THE EFFECTS OF CAFFEINE INTAKE ON MUSCLE PROTEIN SYNTHESIS AND THE CHANGE IN LEAN MASS FOLLOWING RESISTANCE EXCERCISE

A Dissertation

by

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ABSTRACT

Caffeine is a commonly used drug and can be found in many everyday products. It has been established that caffeine has ergogenic properties in aerobic metabolism; however, the effects of caffeine on anaerobic metabolism are still unclear in respect to performance and muscle recovery and some data suggest caffeine may even have inhibitory effects on muscle growth. The purpose of this research was to document the effects of caffeine intake on muscle protein synthesis rates, muscle performance, and changes in lean mass following resistance exercise training. We hypothesized that increased caffeine intake would cause a decrease in muscle protein synthesis rates.

The first study examined the effect of caffeine on rates of muscle protein synthesis rates following an acute bout of resistance exercise in male Sprague Dawley rats. Caffeine intake did not alter the rates of muscle protein synthesis following resistance exercise.

The second study examined muscle protein synthesis rates following resistance exercise in men consuming a caffeine bolus before exercise. Muscle performance was measured using maximal power, total weight, and total repetitions. Activation of cellular proteins (AMPK and p70s6) was measured by Western Blots. Caffeine intake had no effect on 24-hour muscle protein synthesis, power output, and total weight and repetitions performed. There were also no differences in total p70s6K, phosphorylated p70s6K, or phosphorylated AMPK between groups, but an increase in total AMPK expression was observed.

The final study analyzed the effects of habitual caffeine intake on changes in lean mass and muscle performance following 12 weeks of full-body resistance exercise in an untrained population. There were no changes in lean mass; however, the data suggested that high caffeine intake was associated with lower muscle performance in certain exercises (leg press, leg curl, and lat pulldown) and not in others (chest press, bicep curl, leg extension, and triceps extension).

The results of these studies suggest that caffeine intake has no effects on muscle protein synthesis following acute resistance exercise and no effect on changes in lean mass following chronic resistance exercise. Future studies involving different populations and exercise models may clarify the effects of caffeine on exercise performance.

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INTRODUCTION AND LITERATURE REVIEW

Caffeine is one of the most widely used drugs in the world with approximately 80% of the adult population reporting daily use in the United States [1]. Caffeine is found in plants, coffee beans, tea leaves, kola nuts, and cacao pods, and can be synthetically produced as well. Caffeine is classified as a stimulant and can alter the central nervous system resulting in decreased fatigue and increased alertness. Until recently, the International Olympic Committee and the National College Athletic Association had set upper limits (15µg/ml) to traceable urinary caffeine levels during exercise performance [2]. Although the mechanisms behind the effects of caffeine were believed as understood from earlier studies, research during the past two decades has raised more questions about the primary physiological mechanism of caffeine.

Systemic effects of caffeine include increased alertness, respiratory rate, and decreased fatigue, but it is not clear if caffeine has an ergogenic effect on exercise performance. The effects of caffeine on aerobic-endurance exercise have been documented, demonstrating an increased ability to train and perform at longer durations and at higher power outputs [3, 4]. These systemic effects may also enhance anaerobic strength and skeletal muscle performance; however, research results regarding caffeine intake and anaerobic strength performance have been inconsistent [5, 6].

An important aspect of resistance exercise is the muscular recovery period following exercise. While caffeine is currently being used to enhance acute performance

during exercise training, the consequences on the muscular recovery period have not been considered. The majority of muscle protein synthesis and muscle hypertrophy occurs following resistance exercise and peak muscle protein synthesis occurs approximately 24-48 hours post-exercise [7-9]. There have been no studies on the effects of caffeine on muscle protein synthesis rates; although, caffeine has been reported to inhibit key regulatory proteins involved in muscle growth in animal studies [10-13].

The primary goal of this research was to examine the effects of caffeine on muscle recovery, changes in lean mass, and protein synthesis rates following resistance exercise. It has been proposed that increased caffeine consumption will attenuate muscle recovery and protein synthesis by down-regulating key pathways responsible for muscle hypertrophy. Considering the popularity of caffeine in energy drinks, sport drinks, coffee, diet aids, and other products and more recently pre-workout supplements, the notion of caffeine as an ergogenic aid in strength training needs to be validated. The results of this investigation would benefit a wide range of athletes, from recreational to elite, allowing for optimization of training, performance, and recovery. As a secondary objective, this research attempted to clarify the interaction between caffeine supplementation and exercise muscle performance.

Biosynthesis

The chemical nomenclature and structure of caffeine is 1,3,7-trimethylxanthine (**Figure 1**), a xanthine molecule with methyl groups replacing hydrogens. Caffeine is produced by plants within chloroplasts; xanthine is converted to xanthosine, methylxanthosine, methylxanthine, dimethylxanthine, and finally to trimethylxanthine

(caffeine), where it is isolated for human consumption (**Figure 2**). Caffeine cannot be produced in animal tissue [14].

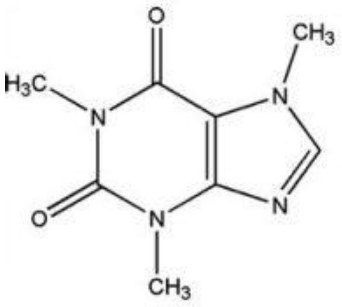

Figure 1. Caffeine molecule. 1, 3, 7-trimethylxanthine.

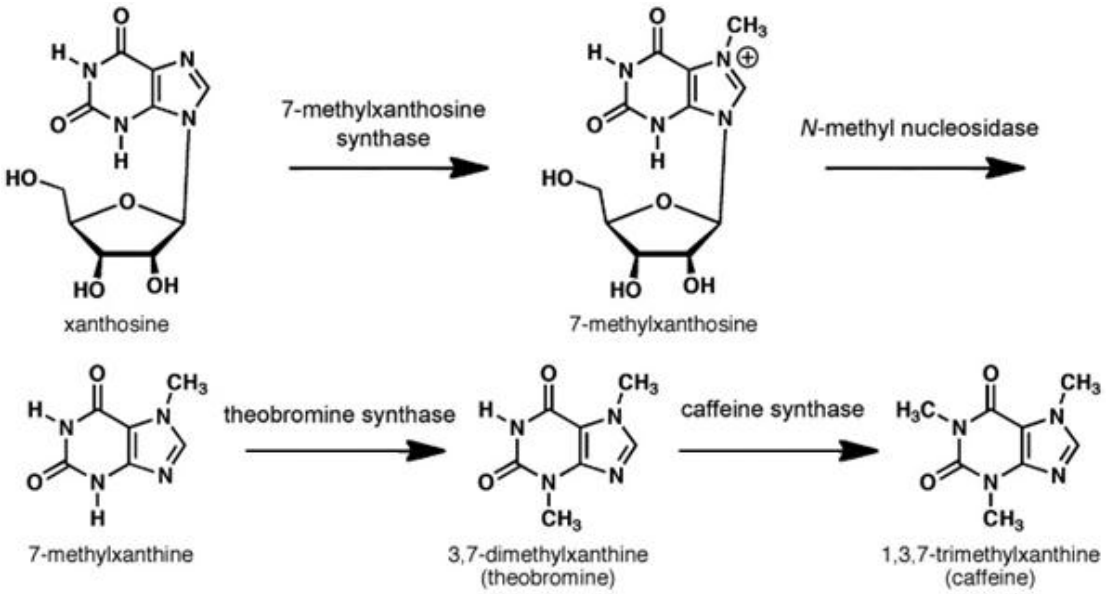

Figure 2. Caffeine biosynthesis pathway.

Absorption & Mode of Action

Absorption

Caffeine is quickly absorbed by the mouth, throat, and gastrointestinal track. Due to its hydrophobic properties, it can diffuse into most tissues without a carrier. Peak plasma concentrations of caffeine occur 40-60 minutes after ingestion and physiological effects last 6-10 hours with a half-life of 3-6 hours [15, 16]. Caffeine is metabolized in the liver by a group of enzymes known as cytochrome P450 (in particular CYP1A2) into three dimethylxanthines: paraxanthine, theophylline, and theobromine, each with similar yet varying effectiveness to that of caffeine. Paraxanthine and theophylline have the strongest potency and can cause physiological changes similar to that of caffeine [16, 17]. Only 1-3% of caffeine is excreted in urine as 1, 3, 7-trimethylxanthine.

Adenosine

Adenosine is involved in the regulation of central nervous system neurotransmission, vasodilation, and catecholamines. Adenosine receptors are found in the brain, heart, smooth muscle, adipocytes, and skeletal muscle [15, 18]. There are four different types of adenosine receptors (A1, A2a, A2b, and A3), and depending on which receptor adenosine binds to is what determines the physiological response [14]. High concentrations of adenosine occur during states of fatigue and low energy, and exercise increases adenosine concentration in skeletal muscle, smooth muscle, and brain [19-21]. As adenosine accumulates, increased pain perception, increased fatigue, decreased arousal, and decreased performance result [22, 23]. Due to the similar structure of caffeine to adenosine and its ability to cross the blood-brain barrier, caffeine acts as a

competitive inhibitor and blocks adenosine receptor binding [24]. When caffeine is bound to adenosine receptors, the brain does not respond to adenosine, which causes an individual to feel less fatigue and more alert [22, 23, 25].

Phosphodiesterase

Another well-known effect of caffeine is through its inhibition of phosphodiesterase [14]. Phosphodiesterase breaks down phosphodiester bonds; in particular, the diester bond of cyclic adenosine monophosphate (cAMP), converting it to adenosine monophosphate (AMP). Similar to adenosine, caffeine acts as a competitive inhibitor of phosphodiesterase, causing cAMP concentrations to increase [14, 26]. Cyclic adenosine monophosphate is synthesized from adenosine triphosphate (ATP) by adenylyl cyclase and acts as a second messenger. Cyclic adenosine monophosphate activates protein kinase A which activates or inhibits certain metabolic pathways (e.g. lipolysis and glycogen synthase) in all cell types. Compared to the effects of adenosine, the potency of caffeine on phosphodiesterase is extremely low. The half maximal effective concentration (EC50) for caffeine to inhibit phosphodiesterase is approximately 20X higher than that for adenosine [26].

Muscle Contraction

Ryanodine receptors are found in muscle cells and once activated cause calcium release for muscle contraction [14]. Ryanodine receptors are caffeine sensitive and exposure to caffeine causes increased activation and increased calcium release [14]. However, the amount of caffeine needed to activate ryanodine receptors is

approximately 100X higher than the amount of caffeine needed to block adenosine from binding to adenosine receptors [14, 26].

Fat Oxidation

A previous study has suggested that caffeine intake increases fat oxidation during exercise [16]. This causes an increase in the free fatty acids from adipose tissue and the muscle is then free to use it as an energy source sparing glycogen and increasing time to fatigue [27]. Some studies have reported conflicting results. Graham et al. 2001 recruited 10 male participants to perform 1-hour of exercise on a cycle ergometer at 70% VO_2 max in a cross-over design. Respiratory exchange ratios were measured at four different times. No significant differences were seen between the placebo and caffeine groups in terms of free fatty acid exchange (**Figure 3**) [16].

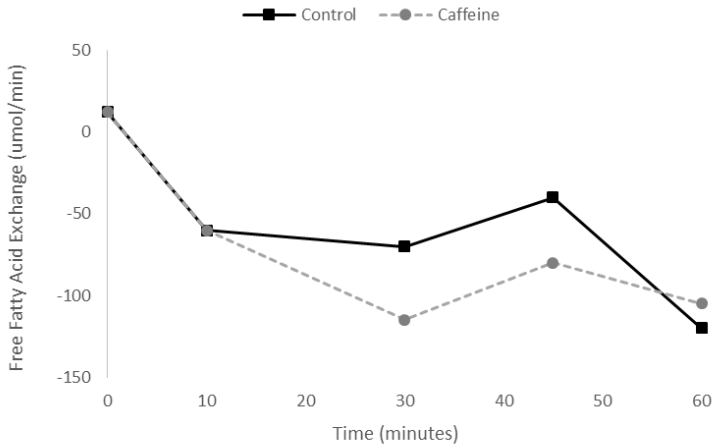

Figure 3. Free fatty acid exchange as a function of caffeine intake and time.

These findings were similar to a previous study that reported no significant differences in free fatty acids exchange were observed when participants exercised at 75% VO_2 max for 30 minutes on a cycle ergometer [28]. The ability of caffeine to increase fatty acid oxidation to increase exercise performance is now widely challenged and not accepted as a likely explanation [24].

Sparing of Muscle Glycogen

Earlier studies [29, 30] reported that caffeine intake before exercise resulted in glycogen sparing. However, more recent studies have indicated otherwise [16, 31, 32]. The more current studies have consistently demonstrated no differences in glycogen levels during exercise between placebo and caffeine groups. Graham et al. [33] accumulated all data from previous studies and analyzed the potential effect of caffeine on muscle glycogen sparing, and concluded that caffeine played no role in muscle glycogen sparing (**Figure 4**).

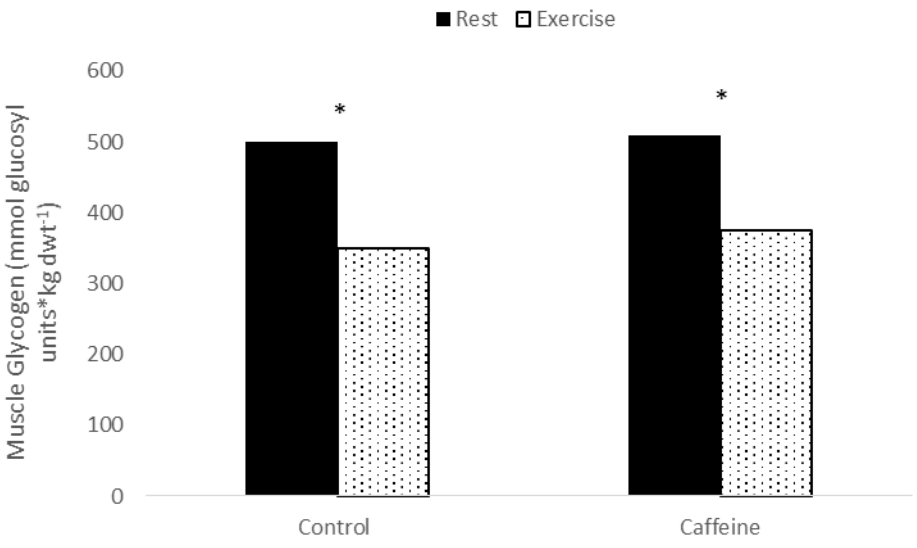

Figure 4. Muscle glycogen levels as a function of caffeine intake. *indicates

statistically significant differences between the control and caffeine group [33].

Increased Sympathetic Activity

Caffeine may increase sympathetic activity during exercise by increasing catecholamine release, in particular adrenaline. Previous studies reported significant increases in circulating levels of adrenaline following caffeine intake [34-36]. It is debatable whether the increase is sufficient to cause a metabolic or performance difference [16, 24]. In one study, tetraplegics, whose adrenal responses to sympathetic nervous system stimulation are not present, were given caffeine supplements, and their muscles were electrically stimulated. It was determined that plasma catecholamine levels remained the same; however, onset of muscle fatigue was still delayed during the stimulated exercise [37]. This brings forth the issue of whether caffeine's ergogenic effect is accomplished through a change in catecholamines or another mechanism.

Blood Flow

As a stimulant, caffeine can cause an increase in heart rate. It has been speculated that the increase in heart rate would also cause an increase of blood flow to the muscles; thus, increasing exercise performance. Daniels et al. [38] determined the rate of forearm blood flow during exercise following a pre-exercise caffeine supplement and concluded that there was no statistically significant differences between caffeine and placebo groups. It was proposed that caffeine's ability to block adenosine, which is also responsible for vasodilation, may have counteracted any observable changes [38-40].

Other Mechanisms

Other suspected physiological mechanisms of caffeine are still not fully understood. It is believed that caffeine may have a direct effect on the sodium-potassium and ATPase pumps in skeletal muscle [16, 41]. It is hypothesized that caffeine ingestion could help prevent potassium loss, which causes a decrease in motor unit activation and less force production. Another possibility involved caffeine's effect on glycogen phosphorylase and the localization and binding of calcium during muscle contraction [16, 41].

Dosage & Timing

The most common form of caffeine intake occurs through coffee consumption. However, one cup (8oz) of coffee can contain anywhere from 100-250mg of caffeine making it difficult to document intake. Until recently, the NCAA and the IOC set their upper limit for caffeine with a urinary test level no higher than 14μm/ml (~800mg) [2]. This can be achieved by ingesting approximately 9+mg/kg of body weight of caffeine. In most research studies, the typical dosage given to the participant has been 2-9mg/kg of body weight (most common being 5-6mg), in powder or tablet form, approximately 1-hour before exercise. This allows for peak caffeine plasma levels during exercise [16, 24, 27, 42]. Based on past studies, the ergogenic potential of caffeine can be seen in dosages much lower than the NCAA and IOC limit. These dosages range anywhere from 400-600mg and in some studies have been as low as 250mg [2]. Important factors to consider are the different rates of caffeine metabolism, sensitivity, and tolerance an individual might have. It has been observed that individuals that consume caffeine can

build up tolerance, requiring more caffeine to cause similar physiological effects, in as little as 2-3 days [16].

Caffeine and Exercise

While caffeine has no nutritional value, previous studies have observed caffeine's ergogenic effects during certain types of exercise. It would be most efficient to organize the effects of caffeine on exercise into two categories: aerobic exercise and anaerobic exercise. Caffeine's impact on each type of exercise will be discussed in more detail in the following section.

Aerobic Exercise

In terms of aerobic exercise, few studies report that caffeine intake has no effect on exercise performance. The majority of studies have shown that caffeine supplements of 3-9mg/kg cause a significant increase in exercise capacity [16, 43-45]. Due to the sheer volume of the studies involving caffeine and aerobic exercise and the consistency of the results, only a few more recent studies will be summarized in this review.

In a study by Bell et al. [3], 21 regularly actively aerobic individuals were given a 5mg/kg of body weight bolus of caffeine or placebo and exercise was performed at three separate time-points following supplementation (1, 3, 6 hours post-supplementation). The individuals were also placed into four groups depending on caffeine intake frequency and served as their own controls. Exercise consisted of peddling on a cycle ergometer at 80% of maximal oxygen consumption until exhaustion. For non-users of caffeine, there was a significant increase in time to exhaustion at all

three test points. For the caffeine user group, significant increases in time to exhaustion were recorded in only the 1 and 3 hour test points (**Figure 5**).

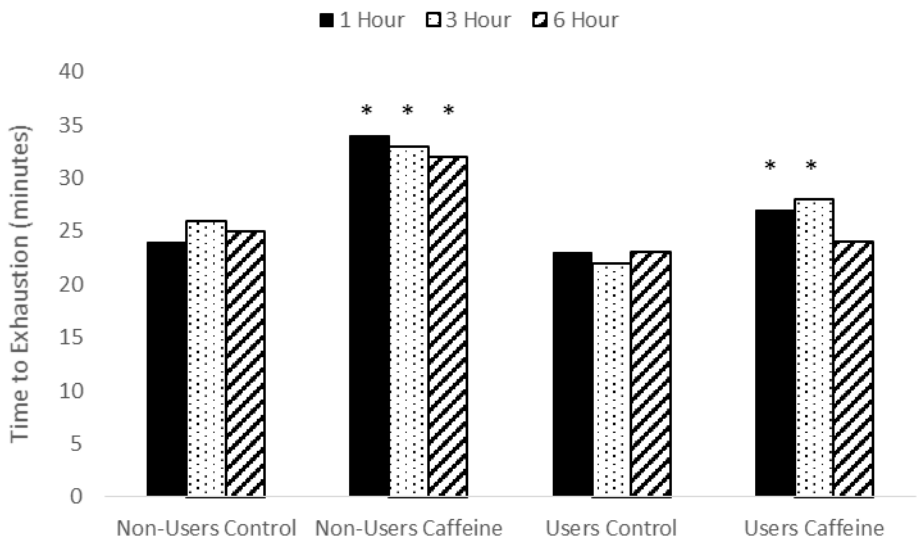

Figure 5. Effects of caffeine on time to exhaustion. *indicates a significant difference between control groups [3].

