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The Effects of Pre-and Post-Exercise Consumption of Multi Ingredient Performance Supplements on Cardiovascular Health and Body Composition in Trained Men after Six Weeks of Resistance Training

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THE EFFECTS OF PRE- AND POST-EXERCISE CONSUMPTION OF MULTI-INGREDIENT PERFORMANCE SUPPLEMENTS ON CARDIOVASCULAR HEALTH AND BODY COMPOSITION IN TRAINED MEN AFTER SIX WEEKS OF RESISTANCE TRAINING

By

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To my mother, Colleen O. Johnson
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ABSTRACT

Background: The cardiovascular (CV) and metabolic health benefits or risks associated with consumption of multi-ingredient performance enhancing supplements (MIPS) in conjunction with periodized resistance training (RT) in resistance trained men are unknown. This population is a major target audience for performance supplements, and therefore, the purpose of this study was to investigate the combined effect of RT and commercially available pre- and post-exercise performance supplements, NO-Shotgun® (SHOT) and NO-Synthesize® (SYN), respectively, on CV risk and body composition in resistance trained men. Methods: Twenty-four resistance trained men completed 6 weeks (3 times/week) of whole-body periodized RT while either ingesting SHOT 30 min pre-exercise and SYN immediately post-exercise or an isocaloric maltodextrin placebo (PL) 30 min pre-exercise and immediately post-exercise. Before and after 6 weeks of RT and supplementation, resting heart rate (HR), blood pressures (BP), total body fat, android fat, gynoid fat, fat-free mass (FFM) and fasting blood measures of glucose, lipids, nitrate/nitrite (NOx), cortisol, and high sensitivity C-reactive protein (CRP) were measured. Statistical analysis was conducted using a 2 x 2 (group x time) repeated measures ANOVA. Significance was set at p<0.05. Results: There was no group x time interaction for HR, BP, blood glucose, lipids, NOx, CRP, cortisol concentrations or body fat. However, there was a time effect where significant decreases in body fat (MIPS: -1.2±1.2%; PL: -0.9±1.1%), android fat (MIPS: -1.8±2.1%; PL: -1.6±2.0%), and gynoid fat (MIPS: -1.3±1.6%; PL: -1.0±1.4%) for both groups were observed. FFM increased in both groups, with MIPS increasing significantly more than the PL group (4.2% vs. 1.9%, p=0.0247). Conclusions: Six weeks of MIPS ingestion and periodized RT does not alter CV health parameters or blood indices of health or body fat more than a PL treatment in healthy, resistance-trained men. However, MIPS significantly increased FFM.
CHAPTER ONE

INTRODUCTION

Nutritional supplements intended for consumption before and after resistance training (RT) to improve performance are extremely popular among young men and athletes [1,2]. Given that RT has been shown to increase muscle fiber size and strength in men and women [3] with a concomitant increase in lipolysis and fat oxidation [4], it is not surprising that performance supplements are consumed in conjunction with RT in an attempt to improve body composition and athletic performance. The components of these popular multi-ingredient performance supplements (MIPS) (e.g. protein, branched-chain amino acids (BCAA's), creatine, β-alanine, caffeine, and L-arginine) have been studied individually for their ergogenic effects [5-8]; however, the health and safety outcomes of these supplements are not well known when consumed in combination. Caffeine alone has been shown to increase resting blood pressure (BP) acutely [9], and potentially chronically [10]. Systolic blood pressure (SBP) and HR have also been shown to increase during RT with acute caffeine supplementation in resistance-trained men. However, recent studies evaluating MIPS that contain caffeine did not document the measurement of BP or HR [11-13].

Multiple side effects have been demonstrated with the use of MIPS. Shelmadine et al. [11] investigated eighteen untrained men following 28 days of RT (4x/week) when consuming a MIPS (NO-Shotgun®, Vital pharmaceuticals, Davie, FL) containing a blend of protein, BCAA’s, creatine, β-alanine, caffeine, and L-arginine consumed 30 minutes before RT compared to an isocaloric placebo (maltodextrin) and found side effects including dizziness, nausea, headache, and shortness of breath. Participants taking the placebo also experienced side effects of nausea and shortness of breath. Interestingly, the authors did not report blood pressure or heart rate data, which may be important given these side effects and anecdotal evidence portraying some of these pre- and post-RT products as dangerous. However, fasting blood lipid profile and glucose were unchanged in either group and significant increases in fat-free mass (FFM) were reported with no change in body fat percentage. This improvement in body composition likely overrides the potential negative consequences to using MIPS.
In a follow-up study, Spillane et al. [12] studied untrained men and investigated the same pre-RT supplement (NO-Shotgun®, Vital Pharmaceuticals, Davie, FL) as Shelmadine et al. [11] and added consumption of an additional MIPS (NO-Synthesize®, Vital Pharmaceuticals, Davie, FL) immediately post-RT and all on non-RT days. The authors reported similar side effects for these untrained participants including nausea, rapid heart rate, and shortness of breath regardless of being in the MIPS or placebo groups [12]. Again, blood pressure and heart rate data were not reported but significant increases in FFM (MIPS: Day 0, 57.8 ± 6.4 kg vs. Day 28, 59.9 ± 7.6 kg; placebo: Day 0, 56.4 ± 10.3 kg vs. Day 28, 57.0 ± 9.9 kg, p=0.23) were found in the MIPS group when compared to both baseline measures and the placebo group after 28 days. In addition, whole blood and serum clinical chemistry markers, regardless of group, remained in the normal ranges in this study [12]. The health implications of MIPS use in trained men are not well documented.

The importance of this research is continually growing as recently, popular, commercially available MIPS containing the pharmaceutical amphetamine derivative 1,3-dimethylamylamine (DMAA), were linked to two cases of myocardial infarction in physically active young men during exercise [14]. Some studies have also linked DMAA to cerebral hemorrhaging [15]. While many consumers may not be aware of its presence, nearly 200 commercially available MIPS contain DMAA [16]. While the MIPS in the present study do not contain DMAA, more research is necessary to examine MIPS which in many cases have an ever growing list of ingredients as supplement companies look to distinguish their products in an effort to boost sales.

Therefore, the purpose of the present study was to evaluate the effects of six weeks of pre- and post-RT MIPS consumption with periodized RT on cardiometabolic health and body composition in resistance-trained men. We hypothesized that the inclusion of a pre- and post-RT MIPS with resistance-trained participants would not alter CV markers of health, but would improve body composition more than a placebo supplement.
Specific Aims

1. To determine changes in cardiometabolic health via circulating blood markers and resting blood pressures and heart rate in response to MIPS and six weeks of resistance exercise training in healthy trained men.
   a. Fasting blood measurements were analyzed using a Cholestech LDX Analyzer® and include total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and glucose.
   b. Measures of nitrate/nitrite, cortisol, and high-sensitivity c-reactive protein were determined by enzyme-linked immunosorbent assays.
   c. Resting systolic and diastolic blood pressures were measured using sphygmomanometry.
   d. Resting heart rate was measured manually for 60 seconds at the radial artery.

2. To evaluate the effects of six weeks of MIPS consumption in combination with periodized resistance training on body composition in resistance-trained men.
   a. Fat-free mass and fat mass were measured using dual energy x-ray absorptiometry (DXA).
Research Hypotheses

1. Both groups would improve body composition with the changes experienced by MIPS being greater than PL due to an increase in FFM.
2. Neither group would experience changes in cardiometabolic variables including fasting blood lipids and glucose, nitrate/nitrite, cortisol, high-sensitivity C-reactive protein (CRP), and resting blood pressures (BP) and heart rate (HR).

Assumptions

The following assumptions were made in this study:

1. Participants consumed their post-exercise supplement on all non-training days.
2. Participants put forth a maximal effort during testing and training sessions.
3. Participants provided truthful answers on all questionnaires.
4. All laboratory equipment provided reliable results and was used properly by researchers.
5. Participants followed instructions provided by researchers prior to testing sessions.
Delimitations

Delimitations included the following:

1. The study included only men aged 18-40 years who had been resistance training at least two times per week for the last year prior to the study.
2. Individuals taking blood pressure or cholesterol medication or diagnosed with hypertension, dyslipidemia, cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction were excluded.
3. Individuals with any musculoskeletal injuries or complications that would prevent resistance training were excluded.
4. Individuals who regularly smoke or chew tobacco, take nutritional supplements with known ergogenic effects, or have allergies to milk products were excluded.

Limitations

The major limitations of the study included:

1. The inability to control participants’ diet and physical activity outside of supervised training. Participants self-reported this information.
2. Participants did not have identical athletic/training backgrounds. However, they met our criteria for inclusion.
3. Participants who had taken performance supplements in the past may have detected whether they were taking the placebo or not.
CHAPTER TWO

LITERATURE REVIEW

Resistance Training

Resistance training (RT) is a form of exercise that has been shown to provide multi-dimensional benefits for its practitioners. Well known benefits include increases in muscle size and strength [3]. However, the spectrum of adaptations arising from RT can range from muscular strength and endurance to improvements in bone density, body composition, and overall health. Exercises completed at 3-5 repetition maximum (RM) cause greater gains in hypertrophy and muscular strength while 20-28 RM is shown to improve muscular endurance [21].

Because myofibers are terminally differentiated, increases in muscle size must occur through increased myofiber size [22]. Protein synthesis facilitates the repair and augmentation of myofibers [23]. However, post-exercise protein synthesis appears to be inversely related to training status, with untrained individuals exhibiting greater protein synthesis after RT [24,25]. Myogenic stem cells, called satellite cells, are also stimulated by exercise-induced muscle damage to increase myonuclei number and repair muscle [22]. Increases in myonuclei concentration may be the central cause of further hypertrophy in resistance-trained individuals [26].

