THE INFLUENCE OF CYP1A2 GENOTYPE ON NEUROMUSCULAR FUNCTION FOLLOWING ACUTE CAFFEINE ADMINISTRATION

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Abstract: The purpose of this study is to examine whether genetic variation in CYP1A2 (-163A>C, rs762551) influences the effects of acute caffeine supplementation on neuromuscular function of the lower body at rest and in response to a fatiguing work bout. Forty-two young, healthy males completed the entire study protocol and were genotyped for CYP1A2 enzyme. Subjects were then classified as AA (FAST: n = 26) or AC/CC (SLOW; n =16). This study consisted of 3 separate visits to the laboratory, a familiarization session and 2 experimental sessions: caffeine (CAF; 6 mg/kg/bw) or placebo (PLA). During each session, neuromuscular function, including motor unit behavior, muscle activation, spinal and supraspinal excitability, and muscle contractile properties were assessed. Additionally, each experimental visit ended with repeated, intermittent submaximal contractions at 50% of the subject's maximum effort to fatigue. The main findings from this investigation were the overall lack of ergogenic effects of caffeine on neuromuscular function of the lower body musculature. Specifically, no significant alterations in motor unit behavior, muscle activation, or spinal or supraspinal excitability were found from pre- to post-testing in either condition. However, the present data suggest that caffeine may augment the decline seen in muscle contractile properties in the placebo condition. The present data also suggests a limited role, if any, for the CYP1A2 genotype in mediating the effects of caffeine on neuromuscular function.

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CHAPTER I

INTRODUCTION

1.1 Introduction

Caffeine (1,3,7-trimethylxanthine) is the most widely consumed, central nervous system stimulant in the world. Its primary mechanism of action is thought to be as an adenosine receptor antagonist, preventing the decline in wakefulness seen throughout the day. Caffeine can also affect muscle activation, potentially through peripheral, spinal, and/or supraspinal pathways (Fimland et al. 2010; Kalmar 2005) , as adenosine receptors are located throughout a variety of tissues (Reppert et al. 1991). At the spinal level, caffeine has been reported to increase motor neuron excitability (Kalmar et al. 2006; Walton et al. 2003) and increase the self-sustained firing rate of motor units (Walton et al. 2002). Additionally, another potential mechanism is a change in calcium handling and kinetics following caffeine supplementation, which has been supported previous work showing an increase in muscular twitch force and twitch time (Bazzucchi et al. 2011; Lopes et al. 1983). Despite the lack of clear mechanism (Allen et al. 2008; Penner et al. 1989) and caffeine's widespread use as an ergogenic aid, its effects on muscle function, and specifically muscle strength, have been relatively mixed. For example, a meta-analysis by Warren et al. (2010) showed a modest effect for caffeine on maximal voluntary contraction strength (effect size = 0.19). However, other studies have

reported no change (Behrens et al. 2015b; Fimland et al. 2010) or even a decrease (Bond et al. 1986) in muscular function following caffeine supplementation. Interestingly, these effects may be muscle specific (Mau-Moeller et al. 2013), as the majority of positive changes in strength have been seen in the quadriceps femoris, with largely equivocal effects observed in the triceps surae musculature.

In terms of maximal strength, Bazzucchi et al. (2011) saw a significant increase in MVIC of the biceps brachii an hour after caffeine supplementation, with no change seen following placebo ingestion, as did Behrens et al. (2015a) in the quadriceps. These results are supported by numerous other investigations (Behrens et al. 2015a; Goldstein et al. 2010; Jacobson et al. 1992; Kalmar and Cafarelli 2006; Kalmar and Cafarelli 1999; Kalmar et al. 2006; Park et al. 2008). However, several other investigations suggest that caffeine has minimal to no effect on MVIC. For example, Fimland et al. (2010) and many others (Astorino et al. 2008; Behrens et al. 2015b; Bond et al. 1986; Jacobson and Edwards 1991; Tarnopolsky et al. 1989; van Duinen et al. 2005) have reported no change in MVIC strength following caffeine ingestion. Interestingly, however, Behrens et al. (2015b) did report a significant improvement normalized electromyography (EMG) and rate of torque development (RTD), which led the authors to suggest that caffeine may have altered the excitability of the spinal α-motor neurons at the onset of contraction. Another potential explanation could be that caffeine increased corticospinal excitability during the initial phase of contraction and in turn, enhanced muscle activity of the triceps surae at the 0-100 and 0-200 time intervals, which is indirectly supported previously by Kalmar and Cafarelli (1999), who reported an increase in voluntary activation with no change in h-reflex, suggesting corticospinal mechanisms. Further support for this hypothesis is found in the work of

Behrens et al. (2015a), who reported a significant increase in MVC strength during eccentric, concentric and isometric muscle actions following caffeine administration, which the authors concluded was due to an increase in voluntary activation. The authors also saw an increase in RTD (0-200), which the authors attributed to increased neural drive. Based on the above described studies, Behrens et al. (2015a) suggested that caffeine is most likely exerting its effect centrally, as opposed to influencing in excitability of spinal α-motor neurons.

It has been shown that central adenosine a2a receptor agonists depress the firing of cerebral cortical neurons and lead to hypoactivity, depression of locomotor activity and impairment of coordination (Phillis et al. 1979). Thus, caffeine may improve force production via antagonism of the adenosine a2a receptors. In support of this hypothesis, Bazzucchi et al. (2011) observed an increase in biceps brachii EMG amplitude following caffeine administration, which the authors attributed to greater recruitment high-threshold MUs, as previously proposed by Kalmar and Cafarelli (1999). Behrens (Behrens et al. 2015a; Behrens et al. 2015b) reported increased neural drive following caffeine supplementation, which was evidenced by increase EMG amplitude during the onset of torque production. Moreover, Bazzucchi et al. (2011) observed increases in torque across the torque-velocity relationship, supporting their hypothesis of increased capacity to recruit higher-threshold MUs with caffeine supplementation. However, they did not observe a change in EMG amplitude during non-explosive contractions. Thus, this may support the conclusions of Warren et al. (2010), who reported that the most likely mechanism for the modest improvement in MVC reported from their review was due to increased voluntary activation. In contrast, however, Fimland et al. (2010) reported no changes in EMG amplitude following caffeine. This is supported by the work of Greer and colleagues (2006), who found no change

in Wingate performance or EMG parameters (mean power frequency, integrated EMG amplitude, etc.) following caffeine supplementation (5 mg/kg), when compared to placebo. However, it is also interesting to point out the potential muscle and/or contraction specific effects of caffeine. For example, the contribution of cortical and spinal centers differ based on contraction type and may be partly responsible for the differences observed (Duchateau and Baudry 2013). This is supported by previous work, where H-reflexes and motor evoked potentials were smaller during eccentric contractions, when compared to isometric or concentric contractions (Duclay and Martin 2005; Gruber et al. 2009). Additionally, the review by Warren et al. (2010) showed the largest effect of caffeine on voluntary activation in the quadriceps femoris (ES = 0.67), which is likely because voluntary activation is often lower for the knee extensors than for other lower body musculature or muscle groups in the upper extremity (Mau-Moeller et al. 2013).

While the literature on the effects of caffeine on neuromuscular function are largely equivocal, the H-Reflex and V-Wave (volitional wave) responses to caffeine are quite uniform. H-reflex and V-wave can be used to analyze modulations at the spinal level (Aagaard et al. 2002; Zehr 2002). H-reflex reflects the activation of the α-motor neurons by the Iα afferent pathways (Schieppati 1987), whereas the V-wave reflects the descending neural drive from the α-motor neuron to the muscle (Aagaard et al. 2002; Schieppati 1987; Seynnes et al. 2010). While Walton and colleagues (2003)found an increase in normalized h-reflex following caffeine administration, the vast majority of investigations consistently report no change in H-reflex at rest (Behrens et al. 2015a; Behrens et al. 2015b; Kalmar and Cafarelli 1999) or during weak contractions (Behrens et al. 2015a; Kalmar and Cafarelli 1999), although these authors have suggested that the lack of change in the soleus may be

due to a relatively high activation at rest (Del Balso and Cafarelli 2007; Racinais et al. 2008). Similarly, an unchanged V-wave (Fimland et al. 2010) response prior to and following caffeine-administration has also been reported. It is worth noting that the H-reflex and V-wave are influenced by presynaptic inhibition, reciprocal inhibition, and recurrent inhibition (Crone and Nielsen 1994; Hultborn and Pierrot-Deseilligny 1979; Zehr 2002). Therefore, neural drive could have increased but inhibitory factors could have increased as well. However, the lack of change in EMG amplitude and/or the modulation of evoked spinal reflex responses because of alterations in presynaptic inhibition seem unlikely.

Finally, peripheral mechanisms cannot be excluded as potential explanations for caffeine's effects, although most of the evidence points towards a CNS-related mechanism. In vitro evidence has suggested that caffeine increases intracellular calcium release from the sarcoplasmic reticulum (Allen et al. 2008), potentially due to the interaction of caffeine and the ryanodine receptors of the sarcoplasmic reticulum (Penner et al. 1989). Caffeine could also increase performance due to increased calcium mobilization and increased sensitivity of myofibrils to calcium (Nehlig and Debry 1994), and/or slower reuptake of calcium to the sarcoplasmic reticulum, leading to greater intracellular calcium availability (Allen et al. 2008). Bazzucchi et al. (2011)found significant changes myoelectric and mechanical responses of the biceps brachii, which they attributed to increases in muscle contractility and conduction velocity following caffeine supplementation. As conduction velocity could potentially reflect changes in MU recruitment strategies, changes in contractile properties, and/or excitability of the sarcolemma (Andreassen and Arendt-Nielsen 1987; Solomonow et al. 1989), although the authors hypothesized that sarcolemmic excitability was the most likely to be influenced by caffeine.

As mentioned previously, much of the data suggest that caffeine's effects on neuromuscular function occurs via changes in the CNS, specifically as an increase in voluntary activation following caffeine supplementation (Behrens et al. 2015a; Meyers and Cafarelli 2005; Plaskett and Cafarelli 2001; Tarnopolsky and Cupido 2000). It has been suggested that the central activation ratio (CAR) and interpolated twitch technique (ITT) are not appropriate to distinguish between supraspinal and spinal activation (Behrens et al. 2015b). However, the overall lack of change in the H-reflex, along with the general increase in voluntary activation, points towards modulation at the supraspinal level (Kalmar and Cafarelli 1999). This is supported by later work from Kalmar and coworkers (2006), who found that motor-evoked potentials and cortically evoked twitches of the VL during weak isometric contractions were increased following caffeine ingestion, which confirmed the hypothesis of Phillis et al. (1979). This hypothesis is also potentially supported by the antagonist effects of methylxanthines on adenosine and the adenine nucleotides enhanced spontaneous firing rates of cerebral cortical neurons (Phillis et al. 1979).

Similar to the data describing the influence of caffeine on force production, conflicting evidence on the influence of caffeine on the manifestation of fatigue exists. For example, Fimland et al. (2010) saw no difference between placebo and caffeine in physiological parameters (MVIC, M-Wave, V-wave, EMG amplitude) immediately following and during recovery from a fatiguing protocol. Caffeine also did not improve time to fatigue (Fimland et al. 2010). However, Meyers and Cafarelli (2005) found a significant increase in time to fatigue during submaximal isometric contractions to fatigue, despite no changes in whole muscle activation. They found that the amplitude of evoked twitches and the instantaneous relaxation rate were significantly correlated in both the placebo and

caffeine conditions, indicating that the increase in time to exhaustion may have been due to caffeine's effect on calcium reuptake and subsequent twitch force (Meyers and Cafarelli 2005). In support of this, previous work by Kalmar and Cafarelli (1999) saw a 25.8% increase in time to fatigue during sustained MVC's following caffeine. The authors attributed the increased neural drive with caffeine and thus, slight increases in strength, with the increased time to fatigue (Kalmar and Cafarelli 1999; Plaskett and Cafarelli 2001). Meyers and Cafarelli (2005) demonstrated significant increase in time to exhaustion in "responders" following caffeine, but not in "non-responders", suggesting that the responses to caffeine are probably subject dependent (Kalmar 2005). Interestingly, the dosage in most of these investigations was similar (i.e. 5-8 mg/kg/bw) and thus is probably not due to insufficient caffeine dosage. As mentioned previously, it has been hypothesized that these differences may be due to muscle tested and/or individual differences in the subject's caffeine metabolism.

Recently, the literature has hypothesized a potential role of genetic polymorphisms in caffeine metabolism, providing a genetic link to the "responder/non-responder" classification laid out previously by Meyers and Cafarelli (2005). Since 95% of caffeine is metabolized by a single nucleotide polymorphism (SNP) at intron 1 of the cyctochrome P450 enzyme (CYP1A2), several investigations have examined the effects of the CYP1A2 genotype on caffeine metabolism and subsequent exercise performance (Algrain et al. 2016; Giersch et al. 2018; Guest et al. 2018; Puente et al. 2018; Salinero et al. 2017; Womack et al. 2012). Interestingly, those who have an A allele in position 734 of the CYP1A2 genotype have increased enzyme activity and experience faster caffeine metabolism when compared to those with a C allele in the same position (Han et al. 2001; Sachse et al. 1999). Therefore,

those with a homozygous A allele are considered ‘fast-metabolizers’, with those with the AC/CC alleles are considered ‘slow metabolizers’. This provides a potentially interesting physiological rationale for the divergent responses reported in a large portion of the caffeine literature and on an individual subject level (Pickering and Kiely 2018). Further, there is evidence to suggest that those who possess the slow genotypes may be at a greater risk for cardiovascular related events following caffeine administration (Cornelis et al. 2006; Sachse et al. 2003; Sachse et al. 1999), providing a potentially useful clinical utility to CYP1A2 genotyping.

1.2 Study Purpose

The purpose of this study is to examine whether genetic variation in CYP1A2 (-163A>C, rs762551) influences the effects of acute caffeine supplementation on neuromuscular function of the lower body at rest and in response to a fatiguing work bout.

1.3 Research Questions & Hypotheses

1.3.1 Does acute caffeine supplementation improve neuromuscular function?

$H_{O1.3.1}$: There will be no difference in neuromuscular function following either caffeine or placebo.

$H_{A1.3.1}$: Acute caffeine supplementation will significantly improve neuromuscular function when compared to placebo.

1.3.2 Does CYP1A2 genotype influence the changes in neuromuscular function following acute caffeine supplementation?

$H_{O1.3.2}$: There will be no difference in neuromuscular function following acute caffeine supplementation between CYP1A2 genotypes.

$H_{A1.3.2}$: Those with the homozygous A (AA) CYP1A2 genotype will exhibit more pronounced improvements in neuromuscular function than those with a C CYP1A2 genotype following acute caffeine administration.

1.3.3 Does acute caffeine supplementation influence the fatigability of the knee extensors?

$H_{O1.3.3}$: There will be no differences in the fatigability of knee extensors between the caffeine and placebo conditions.

$H_{A1.3.3}$: Caffeine will delay the onset of fatigue in the knee extensors, when compared to placebo.

1.3.4 Does CYP1A2 genotype influence the changes in fatigability following acute caffeine supplementation?

$H_{O1.3.4}$: There will be no difference in fatigability following acute caffeine supplementation between CYP1A2 genotypes.

$H_{A1.3.4}$: Those with the homozygous A (AA) CYP1A2 genotype will exhibit a more pronounced delay in fatigue of the knee extensors than those with a C CYP1A2 genotype following acute caffeine administration.

