147}. Nutritional factors may activate mTOR independently of IGF-1/PI3K/Akt. The presence of amino acids, especially that of leucine, has been shown to increase mTOR activity and protein synthesis\textsuperscript{35, 37, 103}. 


While there is a large body of work supporting the NOS activity-enhancing role of l-arginine supplementation in the vasculature, evidence for augmentation of muscle NOS is limited. Yao and colleagues fed neonatal pigs a 0.6% L-arginine supplemented diet for 7 days. The supplement group exhibited increased mTOR and 4E-binding protein-1 (4E-BP1) phosphorylation, as well as increased skeletal muscle protein synthesis and total weight gain.148

**Other mechanisms of ergogenesis**

Not all ingredients in SHOT and SYTH specifically target the molecular pathways of hypertrophy to elicit ergogenesis. While they may have minor influences on hypertrophy pathways, some are included to enhance energy systems, reduce fatigue, or improve muscle pH buffering. Principally, these include βA, creatine, and caffeine. Briefly, βA may improve anaerobic capacity by buffering changes in myocellular pH, creatine may buffer muscle adenosine tri-phosphate (ATP) availability, and caffeine may decrease fatigue by antagonizing adenosine receptors in the brain, muscle, and adipose tissue. To understand the performance benefits offered by these supplements discussed earlier, it is important to understand their mechanisms of action.

**βA supplementation increases augments pH control during exercise through the action of carnosine.**

In order to recognize any potential ergogenic effects βA supplementation may offer, it is critical to understand the role cytosolic pH plays in muscle contraction. Adenosine triphosphate (ATP) is the basic energy currency for all cellular activity, including the action of myocellular contractile proteins. During any exercise, the principal acidifying processes are anaerobic glycolysis, with a net result of 2H⁺ per 2 pyruvate produced (when starting from glycogen, as is predominant during high intensity exercise), and ATP hydrolysis to adenosine diphosphate (ADP), which generates 1H⁺. During low and moderate intensity aerobic exercise, protons generated are shuttled to the mitochondria by NADH to drive the electron transport chain, phosphorylating ADP and consuming H⁺. During higher intensity work, ATP demand outpaces the maximal capacity of the oxygen dependent mitochondrial pathways, instead relying more on glycolysis. The result is a net buildup of H⁺ and a decrease in pH.

The role acidosis plays in muscle fatigue is still a matter of serious debate. Chin and Allen demonstrated in 1998 using intact mouse muscle fibers that maximal force production
decreased 30% when pH was lowered using CO₂ from a resting value of 7.15 ± 0.05 to an exercising value of 0.34 ± 0.07\textsuperscript{154}. The authors attribute this to a decrease in Ca\textsuperscript{2+} sensitivity of the contractile proteins, due to the decreased pH. The argument could be made that because the tests took place at room temperature rather than at physiologic temperatures, these results held less value. Knuth et al. examined this question in 2006 using skinned rat fibers\textsuperscript{155}. The authors suggest that discrepancies in previous studies that had found no reduction or even an increase in force at decreased pH were the result of inconsistent muscle fiber type selection, and as such, they made a deliberate effort separately test type I and type II fibers in their study. They found that a decrease in pH from 7.0 to 6.2 with reduced peak power by 34\% in slow fibers at 18\% in fast fibers. Knuth et al. concur with Chin and Allen that H\textsuperscript{+} must compete with contractile proteins for Ca\textsuperscript{2+}, but also suggest that pH may affect sarcoplasmic reticular release and uptake of Ca\textsuperscript{2+}. Whatever the mechanism, changes in cytoplasmic redox state clearly play a role in the ability of muscle fibers to sustain contractile activity. In the face of the failure of the aerobic metabolism to oxidize excess H\textsuperscript{+}, it then must fall on a number of alternative defense systems muscle cells have in place to mitigate changes in pH.

Pragmatically, the most straightforward solution for the muscle cells faced with decreasing pH is to simply pump protons into the interstitium and pass the responsibility for buffering on to other tissues. Muscle cells have two primary methods for accomplishing this; the Na\textsuperscript{+}/H\textsuperscript{+} exchange system and the Lactate\textsuperscript{-}/H\textsuperscript{+} co-transport system. Activated predominantly in glycolytic fibers, the Na\textsuperscript{+}/H\textsuperscript{+} system becomes more active as pH levels decrease below the resting value of ~7.2\textsuperscript{156}. Because it places a greater load on the Na\textsuperscript{+}/K\textsuperscript{+} pumps by increasing cytosolic [Na\textsuperscript{+}], activation of the Na\textsuperscript{+}/H\textsuperscript{+} increases demand for ATP\textsuperscript{157}. The Lactate\textsuperscript{-}/H\textsuperscript{+} co-transport system, which is found in greater numbers in oxidative fibers, is thought to operate in a dual role. In oxidative fibers which are not stressed by low pH, it transports lactate (along with H\textsuperscript{+}) into the cell to fuel the mitochondria. In glycolytic cells, it acts as a relief valve, venting excess lactate and H\textsuperscript{+}\textsuperscript{157, 158}. The activity of the H\textsuperscript{+} transport systems was demonstrated by Street et al. in 2001, who found that muscle contractile power in humans is decreased during isokinetic leg extensor exercise as the exercise-induced decline in interstitial pH increased. The authors also noted that changes in interstitial pH were greater than that of femoral venous blood\textsuperscript{159}. During high intensity contractions, when intramuscular pressures exceed the perfusion pressure
of the blood, ischemic conditions can exist, reducing or negating the effect of the H⁺ transport systems. 

Aside from proton shuttle systems, muscle cells have several methods of directly buffering changes in pH. The generation of ATP from phosphocreatine (PCr) consumes one proton and yields inorganic phosphate (P₁). One of the free oxygen molecules of P₁ has a pKa of 6.82, allowing it to become protonated at physiologic levels. This would appear to negate the acidifying effect of ATP hydrolysis, except that under conditions of high glycolytic activity, such as during intense exercise, P₁ and ADP are taken up as substrates for the non-metabolic resynthesis of ATP, leaving a free H⁺. As we can imagine in cases of high intensity ischemic contraction, acid-base balance can rapidly deteriorate in muscle cells, as the ability to shuttle H⁺ into the interstitium and the ability of P₁ are reduced. However, there is another remaining potential buffer found in all skeletal muscle; histidine containing residues.

The dipeptide protein carnosine, comprised of the amino acids βA and histidine, was discovered by Russian biochemist Vladimir Gulevich in 1900, who was attempting to discover and classify proteins in meat. Carnosine is found exclusively in animal tissues, and of those about 99% in muscle tissue, with the remainder in brain and eye tissues. Carnosine and other histidine containing residues are found in the muscle tissue of many vertebrates, with the highest concentrations in animals that often experience hypoxic conditions, such as diving whales. The imidazole ring of histidine gives carnosine its pH buffering ability. Free histidine has a pKₐ of 6.21, while histidine in most proteins has a pKₐ of 6.5, both too low to accept H⁺ at even extreme physiological values. However, the imidazole group in carnosine has a pKₐ range of 6.83-7.01, allowing it to become protonated at precisely the values exhibited by vigorously exercising muscle tissue.