In a study by Hogervorst et al. [46], 24 well trained cyclists consumed 100mg of caffeine, a 45g of carbohydrate performance bar or a placebo beverage (control) before exercising for 2.5 hours at 60% VO_2 max, followed by a time to exhaustion trial at 75% VO_2 max on a cycle ergometer. It was concluded (**Figure 6**) that time to exhaustion was significantly longer in the caffeine group compared to the carbohydrate and control groups.

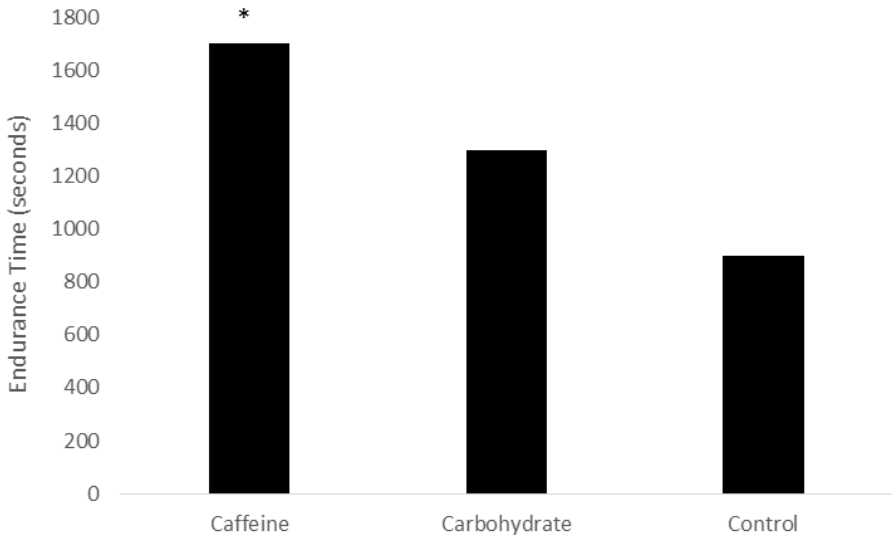

Figure 6. Effects of caffeine take on endurance time. *indicates a significant difference between groups [46].

O'Rouke et al. [47] evaluated caffeine supplementation in regards to 5km runs. Fifteen well trained individuals and fifteen recreationally trained males were recruited and given 5mg/kg of caffeine 1-hour before exercise. Results indicated that 27 out of the 30 runners gained a performance benefit. The trained group experienced a statistically significant ($p<0.05$) 1.1% decrease in time and the recreational group experienced a 1% decrease in time. Similar results were validated in a longer distance (8km) running study as well [48].

In 2011, Backhouse et al. [49] tested the effect of caffeine on the rate of perceived exertion using a double blinded study. Twelve endurance trained athletes were recruited and given 6mg/kg of caffeine following an overnight fast. Athletes performed 90 minutes of exercise on a cycle ergometer at 70% VO_2 max. Perceived exertion was

recorded throughout the exercise bout. A significant decrease in perceived exertion was recorded in the caffeine group compared to the non-caffeine group at six different time-points (**Figure 7**) [49].

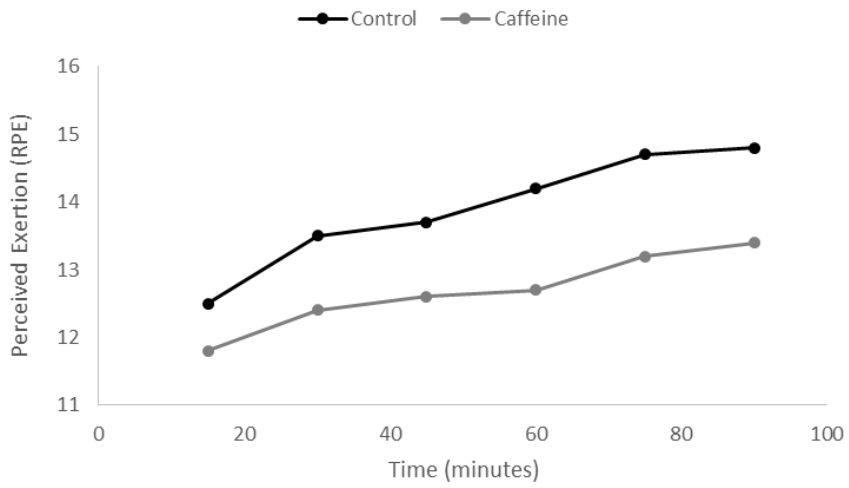

Figure 7. Caffeine intake and the rate of perceived exertion.

Anaerobic Exercise

While the effects of caffeine on aerobic exercise have been examined extensively, studies on the effects of caffeine on anaerobic exercise are limited and unclear. In 2007, Green et al. [50] conducted a study regarding the effects of caffeine on repetitions to failure. Seventeen individuals were tested with a ten repetition maximum in bench press and leg press. After a caffeine (6mg/kg) or placebo supplement, individuals performed 3 sets to failure in each exercise. The number of repetitions were

recorded and analyzed. No significant differences were reported in bench press and only a significant difference was found in the third set of leg press (**Figure 8**).

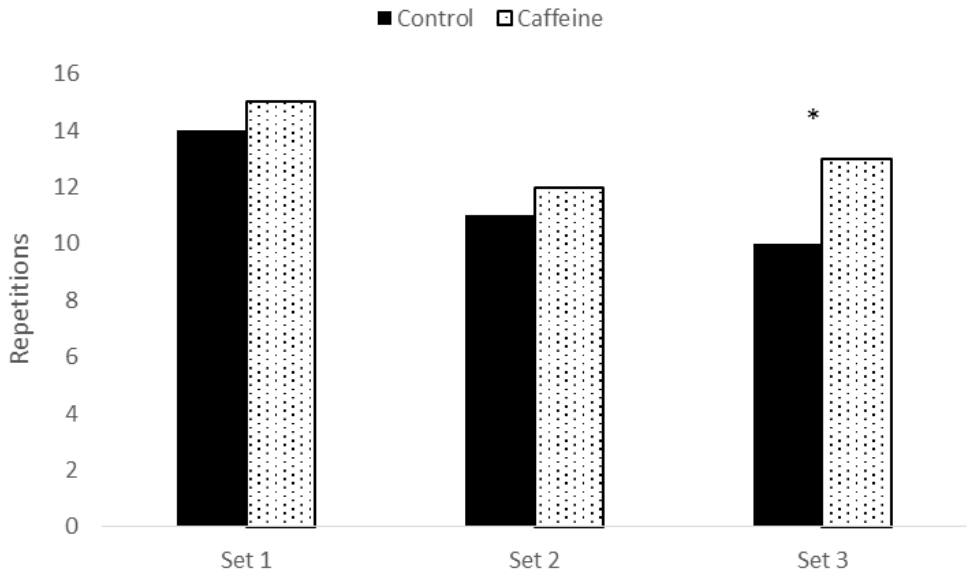

Figure 8. Caffeine intake and leg press repetitions. *indicates a significant difference between control and caffeine groups [50].

In another study by Astorino et al., [51] 22 resistance trained males either ingested a placebo or 6mg/kg of caffeine 1-hour before exercise in a double blind crossover design. Participants then performed bench press and leg press to failure at 60% of their 1 repetition maximum (1RM). Total weight lifted in the caffeine group was approximately 11-12% higher compared to placebo; however, the difference was determined to be not statistically significant. These results were consistent with similar studies [5, 52, 53]. In 2008, Woolf et al. [6] also conducted a similar experiment but

supplemented with 5mg/kg and found a significant difference in bench press (+2.4kg), but not in leg press. Caffeine's ergogenic effect on upper body strength was reaffirmed with the results from a more recent study by Goldstein et al. [54]. There was a significant difference in 1RM weight lifted in 15 resistance trained women who were supplemented with 6mg/kg or placebo, but no differences in the number of repetitions to failure. On the contrary, Astorino et al. [55] followed a similar protocol as previous studies with a 6mg/kg caffeine bolus, but performed 1RM testing in several different exercises: bench press, leg press, bilateral row, and shoulder press. It was concluded that caffeine only had an ergogenic effect on leg press but not upper body exercises [55, 56].

In 2014, Duncan et al. [57] supplemented ten trained males with 6mg/kg of caffeine to determine the effects on peak torque produced in isokinetic knee extensions. Compared to the placebo group, it was concluded that caffeine supplementation significantly increased torque production.

Caffeine and the rate of perceived exertion in resistance exercise were also explored in a study by Duncan et al. [58, 59]. Eleven resistance trained individuals were supplemented with 5mg/kg of caffeine or placebo before performing bench press, deadlift, prone row, and back squat to failure at 60% 1RM. This was one of the few studies that reported differences in the rate of perceived exertion between caffeine and placebo groups [6, 50, 51, 60].

Several researchers have proposed that exercises that are shorter in duration seem to have much more inconsistent results due to the fact that the potential for improvement is small and difficult to measure due to the short nature of the exercise [60, 61].

Adverse Effects

There are several side effects when consuming high amounts of caffeine. Caffeine acts as a mild diuretic; causing increased fluid and electrolyte loss and decreased plasma volume [6, 16]. However, in several studies that quantified caffeine intake in regards to body-weight, sweat rates, plasma volume, and electrolyte loss, there were no differences between those who supplemented with caffeine and those who did not during exercise [44, 62]. One possible explanation given was that it would take several hours for noticeable changes in fluid balance, and that exercise was usually completed before the risk for diuresis occurred [16].

Caffeine dependency is another possible side effect. Dependency can be characterized by increased tolerance, increased substance intake, persistent desire for the substance, and withdrawal symptoms [16]. Common withdrawal symptoms include headaches, mood shifts, drowsiness, decreased alertness, and fatigue. Withdrawal symptoms can occur as little as three days after caffeine exposure in novel users and 12 hours in habitual users [2]. Some studies have also reported an increase in core body temperature after consuming caffeine, a detrimental effect for anyone who exercises at high temperatures [2]. Frequent users of caffeine also experience a higher tolerance compared to non-users and need to consume larger amounts of caffeine to elicit a similar response [36, 63-65].

It is not uncommon for caffeine to be used in combination with other drugs to aid in weight loss, or to further its ergogenic effects. A substance that was commonly used with caffeine was ephedra. Studies have shown this combination can increase weight

loss due to an increased metabolic rate. This increase in metabolic rate can cause an increase in cardiovascular stress and in several cases it has been linked to deaths [66]. Ephedra has now been banned by the Federal Drug Administration.

Caffeine and Muscle Protein Synthesis

While performance based studies are of importance, it is just as important to examine the muscle recovery response to resistance exercise. A typical goal of resistance exercise is to increase muscle mass and the majority of muscle protein synthesis and hypertrophy does not occur until after exercise. Peak muscle protein synthesis has been noted to occur at 24-48 hours post exercise [67, 68].

When discussing cellular and muscle growth, there are several key regulatory pathways that need to be examined. One major pathway consists of the mammalian target of rapamycin, protein kinase B, phosphatidylinositol 3-kinases (an up-regulator of the mammalian target of rapamycin), and AMP-Activated Protein Kinase activity (**Figure 9**) [11, 69-71]. Each will be discussed briefly below.

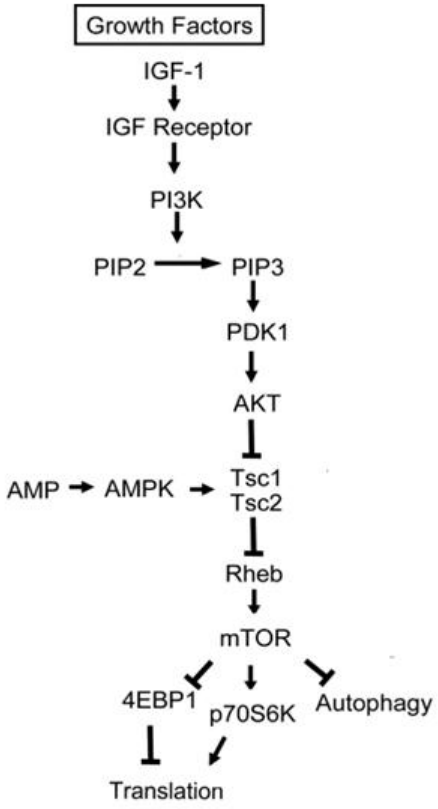

Figure 9. Cellular growth pathway.

Mammalian Target of Rapamycin

The mammalian target of rapamycin (mTOR) is one of the regulatory proteins involved with cellular growth [72]. One method of mTOR activation occurs through upstream signaling of phosphatidylinositol-3-kinase and phosphatidylinositol regulated protein kinase. Once mTOR has been activated, downstream translation factors consisting of 4EBP1 and p70s6K would increase in activation or inhibition causing an increase in translation and cellular proliferation [73] (**Figure 9**).

In previous studies, caffeine exposure has been demonstrated to inhibit mTOR activity [74, 75]. In a study by Scott et al. [13], rat adipocytes and brain tissue were incubated with theophylline (a substance with similar effects to caffeine) and mTOR activity levels were significantly reduced compared to non-theophylline groups (**Figure 10**) [13].

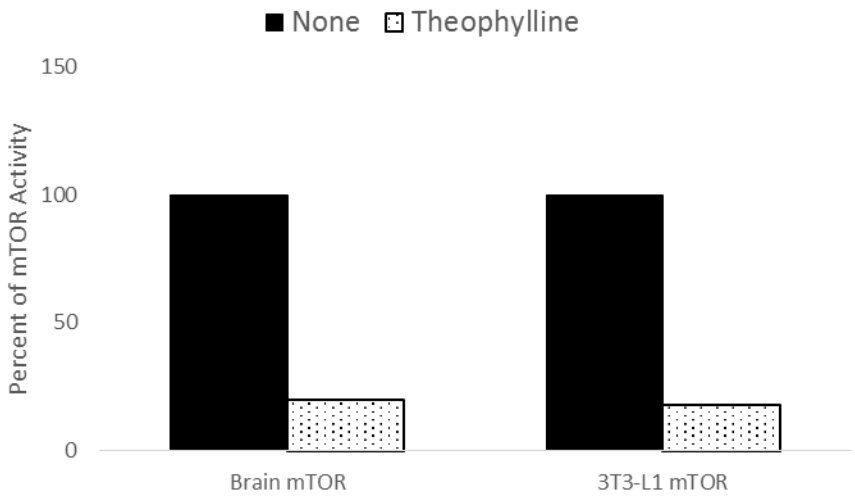

Figure 10. Effects of caffeine on brain and adipocyte mTOR activity levels.

Protein Kinase B

Protein Kinase B, or commonly known as Akt, is a key protein in the mTOR pathway and its activation is also associated with increased cellular growth. In a study by Akiba et al. [76], cells were treated with varying concentrations (0-1.0mM) of caffeine for 1-hour before being stimulated with insulin. It was observed that caffeine significantly decreased Akt activity levels compared to non-caffeine treated cells

(**Figure 11**) [76]. Similar results were noticed in other tissues as well [11, 70, 74, 75, 77].

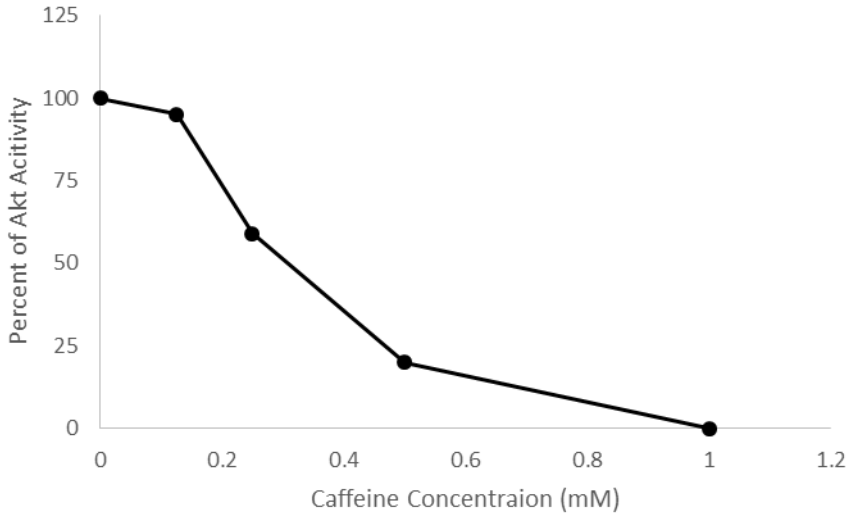

Figure 11. Akt activity with the presence of caffeine.

Phosphatidylinositol 3-Kinase

Phosphatidylinositol 3-Kinase (PI3K) are enzymes responsible for converting phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 acts as an upstream regulator for mTOR and Akt in the cellular proliferation pathway (**Figure 9**). In a study by Foukas et al. [11], caffeine significantly inhibited PI3K activity compared to non-caffeine groups. Similar results were found in additional studies as well [74-77] (**Figure 12**) [11].

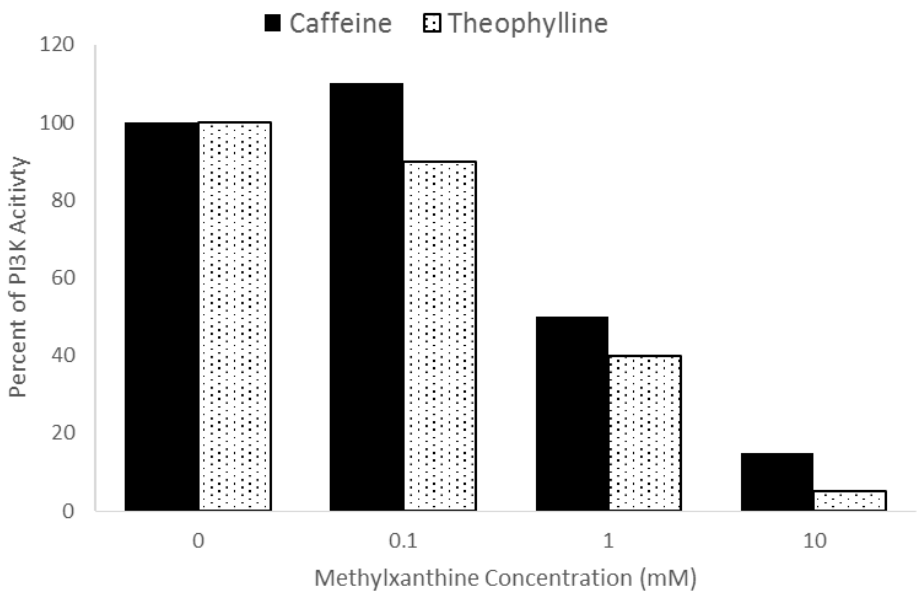

Figure 12. Caffeine on PI3K activity.

AMP-Activated Protein Kinase Activity

AMP-Activated protein Kinase Activity (AMPK) is referred to as the "energy sensor" protein within the cell. It can increase glucose uptake and fatty acid oxidation in skeletal muscle. An important regulator to the activation of AMPK is the presence of AMP. Elevated concentrations of AMP occur during high levels of cellular stress, exercise, or during increased muscle contraction [78]. Given that increased cellular synthesis is a high energy requiring process, it would be characteristic to see decreased protein synthesis during these times of high energy consumption [78]. In a 2002 study by Bolster et al., 5-aminoimidazole-4-carboxamide 1-D-ribonucleoside (AICAR) was used to directly stimulate AMPK without altering any cellular concentrations of ATP, ADP, and AMP [78]. Animals exposed to AICAR had a significant increase in AMPK

activation and as of a result had a significant decrease in protein synthesis rates (**Figure 13**) [78].