1.4 Significance of Study

The present investigation has the potential to elucidate the divergent neuromuscular responses to caffeine widely reported in the literature to date. Additionally, the findings of

this investigation have the potential to help create more accurate and robust caffeine recommendations for improved exercise performance.

1.5 Delimitations

1. Approximately 42 young, healthy males participated in this investigation.

2. Only participants between the ages of 18 and 35 years of age were be recruited for this investigation.

3. All participants were healthy and free from any neuromuscular or musculoskeletal conditions at the time of participation.

4. The participants performed both voluntary and involuntary (i.e. evoked) contractions.

5. Data was only be collected from the musculature of the right leg.

6. All data was collected in the seated position.

7. All muscular contractions collected in this investigation were isometric.

8. Only active males were recruited to participate in this investigation. Thus, activity levels and exercise status may vary within the sample.

1.6 Limitations

1. The recruitment process will not be truly randomized, as participants were recruited by word of mouth.

2. The data presented from this investigation are from a rather homogenous sample, thus, the applicability to other populations may be limited.

3. As the study design involves a number of maximal contractions and a submaximal fatigue protocol, differences in motivation levels between subjects may influence results.

1.7 Assumptions

1. All participants abstained from caffeine during the duration of the investigation.

2. All participants provided accurate information on their health and exercise status.

3. All equipment used in the investigation was calibrated and functioning.

4. All data testing procedures, data entry, and data analysis were performed correctly and without errors.

CHAPTER II

REVIEW OF LITERATURE

The following literature review will include previous research studies that are relevant to the purpose of this study. Each study will be summarized and the results of the study will be provided along with the interpretations of the authors. The aim of this review of the literature is to focus on the neuromuscular responses to caffeine and the influence of the CYP1A2 genotype, and the potential changes in the variables assessed in the methods section.

2.1 CYP1A2 Genotype & Caffeine

Algrain et al. (2016)

The purpose of this study was to determine if a polymorphism in the cytochrome P450 CYP1A2 gene impacts performance following caffeine supplementation in recreational cyclists. Following either caffeine or placebo chewing gum, serum blood samples were taken at baseline, during the warm-up and immediately before and after the trial. The authors found no improvement in performance between conditions, as well as no effect for genotype, defined as AA allele carriers or C carriers. CYP1A2 genotype did not influence the ergogenic effects of caffeine, nor the circulating caffeine concentrations of caffeine.

Cornelis et al. (2006)

The purpose of this investigation was to determine whether the CYP1A2 genotype modifies the association between coffee consumption and the risk of acute nonfatal myocardial infarction. The authors found that fifty-one percent of those that had a nonfatal myocardial infarction and fifty-four percent of controls (i.e. no myocardial infarction) were carriers of the slow allele (*1F). The authors reported that there was an increased risk of myocardial infarction with increased coffee consumption, but only in those who were carriers of the *1F allele.

Giersch et al. (2018)

The purpose of this investigation was to determine whether CYP1A2 polymorphism affects caffeine metabolism and subsequent exercise performance between the difference genotypes. Sixty minutes following the ingestion of either 6 mg/kg of bodyweight or placebo (all-purpose flour), subjects completed a 3 km cycling time trial. Subjects were then divided into slow (AC heterozygous or CC homozygous) or fast (AA homozygous) metabolizers. Slow metabolizers exhibited significantly higher serum caffeine 1-hour post-ingestion. However, no significant differences in measured caffeine metabolite, metabolite: caffeine ratio or paraxanthine:caffeine ratio was seen. Caffeine resulted in a significant decrease in time trial performance (7.1 ± 13.9 s.), with no significant differences between groups. Thus, genotype variation appears to effect serum caffeine metabolism but not exercise performance.

de Souza Gonçalves et al. (2017)

The purpose of this study was to investigate of habitual caffeine intake on aerobic exercise performance responses to acute caffeine supplementation. Participants were allocated into 3 groups based on habitual caffeine intake: low (58 ± 29 $mg \cdot d^{-1}$), moderate (143 ± 25 $mg \cdot d^{-1}$), and high consumers (351 ± 139 $mg \cdot d^{-1}$). Participants then completed 3 cycling time trials following the ingestion: caffeine (6 mg/kg of bodyweight), placebo, and no supplement. Results showed that caffeine significantly improved time trial performance, when compared to both placebo and no supplement conditions. Furthermore, the results showed no effect of habitual caffeine intake on exercise performance. The authors suggested that benefits of caffeine on time trial performance are not influenced by habitual caffeine intake.

Guest et al. (2018)

The purpose of this study was to determine whether CYP1A2 gene variation modifies the ergogenic effects of caffeine in a 10-km cycling time trial. Subjects completed 3 10-km time trials under the following conditions: 0, 2, or 4 mg/kg of bodyweight of caffeine. Results showed a 3% decrease in performance following 4 mg/kg of caffeine. However, those with AA homozygous (i.e. fast metabolizers) genotype exhibited a 4.8% and 6.8% decrease in performance with 2 and 4 mg/kg of caffeine, respectively. Those with a CC homozygous (i.e. slow metabolizers) genotype exhibited a 13.7% increase when compared to placebo. No effects were seen in those with AC heterozygous genotypes. The authors suggested that CYP1A2 genotype plays a significant role in potential effects from caffeine supplementation.

Pataky et al. (2016)

The purpose of this investigation was to examine the efficacy of a caffeine mouth rinse on cycling performance, as well as to determine whether its efficacy was influenced by CYP1A2 genotype. The results of this study indicate that only those with an AC heterozygous genotype of the CYP1A2 gene received a significant increase in performance following 6 mg/kg/bw of caffeine ingestion, although both AA and AC genotypes significantly improved performance following caffeine ingestion plus a caffeine mouth rinse, with no significant differences between AA and AC. Interestingly, the authors reported that both caffeine and caffeine plus caffeine mouth rinse elicited greater improvements before 10:00 am, when compared with after 10:00 am. The authors concluded that both genotype and time of day can influence the efficacy of caffeine to improve time-trial performance.

Puente et al. (2018)

The purpose of this investigation was to examine the influence of the CYP1A2 gene polymorphism on the ergogenic effects of caffeine in elite basketball players. Sixty minutes following the ingestion of either caffeine (3 mg/kg of bodyweight) or placebo, subjects completed a jumping and agility tests, as well as a 20-minute simulated game. Subjects with the AA homozygous genotype improved jump height by 2.9 ± 3.6% following caffeine ingestion, while the CC homozygous group did not improve jump performance. Caffeine did not improve agility in either group, but improved the number of impacts during the simulated game in both groups. Interestingly, the AA group experienced self-reported insomnia following caffeine, while the CC group reported no

side effects. The authors thus reported a moderate beneficial effect from caffeine in those with the AA genotype.

Sachse et al. (1999)

The aim of this investigation was to determine the amount of variability of CYP1A2 activity is explained by a gene polymorphism in intron 1. Following the ingestion of 100 mg of caffeine, a population of 185 healthy and 51 smokers were determined as 46% homozygous for variant A, 44% were heterozygous, and 10% were homozygous for variant C. Significant differences between genotypes in the 5-hour plasma 17X/caffeine ratios were only found in those who were smoker. The authors found that, while no significant differences in CYP1A2 metabolic activity between genotypes were found between non-smokers, smokers exhibiting the homozygous A genotype exhibited a 1.6-fold higher metabolic activity than heterozygous or homozygous C genotypes. The authors concluded that the A/A genotype may be a direct cause of increased CYP1A2 genotype or may be genetically linked to other polymorphisms conferring high inducibility following caffeine administration.

Sachse et al. (2003)

The purpose of this investigation was to examine the influence of CYP1A2 for allele frequencies, linkage disequilibrium and caffeine metabolism in colorectal patients and healthy controls. In the most germane finding of this investigation, the authors found lower caffeine metabolic ratios were detected in colorectal patients than controls, but only in those who were smokers. The authors also found no association between CYP1A2 genotype and caffeine phenotype, based on the caffeine metabolite ratio.

Salinero et al. (2017)

The aim of this investigation was to determine the influence of the CYP1A2 genotype on exercise performance following a moderate dose of caffeine. Participants ingested either 3 mg/kg of caffeine or a placebo, following which they completed a Wingate test. Visual attention and side effects were also measured. Subjects were grouped based on CYP1A2 genotype. Acute caffeine ingestion increased peak power output, with no significant differences between groups. No significant differences in reaction times were seen between caffeine and placebo conditions. Interestingly, 31% of subjects exhibiting the CC allele exhibited nervousness following caffeine ingestion, while none of the subjects in the AA experienced an increase in nervousness. The authors concluded that although caffeine ingestion improved Wingate performance, the effects do not appear to be dependent on CYP1A2 genotype.

Soares et al. (2018)

The purpose of this study was to examine if the influence of CYP1A2 genotype on the blood pressure response to caffeine ingestion was affected by physical activity status and habitual caffeine consumption. Subjects were classified as fast metabolizer (AA genotype) or slow metabolizer (AC) based on their CYP1A2 genotype. Subjects were also stratified based on their physical activity level (i.e. sedentary or physically active) and habitual caffeine consumption (i.e. non-habitual or habitual). Results showed that those classified as slow-metabolizers had increased basal diastolic blood pressure and post-caffeine systolic blood pressure compared to fast-metabolizers. Additionally, physical activity only modulated the acute blood pressure responses to caffeine in slow-

metabolizers. Further, results showed a significant increase in diastolic blood pressure of heavy caffeine users only in those classified as slow metabolizers. These results lead the authors to conclude that basal and post-caffeine blood pressure responses are modified by physical activity and habitual caffeine usage.

Womack et al. (2012)

The purpose of this investigation was the determine the influence of a (C/A) single nucleotide polymorphism at intron 1 of the cytochrome P450 (CYP1A2) genotype on 40-kilometer time trials on a cycle ergometer in trained male cyclists following acute caffeine (6 mg/kg/bw) supplementation. Caffeine resulted in a significantly greater reduction in time trial performance in the AA homozygous group, when compared to C carriers. The authors concluded that caffeine may have a greater ergogenic effect in AA allele carriers, when compared to those with a C allele.

2.2 Neuromuscular Responses to Caffeine

Bazzucchi et al. (2011)

The purpose of this investigation was to examine the effects of caffeine on neuromuscular function during elbow flexion exercise. Fourteen male subjects volunteered to participate in this randomized, repeated measures, double-blind (6 mg/kg/bw of caffeine or placebo) investigation. Maximal voluntary strength, evoked maximal twitch and maximal isokinetic contractions of the elbow flexor musculature was measured both before and after each condition. The results of this investigation found an enhancement in the torque-angular velocity curve, along with an 8.7% increase in conduction velocity, following caffeine supplementation. Additionally, the authors found

a significant increase in peak torque and area under of the curve of a maximal twitch following caffeine, when compared to placebo. The authors concluded that caffeine improves performance during maximal dynamic contractions of the elbow flexor musculature. The authors also hypothesized that caffeine has an effect on motor unit recruitment, as evidenced by the increase in conduction velocity seen post caffeine supplementation.

Behrens et al. (2015a)

The purpose of this study was to examine the effects of caffeine (8 mg/kg/bw) on maximal voluntary strength and voluntary activation of the quadriceps musculature during isometric, concentric and eccentric muscle actions. Further, surface electromyography, h-reflex and v-wave were measured. Fourteen subjects volunteered to participate in this randomized, controlled, counterbalanced, double-blind cross over design in which neuromuscular function was assessed prior to and 1-hour following either caffeine or placebo ingestion. The authors found a significant increase in maximal voluntary strength in all contraction types, along with an increased voluntary activation, following caffeine supplementation. The authors also found an increase in explosive voluntary strength and voluntary activation at the onset of contraction following caffeine administration. The authors concluded that while caffeine does not appear to alter spinal reflexes, the increases in maximal voluntary strength are most likely due to increases in voluntary activation of the musculature.

Behrens et al. (2015b)

The authors aimed to examine the effects of acute caffeine supplementation on neuromuscular function of the plantar flexors. Thirteen subjects volunteered to participate in this randomized, controlled, counterbalanced, double-blind investigation, in which rate of torque development, maximal voluntary isometric torque, h-reflex, v-wave, and neural drive were measured prior to and one-hour post caffeine supplementation. No change in evoked potentials or maximal voluntary torque were seen between conditions. However, the authors reported an enhanced neural drive to the plantar flexors, along with an increase in rate of torque development in the 0-100 ms and 100-200 ms windows following caffeine administration. The authors concluded that only caffeine supplementation increased explosive voluntary strength through enhanced neural activation.

Fimland et al. (2010)

The purpose of this investigation was to examine the effect of caffeine on recovery following intermittent, fatiguing isometric contractions of the plantar flexors. Electromyography, maximal voluntary strength, and evoked v-waves of the gastrocnemius and soleus were measured in 13 males prior to, immediately following, as well as 10 and 20 minutes following fatigue after the ingestion of either caffeine (6 mg/kg/bw) or placebo. Following both caffeine and placebo conditions, there was a substantial reduction in strength with a gradual return towards baseline in the latter time points, with no significant differences between conditions in any measure at any time

point. The authors concluded that caffeine does not offer enhanced recovery following intermittent, fatiguing isometric contractions of the plantar flexors.

Greer et al. (2006)

The purpose of this investigation was examine the effects of caffeine on Wingate performance and neuromuscular parameters following either placebo or caffeine (5 mg/kg/bw). Eighteen young males volunteered to participate in this investigation. Peak power, mean power, and percent decline, as well as surface electromyographic parameters of the vastus lateralis and gastrocnemius were measured. The authors found no significant differences in peak power, mean power, and percent decline during the Wingate test in either condition. Furthermore, the authors found a significant decrease in mean and median power frequency of both muscles in all trials, with no significant differences seen between conditions. The authors concluded that caffeine supplementation does not improve neuromuscular drive, frequency of decline in electromyography, and power output variables, when compared to placebo.

Kalmar and Cafarelli (1999)

The purpose of this investigation was to examine the effects of caffeine on neuromuscular function of the plantar flexors. Eleven males completed 3 conditions: control, placebo, or caffeine (6 mg/kg/bw), in which surface electromyography was collected during h-reflex of the tibial nerve, voluntary activation through the interpolated twitch technique, a maximal voluntary strength test, 6 submaximal isometric contractions, and a submaximal isometric contraction to fatigue at 50% of the subject's max. Additionally, intramuscular recordings of motor unit behavior were collected during

the submaximal contractions. The authors found an increase in voluntary activation during maximal voluntary contractions, with no change in h-reflex, force-electromyographic relationship, or motor unit behavior. Time to fatigue was significantly increased during the caffeine trial, with no significant change during either the control or placebo conditions. Interestingly, the authors reported that the increased time to fatigue was accompanied by an attenuated decline in twitch amplitude during the caffeine condition. The authors concluded that the increase in maximal strength was most likely due to supraspinal factors, while the lack of decline in twitch amplitude following fatigue was most likely due to a peripheral mechanism.

Kalmar and Cafarelli (2004)

The purpose of this study was to examine if the fatigue-related decline in surface electromyography and motor evoked potentials could be attributed to central mechanisms and if so, if this could be offset by caffeine supplementation. Seven volunteers underwent two experimental conditions (6 mg/kg/bw of caffeine or placebo), in which central excitability was measured via transcranial magnetic stimulation and surface electromyography, voluntary activation was measured via twitch interpolation before, during, and after fatigue, and a maximal m-wave was elicited to monitor peripheral transmission. The fatiguing protocol of the first dorsal interosseous consisted of 4 sets of 10 finger abductions at 75% of the subject's max, with 2 seconds of rest between contractions and 12 seconds between sets. The authors reported an increase post-activation potentiation of the motor evoked potentials following caffeine administration, with a decline in motor evoked potential, maximal electromyography, and peripheral transmission with fatigue in both conditions. The authors stressed that the estimates of

central fatigue were greatly reduced when normalized to maximal m-wave, thus, when estimating central fatigue, peripheral transmission must be accounted for. The authors concluded that caffeine may induce increases in post-activation potentiation and could provide utility in the measure of central fatigue.

