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ACUTE EFFECTS OF A MULTI-INGREDIENT PRE-WORKOUT SUPPLEMENT ON
MARKERS OF CLINICAL HEALTH AND EXERCISE
PERFORMANCE IN ACTIVE FEMALES

A Manuscript Style Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science

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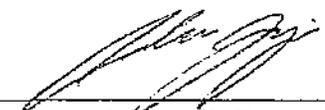
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By Michael J. Cameron

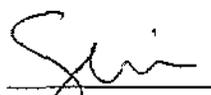
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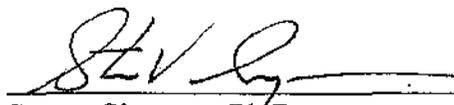
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ABSTRACT

Cameron, M. J. The acute effects of multi-ingredient pre-workout supplement on markers of clinical health and exercise performance in active females. MS in Clinical Exercise Physiology, May 2016, 57pp. (A. Jagim)

Multi-ingredient pre-workout supplements (MIPS) are consumed with the belief of enhancing exercise performance, but research has primarily dealt with male subjects. The purpose of this study was to examine the acute effects of ingesting a MIPS on active females. Fifteen females participated in a randomized, double-blind, placebo controlled cross-over design study. Subjects reported to the laboratory for a familiarization, baseline, and two experimental testing sessions. Experimental testing included REE, heart rate, and blood pressure. Also, muscular endurance was assessed by performing a set to failure on back squat and bench press (BP) at 85% of their 5-repetition maximum. Anaerobic power was assessed using a counter-movement vertical jump test and a non-motorized force treadmill sprint test. Subjective measurements were assessed using a 5-point Likert scale. A significant main effect for condition was observed for REE ($p=0.021$), diastolic blood pressure ($p=0.011$), BP repetitions ($p=0.037$), and total work ($p=0.039$) following ingestion of the MIPS. A significant condition x time interaction was observed regarding feelings of focus at 80-minutes post ingestion ($p=0.046$). In conclusion, the current study suggests that consumption of a MIPS significantly increased REE, diastolic blood pressure, upper body muscular endurance, total work completed, and produced an increase in feelings of focus.

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INTRODUCTION

The use of dietary supplements to enhance exercise performance and improve body composition has long been a popular strategy for active individuals (Baron, 2004). Supplement companies continually release new products each year and compete for consumer's attention. A newer category of dietary supplements referred to as multi-ingredient pre-workout supplements (MIPS), are a class of supplements that typically include a combination of ingredients such as caffeine, taurine, branch-chain amino acids, creatine, glutamine, and β -alanine. They are often manufactured in a proprietary blend, designed for ingestion prior to a workout and purported to enhance exercise performance. Based upon previous findings, the primary active ingredient in most pre-workout supplements appears to be caffeine as it is one of the few ingredients that offers fast-acting performance benefits (Walsh, Gonzalez, Ratamess, & Hoffman, 2010; Spradley et al., 2012; Gonzalez, Walsh, Ratamess, Kang, & Hoffman, 2011), and when taken alone is still a very popular ergogenic aid for athletes (Antonio 2004). More recent studies have focused on the effects of combining caffeine with a variety of ingredients for their potential synergistic benefits, common to pre-workout supplements, such as beta-alanine, creatine, various herbal extracts. Together, these ingredients are purported to enhance energy availability, metabolism and the buffering capacity of skeletal muscle (Beck et al., 2006; Gonzales et al., 2011; Spradley et al., 2012). Previous studies have reported improvements in acute muscular performance in individuals during upper and lower body exercises, however, results are inconclusive (Beck et al., 2006; Spradley et al., 2012;

Forbes, Candow, Little, Magnus, & Chilibeck 2007). For example, Beck et al. (2006) examined the acute effects of a caffeine-containing supplement and discovered a significant increase in upper body maximal strength in male subjects. In addition, Spradley et al. (2012) found that consuming a pre-workout supplement significantly improved leg press endurance. It has also been reported that consuming caffeinated energy drinks alone lead to an increase in upper body muscle endurance, but no effect on peak or mean power (Forbes et al., 2007). Following chronic supplementation, these products may enhance training adaptations when combined with a structured training program.

Further, several of these pre-workout supplements, particularly ones containing caffeine, have purported benefits regarding the enhancement of thermogenesis and increases in lipolysis (Acheson, Zahorska-Markiewicz, Pittet, Anatharaman, & Jequier., 1980; Campbell et al., 2016; Dalbo, Roberts, Stout, & Kersick., 2008; Outlaw et al., 2013). For example, Acheson et al. (1980) observed an increase in metabolic rate after caffeine consumption in both individuals who were normal weight and obese. In a more recent study, Campbell et al. (2016) observed that females who ingested a caffeine-containing supplement containing primary ingredients of caffeine and green tea extract experienced significant increases in resting energy expenditure (REE) for up to 3-hours post ingestion. Over time, these physiological responses may lead to enhanced fat loss and improvements in body composition resulting from repeated increases in resting metabolism leading to a higher total daily energy expenditure.

Pre-workout supplements also make claims of enhanced energy levels and improved general well-being following ingestion. If exercise is more enjoyable and less

exhausting, individuals may participate longer, with greater intensity and more frequently. Several researchers (Duncan, Smith, Cook, & James, 2012; Walsh et al., 2010; Spradley et al., 2012; Hoffman et al., 2009) have observed benefits ranging from improvements in feelings of focus, energy to reductions in fatigue following consumption of a pre-workout supplement during a bout of high-intensity exercise. Further, Walsh et al. (2010) reported significantly improved time to exhaustion in subjects running on a treadmill at 70% of maximal oxygen consumption ($VO_2\text{max}$) following consumption of a pre-workout supplement compared to a placebo. In addition, participants also reported improved feelings of focus, energy, and decreased feelings of fatigue (Walsh et al., 2010).

Although there have been numerous studies showing the benefits of pre-workout supplementation, the majority of the available research has focused primarily on males. There are limited data regarding the effectiveness of pre-workout supplements in female populations. Recently, a new product that was designed specifically for women (Fitmiss™ Ignite™, MusclePharm, Inc., Denver, CO, USA), purported to decrease body fat, increase energy, and improve performance level has been shown to be safe for consumption (Vogel et al., 2015). However, less is known regarding its influence on metabolism and performance. Therefore, the purpose of the current study was to examine the acute effects of a ingesting a MIPS on clinical health markers, REE, and exercise performance in recreationally active females. The secondary purpose of the current study was to examine the effects of MIPS ingestion on subjective markers of focus, energy, and fatigue during exercise. It is hypothesized there will be an increase in REE,

cardiovascular responses, and exercise performance following the supplementation treatment.

METHODS

Subjects

A total of 15 recreationally active college-aged females were recruited to participate in this randomized, double-blind, placebo (PLA) controlled cross-over design study (mean \pm SD, age: 21.5 ± 1.72 y, height: 165 ± 5.3 cm, weight: 61.6 ± 5.10 kg, BF%: 22.9 ± 4.09 %). Recreationally active was defined as participating in at least 150 minutes of moderate activity per week for at least 6 months (Pescatello, Arena, Riebe, & Thompson, 2014) (mean \pm SD, hours/week: 5.6 ± 2.2 hours). Participants provided written informed consent, completed a Physical Activity Readiness Questionnaire (PAR-Q), demographic, and exercise history form in compliance with the Human Subjects Guidelines of the University of Wisconsin-La Crosse and the American College of Sports Medicine. Participants were required to not have taken any nutritional supplements and/or ergogenic aids in the previous 3 weeks before baseline testing, excluding daily vitamin and/or protein supplementation. Caffeine consumption was to be absent within one week of baseline testing until the end of their final testing session.