Synthesis of carnosine in the body is catalyzed by the enzyme carnosine synthase. While histidine is proteinogenic and found in relatively high concentrations throughout muscle tissue, βA is nonproteinogenic and must be obtained through dietary sources, principally from meat. Combined with its much higher Michaelis constant of 1.0-2.3 mM for carnosine synthase (histidine Km=16.8 µM), it would appear that βA is the rate limiting substrate for the formation of carnosine. Harris et al. conducted the earliest studies examining the relationship of dietary supplementation of βA and muscle carnosine concentration in 2006. They found that 4 weeks of supplementation beginning at 4g per day and increasing to 6.4g per day after the first two
weeks resulted in increases in muscle carnosine levels ranging from 42.1% to 65.8%, depending on the dosing method. The placebo group increased muscle carnosine by 9.9%. Frequent, smaller doses of βA seemed to be more effective than larger doses of total isomolar content, although there was no significant difference between groups. Dosing with intact carnosine protein with isomolar amounts of βA showed no significant advantage. Because the action of serum carnosinase quickly cleaves carnosine into its component amino acids its introduction to the bloodstream, it is not surprising that carnosine ingestion offers no advantage.

Training status has been demonstrated to increase muscular buffering capacity and carnosine content. In 2007, Derave and colleagues pioneered a non-invasive NMR spectroscopy method for determining muscle carnosine content in trained sprinters supplemented with βA. Fifteen male 400m sprinters with personal best times under 53s consumed placebo (n=7) or βA (n=8) at 4.8g per day during a 4 week training period. Using their new spectroscopic method, the authors demonstrated a 47% and 37% carnosine increase in soleus and gastrocnemius muscles, respectively. A small, but significant, increase was detected in the placebo gastrocnemius muscles of 16%, while no increase was found in placebo soleus. In summation, βA supplementation increases muscle carnosine concentrations, improving cytosolic acid buffering capacity. Increased ability to moderate exercise induced decreases in muscle pH is the source of ergogenic potential for carnosine, and indirectly, βA.

**Caffeine**

Caffeine is a crystalline xanthine alkaloid, the most commonly used psychoactive drug in the world, and one of the oldest known ergogenic aids. Caffeine exerts its effects in the brain, skeletal muscle, and adipocytes by acting as an antagonist to adenosine receptors. Adenosine normally functions as an inhibitor of neuronal activity by binding to adenosine receptors in the central nervous system and its presence manifests itself as an enhancement of motor activity. Additionally, adenosine stimulation of A2 receptors in peripheral tissues results in increased pain sensation. Caffeine blockade of these receptors has been shown to reduce exercise-induced pain, resulting in increased performance. In adipose tissue, caffeine acts to stimulate lipolysis and increase plasma free fatty acids (FFA) through the inhibition of cyclic AMP-inhibiting phosphodiesterase, increasing its use as a substrate for metabolism (as evidenced by changes in respiratory exchange ratio), and possibly sparing muscle glycogen during extended exercise, although this point is a matter of contention.
Creatine

Creatine is an amino acid found in abundance in animal based foods\textsuperscript{30}, and can be found in human tissues including brain and skeletal muscle\textsuperscript{29}. Skeletal muscle, however, lacks the ability to synthesize creatine. Working in conjunction, the liver and kidneys assume the task of creatine synthesis; the liver generates guanidinoacetate from the transfer of an amidino group from L-arginine to glycine via L-arginine:glycine amidinotransferase (AGAT) and the kidneys methylate it via Guanidinoacetate N-methyltransferase (GAMT) to form creatine\textsuperscript{165}. The creatine generated is then delivered to active skeletal muscle. The ergogenic potential of creatine lies in its ability to bind with inorganic phosphate (P\textsubscript{i}) to form phosphocreatine (PCr). Through the action of the enzyme creatine kinase, PCr can donate its phosphate to adenosine diphosphate (ADP) to form ATP\textsuperscript{29}. In this way, the PCr/ADP/ATP system acts as a reservoir of ATP in the cytosol, buffering against depletion during exercise\textsuperscript{166}. In an oft-cited study, Harris et al. determined that repeated (6 x 5g per day) dietary creatine supplementation enhances muscle creatine levels by as much as 50% in healthy males\textsuperscript{167}. As discussed previously, elevated muscle creatine is associated with increases in strength and power.

Conclusions

With such a wide variety of ingredients included in the SHOT and SYNTH products, each with multiple potential modes of ergogenics, concrete conclusions are difficult to draw. Mechanistically, consumption caffeine, creatine, β-Alanine, and protein/amino acids should augment strength and hypertrophy gains associated with resistance training. However, inter-study variability in training status, test modality, supplement dosages and timing, and others have yielded mixed results. This study seeks to improve on earlier work by using trained subjects and carefully supervising all training sessions, as well as by measuring both traditional weight-lifting and laboratory strength and anaerobic capacity variables. Additionally, few studies to date have combined the wide variety of potential ergogenic ingredients before and after exercise as part of an extended training regimen. In this manner, we hope to provide the most favorable environment for supplement-mediated improvements in strength and hypertrophy.
CHAPTER THREE

METHODS AND MATERIALS

Subject characteristics

Participants in this study could not have uncontrolled hypertension (bp>140/90), uncontrolled cholesterol/blood lipid levels or those that take cholesterol medication, diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, or any musculoskeletal complications that would impede one from exercising with weights were excluded. Prior to beginning the first pre-testing session, subjects were provided with the written informed consent as approved by the Florida State University Institutional Review board on human subject use and complete a health history and fitness questionnaire. All subjects were males between the ages of 18 and 40 years. Participants must have been regularly participating in at least two resistance training sessions per week for the last 12 months, without any gaps totaling more than 4 weeks. Individuals who were currently consuming other workout supplements or ergogenic aids were asked to stop consumption, and a washout period of 1-4 weeks was started depending on the content of those products. A 72-hour food and activity log was maintained at baseline and during the final week of training.

Table 1. Subject characteristics

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Training Status

One principal objective of this study was to evaluate the effectiveness of SHOT and SYNTH in already well trained subjects. Bench and leg press 1RM can be used as a measure of muscular fitness and training status. There were no differences between groups in any exercise variable before training. The average bench press 1RM for all subjects was 253.14±12.08lbs. Relative to body mass, average 1RM were 1.38 and 2.84 for bench press and leg press, respectively. According to the American College of Sports Medicine normative tables, this value places the subjects, on average, above the 80th percentile in bench press and above the 90th percentile for leg press. Based on these values, we can conclude that our subjects were well trained, at least in strength measures, prior to participation in the study.