Kalmar and Cafarelli (2006)

The purpose of this investigation was to examine whether declines in central excitability contribute to the central fatigue post exercise and if this potential decrease in central excitability could be counteracted with caffeine supplementation. Eight men completed two experimental sessions in which knee extensor torque, voluntary activation, peripheral transmission, contractile properties, and central excitability were measured prior to and after caffeine (6 mg/kg/bw) or placebo following an initial hour of rest. Finally, a fatigue protocol consisting of sets of 10 4-s knee extension contractions, in which the first and last contraction were maximal and the middle 8 were 50% of max, was completed until a 35% drop in maximal voluntary torque was seen. The authors found a significant increase in central excitability, as exhibited by an increased pre-fatigue motor evoked potential and cortically evoked twitch, following caffeine administration. The authors also found that caffeine potentiated the motor evoked potential early in the fatigue protocol and offset the sharp decline seen in the placebo condition. However, this was not associated with an increased voluntary activation during fatigue or recovery. The authors thus concluded that caffeine improves central excitability and that changes in voluntary activation are not mediated by central excitability.

Kalmar et al. (2006)

The purpose of this investigation was to examine the potential role of reduced spinal excitability in central activation failure and if this reduction could be mediated by acute caffeine supplementation. Ten male subjects volunteered to participate in two experimental sessions (6 mg/kg/bw of caffeine or placebo), in which contractile and electrical properties of the plantar flexors. Spinal excitability was measured as the ratio of h-reflex to maxima m-wave, while voluntary activation maximal electromyography and interpolated twitch. Both conditions saw a significant reduction in maximal voluntary strength and voluntary activation. However, caffeine offset the reduction in spinal excitability observed during the placebo condition. Interestingly, the decline in spinal excitability was correlated with a decline in maximal electromyography amplitude, but not with decline in maximal voluntary strength or voluntary activation. The authors concluded that the decline in spinal excitability did not limited maximal activation of the plantar flexors following a fatiguing protocol.

Lopes et al. (1983)

The purpose of this investigation was to examine the effects of caffeine on voluntary and electrically stimulated contractions of the adductor pollicis muscle. Five healthy adults completed a series of voluntary and electrically evoked contractions prior to and immediately after caffeine (500 mg) and placebo. The authors reported no difference in maximal voluntary strength prior to either supplement. However, in fresh muscle and after fatigue, the authors reported higher muscle tensions at lower frequency stimulation following caffeine supplementation, resulting in a leftward shift of the

frequency-force curve. The authors concluded that 500 mg of caffeine alters muscle contractile properties in both a fresh and fatigued state.

Meyers and Cafarelli (2005)

The purpose of this investigation was to examine whether the previously reported increase in time to task-failure following caffeine supplementation was a function of increased firing rates of active motor units. Ten male volunteers completed a fatigue protocol consisting intermittent quadriceps contractions at 50% of maximal strength 1-hour after the ingestion of either caffeine (6 mg/kg/bw) or placebo, in a randomized, double-blind, repeated-measures design. The authors found a significant increase in time to fatigue in the caffeine condition, when compared to placebo. However, this increase could not be explained by increase motor unit firing rates or other neuromuscular parameters. Interestingly, in the caffeine condition, the amplitude of evoked twitches and their maximal instantaneous firing rate of relaxation did not decline to the same degree as the placebo condition. The correlation with these variables and the increase in time to task-failure led the authors to suggest that caffeine effects on calcium reuptake and twitch force may be the primary mechanisms for increased time to fatigue following caffeine administration.

Mora-Rodríguez et al. (2012)

The purpose of this study was to examine the efficacy of caffeine to counteract the decline in neuromuscular performance during the morning hours associated with the circadian rhythm. Twelve resistance-trained males volunteered for this double-blind, repeated measures design in which a neuromuscular function was assessed under 3

conditions: 1) morning with caffeine (3 mg/kg/bw); 2) morning with placebo; 3) afternoon with placebo. Bar velocity during 75% 1-repetition maximum bench press and back squat, as well as maximal voluntary contraction strength and electrically evoked contractions of the right quadriceps were measured prior to and post-supplement consumption in each condition. Dynamic strength and power output were significantly enhanced in the afternoon when compared to the morning placebo condition. However, during the morning caffeine condition, participants exhibited significantly higher muscular strength and power output, with the exception of bench press velocity, then the morning placebo condition. Additionally, evoked measures were significantly higher in the morning caffeine condition, when compared to the morning placebo. The authors concluded that morning caffeine administration can bring neuromuscular performance to afternoon levels. Additionally, the authors suggested that due to the increase in evoked contractions, the performance increases most likely occur at the muscle level.

Morse et al. (2016)

The purpose of this investigation was to examine whether a low-dose of caffeine would delay the onset of the electromyographic fatigue threshold in the superficial quadriceps musculature. Ten physically actives males completed 1-hour of single-leg cycling in which electromyographic signals were recorded from the vastus medialis, vastus lateralis, and rectus femoris following either caffeine (200 mg) or placebo in a randomized, double-blind, repeated measures design. The authors found a significant increase in maximal power output and electromyographic fatigue threshold following caffeine, when compared to placebo. The authors concluded that acute low-dose caffeine supplementation delays neuromuscular fatigue during single-leg cycling.

Pereira et al. (2010)

The purpose of this investigation was to examine the effects of acute caffeine supplementation on anaerobic performance and fatigability. Fourteen (7 males and 7 females) recreationally active volunteers completed a Wingate test following either caffeine (6 mg/kg/bw) or placebo in this randomized, double-blind, repeated measures study. Wingate power output variables and median power frequency of the vastus medialis, vastus lateralis, and rectus femoris were recorded during the testing. The results of the investigation showed no improvement in relative peak power, relative mean power, fatigue index, or peak power instant, nor in median power frequency of any quadriceps muscle. The authors concluded that caffeine offered no benefit to the Wingate performance parameters measured in the present investigation.

Pethick et al. (2018)

The purpose of this investigation was to examine the effects of acute caffeine consumption of muscle torque complexity of the knee extensors. Sixty minutes after caffeine consumption (6 mg/kg/bw) or placebo, 11 healthy participants completed intermittent (6 s. work/4 s. rest) isometric contractions at 50% of their maximal voluntary torque in this randomized, double-blind, repeated measures design. Torque complexity and fractal scaling of the torque were measured throughout the fatigue protocol and global, central, and peripheral fatigue (through peripheral nerve stimulation) were measured prior to and immediately post-fatigue. Caffeine significantly increased time to fatigue and complexity significantly decreased as global, peripheral, and central fatigue developed in both conditions. Interestingly, the rate of decrease in complexity, as well

and the rate of development of central and global fatigue were significantly slower following caffeine supplementation. However, there were no differences in the rate of peripheral fatigue between conditions. The authors concluded that caffeine delayed the accumulation of fatigue and loss of torque complexity leading to an increase in time to task-failure, which are most likely due to centrally-mediated mechanisms.

Pires et al. (2018)

The purpose of this investigation was to examine the effect of caffeine and caffeine-perceived placebo on motor performance during a maximal incremental cycling test. Nine participants completed three incremental cycling tests (control, placebo, or caffeine) in a randomized, double-blind, repeated measures design 60 minutes following the ingestion of the substance. Prefrontal cortex oxygenation, motor cortex activation and vastus lateralis and rectus femoris muscle activity were measured throughout each test. Both placebo and caffeine significantly increased rectus femoris muscle activity at maximal effort and enhanced peak power output and time to exhaustion, when compared to control. At 80% and 100% duration of the test, both placebo and caffeine exhibited increase prefrontal cortex deoxygenation, but not motor cortex activation, when compared to control. The authors concluded that both caffeine and a caffeine-perceived placebo can improve motor performance, despite the lack of change in motor cortex activation and a decrease in prefrontal cortex deoxygenation.

Plaskett and Cafarelli (2001)

The purpose of study was to examine the effects of caffeine on neuromuscular parameters during submaximal isometric contractions. In a randomized, double-blind,

repeated measures design, 15 subjects completed repeated 50% maximal effort isometric contractions of the knee extensors to failure 1-hour following the ingestion of caffeine (6 mg/kg/bw) or placebo. Time to task-failure was significantly increased after caffeine when compared to placebo. Interestingly, changes in contractile properties of the quadriceps, motor unit activation and m-wave amplitude could not account from the changes seen with caffeine administration. The authors reported a reduced "force sensation" during the first 10-20 second of the contraction in the caffeine condition, when compared to placebo. The authors thus suggested that caffeine exerts its effects due to neural factors and stated the increase in time to task-failure may have been caused by "a willingness to maintain near-maximal activation longer because of alterations in muscle sensory processes."

Walton et al. (2002)

The purpose of this study was to examine the effect of caffeine on the self-sustained firing of motor units of the tibialis anterior. Seven caffeine naïve male ingested either caffeine (6 mg/kg/bw) or placebo in this randomized, double-blind, repeated measures study. Maximal voluntary contractions, surface electromyography and intramuscular motor unit recordings were recorded prior to and 1-hour following the ingestion of each supplement. The authors found a significant increase in the occurrence of self-sustained firing of motor units in the tibialis anterior following caffeine ingestion.

Walton et al. (2003)

The purpose of this investigation was to examine whether acute caffeine ingestion would cause an increase in spinal excitability and h-reflex amplitude. Seven subjects

completed 2 experimental visits in this double-blind, randomized, repeated measures design. An h-reflex recruitment curve was recorded through tibial nerve stimulation immediately prior to and 1-hour post-caffeine (6 mg/kg/bw) or placebo ingestion. The authors found a significant increase in the slope of the h-reflex (normalized to m-wave) following caffeine administration. The author thus concluded that caffeine can effectively increase spinal excitability following a dosage of 6 nmg/kg/bw.

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CHAPTER III

METHODOLGOY

3.1 Participants

Forty-two young, healthy males completed the entire study protocol and were genotyped for CYP1A2 enzyme. Subjects were then classified as AA (FAST: n = 26) or AC/CC (SLOW; n =16). Table 1 contains the data for descriptive statistics (presented as mean ± (SD), along with the results of the independent samples t-tests. All participants voluntarily participated in each testing session, which took place in the Applied Neuromuscular Physiology Laboratory at Oklahoma State University. This study was approved by the Oklahoma State University Institutional review board for human participant research (Approval #: ED-17-88) prior to any data collection. Prior to any testing, all participants completed an informed consent, pre-exercise health questionnaire, caffeine consumption questionnaire, and a brief exercise history survey to quantify their habitual caffeine consumption and physical activity. Participants were included in the study if they met the inclusion criteria and were free from any musculoskeletal dysfunctions or circulatory/edema pathologies involving the hip, knee, or ankle joints. Participants also reported being free from any neurological disorders.

	FAST	SLOW	Sig.
Age	22 ± 3 yrs	24 ± 4 yrs	p = 0.204
Height	176.0 ± 6.8 cm	179.6 ± 5.0 cm	p = 0.075
Weight	89.0 ± 15.0 kg	87.0 ± 10.6 kg	p = 0.653
Avg. Caffeine Intake	290.6 ± 295.1 mg/day	324.7 ± 276.3 mg/day	P = 0.715

Table 1. Mean ± SD and p-values for descriptive variables between CYP1A2 genotype groups.

3.2 Experimental Design

This study consisted of 3 separate visits to the laboratory, a familiarization session and 2 experimental sessions. The familiarization session lasted approximately 1 hour, while each experimental session lasted approximately 3 hours. Each experimental session was separated by 6 ± 1 days and took place at approximately the same time of day (±1 hour). Additionally, every effort was made to begin experimental sessions in the morning, as caffeine has been shown to have potentially dampened ergogenic effects in the afternoon (Pataky et al. 2016). Participants were instructed to refrain from any structured lower body exercise 48 hours prior to each testing session and abstain from caffeine consumption throughout the duration of their enrollment in the study (i.e. 2 weeks)., Thus participants were withdrawn from caffeine for a minimum of 5 days prior to the first experimental visit. On the first visit, following the explanation of all study procedures, the signing of the informed consent and the completion of the required paperwork, the participant's height and weight were measured. Each participant was then seated in the dynamometer and completed several practice contractions required during future testing sessions, which upon completion, completed the familiarization session. For each experimental condition, participants were instructed to arrive at the laboratory in a fasted state. Upon arrival, participants were instructed to lie in a supine position for 5 minutes. Following this 5-minute period, body composition was measured via

Bioelectrical Impedance Spectroscopy (BIS; ImpediMed, Inc., Carlsbad, CA, USA) in order to quantify each individual's fat mass (FM) and fat free mass (FFM). Following the assessment of body composition, ultrasound (US) images of the right thigh were performed to quantify the size, and quality of each participant's *rectus femoris* (RF) and *vastus lateralis* (VL). All body composition measures and US images were obtained while the participant remained in the supine position. Participants were then seated in a dynamometer in for all neuromuscular function assessments. Specifically, h-reflex and m-wave of the soleus and m-wave of the quadriceps musculature were measured via peripheral nerve stimulation. Evoked twitch properties of the quadriceps musculature were assessed in incremental steps, culminating in the measurement of the compound muscle action potential (CMAP). These previously mentioned evoked involuntary measures occurred prior to any warm-up (Folland et al. 2008). Following a brief warm-up, subjects then performed maximal voluntary isometric contraction (MVIC) testing, in which the subject's maximal voluntary torque (MVT) was recorded. Additionally, voluntary activation (%VA), resting doublet, and potentiated doublet twitch properties were assessed via the interpolated twitch technique (ITT). Finally, subjects completed 2 MVIC ramp contractions at 30%, 50%, and 70% of their MVIC force in order to record motor unit (MU) behavior. Approximately 2 minutes of rest was given between each ramp contraction. Following the completion of neuromuscular function testing, subject's consumed either 6 mg/kg/bw of caffeine anhydrous (CAF) or flour placebo (PLA), which was provided in gelatin capsules. Participants then remained seated in the dynamometer for one-hour of rest following the consumption of the supplement. One-hour post consumption, neuromuscular function was again completed in the manner described

above. Following the completion of post-consumption testing and a 5-minute washout period, subjects completed repeated 50% MVIC ramp contractions to fatigue, followed by an additional ITT to quantify fatigue. MU behavior, muscle activation and torque variables were analyzed during the first, middle and last repetitions of the fatigue protocol. Upon the completion of the fatigue protocol, the experimental visit was completed. Torque and electromyographic (EMG) signals were recorded continuously during all quadriceps measurements. Each experimental visit (i.e. CAF or PLA) was identical in procedures and the order was randomized for each participant. Below is a summary of each visit in Table 2:

Time Point	**Measures Completed**
Pre-Supplementation	1. Body Composition 2. Ultrasound Measures 3. Evoked Measures of Soleus 4. Evoked Measures of Quadriceps 5. Voluntary Strength Measures 6. Voluntary Activation 7. Motor Unit Recordings
Administration of Caffeine (6 mg/kg/bw) or Placebo (6 mg/kg/bw of flour)	
1-hour Post-Supplementation	1. Evoked Measures of Soleus 2. Evoked Measures of Quadriceps 3. Voluntary Strength Measures 4. Voluntary Activation 5. Motor Unit Recordings
5-minutes of rest	
Fatigue Protocol (Repeated 50% MVIC contractions to fatigue)	
Post-Fatigue	1. Voluntary Activation

Table 2. Overview of study procedures.