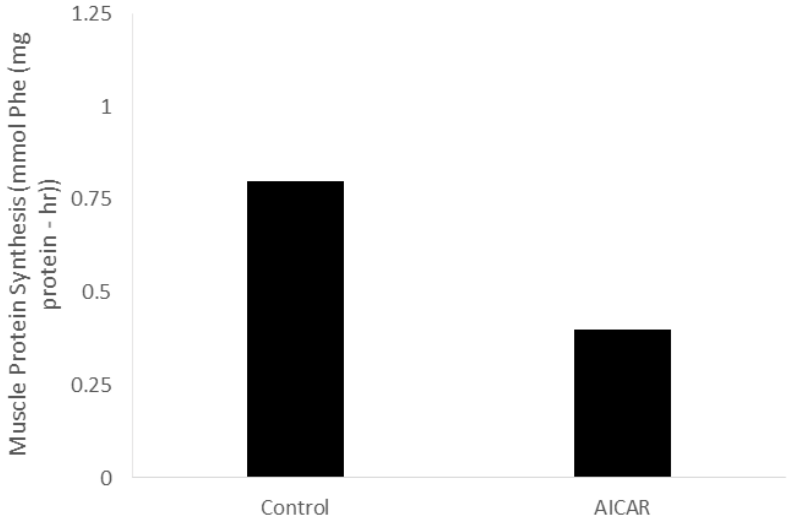

Figure 13. Rates of protein synthesis with the presence of AICAR.

Caffeine has similar stimulatory effects to AMPK at higher concentrations (>3mM). In studies by Egawa et al. [10] and Jensen et al. [12], rat soleus muscle was incubated with caffeine at various concentrations and durations resulting in significant increases in AMPK phosphorylation (**Figure 14**). Currently, no studies have examined the direct effects of caffeine on muscle protein synthesis rates.

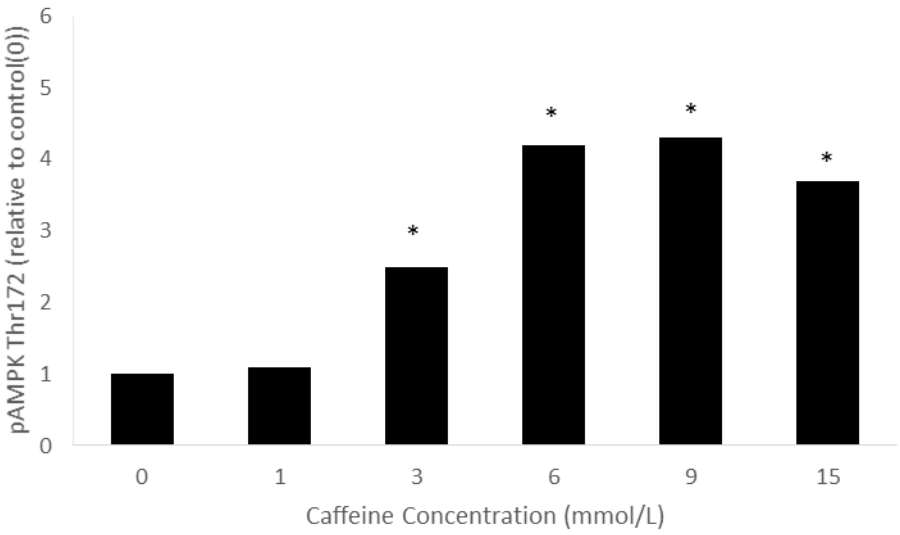

Figure 14. The effects of caffeine concentration on AMPK phosphorylation. *denotes significant difference from control [10].

Hormones

Two hormones of interest involving cellular growth are human growth hormone and cortisol. Growth hormone is associated with the stimulation of cellular growth and reproduction. Cortisol is a systemic catabolic marker, and high levels of cortisol can also cause a decrease in cellular growth. A recent study comparing hormonal levels post-resistance exercise between caffeine and non-caffeine groups noticed a decrease in serum growth hormone in the caffeine group (**Figure 15**) [79]. Additionally, studies by Woolf et al. [6] and Beaven et al. [80] concluded groups that ingested caffeine before exercise experienced a greater increase in cortisol levels immediately post exercise compared to those that did not ingest caffeine (**Figure 16**) [6]**.**

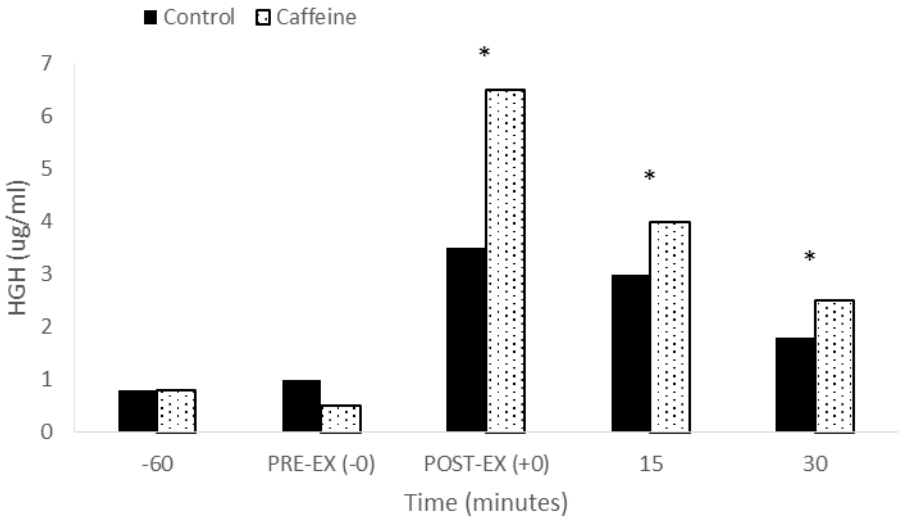

Figure 15. Caffeine and growth hormone levels post exercise. *denotes significant differences between control and caffeine groups [79].

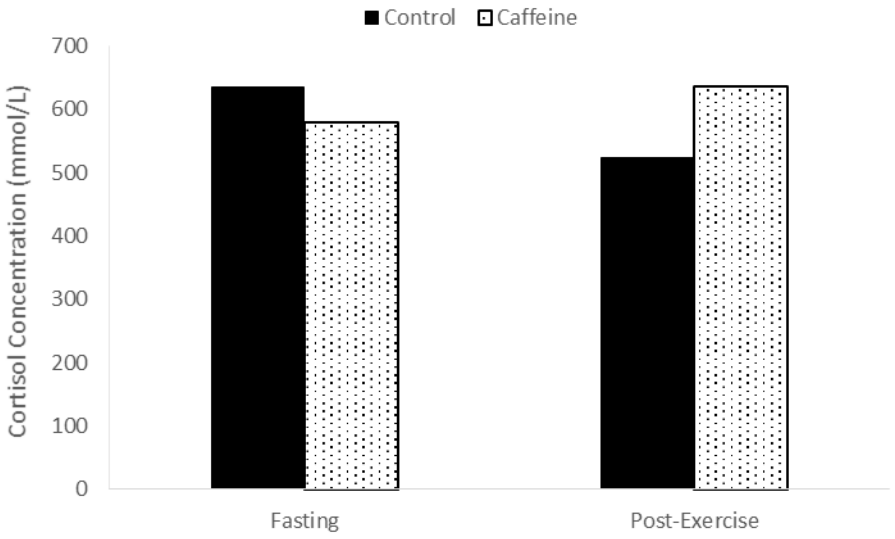

Figure 16. Caffeine and cortisol levels post exercise.

Body Composition

While there have been several studies determining the effects of caffeine on body composition, it has often been supplemented in conjuncture with tea, coffee, ephedra, or ma huang. While some of these supplemental studies have reported an increase in metabolic rate or thermogenesis resulting in an increase in weight loss, it is difficult to determine what role caffeine played in those studies [66, 81-85]. Supplementation of caffeine in animal model studies have consistently reported a reduction in fat mass and total body weight compared to those in the placebo groups [86-89]. In human studies, caffeine supplementation has been reported to decrease total body mass and increase body metabolism in far fewer studies, and no study currently exists in regards to the effect of caffeine on lean body mass [90-92]. Franco et al. [93] used a rat model to determine any effects of caffeine on lean body mass and concluded that caffeine intake played no role.

CHAPTER II

THE EFFECTS OF CAFFEINE INTAKE ON 24-HOUR RATES OF PROTEIN SYNTHESIS IN RAT SKELETAL MSUCLE AFTER AN ACUTE BOUT OF EXERCISE

Introduction

Caffeine is one of the most widely used drugs in the world. Caffeine is classified as a stimulant, and can decrease fatigue and increase alertness by altering the central nervous system [16]. Due to these physiological changes, caffeine has often been used as an ergogenic aid; however, the significance of its use on exercise performance and recovery are still not completely understood.

Past research has concluded that caffeine can function as an effective ergogenic aid in endurance exercise. Individuals who consumed 2-6mg/kg of body weight of caffeine approximately 1-hour before exercise demonstrated increased time to fatigue, increased power output, decreased rates of perceived exertion, and increased running speeds [3, 30, 46, 47, 49]. While caffeine has an ergogenic effect on endurance exercise, current studies regarding caffeine and resistance exercise have reported mixed results. Two studies [5, 94] reported strength increases in chest press in individuals who supplemented with 5-6mg/kg of body weight of caffeine. However, other studies have reported no statistical differences in strength and number of repetitions in leg and chest press in caffeinated (5-6mg/kg) groups [51, 95, 96].

An area of interest, which to our knowledge has not been examined, is the significance of caffeine intake on the rates of muscle protein synthesis following

resistance exercise. Previous studies have reported that following an acute bout of resistance exercise, an increase in muscle protein synthesis can be detected for upwards of 24-48 hours in humans [7, 8]. This same increase occurs in animals engaging in resistance exercise [97-99]. The rise in muscle protein synthesis is an indicator of cellular growth and muscle hypertrophy, important objectives of resistance exercise [7, 8]. Protein synthesis rates are regulated by several proteins including AKT and mTOR. Activation of AKT and mTOR causes an increase in downstream translational factors resulting in increased muscle protein synthesis rates and cellular growth [100].

In vitro studies using incubated caffeine have reported a decrease in AKT and mTOR protein activation [11, 13, 75, 76]. Furthermore, caffeine has been reported to increase levels of AMPK. High levels of AMPK occur during high cellular stress environments such as muscle contraction, exercise, and states of low energy [78]. To determine the response of AMPK on muscle protein synthesis, Bolster et al. [78] incubated cells with AICAR (a compound that synthetically stimulates cellular AMPK levels) [101] and concluded that cells with increased activation of AMPK had statistically significant lower rates of muscle protein synthesis. Further studies by Egawa et al. [10] and Jensen et al. [12] have demonstrated that muscle tissue incubated with caffeine causes increased levels of AMPK similarly to that of a high stress (exercise) environment and AICAR stimulation.

Due to caffeine's ability to activate pathways that have been known to inhibit muscle protein synthesis, it would be of interest to determine if caffeine consumption hinders muscle protein synthesis rates following resistance exercise. The purpose of this

study was to investigate the effects of different dosages of caffeine on 24-hour muscle protein synthesis rates following an acute bout of resistance exercise in the rat model. It was hypothesized that increased caffeine intake would decrease muscle protein synthesis rates.

Methods

Animals

Thirty-six male Sprague-Dawley rats (aged 6 months) were obtained from Harlan Laboratories (Houston, TX), of which thirty-five animals completed the study. Animals were acclimated for seven days to the housing facility at Texas A&M's animal research laboratory (Laboratory Animal Resources and Research). The housing facility was a climate controlled environment with a regulated 12-hour light and dark cycle. Animals were housed individually with free access to water and standard rodent chow (Harlan Teklad 8604). Rat weight was measured every 48 hours along with the amount of food consumed. Animals were then randomly assigned to one of six groups: no-caffeine (NC) no-exercise (NE), NC-exercise (E), low-caffeine (LC) NE, LCE, high-caffeine (HC) NE, or HCE groups. This study was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Operant Conditioning and Resistance Exercise

All rats were operantly conditioned to perform jump squats (resistance exercise) prior to the initiation of the experimental exercise protocol. Exercise was performed within an acrylic glass box (21cm width x 21cm depth x 35cm height) with a single illuminating switch overhead. Whenever the switch was illuminated the rat would

perform a “jump squat” (activating the lower body muscles) to turn off the switch by depressing the light bar [102]. When necessary, a quick foot shock (1mA, 60Hz) would follow after five seconds of non-compliance [10, 97, 103]. Operant conditioning began on Day 8 of the study and consisted of rats performing 50 jump squats for four sessions with 48 hours of rest between sessions (**Figure 17**). The first three operant sessions involved no additional weight. During the final operant session animals wore a Velcro vest while performing resistance exercise; however, no weight was added to the vest. Resistance exercise commenced on Day 16 of the study and consisted of four sessions with five sets of variable vest weights and repetitions (**Table 1**). Resistance was increased by attaching weight to the Velcro vest. Rats were given 2 seconds of rest between repetitions and 1 minute of rest between sets. Forty-eight hours of rest were given between each exercise bout.

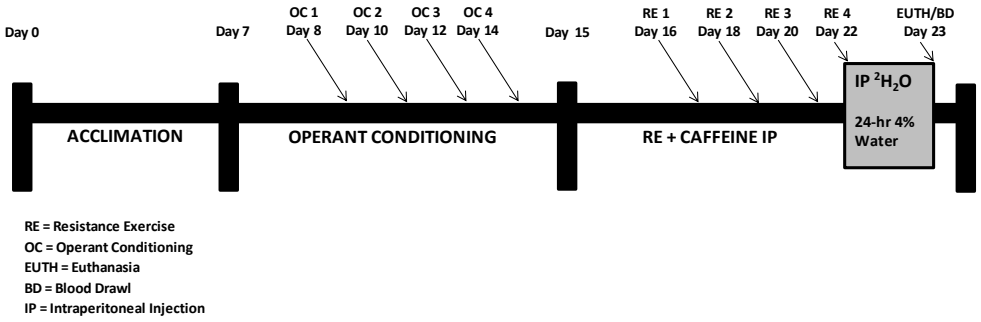

Figure 17. Schematic display of Chapter II study design.

Table 1. Weight and repetitions (reps) used during resistance exercise.

	Resistance Exercise				
	Set 1 (grams-reps)	Set 2 (grams-reps)	Set 3 (grams-reps)	Set 4 (grams-reps)	Set 5 (grams-reps)
Session 1	30-14	80-12	130-10	180-8	230-6
Session 2	80-16	80-16	130-10	180-10	230-8
Session 3	80-18	130-16	180-14	180-14	230-8
Session 4	80-18	130-16	180-14	180-14	230-8

Caffeine Supplementation

Anhydrous caffeine was reconstituted in 4ml of saline at appropriate dosages for the low caffeine (2mg) and high caffeine (6mg) groups. This solution was administered via an intraperitoneal injection (IP) at three time points (-5hr, -1hr, and +5hr from resistance exercise) during each exercise day to replicate chronic and prolonged caffeine ingestion (pre and post workout) in humans. Caffeine solutions used were prepared within 24 hours of injections and non-caffeinated groups were given a sham injection of saline.

Protein Synthesis

Twenty-four hours after the last bout of exercise, animals were euthanized with Ketamine/Medetomidine (50mg/kg; 0.5mg/kg, respectively). Gastrocnemius, soleus, and plantaris muscles were excised and weighed. All muscle samples were flash frozen in liquid nitrogen and stored at -80°C until analysis. Approximately 1ml of blood was drawn via cardiac puncture and centrifuged at 2000g for 20 minutes in order to collect blood plasma, which was frozen until analysis.

Protein synthesis rates were determined using deuterium oxide, a method that has been shown to be comparable to the former phenylalanine protein tracer methodologies by allowing for conditions that better mimic the free-living state [104, 105]. Briefly described, 24 hours prior to tissue harvest, a bolus consisting of 20µl of deuterium oxide per gram of body weight was administered via IP injection into each animal. This initial bolus would allow deuterium oxide enrichment to reach approximately 4% of total body water and cause deuterium (^2H) to rapidly equilibrate with body water leading to intracellular ^2H labeling of alanine via transamination reactions [106, 107]. After the initial injection, all drinking water was replaced with 4% ^2H_2O water to maintain the desired 4% total body enrichment.

Gas chromatography-mass spectrometry (GCMS, Agilent 7890 GC/5975MSD, Santa Clara, CA) was used to determine the rate of ^2H labeled alanine incorporation into gastrocnemius, plantaris, and soleus proteins as well as quantify the available tracer pool (plasma), to determine rates of protein synthesis [104, 108, 109]. Plasma ^2H ratios were calculated by adding 0.002ml of 10N NaOH and 0.004ml of 5% (vol/vol) acetone in acetonitrile to 0.02ml of each plasma sample or standard. After incubation for 24 hours, the reaction was stopped by the addition of 0.6ml of chloroform and 0.5g of $NaSO_4$ (a drying agent). 1µl of this sample was then injected and separated by size (using helium as a carrier gas) in the gas chromatograph. Samples were injected into the mass spectrometer to differentiate between deuterated and non-deuterated acetone.

Muscle ^2H ratios were calculated by homogenizing approximately 30mg of tissue in 0.4ml of 10% trichlorocetic acid (TCA) with a Polytron homogenizer and centrifuged

at 5000g for 15 minutes at 4°C in order to separate tissue proteins (pellet) from unbound amino acids (supernatant). The supernatant was discarded and 0.4ml of TCA was added to the pellet and vortexed. This was repeated three total times to remove all free amino acids in the sample. Each sample was then incubated for 24 hours at 100°C in 0.4ml of 6N HCl to hydrolyze the proteins into free amino acids. Methyl-8 (Fisher Scientific, Waltham, MA) was used along with methanol and acetonitrile to derivatize the samples before analysis. A 1µl of aliquot was injected with a split ratio of 10:1 into the GCMS to determine ratio of protein bound ^{2}H-alanine to unlabeled alanine. The rate of protein synthesis was calculated as using the following equation:

$$\text{Fractional Synthesis Rates} = E_A \cdot [E_{BW} \times 3.7 \times t]^{-1} \times 100$$

E_A represents the quantity of protein bound ^{2}H-labeled alanine (mole % excess), E_{BW} indicates the quantity of $^{2}H_2O$ in body water from plasma (mole % excess), and t represents time in hours or days [108]. The number 3.7 is used because it represents the average number of hydrogen on protein-bound alanine that was exchanged with ^{2}H from $^{2}H_2O$ in body water [108].

Statistical Analysis

The Shapiro-Wilk's test was used to determine whether the assumption of normality had been violated ($p<0.05$). Two-way analysis of variance was used to determine if there were differences and interaction between independent variables caffeine and exercise on protein fractional synthesis rates. If a p-value of <0.05 occurred, a Tukey HSD post-hoc test was conducted to determine the differences among caffeine groups. Similar analyses were used to determine differences in muscle mass, food intake,

and body mass changes within caffeine and exercise groups. All statistics were applied using IBM SPSS Software version 20 (IBM Corporation, New York) and expressed as mean ± standard error (SE).

Results

Body and Muscle Mass

Food intake and body masses were recorded and the final change in body mass (Day 22-Day 8) was noted (**Table 2)**. Change in body mass (BM) and food intake (FI) were not statistically different in caffeine groups (BM $p=0.86$; FI $p=0.22$). However, there were statistically significant differences in BM and daily and total FI between exercise groups (BM NE: 4.8±1.8, E: -21.9±3.1, $p<0.01$; FI NE: 44.1±1.7, E: 37.7±1.2, $p=0.01$).

Gastrocnemius, plantaris, soleus, and total plantarflexor muscle masses were not statistically different ($p>0.05$) among caffeine groups; however, the exercise groups were significantly ($p<0.05$) lower in all muscle masses analyzed **(Figure 18)**.

Mixed Muscle Fractional Synthesis Rates

The effects of exercise and caffeine intake on mixed fractional synthesis rates in gastrocnemius, plantaris, and soleus muscles are presented in **Figure 3.** There were no statistical differences or interaction ($p>0.05$) between exercise and caffeine intake on gastrocnemius, plantaris, and soleus muscle protein synthesis rates.