Experimental Design

Participants first completed a familiarization session to become comfortable with the equipment and testing procedures prior to the experimental sessions. At this time they completed a demographic, health history, and exercise history form. Participants then completed a trial to practice a counter-movement vertical jump (CMVJ) and a maximal

effort sprint test on a non-motorized force treadmill. Also, they completed 10 repetitions for the back squat (BS) and bench press (BP) exercises.

Participants reported to the human performance lab within 4-7 days of their familiarization session for baseline testing which included a body composition assessment, 10-minute dynamic warm-up, 3 CMVJ, 5 repetition maximum for back squat (5RMBS), 5 repetition maximum for bench press, (5RMBP), and a 25-second maximal sprint test on a treadmill. The participants were asked to fast for > 2 hours and abstain from exercise > 24 hours prior to baseline testing.

Within 7 days of baseline testing, participants returned for the first of two experimental testing sessions. Participants completed a 2-day diet history, leading up to the first testing day, which was later assessed for total energy and macronutrient composition. Participant's bodyweight was first determined upon arrival to the laboratory without shoes, followed by a questionnaire to assess their baseline feelings of focus, energy, and fatigue using a 5-point Likert scale (LS). Participants then remained seated for a 3-minute period followed by the assessment for baseline heart rate (HR) and blood pressure (BP). Then participants ingested either a placebo or the supplement. For the next 60 minutes participants were assessed for changes in REE and respiratory exchange ratio (RER) at time points 35 and 60 minutes post-ingestion. The HR and BP measurements were taken at 15-minute intervals following ingestion. At the end of 60 minutes a second questionnaire was administered. Following the questionnaire, participants completed a standardized dynamic warm-up lasting 10 minutes. Participants then completed a CMVJ that was recorded and later converted into power (W). Three minutes following the CMVJ test, subjects completed a set to failure on back squat and bench press at a

resistance of 85% of their predetermined 5RM. The lifts were separated with a 5-minute rest period. Following the bench press, a third questionnaire was given. Ten minutes later, the subjects performed a 25-second maximal sprint test on a non-motorized force treadmill set at 12% of their bodyweight, followed by the completion of a 4th questionnaire to end the testing session.

Four to seven days following the first testing session, participants reported to the lab and completed the same protocol, receiving the opposite treatment. Participants were encouraged to eat similar foods to what they ate before test day number 1 by reviewing their 2-day diet history, however participants were again asked to complete a second 2-day dietary history.

Testing Procedures

Resting Measurements

Height and body mass was determined according to standard procedures using a Healthometer (Telstar LLC, Bridgeview, IL) scale. Resting energy expenditure and RER analysis was conducted using a TrueOne® 2400 metabolic measurement system (ParvoMedics, Sandy, UT). This test is a non-exertional test performed in a fasted state with the participants lying supine on an exam table. A clear, hard plastic hood and soft, clear plastic drape was placed over the participants' head and neck in order to determine resting oxygen uptake and energy expenditure. All participants laid motionless without falling asleep for 60 minutes. Body composition was assessed using air displacement plethysimography (BODPOD, Cosmed USA, Inc.). Heart rate and BP were assessed using standard clinical procedures. To assess subject's feelings of focus, energy, and fatigue, the 5-point Likert scale was displayed to them. Specifically subjects responded verbally

to their rating on a scale of 1-5, which corresponded to: 1 = low, 2= medium-low, 3 = medium, 4 = medium-high, 5 = high as has been previously used (Hoffman et al., 2009).

Baseline Testing

Body composition was assessed using air displacement plethysmography (BODPOD, Cosmed USA, Inc.). Following body composition, participants completed a 10 minute standardized dynamic warm-up consisting body-weight movements. After the warm-up, participants completed 3 attempts at a CMVJ using the Just Jump System (Sports Imports, Columbus, OH) to get them accustomed to the movement. Both the 5RMBS and 5RMBP were performed on an Optima Smith Machine (LifeFitness, Schiller Park, IL). Strength testing to determine participant's 5RMBS and 5RMBP started with a warm-up set of 5 repetitions at approximately 50% of their 5RM. Next participants completed two sets at a load corresponding to 60-80% of their estimated 5RM with three minutes of rest in between. Participants then performed sets of 5 repetitions of increasing weight to determine their 5RM. Three minutes rest was provided between all successful attempts. All 5RM determinations were made within 1-3 attempts. A successful 5RMBS was determined by having the participant's thighs parallel with the floor. A successful 5RMBP was determined if the participant lowered the bar to their chest. Participants were encouraged to not pause at the top of each lift for more than a second for both 5RMBS and 5RMBP. Five minutes later, the participants practiced a 25-second maximal sprint test on the non-motorized force treadmill (Woodway, Waukesha, WI, USA) set at a resistance of 12% of their bodyweight in kg (McClain et al., 2015). Participants completed two trials with the first one lasting 15 seconds and the second 20 seconds.

Performance Testing

Participants completed a 10-minute dynamic warm-up consisting of 4 minutes on a stationary cycle followed by 2 minutes of running on a standard treadmill at a speed of 6.0 mph. Then a set of dynamic stretches of upper and lower body musculature was performed. Following the warm-up, participants completed a CMVJ using the Just Jump System (Sports Imports, Columbus, OH). Average power (W) and peak power (W) were later assessed using the participant's mass (kg), height (cm), and CMVJ height (cm) (Johnson, & Bahamonde, 1996).

Experimental Testing

A resistance set at 85% of the pre-determined 5RM was used for the back squat and bench press to failure exercises on the two experimental testing days (mean \pm SD, BS: 59.1 ± 14.5 kg, BP 35.8 ± 7.28 kg). Participants were instructed to complete as many repetitions as possible while completing each repetition as "explosively" as possible without pausing at the top. There was a 5-minute rest period between the 5RMBS and 5RMBP. Ten minutes later, the participants performed a 25-second maximal sprint test on a non-motorized force treadmill (Woodway, Waukesha, WI, USA) set at a resistance of 12% of their bodyweight in kg (McClain et al., 2015). Participants were given a 3-second count down and were instructed to sprint as fast as possible for the entire 25-seconds. Sprint tests were analyzed for work completed, peak and average velocity and power.

Dietary Analysis

Each subject's 2-day diet history was assessed using a commercially available nutrition analysis program (MyFitnessPal, Inc.) to assess for changes in macronutrient intake prior to each testing session.

STATISTICAL ANALYSIS

All data was analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL). Descriptive statistics were used to assess baseline physical characteristics. A treatment (supplement or placebo) x time for REE, respiratory quotient (RQ) (35, 60 minutes), Likert scale scores (0, 60, 80, 90 minutes), HR and BP (0, 15, 30, 45, 60, minutes) repeated measures analysis of variance (ANOVA) was used to assess differences in REE, RER, cardiovascular responses, subjective feelings of energy, focus, and fatigue between treatment conditions. A paired samples t-test was used to assess differences in muscular endurance and anaerobic capacity between each condition. Data were considered statistically significant when the probability of type I error was $p < 0.05$. If a significant interaction was observed for the ANOVA, a Tukey's honest significant differences (HSD) post-hoc analysis was performed in order to determine where significance occurred between conditions.

RESULTS

Resting Energy Expenditure

There were no significant effects observed for RQ between the conditions ($p > 0.05$). A significant main effect for condition was observed for REE (MIPS: $1,497 \pm 55.7$; PLA: $1,416 \pm 42.2$ kcal/day). Post-hoc analysis revealed the MIPS condition exhibited a higher REE at 35 minutes and 60 minutes post-ingestion as seen in Figure 1. (MIPS35: $1,496.9 \pm 199.5$; PLA35: $1,425.8 \pm 148.5$ kcal/day, $p = 0.043$), (MIPS60: $1,514.8 \pm 233.8$; PLA60: $1,422.6 \pm 181.4$ kcal/day, $p = 0.034$). Due to machine complications, one participant's data was discarded from the 35-minute data time point.