RT also has beneficial effects on body composition and has been shown to increase subcutaneous abdominal adipose tissue lipolysis and whole body fat oxidation after acute bouts [4]. Increases in lean body mass (LBM), via hypertrophy, also improve body composition [27]. Furthermore, increases in LBM with chronic RT can lead to increased resting metabolic rate (RMR) and decreased body fat percentage [28]. Lesser-known benefits of RT include decreased blood pressure [29,30] and improvements in blood lipid profile and mental health status [31,32].
Supplements to Augment Responses to RT

**Protein**

Acute [33] and chronic [34] protein supplementation in conjunction with RT has been shown to stimulate muscle protein synthesis beyond that of RT alone. The basic principle of muscle fiber hypertrophy is a greater rate of muscle protein synthesis compared to degradation [34]. This increase in net protein balance has been shown with both whey and casein protein [33] and certain specific amino acids such as leucine [35]. Muscle protein anabolism is also greater when the protein supplement is taken immediately post-exercise compared to 2 hours post-exercise due to increased amino acid availability [36]. Carbohydrate availability has also been shown to be vital to net protein anabolism [37].

Cribb et al. [38] reported significant improvements in body composition and upper and lower body maximal strength in resistance-trained men supplementing with whey isolate and creatine monohydrate immediately before and after the RT exercise when compared to supplementation in the morning and evening. After 8-12 weeks of familiarization, participants completed 10 weeks of progressive RT designed to induce gains in muscle strength and hypertrophy. The group that consumed the supplement immediately rather than delaying, increased lean mass significantly (Pre, 69.5±2.3; Post, 72.3±2.3kg, p=0.002) while the morning and evening supplementation group did not increase significantly (Pre, 65.2±1.5; Post,66.7±1.5kg ).

Andersen et al. [34] compared RT (3x/wk for 14 week) with protein supplementation vs. a carbohydrate placebo in recreationally active men. These authors concluded that protein supplementation significantly increased muscle hypertrophy of both type I and type II myofibers (18±5%, p <0.01 and 26±5%, p<0.01, respectively). However, the carbohydrate supplementation group had no changes above baseline in muscle hypertrophy. In another study, using resistance-trained men, Cribb et al.[5] reported protein type to be critical to changes in body composition with RT. After 10-weeks of high-intensity, progressive overload RT (3x/week) and supplementation with whey or casein proteins, the whey isolate group gained
significantly more lean mass (+5.0±0.3kg vs. +0.8±0.4kg, p<0.01) and lost significantly more fat mass (FM; -1.5±0.5kg vs. +0.2±0.3kg, p<0.05) than the casein group.

**Branched-chain amino acids**

Similar to the effects of protein supplementation, increased branched-chain amino acid (BCAA) availability dramatically improves muscle protein synthesis [36]. The BCAA’s (leucine, isoleucine, and valine) are among the nine essential amino acids (EAA’s) in humans and comprise one-third of muscle protein [39,40]. A central difference of BCAA’s from other amino acids is their ability to be more readily metabolized in skeletal muscle [39]. Thus, BCAA supplementation can decrease muscle protein degradation that, in turn, improves recovery from exercise [41,42]. Furthermore, it is thought that BCAA’s are the primary amino acids oxidized during intense exercise [43]. For these reasons, BCAA’s have been studied and used for their effects when taken before and/or after RT [35,44].

BCAA’s also reportedly decrease concentrations of the catabolic stress hormone, cortisol [45,46] which is released acutely following RT bouts [47]. BCAA supplementation may also decrease cortisol following a week of intense RT. Sharp and Pearson [46] demonstrated decreased cortisol concentrations in recreationally active young men after three weeks of BCAA supplementation (6g/day) followed by a fourth week of supplementation and four bouts of RT (three sets of 6-8 reps with 80% 1RM and 60 sec between sets and exercises, three lower- and five- upper body exercises). The authors reported significant decreases in cortisol 12 hrs after RT bout two, and 12 and 36 hrs after RT bout four.

While leucine is thought to be responsible for most ergogenic effects of BCAA’s, its effects on body composition are questionable. In one RT study [48] using 26 untrained men, leucine supplementation did not increase LBM or decrease FM any more than the lactose placebo (LBM: leucine, 2.9±2.5% vs. placebo, 2.0±2.1%; FM: leucine 1.6±15.6% vs. placebo 1.1±7.6%). However, leucine supplementation has been shown to increase LBM with 12 weeks of RT (5x/wk) by decreasing protein degradation and increasing nitrogen retention, both leading to positive nitrogen balance [49]. These
researchers implemented a higher-volume, more frequent training protocol, which may account for differences seen in their data when compared to other studies.

**Creatine**

Creatine supplementation shows consistently beneficial but varying results with the implication that individuals respond differently to supplementation [50-52]. Creatine is an amino acid derivative found in the body, with particularly high concentrations in skeletal muscle. However, skeletal muscle cannot synthesize creatine and must be transported from the liver. The ergogenic effect of creatine is centered on its affinity to bind with inorganic phosphate and donate its phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) through creatine kinase. Thus, creatine can provide a rapid source of available ATP to be used for short-duration, explosive activities. Creatine has been demonstrated to increase peak strength in only nine days with three sessions of isokinetic RT in college-aged men [6]. This study suggests creatine augments neural adaptations to RT. Additionally, 16 weeks of RT with creatine supplementation has also been reported to increase muscle satellite cell activity and myonuclei concentration, leading to hypertrophy, more than RT with protein and/or carbohydrate supplementation [53]. Significant increases in lean mass have also been seen in as little as four weeks of creatine supplementation and RT [54]. In this study, college football players participated in intense (8hrs/wk) resistance and agility training with or without creatine monohydrate consumption (15.75 grams/day). Gains in LM were greater in with consumption of creatine monohydrate compared to a placebo (2.43±1.4kg vs. 1.33±1.1kg, p<0.05).

**Beta-Alanine**

Beta (β) -alanine is the limiting amino acid in the synthesis of the dipeptide, carnosine, which is a known H+ buffer [55]. Supplementation of β-alanine increases intra-muscular carnosine concentrations [56], whereas intact carnosine is not transported into the muscle and is instead hydrolysed in plasma by carnosinase [57]. Short-term (30 days) β-alanine supplementation has been shown to increase muscular endurance and decrease feelings of fatigue in resistance-trained males [58,59].
Carnosine’s buffering effects, allow β-alanine supplementation to improve anaerobic performance by controlling pH without changes in body composition [8]. This is likely due to a lack of change in anabolic hormone concentrations. Hoffman et al. [60] reported that 30 days of β-alanine supplementation (4.8 grams/day) did not affect the acute endocrine response to RT in resistance-trained men. Participants performed six sets of 12 repetitions of squats at 70% 1RM. The group taking β-alanine completed 22% more volume over the duration of the study compared to the placebo group (p<0.05). However, growth hormone, testosterone, and cortisol were not different between groups at baseline or post RT bouts. Another recent study demonstrated that 8-weeks of β-alanine supplementation and high-intensity interval training (HIIT; 75-100% of maximal effort for 5-30 seconds with 60-120 seconds rest in between intervals) can potentially improve body composition more than a placebo and HIIT [61]. Both groups of collegiate wrestlers in this study experienced similar improvements in body fat (supplement, -1.9±1.5%, vs. placebo, -2.2±2.8%). However, the experimental group gained 2.2±9.5kg of LBM versus the placebo group, which decreased LBM (-2.2±6.4kg). Although statistically insignificant, this gain in LBM is still important to consider, especially in a weight-dependent sport. It is also imperative to note that the training was part of the collegiate wrestling pre-season and not a laboratory training intervention.

**Caffeine**

Under normal physiological conditions, caffeine blocks central nervous system (CNS) adenosine receptors, which in turn delays fatigue [62]. This is the main mechanism that results in performance benefits with caffeine ingestion as opposed to the mixed results of studies surrounding fat-metabolism and muscle-glycogen sparing [7,62,63]. Wu et al. [64] reported that an acute dose of 6g of caffeine/kg of body weight attenuates post-exercise GH response while increasing plasma free fatty acid (FFA) concentration in resistance-trained men. Acute caffeine supplementation has also been shown to increase upper body strength (bench press 1RM) [17] and delay fatigue and possibly blunt pain responses during high-intensity resistance training [7]. Beck et al. [17] used an acute dose of 200mg of caffeine one-hour prior to exercise in resistance-trained men to elicit a 2.1% improvement in bench press 1RM while the placebo had no
effect. There was also a trend (p=0.074) for the caffeine group to bench press more total volume. However, neither group showed any change in leg press 1RM or leg press volume lifted.

There are also known placebo effects of caffeine. Duncan et al. [65] reported that individuals who thought they were ingesting caffeine lifted more total weight than when compared to the control condition of expecting placebo and receiving placebo (caffeine, 713.8±120.8kg vs. control, 576.9±100.8kg). Participants who expected to receive caffeine also reported significantly lower Rating of Perceived Exertion (RPE) values compared to the group expecting the placebo solution (p=0.04). Caffeine has also been shown to improve mood state in acute bouts of RT to failure [66].