Table 2. Changes in food intake and body mass. Data values are presented as mean±SE. *denotes statistically significant differences between no exercise and exercise groups ($p<0.05$)

	No Caffeine		Low Caffeine		High Caffeine	
	No Exercise	Exercise	No Exercise	Exercise	No Exercise	Exercise
Number	6	6	6	6	5	6
Food Intake (g)						
Daily Food Intake	46.7±4.0	38.9±3.1	45.7±1.9	37.4±1.1	40.1±2.4	36.7±2.2
Total Food Intake	186.8±16.0*	155.7±12.4*	182.7±7.4*	145.0±4.5*	160.2±9.4*	146.8±8.6*
Body Mass (g)						
Day 8	500.8±21.2	463.8±29.0	464.5±9.4	458.0±6.4	451.8±15.1	428.5±3.7
Day 16	507.4±21.9	451.6±28.0	476.3±9.7	450.5±3.5	452.9±17.8	418.0±9.6
Day 18	510.8±19.3	446.7±29.5	471.1±10.5	442.5±2.5	459.1±18.5	407.8±9.0
Day 20	504.3±20.9	442.0±28.9	470.3±10.4	435.6±2.3	449.9±16.8	403.6±10.4
Day 22	505.0±12.2	434.1±19.5	475.5±7.2	432.4±5.3	468.6±19.3	411.2±7.4
Change Body Mass	4.0±4.4*	-18.6±4.4*	7.7±2.6*	-24.7±6.0*	2.7±2.1*	-22.4±6.6*
Plantarflexor Muscle Masses (g)						
Gastrocnemius	2.3±0.2*	2.3±0.09*	2.5±0.1*	2.3±0.06*	2.4±0.1*	2.2±0.04*
Plantaris	0.54±0.02*	0.5±0.02*	0.5±0.01*	0.5±0.01*	0.5±0.03*	0.5±0.01*
Soleus	0.22±0.02*	0.2±0.01*	0.2±0.07*	0.2±0.06*	0.2±0.02*	0.18±0.08*
Total Plantarflexor Mass	3.1±0.2*	3.0±0.1*	3.2±0.1*	3.1±0.1*	3.1±0.1*	2.9±0.04*

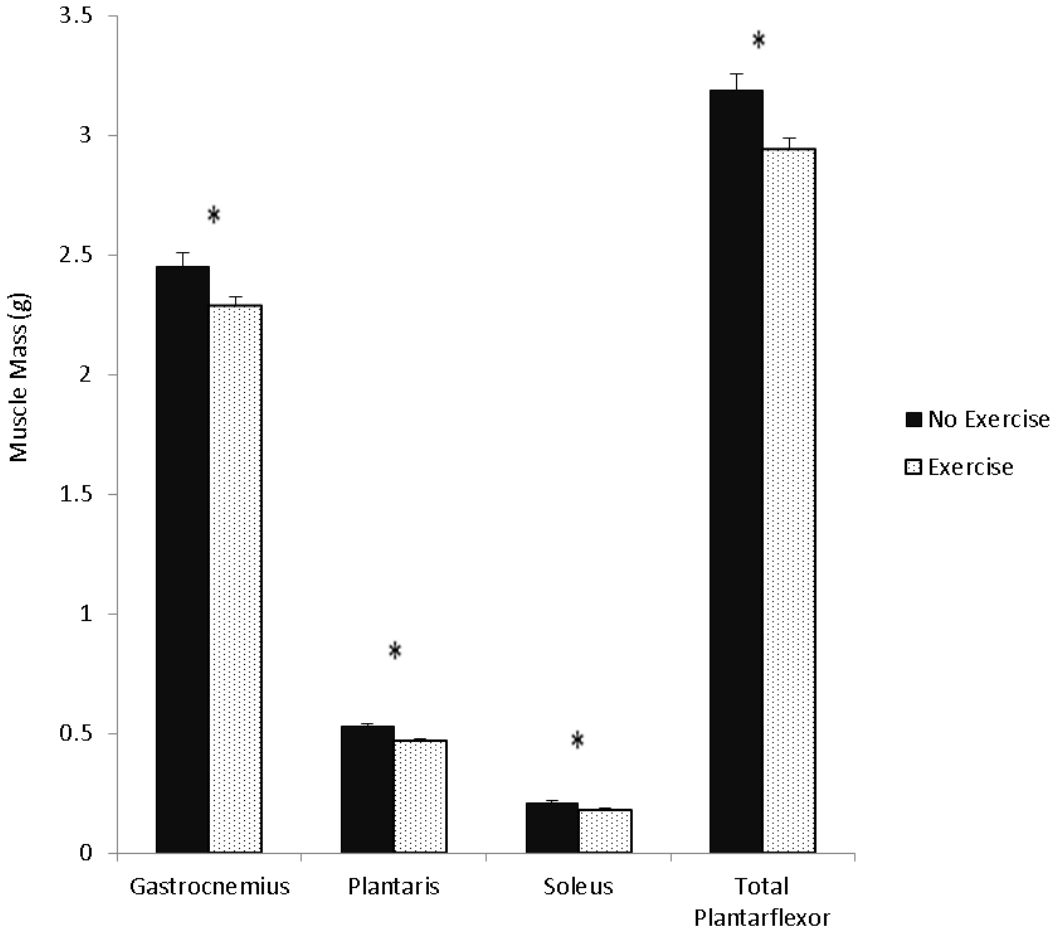

Figure 18. Gastrocnemius, plantaris, soleus, and total plantarflexor muscle weights.

*indicates a significant difference between exercise and non-exercise groups.

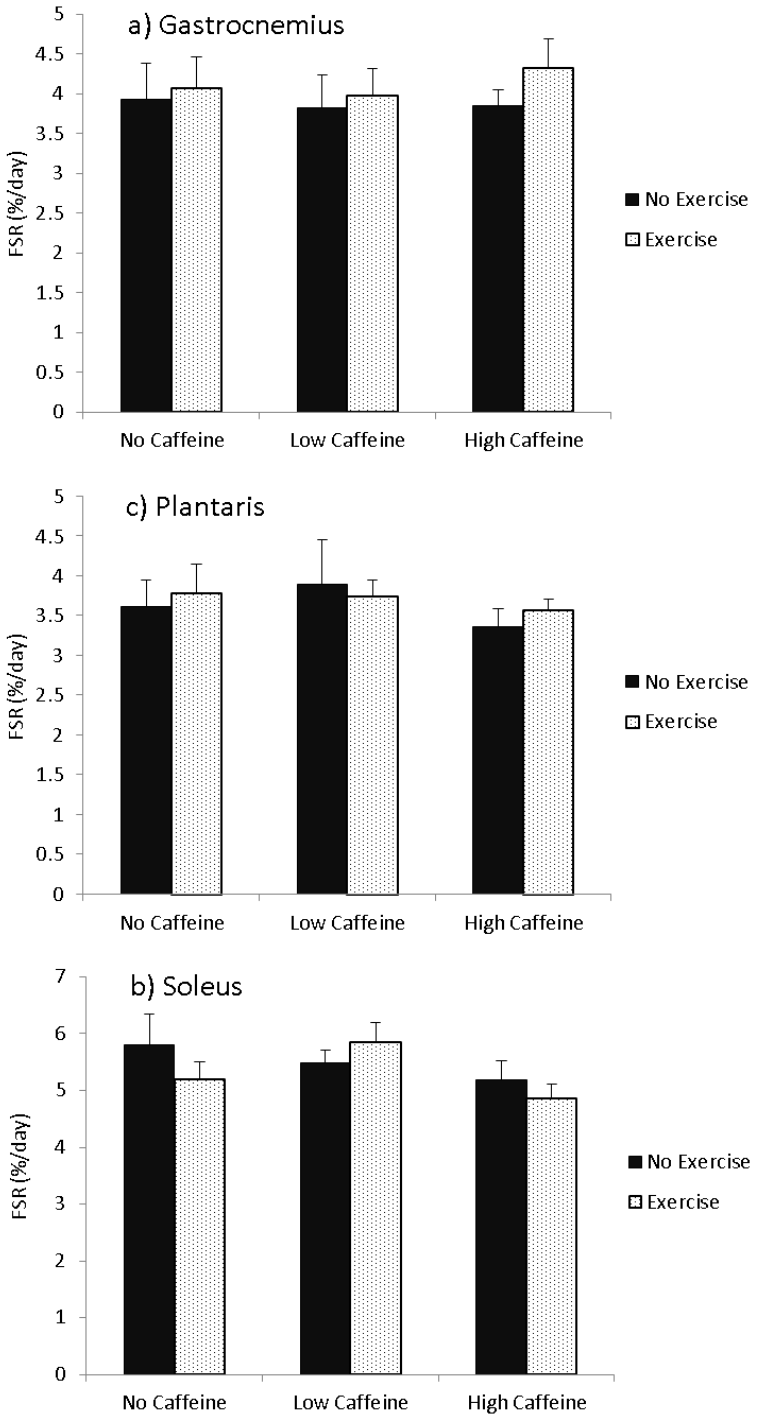

Figure 19. The effects of exercise and caffeine intake on mixed fractional synthesis rates in gastrocnemius, plantaris, and soleus muscles.

Discussion

To our knowledge, this is the first study to investigate the impact of caffeine on muscle anabolism following resistance exercise. The purpose of this study was to evaluate the significance of caffeine intake on 24 hour rates of muscle protein synthesis in rat skeletal muscle following an acute bout of resistance exercise. With caffeine's capacity to inhibit particular growth pathways, it was hypothesized that increased caffeine intake would cause a decrease in synthesis rates. The most important finding in the present work is that caffeine ingestion does not influence protein anabolism in skeletal muscle.

The exercise protocol for this study was slightly modified from previous rat studies involving similar resistance exercise training [97, 98, 103, 108, 109]. In an attempt to elicit a greater muscle protein synthesis response, this study increased the number of repetitions and the total weight added to the vests by 20% compared to the previous studies. Prior work by Fluckey et al. [98] and Farrell et al. [97] reported statistically significant increases in rates of protein synthesis in the exercise group using acute assessments during a period of time that represents "peak anabolism" post exercise; however, no differences in muscle mass were reported, likely due to the brevity of the training response (4 sessions over 8 days). In this study, even with the increased repetitions and weight, no differences in muscle protein synthesis occurred between the exercise and non-exercise groups. Furthermore, exercise animals had statistically significant decreases in BM, FI, and gastrocnemius, plantaris, and soleus, and total plantarflexor mass compared to non-exercise animals. This decrease in total muscle

mass suggests that muscle protein breakdown may have been higher than muscle protein synthesis rates in the exercise animals [110].

One possible explanation as to why exercising animals had a decrease in muscle mass may be due to overtraining. Previous studies that adequately induced an overtraining state in animals are limited; however, increased weight loss and decreased dietary intake were commonly reported symptoms [111-113]. Due to the analogous symptoms present in this study (as evident by the lowered muscle weights and food intake), the animals in the present study may have exhibited "overtraining." Further, given that muscle protein synthesis was not different among exercise animal groups when compared to non-exercise controls, it is possible that the significant decrease in BM is not only due to increased energy expenditure from the exercise, but from overtraining induced increases of muscle protein breakdown [114, 115]. The higher repetitions and weight used in this study combined with the electrical compliance stimulation and low recovery time (48 hours), may have further contributed to an overly stressful environment for the animals.

When observing the muscle protein synthesis results, the caffeine groups had no significant differences in muscle protein synthesis rates and no significant interaction between caffeine and exercise groups. Thus, it is doubtful that caffeine had any impact on muscle protein synthesis, with or without exercise or overtraining. Previous studies have reported an inhibitory role of caffeine on regulatory proteins in the Akt and mTOR pathway, which is typically indicative of reduced muscle protein synthesis [11, 13, 75, 76]. While no studies exist related to muscle protein synthesis in response to caffeine

intake, it could be reasonably inferred that caffeine should decrease protein synthesis rates based on how it impacts the mTOR pathway. However, data from the present study suggest that caffeine plays no role in 24-hour muscle protein synthesis rates. This disparity in conclusions may be due to differences in study methodologies. Past studies observing caffeine's effect on Akt and mTOR inhibition have all been conducted in an in-vitro environment with several different cells or tissues [75, 76, 98, 116]. The differences in cell types or cultures may have impacted tissue-specific outcomes, particularly when comparing against the present studies in intact animals.

It must be noted; however, that even when comparing prior in-vitro studies to the present study, differences in the absolute control of caffeine concentration and the time of caffeine exposure must be considered. In-vivo studies have used anywhere from 1mM to 15mM (approximately 2-3 times higher concentration than this study) of caffeine [75, 76, 98, 116]. Such high amounts of caffeine would be impractical in a live specimen and would increase the risk of approaching lethal doses. Due to the high concentrations of caffeine used in these in vitro studies, cells were also incubated for a much shorter time period (approximately 15-30 minutes). Compared to human and animal metabolism, where the half-life of caffeine is several hours, caffeine exposure is substantially longer in vivo when compared to that of an in vitro environment. Further, it has been suggested that actual administration routes (i.e. perfusion vs. incubation) may impact the tissue's response to caffeine exposure [10]. The differences in methodological approaches coupled with widely varied design regiments make comparisons among studies problematic. While we agree that there are benefits to in-vitro studies, it does not

represent a normal biological context and it is difficult to extrapolate the results to a live animal setting inevitably producing some variability. The present study chose to assess the impact of caffeine on muscle protein synthesis, with or without physical activity, in intact animals using doses that are consistent with moderate caffeine use. The present data indicate that caffeine ingestion is not detrimental to anabolic responses in muscle.

One advantage of this study and the ^{2}H methodology is that muscle protein synthesis rates were measured over a 24-hour time period. Past studies were typically only able to measure protein synthesis rates for a few hours due to methodological restrictions [97, 98, 103]. The deuterium oxide methodology used in the present study offers a relatively non-invasive approach to assess muscle protein synthesis and maximizes flexibility with regard to food intake and implementation of the experimental treatments (exercise or caffeine). Since peak protein synthesis rates do not occur until 24-48 hours following exercise, this study more closely resembles real-life conditions compared to past studies on muscle protein synthesis [7, 8]. This is not the first study to use deuterium oxide to measure muscle protein synthesis rates and previous studies with similar ^{2}H methodologies have been conducted with positive and replicable results [104, 108, 109].

One limitation of this study is that it was performed in rats, which makes extrapolations to human models problematic. The half-life for caffeine is 2.5-4.5 hours in adult humans while rats experience a faster half-life at 0.7-1.2 hours [22]. It would be of great interest to conduct a similar study using human participants to determine any differences in metabolism. Another limitation of this study involves the fact that there

was no quantitative measure for caffeine in each animal. Due to the controlled administration of caffeine via intraperitoneal injections, no measurement or analysis of blood caffeine levels during and after exercise were deemed necessary. Lastly, due to limitations in study scheduling, animals were typically exercised during the ninth hour of their dark cycle, which was not a representation of tangible training conditions. This may have further exacerbated any stress responses from the animals.

Conclusions

The data suggest that caffeine intake has little impact on 24-hour muscle protein synthesis rates, with or without an acute bout of resistance exercise in rat skeletal muscle. While the strenuous resistance exercise protocol of the present study may have masked any potential effects of caffeine, it is our conclusion that caffeine use in large or small amounts has no significant effect on muscle anabolism following resistance exercise. This is potentially valuable information to the many who use caffeine to increase training intensity and duration without any previous knowledge of whether it had negative consequences on recovery where all the adaptation occurs. Future work with a different resistance exercise protocol may help confirm the results of this study.

CHAPTER III

THE EFFECTS OF PRE-EXERCISE CAFFEINE ADMINISTRATION ON 24-HOUR RATES OF PROTEIN SYNTHESIS IN HUMAN SKELETAL MUSCLE

Introduction

Caffeine is one of the most widely used drugs in the world with approximately 80% of the United States adult population reporting everyday use [1]. Caffeine is classified as a stimulant and can decrease fatigue and increase alertness by affecting the central nervous system. Caffeine has also been recognized as an effective ergogenic aid in endurance exercise and until recently, the IOC and the NCAA both held upper limits for detectable caffeine levels resulting in disqualification [2]. Previous human studies involving aerobic exercise have concluded that individuals who consumed 2-6mg/kg of body weight of caffeine approximately 1-hour before exercise would report increased times to fatigue, increased power, increased running speeds, and decreased rates of perceived exertion [3, 30, 46, 47, 49]. Studies on the effects of caffeine and resistance exercise; however, have reported mixed results including increased or decreased strength, power, repetitions, and performance suggesting potential benefits to acute performance remains unresolved [5, 6, 50, 51, 117].

An important intermediate response to resistance exercise, that to our knowledge has not been examined, is the impact of caffeine intake on rates of muscle protein synthesis during the recovery phase of exercise. Previous studies have concluded that an increase in protein synthesis rates following an acute bout of resistance exercise can

persist for 24 hours and upwards of 48 hours and are an indicator of cellular growth and muscle hypertrophy [7, 8]. This may be of considerable importance due to the increasingly popular use of caffeinated energy drinks, diet products, and pre-workout supplements in the past several years. Some over the counter pre-workout supplements contain upwards of 200mg of caffeine per serving with the purpose of increasing training intensity and volume and decreasing fatigue without knowledge of the consequences on cell signaling and protein synthesis during the muscle recovery period.

Protein synthesis rates are regulated by several key proteins including protein kinase B (AKT) and mammalian target of rapamycin (mTOR). Activation of AKT and mTOR causes phosphorylation of downstream proteins 4E-BP1 and ribosomal protein s6 (p70s6); ultimately, increasing translational factors and protein synthesis [100]. In vitro studies have demonstrated decreased activation of AKT and mTOR proteins with caffeine present, and caffeine can also increase levels of AMP-activated protein kinase (AMPK) [11, 13, 75, 76]. High levels of AMPK are typically present during high cellular stress environments such as muscle contraction and exercise. In a study by Bolster et. al [78] 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) and it was concluded that high levels of cellular AMPK decreased muscle protein synthesis. In addition, studies by Egawa et al. [10] and Jensen et al. [12] have demonstrated that rat muscle tissue incubated in caffeine increases levels of AMPK similarly to that of a high stress environment and AICAR stimulation. It would be important to compare these previous animal studies with an equivalent human model to determine if caffeine has

similar inhibitory effects on these cellular growth pathways and ultimately muscle growth.

The purpose of this study was to investigate the effects of a pre-exercise caffeine supplement on 24-hour muscle protein synthesis rates following an acute bout of resistance exercise in recreationally resistance trained men. Due to caffeine's ability to down regulate key proteins in cellular growth, we hypothesized that a pre-exercise caffeine supplement would result in decreased cumulative protein synthesis rates. A secondary objective was to determine if caffeine will alter acute resistance exercise performance or regulatory proteins in the mTOR pathway.

Methods

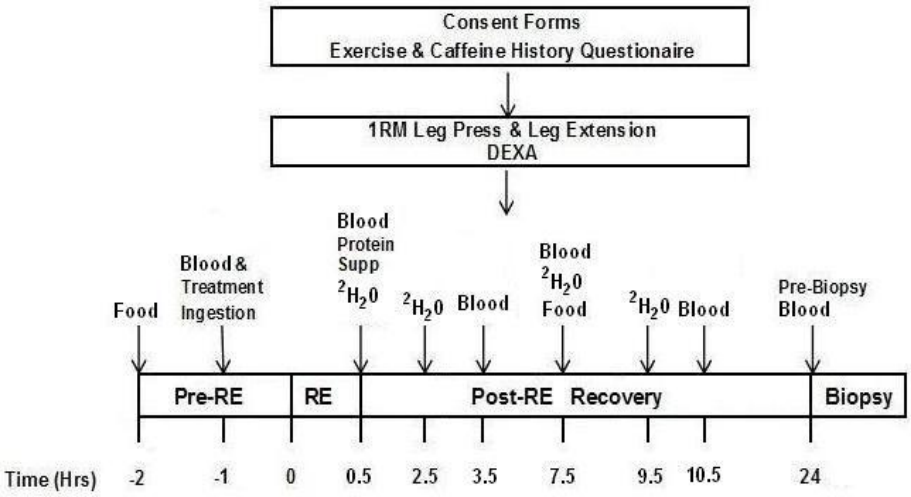

Figure 20. Schematic display of Chapter III study design.