Experimental design

This study used a longitudinal and double-blind design with placebo control. Following the initial collection of pre-testing data and before the start of training, subjects were placed into performance supplement supplement (PL) or placebo (PLA) groups. The ratio of initial maximal voluntary contraction (MVC, isometric 60° knee extension) to lean mass (LM) was used as a grouping method to balance the experimental groups for relative strength. Following pre-testing data collection, subjects participated in a periodized 6-week resistance training program under supervision of research personnel. Subjects consumed one 27g serving of NO-Shotgun® SHOT or isocaloric maltodextrin placebo (PLA) 15 minutes prior to exercise. Upon the conclusion of each training session, subjects immediately consumed one 27g serving of NO-Synthesize (SYNTH) or placebo (PL). Subjects were also given single servings of SYNTH or PL to consume on non-training days. The subjects returned for post-testing at least 36 hours following the final training session. This time frame was chosen in order to minimize the effects of delayed onset muscle soreness (DOMS) and post exercise reduced maximal torque on testing data, as well as to ensure that any changes were due to training and not only from the final session.
Training protocol

The overall course of the resistance exercise program utilized by this study progressed from high repetitions at moderate resistance to low repetitions at high resistance. For the duration of the study, 3 sets of each exercise were completed. For the first two weeks, subjects completed 10 repetitions at 70-75% of 1RM. For weeks 3-4 resistance was increased to 6 repetitions at 80-85% 1RM. For the final two weeks subjects completed 4 repetitions per set at 85-90% of 1RM. Each major muscle group was trained once per week using at least one exercise. The specific exercises performed during each training session can be seen in Appendix A, Figure 1. The six week training program was designed to target every major muscle group in a three day split. The exercises for day 1, designed to work the biceps, triceps, and shoulders were performed in the following order: shoulder military press, dumbbell incline biceps curl, cable overhead French press, straight bar curls, cable triceps press down, and dumbbell reverse fly. The exercises for day 2, designed to work the muscles of the legs and core, were (in order): leg press, straight leg dead lift, dumbbell lunge, leg curls, standing calf raises, abdominal crunch, and core planks. The third and final day of the rotation was designed to work the muscles of the chest and back with the following exercises (in order): flat bench press, cable pull down, incline bench press, cable low row (neutral grip), dumbbell chest flys, and dumbbell shrugs. 3 sets of each exercise were performed for prescribed number of repetitions or to failure, with resting times of 60-90 seconds between sets. If a subject was unable to perform the prescribed weight for an exercise, the weight was adjusted to yield failure at or near the correct number of repetitions.

Testing sessions

Pre- and post-testing sessions were identical in terms of order and type of measurements taken. The participants were then be asked to complete a Profile of Mood States (POMS) questionnaire. Heart rate (60 second) was measured using the radial pulse, and blood pressure was measured using the auscultatory method. Each measurement was taken twice and the average was recorded. The same researcher measured these variables at the post-testing session.
Profile of mood states (POMS)

POMS scores are divided into 5 categories; tension, depression, anger, vigor, fatigue, and confusion) which are composed of the aggregate values of 65 mood descriptors. Subjects rate each descriptor on a scale from 0 to 4, with 4 implying that a descriptor very accurately describes how they have been feeling, and 0 suggesting no accuracy. The subjects were instructed to consider their feelings over the past week and apply that to the form. See appendix A, figure 2 for an example of the POMS form used in this study.

Blood measures

Prior to beginning blood collection each session, a Cholestech LDX Analyzer (Cholestech Corp, Hayward, CA) was activated and calibrated using an Optics Check Cassette (MDSS GmH, Hannover, Germany). Calibration values were recorded to confirm that the instrument conforms to the manufacturer suggested standard range of between 85-105. Approximately 15mL of blood were drawn via venipuncture of the median cubital or cephalic veins in the antecubital space of the subject-preferred forearm. Briefly, the antecubital space was cleaned thoroughly with alcohol pads before an elastic tourniquet is applied to the upper arm in order to engorge the lower veins and enhance visualization. A butterfly style BD Vacutainer Push Button blood collection set (Becton, Dickinson & Company, Franklin Lakes, New Jersey) was inserted into the target vein. One 10mL BD Vacutainer Serum (red top) tube was filled first, followed by two 2mL BD Vacutainer K2 EDTA 3.6mg (purple top) vials. Following completion of the blood draw, the tourniquet was removed and the needle quickly retracted from the subject’s arm for proper disposal. The subject was instructed to firmly press a piece of sterile gauze to the puncture site. A small amount of blood was drawn via a capillary tube and plunger system from the 10mL serum vial for analysis on the Cholestech instrument using Lipid Profile · GLU (MDSS GmH, Hannover, Germany) cassettes, which report Total Cholesterol, Triglycerides, HDL, LDL, non-HDL, and blood glucose. Following collection, all blood vials were stored on ice until centrifuging at 3500RPM at 4°C for 15 minutes using an IEC CL3R Multispeed Centrifuge (Thermo Electron Corporation, Needham Heights, Massachusetts). Aliquots of 300µL each were transferred into microtubes and frozen at -80 degrees C for later blood chemistry analysis. Following blood collection, the subject consumed one 8oz box of apple juice (140kcal, 27g sugar).
Body composition

Circumference measurements of the upper arm, chest, gluteal, and thigh were taken using a measuring tape with strain gauge (Creative Health Products, Ann Arbor, Michigan). For the chest measurement, the tape was run horizontally across the nipples and around the back, and the subject was instructed to exhale fully. For the upper arm measurement, the subject was instructed to raise his dominant arm until the elbow is at shoulder height. The subject was then instructed to contract the biceps brachii maximally until the measurement is completed. The measurement was taken at the thickest part of the contracting biceps brachii. The gluteal measurement was taken around the widest part with the subject standing with his feet together. The thigh measurement was taken while the subject stands with the heel of the dominant leg placed on the toes of the opposite foot. The measurement was then taken at the widest part of the dominant leg. A measurement from the top of the patella to the point of circumference measurement was made and recorded to be repeated in the post-test.

After circumference measurements are taken, subject body mass was recorded using a SECA electronic scale (SECA, Hamburg, Germany) and height was taken using a SECA 216 wall mounted measuring rod. All measurements were taken with shoes removed wearing only underwear. Subjects were then asked to lie on the platform of a GE Lunar iDXA (General Electric Company, Fairfield, Connecticut) with all metal objects removed. The iDXA instrument is previously calibrated before each session using a standard calibration program with a phantom block of known density. Results were analyzed with enCORE Software, version 11.0 (GE Lunar).

Performance measures

Isokinetic and isometric strength

After completion of the blood draw and body composition measures, subjects were escorted to the human performance laboratory to begin performance testing. The order of performance testing was uniform for each subject and maintained between pre- and post-testing sessions. The first tests were conducted using the Biodex System 3 (Biodex Medical Systems, Shirley, New York) exercise dynamometers. The subject was placed in the upright seated position in the instrument, and the seat height and position was adjusted in order to align the
instrument’s axis of rotation with that of the subject’s knee. Once the subject is correctly positioned and strapped in, range of motion (ROM) was determined and limb weight was measured. Seat, leg arm, and ROM positions were all recorded for repetition during the post-testing session. The subject’s dominant leg was used for all Biodex testing. Subjects were instructed to cross their arms over their chests, but not to grab the restraints. The first test conducted using the Biodex was an isokinetic 90°/sec unilateral knee extension/flexion followed by a 180°/sec unilateral knee/extension flexion. Five sets of consecutive maximal extension and flexion were performed during each test, with a one minute rest interval between tests. Following the isokinetic test, a 60° isometric knee extension/flexion test was performed. This test involves three maximal extension and flexion exertion against an immovable arm, with 10 second rest periods between exertions. Continuous verbal encouragement was provided by the research team throughout the duration of all tests. Subjects were allowed to view the data reporting screen and all values as they are recorded.