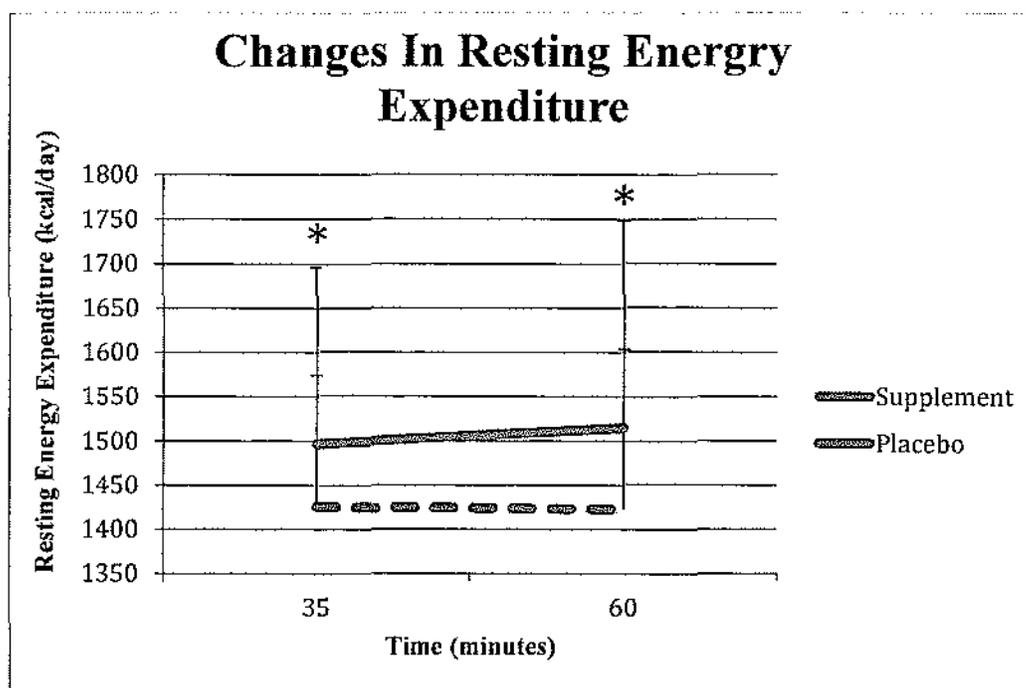


Figure 1. Changes in resting energy expenditure (mean \pm SD)

* Significantly different ($p < 0.05$)

Heart Rate and Blood Pressure

There was a significant main effect for time observed regarding HR responses as seen in Figure 2 ($p = 0.002$). Heart rate was significantly higher at baseline compared to each time point post-ingestion in each condition. There was an overall main effect for time regarding mean diastolic blood pressure following ingestion of the MIPS compared to the PLA (MIPS: 84 ± 1.1 ; PLA: 64.8 ± 1.3 mmHg, $p = 0.011$) however no significant group x condition interactions ($p = 0.44$) were observed as seen in Figure 3. There were no significant differences observed for systolic blood pressure overall or between conditions following ingestion of the MIPS or PLA as seen in Figure 4 ($p > 0.05$).

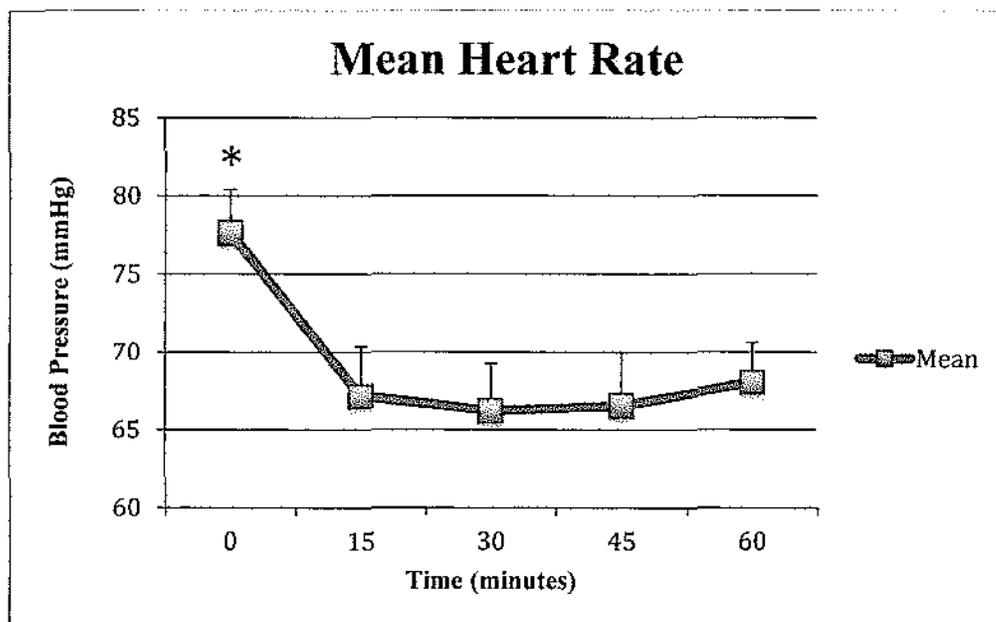


Figure 2. Mean HR response at 0, 15, 30, 45, and 60 minutes post-ingestion (mean \pm SE)
* Significantly different ($p < 0.05$)

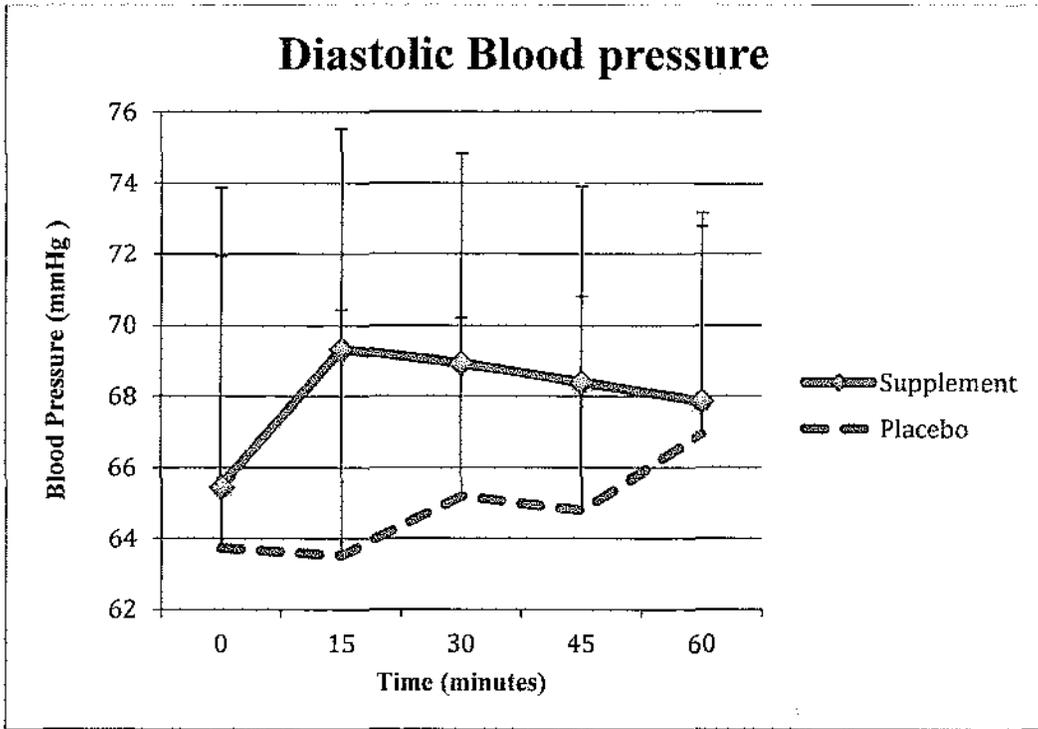


Figure 3. Mean diastolic blood pressure response at 0, 15, 30, 45, and 60 minutes post-ingestion (mean \pm SD)