Arciero et al. [67] reported no changes in mood state in healthy young men (ages 19-26 years) after caffeine supplementation (5mg/kg fat-free mass) without involvement of exercise. The authors also report no changes in systolic or diastolic blood pressures. However, these participants were habitual users of caffeine who were tested after 48 hours of abstaining from caffeine consumption. This supports the work by Robertson et al. [68] showing a cardiovascular tolerance in habitual caffeine consumers.

Acutely, caffeine is known to increase resting BP in some healthy men and women [9]. Additionally, systolic blood pressure (+ 8-10 mmHg) and HR (+ 10 beats/min) are elevated during RT in resistance-trained men. It has been suggested that the acute and chronic cardiovascular effects of caffeine are greatly influenced by the variability of caffeine metabolism among individuals [70]. The mechanisms influencing blood pressure increases from caffeine consumption may include its antagonistic effects on the adenosine receptor, the inhibition of phosphodiesterase, the release of catecholamines from the adrenal medulla, the release of corticosteroids from the adrenal cortex, and renal effects including the activation of the renin-angiotensin-aldosterone system [70].

Supplementation with caffeine has also been investigated for its thermogenic effect [71]. Acheson et al. [72] reported an increased metabolic rate three-hours following ingestion of 8g of caffeine/kg of body weight compared to a placebo in normal weight individuals (p<0.02). The subsequent trials in this study used a smaller dose of
4g/kg to compare coffee’s metabolic affects in normal weight and obese individuals. Metabolic rate increased in both groups, however, increased fat oxidation and plasma FFA were only seen in the normal weight individuals. In comparing caffeine’s effects in normal weight individuals, the 8g/kg dose increased metabolic rate by 16% while half that dose (4g/kg) consumed in coffee also increased metabolic rate by 12%. The authors also report that individual responses varied greatly. Thus, it is likely that caffeine may improve RT performance and increase thermogenesis resulting in alterations to weight lifted and body composition over time.

**L-Arginine**

L-arginine is a conditionally EAA integral to the production of nitric oxide (NO) [73]. The NO synthase enzymes convert L-arginine into NO and L-citrulline. NO is known for its vasodilatory effects caused by elevating intracellular concentrations of cyclic guanosine monophosphate (cGMP) thus, relaxing the smooth muscle [74]. This is thought to increase blood flow to muscle tissue leading to increased delivery of nutrients to the muscles. Subsequently, L-arginine is purported to improve anaerobic power but not aerobic capacity in resistance-trained men [75]. In this study, 1-RM bench press and cycle ergometer peak power significantly improved while systolic and diastolic blood pressures, oxygen consumption, ventilation, and respiratory exchange ratio were no different compared to the placebo after 8-weeks of L-arginine supplementation [75]. Other studies have reported L-arginine to decrease muscle endurance [76]. It is evident that RT intensity and duration must be considered concerning acute supplementation with L-arginine. Chronic supplementation with L-arginine should also be considered for possible links to NO-mediated activation of muscle satellite cells to induce skeletal muscle repair following intense RT [77].

**Multi-Ingredient Performance Supplements (MIPS)**

Product-specific multi-ingredient supplement research is a necessary aspect of exercise and nutrition performance science. It allows consumers to review and compare actual supplements as opposed to their individual ingredients. It also allows
supplement companies to support claims based on placebo-controlled, double blind, and randomized scientific data instead of anecdotal evidence.

Recently, two studies examined the acute [19] and chronic [13] effects of pre-exercise supplementation with MPIS including whey protein, BCAAs, creatine, and caffeine (40kcal, 8g PRO, 2g CHO, 2100mg proprietary blend; Game Time®, Corr-Jensen Laboratories Inc., Aurora CO). Both studies used treadmill running as the mode of exercise. The authors reported a 10-12% longer time to exhaustion (TTE) at speeds of 100, 105, and 110% VO₂max with an acute dose of the multi-ingredient supplement compared to the placebo group in recreationally active young men and women. A non-significant trend (p=0.08) in TTE was also observed at 90% VO₂max following acute ingestion of the multi-ingredient supplement. The authors concluded that the multiple ingredients of this supplement combined to have this ergogenic effect with creatine and caffeine playing the primary roles of augmenting the phosphagen system and delaying fatigue [19].

Supplementation of the same combined supplement (Game Time®) with three-weeks [13] of high-intensity interval training (HIIT; 5 sets of a 2:1, work:rest ratio) resulted in significant improvements in LBM (1.2kg, p=0.035), and training volume (11.6% higher than placebo, p=0.041). Conversely, the placebo group only maintained LBM over the three-week period. This suggests MIPS help to improve running performance as well as body composition with three-weeks of HIIT in moderately-trained young men. Previous literature supports the use of BCAA supplementation to improve body composition [41,42]. Similar to increased TTE in the acute study mentioned above [19], Smith et al. [13] suggest that caffeine plays a central role increasing training volume by decreasing fatigue and blunting pain response [7,62].

Bloomer and colleagues [20] investigated a MIPS containing glycine propionyl-L-carnitine (GlycoCarn, Sigma-tau Health Science, Gaithersburd, MD) along with three other pre-exercise supplements also purported to increase NO concentrations. Each of the three other supplements contained proprietary blends of creatine, BCAA’s, caffeine, and L-arginine. Subjects ingested one of the four supplements or a carbohydrate placebo before each acute exercise testing session. Bench press power (bench press throw) and endurance (10 sets to failure of bench press) were measured to evaluate
performance while muscle tissue oxygen saturation was measured, through near infrared spectroscopy, to indicate blood flow and oxygen delivery. Plasma nitrate/nitrite levels and subjective muscle pump were also measured to estimate serum NO values and subjective feelings of workout impact, respectively. The data indicated no significance differences between the four pre-exercise supplements and a carbohydrate-placebo. This study refutes the specific claims that these four supplement manufacturers make, including, improved performance, blood flow, muscle pump, and NO concentrations.

Hoffman et al. [78] used supplementation with MIPS (BCAA’s, creatine, and caffeine) to acutely augment post-exercise serum growth hormone concentrations in resistance-trained men. In this study, participants performed six sets of no more than 10 repetitions at 75% of their 1RM for squat. Participants were given relatively short rest periods of two minutes in between sets. The group consuming the supplement tended to complete more repetitions (p=0.08) and have a higher training volume (p=0.09) compared to the maltodextrin placebo. These authors suggest the increased volume lifted lead to increased serum growth hormone concentrations immediately and 15-minutes post RT. It is interesting to note testosterone was also measured but no difference was found between the two groups.

Another recent study examined the effects of a pre-exercise supplement, containing whey protein, creatine, BCAA’s, caffeine, β-alanine, and L-arginine, compared to an isocaloric placebo (maltodextrose) during four weeks of RT in young, sedentary men [11]. The RT program consisted of two upper and two lower body RT days each week for a total of 16 RT workouts over 28 days. Subjects were instructed to perform 10 repetitions of each exercise at 70-80% 1RM with two-minute rest intervals between each exercise and set. However, subjects were not supervised during RT. Significant increases in total body mass were reported in both groups, however, the supplement provided a larger, albeit non-significant change (placebo, 1.4±0.9%, vs. supplement, 2.6±1.7%; p=0.06). These authors also reported significant increases in fat-free mass (4.8±1.5%, p=0.001) and upper body strength (8.8±5.3%, p=0.003) after RT with the MIPS when compared to the placebo. Increases in lower body strength
were quantitatively greater with the MIPS compared to the placebo, but still statistically insignificant (p=0.10).

Other promising findings from this study include significant increases in myogenic regulatory factors involved in satellite cell proliferation (Myo-D) and differentiation (MRF-4). Satellite cell proliferation and differentiation has been shown to increase muscle hypertrophy by increasing the number of myonuclei [26,79].

Spillane et al. [12] employed an identical study design and RT regimen as the aforementioned study conducted by Shelmadine et al. [11] to investigate the effects of the same pre-exercise MIPS in combination with a post-exercise MIPS containing whey protein, BCAA’s, creatine, β-alanine, and L-arginine. On training days, participants consumed the pre- and post-exercise supplements 30 minutes prior and immediately after RT. Participants in the placebo group consumed the isocaloric matodextrose supplement in the same fashion. The experimental group yielded a 3.7% gain in fat-free mass (Pre, 57.8±8.0 vs. Post, 59.9±7.6kg) that was significantly greater (p=0.023) than the gains of the placebo group (Pre, 56.4±10.3 vs. Post, 57.0±9.9kg). Participants consuming the pre- and post-exercise MIPS also preferentially improved fat mass and body fat percentage over the placebo group (p=0.026, p=0.014, respectively). Identical to the work by Shelmadine et al [11], participants were non-resistance-trained and unsupervised during supplementation and RT.

Interestingly, changes in blood pressure and heart rate were not reported in either of these previous studies, however, no significant differences occurred in TC, HDL, LDL, VLDL, TRG, or glucose as a result of training or supplementation in either group. Therefore, the use of these MIPS in untrained individuals appears to be safe for blood lipids and glucose, however, the impact on blood pressure and heart rate are unknown, particularly among trained individuals.

**Side Effects**

Some studies investigating MIPS have seen side effects associated with supplementation in a small number of participants. Supplementation with protein and creatine during a 12-week RT program has been shown to cause mild symptoms of bloating, cramps, and diarrhea [80]. In a study by Shelmadine et al. [14], participants
supplementing with a MIPS including whey protein, creatine, BCAA’s, caffeine, β-alanine, and L-arginine prior to RT reported dizziness, nausea, headache, rapid HR, shortness of breath, and nervousness. However, participants supplementing with the carbohydrate placebo also reported nausea, rapid HR, and shortness of breath. Spillane et al. [15] reported feelings of dizziness, nausea, headache, rapid HR, shortness of breath, and nervousness in participants supplementing with MIPS before and after RT. Again, the placebo group also reported feelings of nausea, rapid HR, and shortness of breath. It should be noted that the authors in each of the three previously discussed studies all reported that side effects did not cause participants to miss RT or supplementation.