**Wingate test**

Following the Biodex isokinetic and isometric tests, a Wingate test was performed to evaluate anaerobic capacity. All tests were performed using a plate loaded and friction braked Monark Ergomedic 874-E (Monark Exercise AB, Vansbro, Sweden) cycle ergometer. Resistance (r) was set as 7.5% of body mass (kg). Each subject was fitted to the ergometer by adjusting the seat height to ensure 5-10° of knee flexion at the bottom of each cycle. The subject performed a two-minute warm-up at 75 rpm with only the resistance added by the weight basket (0.5kg), with two brief (~10sec) bouts of practice sprinting. Following the warm-up period, a 5-second countdown period was begun where the subject spun up to full speed. When the subject was cycling at full speed, the resistance was added and the 30-second test timer was started. Throughout the test the subjects were given verbal encouragement for them to be aware of the time remaining. At the end of the 30-second test period, the resistance was removed and the subject was instructed to cycle slowly for at least two minutes to cool down. Video of the exercise bout was recorded using a Pentax Optio W90 (Pentax Imaging Company, Golden, Colorado) camera. The video was later analyzed to determine total revolutions ($R_{total}$) and peak revolutions ($R_{max}$). If the exercise is broken down into 5-second intervals (i.e. 0-5 seconds, 5-10 seconds, 10-15 seconds, etc.), $R_{max}$ is defined as the maximal number of revolutions achieved.
during an interval. From these values, total work (W) was calculated as \( r \times R_{total} \), where \( r \) is the resistance in kg and \( R_{total} \) is the total number of revolutions completed in the 30 second testing period. Peak anaerobic power is calculated as \( \left( \frac{r \times 6m \times R_{max}}{5 \text{ seconds}} \right) \), where \( R_{max} \) is the number of revolutions completed in the first 5-seconds of the test and \( 6m \) corresponds to the distance traversed by the flywheel in one revolution (6 meters). Mean anaerobic power is calculated as \( \left( \frac{W}{30 \text{ seconds}} \right) \). Further, relative values can be obtained by dividing these numbers by body mass or fat free mass.

**One repetition maximum (1-RM)**

On a separate day from the laboratory pretesting, but prior to the first training session, subjects reported to the training location for the determination of one repetition maximum (1-RM) in the bench press and 45° leg press exercises. For the purposes of this study, 1-RM is defined as the maximum weight an individual is able to perform in a given exercise, with good form, through the full range of motion. The protocol used by this study is derived from that of the National Strength and Conditioning Association (NSCA) and is as follows: The subject began by warming up with a low resistance that allows for an easy 5-10 repetitions, followed by a one minute rest. A second warm-up load was estimated to allow the subject to complete 3-5 repetitions, followed by a two-minute rest. Following the second warm-up, weight was increased by 5-10% for bench press, or 10-20% for leg press and a single repetition was performed, followed by a 2-4 minute rest period. This process continued until the subject failed the attempt or could not maintain proper form. Upon failure, weight was reduced by 2.5-5% for bench press, or 5-10% for leg press and the subject made another, final attempt after a 4-minute rest period. The maximum weight successfully lifted was recorded as the subject’s 1-RM for that exercise. The form cues used for the 1-RM and training sessions for each exercise did not differ. For the bench press, the subject was to lie flat on the bench with the eyes approximately at the level of the bar as it rests in the rack. The subject was to grasp the bar so that the wrists were situated directly above the elbows for the duration of each repetition. The subject’s back was to maintain contact with the bench at all times, and did become unnaturally arched. The subject’s feet were to stay flat on the floor so that the heels would not rise during the exercise. The subject lowered the weight until the upper arms are parallel with the floor, and the elbows were flexed at
approximately 90°, at which point the subject was to press the weight back to full extension. At no point did the bar touch the chest, as is common in competition lifting.

The form cues for the leg press were somewhat less extensive. The subject was to seat himself in the machine and place his feet on the plate so that they are just wider than shoulder width and his knees are were flexed to approximately 90°. The subject lowered the weight until the tops of his thighs were just touching his chest, at which point he should press out to full extension.

Following the completion of all pre-testing, subjects began the RE training program detailed previously. Subjects consumed their assigned pre-workout supplement under the supervision of research staff 15 minutes prior to the beginning of resistance training. During this time, the subjects were instructed to perform a light warm-up on the cardiovascular exercise machine of their choice. Immediately upon the completion of each training session, the subjects consumed their assigned post workout supplement and be given single serving baggies of supplement to consume on non-training days. Upon completion of the training sessions, the subjects reported back to the lab for a round of post-testing, identical to that of the pre-testing sessions.

Statistical analysis

Descriptive data were generated for all variables and expressed as mean ± standard error of the mean. A 2 (group) × 2 (time) analysis of variance (ANOVA) with repeated measures was used to analyze performance, body composition, and mood data. Tukey LSD post hoc tests were used to examine pairwise difference. Significant interactions were analyzed by simple main effects. Significance was set at (p < 0.05). PASW Statistics for Windows version 18.0.0 (International Business Machines Corporation, Armonk, New York, United States) and Statistica (Statsoft, Tulsa, Oklahoma, USA) software were used to perform the analyses.
CHAPTER FOUR

RESULTS

Subject Nutrition

After the pre-testing session, subjects were required to complete a 3 day food and activity log. A subset of these data has been analyzed (n=8). There were no significant differences between groups in total kilocalories consumed, grams of protein, carbohydrate, and fat, or caffeine consumed before or after training.

Table 2. Subject Nutrition

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<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Kilocalories (kcal/day/kg)</td>
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<td>Carbohydrate (g/day/kg)</td>
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<tr>
<td>Fat (g/day/kg)</td>
<td>1.78±0.80</td>
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<tr>
<td>Caffeine (mg/day/kg)</td>
<td>2.22±0.75</td>
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Wingate test: anaerobic power

There were no group x time interactions for any Wingate variables. There was a main time effect for peak anaerobic power (ANP, \( p = 0.001 \)), relative peak anaerobic power (RANP, \( p = 0.001 \)), mean anaerobic power (MANP, \( p = 0.007 \)), relative mean anaerobic power (RMANP, \( p = 0.016 \)), and total work (\( p = 0.006 \)).

Post-hoc analysis revealed that the PS group significantly increased ANP by 16.22% (PRE 77.69±3.81W, POST 93.22±4.11W, \( p = 0.002 \)), RANP by 9.38% (PRE 0.92±0.04W·kg\(^{-1}\), POST 1.09±0.05W·kg\(^{-1}\), \( p = 0.003 \)), MANP by 9.94% (PRE 112.74±5.74W, POST 125.19±6.58W, \( p = 0.015 \)), RMANP by 8.21% (PRE 1.33±0.04W·kg\(^{-1}\), POST 1.45±0.049·kg\(^{-1}\), \( p = 0.03 \)) while PLA remained unchanged. There were no changes in fatigue index for either group.

Figure 1. Wingate Mean Anaerobic Power (W). Post-hoc analysis indicates Main Time Effect (\( p = 0.007 \)) main time effect was driven by supplement group.
One repetition maximum (1-RM)

There were no group x time interactions observed for any maximal strength variable. Time effects were noted for all 1-RM measures (p =0.001). Post-hoc analysis indicated that in leg press (LP), the PS group increased with training by 19.56% (PRE 740.00±51.85lbs, POST 920.00±54.34lbs, p < 0.001) and the PLA group increased by 25.94% (PRE 699.44±62.36, POST 944.44±65.31lbs, p < 0.001). In bench press (BP), PS group increased by 8.40% (PRE 247.31±15.44lbs, POST 270.00±15.23lbs, p = 0.001) and the PLA group increased by 6.96%, (PRE 260.00±18.56, POST 279.44±18.31lbs, p = 0.001).