3.3 Instrumentation and Procedures

3.3.1 Ultrasonography

Cross-sectional area (mCSA) and echo intensity (EI) of the right VL and RF musculature were obtained using a portable brightness mode (B-Mode) diagnosticultrasound imaging device (GE Logic S8, Milwaukee, WI, USA) with a linear array probe (model ML6-15-D, 4-15 MHz, 50-mm field view) via transverse images. All US images were taken with the participants laying on their left side on an adjustable padded plinth with their legs completely relaxed and knees bent at approximately 10°. All US images were taken at 50% of the distance between the right greater trochanter and the lateral femoral epicondyle. During each panoramic US scan, the probe was placed perpendicular to the skin and advanced laterally along the skin above the musculature in a slow, consistent manner, with great care taken to ensure minimal and consistent pressure. A generous amount of water-soluble transmission gel was applied to the skin to enhance acoustic coupling (Wilhelm et al. 2014). In order to maintain consistency between subjects and visits, the gain and frequency settings were recorded and held constant, at 50 dB and 12Hz, respectively. Depth was also held constant between each participant and visit to keep the pixels per cm standardized. Panoramic US images were captured until three uniform scans with acceptable image quality were collected and recorded for future analyses (Jenkins et al. 2015b).

A single experienced investigator performed all US scans in order to minimize the inter-rater variability. All recorded US images were analzyed using Image-J software (National Institutes of Health, USA, Version 1.50i) and were performed by a single experienced investigator. Each image was individually calibrated from pixels to cm using

the straight-line function available in Image-J. VL_{mCSA}, VL_{EI}, RF_{mCSA}, and RF_{EI} of the three images were analyzed by defining a region of interest by including as much muscle as possible, without including any bone or fascia, using the polygon function available in the Image-J software. EI for each muscle was quantified using computer-aided gray scale analysis using the standard histogram function and was recorded in arbitrary units (au) with values ranging from 0 (black) to 255 (white). All VL_{mCSA}, VL_{EI}, RF_{mCSA}, and RF_{EI} were recorded, stored, and used in the final analysis.

3.3.2 Evoked Measures

Evoked measures of the quadriceps and soleus musculature were assessed in the present investigation. During all testing, participants were seated with straps securing the trunk and hips on a calibrated isokinetic dynamometer (Biodex System 4; Biodex Medical Systems, Inc. Shirley, NY, USA) with the axis of rotation of the dynamometer head aligned with the lateral epicondyle of the subject's right femur. For all quadriceps muscle actions, the lower right leg was secured to the dynamometer lever arm approximately 3 cm above the lateral malleolus. Additionally, all participant's hip and knee angle was held constant at approximately 90° and 120°, respectively, which was held constant throughout all testing.

Maximal M-wave of the soleus (SOL_M) musculature was assessed via transcutaneous electrical stimulation of the tibial nerve. Briefly, the stimuli were delivered via a cathode-anode arrangement using high voltage (maximal voltage = 400 V) stimulus from a constant-current electrical stimulation cart (Cadwell Sierra Summit, Cadwell Industries, Inc., Kennewick, WA, USA). The anode and cathode of the probe (Cadwell Stimtroller *Plus*, Cadwell Industries, Inc., Kennewick, WA, USA) were placed

on either side of the tibial nerve, with a recording electrode placed on the muscle belly of the soleus, a reference electrode on the Achilles tendon, and a ground electrode on the medial malleolus. The optimal stimulation probe position was determined by delivering single low-voltage exploratory stimuli (20-30 mV) with the cathode probe. Final probe location was selected based on visual inspection of the CMAP amplitudes. Once the optimal probe position was obtained, the spot was marked with permanent marker and used for both SOL_M and soleus h-reflex (SOL_H) assessments. This mark was maintained throughout the duration of the investigation to obtain consistent probe placement across visits. For all m-wave assessments, incremental increases in intensity were made until a plateau in the CMAP was found. Following the successful measurement of the CMAP, a baseline measure where no m-wave was present was located and recorded. Once this was recorded, a step-wise increase (i.e. 5 mV) from baseline back to CMAP was completed in order to obtain a recruitment curve for each subject. The muscle activation and the torque produced by each incremental evoked twitch were recorded. Following the CMAP plateau, two supramaximal (i.e. 120% of maximal CMAP stimulation) stimulations were delivered, if possible. Throughout the duration of the protocol, 10 seconds were given between each stimulation to ensure complete neuromuscular recovery. The mean m-wave peak-to-peak (M_{MAX}) amplitude of the two stimulations was defined as the maximal m-wave or SOL_M. SOL_M was used for the normalization of the voluntary EMG variables and SOL_H.

Following the completion of the SOL_M assessment, SOL_H was assessed in a similar manner. Briefly, the subject was seated and relaxed in the dynamometer, with their head resting on the cushion and eyes closed to further facilitate the reflex. The

polarity of the probe was reversed and stimulation width increased to 1k. Incremental increases in stimulation were applied to the tibial nerve until a maximal SOL_H was achieved, as visually observed in real time. An additional SOL_M was then measured and recorded. M-wave of the RF (RF_M) and VL (VL_M) were then assessed via transcutaneous electrical stimulation of the femoral nerve, with the procedures remaining consistent from the SOL_M. The recording electrode was placed on the belly of the VL, with the reference electrode on the distal quadriceps tendon and the ground electrode remaining on the medial malleolus. However, RF_M, VL_M, and EMG variables were recorded through separate bipolar electrodes (discussed further in section 3.3.4). Torque and EMG were recorded continuously throughout the protocol and further analyzed offline The cathode of the probe was placed in the femoral triangle, with the anode (40 x50mm, Technomed Medical Accessories, Amerikalaan 71, Netherlands) on the greater trochanter of the right femur. The placement of the cathode was marked with permanent marker in order to ensure consistent placement between visits

Evoked twitch properties measures included resting peak twitch torque (pTT), resting peak rate of twitch torque development (+dt/dt), resting peak rate of twitch relaxation (-dt/dt), resting doublet peak twitch torque (pTT_D), potentiated doublet peak twitch torque (pTT_{POT}), resting doublet peak rate of twitch torque development ($+dt/dt_D$), potentiated double peak rate of twitch torque development ($+dt/dt_{POT}$), resting doublet peak rate of relaxation ($-dt/dt_D$), and potentiated doublet peak rate of relaxation ($-dt/dt_{POT}$).

3.3.3 Maximal Strength and Voluntary Activation

For all voluntary isometric testing, participants were seated with straps securing the trunk, and hips on a calibrated isokinetic dynamometer with the axis of rotation of the dynamometer head aligned with the lateral epicondyle of the subject's right femur, with the lower right leg was secured to the dynamometer lever arm approximately 3 cm above the lateral malleolus. Each participant's hip and knee angle were held constant at 120°, respectively, which were held constant throughout all testing. Participants completed a submaximal isometric warm-up by performing 3, 3-second contractions at 25%, 50%, and 75% of their perceived effort, with approximately 30 seconds of rest between contractions. Following the warm up and 1 minute of rest, 2 separate 3-5 second MVICs of the knee extensors were performed. One minute of rest was given between each attempt in order to avoid any undue fatigue. For each MVIC contraction, the participant was instructed to kick out "as hard as possible" during the entire contraction (Tomko et al. 2018). MVT was defined as the highest instantaneous torque produced during a 1000 ms epoch of the MVIC contractions and recorded to normalize force during the MU ramp contractions.

Finally, an additional MVIC was performed in order to assess %VA via ITT. Specifically, prior to the contraction, a doublet stimulus was applied to the femoral nerve in order to obtain a resting doublet twitch. The subject then completed an MVIC, in which another doublet stimulus was applied during the force plateau of the contraction (i.e. ITT), with an additional doublet stimuli applied 3 to 5 seconds following the completion of the contraction (i.e. potentiated twitch). %VA was calculated as (1-

[superimposed twitch/potentiated twitch])*100 (Behm et al. 1996). Loud verbal encouragement was given during each voluntary contraction.

3.3.4 Surface Electromyography

Surface EMG signals were collected from bipolar bar electrodes (Delsys, Inc., Natick, MA, USA) placed over the VL and RF of the right leg using a 16-channel Bagnioli acquisition system (Delsys, Inc., Natick, MA, USA). EMG variables analyzed included normalized (to VL_M) EMG amplitude (VL_{AMP}), VL median power frequency (VL_{MDF}), VL integrated EMG (VL_{iEMG}), normalized (to RF_M) EMG amplitude (RF_{AMP}), RF median power frequency (RF_{MDF}) and RF integrated EMG (RF_{iEMG}). Additionally, 4 channels of EMG were recorded from a specialized five-pin array that was placed over the distal portion of the VL in accordance with the recommendations set forth by Zaheer et al. (2012). In order to minimize skin impedance and improve signal quality, the skin was shaved, abraded and cleansed with isopropyl alcohol prior to the placement of the surface electrodes (Beck and Housh 2008). Each sensor was secured to the skin with hypoallergenic tape directly and was placed over the muscle belly in line with the muscle fiber orientation in a bipolar fashion (Lieber and Friden 2000) in accordance with SENIAM guidelines (Hermens et al. 1999). A reference electrode (Dermatrode; American Imex, Irvine, CA, USA) was placed over the spinous C7 process. All sensor locations were marked with permanent marker to ensure consistent placement between visits.

3.3.5 Motor Unit Decomposition

MU behavior was recorded through the 4 channels of raw EMG signal recorded from the 5-pin array placed over the distal portion of the VL. In order to record MU behavior, isometric ramp contractions were completed in a randomized order at 30%, 50%, and 70% of each subject's previously recorded MVT. Two contractions were completed at each intensity and 2 minutes were given between each contraction to minimize the effects of fatigue. Each contraction featured a 10% MVT/s ramp up to the target torque level, a hold at the target torque, and a 10% MVT/s ramp down to baseline (Colquhoun et al. 2018b). All recorded signals were stored on a personal computer and decomposed offline using the Precision Decomposition III Algorithm first described by De Luca et al. (2006) and improved upon by Nawab et al. (2010). Following the decomposition process, only MUs demonstrating at least 90.0% accuracy, as determined by the Decompose-Synthesize-Decompose-Compare test, were retained and utilized in the final analyses. Additionally, as the purpose of these recordings was to characterize MU behavior at rest, all MUs recruited during the plateau of the contraction were excluded from final analysis (Miller et al. 2019). Finally, only contractions following the recommendations set forth by Colquhoun et al. (2018b) were utilized in the final analyses.

All MU firing rate curves were smoothed prior to calculation by low-pass filtering each MUs impulse train with a 2-second Hanning window. Custom-written LabVIEW programs were used to analyze all of the MUs that met the inclusion criteria described previously. The MU properties calculated by the LabVIEW programs from each contraction were those previously described by Colquhoun et al. (2018a; 2018b).

Specifically, these variables included: [1] Recruitment threshold (RT), which is defined as the relative force (% MVIC) at which the MU first discharged; [2] Mean firing rate (MFR), which was calculated of as the average firing rate (pulses·s^{-1} (pps)) during the plateau in each individual MU's firing curve; and [3] MU action potential amplitude (MUAP) , defined as the average peak-to-peak amplitude (mV) of the waveforms across the 4 EMG channels. MU behavior was expressed as a function of RT and MUAP. For the MFR vs. RT relationship, linear regression was utilized (Colquhoun et al. 2018a; Colquhoun et al. 2018b). For all other relationships, exponential regressions were run (Miller et al. 2019).

3.3.6 Fatigue Protocol

During both experimental conditions, subject's completed an isometric fatigue protocol consisting of repeated 50% MVT isometric ramp contractions until failure. Each ramp contraction began and ended with a 3 second quiescent period, with a 5 second ramp to target torque, a 10 second hold at target torque, and a 5 second ramp back to baseline. Contractions were repeated until the subject if not longer able to maintain at least 45% of MVT for at least 80% of the plateau (Pethick et al. 2018). During the fatiguing exercise protocol, average torque (TQ_{AVG}), standard deviation of torque (TQ_{SD}), coefficient of variation of torque (TQ_{CV}), and torque impulse (TQ_{IMP}) were quantified.

3.4 Torque and EMG Signal Processing

EMG and Torque signals were recorded simultaneously during all voluntary and evoked isometric contractions of the quadriceps described previously and analyzed using custom-written LabVIEW programs (LabVIEW 2017; National Instruments, Austin, TX,

USA). The torque and EMG signals were preamplified with a common mode rejection ratio of 110 dB min and an impedance of 2M Ω and sampled at 20 kHz with through the Bagnioli acquisition system. The signals were zero-meaned and digitally filtered using a zero-phase shift 4th-order Butterworth filter with a band pass of 10 – 499 Hz. The torque signals were zero-meaned, low-pass filtered using a zero-phase shift 4th-order Butterworth filter with a 15 Hz cutoff. Torque and EMG onset were manually detected from the filtered signals to provide a more accurate analysis of torque and EMG variables (Folland et al. 2014; Tillin et al. 2013). All onsets were manually determined by the same investigator to avoid inter-rater reliability bias. Further, all recorded signals were stored on a personal computer and processed off-line with a custom written LabVIEW program. All analyses were completed using only filtered signals.

3.5 Saliva Analysis

Saliva samples were collected from each participant on the last experimental visit to quantify their CYP1A2 genotype, among others. Saliva samples were collected using an Oragene ON-500 saliva collection kit (DNA Genotek, Ottawa, Ontario, Canada) for DNA analysis. All DNA samples were shipped to the University of Toronto for analysis and were stored at -80ºC until final analyses. Briefly, genotyping was performed using the iPLEX Gold assay with mass-spectrometry-based detection on the Sequenom MassARRAY® platform (Agena Bioscience, San Diego, CA, USA) as previously described by Guest et al. (2018). Further detail on DNA analysis can be found in Jenkins et al. (2018).

3.6 Statistical analysis

Descriptive statistics of the participants are displayed in Table 1 as means ± standard deviations to describe the between-subject variability. Body composition and US measures for individual subjects were averaged across days and independent samples t-tests were run to quantify and potential differences between genotypes. All statistical analysis was performed using SPSS v. 24 (SPSS Inc., Armonk, New York, USA) and the type-I error rate was set a-priori at 5%.