**Summary**

Often, a wide variety of ingredients are included in commercial pre- and post-exercise supplements. Due to differences in study design, laboratory measures, participant training status, supplement composition, dosage and timing, it is difficult to say with certainty how a MIPS may affect participants that are already resistance-trained. The limited pool of research on MIPS suggests that they may be beneficial and safe for novice or untrained individuals. However, their efficacy in individuals who are already resistance-trained remains to be studied.
CHAPTER THREE

METHODS AND MATERIALS

Participants

Twenty-four healthy, resistance trained (≥3x per week ≥12 months) men (24.0 ± 2.3 yrs, height of 180.5 ± 5.8 cm, body mass of 83.7 ± 0.5 kg, and body mass index, BMI, of 25.5 ± 2.2 kg/m²) were recruited through advertisements and flyers. Participants were non-smokers and had no known existing diseases, musculoskeletal disorders or injuries, uncontrolled hypertension (BP>140/90 mmHg), uncontrolled cholesterol/blood lipid levels, use of cholesterol medication, or dairy allergies as assessed by a medical history questionnaire. Those taking anabolic steroids or supplements were excluded because they intended to alter androgen levels. Use of other performance supplements (e.g. caffeine and creatine monohydrate) required a 4-week washout period\[81\] prior to participation (excluding multivitamins). Participants were instructed not to take any supplement, other than the provided pre- and post-RT supplements, for the duration of the study. The study was approved by The Florida State University’s Human Subjects Institutional Review Board (Appendix F), and each participant provided written informed consent prior to participation (Appendix G).

Study design and supplementation protocol

Participants were assigned to one of two groups in this placebo-controlled, double blind, randomized 6-week protocol after being matched for isometric maximal voluntary contraction strength to fat-free mass ratio. Participants consumed either 21 g of NO-Shotgun® (SHOT: containing whey protein, BCAA’s, creatine, caffeine, β-alanine, and L-arginine; Appendix A) and 21 g of NO-Synthesize® (SYN: containing whey protein, BCAA’s, creatine, β-alanine, and L-arginine; Appendix B) or 42 g of an isocaloric, flavor-matched maltodextrin placebo (PL). MIPS consumed 21 g/day of SHOT 20-30 minutes before RT and 21 g/day of SYN immediately post-RT. The PL group consumed 21 g 20-30 minutes before RT and 21 g immediately post-RT. On non-training days, MIPS consumed 21 g/day of SYN and the PL group consumed 21 g/day of placebo. To increase compliance, all supplements were distributed in identical
single-serving containers before and after each workout and ingested in the presence of research personnel. On non-workout days, participants were given identical single-serving containers to take home and consume. Empty containers were collected and participants were asked to verbally verify supplement compliance on each workout day. An example of the empty container collection verification may be found at the bottom of the training log in Appendix C.

**Resistance training protocol**

All participants completed three-days per week of RT for six weeks. The RT protocol was designed to target all of the major muscle groups (chest, back, trapezius, biceps, triceps, shoulders, legs, and abdominals) and was modified from our previous research [4,82] and that of others [12]. Prior to each exercise session, participants performed a standardized 5-minute warm-up on a treadmill. For weeks one and two, three sets of 10 repetitions of each exercise were completed at 70-75% 1RM. For weeks three and four, three sets of six repetitions of each exercise were completed at 80-85% 1RM. For weeks five and six, three sets of four repetitions of each exercise were completed at 85-90% 1RM. Day one exercises included flat bench press, incline bench press, chest fly, latissimus pull down, seated row, and shrugs. Day two exercises included leg press, step-ups, leg curl, heel raise, lunge, abdominal crunch, and plank (held 60 seconds). Day three exercises included biceps curl, alternate curl, overhead triceps extension, triceps press down, shoulder press, and reverse fly. Rest intervals were 60-90 seconds between sets and 120 seconds between exercises. An example of the training log for weeks one and two may be found in Appendix C.

**Laboratory testing**

Body composition, CV, and blood measures took place following a 10- to 12-hour fast and a 24- to 48-hour restriction of physical activity, caffeine, and alcohol intake at baseline and after 6 weeks of RT and dietary supplement intervention. Six-week testing (post) took place 24-48 hours after conclusion of RT to avoid any residual impact of the last training session on dependent variables.
Anthropometrics and body composition

Height and body mass were measured using a wall-mount stadiometer and electronic scale, respectively (SECA, Birmingham, UK). Total fat mass (FM), FFM, and regional (android and gynoid mass) body composition was determined by dual energy x-ray absorptiometry (DXA; model DPX-IQ; GE Medical Systems; Madison, WI) with subjects in the supine position as previously described [83]. Coefficient of variation for body composition analysis using DXA in our laboratory for lean body mass (LBM) and FM was 1.9% and 1.5% respectively, based on three repeated measures of 10 physically active young men. The quality analysis for the densitometer was conducted on a daily basis using a standard aluminum spine block (phantom) provided by the manufacturer. Measurements of the phantom were within the manufacturer’s precision standard with a coefficient of variation <0.5%.

Heart rate and blood pressure

Systolic and diastolic blood pressure was measured twice by the same investigator and averaged using a manual sphygmomanometer (American Diagnostic Corp., Hauppauge, NY) and stethoscope. Heart rate was measured manually at the radial artery for 60 seconds.

Cardiovascular and metabolic biomarkers

Venous blood samples (~10 ml) were obtained before and after six weeks of RT and supplementation with MIPS or PL. Blood samples were collected into either no preservative (serum) or EDTA-coated (plasma) vacutainer tubes (Becton, Dickinson & Company, Franklin Lakes, NJ) and centrifuged (IEC CL3R Multispeed Centrifuge, Thermo Electron Corporation, Needham Heights, MA) for 15 minutes at 3500 rpm at 4°C. Plasma and serum was then separated and subsequently stored at -80°C in 300 µL aliquots until analyzed. Blood was analyzed for total cholesterol (TC), high-density lipoproteins (HDL), TC/HDL ratio, non-HDL (VLDL + LDL), low-density lipoproteins (LDL), triglycerides (TRG) and blood glucose (Cholestech LDX Analyzer; Cholestech Corp, Hayward, CA). Total nitrate/nitrite (NOx), high-sensitivity C-reactive protein (CRP), and cortisol were determined in triplicate (NOx was in duplicate) using

**Profile of Mood States (POMS)**

POMS scores were used to measure mood before and after the six-week intervention. These scores are compiled based on responses to 65 mood descriptors and divided into six categories describing tension, depression, anger, vigor, fatigue, and confusion. Participants were asked to consider their mood over the previous seven days in their responses. The POMS questionnaire may be found in Appendix D.

**Dietary analysis**

Dietary analysis was measured using three-day food records before training and during the last week of the study. Participants were asked to maintain their normal eating patterns and habits. FoodWise™ dietary analysis software (McGraw-Hill, New York, NY) was used for analysis. Each participant was asked to mimic their pre-baseline day of eating prior to post-testing to the best of their ability based off of their dietary food logs. The instructions for the three-day food may be found in Appendix E.

**Reported side effects from supplements**

Participants were asked at each exercise session about any supplement side effects. In addition, they were asked to fill out a questionnaire regarding any side effects at the end of testing (six weeks).

**Supplement analysis**

To verify the absence of anabolic steroids, other illicit substances, and concentration of caffeine, the supplements and placebo were sent to a third-party laboratory for analysis (West Chester University of Pennsylvania, West Chester, PA). The samples submitted for analysis were mixed thoroughly and test samples weighed out. Each of these test samples was treated independently and studied in replicate. The analytes of interest were extracted into methanol using liquid-liquid extraction and quantitatively analyzed using gas chromatography-mass spectrometry (GC-MS). A
Varian CP 3800 gas chromatograph- Saturn 2000 mass spectrometer with a Rxi-5Sil MS (30 m x 0.25 mm ID x 0.25 µm) column was used for the analyses. Standards of caffeine purchased from Cerilliant (Round Rock, TX) were used to generate instrument mass-response graphs used for quantitation.

Statistical analysis

Data were analyzed using a 2 x 2 (group x time) analysis of variance (ANOVA) with repeated measures. A Tukey post hoc test was used to identify significant differences when a significant F-ratio was obtained. Significance is reported at p < 0.05, and all values are reported as means ± standard deviation (SD). JMP Pro 9 statistical software (SAS Institute Inc., Cary, NC) was used for all analyses.
CHAPTER FOUR

RESULTS

Participant characteristics

Twenty-nine participants began the study; however, five withdrew over the course of the six-week protocol (three for personal reasons, one was found to be a smoker, and one did not follow the RT protocol). Therefore, 24 participants completed the study. MIPS (n=13) had an average (mean ± SD) age of 23.6 ± 3.5 yr, height of 180.6 ± 6.7 cm, and body mass of 83.4 ± 11.5 kg. PL (n=11) had an average age of 23.6 ± 4.6 yr, height of 181.0 ± 4.7 cm, and body mass of 82.2 ± 7.2 kg. Groups were not different at baseline (Table 1).