![Figure 3. Leg press 1-RM and Bench press 1-RM. * indicates main time effect (p = 0.001). Values are means ± SE.](image)

Body composition

Group x time interactions were observed for lean mass (LM, p = 0.017) but not for any fat measures. The PS increased LM by 4.72% (PRE 62.96±20.19kg, POST 65.77±20.81kg, p < 0.001). No significant changes were observed in LM in the PLA group over time with training, although there was a trend for increases in LM (p = 0.085) A main time effect was detected for
percent body fat (%BF), percent android fat (ANDRO, p = 0.001), and percent gynoid fat (GYNO, p = 0.002). No changes were observed in fat mass (kg). Post-hoc analysis revealed that the PS group decreased percent body fat from 21.64±1.36% to 20.46±1.30% (p = 0.004), ANDRO from 23.69±2.23% to 21.88±2.20% (p = 0.017) and GYNO from 21.85±1.54% to 20.54±1.45% (p = 0.021). There was no significant decrease in overall fat mass (kg). There were no significant changes in fat variables for the PLA group.

Figure 4. Lean Mass (kg) * indicates Group x Time effect (p = 0.017) with main time effect (p = 0.001)

Circumferences

Circumferences of the brachial region of the arm (ARM), chest (CHE), thigh (THI) and gluteals (GLT) were measured pre and post training. There were no group x time interactions for any variable. Time effects were observed in CHE (p = 0.005), ARM (p = 0.001), and GLUT (p = 0.004).

Post-hoc analysis indicated that the PS group increased ARM by 2.2% (PRE 37.61±0.80cm, POST 38.49±0.74cm, p = 0.002) and THI by 2.5% (PRE 55.12±1.16, POST56.57±1.45 cm, p = 0.021). The PLA group increased ARM by 2.6% (PRE 36.78±0.90cm, POST 37.79±0.92cm, p = 0.001). There were no other significant changes in circumference for either group.
Figure 5. Thigh Circumference (cm). * indicates Main Time Effect (p = 0.001)

Profile of mood states (POMS)

No group x time interactions were observed for any variable. A time effect was noted for vigor (p = 0.02). Post hoc analysis indicated that the PS group increased Vigor scores by 13.67% (PRE 15.25±1.70, POST 17.67±1.83, p = 0.019) while PLA remained unchanged.

Training volume

There were no differences in training volume (weight x successful repetitions x sets) between groups for any week for any exercise. When the values were adjusted for lean mass there were still no differences.

Isokinetic and isometric strength measures

Isokinetic strength

There were no group x time interactions observed for any isokinetic variable. Time effects were observed for 30°sec⁻¹ extension average power (p = 0.018), 30°sec⁻¹ flexion average power (p = 0.009), 30°sec⁻¹ agonist/antagonist ratio (p = 0.029). For 60°sec⁻¹ extension, time
effects were observed for average power (p =0.020) and maximum repetition total work (p = 0.025). For 60°sec⁻¹ flexion, time effects were noted for peak power (p = 0.015), maximum repetition total work (p = 0.025), average power ( p = 0.004), and average peak torque (p = 0.019).

Post hoc analysis revealed that during 30°sec⁻¹ extension the PS group decreased relative peak torque -3.45% (PRE 254.54±16.52N-M·kg⁻¹, POST 245.870±12.23 N-M·kg⁻¹, p = 0.09) while average power increased 6.18% (PRE 72.100±3.715W, POST 76.85±3.587W, p= 0.023) and acceleration time decreased 52.16% (PRE 29.17±3.98ms, POST 19.17±1.93ms, p= 0.032). During 60°sec⁻¹ flexion peak torque increased 14.51% (PRE 108.73±4.56N·M, POST 121.03± 6.51 N·M, p = 0.048), maximum repetition total work from increased 15.21% (PRE103.56±6.91J, POST 122.13±8.33J, p = 0.032), and average power increased 13.31% (PRE 68.80±3.01W, POST 79.36±75.45W, p = 0.028). There were trends during 60°sec⁻¹ extension for an increase in maximum repetition total work (p = 0.053) and average peak torque (p = 0.052). There was also a trend for improved agonist/antagonist ratio during 30°sec⁻¹ isokinetic exercise (p = 0.053).

During 30°sec⁻¹ flexion the PLA group increased average power 17.14% (PRE 40.62±2.67W, POST 49.01±2.11W, p = 0.002), decreased deceleration time 49.14% (PRE 261.00±0.62ms, POST 175.00±38.04 ms, p = 0.029), and improved average peak torque 9.60% (PRE 115.29±6.66N·M, POST 127.54±6.10N·M, p = 0.027). There were trends for improvement in average power (p = 0.058) and average peak torque (p = 0.065) during 30°sec⁻¹ flexion.

**Isometric strength**

Group x time interactions were observed for relative average peak torque during isometric flexion (p = 0.028). There were also similar trends during isometric flexion for average peak torque (p = 0.053) and relative peak torque (p = 0.057).

Post hoc analysis revealed that there were no changes in any isometric variables for the PS group. However, the PLA group improved peak torque by 12.69% (PRE 123.55±8.10N·M, POST 141.502±6.87N·M, p = 0.033), and average peak torque by 12.21% (PRE 114.191±8.20N·M, POST 130.918±6.33N·M, p= 0.047). There was also a trend for improvement in relative peak torque in the PLA group (p = 0.053).
CHAPTER FIVE

DISCUSSION

The principal objectives of this investigation were to examine the effectiveness of combined supplementation with SHOT and SYNTH in trained males over the course of a 6 week resistance training period at increasing measures of muscle strength and power, as well as augmenting body composition. Secondarily, we sought to determine if SHOT and SYNTH consumption increased training volume or altered mood state in this population. Group x time interactions were observed for lean mass (LM), and fat free mass (FFM). The PS increased LM by 4.72%, and FFM by 4.05%, while the PLA group was unchanged. Both groups increased leg press and bench press to an equal degree. Post-hoc analysis indicated that the PS group significantly increased measures of anaerobic power, while the PLA group showed no changes. Isokinetic flexion and extension results were mixed, with improvements and declines noted by both groups in select variables. The supplement had no effect on training volume for any week or exercise, but may have elevated feelings of vigor in PS group.

Body composition

Changes in body composition were perhaps the most remarkable results of the current study. As can be seen in Figure 2, The PS group increased LM and FFM, while decreasing body fat %. Because there were no changes in fat mass, the decreased body fat % observed in the PS group was due to increased LM and overall body mass. The PLA group made no significant changes in any body composition variable, although there were trends for improved LM and FFM. The lack of change in fat mass (kg) demonstrated in this study reflects the findings of other similar studies, but is at odds with popular claims made about these products. Indeed, one the proprietary blends listed on the SHOT label contains 376mg of a combination of caffeine, β-phenylethylamine HCL, hordeum vulgare bud, and L-tyrosine, and is marketed in SHOT and in other similar products as a “fat burning” component. However, because participants were asked to consume their normal dietary intake rather than being fed specific meals with specific caloric restrictions, we cannot draw the conclusion that SHOT and SYNTH consumption pre- and post-exercise are ineffective at reducing fat mass. However, it is worth noting that no changes in dietary intake were reported from baseline (week 0) to post-testing.
(week 6), therefore, our lack of change in body mass (kg) is likely real. Perhaps more valuable to consumers, limb circumferences increased in some measures for the PS group, but not for the PLA group. Thigh circumference changes are depicted in Figure 5.