Participants

Twenty-four healthy, recreationally trained males were recruited for this study with a target age of 18-28 years. During the first meeting, all participants were asked to complete a consent form, exercise history questionnaire (how many months of resistance training experience they had), and a caffeine consumption report adapted and modified from Shohet et al. [118]. To be eligible for the study, participants must have been consuming less than 50mg/day of caffeine. Furthermore, all participants were required to be recreationally trained with a minimum of 1 month upper and lower body weight training experience. These two prerequisites ensured that participants were similarly trained and had limited caffeine usage. This study was approved by the Institutional Review Board at Texas A&M University.

Dietary Control

All participants were required to attend a nutrition education session in which products that commonly contained caffeine were discussed, and participants were instructed to refrain from ingesting any dietary or supplemental caffeine found in beverages, drugs, or foods seven days prior to the experimental exercise and throughout the entire study. During the nutrition education session, the NutriBase 9.0 software (Phoenix, AZ) was also introduced and individuals practiced logging dietary intake until they were proficient to ensure accurate and consistent reporting of their food intake. Food logs were required on three separate days the week before commencement of the study and were collected the day of experimental exercise.

All caloric intake and meals were standardized on the day of experimental exercise. Caloric requirements were calculated using the Harris-Benedict equation and meals were provided following a macronutrient ratio of 55% carbohydrates, 20% fat, and 25% protein. Meals consisted of a Lean Cuisine Culinary Collection (Wilkes-Barre, PA) frozen meal for breakfast and dinner and any additional caloric needs were supplemented throughout the day with Boost High Protein (Fremont, MI) drinks.

Exercise Testing and Exercise Protocol

Following the initial meeting, participants were asked to return and perform a 1RM test for leg press and leg extension 9 (**Figure 20**). Resistance exercise was performed using Keiser (Fresno, CA) pneumatic leg press and leg extension machines. All exercises were demonstrated and participants were instructed to perform three sets of five repetitions, adjusting weights to a perceived exertion score of four on the OMNI scale which approximates 40% of 1RM effort [119-121]. Resistance weight was then increased proportionally to the reported level of perceived exertion and if the participant successfully completed one repetition, resistance was increased each trial until the participant was unable to complete more than one repetition. Three to five trials were conducted with 3 minute rests between trials and 5 minute rests between exercises.

On the day of the experimental exercise, participants started with a 10 minute warm-up on a cycle ergometer followed by resistance exercise training. A unilateral leg exercise protocol was used where the dominant leg performed exercise and the opposing leg was used to control for the effects of caffeine without exercise. This unilateral design has effectively induced muscle hypertrophy in past studies, and can effectively reduce

variability between exercise and control sample measurements [94, 122, 123]. For leg press and leg extension, participants performed five sets to failure at 80% 1RM with 2minute rests between sets and 4 minute rests between exercises. Power outputs were documented, and the total repetitions were also recorded. Participants performed the experimental exercise session in the morning (7-9AM), and immediately after exercise, participants were given 24 grams of a protein supplement (117 total calories, 10% carbohydrates, 8% fat, 82% protein) (Optimum Nutrition; Aurora, IL) to aid in muscle recovery.

Caffeine Administration

For this study a randomized, double-blind, single treatment design was implemented. On the day of exercise intervention, a supplement composed of caffeine, natural zero-calorie sweetener (Truvia; Boulder, Colorado), and water (caffeine group) or simply water and Truvia (control group) was administered 60 minutes before exercise (to allow for peak caffeine plasma levels to occur). The caffeine dose was 6mg/kg of body weight based on previous caffeine studies [6, 51, 79].

Blood Sampling and Analysis

Blood samples were collected six times during the testing day: immediately before caffeine or placebo ingestion, immediately following resistance exercise, 3 hours post-exercise, 5 hours post-exercise, 10 hours post-exercise, and immediately before the biopsy (**Figure 20**). All blood samples were collected from the antecubital vein using purple top vacutainers containing EDTA. Blood samples were then centrifuged at 4°C and the remaining plasma was collected and stored at -80°C until analysis. Blood plasma

samples were sent to LabCorp (Conroe, TX) to have caffeine levels analyzed at a later date.

Muscle Biopsy and Protein Synthesis

To determine muscle protein synthesis, deuterium oxide (heavy water), a relatively non-invasive and safe procedure was used. This method of protein synthesis measurement has been effectively implemented in several studies and is comparable to a ^{2}H-Phenylalanine tracer and sometimes preferred due to its ability to replicate a free-living state [108, 109, 124]. Deuterium oxide (Cambridge Isotopes, Andover, MA) was provided in four servings of 6-6.5ml of 70% $^{2}H_2O$/kg lean body mass at four separate time points: immediately after exercise, 2 hours post- exercise, 7 hours post-exercise, and 9 hours post-exercise (**Figure 20**). The administration of deuterium oxide allowed for approximately 4% of total body water enrichment. Furthermore, ^{2}H rapidly equilibrates with body water leading to intracellular ^{2}H labeling of alanine via transamination reactions [108]. Biopsy samples were obtained using a local anesthetic (1% Xylocaine HCl) from the vastus lateralis muscles of both the exercised and control legs and stored at -80°C until analysis. All muscle samples were cleaned of visible fat, connective tissue, and blood. Gas chromatography-mass spectrometry (GCMS, Agilent 7890 GC/5975MSD, Santa Clara, CA) was used to determine ^{2}H labeled alanine incorporated into skeletal muscle and plasma as the marker of muscle protein synthesis [108].

Plasma ^{2}H ratios were calculated by adding 0.002ml of 10N NaOH and 0.004ml of 5% (vol:vol) acetone in acetonitrile to 0.02ml of each plasma sample. After

incubation for 24 hours, the reaction was stopped by the addition of 0.6ml of chloroform and 0.5g of NaSO4 (a drying agent). A 1µl of this sample was injected and separated by size (using helium) in the gas chromatograph. Samples were then injected into the mass spectrometer to differentiate between deuterated and non-deuterated acetone.

Muscle ^{2}H ratios were calculated by homogenizing approximately 30mg of tissue in 0.4ml of 10% TCA with a Polytron homogenizer and centrifuged at 5000g for 15 minutes at 4°C. The supernatant was discarded and 0.4ml of TCA was added to the pellet and vortexed. This was repeated for three additional times to remove all free amino acids in the sample. Each sample was then incubated for 24 hours at 100°C in 0.4ml of 6N HCl to hydrolyze the proteins into free amino acids. Methyl-8 (Fish Scientific, Waltham, MA) along with methanol and acetonitrile was used to derivatize the samples before analysis. A 1µl of aliquot was injected with a split ratio of 10:1 into the GCMS to determine ratio of protein bound ^{2}H-Alaine to unlabeled alanine. The rate of protein synthesis was calculated as using the following equation:

$$\text{Protein Synthesis Rates} = E_A \cdot [E_{BW} \times 3.7 \times t]^{-1} \times 100$$

E_A represents the quantity of protein bound ^{2}H-labeled alanine (mole % excess), E_{BW} indicates the quantity of $^{2}H_2O$ in body water from plasma (mole % excess), and t represents time in hours or days [108]. The number 3.7 was used in the equation because it represented the average number of H on protein-bound alanine that was exchanged with ^{2}H from $^{2}H_2O$ in body water [108].

Western Blotting

Approximately 30mg of pulverized skeletal muscle tissue was homogenized (Polytron homogenizer) in 0.4ml of a premade lysis buffer (25mM hepes, 5mM B-glycerophosphate, 200uM ATP, 0.5% protease inhibitor cocktail, 25mM benzamidine, 2mM phenylmethansesulfonyl fluoride, dimethyl sulfoxide, 4mM ethylenediaminetetraacetic acid, 10mM magnesium chloride). Samples were then placed on ice for 1-hour (vortexing every 15 minutes) before being centrifuged at 4°C and 14,000 RPM for 30 minutes. Supernatant were collected and used for western blotting. A bicinchoninic acid assay kit was used to determine protein concentration in samples. This allowed for similar amounts of total protein to be loaded in each gel well. Proteins were then separated using PAGEr Gold Tris-Glycine Precast Gels with a 4-20% gradient (Lonza). Gels were run at 40mA and maximum volts and watts for approximately 45 minutes. Separated proteins were then transferred to a nitrocellulose membrane using a semi-dry transfer process at 300mA, maximum volts, and maximum watts for an additional 40 minutes. A 1% non-fat dry milk solution was used for all blocking steps. Primary antibodies were incubated for 24 hours in a cold room and washed three times in TBS for 5 minutes each before secondary antibodies were added and incubated for 1-hour at room temperature (1:1000 dilution; Cell Signaling, Beverly, MA). Membranes were then washed again three times in TBS for five minutes each, and imaged using enhanced chemiluminescence to determine the protein bands of interest. Phosphorylated AMPK, phosphorylated p70s6k, total AMPK, and total p70s6k protein bands were

quantified by calculating the bands' integrated density values (IDV) and expressed as arbitrary units (AU).

Statistical Analysis

The data was analyzed on IBM SPSS Software version 20 (IBM Corporation, New York). The Shapiro-Wilk's test was used to determine whether the assumption of normality had been violated ($p<0.05$). Two-way analysis of variance was used to establish significant differences and interactions between independent variables (caffeine and exercise) on protein synthesis rates and AMPK and p70s6k and total AMPK and p70s6k expression. Post-hoc analysis using Tukey's Honestly Significant Difference test was used to assess significant differences between groups if needed. T-tests were conducted to determine differences between caffeine groups in baseline demographics, exercise performance, and blood caffeine levels. Significance was set at a p-value of <0.05 for all analysis and all results were expressed as mean ± SE.

Results

Baseline Demographics

Twenty-four male participants completed the study. Participant demographics are represented in **Table 3**. Baseline characteristics, age, BM, lean mass, percent body fat, training history (months), 80% 1RM leg press and 80% 1RM leg extension, were not significantly different between the caffeine and non-caffeine groups. Total caloric intake and ratio of macronutrient intake during the experiment was also similar between groups and participants reported consuming on average less than 1mg of caffeine per day in both groups during the seven day experimental lead-in period.

Table 3. Baseline participant demographics. Data is presented as mean±SE.

	No Caffeine	Caffeine	p-value
Demographics			
Number	13	11	
Age (yr)	20.3±0.4	19.9±0.3	p=0.42
Body Mass (kg)	79.3±4.5	76.6±2.8	p=0.59
Lean Mass (kg)	61.3±1.8	59.9±2.8	p=0.66
% Body Fat	18.2±2.0	18.2±2.0	p=0.97
Nutrition/Diet			
Calories (kcal)	3334.8±464.3	2731.0±534.5	p=0.41
% CHO	47.3±2.8	40.8.7±2.2	p=0.09
% Protein	18.2±0.8	19.8±2.4	p=0.54
% Fat	34.6±2.8	39.4±1.9	p=0.16
Caffeine Intake (mg)	0.67±0.51	0.52±0.41	p=0.82
Exercise			
Training History (months)	6.5±1.9	11.5±3.7	p=0.25
Leg Press 80% 1RM (lbs)	492.9±43.2	497.5±44.6	p=0.94
Leg Extension 80% 1RM (lbs)	175.5±6.9	163.5±12.2	p=0.41

Plasma Caffeine Levels

Blood plasma caffeine levels immediately after exercise for the non-caffeine group were 0.0±0.0µg/ml of plasma while the caffeine group averaged 8.1±0.4µg/ml of plasma ($p<0.01$).

Strength Output

Muscle performance was reported for maximum power produced, total repetitions performed, and total weight lifted for both leg press (LP) and leg extension (LE) (**Figure 21**)**.** There were no significant differences in maximal power (LP: p=0.38,

LE: p=0.98), total repetitions (LP: p=0.88; LE: p=0.73), and total weight (LP: p=0.62; LE: p=0.18) with LP and LE between caffeine and non-caffeine groups.

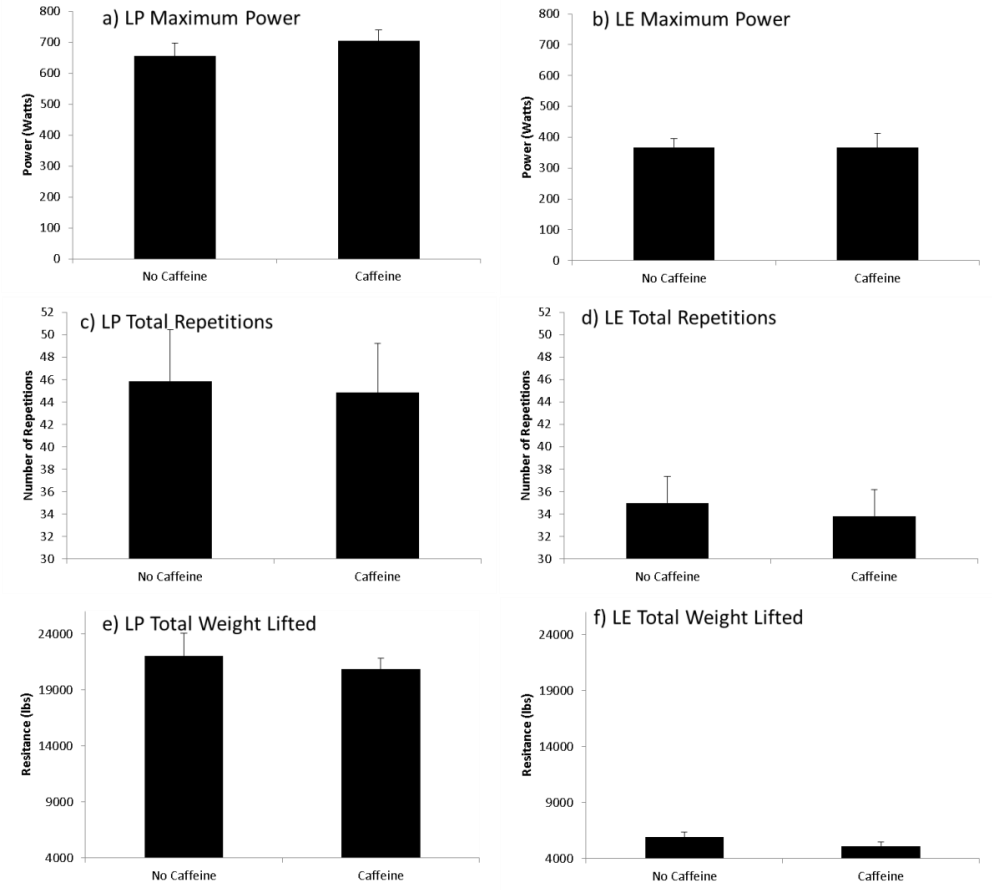

Figure 21. Strength performance for leg press and leg extension in caffeine and non-caffeine groups.

Muscle Protein Synthesis Rates

Total muscle protein synthesis rates were determined in both mixed and myofibrillar muscle. There were no statistical differences or interactions between

exercise and caffeine intake for mixed or myofibrillar muscle muscle protein synthesis rates ($p>0.05$) (**Figure 22**).

Percent change in muscle protein synthesis was not significantly different nor was there a significant interaction between caffeine and exercise groups for both mixed and myofibrillar fractions (**Figure 23**).

AMPK and p70s6K Expression

No significant interactions or differences were determined between exercise and caffeine groups for total p70s6k, phosphorylated p70s6k, or the ratio of phosphorylated to total p70s6K ($p=0.154$; $p=0.450$; $p= 0.908$) (**Figure 24**). There was a statistically significant difference in total AMPK expression for the no caffeine, no exercise group and the caffeine, no exercise group ($p=0.038$) and the no caffeine, no exercise group, and the no caffeine, exercise group ($p= 0.021$). Phosphorylated AMPK was not significantly active at the 24-hour time-point.

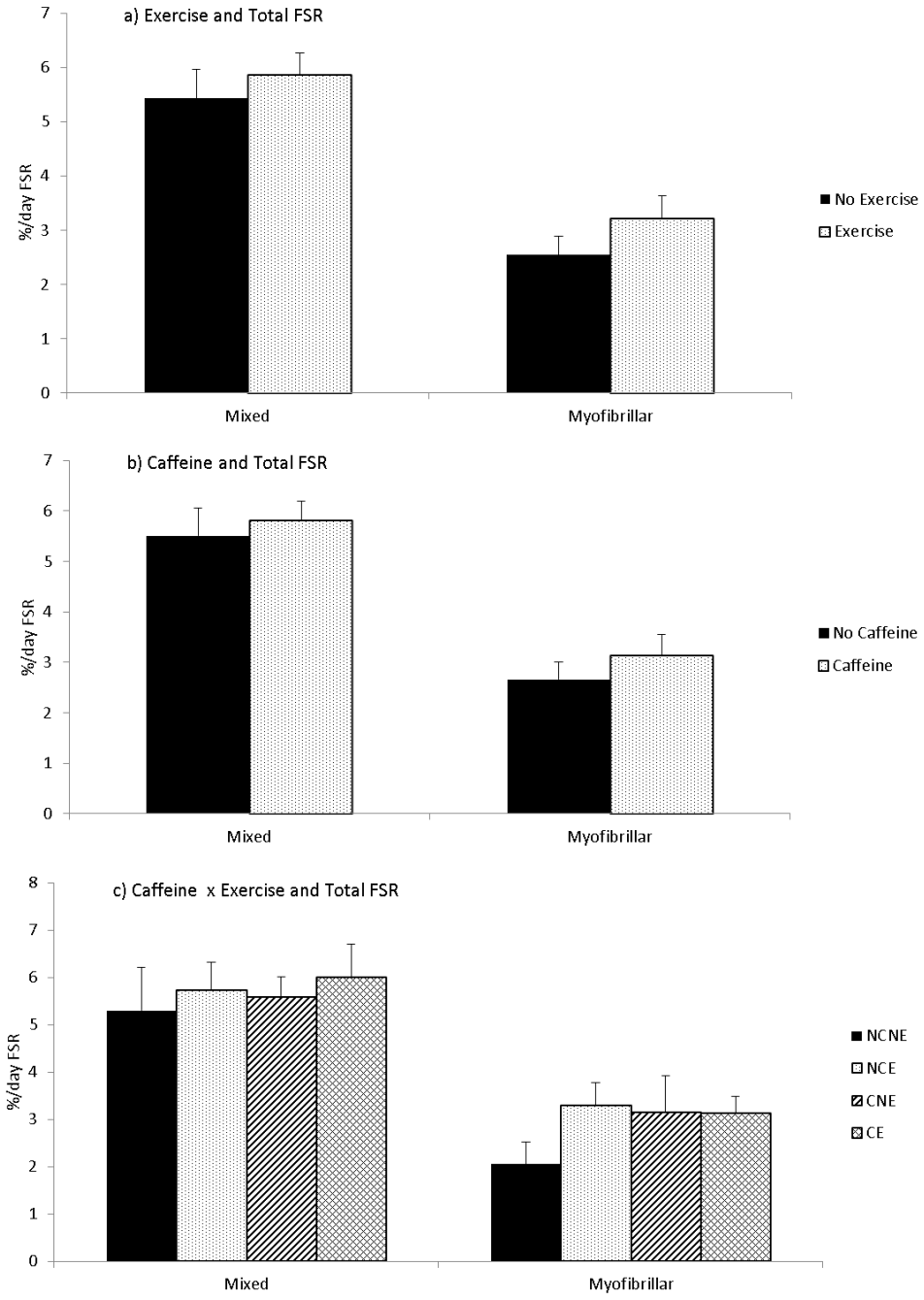

Figure 22. The effects of exercise and caffeine intake on total FSR.