Table 1. Participant characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>MIPS</th>
<th>PL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.6 ± 3.5</td>
<td>23.6 ± 4.6</td>
<td>0.9576</td>
</tr>
<tr>
<td>Training (years)</td>
<td>6.1 ± 3.4</td>
<td>4.5 ± 3.7</td>
<td>0.2914</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.6 ± 6.7</td>
<td>181.0 ± 4.7</td>
<td>0.8557</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>83.4 ± 11.5</td>
<td>82.2 ± 7.2</td>
<td>0.7674</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 2.2</td>
<td>25.1 ± 2.0</td>
<td>0.6641</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Multi-Ingredient Performance Supplement, MIPS. Carbohydrate placebo, PL. Level of statistical significance, p.
Body composition

Total body mass was increased in both groups (p< 0.0001) but more so in MIPS than PL (p= 0.0238). Fat-free mass was increased in both groups (p< 0.0001); however, the increases were greater with MIPS (p= 0.0247; Figure 1). Total body (p= 0.0005), android (p= 0.0009), and gynoid (p= 0.0019) fat percentages all significantly decreased with training, with no group by time differences (Figure 1 and Table 2). However, post-hoc analysis indicated significant (p<0.05) decreases for MIPS while PL remained unchanged.

Figure 1. Body composition. Data are mean ± SE. * Indicates a significant difference from baseline. † Indicates a significant difference between groups (p < 0.05) Multi-Ingredient Performance Supplement, MIPS. Carbohydrate Placebo, PL.
Table 2. Body composition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Post</th>
<th>Time</th>
<th>Group x Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass</td>
<td>MIPS*</td>
<td>83.4 ± 11.5</td>
<td>85.8 ± 11.8</td>
<td>&lt;.0001*</td>
<td>0.0238†</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>82.2 ± 7.2</td>
<td>83.3 ± 7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>MIPS*</td>
<td>66.8 ± 9.2</td>
<td>69.6 ± 9.2</td>
<td>&lt;.0001*</td>
<td>0.0247†</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>66.9 ± 5.3</td>
<td>68.2 ± 6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat %</td>
<td>MIPS</td>
<td>20.7 ± 4.0</td>
<td>19.5 ± 3.8</td>
<td>0.0005*</td>
<td>0.6820</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>20.2 ± 5.0</td>
<td>19.2 ± 4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Android Region Fat %</td>
<td>MIPS</td>
<td>22.3 ± 7.0</td>
<td>20.5 ± 6.8</td>
<td>0.0009*</td>
<td>0.8459</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>20.6 ± 8.7</td>
<td>19.0 ± 8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gynoid Region Fat %</td>
<td>MIPS</td>
<td>20.8 ± 4.3</td>
<td>19.5 ± 3.7</td>
<td>0.0019*</td>
<td>0.6662</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>21.1 ± 6.1</td>
<td>20.1 ± 5.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD. * Indicates a significant difference from baseline. † Indicates a significant difference between groups (p < 0.05). Multi-Ingredient Performance Supplement, MIPS. Carbohydrate placebo, PL.

Heart rate and blood pressure

Systolic (MIPS: pre 120 ± 10, post 117 ± 14; PL: pre 116 ± 7, post 115 ± 10 mmHg) and diastolic (MIPS: pre 74 ± 10, post 72 ± 10; PL: pre 69 ± 8, post 67 ± 9 mmHg) blood pressures were unchanged as a result of training in either group. Resting heart rate also did not change as a result of training or supplementation (MIPS: pre 62 ± 8, post 66 ± 12; PL: pre 59 ± 6, post 61 ± 11 beats per minute).

Cardiovascular and metabolic biomarkers

Blood markers of CV and metabolic health were within normal clinical ranges for the entirety of this study. There were no time or group x time (p>0.05) effects observed for any of the measures (Table 3).
Table 3. Serum measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Post</th>
<th>Time</th>
<th>Group x Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>MIPS</td>
<td>90.6 ± 27.1</td>
<td>118.5 ± 41.6</td>
<td>0.1124</td>
<td>0.5709</td>
</tr>
<tr>
<td>mg/dL</td>
<td>PL</td>
<td>89.3 ± 26.9</td>
<td>102.9 ± 47.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>MIPS</td>
<td>150.4 ± 31.4</td>
<td>145.0 ± 18.6</td>
<td>0.9464</td>
<td>0.2731</td>
</tr>
<tr>
<td>mg/dL</td>
<td>PL</td>
<td>162.8 ± 47.5</td>
<td>167.6 ± 41.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-Density Lipoprotein</td>
<td>MIPS</td>
<td>44.5 ± 7.4</td>
<td>42.6 ± 8.2</td>
<td>0.3284</td>
<td>0.9779</td>
</tr>
<tr>
<td>mg/dL</td>
<td>PL</td>
<td>45.0 ± 13.2</td>
<td>43.1 ± 6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-Density Lipoprotein</td>
<td>MIPS</td>
<td>89.5 ± 29.0</td>
<td>82.7 ± 19.4</td>
<td>0.9253</td>
<td>0.2013</td>
</tr>
<tr>
<td>mg/dL</td>
<td>PL</td>
<td>103.7 ± 29.7</td>
<td>109.5 ± 46.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-HDL</td>
<td>MIPS</td>
<td>105.9 ± 30.0</td>
<td>101.2 ± 21.1</td>
<td>0.9725</td>
<td>0.2497</td>
</tr>
<tr>
<td>mg/dL</td>
<td>PL</td>
<td>117.8 ± 47.6</td>
<td>122.2 ± 43.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>MIPS</td>
<td>94.4 ± 4.0</td>
<td>97.1 ± 10.1</td>
<td>0.2655</td>
<td>0.8211</td>
</tr>
<tr>
<td>mg/dL</td>
<td>PL</td>
<td>92.1 ± 10.4</td>
<td>94.0 ± 6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>MIPS</td>
<td>123.4 ± 57.2</td>
<td>123.4 ± 65.8</td>
<td>0.5071</td>
<td>0.5029</td>
</tr>
<tr>
<td>nmol/L</td>
<td>PL</td>
<td>116.2 ± 37.1</td>
<td>98.4 ± 31.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous Nitrite</td>
<td>MIPS</td>
<td>5.3 ± 1.6</td>
<td>5.2 ± 1.1</td>
<td>0.9663</td>
<td>0.7607</td>
</tr>
<tr>
<td>µmol/L</td>
<td>PL</td>
<td>5.3 ± 1.2</td>
<td>5.4 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Nitrite</td>
<td>MIPS</td>
<td>18.4 ± 18.5</td>
<td>17.9 ± 18.0</td>
<td>0.7688</td>
<td>0.9568</td>
</tr>
<tr>
<td>µmol/L</td>
<td>PL</td>
<td>20.6 ± 20.6</td>
<td>20.2 ± 20.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>MIPS</td>
<td>13.1 ± 6.5</td>
<td>12.7 ± 11.0</td>
<td>0.7782</td>
<td>0.9781</td>
</tr>
<tr>
<td>µmol/L</td>
<td>PL</td>
<td>15.3 ± 6.5</td>
<td>14.8 ± 9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate / Nitrite</td>
<td>NOS</td>
<td>0.5 ± 0.4</td>
<td>0.7 ± 0.7</td>
<td>0.3325</td>
<td>0.6285</td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>NOS</td>
<td>1.015 ± 0.87</td>
<td>1.1 ± 0.97</td>
<td>0.1478</td>
<td>0.1992</td>
</tr>
<tr>
<td>mg/L</td>
<td>PL</td>
<td>0.778 ± 0.89</td>
<td>2.124 ± 2.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD. Non-HDL, total cholesterol minus HDL; CRP, high-sensitivity C-reactive protein. Multi-Ingredient Performance Supplement, MIPS. Carbohydrate placebo, PL.
Profile of mood states

No group x time interactions were observed for any profile of mood state variables (p>0.05). However, training resulted in an increase in vigor (p = 0.0139) for both groups (MIPS: pre 15.6 ± 5.8, post 18.1 ± 6.9, 16% vs. PL: pre 17.3 ± 6.9, post 20.1 ± 4.7, 16%).

Dietary analysis and supplement compliance

While all participants were asked to complete a three-day food record before RT and during the final week of RT, only eight of the participants (n=5 for MIPS and n=3 for PL) turned in completed food records to the research staff for analysis. However, all participants were questioned personally and reported no change in dietary habits for the duration of the six-week study. In addition, the participant population included only highly resistance-trained individuals and thus, they verbally reported monotonous eating patterns and were instructed to replicate pre-baseline testing nutritional intake for post-testing. Nutrient intake for this subgroup (n=8) was not different at a baseline and remained unchanged for the duration of the study and was not different between groups (Table 4).

Participants were monitored by research personnel for all training and supplement consumption on training days. As a result, 100% compliance was observed for these days with respect to both RT and supplement consumption. On non-training days, participants verbally reported 100% compliance for supplement ingestion and returned all empty single-serving supplement containers as evidence.
Table 4. Nutritional composition for a subsample of MIPS and PL participants

<table>
<thead>
<tr>
<th></th>
<th>MIPS</th>
<th>PL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Kilocalories</strong></td>
<td><strong>(kcal/kg)</strong></td>
<td><strong>(g/kg)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td><strong>1.85 ± 0.48</strong></td>
<td><strong>1.43 ± 0.26</strong></td>
<td><strong>0.555</strong></td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td><strong>3.21 ± 0.67</strong></td>
<td><strong>2.46 ± 0.78</strong></td>
<td><strong>0.49</strong></td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td><strong>1.78 ± 0.80</strong></td>
<td><strong>1.02 ± 0.94</strong></td>
<td><strong>0.514</strong></td>
</tr>
<tr>
<td><strong>Caffeine</strong></td>
<td><strong>2.22 ± 0.75</strong></td>
<td><strong>1.94 ± 0.70</strong></td>
<td><strong>0.879</strong></td>
</tr>
</tbody>
</table>

Data are mean ± SD. Intake does not include supplementation. Multi-Ingredient Performance Supplement, MIPS. Carbohydrate placebo, PL. Level of statistical significance, p.