The PS group increased lean mass significantly more than the PLA group. This is in concurrence with many similar studies\textsuperscript{18, 19, 32, 59, 173}. As muscle mass is one of the main determinants of strength and power\textsuperscript{174}, it is somewhat unexpected that the PS group did not experience greater improvements in 1-RM strength, although 1-RM tests may not be sensitive enough to detect the modest difference in LM improvement exhibited by the PS group. The same explanation is most likely explains the lack of group x time effects in circumference measurements. One remarkable finding of this study is that the increase noted in LM by the PS group in this study (+4.71\%) was very similar to that of the supplement group in Shelmadine et al. (+4.75\%)\textsuperscript{19}, despite the increased training status of our subjects.

**Wingate test: anaerobic power**

While the present study noted a time effect for peak and average anaerobic power and total work performed, there were no differences between the two groups. There was, however, a strong trend (p = 0.06) for the PS group to improve peak ANP. We also noted an increase in mean anaerobic power for the PS group, but not for the PLA group, as can be seen in figure 1. These findings are similar to those of Beck et al.\textsuperscript{18}, who demonstrated increases peak and mean ANP following 10 weeks of resistance training using untrained males consuming a pre-exercise supplement containing protein, creatine, and BCAAs. The protocol used by Beck et al. called for two consecutive 30 second cycling bouts, whereas this study only used a single bout. The differences in training duration (6 weeks vs. 10 weeks), number of cycling bouts, and training status may explain why Beck et al. were able to elicit significant group x training effects while we were not.

It was also expected that the inclusion of βA in the SHOT and SYNTH supplements would yield improvements in fatigue index (FI) through the acid buffering effects of carnosine. Instead, we found no significant time t or group x training effect for FI, in contrast to the findings of others. Hoffman et al. noted improvements in FI following 30 days of βA supplementation in American football players during offseason training\textsuperscript{10}. Some of this discrepancy may be explained by βA dosages. In studies that have demonstrated improvements in performance, βA
dosages tend to range from 4.8 to 6.0g·day\(^{-1}\). Unfortunately, the performance supplement in the present study included βA as part of a proprietary blend, rather than labeling it independently and, therefore, there is no accurate content claim for βA. We can only speculate, therefore, that our PS group may have been consuming less than the 4.8g/day that has been shown to elicit training enhancements.

**One repetition maximum (1-RM)**

Figures 3 and 4 depict that the present study demonstrated a significant effect of time for both BP and LP strength in both groups; however, there was no group x time effect. Shelmadine et al. also noted a training effect for both groups in BP and LP following 28 days of resistance training with SHOT supplementation. Conversely, the group supplemented with SHOT improved BP to a significantly greater degree than the placebo (18.40% vs. 8.82%, p = 0.003\(^{19}\)). In contrast to Shelmadine et al., Beck et al. found no differences in training-induced enhancements in BP or LP between the CR-PRO supplement group and placebo groups in their 10-week resistance training study\(^{18}\). Cribb et al. were able to elicit 1-RM group x training effects in trained males following 10 weeks of RE training and consumption of whey protein\(^{64}\) or whey protein and creatine\(^{80}\). With so much conflicting evidence and confounding variables, it is difficult to draw conclusions about the effectiveness of PS on 1-RM strength in trained males. It is worth noting, however, that in all of these studies the supplement group increased lean mass significantly more than the placebo.

**Isokinetic and isometric strength**

Isokinetic leg exercise results were somewhat mixed. There appeared to be a pattern for both groups to improve strength and power during flexion but to make little improvement or even decrease performance in extension, as was the case with 30°sec\(^{-1}\) extension in the PS group. However, the PS group did exhibit trends (p = 0.054) for improvements in some 60°sec\(^{-1}\) extension variables. Training specificity is one explanation for these data; our training program included seated hamstring curls, but not knee extensions. Thus, each participant spent 6 weeks without doing seated extension types of exercise.

Results of the isometric tests are particularly puzzling, as the PS group made no improvements while the PLA group improved in several measures during flexion. This is in contrast to other studies using supplement combined with training\(^{176, 177}\) and correlations of muscle mass and
isometric force production. Neither group in the present study performed isometric exercise as part of training, so changes in performance should be due to supplementation or changes in muscle mass. There are a number of possible explanations for these findings. Isometric exercise performance is somewhat sensitive to innate muscle fiber type distribution, which was not tested or controlled for in this investigation. Additionally, while there were no differences before training, some individuals in the PLA group showed much higher increases in performance than the median, which might have skewed the results.

### Training volume

We observed no differences in volume (weight lifted x repetitions x sets) for any exercise over the course of the training period. This was in contrast to common findings of other supplement plus training studies involving caffeine, βA, and creatine, but not all studies. The lack of difference between groups in training volume may have been a result of our study design rather than supplement effects. All subjects were instructed that the goal of every set should be failure and they were to achieve this by selecting weights that caused them to fail at a specific number of repetitions (10 for weeks 1-2, 6 for weeks 3-4, and 4 for weeks 5-6). The number of repetitions was controlled in order to facilitate the periodized training goals. If subjects had been given control of the number of repetitions or sets, those receiving supplement may have performed a higher number allowing them to increase training volume. On the other hand, eliminating training volume as a variable leaves manipulation of hypertrophic pathways by the supplement ingredients as the most probable explanation for increased muscle mass in the PS group.

### Profile of mood states (POMS)

The lack of change in mood as detected by POMS is in agreement with other studies involving similar energy-enhancing beverages. The main time effect for increased vigor scores in the PS group over the course of training is interesting, as this measure is typically decreased with training, especially during that of high intensity. It appears that consumption of SHOT and SYNTH may be beneficial for those seeking to improve feelings of vigor over the course of resistance training. More investigation of these and similar products is warranted, especially in the area of training volume.
Conclusion

Consumption of SHOT and SYNT in close proximity to resistance exercise during the course of a periodized 6 week exercise training program resulted in significant improvements in lean mass in trained males, whereas the consumption of isocaloric carbohydrate beverage did not. At the dosages consumed and with the specific population in this study, these products do not appear to offer advantages in measures of muscle strength and power. Sustained SHOT and SYNT consumption has no negative effect on mood, and may improve feelings of vigor. Continued investigation of these or similar products is warranted as questions about the influence of performance supplements on volitional training volume should be answered. Additionally, an investigation of the supplementation effects with multi-ingredient performance supplements on health variables and endocrine responses as well as in comparison to simple whey protein and BCAAs, and in different populations or following different exercise types would be valuable to understanding more about performance supplements and would greatly improve the field of sports nutrition.
APPENDIX A

WEEKS 1-2
(Circle)

3 sets of 10 at 70-75% 1RM

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<tr>
<th>Day 1</th>
<th>Biceps, Triceps, &amp; Shoulders</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight</td>
<td>Reps</td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td>Shoulder Military Press</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB Incline Biceps Curl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cable Overhead French Press</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Straight Bar Curls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cable Triceps Press Down</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB Reverse Fly</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Legs &amp; Abdominals/Core</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight</td>
<td>Reps</td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td>Leg Press</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Straight Leg Dead Lift</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB Lunge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cybex Leg Curls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cybex Standing Calf Raise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cybex Abdominal Crunch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plank (1 min)</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 3</th>
<th>Chest, Back, and Traps</th>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight</td>
<td>Reps</td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td>Flat Bench Press</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cable Lat Pull Down</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incline Bench Press</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cable Low Row (Neutral Grip)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB Chest Flys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DB Shrugs</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Supplement Baggie Returned</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Example of workout data log from weeks 1 and 2.
Below is a list of words that describe feelings people have. Please read each one carefully. Then check ONE space to the right of each feeling that best describes how you have felt DURING THE PAST WEEK.