Separate two (Condition) x 2 (Time) x 2 (Genotype) mixed-model anaylsees of variances (ANOVs) were run to examine resting changes in MVT, SOL_M, SOL_H, RF_M, VL_M, %VA, pTT, +dt/dt, -dt/dt, pTT_D, pTT_{POT}, $+dt/dt_D$, $+dt/dt_{POT}$, $-dt/dt_D$, $-dt/dt_{POT}$, 30% MFR vs. RT slope, 30% MFR vs. RT y-intercept, 30% MUAP vs. RT A term, 30% MUAP vs. RT b term, 30% MFR vs. MUAP A term, 30% MFR vs. MUAP b term, 50% MFR vs. RT slope, 50% MFR vs. RT y-intercept, 50% MUAP vs. RT A term, 50% MUAP vs. RT b term, 50% MFR vs. MUAP A term, 50% MFR vs. MUAP b term, 70% MFR vs. RT slope, 70% MFR vs. RT y-intercept, 70% MUAP vs. RT A term, 70% MUAP vs. RT b term, 70% MFR vs. MUAP A term, and 70% MFR vs. MUAP b term. Additionally, separate 2 (Condition) x 2(Time) x 2 (Genotype) mixed-model ANOVAs were utilized to examine potential changes from post-supplementation to post-fatigue in %VA, pTT_D, pTT_{POT}, $+dt/dt_D$, $+dt/dt_{POT}$, $-dt/dt_D$, and $-dt/dt_{POT}$.In order to quantify changes during the fatiguing protocol at the first, middle, and last repetition, separate 2 (Condition) x 3 (Time) x 2 (Genotype) mixed-model ANOVAs were run to analyze TQ_{AVG}, TQ_{SD}, TQ_{CV}, TQ_{IMP}, VL_{iEMG}, VL_{MDF}, VL_{AMP}, RF_{iEMG}, RF_{MDF}, RF_{AMP}, MFR vs. RT slope, MFR vs. RT y-intercept, MUAP vs. RT A term, MUAP vs. RT b term, MFR

vs. MUAP A term, and MFR vs. MUAP b term. Significant interactions were decomposed with follow up, lower-order ANOVAs and Sidak corrected dependent samples t-tests on the simple main effects. Simple main effects that were not involved in the interaction were analyzed with Sidak corrected dependent samples t-tests on the marginal means. Finally, simple linear regression analyses were performed to quantify the relationship between twitch variables and change in MVIC across conditions. The partial eta-squared effect sizes were calculated for each ANOVA**.**

CHAPTER IV

RESULTS

4.1 Body Composition & Ultrasonography

Table 3 presents the body composition and US data, along with the results of the independent samples t-tests. All data are presented as mean ± SD.

	FAST	**SLOW**	**Sig.**
Rectus Femoris Cross-Sectional Area (RF_{mCSA})	11.6 ± 3.2 cm^2	11.8 ± 1.9 cm^2	p = 0.852
Rectus Femoris Echo Intensity (RF_{EI})	37.9 ± 5.3 au	40.6 ± 5.3 au	p = 0.115
Vastus Lateralis Cross-Sectional Area (VL_{mCSA})_	35.3 ± 5.1 cm^2	32.4 ± 9.0 cm^2	p = 0.179
Vastus Lateralis Echo Intensity (VL_{EI})	45.6 ± 7.3 au	50.2 ± 5.0 au	p = 0.032*
Fat-Free Mass (FFM)	73.6 ± 10.1 kg	75.1 ± 8.3 kg	p = 0.635
Fat Mass (FM)	14.2 ± 8.4 kg	17.7 ± 8.8 kg	p = 0.213

Table 3. Mean ± SD and p-values for ultrasound and body composition measures between genotype groups.
*Indicates significant difference between groups

The results of the independent samples t-tests indicated a significantly higher VL_{EI} in SLOW when compared to FAST (50.2 ± 5.0 au vs. 45.6 ± 7.3 au; p = 0.032).

4.2 Neuromuscular Function: Pre vs. Post Supplementation

The following results represent data examining changes in neuromuscular function prior to- (PRE) and 1-hour following (POST) caffeine (CAF) and placebo (PLA) supplementation. Neuromuscular changes associated with the fatiguing exercise protocol are discussed later in the section 4.3.

4.2.1 Maximal Voluntary Isometric Contraction (MVIC) Strength

A significant Condition × Time interaction ($F_{1,37} = 7.563$; $p = 0.009$; $\eta^2 = 0.170$) was observed. Follow-up analyses indicated that there was a significant decrease in MVIC strength from PLA_{PRE} to PLA_{POST} (295.6 ± 65.2 Nm vs. 268.7 ± 73.5 Nm; p = <0.001), but not from CAF_{PRE} to CAF_{POST} (295.7 ± 68.9 Nm vs. 286.6 ± 75.4 Nm; p = 0.094). There were no other main effects or interactions (all $p > 0.05$). This data is visually depicted below in Figure 1

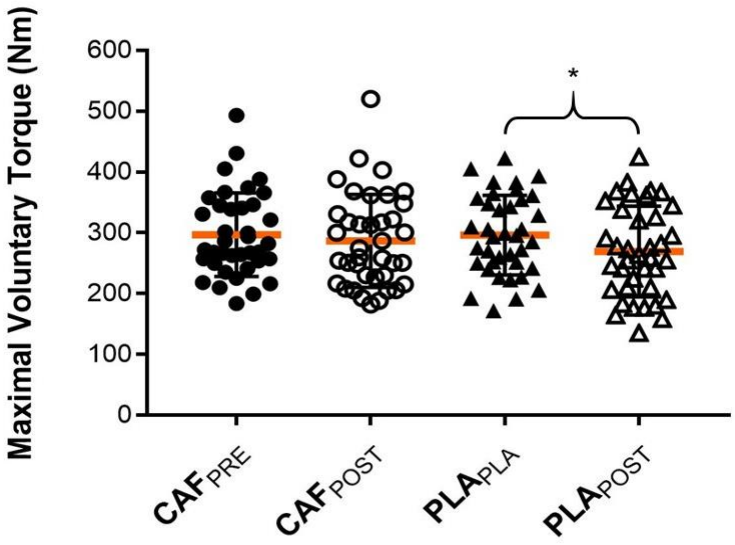

Figure 1. Individual plots of Maximal Voluntary Torque (MVT) obtained during Maximal Voluntary Isometric Contractions (MVIC) prior to and following caffeine (CAF) and placebo (PLA) ingestion. The orange bars represent the mean MVT during each condition.
*Indicates significant decrease in MVT from PLA_{PRE} to PLA_{POST} (p = <0.001).

4.2.2 Motor Unit (MU) Behavior

The following results represent data examining the relationship between mean firing rate (MFR), recruitment threshold (RT), and MU action potential (MUAP) amplitude at PRE and POST in each condition. Twenty-six subjects met the MU criteria

for all 3 contraction intensities and were utilized in the following analyses. The genotype breakdown was: FAST (n = 15) and SLOW (n = 11). Changes in MU behavior during the fatiguing exercise protocol will be discussed separately in section 4.2.1.

4.2.2.1 30% MVIC

The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize MU behavior at 30% MVIC can be found below in Table 4. The average number of MUs analyzed per subject were: CAF_{PRE}: 20.9 MUs (95% CI: 18.7-23.2 MUs), CAF_{POST}: 22.2 MUs (95% CI: 20.1-24.2 MUs), PLA_{PRE}: 21.0 MUs (95% CI: 18.8-23.2 MUs), and PLA_{POST}: 21.9 MUs (95% CI: 19.9-23.9 MUs). The mean RT range (% MVIC) for analyzed MUs were: CAF_{PRE}: 4.9-23.5%, CAF_{POST}: 6.7-25.1%, PLA_{PRE}: 5.2-24.4%, and PLA_{POST}: 5.6-25.3%. Significant main and interaction effects are discussed below.

	MFR vs. RT		**MUAP vs. RT**		**MFR vs. MUAP**	
	Slope	*Y-Int*	*A*	*b*	*A*	*b*
Condition	0.231	0.986	0.474	0.192	0.297	0.336
Condition x Genotype	0.290	0.646	**0.033***	0.254	0.432	0.427
Time	0.627	0.758	0.492	0.280	0.356	0.748
Time x Genotype	0.951	0.680	0.626	0.924	0.836	0.325
Condition x Time	0.055	0.063	0.134	0.172	0.895	0.574
Condition x Time x Genotype	0.573	0.676	0.139	0.530	0.825	0.574
Genotype	0.737	0.652	0.094	0.491	0.245	0.273

Table 4. P-values for interaction and main effects for motor unit behavior at 30% MVIC pre- and post-supplementation.
*Indicates significant interaction

There was a significant Condition × Genotype interaction ($F_{1,24} = 5.122$; $p = 0.033$; $\eta^2 = 0.176$) for the A term in the MUAP vs. RT relationship. Follow-up independent samples t-tests indicated that the A term (collapsed across condition) was significantly greater in the SLOW versus FAST metabolizers (43.5 ± 21.1 mV vs. 29.0 ±

11.9 mV; p = 0.036) in the PLA condition. No other significant differences were observed (all p = 0.106-0.563).

4.2.2.2 50% MVIC

The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize MU behavior at 50% MVIC can be found below in Table 5. The average number of MUs analyzed per subject were: CAF_{PRE}: 20.6 MUs (95% CI: 18.3-22.9 MUs), CAF_{POST}: 22.7 MUs (95% CI: 20.4-25.0 MUs), PLA_{PRE}: 22.3 MUs (95% CI: 20.4-24.2 MUs), and PLA_{POST}: 21.0 MUs (95% CI: 18.3-23.6 MUs). The mean RT range (% MVIC) for analyzed MUs were: CAF_{PRE}: 10.8-41.6%, CAF_{POST}: 12.6-42.0%, PLA_{PRE}: 11.8-41.3%, and PLA_{POST}: 13.9-40.4%. Significant main and interaction effects are discussed below.

	MFR vs. RT		**MUAP vs. RT**		**MFR vs. MUAP**	
	Slope	*Y-Int*	*A*	*b*	*A*	*b*
Condition	**0.014†**	**0.022†**	0.402	**0.049†**	0.335	0.498
Condition x Genotype	0.196	0.646	0.366	0.870	0.229	0.427
Time	0.065	**0.022†**	0.055	0.266	0.288	0.394
Time x Genotype	0.691	0.657	0.567	0.998	0.383	0.467
Condition x Time	0.622	0.648	0.069	**0.019***	0.141	0.544
Condition x Time x Genotype	0.566	0.908	0.322	0.838	0.434	0.338
Genotype	0.211	0.645	0.086	0.739	0.057	0.317

Table 5. P-values for interaction and main effects for motor unit behavior at 50% MVIC pre- and post-supplementation.
†Indicates significant main effect
*Indicates significant interaction

There was a significant main effect for Condition ($F_{1,24} = 7.007$; p = 0.014; $\eta^2 = 0.226$) for the slope of the MFR vs. RT relationship. Follow-up t-tests revealed that the slope was significantly lower (-0.51 ± 0.20 pps·RT^{-1} vs. -0.45 ± 0.17 pps·RT^{-1}; p = 0.014) and that the y-intercept was significantly greater (27.5 ± 7.2 pps vs. 25.6 ± 5.8 pps; p =

0.022) in the PLA versus CAF condition. Additionally, there was a main effect for Time ($F_{1,24} = 6.027$; $p = 0.022$; $\eta^2 = 0.201$) the y-intercept of the MFR vs. RT relationship. Post-hoc t-tests indicated that the y-intercept was significantly greater at POST than at PRE (27.7 ± 6.3 pps vs. 25.5 ± 5.5 pps; $p = 0.018$), when collapsed across conditions. Finally, a significant Condition x Time interaction ($F_{1,24} = 6.344$; $p = 0.019$; $\eta^2 = 0.209$) was observed for the MUAP vs. RT b term. Post hoc t-tests found significantly higher b term of the MUAP vs. RT relationship in the PLA_{POST} condition, when compared to CAF_{POST} (0.055 ± 0.017 mV vs. 0.046 ± 0.017 mV; $p = 0.005$).

4.2.2.3 70% MVIC

The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize MU behavior at 70% MVIC can be found below in Table 6. The average number of MUs analyzed per subject were: CAF_{PRE}: 22.5 MUs (95% CI: 20.1-24.8 MUs), CAF_{POST}: 24.7 MUs (95% CI: 21.9-27.5 MUs), PLA_{PRE}: 22.7 MUs (95% CI: 20.1-25.3 MUs), and PLA_{POST}: 22.3 MUs (95% CI: 19.9-24.8 MUs). The mean RT range (% MVIC) for analyzed MUs were: CAF_{PRE}: 21.3-58.9%, CAF_{POST}: 21.0-55.5%, PLA_{PRE}: 22.4-58.4%, and PLA_{POST}: 23.6-56.6%. No significant interaction or main effects were found for any relationship (all p = >0.05).

	MFR vs. RT		**MUAP vs. RT**		**MFR vs. MUAP**	
	Slope	*Y-Int*	*A*	*b*	*A*	*b*
Condition	0.093	0.053	0.907	0.832	0.694	0.872
Condition x Genotype	0.195	0.571	0.764	0.459	0.107	0.468
Time	0.243	0.470	0.198	0.152	0.611	0.191
Time x Genotype	0.613	0.350	0.721	0.636	0.704	0.472
Condition x Time	0.210	0.268	0.463	0.535	0.321	0.875
Condition x Time x Genotype	0.876	0.626	0.313	0.102	0.322	0.573
Genotype	0.416	0.660	0.127	0.353	0.251	0.102

Table 6. P-values for interaction and main effects for motor unit behavior at 70% MVIC pre- and post-supplementation.

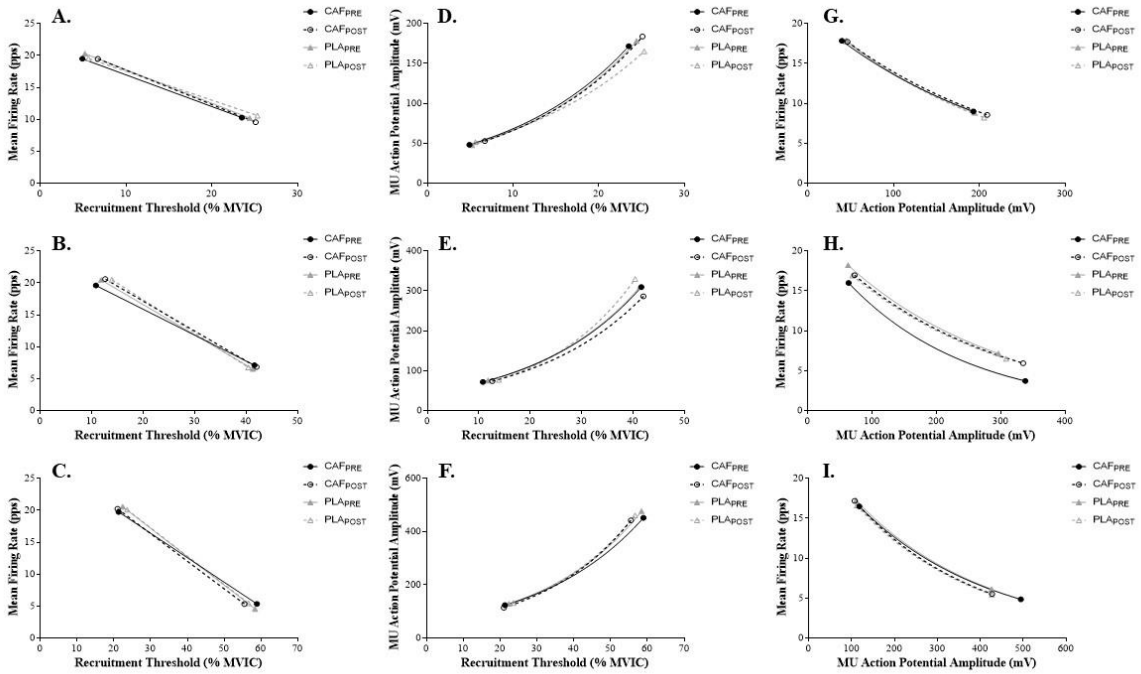

Figure 2. A-C) Mean firing rate (MFR) vs. recruitment threshold (RT) relationships across contractions intensities at pre- (CAF_{PRE}) and post-caffeine (CAF_{POST}), as well as pre- (PLA_{PRE}) and post-placebo (PLA_{POST}). D-F) Motor unit action potential amplitude (MUAP) vs. RT relationships across contractions intensities at CAF_{PRE}, CAF_{POST}, PLA_{PRE}, and PLA_{POST}. G-I) MFR vs. MUAP relationships across contractions intensities at CAF_{PRE}, CAF_{POST}, PLA_{PRE}, and PLA_{POST}.