**Reported side effects**
Over the 6-week study nausea and dry-mouth was reported by one participant in MIPS. Feelings of paresthesia were reported by two participants in MIPS and two participants in PL. No other side effects were reported.

**Supplement analysis**
Caffeine was only present in the pre-workout supplement (SHOT; 190 mg ± 10 mg per 21 g serving). No additional stimulants were detected in either supplement. No quantifiable amounts of caffeine were detected in the post exercise supplement. These data are in agreement with the supplement fact sheet. No other adulterants such as commonly abused stimulants and known anabolic steroids were detected.
CHAPTER FIVE

DISCUSSION

The primary findings from this investigation were that six weeks of MIPS before and after RT and on all non-RT days did not alter blood lipid profile, SBP, DBP, HR, glucose, cortisol, NOx, or CRP in resistance-trained men. In addition, six weeks of MIPS in resistance-trained men appears to improve body composition to a greater degree than PL. This is in agreement with the results of others who have reported similar findings in untrained participants [11,12]. These studies also reported an improvement in muscle mass and body composition has been demonstrated in untrained men without any deleterious effect on certain blood safety markers when consuming MIPS either before or before and after RT for four-weeks [11,12]. It has not been determined, however, whether MIPS impact CV (e.g. blood lipids, blood pressure, and heart rate) and/or metabolic (e.g. cortisol) health and body composition in resistance-trained men (a primary marketing target of this class of ergogenic aid). For this reason, we sought to investigate the result of six-weeks of pre- and post-RT MIPS consumption with periodized RT on CV and metabolic health and body composition in resistance-trained men.

Body composition

Changes in body composition in the current study were dramatic and in some variables, greater than expected based on the current literature. We observed an increase in total body mass in both MIPS (2.9%) and PL (1.3%) with MIPS exhibiting a significantly greater gain (p = 0.02).

Fat free mass (FFM)

Participants in MIPS also increased FFM significantly more than PL (+4.2% vs. +1.9%, respectively, p=0.02). Similar to Shelmadine et al. [11], these gains mirror increases in FFM measured in untrained men after 4 weeks of a pre-workout only performance supplement (SHOT) and RT (SHOT, +4.8% vs. PL, + 1.7%). In addition, Spillane et al. [12] reported similar findings of increased FFM in untrained men after 4
weeks of RT when supplementing both pre- and post-RT with the identical performance supplement used in the present study (+3.7%) compared to placebo (+1.0%). It is surprising that our findings so closely results reported in untrained populations despite our participants averaging 4-6 years of RT experience and substantially higher baseline FFM (+9-13 kg) than participants in the aforementioned studies [11,12].

**Body fat**

Body fat % decreased significantly in both groups and this was not solely a function of increased FFM. Total body fat decreased by 2.66 and 3.54% in MIPS and PL, respectively. In addition, android (MIPS: -1.8; PL: -1.4%) and gynoid fat % (MIPS: -1.3; PL: -1.0%) decreased significantly in both groups. Android fat is highly associated with increased risk of CV mortality and diabetes mellitus and thus, it is notable that our RT protocol was successful in reducing both android and gynoid fat. Our findings are supported by reseach demonstrating that exercise can decrease body fat independent of supplementation [83,84]. Our prior work has shown increased lipolysis with just one bout of RT in resistance-trained men [4] and in overweight/obese sedentary men [82]. A gain in FFM with concurrent decreases in body fat is a highly desirable goal of athletes in many different sports as well as recreational weight-lifters. Our findings support the supplementation of MIPS in combination with periodized RT as an effective method of improving body composition.

**Similar results using MIPS**

Regarding the multitude of individual ingredients in SHOT and SYN, including whey protein, BCAA’s, creatine, caffeine, β-alanine, and L-arginine, it is difficult to isolate their specific effects. In fact, some research suggests a synergistic effect of ingredients in the investigated supplements [58,85,86]. The increases in FFM we observed may likely be explained by studies that used supplementation of creatine with β-alanine [58] or whey protein and amino acids [85]. Hoffman et al. [58] demonstrated RT in combination with supplementation of creatine and β-alanine decreased body fat by 1.21% through an increase in LBM (1.74 ± 1.72 kg) greater than creatine alone (data not reported) or a carbohydrate placebo (-0.44 ± 1.62 kg) in collegiate football players.
Willoughby et al. [85] observed an increase in FFM of 5.6% when combining 14g of whey and casein protein with 6g of free amino acids as compared to a 2.7% increase with a carbohydrate placebo supplement in untrained men following 10 weeks of heavy RT (3 sets of 6-8 reps with 85-90% 1RM, 4x/wk). Perhaps most relevant to the present study, Schmitz et al. [86] recorded 2.4% and 0.27% (p= 0.049) increases in LBM in trained participants (≥ two years RT experience) supplementing with MIPS with and without BCAA’s, respectively. The investigators implemented a nine-week progressive overload RT regimen with participants training four times a week. The two supplements were matched for creatine (4g), protein (7g), and carbohydrate (39g). The supplements from the present study include the primary ingredients from these studies [58,85,86] and alternative ingredients in a proprietary blend that are purported to improve body composition. Our results support that the multitude of ingredients in SHOT and SYN may be working synergistically to improve body composition compared to PL.

**Cardiovascular and metabolic biomarkers**

All measured CV and metabolic variables were within normal clinical ranges at baseline and post six weeks in the present study. Interventions using various intensities of RT with untrained subjects have significantly lowered LDL cholesterol in the same six-week span as the present study [87]. While RT may also improve triglycerides in untrained individuals [88], our participants were experienced with RT, which likely contributed to the lack of difference measured in these variables. Our findings are supported by previous studies in untrained men [11,12] using SHOT and SYN, which also report no changes in CV health variables after 4 weeks of RT in men of similar age to participants in the current study.

**Cortisol**

Cortisol is widely accepted as both a catabolic biomarker and an indicator of overall stress. Based on the current literature, we hypothesized a decrease in cortisol concentration in MIPS but saw no changes. Bird et al. [45] reported that both carbohydrate and carbohydrate with essential amino acid supplementation (6% carbohydrate & 6% carbohydrate + 6g essential amino acids; 8.5mL/kg) during an acute
bout of RT suppressed cortisol response in untrained men. This may have been due to a glucoregulatory effect in which carbohydrate was an adequate source of energy to prevent the need for cortisol to stimulate the liver to release glucose. Sharp and Pearson [46] demonstrated decreased cortisol concentrations in recreationally active young men after three weeks of branch chain amino acids supplementation (6g/day) followed by a fourth week of supplementation and four bouts of RT (three sets of 6-8 reps with 80% 1RM and 60 sec between sets and exercises, three lower- and five-upper body exercises). The authors reported significant decreases in cortisol 12 hrs after RT bout two, and 12 and 36 hrs after RT bout four. Because of the low-training status of the participants, the RT was deemed an over-reaching training week. Two factors that may have contributed to the differences in our data are the experienced training status of our subjects and the slightly later (36 vs. 48 hrs) post-training cortisol measurements in the present study. Our data indicate training-status may be a determining factor in resting cortisol concentrations with performance supplementation.

**Nitric oxide production**

Total nitrate/nitrite (NOx), which is indicative of production of the potent vasodilator nitric oxide, was also measured to evaluate the impact on CV health. Nitric oxide, is synthesized from L-arginine and L-citrulline, two amino acids present in the MIPS. Nitric oxide has been reported to improve CV health through vasodilation and prevention of lipid build-up on the arterial walls [89]; however, this finding is specific to diseased populations [90]. We recorded no change in resting NOx within or between groups during the intervention, which agrees with most previously published research [13,36] in healthy individuals. Interestingly, L-arginine has recently been shown to increase blood volume within the muscle without changing NOx [91]. It is possible that our participants experienced similar changes in blood volume without us detecting any change in NOx. In addition, by design, we sampled blood 48 h following the last training bout to measure the impact of supplementation and training over time rather than acute response to RT.
**C-reactive protein (CRP)**

The inflammatory marker CRP was measured to reflect total body inflammation. Clinical data indicate values of <1, 1-3, >3 mg/L as low, moderate, and high risk, respectively, for CV disease. We recorded no significant changes in CRP within or between groups. Kadaglou et al. [92] recently reported no change in CRP following three months of RT (3x/week) in type 2 diabetic patients. Conversely, Sheikholeslami et al. [87] demonstrated significantly lower CRP values in untrained men following 6 weeks of moderate (45-55% 1RM) or high intensity (80-90% 1RM) RT in healthy males compared to a non-intervention control group. The discrepancy in results may be due to the experienced training status of our participants compared to diabetic and untrained individuals. In addition, CRP is notorious for its high intra-subject variability [93] and may have confounded our results.