<table>
<thead>
<tr>
<th>0 = NOT AT ALL</th>
<th>1 = A LITTLE</th>
<th>2 = MODERATELY</th>
<th>3 = QUITE A BIT</th>
<th>4 = EXTREMELY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Friendly</td>
<td>34. Nervous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Tense</td>
<td>35. Lonely</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Angry</td>
<td>36. Miserable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Worn-out</td>
<td>37. Muddled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Unhappy</td>
<td>38. Cheerful</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Lively</td>
<td>40. Exhausted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Confused</td>
<td>41. Anxious</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Sorry for things done</td>
<td>42. Ready to Fight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Shaky</td>
<td>43. Good Natured</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Listless</td>
<td>44. Gloomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Peeved</td>
<td>45. Desperate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Considerate</td>
<td>46. Sluggish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Sad</td>
<td>47. Rebellious</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Active</td>
<td>48. Helpless</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. On Edge</td>
<td>49. Weary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. Grouchy</td>
<td>50. Bewildered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Blue</td>
<td>51. Alert</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Energetic</td>
<td>52. Deceived</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. Panicky</td>
<td>53. Furious</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. Hopeless</td>
<td>54. Efficient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. Relaxed</td>
<td>55. Trusting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. Unworthy</td>
<td>56. Full of Pep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. Spiteful</td>
<td>57. Bad Tempered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. Sympathetic</td>
<td>58. Worthless</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26. Uneasy</td>
<td>59. Forgetful</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27. Restless</td>
<td>60. Carefree</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28. Unable to concentrate</td>
<td>61. Terrified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. Fatigued</td>
<td>62. Guilty</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. Helpful</td>
<td>63. Vigorous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31. Annoyed</td>
<td>64. Uncertain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32. Discouraged</td>
<td>65. Bushed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33. Resentful</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Figure 7. Profile of Mood States (POMS) questionnaire
Approval Memorandum

Date: 1/26/2011

To: Michael Ormsbee

Address: 1493
Dept.: NUTRITION FOOD AND MOVEMENT SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anabolic hormones, and blood lipids in trained men during 6-weeks of resistance training.

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 01/12/2011. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals, which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 1/11/2012 you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the Chair of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.
This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB00000446.

Cc: Bahram Arjmandi, Chair
HSC No. 2010.5392
Informed Consent Form

Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anabolic hormones, and blood lipids in trained men during 8 weeks of resistance training.

Informed Consent Form

1. I voluntarily and without element of force or coercion, consent to be a participant in the research project entitled “Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anabolic hormones, and blood lipids in trained men during 8 weeks of resistance training.” This study is being conducted by Dr. Mike Ormsbee, Dr. Lynn Panton, and Dr. Jeong-Su Kim who are faculty members and D. David Thomas, Amber Kinsey, and Kyle Mander who are students in the department of Nutrition, Food & Exercise Sciences at The Florida State University.

2. The purpose of the proposed study is to examine how commercially available pre- and post-workout supplements affect body composition, muscle strength and power, anabolic hormones and blood lipids during a 8-week resistance training program. Forty resistance trained men (18 to 40 years of age) will be recruited for this study.

3. My participation in this study will require coming to the Human Performance Laboratory at The Florida State University for testing on four different occasions over 8 weeks to complete the measurements and assessments as described below.

On my first visit, I will be given an informed consent document to sign and a medical history form to complete before I can participate in the study. I cannot participate in this study if I have not been resistance training at least 2 times per week for the past year (with no more than a month break), have uncontrolled hypertension (BP>140/90 mmHg), uncontrolled cholesterol/blood lipid levels or currently take cholesterol medication, diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, or any musculoskeletal complications that would impede me from exercising with weights. In addition, I will be excluded if I currently smoke, take cholesterol medication, nutritional supplements (except for a multivitamin without known ergogenic enhancers), or have any allergies to milk products. If I do take a supplement I will have to go off the supplement for four weeks before I can participate. During the course of the study I cannot go on any additional supplements, will maintain my normal dietary intake pattern, and will not partake in any planned physical activity outside of the research training protocol. I will arrive to the laboratory in a fasted state meaning that I will not eat or drink anything (except for water) for 8 to 10 hours before my appointment.

During this visit, I will then answer questionnaires regarding my mood-state. I will have my blood pressure (BP), height, weight, circumferences, and body composition measured. Height

FSU Human Subjects Committee Approved on 1/26/11. Void after 1/11/12. HSC# 2010.5392
Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anaerobic hormones, and blood lipids in trained men during 8 weeks of resistance training.

and weight will be assessed using a standardized scale. Shoulders, chest, waist, abdominal, hip, calf, thigh and biceps circumference measures will be taken a minimum of two times. My body composition and bone mineral density will be measured using dual-energy X-ray absorptiometry (DXA). Very low doses of radiation are used; however, this test is non-invasive. I will lie on a padded table for approximately 10 minutes while the scan is being completed. Testing will be completed according to the manufacturer's instructions and specifications by a certified X-ray technician. Blood will be drawn under sterile conditions in the amount of 20 milliliters from a forearm vein and finger prick and stored for later analysis. The blood samples will not be used for any other research or testing purposes other than those specified in the research proposal. Peak force and anaerobic power will be measured using the BiodeX™ machine at 180° of isokinetic flexion and extension and the Wingate protocol on a cycle ergometer, respectively. The Wingate protocol requires me to pedal as quickly as possible against resistance for a 30-second sprint.

I will be given food and physical activity record forms (to list all foods and beverages consumed and physical activity completed over 3 days) to fill out and turn in at the start of my exercise training and I will receive instructions on how to complete these forms. This first visit will take approximately 2 hours.

On the second visit, both upper and lower body strength will be assessed using the bench press and leg press exercises, respectively. After warm-up, I will be progressed towards the maximum weight that I can lift 1-time through a full range of motion, also called a 1-repetition maximum (1RM). All measurements will be recorded within three and five attempts and will be supervised by trained personnel. The other lifts that I will be completing during the 8-week training period will be demonstrated to me at this time and I will complete these lifts in order to assess my resistance to be lifted for the training period of this study. This visit will take approximately 60 minutes.