4.2.3 Muscle Activation & Spinal Reflexes

The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize muscle activation & spinal reflexes can be found below in Table 7. Significant main effects are discussed below.

	%VA	VL_M	VL_{RMS}	RF_M	SOL_M	SOL_H
Condition	0.653	0.354	0.692	0.514	0.725	0.265
Condition x Genotype	0.998	0.747	0.936	0.866	0.652	0.571
Time	0.792	0.131	0.726	0.146	0.067	0.068
Time x Genotype	0.225	0.410	0.452	0.748	0.842	0.512
Condition x Time	0.406	0.991	0.931	0.504	0.541	0.563
Condition x Time x Genotype	0.234	0.139	0.107	0.053	0.117	0.762
Genotype	**0.003†**	0.593	0.302	0.287	0.149	0.450

Table 7. P-values for interaction and main effects for muscle activation and spinal excitability from pre- to post-supplementation.
†Indicates significant main effect

4.2.3.1 Voluntary Activation (%VA)

There was a significant main effect for Genotype ($F_{1,33} = 10.692$; $p = 0.003$; $\eta^2 = 0.256$) for %VA. Post-hoc analyses revealed a significantly greater %VA in the SLOW metabolizers when compared to FAST metabolizers (96.4 ± 3.0% vs. 93.9 ± 2.6%; $p = 0.003$). No other significant interactions or main effects were found.

4.2.3.2 Vastus Lateralis (VL) M-Wave (VL_M)

No significant interaction or main effects were found ($p = >0.05$).

4.2.3.3 Normalized VL Electromyographic Amplitude (VL_{AMP})

No significant interaction or main effects were found ($p = >0.05$).

4.2.3.4 Rectus Femoris (RF) M-Wave (RF_M)

No significant interaction or main effects were found ($p = >0.05$).

4.2.3.5 Normalized Soleus H-Reflex (SOL_H)

No significant interaction or main effects were found ($p = >0.05$).

4.2.3.6 Soleus M-Wave (SOL_M)

No significant interaction or main effects were found ($p = >0.05$).

4.2.4 Muscle Contractile Properties

The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize muscle contractile properties can be found below in Tables 8-10. Due to difficulties with recording, only thirty-five subjects (FAST = 22; SLOW= 13) exhibited useable data at all time points (PRE, POST, and FATIGUE) for both conditions and were utilized in the final analyses. Significant main effects are discussed below.

	pTT	+dt/dt	-dt/dt
Condition	0.072	0.579	0.728
Condition x Genotype	0.903	0.710	0.223
Time	**0.033**†	**0.007**†	0.419
Time x Genotype	0.339	0.157	0.445
Condition x Time	0.384	0.576	0.507
Condition x Time x Genotype	0.723	0.761	0.347
Genotype	0.191	0.303	0.672

Table 8. P-values for interaction and main effects for evoked singlet properties pre- and post-supplementation.
†Indicates significant main effect

4.2.4.1 Singlet Peak Twitch Torque (pTT)

There was a significant main effect for Time ($F_{1,33} = 4.927$; $p = 0.033$; $\eta^2 = 0.130$) for pTT. Post-hoc paired samples t-tests found a significantly higher pTT at PRE when compared to POST (48.4 ± 11.1 Nm vs. 44.5 ± 12.4 Nm; $p = 0.033$), when collapsed across condition. No other significant interaction or main effects were found ($p = >0.05$).

4.2.4.2 Singlet Peak Rate of Torque Development (+dt/dt)

There was a significant main effect for Time ($F_{1,33} = 8.318$; $p = 0.007$; $\eta^2 = 0.201$) for +dt/dt. Post-hoc paired samples t-tests revealed a significantly greater +dt/dt at PRE when compared to POST (794.3 ± 298.3 $Nm \cdot s^{-1}$ vs. 700.9 ± 270.3 $Nm \cdot s^{-1}$; $p = 0.007$), when collapsed across condition. No other significant interaction or main effects were found ($p = >0.05$).

4.2.4.3 Singlet Peak Relaxation Rate (-dt/dt)

No significant interactions or main effects were found ($p = >0.05$).

	pTT_D	$+dt/dt_D$	$-dt/dt_D$
Condition	**0.003†**	**0.002†**	**0.017†**
Condition x Genotype	0.907	0.904	0.651
Time	**<0.001†**	**0.025†**	0.088
Time x Genotype	0.655	0.718	0.945
Condition x Time	0.864	0.357	**0.029***
Condition x Time x Genotype	0.651	**0.027***	0.143
Genotype	0.253	0.612	0.117

Table 9. P-values for interaction and main effects for evoked resting doublet properties pre- and post-supplementation.
*Indicates significant interaction effect
†Indicates significant main effect

4.2.4.4 Resting Doublet Peak Twitch Torque (pTT_D)

There was a significant main effect for Condition ($F_{1,33}$ = 10.560; p = 0.003; η^2 = 0.242) for pTT_D. Post-hoc paired samples t-tests found a significant greater during CAF when compared to PLA (53.4 ± 12.3 Nm vs. 49.7 ± 13.0; p = 0.003), when collapsed across time. There was also a significant main effect for Time ($F_{1,33}$ = 16.362; p = <0.001; η^2 = 0.331) for PTT_D. Paired samples t-tests found that PRE was significantly greater than POST (54.8 ± 12.5 Nm vs. 48.3 ± 13.5 Nm; p = <0.001), when collapsed across condition. No other significant interaction or main effects were found (p = >0.05).

4.2.4.5 Resting Doublet Peak Rate of Torque Development ($+dt/dt_D$)

There was a significant Condition x Time x Genotype interaction ($F_{1,33}$ = 95.345; p = 0.027; η^2 = 0.139) for $+dt/dt_{POT}$. Post-hoc paired samples t-tests found a significantly greater $+dt/dt_{POT}$ in FAST during the CAF_{PRE} condition when compared to PLA_{POST} (1040.5 ± 217.5 $Nm \cdot s^{-1}$ vs. 896.9 ± 209.7 $Nm \cdot s^{-1}$; p = <0.001). No other significant interaction or main effects were found (p = >0.05).

4.2.4.6 Resting Doublet Peak Relaxation Rate (-dt/dt$_D$)

There was a significant Condition x Time interaction ($F_{1,33}$ = 5.196; p = 0.029; η^2 = 0.136) for -dt/dt$_D$. Post-hoc paired samples t-tests found that PLA$_{POST}$ had significantly slower -dt/dt$_D$ than all other conditions (p = 0.002-0.011). No other significant interaction or main effects were found (p = >0.05).

	pTT$_{POT}$	+dt/dt$_{POT}$	-dt/dt$_{POT}$
Condition	**0.013†**	**0.013†**	**0.004†**
Condition x Genotype	0.446	0.776	0.406
Time	**<0.001†**	**0.002†**	**0.036†**
Time x Genotype	0.843	0.775	0.447
Condition x Time	0.825	0.854	0.393
Condition x Time x Genotype	0.206	0.103	0.427
Genotype	0.270	0.637	0.252

Table 10. P-values for interaction and main effects for evoked potentiated doublet properties pre- and post-supplementation.
*Indicates significant interaction effect
†Indicates significant main effect

4.2.4.7 Potentiated Doublet Peak Twitch Torque (pTT$_{POT}$)

There was a significant main effect for Condition ($F_{1,33}$ = 6.963; p = 0.013; η^2 = 0.174) for pTT$_{POT}$. Post-hoc paired samples t-tests found a significantly greater pTT$_{POT}$ during CAF when compared to PLA (69.1 ± 14.7 Nm vs. 64.6 ± 15.8; p = 0.013), when collapsed across time. There was also a main effect for Time ($F_{1,33}$ = 23.138; p = <0.001; η^2 = 0.412) for pTT$_{POT}$. Post-hoc paired samples t-tests found that PRE was significantly greater than POST (71.5 ± 14.3 Nm vs. 62.1 ± 16.8 Nm; p = <0.001), when collapsed across condition. No other significant interaction or main effects were found (p = >0.05).

4.2.4.8 Potentiated Doublet Peak Rate of Torque Development (+dt/dt$_{POT}$)

There was a significant main effect for Condition ($F_{1,33}$ = 6.844; p = 0.013; η^2 = 0.172) for +dt/dt$_{POT}$. Post-hoc paired samples t-tests found significantly greater +dt/dt$_{POT}$

values during the CAF condition when compared to PLA (1305.0 ± 255.8 $Nm \cdot s^{-1}$ vs. 1217.9 ± 282.4 $Nm \cdot s^{-1}$; $p = 0.013$), when collapsed across time. There was also a significant main effect for Time ($F_{1,33} = 11.581$; $p = 0.002$; $\eta^2 = 0.260$) for $+dt/dt_{POT}$. Post-hoc paired samples t-tests found significantly greater $+dt/dt_{POT}$ values at PRE when compared to POST (1318.1 ± 262.7 $Nm \cdot s^{-1}$ vs. 1204.9 ± 279.1 $Nm \cdot s^{-1}$; $p = 0.002$), when collapsed across conditions. No other significant interaction or main effects were found ($p = >0.05$).

4.2.4.9 Potentiated Doublet Peak Relaxation Rate (-dt/dt$_{POT}$)

There was a significant main effect for Condition ($F_{1,33} = 9.364$; $p = 0.004$; $\eta^2 = 0.221$) for $-dt/dt_{POT}$. Post-hoc paired samples t-tests found a significantly faster $-dt/dt_{POT}$ during CAF when compared to PLA (-701.1 ± 191.8 $Nm \cdot s^{-1}$ vs. -631.3 ± 178.2 $Nm \cdot s^{-1}$; $p = 0.005$), when collapsed across time. There was also a significant main effect for Time ($F_{1,33} = 4.798$; $p = 0.036$; $\eta^2 = 0.127$) for $-dt/dt_{POT}$. Post-hoc paired samples t-tests revealed a significantly faster $-dt/dt_{POT}$ at PRE when compared to (-691.7 ± 187.1 $Nm \cdot s^{-1}$ vs. -640.7 ± 191.7 $Nm \cdot s^{-1}$; $p = 0.005$), when collapsed across conditions. No other significant interaction or main effects were found ($p = >0.05$).

4.3 Neuromuscular Function & Fatigue

4.3.1 MU Behavior

The following results represent data examining the relationship between MFR, RT, and MUAP amplitude during the first (FIRST), middle (MID), and last (LAST) repetition during the fatigue protocol of both the CAF and PLA conditions. The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize MU behavior during fatigue can be found below in Table 11. The average

number of MUs analyzed per subject were: CAF_{FIRST}: 21.8 MUs (95% CI: 19.1-24.5 MUs), CAF_{MID}: 21.0 MUs (95% CI: 18.2-23.8 MUs), CAF_{LAST}: 19.4 MUs (95% CI: 16.6-22.2 MUs), PLA_{FIRST}: 21.3 MUs (95% CI: 18.9-23.6 MUs), PLA_{MID}: 19.5 MUs (95% CI: 17.1-21.9 MUs), and PLA_{LAST}: 18.8 MUs (95% CI: 16.3-21.2 MUs). The mean RT range (% MVIC) for analyzed MUs were: CAF_{FIRST}: 14.9-38.4%, CAF_{MID}: 15.0-39.7%, CAF_{LAST}: 17.5-42.3%, PLA_{FIRST}: 15.1-39.3%, PLA_{MID}: 18.6-43.3%, and PLA_{LAST}: 18.1-41.5%. Significant main and interaction effects are discussed below.

	MFR vs. RT		**MUAP vs. RT**		**MFR vs. MUAP**	
	Slope	*Y-Int*	*A*	*b*	*A*	*b*
Condition	0.794	0.870	0.861	0.253	0.381	0.186
Condition x Genotype	0.283	0.203	0.102	0.102	0.691	0.808
Time	0.480	0.748	**<0.001***	**<0.001***	0.101	0.711
Time x Genotype	0.987	0.627	0.132	0.821	0.758	0.457
Condition x Time	0.620	0.977	0.468	0.122	0.139	0.204
Condition x Time x Genotype	0.977	0.867	0.560	0.641	0.345	0.590
Genotype	0.314	0.227	0.797	0.535	0.370	0.722

Table 11. P-values for interaction and main effects for motor unit behavior during fatigue.
*Indicates significant main effect

4.3.1.1 RT vs. MFR slope

No significant interaction or main effects were found (p = >0.05).

4.3.1.2 RT vs. MFR y-intercept

No significant interaction or main effects were found (p = >0.05).

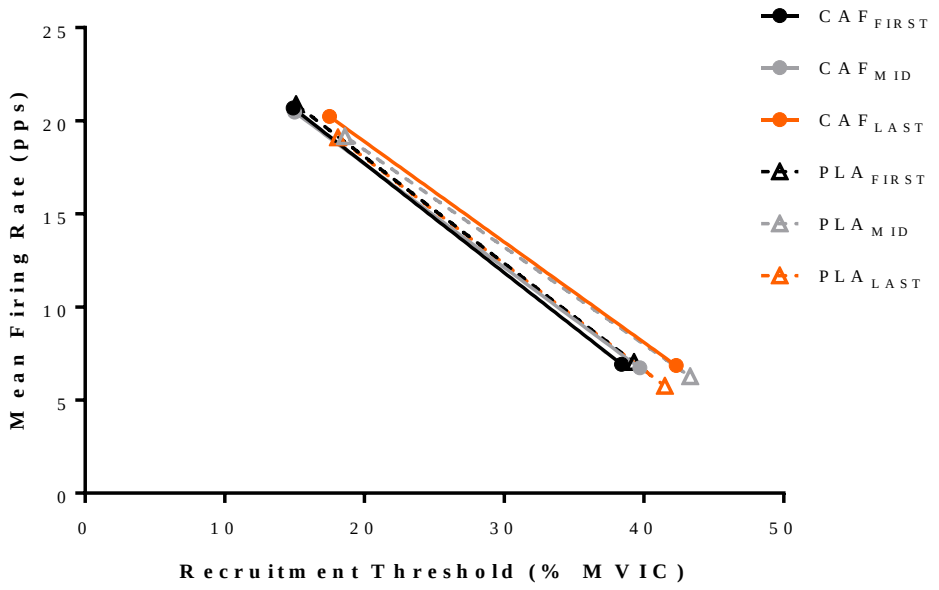

Figure 3. Mean firing rate (MFR) vs. recruitment threshold (RT) relationship during the fatigue protocol during both the caffeine (CAF) and placebo (PLA) conditions.

4.3.1.3 RT vs. MUAP A Term

There was a significant main effect for Time ($F_{1.4,35.1} = 16.388$; $p = <0.001$; $\eta^2 = 0.396$) in the RT vs. MUAP A term during fatigue. Post-hoc paired samples t-tests revealed a significantly lower RT vs. MUAP A term during FIRST when compared to MID (34.8 ± 27.9 mV vs. 46.1 ± 25.5 mV; $p = <0.001$), during FIRST when compared to LAST (34.8 ± 27.9 mV vs. 58.1 ± 38.4 mV; $p = <0.001$) and MID when compared to LAST (46.1 ± 25.5 mV vs. 58.1 ± 38.4 mV; $p = <0.001$), when collapsed across condition. No other significant interaction or main effects were found ($p = >0.05$).