**Heart rate and blood pressure**

While RT may independently improve BP in untrained normotensive and pre-hypertensive individuals [88], our data did not indicate changes in BP, perhaps due to our participants’ RT history. Similarly, we saw no changes in HR, likely due to their training status. Research shows caffeine, a central ingredient in SHOT, the pre-exercise MIPS used in the present study, increases HR, SBP, and DBP acutely [9]. Energy drinks with less caffeine than SHOT (80 vs. ~190mg, respectively) have been reported to elevate BP up to 24 hours after consumption in nine healthy (aged 18-45 years), nonsmoking, normotensive men (n=4) and women (n=5) who self-reported to consume between 4 and 379 mg/day of caffeine (four participants reported habitual caffeine intake) [94]. However, some research suggests chronic coffee drinkers are at a decreased risk for developing CV disease [95]. No published research indicates the HR and BP response to caffeine-containing MIPS.

**Profile of mood states**

Regarding mood states, RT elicited an increase in vigor for both MIPS and PL. This indicates that it was unlikely our participants were over-trained at the post-testing
time point. Bresciani et al. [96] reported a non-significant decrease in vigor and a significant decrease in total mood as being indicative of overtraining in active men.

**Side effects**

Resistance-trained men appear to tolerate SHOT and SYN better than their untrained counterparts. Untrained men participating in the study conducted by Shelmadine et al. [11] reported feelings of dizziness, nausea, headache, rapid heart rate, shortness of breath, and nervousness after consuming SHOT. Similarly, Spillane et al. [12] reported select untrained subjects experiencing the same side-effects while supplementing with both SHOT and SYN. Only one individual taking SHOT and SYN in the present study reported feeling nauseous while two others reported feelings of paresthesia (although two people in PL also reported feeling paresthesia). It is probable that individuals experienced in RT are also experienced with a variety of MIPS and may be more tolerant or less likely to report side-effects if any were present.

**Limitations**

The present study was limited by the accuracy of self-reported dietary intake and supplement consumption on non-training days. However, our research staff collected empty supplement single-serving containers three times per week in an effort to verify compliance and all participants verbally reported no change in eating patterns. In addition, differences in prior training status of our participants may have limited our findings; however, even though MIPS had a longer training history, they still were able to increase FFM more than PL. The possibility also exists that participants may have detected which group they were assigned to based on prior experiences taking MIPS or similar supplements.

**Conclusions**

Considering the popularity of MIPS, and potential harmful side effects, it is important to confirm their safety and efficacy. Six weeks of MIPS before and after RT does not alter HR, SBP, DBP, fasted blood lipids, glucose, NOx, or CRP in resistance-trained men. However, FFM was increased in MIPS, and regional body fat was reduced
regardless of treatment group. Thus, a six-week periodized RT program combined with MIPS does not alter CV and metabolic health and appears to improve body composition more than an isocaloric placebo supplement in healthy resistance-trained men. Future studies investigating the effects of MIPS on cardiometabolic health variables and body composition should consider closely controlling diet, extending supplementation beyond six weeks, and including other populations such as females and individuals older than 40 years.
APPENDIX A

NO-SHOTGUN® SUPPLEMENT FACTS

CONTAINS NO FRUIT JUICE

**SUPPLEMENT FACTS**

<table>
<thead>
<tr>
<th>Serving Size</th>
<th>21 g (1 Scoop)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Servings Per Container</td>
<td>28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount Per Serving</th>
<th>%DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>33 mg</td>
<td>1%*</td>
</tr>
<tr>
<td>Protein</td>
<td>16 g</td>
<td>36%*</td>
</tr>
<tr>
<td>Vitamin B6 (Pyridoxine HCl)</td>
<td>100 mcg</td>
<td>5%</td>
</tr>
<tr>
<td>Folate (Folic Acid)</td>
<td>40 mcg</td>
<td>10%</td>
</tr>
<tr>
<td>Calcium</td>
<td>11 mg</td>
<td>1%</td>
</tr>
</tbody>
</table>

Protein Hydrolysate Matrix Yielding 22% Glutamine
Peptide, 21% BCAA Peptide, & 41% EAA’s, 3.50 g
Casein Protein Hydrolysate, Whey Protein Isolate, Whey Protein Hydrolysate
Proprietary Branched Chain Ethyl Ester Amino Acid Matrix 6.16 g **
L-Leucine, L-Isoleucine, L-Leucine Ethyl Ester HCl, L-Alanyl-L-Glutamine,
L-2-Aminopentanoic Acid (L-Orn-Valine), L-Valine Ethyl Ester HCl,
L-Isoleucine Ethyl Ester HCl, L-Arginine Ethyl Ester diHCl, L-Arginine, L-Valine
Proprietary Muscle Volumizing, NO2, Insulinotropic and Glutamine Matrix 4.89 g **
Creatine Gluconate, Creatine Taurinate,
Creatine Monohydrate, L-Citrulline Malate,
Bisa Picolinato Oxo Vanadium (BPOV), CEX® (Creatine Ethyl Ester HCl),
Di-L-Arginine-L-Malate, MTB Pump (Magnesium Tanninato B),
Di-Na Creatine Phosphate Tetrahydrate, Gamma-Butyrobetaine HCl,
Gamma-Butyrobetaine Ethyl Ester Chloride, COP® (Creatinol-O-Phosphate)
Power, Speed, Strength and Endurance Matrix 3.17 g **
Beta-Alanine, Creatine Magnesium Chelate,
Monosodium Phosphate Anhydrous, Trisodium Phosphate Dodecahydrate,
L-Histidine, KIC (Ketolactoacidoic Acid Calcium),
Guanidinopropionic Acid (GPA), Beta-Alanine Ethyl Ester HCl
Redline® Energy & Meltdown® Fat Burning Technology 376 mg **
Caffeine Anhydrous, Beta-Phenylethylamine HCl,
Barley (Hordeum vulgare) (bud) [std. to hordeinone HCl],
K-beta-Methylphenylalanine HCl, L-Tyrosine

Not a significant source of vitamin A, vitamin C, and iron.
* Percent Daily Values (DV) are based on a 2,000 calorie diet.
** Daily Value not established

OTHER INGREDIENTS: natural & artificial flavors, malic acid, Sucralose, citric acid anhydrous, glycine (food grade).

Contains: Milk
### APPENDIX B

**NO-SYNTHESIZE® SUPPLEMENT FACTS**

#### SUPPLEMENT FACTS

<table>
<thead>
<tr>
<th>Serving Size</th>
<th>Amount Per Serving</th>
<th>%DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 g (1 Scoop)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Servings Per Container</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

**Calories** | 68
---|---
**Sodium** | 33 mg | 1%
**Protein** | 17 g | 34%
**Folate (Folic Acid)** | 40 mcg | 10%
**Calcium** | 11 mg | 1%

**Protein Hydrolysate Matrix Yielding 22% Glutamine Peptide, 21% BCAA Peptide, & 41% EAA’s** | 3.50 g
---|---
**Casein Protein Hydrolysate, Whey Protein Isolate, Whey Protein Hydrolysate**
**Proprietary Branched Chain Ethyl Ester Amino Acid Matrix** | 6.21g **
---|---
**L-Leucine** | **L-Threonine**, **L-Leucine Ethyl Ester HCl**, **L-Histidine**, **L-Lysine**, **L-Methionine**, **L-Phenylalanine**, **L-Isoleucine**, **L-Valine**, **L-Valine Ethyl Ester HCl**, **L-Isoleucine Ethyl Ester HCl**, **L-2-Aminopentanoic Acid (L-Nor-Valine)**
---|---
**MHF-1™ (Myogenic Hyperplasia Factors™), NO2, Insulinotrophic and MyoBlast Amplification Matrix** | 7.11 g **
---|---
**Creatine Gluconate**, **Creatine Taurinate**, **Creatine Magnesium Chelate**, **Monosodium Phosphate Anhydrous**, **Trisodium Phosphate Dodecahydrate**, **L-Citrulline Malate**, **Creatine Monohydrate**, **Bis Picolinate Oxo Vanadium (BPOV)**, **CEX (Creatine Ethyl Ester HCl)**, **Beta-Alanine Ethyl Ester HCl**, **Di-L-Arginine-L-Malate**, **MTB Pump (Magnesium Tanshinoate B)**, **Di-Na Creatine Phosphate Tetrahydrate**, **Gamma Butyrobetaine HCl**, **Gamma-Butyrobetaine Ethyl Ester Chloride**, **COP (Creatinol-O-Phosphate)**
---|---
**OTHER INGREDIENTS**: natural & artificial flavors, malic acid, Sucralean® brand sucrose, and citric acid anhydrorous.