After finishing the baseline testing on visit two, I will be randomly assigned to one of two intervention groups for the duration of the 8-week intervention: 1) Commercial supplementation (S) of NO-Shotgun® ingested 30 minutes prior to each exercise session and Synthesize® ingested immediately after completion of the exercise session and one time per day when convenient on non-training days (at least an additional 1 times per week). 2) Placebo (P) consumption 30 minutes prior to each exercise session, immediately post-exercise, and one time per day when convenient on non-training days (at least an additional 1 times per week). Following each week of the study, I will return all empty containers to the research staff to help verify compliance.

Please Initial

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Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anabolic hormones, and blood lipids in trained men during 6 weeks of resistance training.

The primary ingredients in the pre-workout supplement (NO-Shotgun®) are protein including essential amino acids and branch-chain amino acids, creatine, L-arginine, L-citrulline, beta-alanine, and caffeine (see product label below). The primary ingredients in the post-workout supplement (Synthesiz®) include all of the same ingredients as the pre-workout supplement; however, it does not contain any caffeine (see product label below).

NO-Shotgun® Supplement Label.
Both groups will complete resistance training exercises 3 days per week for 6 weeks. Day 1 will target chest, back, and trapezius muscles with the following exercises: bench press, incline bench press, chest flies, lat pull down, row, and shrugs. Day 2 will target biceps, triceps, and shoulders with the following exercises: biceps curl, alternate curls, overhead triceps extension, triceps press down, shoulder press, and reverse fly. Day 3 will target legs and abdominals with the following exercises: squats, leg press, step-ups, leg curls, knee raise, lunges, abdominal crunch and core plank. Each exercise session will last for approximately 60 minutes and rest periods will be set to no more than 2 minutes between all exercises and sets. The intensity of each workout will progress every 2 weeks. For weeks 1 and 2, 3 sets of 10 repetitions with a load equaling ~75% of 1RM will be used. For weeks 3 and 4, 3 sets of 6 repetitions with a load equaling ~80% of 1RM will be used. For weeks 5 and 6, 3 sets of 4 repetitions will a load equaling ~85% of 1RM will be used. I will record all of my resistance training exercises, weight used, repetitions and sets performed in a weekly training log and return this to the research personnel who will be monitoring my exercise workouts.

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Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anabolic hormones, and blood lipids in trained men during 6 weeks of resistance training.

I will repeat my 3-day food and physical activity diary during the last week of exercise training (week 6) and turn the forms into the research staff.

For visit three, I will arrive to the human performance laboratory in a fasted state between 48 and 60 hours following my last workout and bring with me my completed 3-day food diary. The same testing procedures as were completed during visits one and two will be replicated for visits three and four following the 6-week intervention.

4. I understand there is a minimal level of risk involved if I agree to participate in this study. I may experience some muscle soreness from the 1RM and resistance training sessions. The risks associated with 1RM and exercise training are minimal and the selected protocols have been previously used in other studies. There is the possibility of muscle fatigue or soreness related with resistance training or testing. Although there is a potential risk of muscle injury with maximal strength testing (1RM), the risk will be reduced by including a proper warm-up and rest intervals and by using qualified exercise instructors to supervise testing and training and ensure proper exercise techniques and intensity.

The risk of drawing blood is small and there may be some local discomfort at the site of needle placement with possible bruising or swelling. The risk of local infection is also small. These risks will be minimized by the use of skilled technicians using sterile techniques and equipment.

Body composition will be evaluated by DXA. This involves some radiation of approximately 0.5 millirem (mREM) per total body scan or 1 mREM for both scans. This is much less than a traditional chest x-ray (20-50 mREM) or full dental x-ray (300 mREM). The measurement of body composition using DXA is non-invasive.

The risk of adverse events from these commercially available supplements is also small. NO-Shotgun® and Synthesized® (Vital Pharmaceuticals, Inc, Davie, FL) contain a proprietary blend of a number of potentially hypertrophic compounds including creatine monohydrate, beta-alanine, arginine, alpha-ketoglutarate (KIC), and leucine. Published research on Shotgun® has indicated minor side effects (nausea, rapid heart rate, headache, and shortness of breath) in 4% of people taking either the supplement or the placebo. Concern has also been raised regarding the long-term safety of creatine supplementation; however, research indicates no clinically significant changes from normal values in renal, hepatic, or cardiac safety in studies up to 5 years in length. Previous research on the other primary compounds in the supplement indicates no negative effects on clinical safety markers in whole blood or serum and the...
Effects of pre- and post-exercise intake of performance supplements on body composition, muscle strength and power, anabolic hormones, and blood lipids in trained men during 6 weeks of resistance training.

Compounds are found naturally in whole foods. The primary difference between Shotgun® and Synthesize® is that the pre-workout supplement Shotgun® contains caffeine. The dose of caffeine provided by one serving does not exceed 200 mg per serving which is equal to or less than the amount of caffeine in one cup of coffee. Thus, the risk of supplementation is quite minimal. I am aware that the facility that produces the supplements for this study also manufacture products made from soy, wheat, and grain at the facility. It is possible that cross-contamination could occur, but is unlikely. If I have an allergy to soy, wheat, or grain I must make this known to the research team.

5. The possible benefits of my participation in this research project include knowledge about my body composition, bone mineral density, resting vital measures, body circumferences, upper and lower body muscular strength, anaerobic power, blood lipoic profile and hormone status. Participants in both groups will have the potential to improve metabolic, cardiovascular and muscular health and may improve body composition, physical functioning, and quality of life.

6. The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with the participants name and code number will be kept by the principal investigator, Dr. Michael Ormsbee, in a locked drawer in his office.

7. In case of an injury, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any other treatment or care will be provided at my expense. The researchers involved in this study, the Department of Nutrition, Food, and Exercise Sciences, the Florida State University Athletic Department and Gold’s Gym disclaims any and all liability from and in connection with this exercise training program undertaken in the Tully Gymnasium and Gold’s Gym and in no way will they be held responsible for any injuries that may occur as a result of the exercise training completed for this study.

8. Any questions I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Dr. Michael Ormsbee at (850) 644-4793 (ormsbee@fsu.edu), Dr. Lynn Pantale at (850) 644-4685 (lpantale@fsu.edu) or Dr. Jeong-Soo Kim at (850) 644-4795 (kimjs@fsu.edu) for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

Please Initial

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9. In case of an injury or if I have questions about my rights as a subject/participant in this research, or I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633 (humansubjects@magnet.fsu.edu).

10. The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved.

11. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

__________________________________________________________
Print name

__________________________________________________________
Signature Date

__________________________________________________________
Please Initial

FSU Human Subjects Committee Approved on 1/26/11. Void after 1/11/12. HSC# 2010.5392
REFERENCES


82. McNair DM. Profile of mood states (poms) in evaluation of antianxiety and antidepressant drugs. *Journal De Pharmacologie* 1974; 5:10-10.


BIOGRAPHICAL SKETCH

Personal

William Kyle Mandler
Born October 6, 1986 in Columbus, IN

Education

Fall 2011 M.S. in Exercise Physiology
Florida State University, Tallahassee, FL
May, 2009 B.A. Chemistry, Exercise Science
Hanover College, Hanover, IN
June, 2005 Columbus East High School
Columbus, IN

Professional Experience

2010-2011 Graduate Teaching Assistant
Department of Nutrition, Food, and Exercise Sciences, Florida State University