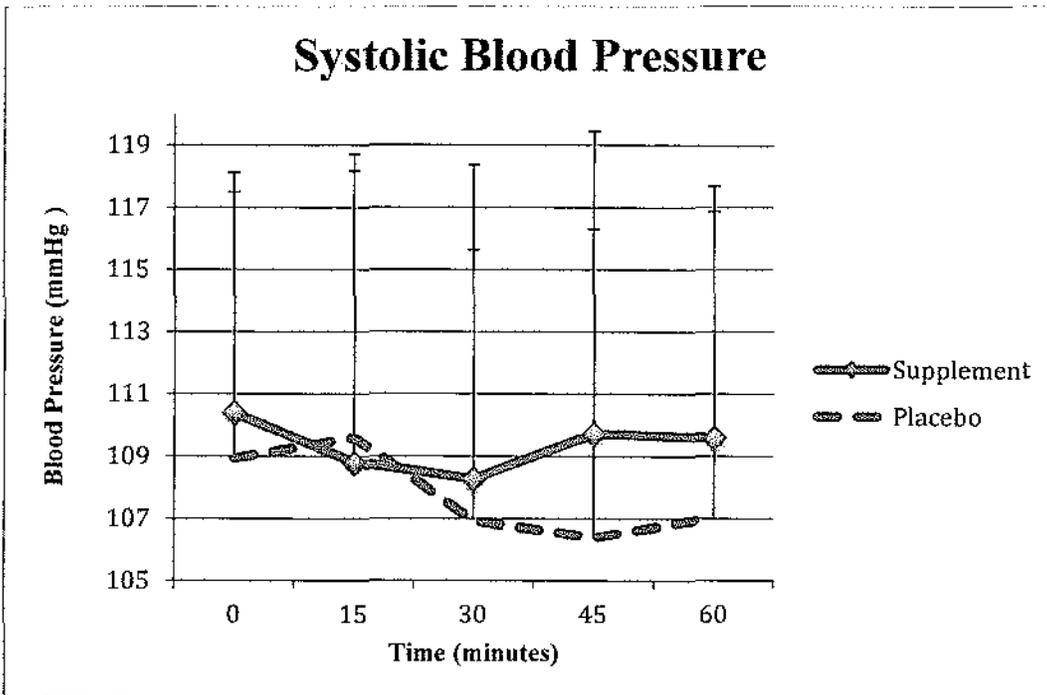


Figure 4. Mean systolic blood pressure response at 0, 15, 30, 45, and 60 minutes post-ingestion (mean \pm SD)

Strength Performance

There was no significant difference in squat performance between the MIPS and PLA (MIPS: 13.5 ± 3.7 ; PLA: 12.8 ± 3.1 reps, $p = 0.28$). Participants completed a significantly higher number of bench press repetitions to failure following ingestion of the MIPS as seen in Figure 5 condition compared to PLA (MIPS: 12.1 ± 2.4 ; PLA: 11.2 ± 2.5 reps, $p = 0.037$).

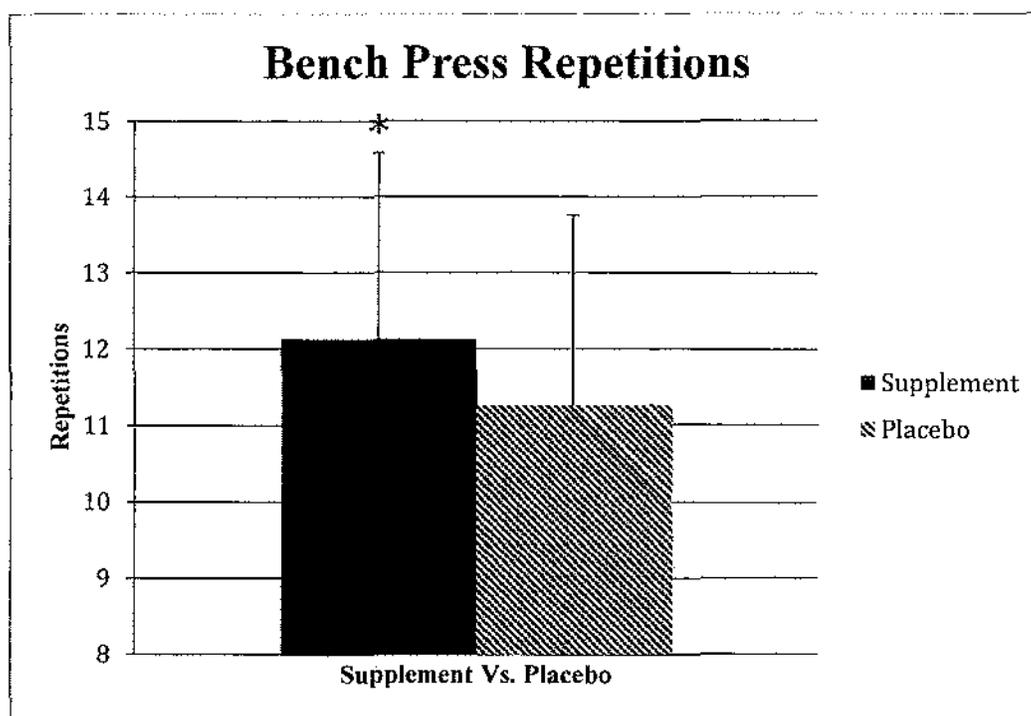


Figure 5. Mean bench press repetitions to failure at 85% of 5RMBP (mean \pm SD)
* Significantly different ($p < 0.05$)

Performance Measurements

No significant improvements in CMVJ performance were observed following ingestion of the MIPS as seen in Table 1 ($p > 0.05$). Participants completed a significantly greater amount of total work (m) during the 25-second treadmill sprint following ingestion of the MIPS compared to the PLA (MIPS: 93.97 ± 6.63 ; PLA: 93.01

± 7.49 m, $p = 0.039$). No other significant improvements in anaerobic performance measurements were observed.

Table 1. CMVJ and treadmill performance data (mean \pm SD)

Time (minutes)	MIPS (n=15)	Placebo (n=15)
Countermovement Vertical Jump		
Height (cm)	47.3 \pm 3.95	47.3 \pm 3.35
Average Power (W)	2012.8 \pm 222.31	2016.3 \pm 208.91
Peak Power (W)	3607.8 \pm 451.97	3614.5 \pm 409.68
Treadmill Performance		
Average Velocity (m/s)	3.7 \pm 0.38	3.6 \pm 0.29
Peak Velocity (m/s)	4.3 \pm 0.45	4.2 \pm 0.43
Average Power (W)	421.6 \pm 61.13	419.8 \pm 63.51
Peak Power (W)	1481.5 \pm 235.65	1454.9 \pm 331.20
Total Work (m) (n = 14)	93.97 \pm 6.627 *	93.01 \pm 7.489

* Significantly different ($p < 0.05$)

Food Logs

There were no significant differences between the subject's mean calorie and macronutrient intakes prior to each testing session as seen in Table 2 ($p > 0.05$).

Table 2. Descriptive Statistics of 2-day Diet History (mean \pm SD)

Measurement	MIPS (n=15)	Placebo (n=15)
Calories (kcal)	1,597.7 \pm 247.79	1,638.1 \pm 243.96
Carbohydrates (g)	219.2 \pm 49.26	215.9 \pm 48.07
Fat (g)	53.6 \pm 20.97	55.8 \pm 21.35
Protein (g)	69.7 \pm 24.02	71.5 \pm 22.97

Questionnaires

A significant main effect for time regarding feelings of fatigue was observed for both conditions beginning 90 minutes post-ingestion ($p < 0.001$). A significant condition x time interaction was observed regarding feelings of focus. Post-hoc analysis revealed

MIPS exhibited greater feelings of focus at 80 minutes post ingestion as seen in Figure 6. (MIPS: $4.1 \pm .6$; PLA: $3.6 \pm .8$, $p = 0.046$). There was a main effect for time regarding feelings of energy throughout the workout for both conditions as subjects reported greater energy at 80 minutes post ingestion compared to energy levels at 90 minutes post ingestion ($p = 0.005$).