4.3.1.4 RT vs. MUAP b Term

There was a significant main effect for Time ($F_{2,50} = 9.803$; $p = <0.001$; $\eta^2 = 0.282$) in the RT vs. MUAP b term during fatigue. Post-hoc paired samples t-tests revealed a significantly greater RT vs. MUAP b term during FIRST when compared to MID (0.058 ± 0.022 mV/%$_{MVIC}$ vs. 0.046 ± 0.019 mV/%$_{MVIC}$; $p = 0.001$) and during

FIRST when compared to LAST (0.058 ± 0.022 mV/%$_{MVIC}$ vs. 0.044 ± 0.019 mV/%$_{MVIC}$; p = 0.003), when collapsed across conditions. No other significant interaction or main effects were found (p =0 >0.05).

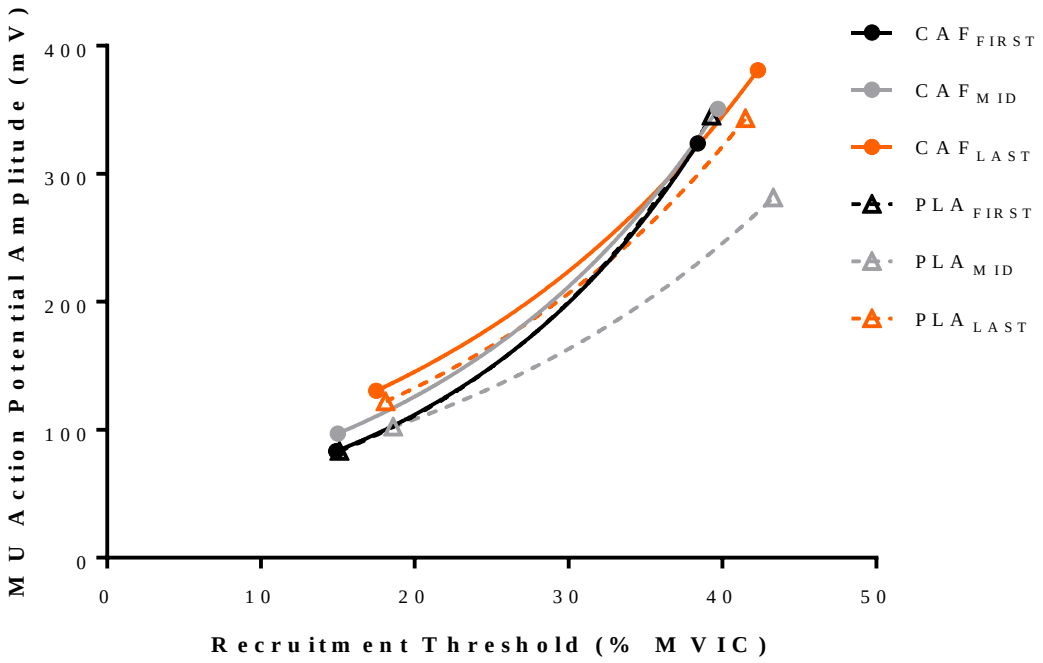

Figure 4. Motor unit action potential amplitude (MUAP) vs. recruitment threshold (RT) relationship during the fatigue protocol during both the caffeine (CAF) and placebo (PLA) conditions. Significant main effects are discussed above in section 4.1.3.3 and 4.1.3.4.

4.3.1.5 MUAP vs. MFR A term

No significant interaction or main effects were found (p = >0.05).

4.3.1.6 MUAP vs. MFR b term

No significant interaction or main effects were found (p = >0.05).

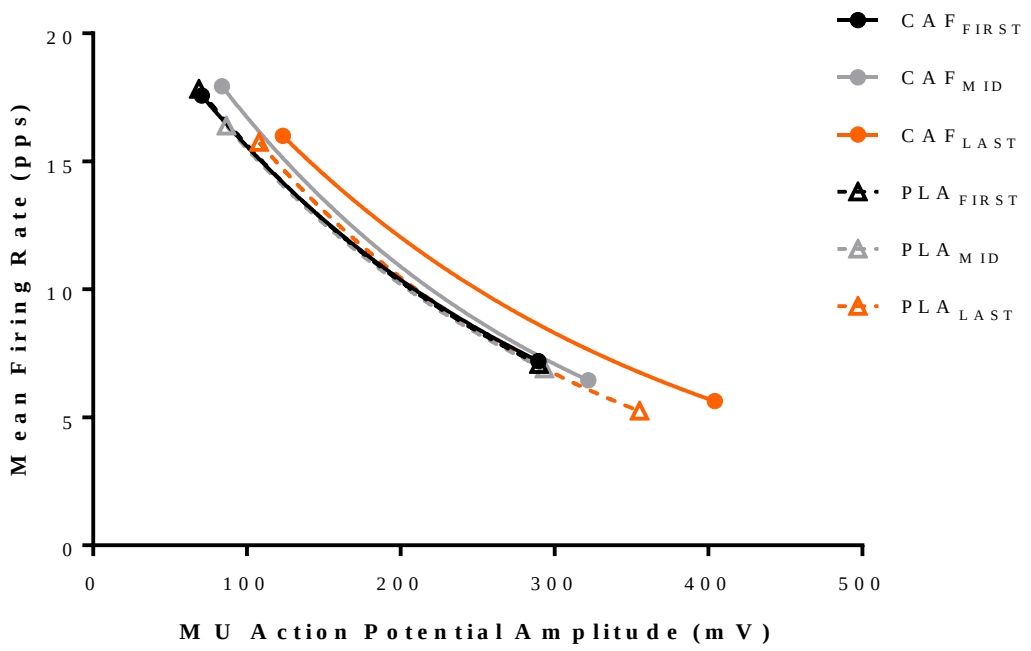

Figure 5. Mean firing rate (MFR) vs. motor unit action potential amplitude (MUAP) relationship during the fatigue protocol during both the caffeine (CAF) and placebo (PLA) conditions.

4.3.2 Muscle Activation

4.3.2.1 %VA

There was a significant main effect for Time ($F_{1,33} = 9.016$; $p = 0.005$; $\eta^2 = 0.231$) for Voluntary Activation. Post-hoc paired samples t-tests revealed a significantly lower VA post-fatigue (89.9 ± 7.5% vs. 94.8 ± 3.8%; $p = 0.005$) when collapsed across conditions. No other interaction or main effects were found ($p = >0.05$).

4.3.3 Muscle Contractile Properties

The p-values for the main and interaction effects from each of the ANOVAs for the relationships used to characterize muscle contractile properties can be found below in Tables 12-13. Significant main interactions and effects are discussed below.

	pTT_D	$+dt/dt_D$	$-dt/dt_D$
Condition	0.226	0.137	**0.008†**
Condition x Genotype	0.788	0.349	0.378
Time	**<0.001†**	**<0.001†**	**<0.001†**
Time x Genotype	0.176	0.361	0.069
Condition x Time	**0.029***	**0.010***	**0.014***
Condition x Time x Genotype	0.915	0.598	0.580
Genotype	0.132	0.873	0.399

Table 12. P-values for interaction and main effects for evoked resting doublet properties pre- and post-fatigue.
*Indicates significant interaction effect
†Indicates significant main effect

4.3.3.1 pTT_D

There was a significant Condition x Time ($F_{1,33} = 5.208$; $p = 0.029$; $\eta^2 = 0.136$) for pTT_D. Post-hoc paired samples t-tests revealed pTT_D was significantly greater at CAF_{POST} when compared to PLA_{POST} (50.1 ± 14.2 Nm vs. 46.5 ± 13.9 Nm; $p = 0.007$), $CAF_{FATIGUE}$ (50.1 ± 14.2 Nm vs. 20.2 ± 11.4 Nm; $p = <0.001$) and $PLA_{FATIGUE}$ (50.1 ± 14.2 Nm vs. 21.4 ± 12.3 Nm; $p = <0.001$). Analyses also revealed significantly higher pTT_D at PLA_{POST} than $CAF_{FATIGUE}$ (46.5 ± 13.9 Nm vs. 20.2 ± 11.4 Nm; $p = <0.001$) and $PLA_{FATIGUE}$ (46.5 ± 13.9 Nm vs. 21.4 ± 12.3 Nm; $p = <0.001$). No other significant interaction or main effects were found ($p = >0.05$).

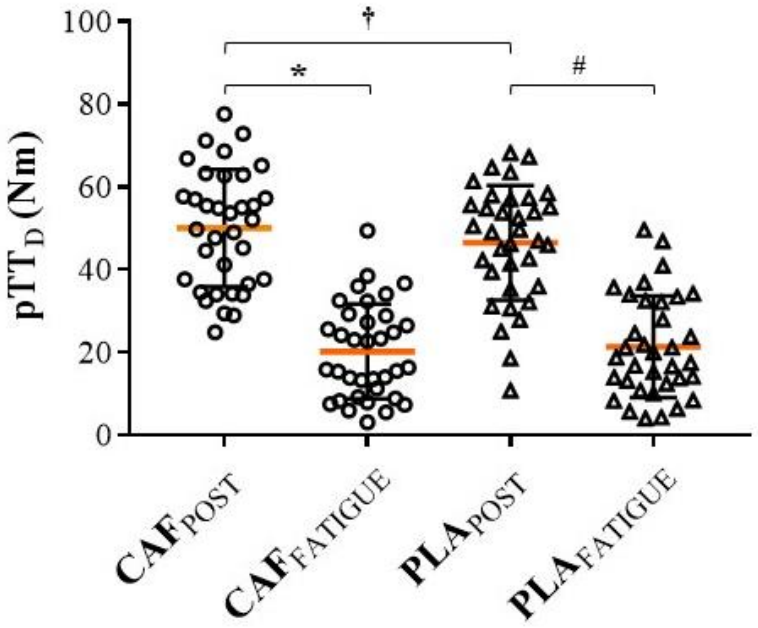

Figure 6. Individual subject plots for resting doublet peak twitch torque (pTT_D) prior to- and post-fatigue in the caffeine (CAF) and placebo (PLA) conditions. The orange bars represent the groups mean at that time point.
*Indicates significantly greater pTT_D at CAF_{POST} when compared to $CAF_{FATIGUE}$
# Indicates significantly greater pTT_D at PLA_{POST} when compared to $PLA_{FATIGUE}$
† Indicates significantly greater pTT_D at CAF_{POST} when compared to PLA_{POST}

4.3.3.2 +dt/dt$_D$

There was a significant Condition x Time ($F_{1,33} = 7.534$; $p = 0.010$; $\eta^2 = 0.186$) for $+dt/dt_D$. Post-hoc paired samples t-tests revealed $+dt/dt_D$ was significantly greater at CAF_{POST} when compared to PLA_{POST} (955.0 ± 234.6 Nm·s^{-1} vs. 875.4 ± 234.1 Nm·s^{-1}; $p = 0.004$), $CAF_{FATIGUE}$ (955.0 ± 234.6 Nm·s^{-1} vs. 426.0 ± 214.5 Nm; $p = <0.001$) and $PLA_{FATIGUE}$ (955.0 ± 234.6 Nm·s^{-1} vs. 445.9 ± 234.8 Nm·s^{-1}; $p = <0.001$). Analyses also revealed significantly higher $+dt/dt_D$ at PLA_{POST} than $CAF_{FATIGUE}$ (875.4 ± 234.1 Nm·s^{-1} vs. 426.0 ± 214.5 Nm·s^{-1}; $p = <0.001$) and $PLA_{FATIGUE}$ (875.4 ± 234.1 Nm·s^{-1} vs. 445.9 ±

234.8 Nm·s^{-1}; p = <0.001). No other significant interaction or main effects were found (p = >0.05).

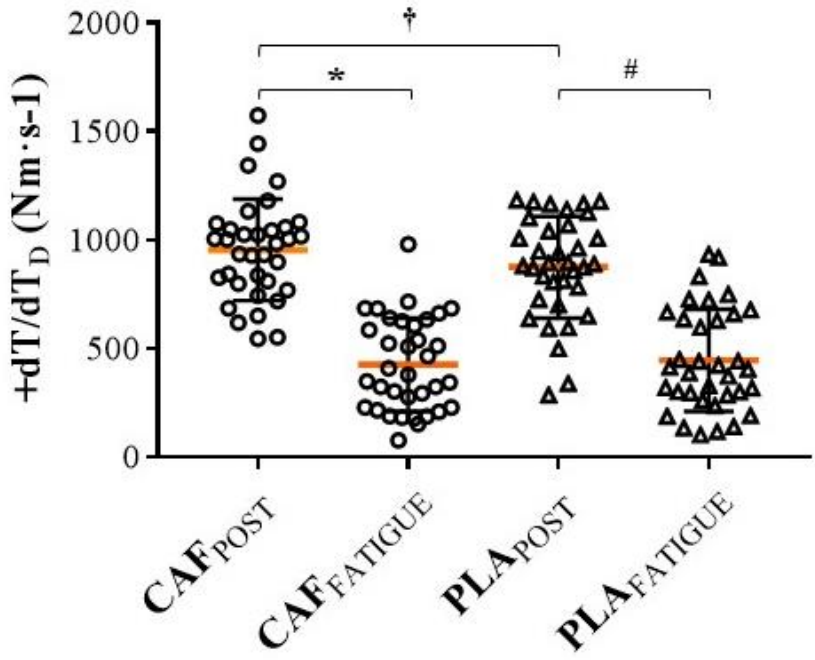

Figure 7. Individual subject plots for resting doublet peak rate of twitch torque development (+dt/dt$_D$) prior to- and post-fatigue in the caffeine (CAF) and placebo (PLA) conditions. The orange bars represent the groups mean at that time point.
*Indicates significantly greater +dt/dt$_D$ at CAF$_{POST}$ when compared to CAF$_{FATIGUE}$
# Indicates significantly greater +dt/dt$_D$ at PLA$_{POST}$ when compared to PLA$_{FATIGUE}$
† Indicates significantly greater +dt/dt$_D$ at CAF$_{POST}$ when compared to PLA$_{POST}$

4.3.3.3 -dt/dt$_D$

There was a significant Condition x Time ($F_{1,33}$ = 6.729; p = 0.014; η^2 = 0.169) for -dt/dt$_D$. Post-hoc paired samples t-tests indicated significantly faster -dt/dt$_D$ during CAF$_{POST}$ when compared to PLA$_{POST}$ (-447.5 ± 134.5 Nm·s^{-1} vs. -389.5 ± 119.0 Nm·s^{-1}), CAF$_{FATIGUE}$ (-447.5 ± 134.5 Nm·s^{-1} vs. -173.9 ± 83.3 Nm·s^{-1}), and PLA$_{FATIGUE}$ (-447.5 ± 134.5 Nm·s^{-1} vs. -167.8 ± 86.9 Nm·s^{-1}). Analyses also revealed significantly faster -dt/dt$_D$ at PLA$_{POST}$ when compared to CAF$_{FATIGUE}$ (-389.5 ± 119.0 Nm·s^{-1} vs. -173.9 ± 83.3

$Nm \cdot s^{-1}$) and $PLA_{FATIGUE}$ (-389.5 ± 119.0 $Nm \cdot s^{-1}$ vs. -167.8 ± 86.9 $Nm \cdot s^{-1}$). No other significant interaction or main effects were found (p = >0.05).