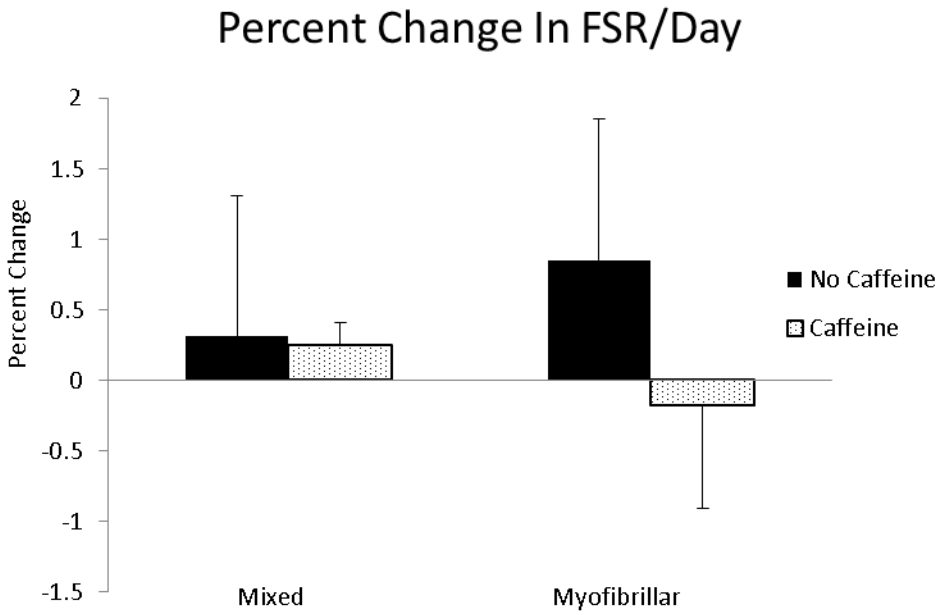

Figure 23. Percent change in FSR per day between caffeine and non-caffeine groups.

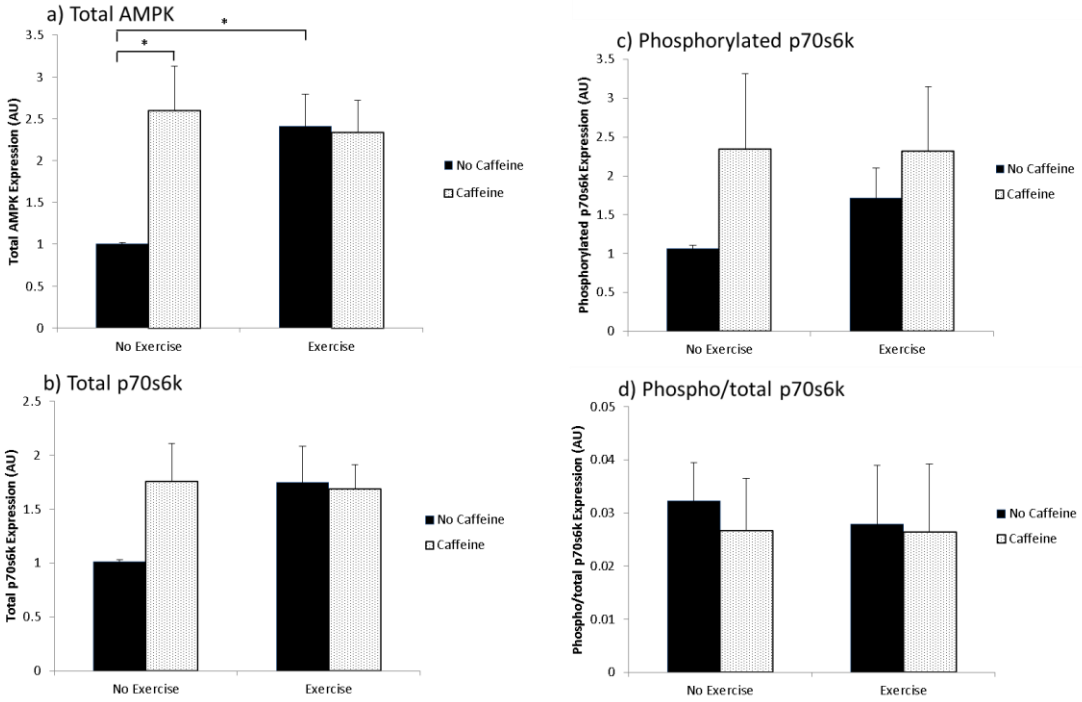

Figure 24. Western blotting expression for total AMPK, total p706sk, and phosphorylated p70s6k. *indicates a statistically significant difference between groups.

Discussion

The purpose of this study was to evaluate the response of caffeine intake on resistance exercise performance and muscle protein synthesis rates 24 hours following resistance exercise. To our knowledge, this was the first study to examine the effects of caffeine with this approach. It was hypothesized that a caffeine supplement before exercise would increase exercise performance but inhibit 24-hour muscle recovery responses due to its inhibitory effects on muscle protein synthesis.

To quantify exercise performance, this study examined maximum power produced, total number of repetitions performed, and total weight lifted (repetitions x weight). However, no differences were observed. These findings were consistent with previous studies that reported no significant differences in strength and performance in both LP and chest press while using 5-6mg/kg of body weight of caffeine [51, 95, 96, 125]. Only two studies [5, 6] reported differences in chest press strength, but they reported no differences in LP strength. Beck et al. [5] proposed that caffeine may have differing responses on upper body muscle groups compared to lower body muscles while Graham et al. [16] suggested that exercise duration may play a role in whether caffeine can cause a detectable change. Graham et al. [16] further proposed that results from shorter and highly intense exercise (similar to weight training) were inconsistent because the potential for improvement was small and difficult to measure.

When analyzing muscle protein synthesis rates in exercising and non-exercising legs, this study found no detectable differences in either mixed or myofibrillar muscle. This lack of significance may be explained by the training status of the participants in this study. Phillips et al. [126] recorded muscle protein turnover in both trained and untrained individuals and noticed that the muscle protein synthesis response in untrained individuals was more than 50% greater than those that were trained. Another possibility, as suggested by Phillips et al. [126], was that the given exercise training stimulus was insufficient to induce a high enough degree of muscle damage that is required to cause protein synthesis rate differences in a trained population. Future experiments involving untrained or highly trained individuals would help increase the applicability of the findings in this study due to the different responses in each type of individual.

No significant differences in muscle protein synthesis rates and percent change in muscle protein synthesis rates between caffeine groups in both mixed and myofibrillar muscle were determined in this study (**Figure 22**). While not statistically significant, there was a large drop in relative muscle protein synthesis rates in the myofibrillar caffeine group compared to the non-caffeine group which had a large increase. A slightly higher increase in the non-caffeine group muscle protein synthesis rates compared to that of the caffeine group was also determined in mixed muscle. The differences between the mixed and myofibrillar percent change may be due to the influence of changes in sarcoplasmic or mitochondrial proteins when mixed muscle protein responses are measured [123].

To our knowledge there have been no studies that have investigated the effect of caffeine on total and phosphorylated AMPK and p70s6K expression in response to resistance exercise in human skeletal muscle. Previous in vitro animal studies have concluded that the presence of caffeine can cause an increase in AMPK and phosphorylated AMPK while decreasing the expression of p70s6k and phosphorylated p70s6k [10, 12, 75, 127]. In this current study, AMPK expression was significantly higher in the no caffeine exercise group and the caffeine no exercise group compared to the control group. These findings further confirm exercise and caffeine intake increases the total presence of AMPK. No differences total p70s6k, and phosphorylated p70s6k expression between caffeine or exercise groups were reported in this study. The disparities in the p70s6K results may have been due to several differences in study methodologies. In several of the previous studies, non-muscle (adipocyte, brain, and adrenal) cell cultures were used which may have resulted in different cellular responses [13, 75]. Furthermore, cells were incubated with caffeine for long time periods (.25-9-hours) and at high caffeine concentrations (3mM-10mM/L). To achieve similar concentrations, plasma caffeine levels would need to be approximately 72-240x higher (582.57-1941.9µg/ml) than the average plasma concentration detected in this study. While at higher concentrations, caffeine may have a significant inhibitory impact on the protein synthesis pathway and ultimately protein synthesis rates; it is unlikely it can be achieved through ordinary caffeine intake or at the supplementation amounts of this study. Furthermore, changes in p70s6K expression may require more than a 24-hour time period.

The lack of detectable phosphorylated AMPK may be due to its rapid return to baseline values. In 2006, Dreyer et al. [9] reported the impact of resistance exercise on AMPK activity and muscle protein synthesis rates. It was concluded that the increase in AMPK activity immediately after exercise was responsible for decreases in muscle protein synthesis rates following exercise; however, the significant increase in AMPK activity was only detected for 1-hour after exercise and was no longer significant by the second hour. Considering phosphorylated AMPK in this study was evaluated at the 24-hour time point, phosphorylated AMPK may have returned to baseline values regardless of exercise and caffeine stimulation.

Limitations

The variability in participant training history may have caused inconsistent cellular responses to the resistance exercise resulting in the lack of significant differences. Furthermore, no quantitative (only qualitative) method was used to determine participant tolerance to caffeine. Depending on caffeine sensitivity, hyper or hypo responders may have had differing responses and increasing variability in the results.

Conclusions

Based on the results in this study, a caffeine bolus of 6mg/kg of body weight 1-hour before exercise increased total AMPK content; however, had no effect on resistance exercise performance, 24-hour post-exercise muscle protein synthesis rates, and 24-hour p70s6K expression in recreationally trained individuals. These results would be

informative to resistance training individuals who consume caffeine daily or as a pre-work supplement and are trying to maximize exercise performance and muscle recovery.

CHAPTER IV

THE EFFECTS OF HABITUAL CAFFEINE INTAKE ON LEAN BODY MASS AND MUSCLE PERFORMANCE WITH CHRONIC RESISTANCE EXERCISE

Introduction

The average adult consumes 200mg of caffeine per day making caffeine one of the most commonly used drugs in the United States [1]. Caffeine has been used for various reasons including increased weight loss and exercise performance [66, 81, 84, 92]. Previous studies have reported an increase in metabolic rate and thermogenesis after caffeine consumption leading to a small decrease in weight and fat mass [25, 90, 128, 129]. For aerobic exercise performance, individuals who consumed 2-6mg/kg of body weight of caffeine approximately 1-hour before exercise increased their time to fatigue, power, and running speeds while decreasing perceived exertion [3, 30, 46, 47, 49]. Differing from aerobic exercise, the acute effects of caffeine on resistance exercise have had mixed results with some studies reporting an increase in leg and chest presses performance while others had no differences [5, 6, 50, 51, 117].

Currently, the effect of caffeine intake on changes in lean mass with chronic resistance training has not been thoroughly investigated. Chronic resistance exercise can improve skeletal muscle function by stimulating muscle hypertrophy and thus increasing lean mass [7, 8, 67, 110, 130, 131]. An important regulator of muscle hypertrophy is the Akt and mTOR pathway. Activation of Akt and mTOR increases downstream translational factors and ultimately cellular growth [100]. The presence of caffeine can

inhibit AKT and mTOR proteins and caffeine has further been reported to stimulate AMPK; an inhibitor of cellular growth at high concentrations [10-13, 75, 76, 78].

The purpose of this study was to investigate the effects of habitual caffeine intake on lean mass changes following a 12 week (chronic) resistance exercise program. Furthermore, an analysis of caffeine intake and muscle performance may also further clarify current mixed conclusions regarding caffeine and resistance exercise performance. Due to the ability of caffeine to down regulate cellular growth, it was hypothesized that habitual caffeine intake would hinder changes in lean mass.

Methods

Participants

Study participants were recruited with the use of advertisements, flyers, and mailings to local community and senior centers. Both genders were eligible to participate and were required to be generally healthy, non-smoking, and able to perform exercise testing and training. Participants were excluded if an initial health assessment indicated they had high blood pressure (>160/100), cardiac arrhythmias, cancer, hernias, aortic aneurysms, diabetes, kidney disease, or lung disease. Furthermore, to limit variances in training history, those who participated in one or more hours of resistance exercise per week (determined by an activity questionnaire) were disqualified [132]. Participants were categorized into low caffeine consumers (<1.2mg/kg of body weight; less than 1 cup of coffee per day), and high caffeine consumers (>1.2mg/kg of body weight; more than 1 cup of coffee per day) [16]. This study was approved by the Institutional Review Board at Texas A&M University.

Nutrition and Exercise Orientation

Prior to the exercise intervention, participants were required to attend six orientation sessions over a span of 2 weeks. In addition, a combined 2 hours of nutrition education was provided by a registered dietician during the orientation sessions. It was emphasized that participants should aim to consume >1.0g/kg of protein, with a macronutrient ratio of 50% carbohydrates, 30% fat, 20% protein; saturated fat <10% of total calories, and 25-30g of fiber per day as recommended by the American Dietetic Association and National Academy of Sciences. Informational handouts on healthy food selections and suggested behavior modifications were also distributed. Additionally participants were given the opportunity to practice completing dietary logs using Nutribase 7.0 (Cybersoft, Arizona) to increase their proficiency with the software. This was the same software used throughout the study to determine all food and caffeine intake. Portion size samples of foods were also provided to improve accuracy of food measurement.

During the study, participants were required to document all food intake (via Nutribase 7.0, Cybersoft, Arizona) for a minimum of 4 days/wk on non-consecutive days including non-training days for all weeks. On exercise days participants were given a 0.4 g/kg of body weight protein supplement (METRX original) drink immediately after exercise to ensure proper muscle recovery. Feedback from the food log analyses was provided every two weeks throughout the study, more often if necessary, to ensure adherence to recommended intakes.

Prior to initiating exercise training, participants engaged in six 1-hour sessions of resistance exercise orientation using Keiser 300 Series Pneumatic Equipment (Keiser, Fresno, CA). All exercises were demonstrated and participants were instructed to perform three sets of eight repetitions. Weights were adjusted to achieve a perceived exertion score of four on the OMNI scale which approximates to 40 percent of 1-RM effort [119-121].

Baseline Testing

Upon completing all of the orientation sessions one repetition maximum (1RM) was determined for all exercises. Following a 10 minute warm-up on a cycle ergometer and 5 minutes of dynamic full body stretching, 1RM was determined by increasing resistance proportionally to the reported level of perceived exertion after each attempt (Schwinn Fitness, Inc, Denver, CO). Three-minute rest intervals were given after each attempt and resistance was increased until the participant was unable to complete one repetition.

Body composition was determined using dual energy x-ray absorptiometry (DEXA) (GE/Lunar Prodigy). Other baseline measurements included resting metabolic rate (ParvoMedics TrueMax 2400 Metabolic Measurement System; Sandy, UT), and blood pressure (DINAMAP 400 automated NIBP; GE Medical, Piscataway, NJ).

Exercise Intervention

All exercise sessions were supervised by research assistants and were performed in the following order: chest press, LP, lat pulldown, leg curl, biceps curl, LE, and triceps pushdown. Exercise sessions took place from 7-9am, three times per week (non-

consecutive days) for 12 weeks, and included a 10 minute warm-up on a cycle ergometer, 5 minutes of stretching, and finally three sets of 8-12 repetitions for each exercise. Resistance was set at 75% of 1RM and participants were instructed to perform as many repetitions as possible each set until muscle failure or until 12 repetitions had been completed. Resistance was increased for a particular exercise the following session if the participant was able to perform 12 repetitions for all three sets of the exercise. Participants were given 60 seconds of rest between sets and 2 minutes of rest between exercises. Increasing the resistance allowed for a consistent relative intensity throughout the study. Participants were also instructed to not perform any additional resistance exercise training and to maintain any non-resistance exercise training activities at the same level as prior to the study.

Statistical Analysis

Both independent and paired t-tests were completed to determine exercise and caffeine effects. Spearman's coefficient was also calculated to determine any correlations between caffeine intake and changes in lean mass and exercise performance. Significance was reported when $p<0.05$ was determined. The Shapiro-Wilk's test was used to determine whether the assumption of normality had been violated ($p<0.05$). All statistical analysis was completed using IBM SPSS Software version 20 (IBM Corporation, New York) and expressed as mean ± SE.

Results

Baseline Demographics

A total of 37 participants completed the study. Baseline participant demographics are represented in **Table 4**. No significant differences were observed between the caffeine groups in baseline age, BM, body mass index (BMI), percent body fat (%BF), or lean mass (LM) ($p>0.05$); however, there were statistically significant gender differences in BM, %BF, and LM ($p<0.05$).

Exercise Training Effects

Pre and post-training values for independent variables are listed in **Table 5**. Statistically significant ($p<0.05$) increases in LM and muscle performance (in all exercises: LP, chest press (CP), leg curl (LC), lat pulldown (LTP), LE, triceps pushdown (TP), and biceps curl (BC) and decreases in %BF were observed in both caffeine groups following 12 weeks of training. Statistically significant ($p>0.05$) increases in BM and BMI were observed in the low caffeine groups but not the in the high caffeine group. A significant increase from baseline resting metabolic rate (RMR) was observed in the high caffeine group, but not in the low caffeine group ($p<0.05$).

Table 4. Pre-study participant demographics. Data is presented as mean±SE.

	Low Caffeine (<1.2 mg/kg)			High Caffeine (>1.2 mg/kg)		
	Men	Women	Total	Men	Women	Total
Number	10	15	25	5	7	12
Age (years)	61.4±1.8	58.2±1.1	59.5±1.0	59.5±3.5	61.1±2.3	60.5±1.9
Weight (kgs)	92.9±5.7	72.8±4.2	80.9±3.9	86.4±2.9	77.4±6.4	81.1±4.0
BMI	29.9±1.9	27.2±1.3	28.3±1.1	27.4±1.6	29.6±2.2	28.7±1.4
%Body Fat	33.4±2.4	42.8±1.9	39.0±1.7	35.4±2.3	44.3±3.3	40.6±2.5
Lean Mass (kgs)	57.8±2.5	39.0±1.2	46.5±1.1	53.1±2.2	40.2±1.2	45.6±7.6

There were no significant differences between low and high caffeine groups.

Table 5. Pre and Post-Training Data. Values are represented as means±SE. Post-training values were determined following 12 weeks of resistance exercise.

	LOW CAFFEINE (N=25)			HIGH CAFFEINE (N=12)		
Variable	Pre Training	Post Training	p-value	Pre Training	Post Training	p-value
Diet and Metabolism						
Caloric Intake (kcal/day)	1740 ± 87	1610 ± 65	p = 0.18	1905 ± 81	1746 ± 113	p = 0.07
Relative Protein Intake (g/kg)	2.0 ± 0.4	1.5± 0.1	p = 0.23	1.7±0.1	1.5±0.1	p = 0.04*
Relative Carb Intake (g/kg)	4.8±0.7	2.9±0.1	p = 0.02*	3.7±0.5	2.8±0.2	p = 0.04*
Relative Fat Intake (g/kg)	1.5±0.1	1.3±0.1	p = 0.11	1.4±0.1	1.2±0.2	p = 0.14
Total Caffeine Intake (mg)	58.8±17.2	19.3±5.2	p = 0.04*	201.3±41.3	207.7±32.0	p = 0.75
Caffeine Intake (mg/kg)	0.8±0.3	0.2±0.1	p = 0.05*	2.5±0.5	2.5±0.4	p = 0.74
Resting Metabolic Rate (kcal)	1393.0±60.0	1510.1±88.5	p = 0.15	1410.6±53.6	1572.8±77.7	p < 0.01*
Whole Body Composition						
Body Mass (kg)	80.9±3.9	81.8±4.1	p = 0.03*	81.1±4.0	81.6±3.8	p = 0.39
BMI (kg/m)	28.3±1.1	28.6±1.1	p = 0.05*	28.7±1.4	28.8±1.3	p = 0.35
Lean mass (kg)	46.1±22.4	47.5±23.0	p < 0.01*	45.6±22.0	47.5±22.9	p < 0.01*
Regional Fat Mass						
% Body Fat	39.0±1.7	37.6±1.7	p < 0.01*	40.6±2.5	38.6±2.9	p < 0.01*
Android % Fat	45.3±1.9	43.8±1.9	p < 0.01*	47.9±2.4	45.3±2.8	p < 0.01*
Gynoid % Fat	44.5±1.9	43.1±1.9	p < 0.01*	45.3±3.0	43.4±3.2	p < 0.01*
Muscle Performance						
Leg Press (lbs)	380.7±24.1	548.5±33.3	p < 0.01*	524.6±54.2	623.7±55.1	p < 0.01*
Chest Press (lbs)	76.2±7.8	94.1±9.2	p < 0.01*	75.4±9.1	94.3±10.1	p < 0.01*
Leg Curl (lbs)	113.1±7.6	151.2±9.3	p < 0.01*	123.6±12.3	143.2±12.4	p < 0.01*
Lat Pull (lbs)	106.9±8.1	135.1±8.9	p < 0.01*	123.8±11.4	136.3±12.6	p = 0.01*
Leg Extension (lbs)	86.6±8.4	107.0±8.0	p < 0.01*	98.2±12.6	113.2±12.6	p = 0.01*
Triceps Pushdown (lbs)	150.5±11.4	199.5±14.6	p < 0.01*	168.3±12.2	208.4±18.2	p < 0.01*
Biceps Curl (lbs)	34.6±4.2	43.3±4.0	p < 0.01*	33.0±5.4	42.7±5.8	p < 0.01*

*denotes significant differences between pre and post values with a p-value <0.05

Effect of Caffeine Intake on Muscle Performance, Metabolism, and Body Mass and Composition

Relative (percent change in pre and post values) differences in muscle performance between the caffeine groups are reported in **Figure 25**. Relative

performance gains for LP, LC, and LTP were significantly (LP: p=0.01; LC: p=0.03; LTP: p<0.01) higher in the low caffeine group, while CP, LE, TP, and BC were not statistically different (CP: p=0.931; LE: p=0.579; TP: p=0.132; BC: p=0.690) between low and high caffeine groups. Change in total muscle performance (percent change of total weight lifted) was also statistically higher in the low caffeine group compared to the high caffeine group (p=0.004). Correlation coefficients between caffeine intake (g/kg of body weight) and exercise performance (LP: r_s=-0.06, LC: r_s =-0.28, LTP: r_s =-0.28, LE: r_s =-0.03, TP: r_s =-0.04, BC: r_s =-0.21; p>0.05) were negative but not significant, while CP had a weak positive correlation (r_s =0.25). There were no gender differences in exercise performance (p>0.05).