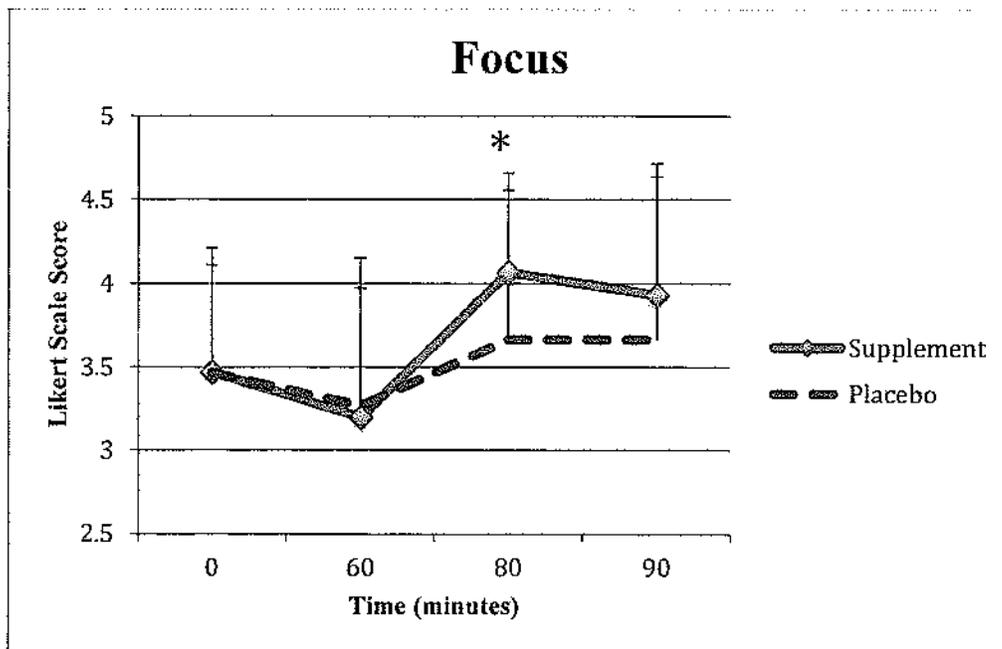


Figure 6. Mean LS scores at 0, 60, 80, and 90 minutes post-ingestion (mean \pm SD)
* Significantly different ($p < 0.05$)

Reported Side Effects

The side effects reported by the subjects included symptoms of having a “flush face”, “tingly hands”, and “jitteriness” approximately 30 minutes post-ingestion of the MIPS but not the PLA ($n = 4$).

DISCUSSION

The purpose of the current investigation was to examine the acute effects of ingesting a MIPS, (FitMiss™ Ignite™), on markers of clinical health, REE and exercise performance in recreationally active females. A secondary aim of the current study was to examine the effects of MIPS ingestion on subjective markers of focus, energy, and fatigue during exercise. The primary findings from the current study suggest that ingestion of a MIPS appears to increase REE following ingestion. Specifically, REE was significantly greater at 35 minutes post ingestion of the MIPS compared to the PLA. These results are in accordance with previous studies that have also observed significant increases in REE following ingestion of a MIPS (Dalbo et al., 2008; Outlaw et al., 2013). For example, Dalbo et al. (2008) reported significant increases in REE following ingestion of a commercially available caffeine-containing supplement (200 mg) for 3 hours post ingestion in males and females. This phenomenon has also been observed in females as well by Campbell et al. (2016) who reported a significant increase in REE in healthy females for up to three hours post ingestion of a MIPS thermogenic supplement that contained 150 mg of caffeine plus green tea extract. However, one study did report opposing findings and did not observe a significant increase in REE following ingestion of a caffeine-containing supplement (Rashti et al. 2009). Although, it should be noted that the participants from that study were only in a 3-hour post absorption state suggesting that increases in REE may only occur during an extended fast (>3 hours). It is likely that the increases in REE observed in the current study are likely a result of the caffeine

contained within the product as increases in REE have been shown to occur following consumption of caffeine alone (Acheson et al., 1980; Outlaw et al., 2013). Specifically Outlaw et al. (2013) found REE was significantly increased at 60 minutes post ingestion of a caffeine-containing supplement with 340 mg of caffeine, even though the participants were regular caffeine consumers. Caffeine acts as an adenosine antagonist thus interfering with adenosines inhibiting influence on metabolic activity (Goldstein et al. 2010). Therefore, when caffeine binds to the adenosine receptors instead of adenosine, it may be the cause of the acute increases in REE observed.

Furthermore, results from the current study also indicate that MIPS ingestion does not appear to influence HR in recreationally active females as this variable remained stable and did not increase following consumption of a MIPS compared to a PLA. This finding is in accordance with previous studies that observed minimal changes in HR following ingestion of a MIPS or a caffeine-containing supplement (Campbell et al., 2016; Rashti et al., 2014; Vogel et al. 2015). Conversely, some studies (Astronio, Rohmann, & Fith, 2008; Kedia et al., 2014) have observed an increase in HR following consumption of a MIPS.

The current investigation observed that systolic BP was not significantly increased following ingestion of a MIPS, as it remained stable compared to the PLA condition. These findings are in opposition to previous studies, which have observed significant increases in systolic BP following consumption of MIPS or caffeine-containing beverages (Astorino et al., 2008; Campbell et al., 2016; Kedia et al., 2014; Rashti et al., 2009). For example, a significant increase in both systolic and diastolic BP has been observed in female participants (Campbell et al. 2016) following ingestion of a

caffeine-containing thermogenic supplement. The opposing findings for systolic BP observed in the current investigation may be due to the differences in caffeine dosage. For the current investigation, the MIPS contained a blend of caffeine and various other ingredients, however, the exact amount is unknown. Previous studies that have observed changes in HR and systolic BP reported these increases following ingestion of a caffeine-containing beverage with dosages from 230mg up to 495mg which may be the cause for eliciting the exaggerated hemodynamic responses (Astorino et al., 2008; Campbell et al., 2016; Kedia et al., 2014; Rashti et al., 2009).

The current investigation observed a significant increase in upper body muscular endurance, which is in accordance with the previous findings (Duncan et al., 2012; Gonzalez et al., 2011; Spradley et al., 2012). A recent meta-analysis analyzed the data from 34 studies of which the authors concluded that caffeine ingestion appears to improve muscular endurance (overall ES = 0.28, $p < 0.01$) and increase maximum voluntary contraction, particularly during lower body exercises (overall ES = 0.67, $p < 0.01$) (Warren, Park, Maresca, Mckibans, & Millard-Stafford, 2009). Caffeine may also be able to increase muscular endurance by its direct effect on muscle anaerobic energy provision and its ability to increase muscle contractility (Graham, & Spriet, 1996). Caffeine also acts as a central nervous stimulant and therefore may delay the onset of fatigue or allow individuals to better tolerate a higher training intensity (Graham, & Spriet, 1996). However, the current investigation did not observe a statistical increase in lower body muscular endurance. These findings are in opposition to a prior investigation, which observed a significant increase in lower body endurance, but not upper body (Spradley et al., 2012) following ingestion of a MIPS in males. These differences in

performance outcomes may be explained by differences in instrumentation utilized (i.e., leg press versus the back squat) or too low of a caffeine amount to elicit a positive improvement in performance.

Ingestion of the MIPS had no effect on anaerobic performance when performing a CMVJ in the current investigation, which is in accordance with a previous study (Jagim et al., 2016) which failed to detect any improvement in lower body power. Also, anaerobic power measurements during the maximal sprint test were not significantly different following ingestion of a MIPS compared to the PLA. These results are also in accordance with previous studies (Forbes et al., 2007, Hoffman et al., 2009, and Jagim et al., 2016). However, Jagim et al. (2016) did observe a significant increase in mean power. Adversely, the current investigation observed a significant increase in total work during the maximal sprint following ingestion of the MIPS.