**CONTAINS**: Milk

---

* Percent Daily Values (%DV) are based on a 2,000 calorie diet.
** Daily Value not established.
# APPENDIX C

## TRAINING LOG (WEEKS 1&2)

Note: Supplement compliance verification at bottom of training log

<table>
<thead>
<tr>
<th>WEEKS 1-2 (Circle)</th>
<th>3 sets of 10 at 70-75% 1RM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td><strong>Biceps, Triceps, &amp; Shoulders</strong></td>
</tr>
<tr>
<td>Supplement</td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shoulder Military Press</td>
</tr>
<tr>
<td></td>
<td>DB Incline Biceps Curl</td>
</tr>
<tr>
<td></td>
<td>Cable Overhead French Press</td>
</tr>
<tr>
<td></td>
<td>Straight Bar Curls</td>
</tr>
<tr>
<td></td>
<td>Cable Triceps Press Down</td>
</tr>
<tr>
<td></td>
<td>DB Reverse Fly</td>
</tr>
</tbody>
</table>

| **Day 2** | **Legs & Abdominals/Core** | **Set 1** | **Set 2** | **Set 3** |
| Supplement | | Weight | Reps | Weight | Reps | Weight | Reps |
| Pre | | | | | | | |
| Post | | | | | | | |
| | Leg Press | | | | | | |
| | Straight Leg Dead Lift | | | | | | |
| | DB Lunge | | | | | | |
| | Cybex Leg Curls | | | | | | |
| | Cybex Standing Calf Raise | | | | | | |
| | Cybex Abdominal Crunch | | | | | | |
| | Plank (1 min) | | | | | | |

| **Day 3** | **Chest, Back, and Traps** | **Set 1** | **Set 2** | **Set 3** |
| Supplement | | Weight | Reps | Weight | Reps | Weight | Reps |
| Pre | | | | | | | |
| Post | | | | | | | |
| | Flat Bench Press | | | | | | |
| | Cable Lat Pull Down | | | | | | |
| | Incline Bench Press | | | | | | |
| | Cable Low Row (Neutral Grip) | | | | | | |
| | DB Chest Flys | | | | | | |
| | DB Shrugs | | | | | | |

Supplement Baggie Returned
Pre
Post

Notes:
APPENDIX D

PROFILE OF MOOD STATES QUESTIONNAIRE

PROFILE OF MOOD STATES

ID#: ______________________
DATE: ________________

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></td>
<td></td>
<td></td>
<td>34. Nervous</td>
</tr>
<tr>
<td>2. Tense</td>
<td></td>
<td></td>
<td></td>
<td>35. Lonely</td>
</tr>
<tr>
<td>3. Angry</td>
<td></td>
<td></td>
<td></td>
<td>36. Miserable</td>
</tr>
<tr>
<td>4. Worn-out</td>
<td></td>
<td></td>
<td></td>
<td>37. Muddled</td>
</tr>
<tr>
<td>5. Unhappy</td>
<td></td>
<td></td>
<td></td>
<td>38. Cheerful</td>
</tr>
<tr>
<td>7. Lively</td>
<td></td>
<td></td>
<td></td>
<td>40. Exhausted</td>
</tr>
<tr>
<td>8. Confused</td>
<td></td>
<td></td>
<td></td>
<td>41. Anxious</td>
</tr>
<tr>
<td>9. Sorry for things done</td>
<td></td>
<td></td>
<td></td>
<td>42. Ready to Fight</td>
</tr>
<tr>
<td>10. Shaky</td>
<td></td>
<td></td>
<td></td>
<td>43. Good Natured</td>
</tr>
<tr>
<td>11. Listless</td>
<td></td>
<td></td>
<td></td>
<td>44. Gloomy</td>
</tr>
<tr>
<td>12. Peeved</td>
<td></td>
<td></td>
<td></td>
<td>45. Desperate</td>
</tr>
<tr>
<td>13. Considerate</td>
<td></td>
<td></td>
<td></td>
<td>46. Sluggish</td>
</tr>
<tr>
<td>14. Sad</td>
<td></td>
<td></td>
<td></td>
<td>47. Rebellious</td>
</tr>
<tr>
<td>15. Active</td>
<td></td>
<td></td>
<td></td>
<td>48. Helpless</td>
</tr>
<tr>
<td>16. On Edge</td>
<td></td>
<td></td>
<td></td>
<td>49. Weary</td>
</tr>
<tr>
<td>17. Grouchy</td>
<td></td>
<td></td>
<td></td>
<td>50. Bewildered</td>
</tr>
<tr>
<td>18. Blue</td>
<td></td>
<td></td>
<td></td>
<td>51. Alert</td>
</tr>
<tr>
<td>19. Energetic</td>
<td></td>
<td></td>
<td></td>
<td>52. Deceived</td>
</tr>
<tr>
<td>20. Parachute</td>
<td></td>
<td></td>
<td></td>
<td>53. Furious</td>
</tr>
<tr>
<td>21. Hopeless</td>
<td></td>
<td></td>
<td></td>
<td>54. Efficient</td>
</tr>
<tr>
<td>22. Relaxed</td>
<td></td>
<td></td>
<td></td>
<td>55. Trusting</td>
</tr>
<tr>
<td>23. Unworthy</td>
<td></td>
<td></td>
<td></td>
<td>56. Full of Pep</td>
</tr>
<tr>
<td>24. Spiteful</td>
<td></td>
<td></td>
<td></td>
<td>57. Bad Tempered</td>
</tr>
<tr>
<td>25. Sympathetic</td>
<td></td>
<td></td>
<td></td>
<td>58. Worthless</td>
</tr>
<tr>
<td>26. Uneasy</td>
<td></td>
<td></td>
<td></td>
<td>59. Forgetful</td>
</tr>
<tr>
<td>27. Restless</td>
<td></td>
<td></td>
<td></td>
<td>60. Carefree</td>
</tr>
<tr>
<td>28. Unable to concentrate</td>
<td></td>
<td></td>
<td></td>
<td>61. Terrified</td>
</tr>
<tr>
<td>29. Fatigued</td>
<td></td>
<td></td>
<td></td>
<td>62. Guilty</td>
</tr>
<tr>
<td>30. Helpful</td>
<td></td>
<td></td>
<td></td>
<td>63. Vigorous</td>
</tr>
<tr>
<td>31. Annoyed</td>
<td></td>
<td></td>
<td></td>
<td>64. Uncertain</td>
</tr>
<tr>
<td>32. Discouraged</td>
<td></td>
<td></td>
<td></td>
<td>65. Bushed</td>
</tr>
<tr>
<td>33. Resentful</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX E

DIETARY RECALL INSTRUCTIONS

3 Day Food and Activity Record

Directions for 3-Day Food and Activity Record

1. Keep your 3-day food record on three consecutive days. Try to have at least one of those days be on the weekend.

2. Please record each food you eat immediately after you eat it.

3. Record only one food item per line.

4. Be as specific as possible when describing a food eaten: how it was cooked and the amount you ate. Don’t forget to include all beverages you drink. For example: Coffee with 1 tsp. Cream, 12 oz. Regular Coke, or 8 oz. Sweetened Tea.

5. Include brand names or labels from food items whenever possible.

6. Record amounts eaten in household measures. For example: one cup nonfat milk, 3 ounces grilled chicken, 2 tablespoons ranch dressing, 1 medium fruit, 2 slices cheese.

7. Include the method used to prepare the food item. For example: fresh, frozen, stewed, fried, baked, canned, broiled, raw, braised.

8. For canned foods, include the liquid in which it was canned. For example: Sliced peaches in heavy syrup or Fruit cocktail in light syrup.

9. If you eat at a restaurant, do your best to estimate portion size and list the restaurant you ate at. List any visible fat, oil, or sauces added to your food.

10. List amount and type of oil or butter you use in the preparation of your food.

11. Do not alter your diet while you are keeping a food record.

12. Please indicate the activities you participated in during each of the days that you record your diet along with the duration of activity.
APPENDIX F

APPROVAL MEMORANDUM (2 PAGES)

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
APPENDIX G
INFORMED CONSENT LETTER (7 PAGES)

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.

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 6 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 Mandler 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 6-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 6 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 patterns, 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

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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 Biodesitex™ machine at 180° of lackinetic 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 6-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 6-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 3 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 3 times per week).

Following each week of the study, I will return all empty containers to the research staff to help verify compliance.

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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 (Synthesize®) include all of the same ingredients as the pre-workout supplement; however, it does not contain any caffeine (see product label below).

CONTAINS NO FRUIT JUICE

SUPPLEMENT FACTS

Serving Size 21 g (1 Scoop)
Servings Per Container 20

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount Per Serv.</th>
<th>% Daily Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>253</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>26 g</td>
<td>52%</td>
</tr>
<tr>
<td>Vitamin B3 (Niacin)</td>
<td>160 mg</td>
<td>8%</td>
</tr>
<tr>
<td>Potassium</td>
<td>40 mg</td>
<td>1%</td>
</tr>
<tr>
<td>Caffeine</td>
<td>17 mg</td>
<td>1%</td>
</tr>
</tbody>
</table>

Other Ingredients: natural & artificial flavors, malt acid, sucralose, and xanthan gum.

NO-Shotgun® Supplement Label.

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SYNTHESIZE® Supplement Label.

Both groups will complete resistance training exercises 3 days per week for 8 weeks. Day 1 will target chest, back, and trapezius muscles with the following exercises: bench press, incline bench press, chest flys, 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: squat/leg press, step-ups, leg curls, heel raise, lunge, 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 with 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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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 Synthesize® (Vital Pharmaceuticals, Inc, Davie, FL) contain a proprietary blend of a number of potentially hypertrophic compounds including creatine monohydrate, beta-alanine, arginine, alpha ketoscaprate (KIC), and leucine. Published research on Shotgun® has indicated minor side effects (nausea, rapid heart rate, headache, and shortness of breath) in 4 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

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Compounds are all 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 205 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 lipid 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 participant’s 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 (mormsbee@fsu.edu), Dr. Lynn Panton at (850) 644-4685 (lpanton@fsu.edu) or Dr. Jeong-Su Kim at (850) 644-4795 (kimS@fsu.edu) for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

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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

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REFERENCES


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BIOGRAPHICAL SKETCH

Personal

Dennison David Thomas
Born June 26th, 1988 in Fayetteville, NC

Education

August 2012 M.S. in Exercise Physiology
Florida State University, Tallahassee, FL
May 2010 B.S. in Physical Education, concentration in Exercise Science
College of Charleston, Charleston, SC

Professional Experience

2011-2012 Graduate Research Assistant
Department of Nutrition, Food, and Exercise Sciences
Florida State University, Tallahassee, FL