Percent change in LM (kg), BM (kg), %BF, BMI, and RMR were not significantly different between the caffeine groups (LM: p=0.43; BM: p=0.56; %BF: p=0.17; BMI: p=0.86; RMR: p=0.72) (**Figure 26**) or genders (p>0.05). Percent change in LM, BM, %BF had a weak statistically non-significant negative correlation (LM: r_s=-0.12, BM: r_s =-0.02, %BF: r_s =-0.04; p>0.05) while percent changes in BMI and RMR had a weak non-significant positive correlation (BMI: r_s =0.02, RMR: r_s =0.06; p>0.05).

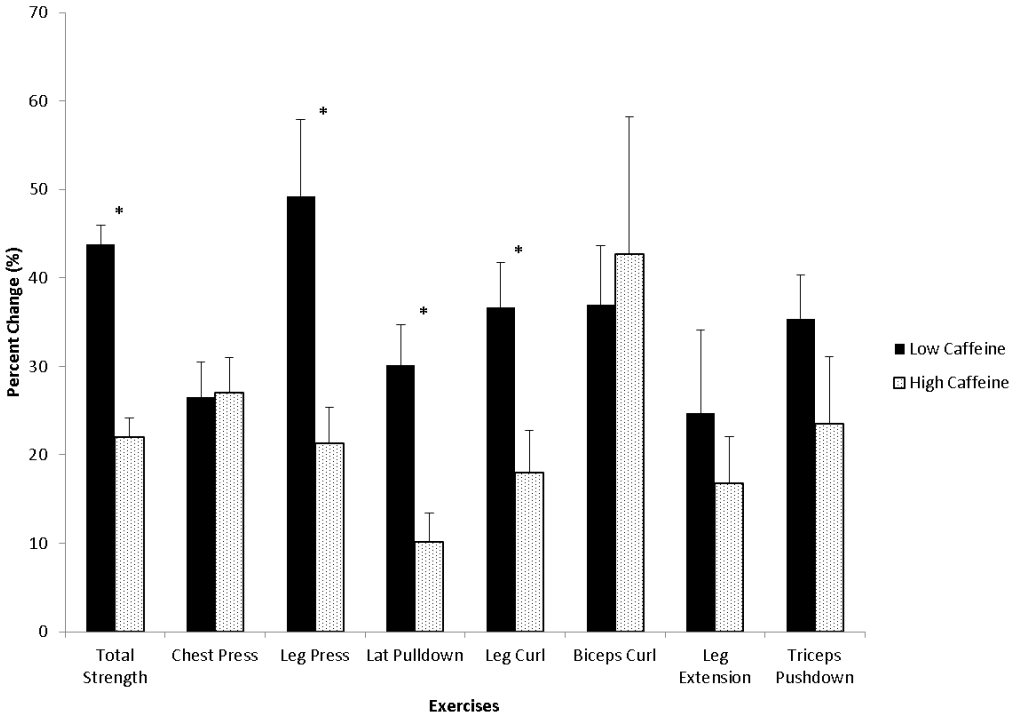

Figure 25. Percent change in weight lifted for different exercises following resistance exercise intervention. Values are represented as ± SE. *denotes significant differences between low and high caffeine groups ($p<0.05$).

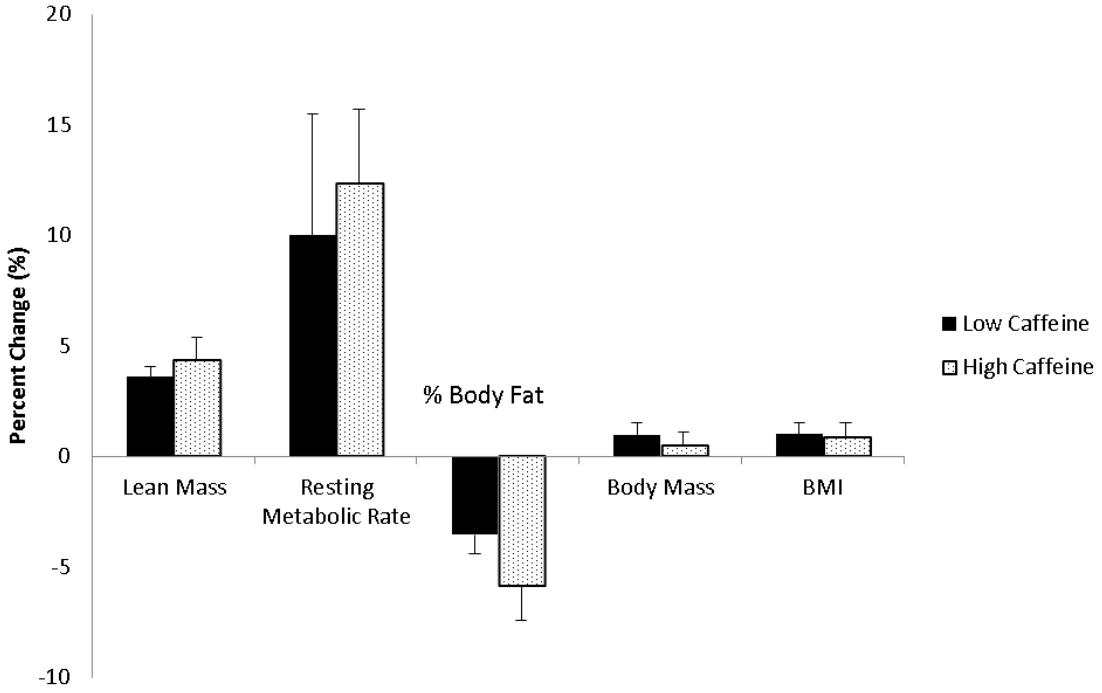

Figure 26. Percent change for body weight, lean mass, body mass index, resting metabolic rate, and percent body fat. No statistical differences were observed between caffeine groups ($p>0.05$).

Discussion

To our knowledge, this was the first study that examined changes in muscle performance and LM with habitual caffeine intake and prolonged resistance exercise (12 weeks). It was hypothesized that higher caffeine intake would decrease gains in LM due to caffeine's ability to inhibit the cellular growth pathways. As a result, there would be a significant decrease in exercise performance.

The only study, to our knowledge, that directly investigated the effects of caffeine and LM with resistance exercise was completed using a rat model. Franco et al.

[93] supplemented rats with caffeine and creatine in an attempt to test for its effect on LBM during vertical jump training. Franco et al. [93] concluded that high dosages of creatine and caffeine did not affect the LBM composition for either sedentary or exercised rats. An additional study by Malek et al. [133] determined the effects of caffeine supplementation on body composition with endurance exercise training and concluded that caffeine supplementation had no effect on body weight, %BF, or fat free mass following eight weeks of endurance training. Similarly to both of the previous studies, the results of the present study indicated that caffeine played no role in the change of LM following chronic resistance exercise in older adults. While there have been few studies examining the effects of caffeine on LM, various studies have determined the effects of caffeine on related measurements: metabolic rate, weight loss, and %BF. Previous studies have reported increases in metabolic rate, weight loss, and decreases in %BF with as little as 150mg of caffeine [84, 87, 92]. The results of this current study differ with past results, indicating no significant differences in metabolic rate, weight loss, or %BF. It is possible the disparity in results may be attributed to the differences in study length. As previously mentioned, past caffeine research has typically been focused on its short term effects (acute exercise). In a long-term study with habitual caffeine consumption, such as this one, caffeine tolerance may develop rapidly and cause decreased physiological effects [134].

While it appeared that caffeine has no effect on changes in LM, differences in muscle performance were determined between caffeine groups in several exercises. Past studies regarding caffeine and resistance exercise have only consisted of LP and CP [5,

6, 50, 51]. This study incorporated multiple exercises involving all major muscle groups. This exercise protocol is more representative of typical training programs and thus the results would be more applicable to those who are currently training. In this study, the low caffeine group had greater increases in muscle performance compared to the high caffeine group for the following measures: total performance, LP, LTP, and LC. This would initially agree with our hypothesis that high caffeine intake decreases muscle performance; however, no differences were determined for CP, LE, TP, and bicep curl. Both Woolf et al. [6] and Beck et al. [5] found that caffeine intake significantly increased muscle performance in CP but not LP. Hudson et al. [117] reported opposite findings with an increase in LE repetitions in caffeine groups. Further studies [51, 95, 96, 125] reported no significant differences in both leg and CP performance. Beck et al. [5] suggested that caffeine may have differing effects on upper body muscle groups compared to lower body muscles though a plausible mechanism is not clear. It is also possible that the differences in performance were affected by the order in which the exercises were performed. In this study, three of the first four exercises performed exhibited a significant increase in performance between low and high caffeine groups. However, in Astorino's study [51], this premise was addressed by randomizing the order of LP and CP with no differences in recorded performance. It must be re-emphasized that all aforementioned studies are not an ideal comparison to this current study since they only studied a single acute session of resistance exercise. Currently there are no other studies exist that assessed the effect of caffeine intake on multiple exercises and

chronic resistance exercise. It would appear that the exact mechanism for why some exercises had a significant increase in performance while others did not is still uncertain.

There were several limitations to this study that may have affected the conclusions. Due to the nature of the study, it was difficult to determine the exact time of caffeine intake in relation to the time of resistance exercise during the 12 week span. It takes approximately 1-hour for caffeine to peak in blood plasma and the half-life of caffeine is approximately 4.5-6.5 hours [16]**.** Depending on when the participants consumed their caffeine, it may have had minimal or no effects on exercise performance and cellular growth. Furthermore, the amount of caffeine consumed in this study was considerably different than previous studies. The average intake for the low caffeine group in this study was 19.25±5.20mg/day. This is equivalent to less than a quarter of a cup of coffee and 0.25mg/kg of body weight for an average individual (75 kg). The high caffeine group consumed an average of 207.73±31.99mg/day of caffeine, which is approximately equivalent to 2-3 cups of coffee and 2.76 mg/kg of body weight for an average individual. While the ergogenic benefits of caffeine has been seen with as low as 2 mg/kg of body weight in aerobic exercise, it is still unclear what amount of caffeine is needed to stimulate an ergogenic response, if any, in resistance exercise. Presently, most studies involving resistance exercise and caffeine supplementation have used 5-6mg/kg of caffeine per kg of body weight which is approximately 2 times higher than the average intake (of the high caffeine group) in this study. The amount of caffeine consumed in this study, may simply have not been sufficient to elicit the physical and cellular effects of caffeine.

Conclusion

The data suggests that caffeine intake does not influence LM changes after 12 weeks of resistance exercise in the older adult population. While caffeine intake does not affect LM, high caffeine intake may play a negative role in exercise muscle performance; in particular, LP, LC, and LTP. Considering this is the first study that investigated full-body muscle exercise protocol and incorporated a chronic resistance exercise program, these results would be beneficial and most applicable to those who participate in similar training. It is recommended that additional chronic studies regarding caffeine and resistance exercise performance be conducted to confirm the results of this study.

CHAPTER V

CONCLUSIONS

Overall Conclusions

For as long as there will be competitive sports and athletic events, there will always be a drive to discover new methods to improve training and performance. The use of caffeine as an ergogenic aid is not a new idea; however, there is still much obscurity regarding its effectiveness on certain training paradigms. The objective of the studies in this dissertation was to determine whether consuming caffeine has a negative impact on muscle performance and muscle growth during and following resistance exercise training.

Muscle Protein Synthesis

While both Chapters II and III evaluated the effects of caffeine intake on muscle protein synthesis rates, Chapter II examined chronic caffeine intake in an animal model while Chapter III comprised of a single bolus caffeine supplement in a recreationally trained, non-caffeine user human population. Past in-vitro animal studies suggested that cellular caffeine exposure would limit cellular growth; however, in both the animal and human models in this dissertation, there were no statistically significant differences in muscle protein synthesis rates in mixed and myofibrillar muscle subfractions [11, 75, 76]. Chapter III further investigated caffeine's effect on skeletal cellular muscle protein content following resistance exercise and it was determined that caffeine intake and exercise increased total AMPK expression; however, no statistically significant differences were found in total p70s6K, phosphorylated p70s6K, or phosphorylated

AMPK. Based on the results of this dissertation, it was concluded that caffeine intake upwards of 6mg/kg of body weight has no inhibitory effects on muscle protein synthesis. It was further concluded that to achieve the cellular inhibitory effects as seen in previous in-vitro studies, caffeine supplementation or intake would need to be near the lethal human or animal dose; something that is not likely with ordinary caffeine intake. Future studies involving different training histories (untrained or elite trained) as well as measuring cellular protein activity at different time points would help further clarify additional information on caffeine. To summarize, the data in this dissertation suggests that normal caffeine intake or supplementation has no negative effects on muscle protein synthesis and thus limiting caffeine intake in an attempt to maximize muscle gains is not supported by the evidence presented here.

Lean Body Mass, RMR, Body Fat, Body Mass, and BMI

Chapter IV investigated the effects of caffeine on lean body mass, RMR, %BF BM, and BMI following 12 weeks of resistance exercise training. This was the first study of its kind to examine the effects of caffeine in an extended training model. No statistically significant differences were observed for lean body mass, RMR, %BF, total BM, or BMI. It was difficult in this study to control for variable changes in caffeine tolerance for each participant throughout the 12 weeks, and thus a future more controlled study in terms of caffeine tolerance would be beneficial to corroborate these results.

Muscle Performance

Currently the effect of caffeine on muscle and exercise performance is an area that remains ambiguous. Caffeine use has been associated with improving resistance

exercise performance acutely and in an attempt to clarify this issue, Chapter III and IV measured muscle performance in terms of maximal power produced, number of repetitions performed, and percent change in weight lifted in regards to caffeine intake. The results remain ambiguous as reported in previous studies and while no statistically significant differences were observed in maximal power produced and the number of repetitions performed, Chapter IV had mixed results in percent change in weight lifted [50, 117]. In particular, LP, LTP, and LC had significantly lower percent changes in weight lifted in high caffeine groups than those from the low caffeine group. Chest press, BC, LE, and TP had no differences between the caffeine groups. These results, while novel due to the wide range exercises compared to past studies reporting just leg and CP, are similar to past studies which also had mixed results in terms of exercise performance [5, 6, 51]. At this point it is uncertain why in certain exercises caffeine was associated with a decrease in muscle performance while others did not. With still such uncertainty, it would be essential to conduct further researching regarding caffeine and muscle performance. Different methods of training (different exercises, number of sets, number of repetitions, and exercise intensities) and including more sensitive performance measurements (electromyography or force measurements) might be helpful in further clarifying this aspect of caffeine ad resistance exercise.

Final Thoughts

While there is still need for further research, it can presently be concluded that any habitual or regular consumption of caffeine will unlikely cause decreases in muscle protein synthesis rates following acute resistance exercise or changes in lean body mass,

RMR, %BF, total body mass, or BMI following chronic resistance exercise. While it is still unclear whether caffeine affects resistance exercise muscle performance, all findings in these studies are beneficial and applicable to any population consuming caffeine and attempting to maximize their resistance exercise training results.

REFERENCES

[1] Somogyi, L.P. Caffeine Intake By The US Population. 2010; Available from: http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofFoods/CFSAN/CFSANFOIAElectronicReadingRoom/UCM333191.pdf.

[2] Keisler, B.D. and T.D. Armsey, 2nd, Caffeine as an ergogenic aid. Curr Sports Med Rep, 2006. **5**(4): p. 215-9.

[3] Bell, D.G. and T.M. McLellan, Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers. J Appl Physiol (1985), 2002. **93**(4): p. 1227-34.

[4] Jacobson, T.L., et al., Effect of caffeine co-ingested with carbohydrate or fat on metabolism and performance in endurance-trained men. Exp Physiol, 2001. **86**(1): p. 137-44.

[5] Beck, T.W., et al., The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. J Strength Cond Res, 2006. **20**(3): p. 506-10.

[6] Woolf, K., W.K. Bidwell, and A.G. Carlson, The effect of caffeine as an ergogenic aid in anaerobic exercise. Int J Sport Nutr Exerc Metab, 2008. **18**(4): p. 412-29.

[7] Fedele, M.J., et al., Severe diabetes prohibits elevations in muscle protein synthesis after acute resistance exercise in rats. J Appl Physiol (1985), 2000. **88**(1): p. 102-8.

[8] Hernandez, J.M., M.J. Fedele, and P.A. Farrell, Time course evaluation of protein synthesis and glucose uptake after acute resistance exercise in rats. J Appl Physiol (1985), 2000. **88**(3): p. 1142-9.

[9] Dreyer, H.C., et al., Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. J Physiol, 2006. **576**(Pt 2): p. 613-24.

[10] Egawa, T., et al., Caffeine acutely activates 5'adenosine monophosphate-activated protein kinase and increases insulin-independent glucose transport in rat skeletal muscles. Metabolism, 2009. **58**(11): p. 1609-17.

[11] Foukas, L.C., et al., Direct effects of caffeine and theophylline on p110 delta and other phosphoinositide 3-kinases. Differential effects on lipid kinase and protein kinase activities. J Biol Chem, 2002. **277**(40): p. 37124-30.

[12] Jensen, T.E., et al., Caffeine-induced Ca(2+) release increases AMPK-dependent glucose uptake in rodent soleus muscle. Am J Physiol Endocrinol Metab, 2007. **293**(1): p. E286-92.

[13] Scott, P.H. and J.C. Lawrence, Jr., Attenuation of mammalian target of rapamycin activity by increased cAMP in 3T3-L1 adipocytes. J Biol Chem, 1998. **273**(51): p. 34496-501.

[14] Daly, J.W., Caffeine analogs: biomedical impact. Cell Mol Life Sci, 2007. **64**(16): p. 2153-69.

[15] Tarnopolsky, M.A., Caffeine and creatine use in sport. Ann Nutr Metab, 2010. **57 Suppl 2**: p. 1-8.

[16] Graham, T.E., Caffeine and exercise: metabolism, endurance and performance. Sports Med, 2001. **31**(11): p. 785-807.

[17] Nehlig, A., J.L. Daval, and G. Debry, Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev, 1992. **17**(2): p. 139-70.

[18] Ribeiro, J.A. and A.M. Sebastiao, Caffeine and adenosine. J Alzheimers Dis, 2010. **20 Suppl 1**: p. S3-15.

[19] Davis, J.M., et al., Central nervous system effects of caffeine and adenosine on fatigue. Am J Physiol Regul Integr Comp Physiol, 2003. **284**(2): p. R399-404.

[20] Kalmar, J.M. and E. Cafarelli, Caffeine: a valuable tool to study central fatigue in humans? Exerc Sport Sci Rev, 2004. **32**(4): p. 143-7.

[21] Lorist, M.M. and M. Tops, Caffeine, fatigue, and cognition. Brain Cogn, 2003. **53**(1): p. 82-94.