A claim of the current MIPS is that consumption of the product may enhance consumer feelings of focus, energy, and fatigue following ingestion particularly during exercise. The results of the current investigation support these claims in that a statistically significant increase in reported feelings of focus were observed following a bout of high-intensity exercise. The stimulatory effects of a MIPS are suggested to be from the combination of caffeine, taurine, branched-chain amino acids, creatine, glutamine, and β -alanine. The findings of the current investigation are in agreement with the observations from a previous study (Walsh et al., 2010) during which participants reported a statistically greater feeling of focus and energy 10-minutes into running on a treadmill at 70% of VO_2 max following ingestion of a MIPS. However, the current study failed to observe a statistical difference in subjective feelings of energy or fatigue levels post

exercise following ingestion of a MIPS compared to the PLA. These findings are in opposition of Jagim et al. (2016) who reported reductions of fatigue levels throughout testing following ingestion of a MIPS during a strength training protocol. This disparity between the two conditions may be more drastic due to the greater workload of a strength training protocol consisting of 5x5 with a 6th set to failure.

Based upon the results of the current study, ingestion of a MIPS appears to increase REE compared to the PLA for up to 60 minutes post ingestion in recreationally active females. These elevations were accompanied with minimal adverse hemodynamic responses, suggesting a low health risk. Over time, ingestion of a MIPS could possibly lead to reductions in body weight as a result of repeated elevations in REE. Further, the current MIPS appears to positively influence upper body muscular endurance, which could also lead to enhance training adaptations overtime by allowing for a greater training volume. The added improvement of the subjective measure of focus could improve quality of a training session as well. With minimal side-effects in the current study and results from a previous study utilizing the same MIPS, the authors found it to be physiologically acceptable for females to consume 1 or 2 servings of a MIPS daily for 28 days (Vogel et al., 2015). Specifically, the results indicated that consuming as much as 2 servings daily for 28 days did not affect hematological markers or resting vitals among in recreationally active females (Vogel et al., 2015). Together, these findings in addition to those observed in the current study support the use of this particular MIPS product for acute improvements in metabolism and exercise performance with minimal side-effects after multiple weeks of supplementation even when consuming twice the recommended serving size. Additional research is needed to examine the long-term effects of MIPS

ingestion on REE, exercise performance, hemodynamic responses, and subjective feelings of fatigue in recreationally active females.

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APPENDIX A
INFORMED CONSENT

CONSENT FORM

The effects of ingesting a commercially available multi-ingredient pre-workout supplement on resting energy expenditure and exercise performance in recreationally active females.

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. If you decide to participate in this study, this form will also be used to record your consent.

You have been asked to participate in a research study examining the acute effects of ingesting a commercially available pre-workout supplement on resting energy expenditure and exercise performance. You were selected to be a possible participant because you met all entrance criteria for the study. You will be asked to complete a baseline testing session followed by two additional testing sessions, separated by one week.

What will I be asked to do?

If you agree to participate in this study, you will be asked to sign an Informed Consent statement in compliance with the Human Subjects Guidelines at The University of Wisconsin – La Crosse and the American College of Sports Medicine. You will then be familiarized to the study requirements and details of your participation. You will also be asked to complete medical history and exercise/activity history questionnaires. If you fit the entrance criteria you will report the Human Performance Laboratory in the Department of Exercise & Sport Science at The University of Wisconsin La Crosse for baseline testing. At this time, baseline information will be collected including body composition, and exercise performance. You will be asked to refrain from any strenuous exercise 48 hours and be in a post-absorptive state for at least 8 hours prior to each testing session. Body composition will be assessed using air-displacement plethysmography (*BODPOD, Cosmed, USA*). Following the body composition assessment you will begin a standardized warm up consisting of dynamic movements and then complete a counter movement vertical jump height test followed by a five-repetition maximum (5RM) back squat and bench press test using a Smith Machine. You will also perform 2 practice trials on the force treadmill. You will then report the Human Performance Laboratory within 7 days and will complete the first of two experimental testing sessions. During these testing sessions, you will ingest either a placebo (maltodextrin) or supplement (*Fitmiss Ignite™, MusclePharm*). Following ingestion of the supplement, participants you will be monitored for any changes in resting energy expenditure, heart rate, blood pressure and subjective feelings of energy and focus at 30 and 60 minutes. You will then complete a vertical jump test followed by a set of back squat and bench press to failure at 85% of your 5RM. Participants will rest for approximately 10 minutes and complete a maximal effort 25 second sprint test on a force treadmill. Within one week, you will return to the lab to receive the opposite treatment and complete the identical testing protocol.

You will also be asked to complete a 4-day food log prior to each testing session which will be analyzed for total energy and macronutrient intake using the Food Processor Nutrition Analysis Software Version 9.1.0 (*ESHA Nutrition Research, Salem, OR*). You will also be asked to complete two questionnaires to assess feelings of energy, fatigue, focus desire to complete task in addition to hunger, satiety and desire to eat using 5-pt Likert scales.

All data will be analyzed using SPSS statistical software. A 2-factor within-subjects repeated measures ANOVA using a treatment (supplement or placebo) x time (30, 60 minutes) will be used to assess differences in resting energy expenditure, cardiovascular response and subjective feelings of energy and focus between treatment conditions. A paired samples T-test will be used to assess changes in muscular endurance and anaerobic capacity. All analysis will be conducted using the Statistical Package for the Social Sciences (SPSS, Version 20; SPSS Inc., Chicago, IL). All data will be presented as mean \pm SD.

What are the possible risks of the study?

For this study, you will be asked to participate in three testing sessions consisting of minimal risk assessments. The vertical jump, back squat and bench press tests are common exercises and movements used in recreational fitness programs. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following testing. There is also the risk of muscle strains/pulls during the exercise testing. However, all testing will be supervised by trained personnel and monitored to ensure the participants follow appropriate exercise guidelines. The supplement as little known side-effects however it does contain caffeine and therefore if you are sensitive to caffeine you may experience increased feelings of energy and alertness. Furthermore, the supplement is manufactured in a facility that also produces products containing milk, egg, soybeans, shellfish, fish, tree nuts and peanuts and if you have a known allergy to any of these it is not recommended that you participate in the study.

What are the possible benefits of this study?

The possible benefit you may receive from participation in this study is an increased knowledge of your muscular strength, endurance and anaerobic power that may be applied to future training programs. You may also gain insight about your health and fitness status from the assessments that will be performed. For example, by completing in the study you will be aware of your dietary habits and whether or not they influence your body composition throughout the course of an exercise program.

Do I have to participate?

No. Your participation is voluntary. You may decide not to participate or to withdraw at any time without your current or future relations with The University of Wisconsin – La Crosse being affected.

Will I be compensated?

No, you will not be compensated for participation in this study.

Who will know about my participation in this research study?

This study is confidential. The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published. Research records will be stored securely and only Dr. Andrew Jagim will have access to the records.

Whom do I contact with questions about the research?

If you have questions regarding this study, you may contact Dr. Andrew Jagim at ajagim@uwlax.edu

Whom do I contact about my rights as a research participant?

This research study has been reviewed by the Exercise & Sport Science Department at The University of Wisconsin – La Crosse and. For research-related problems or questions regarding your rights as a research participant, you can contact these offices at The University of Wisconsin – La Crosse Institutional Review Board Committee for Protection of Human Subjects Member.

Signature

Please be sure you have read the above information, asked questions and received answers to your satisfaction. You will be given a copy of the consent form for your records. By signing this document, you consent to participate in this study.

Signature of Participant: _____

Date: _____

Printed Name:

Signature of Person Obtaining Consent: _____

Date: _____

Printed Name:

APPENDIX B
MEDICAL HISTORY QUESTIONNAIRE

MEDICAL HISTORY QUESTIONNAIRE

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement**.

Name: _____ Age _____ Date of Birth _____

Name and Address of Your Physician: _____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in the blank).

- | | |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------|
| <input type="checkbox"/> Heart murmur, clicks, or other cardiac findings? | <input type="checkbox"/> Bronchitis/Chest Cold? |
| <input type="checkbox"/> Asthma/breathing difficulty? | <input type="checkbox"/> Cancer, Melanoma, or Suspected Skin Lesions? |
| <input type="checkbox"/> Frequent extra, skipped, or rapid heartbeats? | <input type="checkbox"/> Stroke or Blood Clots? |
| <input type="checkbox"/> Chest Pain of Angina (with or without exertion)? | <input type="checkbox"/> Emphysema/lung |
| <input type="checkbox"/> High cholesterol? | <input type="checkbox"/> Epilepsy/seizures? |
| <input type="checkbox"/> Diagnosed high blood pressure? | <input type="checkbox"/> Rheumatic fever? |
| <input type="checkbox"/> disease? | <input type="checkbox"/> Scarlet fever? |
| <input type="checkbox"/> Heart attack or any cardiac surgery? | <input type="checkbox"/> Ulcers? |
| <input type="checkbox"/> Leg cramps (during exercise)? | <input type="checkbox"/> Pneumonia? |
| <input type="checkbox"/> Chronic swollen ankles? | <input type="checkbox"/> Anemias? |
| <input type="checkbox"/> Varicose veins? | <input type="checkbox"/> Liver or kidney disease? |
| <input type="checkbox"/> Frequent dizziness/fainting? | <input type="checkbox"/> Autoimmune disease? |
| <input type="checkbox"/> Muscle or joint problems? | <input type="checkbox"/> Nerve disease? |
| <input type="checkbox"/> High blood sugar/diabetes? | <input type="checkbox"/> Psychological Disorders? |
| <input type="checkbox"/> Thyroid Disease? | |
| <input type="checkbox"/> Low testosterone/hypogonadism? | |
| <input type="checkbox"/> Glaucoma? | |
| <input type="checkbox"/> Bleeding Disorders | |

Do you have or have you been diagnosed with any other medical condition not listed?

Please provide any additional comments/explanations of your current or past medical history.

Please list any recent surgery (i.e., type, dates etc.).

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 weeks.

What was the date of your last complete medical exam?

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study? (including strength and maximal exercise tests) . If yes, please explain:

Recommendation for Participation (for Staff use only):

No exclusion criteria presented. Subject is *cleared* to participate in the study.

Exclusion criteria is/are present. Subject is *not cleared* to participate in the study.

Signed: _____ Date: _____

APPENDIX C
PERSONAL INFORMATION

PERSONAL INFORMATION WORKSHEET

Personal Information

Name: _____

Cell Phone :(____) _____ E-mail address: _____

Birth date: ____ / ____ / ____ Age: ____ Height: ____ Weight: ____

Exercise History/Activity Questionnaire

1. Describe your typical occupational activities.
2. Describe your typical recreational activities
3. Describe any exercise training that you routinely participate.
4. How many days per week do you exercise/participate in these activities?
5. How many hours per week do you train?
6. How long (years/months) have you been consistently training?

APPENDIX D
REVIEW OF LITERATURE

REVIEW OF LITERATURE

Introduction

The use of dietary supplements to enhance exercise performance and improve body composition has long been a popular strategy for active individuals (Baron 2004). Caffeine is one of the earliest ingredients observed to improve body composition by increasing resting energy expenditure (REE) (Acheson, Zahorska-Markiewicz, Pittet, Anantharaman, & Jequier, (1980). Since then multi-ingredient pre-workout supplements (MIPS) have been produced using a proprietary blend of ingredients such as beta-alanine, creatine, and various herbal extracts, with the thought that adding these ingredients may have a greater effect on muscular strength, endurance and energy compared to simply ingesting caffeine. Although supplement companies are continuing to produce new products each year, caffeine alone is still used as a popular ergogenic aid in athletes (Antonio, 2004). The many claims of companies that produce MIPS are their ability to enhance exercise performance, REE, and subjective feelings (FitMiss™ Ignite™, MusclePharm, Inc., Denver, CO, USA.). From previous research other MIPS have been studied on acute ingestion and its effects (Campbell et al., 2016; Jagim et al., 2016; Kedia et al., 2014) Many studies have been conducted and overall observed that maximal strength exercise is comparatively unlikely to be altered with acute ingestion of caffeine, but muscular endurance has been shown to increase (Warren, Park, Maresca, Mckibans, & Millard-Stafford, 2009). Although there have been numerous studies showing the

benefits of pre-workout supplementation, the majority of the available research has focused primarily on males. There are limited data, however, regarding the effectiveness of pre-workout supplements in female populations. In a more recent study, Campbell et al. (2016) observed that females who ingested a caffeine-containing supplement with green tea extract significantly increased REE for up to 3-hours post ingestion. Over time these responses may lead to enhanced fat loss and improvements in body composition from repeated increases in resting metabolism. Due to the wide variety of outcomes after ingestion of a MIPS, along with new products coming out each year, it is practical to study the effectiveness and safety in both male and female populations.

Caffeine

Acheson et al. (1980) observed that caffeine alone may lead to improvement body composition by increasing resting energy expenditure (REE) regardless of a participant's weight. More recently, a study tested the effects of 6mg/kg of caffeine consumption 1 hour before exercise (Astorino, Rohmann, & Firth, 2008). The study consisted of 22 resistance-trained male subjects to determine if an acute ingestion of caffeine had any effect on their one repetition maximum (1RM) performance and their muscular endurance. Heart rate (HR) and systolic blood pressure (BP) both significantly increased when participants took the caffeine. The data reported no significant difference in 1RM or endurance for both the leg press and bench press, but there was an 11-12% increase for muscular endurance test. Even though these findings weren't significant, there was a trend toward a positive outcome from caffeine consumption on muscular endurance that needs to further researched.

With a lot of contradiction on the topic, a meta-analysis was done to clarify the effect caffeine has on maximal voluntary contraction (MVC) and muscular endurance (Warren et al., 2009). The research analyzed data from 34 studies conducted from 1939-2008. The results from all the studies showed that lower body MVC was slightly increased with caffeine ingestion and muscular endurance was also improved.

Goldstein¹ et al. (2010) performed a more recent analysis of research addressing the most efficient way to apply caffeine supplementation to enhance performance. In other words, is it more powerful in a capsule or powder compared to coffee, and is it more effective when consumed between 15-60 minutes before exercise? A low to moderate dosage (3-6 mg/kg) is adequate because higher dosages did not bring further benefits. A major finding is that caffeine supplementation is a proven ergogenic aid for aerobic activity, not just anaerobic. It also shows that most literature is inconsistent with regards to strength and power exercises, making it beneficial to further research the effect.

Very little research has been done on women and caffeine consumption. However, Goldstein², Jacobs, Whitehurst, Penhollow, & Antonio, (2010) tested 15 females on their 1RM and muscular endurance for bench press after consuming 6mg/kg of caffeine. The double blind, placebo-controlled, crossover study showed that caffeine consumption positively increased muscular strength, but not muscular endurance in women, which is in opposition of previous findings in males and the meta-analysis mentioned above (Goldstein¹ et al., 2010 & Warren et al., 2009).

Energy Expenditure

Many supplement companies make claims about burning fat, a desirable outcome for our obese population. One of the first studies to look at how caffeine and coffee affected an individual's metabolic rate in normal weight and obese individuals was done by Acheson et al (1980). Four different trials were conducted to test metabolic rate. The first trial was done on six participants that were prescribed 8mg/kg of caffeine. The second trial included a 4mg/kg dose of caffeine. Trial 3 was identical to trial 2, but in obese subjects. The fourth trial compared drinking coffee and decaf coffee after eating a meal. Each trial showed an increase in metabolic rate and in the fourth trial showed a significant increase in thermic effect compared to the decaf coffee.