[22] Fredholm, B.B., et al., Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev, 1999. **51**(1): p. 83-133.

[23] Sawynok, J., Adenosine receptor activation and nociception. Eur J Pharmacol, 1998. **347**(1): p. 1-11.

[24] Davis, J.K. and J.M. Green, Caffeine and anaerobic performance: ergogenic value and mechanisms of action. Sports Med, 2009. **39**(10): p. 813-32.

[25] Barraco, R.A., et al., Central effects of adenosine analogs on locomotor activity in mice and antagonism of caffeine. Brain Res, 1983. **272**(2): p. 392-5.

[26] Fredholm, B.B., On the mechanism of action of theophylline and caffeine. Acta Med Scand, 1985. **217**(2): p. 149-53.

[27] Juhn, M., Popular sports supplements and ergogenic aids. Sports Med, 2003. **33**(12): p. 921-39.

[28] Raguso, C.A., et al., Effect of theophylline on substrate metabolism during exercise. Metabolism, 1996. **45**(9): p. 1153-60.

[29] Costill, D.L., G.P. Dalsky, and W.J. Fink, Effects of caffeine ingestion on metabolism and exercise performance. Med Sci Sports, 1978. **10**(3): p. 155-8.

[30] Ivy, J.L., et al., Influence of caffeine and carbohydrate feedings on endurance performance. Med Sci Sports, 1979. **11**(1): p. 6-11.

[31] Chesley, A., et al., Regulation of muscle glycogenolytic flux during intense aerobic exercise after caffeine ingestion. Am J Physiol, 1998. **275**(2 Pt 2): p. R596-603.

[32] Laurent, D., et al., Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. J Clin Endocrinol Metab, 2000. **85**(6): p. 2170-5.

[33] Graham, T.E., et al., Does caffeine alter muscle carbohydrate and fat metabolism during exercise? Appl Physiol Nutr Metab, 2008. **33**(6): p. 1311-8.

[34] Graham, T.E. and L.L. Spriet, Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. J Appl Physiol (1985), 1995. **78**(3): p. 867-74.

[35] Jackman, M., et al., Metabolic catecholamine, and endurance responses to caffeine during intense exercise. J Appl Physiol (1985), 1996. **81**(4): p. 1658-63.

[36] Van Soeren, M.H., et al., Caffeine metabolism and epinephrine responses during exercise in users and nonusers. J Appl Physiol (1985), 1993. **75**(2): p. 805-12.

[37] Mohr, T., et al., Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling. J Appl Physiol (1985), 1998. **85**(3): p. 979-85.

[38] Daniels, J.W., et al., Effects of caffeine on blood pressure, heart rate, and forearm blood flow during dynamic leg exercise. J Appl Physiol (1985), 1998. **85**(1): p. 154-9.

[39] Graham, T.E., et al., Caffeine ingestion does not alter carbohydrate or fat metabolism in human skeletal muscle during exercise. J Physiol, 2000. **529 Pt 3**: p. 837-47.

[40] Higgins, J.P. and K.M. Babu, Caffeine reduces myocardial blood flow during exercise. Am J Med, 2013. **126**(8): p. 730 e1-8.

[41] Maughan, R.J., Nutritional ergogenic aids and exercise performance. Nutr Res Rev, 1999. **12**(2): p. 255-80.

[42] Sokmen, B., et al., Caffeine use in sports: considerations for the athlete. J Strength Cond Res, 2008. **22**(3): p. 978-86.

[43] Desbrow, B., et al., The effects of different doses of caffeine on endurance cycling time trial performance. J Sports Sci, 2012. **30**(2): p. 115-20.

[44] Kovacs, E.M., J. Stegen, and F. Brouns, Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. J Appl Physiol (1985), 1998. **85**(2): p. 709-15.

[45] Pasman, W.J., et al., The effect of different dosages of caffeine on endurance performance time. Int J Sports Med, 1995. **16**(4): p. 225-30.

[46] Hogervorst, E., et al., Caffeine improves physical and cognitive performance during exhaustive exercise. Med Sci Sports Exerc, 2008. **40**(10): p. 1841-51.

[47] O'Rourke, M.P., et al., Caffeine has a small effect on 5-km running performance of well-trained and recreational runners. J Sci Med Sport, 2008. **11**(2): p. 231-3.

[48] Bridge, C.A. and M.A. Jones, The effect of caffeine ingestion on 8 km run performance in a field setting. J Sports Sci, 2006. **24**(4): p. 433-9.

[49] Backhouse, S.H., et al., Caffeine ingestion, affect and perceived exertion during prolonged cycling. Appetite, 2011. **57**(1): p. 247-52.

[50] Green, J.M., et al., Effects of caffeine on repetitions to failure and ratings of perceived exertion during resistance training. Int J Sports Physiol Perform, 2007. **2**(3): p. 250-9.

[51] Astorino, T.A., R.L. Rohmann, and K. Firth, Effect of caffeine ingestion on one-repetition maximum muscular strength. Eur J Appl Physiol, 2008. **102**(2): p. 127-32.

[52] Eckerson, J.M., et al., Acute ingestion of sugar-free red bull energy drink has no effect on upper body strength and muscular endurance in resistance trained men. J Strength Cond Res, 2013. **27**(8): p. 2248-54.

[53] Hendrix, C.R., et al., Acute effects of a caffeine-containing supplement on bench press and leg extension strength and time to exhaustion during cycle ergometry. J Strength Cond Res, 2010. **24**(3): p. 859-65.

[54] Goldstein, E., et al., Caffeine enhances upper body strength in resistance-trained women. J Int Soc Sports Nutr, 2010. **7**: p. 18.

[55] Astorino, T.A., et al., Minimal effect of acute caffeine ingestion on intense resistance training performance. J Strength Cond Res, 2011. **25**(6): p. 1752-8.

[56] Astorino, T.A., et al., Effect of two doses of caffeine on muscular function during isokinetic exercise. Med Sci Sports Exerc, 2010. **42**(12): p. 2205-10.

[57] Duncan, M.J., C.D. Thake, and P.J. Downs, Effect of caffeine ingestion on torque and muscle activity during resistance exercise in men. Muscle Nerve, 2014. **50**(4): p. 523-7.

[58] Duncan, M.J., et al., Acute caffeine ingestion enhances strength performance and reduces perceived exertion and muscle pain perception during resistance exercise. Eur J Sport Sci, 2013. **13**(4): p. 392-9.

[59] Duncan, M.J., M. Lyons, and J. Hankey, Placebo effects of caffeine on short-term resistance exercise to failure. Int J Sports Physiol Perform, 2009. **4**(2): p. 244-53.

[60] Duncan, M.J. and S.W. Oxford, The effect of caffeine ingestion on mood state and bench press performance to failure. J Strength Cond Res, 2011. **25**(1): p. 178-85.

[61] Goldstein, E.R., et al., International society of sports nutrition position stand: caffeine and performance. J Int Soc Sports Nutr, 2010. **7**(1): p. 5.

[62] Wemple, R.D., D.R. Lamb, and K.H. McKeever, Caffeine vs caffeine-free sports drinks: effects on urine production at rest and during prolonged exercise. Int J Sports Med, 1997. **18**(1): p. 40-6.

[63] Bangsbo, J., et al., Acute and habitual caffeine ingestion and metabolic responses to steady-state exercise. J Appl Physiol (1985), 1992. **72**(4): p. 1297-303.

[64] Tarnopolsky, M. and C. Cupido, Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers. J Appl Physiol (1985), 2000. **89**(5): p. 1719-24.

[65] Zhang, Y. and J.N. Wells, The effects of chronic caffeine administration on peripheral adenosine receptors. J Pharmacol Exp Ther, 1990. **254**(3): p. 757-63.

[66] Greenway, F.L., et al., Effect of a dietary herbal supplement containing caffeine and ephedra on weight, metabolic rate, and body composition. Obes Res, 2004. **12**(7): p. 1152-7.

[67] Chesley, A., et al., Changes in human muscle protein synthesis after resistance exercise. J Appl Physiol (1985), 1992. **73**(4): p. 1383-8.

[68] MacDougall, J.D., et al., The time course for elevated muscle protein synthesis following heavy resistance exercise. Can J Appl Physiol, 1995. **20**(4): p. 480-6.

[69] Ballou, L.M. and R.Z. Lin, Rapamycin and mTOR kinase inhibitors. J Chem Biol, 2008. **1**(1-4): p. 27-36.

[70] Nomura, M., et al., Inhibition of epidermal growth factor-induced cell transformation and Akt activation by caffeine. Mol Carcinog, 2005. **44**(1): p. 67-76.

[71] Reinke, A., et al., Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p. J Biol Chem, 2006. **281**(42): p. 31616-26.

[72] Zanchi, N.E. and A.H. Lancha, Jr., Mechanical stimuli of skeletal muscle: implications on mTOR/p70s6k and protein synthesis. Eur J Appl Physiol, 2008. **102**(3): p. 253-63.

[73] Asnaghi, L., et al., mTOR: a protein kinase switching between life and death. Pharmacol Res, 2004. **50**(6): p. 545-9.

[74] Miwa, S., et al., Caffeine induces apoptosis of osteosarcoma cells by inhibiting AKT/mTOR/S6K, NF-kappaB and MAPK pathways. Anticancer Res, 2012. **32**(9): p. 3643-9.

[75] Saiki, S., et al., Caffeine induces apoptosis by enhancement of autophagy via PI3K/Akt/mTOR/p70S6K inhibition. Autophagy, 2011. **7**(2): p. 176-87.

[76] Akiba, T., et al., Inhibitory mechanism of caffeine on insulin-stimulated glucose uptake in adipose cells. Biochem Pharmacol, 2004. **68**(10): p. 1929-37.

[77] Kolnes, A.J., et al., Caffeine and theophylline block insulin-stimulated glucose uptake and PKB phosphorylation in rat skeletal muscles. Acta Physiol (Oxf), 2010. **200**(1): p. 65-74.

[78] Bolster, D.R., et al., AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J Biol Chem, 2002. **277**(27): p. 23977-80.

[79] Wu, B.H. and J.C. Lin, Caffeine attenuates acute growth hormone response to a single bout of resistance exercise. J Sports Sci Med, 2010. **9**(2): p. 262-9.

[80] Beaven, C.M., et al., Dose effect of caffeine on testosterone and cortisol responses to resistance exercise. Int J Sport Nutr Exerc Metab, 2008. **18**(2): p. 131-41.

[81] Boozer, C.N., et al., Herbal ephedra/caffeine for weight loss: a 6-month randomized safety and efficacy trial. Int J Obes Relat Metab Disord, 2002. **26**(5): p. 593-604.

[82] Ramsey, J.J., et al., Energy expenditure, body composition, and glucose metabolism in lean and obese rhesus monkeys treated with ephedrine and caffeine. Am J Clin Nutr, 1998. **68**(1): p. 42-51.

[83] Wang, H., et al., Effects of catechin enriched green tea on body composition. Obesity (Silver Spring), 2010. **18**(4): p. 773-9.

[84] Westerterp-Plantenga, M.S., M.P. Lejeune, and E.M. Kovacs, Body weight loss and weight maintenance in relation to habitual caffeine intake and green tea supplementation. Obes Res, 2005. **13**(7): p. 1195-204.

[85] Greenberg, J.A., et al., Coffee, tea and diabetes: the role of weight loss and caffeine. Int J Obes (Lond), 2005. **29**(9): p. 1121-9.

[86] Chen, M.D., et al., Effect of caffeine on the levels of brain serotonin and catecholamine in the genetically obese mice. Zhonghua Yi Xue Za Zhi (Taipei), 1994. **53**(5): p. 257-61.

[87] Kobayashi-Hattori, K., et al., Effect of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. Biosci Biotechnol Biochem, 2005. **69**(11): p. 2219-23.

[88] Michna, L., et al., Stimulatory effect of oral administration of green tea and caffeine on locomotor activity in SKH-1 mice. Life Sci, 2003. **73**(11): p. 1383-92.

[89] Zheng, G., et al., Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice. In Vivo, 2004. **18**(1): p. 55-62.

[90] Arciero, P.J., et al., Influence of age on the thermic response to caffeine in women. Metabolism, 2000. **49**(1): p. 101-7.

[91] Dulloo, A.G., et al., Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. Am J Clin Nutr, 1989. **49**(1): p. 44-50.

[92] Lopez-Garcia, E., et al., Changes in caffeine intake and long-term weight change in men and women. Am J Clin Nutr, 2006. **83**(3): p. 674-80.

[93] Franco, F.S., et al., The effects of a high dosage of creatine and caffeine supplementation on the lean body mass composition of rats submitted to vertical jumping training. J Int Soc Sports Nutr, 2011. **8**: p. 3.

[94] Wilkinson, S.B., et al., Hypertrophy with unilateral resistance exercise occurs without increases in endogenous anabolic hormone concentration. Eur J Appl Physiol, 2006. **98**(6): p. 546-55.

[95] Jacobson, B.H., et al., Effect of caffeine on maximal strength and power in elite male athletes. Br J Sports Med, 1992. **26**(4): p. 276-80.

[96] Williams, A.D., et al., The effect of ephedra and caffeine on maximal strength and power in resistance-trained athletes. J Strength Cond Res, 2008. **22**(2): p. 464-70.

[97] Farrell, P.A., et al., Regulation of protein synthesis after acute resistance exercise in diabetic rats. Am J Physiol, 1999. **276**(4 Pt 1): p. E721-7.

[98] Fluckey, J.D., et al., Augmented insulin action on rates of protein synthesis after resistance exercise in rats. Am J Physiol, 1996. **270**(2 Pt 1): p. E313-9.

[99] Kubica, N., et al., Resistance exercise increases muscle protein synthesis and translation of eukaryotic initiation factor 2Bepsilon mRNA in a mammalian target of rapamycin-dependent manner. J Biol Chem, 2005. **280**(9): p. 7570-80.

[100] Kimball, S.R. and L.S. Jefferson, Control of translation initiation through integration of signals generated by hormones, nutrients, and exercise. J Biol Chem, 2010. **285**(38): p. 29027-32.

[101] Nakamaru, K., et al., AICAR, an activator of AMP-activated protein kinase, down-regulates the insulin receptor expression in HepG2 cells. Biochem Biophys Res Commun, 2005. **328**(2): p. 449-54.

[102] Fluckey, J.D., et al., Insulin stimulation of protein synthesis in rat skeletal muscle following resistance exercise is maintained with advancing age. J Gerontol A Biol Sci Med Sci, 1996. **51**(5): p. B323-30.

[103] Farrell, P.A., et al., Hypertrophy of skeletal muscle in diabetic rats in response to chronic resistance exercise. J Appl Physiol (1985), 1999. **87**(3): p. 1075-82.

[104] Gasier, H.G., et al., Acute resistance exercise augments integrative myofibrillar protein synthesis. Metabolism, 2012. **61**(2): p. 153-6.

[105] Gasier, H.G., et al., A novel approach for assessing protein synthesis in channel catfish, Ictalurus punctatus. Comp Biochem Physiol B Biochem Mol Biol, 2009. **154**(2): p. 235-8.

[106] Belloto, E., et al., Determination of protein replacement rates by deuterated water: validation of underlying assumptions. Am J Physiol Endocrinol Metab, 2007. **292**(5): p. E1340-7.

[107] Oshima, T. and N. Tamiya, Mechanism of transaminase action. Biochem J, 1961. **78**: p. 116-9.

[108] Gasier, H.G., et al., A comparison of 2H2O and phenylalanine flooding dose to investigate muscle protein synthesis with acute exercise in rats. Am J Physiol Endocrinol Metab, 2009. **297**(1): p. E252-9.

[109] Nilsson, M.I., et al., Insulin resistance syndrome blunts the mitochondrial anabolic response following resistance exercise. Am J Physiol Endocrinol Metab, 2010. **299**(3): p. E466-74.

[110] Phillips, S.M., et al., Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol, 1997. **273**(1 Pt 1): p. E99-107.

[111] Hohl, R., et al., Development and characterization of an overtraining animal model. Med Sci Sports Exerc, 2009. **41**(5): p. 1155-63.

[112] Moraska, A., et al., Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. Am J Physiol Regul Integr Comp Physiol, 2000. **279**(4): p. R1321-9.

[113] Pereira, B.C., et al., A new overtraining protocol for mice based on downhill running sessions. Clin Exp Pharmacol Physiol, 2012. **39**(9): p. 793-8.

[114] Armstrong, L.E. and J.L. VanHeest, The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. Sports Med, 2002. **32**(3): p. 185-209.

[115] Lehmann, M., C. Foster, and J. Keul, Overtraining in endurance athletes: a brief review. Med Sci Sports Exerc, 1993. **25**(7): p. 854-62.

[116] Purvis, D., S. Gonsalves, and P.A. Deuster, Physiological and psychological fatigue in extreme conditions: overtraining and elite athletes. PM R, 2010. **2**(5): p. 442-50.

[117] Hudson, G.M., et al., Effects of caffeine and aspirin on light resistance training performance, perceived exertion, and pain perception. J Strength Cond Res, 2008. **22**(6): p. 1950-7.

[118] Shohet, K.L. and R.E. Landrum, Caffeine consumption questionnaire: a standardized measure for caffeine consumption in undergraduate students. Psychol Rep, 2001. **89**(3): p. 521-6.

[119] Gearhart, R.F., Jr., et al., RPE at relative intensities after 12 weeks of resistance-exercise training by older adults. Percept Mot Skills, 2008. **106**(3): p. 893-903.

[120] Gearhart, R.F., Jr., et al., Strength tracking using the OMNI resistance exercise scale in older men and women. J Strength Cond Res, 2009. **23**(3): p. 1011-5.

[121] Gearhart, R.F., Jr., et al., Safety of using the adult OMNI Resistance Exercise Scale to determine 1-RM in older men and women. Percept Mot Skills, 2011. **113**(2): p. 671-6.

[122] Hubal, M.J., et al., Variability in muscle size and strength gain after unilateral resistance training. Med Sci Sports Exerc, 2005. **37**(6): p. 964-72.

[123] Kim, P.L., R.S. Staron, and S.M. Phillips, Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. J Physiol, 2005. **568**(Pt 1): p. 283-90.

[124] Gasier, H.G., et al., Cumulative responses of muscle protein synthesis are augmented with chronic resistance exercise training. Acta Physiol (Oxf), 2011. **201**(3): p. 381-9.

[125] Jacobs, I., H. Pasternak, and D.G. Bell, Effects of ephedrine, caffeine, and their combination on muscular endurance. Med Sci Sports Exerc, 2003. **35**(6): p. 987-94.

[126] Phillips, S.M., et al., Resistance training reduces the acute exercise-induced increase in muscle protein turnover. Am J Physiol, 1999. **276**(1 Pt 1): p. E118-24.

[127] Egawa, T., et al., Caffeine modulates phosphorylation of insulin receptor substrate-1 and impairs insulin signal transduction in rat skeletal muscle. J Appl Physiol (1985), 2011. **111**(6): p. 1629-36.

[128] Astrup, A., et al., Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. Am J Clin Nutr, 1990. **51**(5): p. 759-67.

[129] Bracco, D., et al., Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women. Am J Physiol, 1995. **269**(4 Pt 1): p. E671-8.

[130] Figueroa, A., et al., Effects of exercise training and hormone replacement therapy on lean and fat mass in postmenopausal women. J Gerontol A Biol Sci Med Sci, 2003. **58**(3): p. 266-70.

[131] Irwin, M.L., et al., Exercise improves body fat, lean mass, and bone mass in breast cancer survivors. Obesity (Silver Spring), 2009. **17**(8): p. 1534-41.

[132] Kriska, A.M., et al., Development of questionnaire to examine relationship of physical activity and diabetes in Pima Indians. Diabetes Care, 1990. **13**(4): p. 401-11.

[133] Malek, M.H., et al., Effects of eight weeks of caffeine supplementation and endurance training on aerobic fitness and body composition. J Strength Cond Res, 2006. **20**(4): p. 751-5.

[134] Nehlig, A., Are we dependent upon coffee and caffeine? A review on human and animal data. Neurosci Biobehav Rev, 1999. **23**(4): p. 563-76.