Dalbo, Roberts, Stout, & Kerksick, (2008) studied the acute effects of ingesting a commercial caffeine-containing drink on changes in energy expenditure and markers of lipolysis. Thirty males and thirty females participated in the study and were placed into a supplement group or placebo group. Resting energy expenditure was assessed using respiratory metabolic measures. Results showed a significantly increase in REE with the supplement ingestion compared to placebo. Free fatty acids were also significantly greater post-consumption of the supplement compared to the placebo, suggesting increased lipolysis.

Outlaw et al. (2013) studied the effects of a caffeine-containing dietary supplement on energy expenditure, mood state, and cardiovascular measures. In a double-blind, crossover design, 6 males and 6 females participated. Results demonstrated that the caffeine-containing supplement significantly increased REE, alertness, focus, and energy

compared to the placebo. Also, there were no unsafe side effects related to supplement consumption.

In the most recent literature, Campbell et al. (2016) studied the effects of a caffeine-containing dietary supplement in healthy women. In a randomized, double-blind, placebo-controlled, cross-over design involving 13 females. Participant's fasted overnight and were assessed the following morning for resting metabolic rate (RMR), HR, and BP following ingestion of the supplement or a placebo for 60, 120, and 180 minutes. Results indicate that the supplement significantly increased RMR at each of the time points previously listed. Systolic BP and diastolic BP had a main effect for time following ingestion of the supplement, but HR was not affected.

Muscular Strength

Athletes are always striving to be the best they can be and supplementation as an ergogenic aid has been a popular phenomenon (Baron, 2004). Beck et al. (2006) studied the acute effects of a caffeine supplement on strength, muscular endurance, and anaerobic capabilities. Thirty-seven male subjects participated in the study comprising of a Wingate cycle test, leg extension 1RM, leg extension endurance test, bench press 1RM, and bench press endurance test. The only significant finding was the supplement increased upper body maximal strength. This evidence illustrates the reason why max testing during experiments is still reasonable even when most research articles just show an increase in muscular endurance rather than maximal strength.

Muscular Endurance

Muscular endurance has been the major focus of the effect of caffeine and has been shown to increase in many studies (Warren et al., 2009). A study done on Red Bull

energy drink was designed to determine the acute effects it had on the Wingate Cycle test and muscle endurance (bench press) (Forbes, Candow, Little, Magnus, & Chilibeck, 2007). Twelve men and 4 women participated in a double-blind crossover placebo study with a 7-day break in between testing sessions. The results showed that consumption of Red Bull had a significant effect on upper body muscular endurance compared to the placebo, but no effect for anaerobic power.

Similarly a study was done on the acute effects of the energy drink, which contained 179 mg of caffeine (Duncan, Smith, Cook, & James, 2012). Thirteen resistance trained men consumed either a placebo or the supplement an hour before they performed repetitions to failure on the bench press, prone row, deadlift, and back squat set at 60% of 1RM. The participants significantly increased their repetitions and significantly decreased their rate of perceived exertion (RPE) when consuming the energy drink.

Gonzalez, Walsh, Ratamess, Kang, & Hoffman. (2011) supported the previous two studies with their results. They studied the effects a pre-workout supplement on acute multi-joint resistance exercise with 8 male subjects. They split the sample evenly to perform back squat and bench press exercises. The total number of repetitions was significantly greater when participants took the supplement compared to the placebo. Average peak and mean power during exercise were also significantly greater with supplement consumption.

Spradley et al. (2012) conducted a study on a pre-workout supplement and its effects on fatigue, reaction time, and muscular endurance. Over 3 weeks, 12 recreationally trained males participated in the randomized, double blind, placebo

controlled crossover study. They were tested on a choice reaction machine, bench press endurance, leg press endurance, and maximal oxygen consumption ($VO_2\text{max}$). The results showed that consuming the supplement may lead to significant improvement in leg press endurance, perceived energy, alertness, focus, and choice reaction time, but had no effect on $VO_2\text{max}$.

Anaerobic Power

Hoffman et al. (2009) analyzed the effects of a caffeine-containing energy drink on feelings of energy, fatigue, alertness, focus, quickness and anaerobic power. Twelve males participated in the study where a questionnaire, reaction test, and a 20-second Wingate cycle test were performed three trials in one session with a 10-minute rest period in between each trial. They either consumed the supplement or the placebo 10 minutes before they performed the first trial. After ingestion, participants had significantly higher energy for all trials along with a significantly greater mean focus with the supplement compared to the placebo. Similar to other studies, there were no significant differences for the anaerobic tests.

A more recent study (Jagim et al., 2016) observed the acute effects of a MIPS with college athletes. Ingestion of a MIPS significantly increased participants mean power when performing a maximal sprint test to assess for anaerobic performance. Other markers such as total work, average power, and mean and average velocity were not significantly enhanced with consumption of a MIPS. Jagim et al. (2016) also observed the effects the MIPS had no significance on a counter-movement vertical jump.

Subjective Feelings

The easier a workout is, the longer an athlete can tolerate it. Many studies have shown higher energy levels and greater focus, but only one study has specifically tested time to exhaustion. Walsh, Gonzalez, Ratamess, & Hoffman, (2010) examined how the energy drink Amino Impact would affect the time to exhaustion on a treadmill run at 70% VO₂max. Nine men and 6 women performed two runs while consuming either the supplement or the placebo, on separate days. Supplement consumption lead to a significantly greater time to exhaustion, along with significantly greater focus, energy, and less fatigue before trials. Also, there was greater focus and energy 10 minutes into exercise. The fatigue after exercise was not significant.

In a more recent study, Jagim et al. (2016) studied the acute effects of a multi-ingredient pre-workout supplement on strength performance, lower body power, anaerobic capacity, and subjective feelings. Twelve male subjects were assessed for 5RM's for both back squat and bench press. Subjects reported to the laboratory on two testing days and were first asked a questionnaire based on their feelings of focus, energy, and fatigue. Then subjects ingested either a placebo or a supplement. Thirty minutes following ingestion a counter-movement vertical jump (CMVJ) was performed, followed by 5x5 protocol at 85% of the subject's 5RM for back squat and then bench press with another questionnaire following. Finally, subjects performed a maximal sprint test with another questionnaire to end the testing session. Results indicate that subject feelings of focus were significantly greater following the resistance training with supplement ingestion, along with a reduction in fatigue at the same time point.

Safety Dosage

Kedia et al. (2014) did an acute study on hemodynamic safety of a pre-workout supplement. Forty men and women participated in the study and consumed one dose of the supplement following an 8-hour fasting state. Heart rate and BP were assessed before ingestion and then multiple times after the supplement was ingested. An electrocardiogram (ECG) was performed at baseline and after 180 minutes ingestion to look for abnormalities with the heart's electrical activity. Blood samples were taken before and after ingestion. Following supplementation, systolic and diastolic BPs were raised compared to the placebo, and from a resting state. No changes in the ECG occurred following supplementation.

Vogel et al. (2015) examined the safety of the pre-workout supplement, FitMiss™ Ignite™, on hematological and resting vital signs. They took 34 recreationally active women and put them into two groups: 1 serving or 2 servings, lasting 28 days. They were instructed to keep the same lifestyle based on diet and exercise. Blood samples were recorded before and after the experiment. They were instructed to take the supplement 30 minutes before their workout on exercise days and take it at approximately the same time on rest days. Overall, the changes in the blood samples received were within clinically safe levels and resting vitals were not significantly different or elevated into a high-risk category.

CONCLUSION

In conclusion, more research needs to be done on these highly consumed supplements. There is sufficient research on some of the products, but due to new formulas and products, it is appropriate to do further studies. Also, very little research has been done on female subjects. By completing this study, we hope to help consumers and companies know the effects of their products they either consume or sell.

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