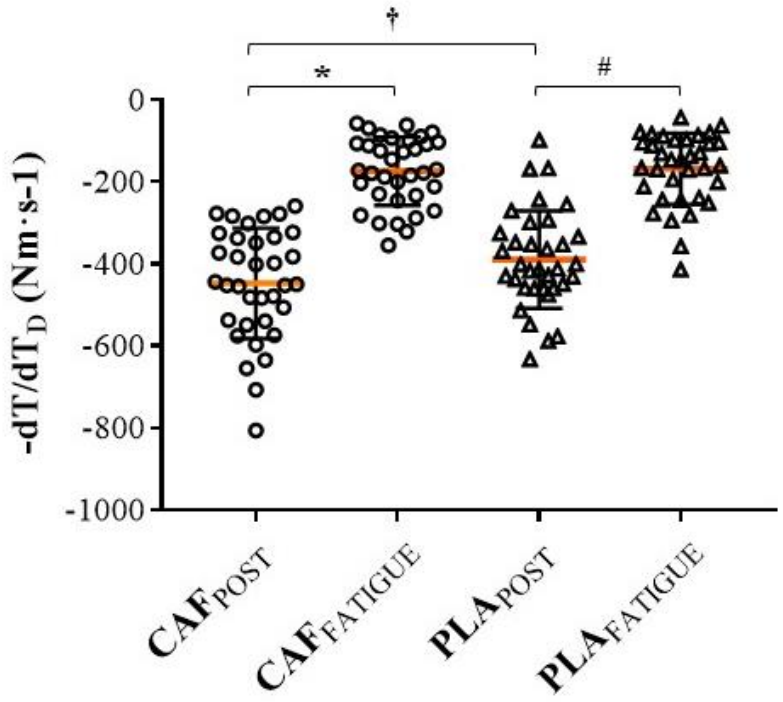

Figure 8. Individual subject plots for resting doublet peak rate of relaxation(-dt/dt$_D$) prior to- and post-fatigue in the caffeine (CAF) and placebo (PLA) conditions. The orange bars represent the groups mean at that time point.
*Indicates significantly faster -dt/dt$_D$ at CAF_{POST} when compared to $CAF_{FATIGUE}$
# Indicates significantly faster -dt/dt$_D$ at PLA_{POST} when compared to $PLA_{FATIGUE}$
† Indicates significantly faster -dt/dt$_D$ at CAF_{POST} when compared to PLA_{POST}

	pTT_{POT}	$+dt/dt_{POT}$	$-dt/dt_{POT}$
Condition	0.223	0.170	**0.013†**
Condition x Genotype	0.672	0.420	0.973
Time	**<0.001†**	**<0.001†**	**<0.001†**
Time x Genotype	0.202	0.376	0.142
Condition x Time	**0.026***	**0.019***	**0.010***
Condition x Time x Genotype	0.818	0.788	0.694
Genotype	0.561	0.909	0.316

Table 13. P-values for interaction and main effects for evoked potentiated doublet properties pre- and post-fatigue.
*Indicates significant interaction effect
†Indicates significant main effect

4.3.3.4 pTT_{POT}

There was a significant Condition x Time ($F_{1,33} = 5.436$; $p = 0.026$; $\eta^2 = 0.141$) for pTT_{POT}. Post-hoc paired samples t-tests revealed pTT_{POT} was significantly greater at CAF_{POST} when compared to $CAF_{FATIGUE}$ (64.1 ± 17.2 Nm vs. 23.3 ± 12.4 Nm; p = <0.001) and $PLA_{FATIGUE}$ (64.1 ± 17.2 Nm vs. 24.3 ± 12.8 Nm; p = <0.001). Analyses also revealed significantly higher pTT_{POT} at PLA_{POST} than $CAF_{FATIGUE}$ (60.2 ± 17.6 Nm vs. 23.3 ± 12.4 Nm; p = <0.001) and $PLA_{FATIGUE}$ (60.2± 17.6 Nm vs. 24.3 ± 12.8 Nm; p = <0.001). No other significant interaction or main effects were found (p = >0.05).

4.3.3.5 $+dt/dt_{POT}$

There was a significant Condition x Time ($F_{1,33} = 6.033$; $p = 0.019$; $\eta^2 = 0.155$) for $+dt/dt_{POT}$. Post-hoc paired samples t-tests revealed $+dt/dt_{POT}$ was significantly greater at CAF_{POST} when compared to $CAF_{FATIGUE}$ (1245.7 ± 268.6 $Nm \cdot s^{-1}$ vs. 493.9 ± 235.9 $Nm \cdot s^{-1}$; p = <0.001) and $PLA_{FATIGUE}$ (1245.7 ± 268.6 $Nm \cdot s^{-1}$ vs. 506.9 ± 252.9 $Nm \cdot s^{-1}$; p = <0.001). Analyses also revealed significantly higher $+dt/dt_{POT}$ at PLA_{POST} than $CAF_{FATIGUE}$ (1164.1 ± 318.2 $Nm \cdot s^{-1}$ vs. 493.9 ± 235.9 $Nm \cdot s^{-1}$; p = <0.001) and $PLA_{FATIGUE}$ (1164.1 ± 318.2 $Nm \cdot s^{-1}$ vs. 506.9 ± 252.9 $Nm \cdot s^{-1}$; p = <0.001). No other significant interaction or main effects were found (p = >0.05).

4.3.3.6 $-dt/dt_{POT}$

There was a significant Condition x Time ($F_{1,33} = 7.496$; $p = 0.010$; $\eta^2 = 0.185$) for $-dt/dt_{POT}$. Post-hoc paired samples t-tests indicated significantly faster $-dt/dt_{POT}$ during CAF_{POST} when compared to PLA_{POST} (-680.6 ± 215.4 $Nm \cdot s^{-1}$ vs. -600.7 ± 191.5 $Nm \cdot s^{-1}$), $CAF_{FATIGUE}$ (-680.6 ± 215.4 $Nm \cdot s^{-1}$ vs. -197.3 ± 105.4 $Nm \cdot s^{-1}$), and $PLA_{FATIGUE}$ (-680.6 ± 215.4 $Nm \cdot s^{-1}$ vs. -190.8 ± 98.4 $Nm \cdot s^{-1}$). Analyses also revealed significantly faster -

dt/dt$_{POT}$ at PLA$_{POST}$ when compared to CAF$_{FATIGUE}$ (-600.7 ± 191.5 Nm·s^{-1} vs. -197.3 ± 105.4 Nm·s^{-1}) and PLA$_{FATIGUE}$ (-600.7 ± 191.5 Nm·s^{-1} vs. -190.8 ± 98.4 Nm·s^{-1}). No other significant interaction or main effects were found (p = >0.05).

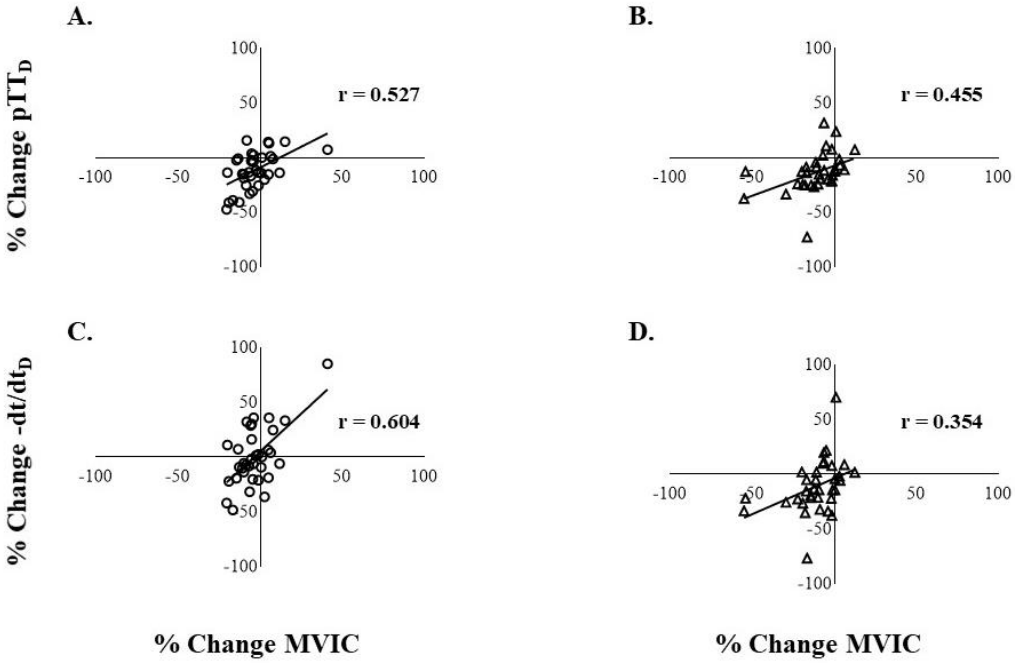

Figure 9. A/B) Relationship between % Change in MVIC and % Change in peak resting doublet twitch torque (pTT$_D$) from pre- to 1-hour post-supplementation in the caffeine and placebo conditions, respectively. **C/D)** Relationship between % Change in MVIC and % Change in peak resting doublet peak relaxation rate (-dT/dt$_D$) from pre- to 1-hour post-supplementation in the caffeine and placebo conditions, respectively.

4.4 Time-Course of Fatigue

4.4.1 *Average Torque (TQ$_{AVG}$)*

There was a significant main effect for Condition ($F_{1,26} = 8.645$; $p = 0.007$; $\eta^2 = 0.250$) for TQ$_{AVG}$. Post-hoc paired samples t-tests indicated a significantly greater TQ$_{AVG}$ during CAF when compared to PLA (140.1 ± 36.0 Nm vs. 131.7 ± 38.7 Nm; p = 0.007), when collapsed across time. There was also a significant main effect for Time ($F_{2,52}$ =

6.585; p = 0.003; η^2 = 0.202) for TQ_{AVG}. Follow-up paired samples t-tests found a significantly higher TQ_{AVG} at FIRST versus LAST (136.5 ± 36.7 Nm vs. 135.0 ± 36.6 Nm; p = 0.010), when collapsed across condition. No other significant interaction or main effects were found (P = >0.05).

4.4.2 Torque SD (TQ_{SD})

There was a significant Condition x Time interaction ($F_{1.3,34.7}$ = 3.644; p = 0.033; η^2 = 0.123) for TQ_{SD}. Follow-up paired samples t-tests revealed significantly lower TQ_{SD} values during CAF_{FIRST} when compared to CAF_{LAST} (3.4 ± 1.2 Nm vs. 4.8 ± 1.7 Nm; p = 0.001) and during CAF_{MID} when compared to CAF_{LAST} (3.3 ± 0.9 Nm vs. 4.8 ± 1.7 Nm; p = <0.001). Follow-up analyses also revealed a significantly lower TQ_{SD} during PLA_{FIRST} when compared to PLA_{LAST} (3.1 ± 1.0 Nm vs. 5.6 ± 2.2 Nm; p = <0.001) and during PLA_{MID} when compared to PLA_{LAST} (3.6 ± 1.3 Nm vs. 5.6 ± 2.2 Nm; p = <0.001). Analyses also found significantly lower TQ_{SD} during CAF_{FIRST} when compared to PLA_{LAST} (3.4 ± 1.2 Nm vs. 5.6 ± 2.2 Nm; p = <0.001), CAF_{MID} when compared to PLA_{LAST} (3.3 ± 0.9 Nm vs. 5.6 ± 2.2 Nm; p = <0.001), and PLA_{FIRST} when compared to CAF_{LAST} (3.1 ± 1.0 Nm vs. 4.8 ± 1.7 Nm; p = <0.001), and PLA_{MID} when compared to CAF_{LAST} (3.6 ± 1.3 Nm vs. 4.8 ± 1.7 Nm; p = 0.001). No other significant interaction or main effects were found (p = >0.05).

4.4.3 CV Torque (TQ_{CV})

There was a significant Condition x Time interaction ($F_{1.3,34.4}$ = 3.818; p = 0.048; η^2 = 0.128) for TQ_{CV}. Follow-up paired samples t-tests found a significantly lower TQ_{CV} at CAF_{FIRST} when compared to CAF_{LAST} (2.5 ± 0.8% vs. 3.7 ± 1.8%; p = 0.001) and at CAF_{MID} when compared to CAF_{LAST} (2.4 ± 0.7% vs. 3.7 ± 1.8%; p = <0.001).

Significantly lower TQ_{CV} values were found during CAF_{FIRST} when compared to PLA_{LAST} (2.5 ± 0.8% vs. 4.5 ± 1.8%; p = <0.001), CAF_{MID} when compared to PLA_{LAST} (2.4 ± 0.7% vs. 4.5 ± 1.8%; p = <0.001), PLA_{FIRST} when compared to PLA_{LAST} (2.5 ± 0.9% vs. 4.5 ± 1.8%; p = <0.001) and PLA_{FIRST} when compared to CAF_{LAST} (2.5 ± 0.9% vs. 3.7 ± 1.8%; p = 0.001). No other interaction or main effects were found (p = >0.05).

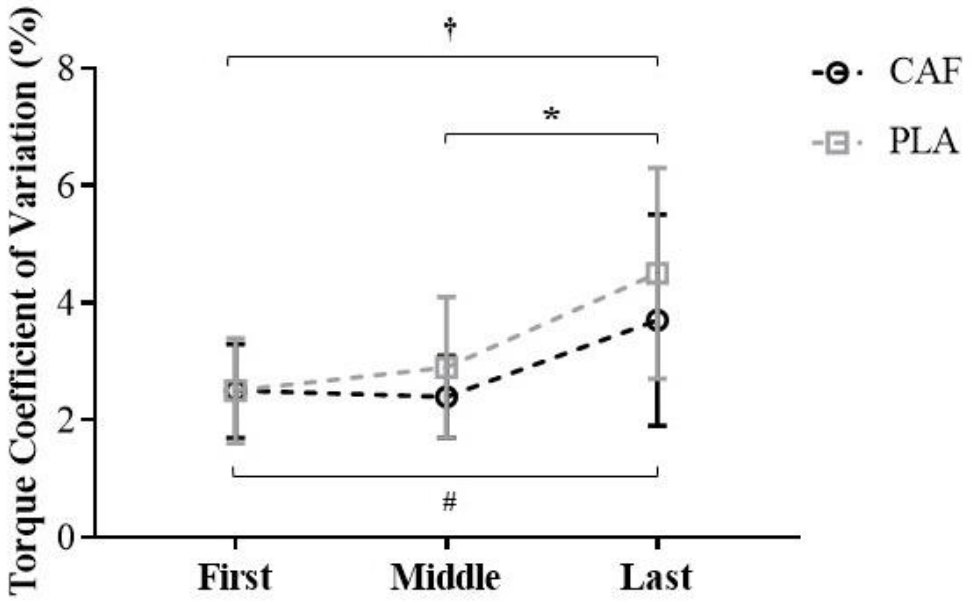

Figure 10. Visual representative of the mean torque coefficient of variation (TQ_{CV}) ± SD at the first, middle, and last repetition of the fatigue protocols in the caffeine (CAF) and placebo (PLA) conditions.
†Indicates significantly lower TQ_{CV} at CAF_{FIRST} when compared to CAF_{LAST}
*Indicates significantly lower TQ_{CV} at CAF_{MID} when compared to CAF_{LAST}
#Indicates significantly lower TQ_{CV} at PLA_{FIRST} when compared to PLA_{LAST}

4.4.4 TQ Impulse (TQ_{IMP})

There was a significant Condition x Time interaction ($F_{2,52}$ = 5.501; p = 0.010; η^2 = 0.163) for TQ_{IMP}. Follow-up paired samples t-tests found a significantly greater TQ_{IMP} at CAF_{FIRST} when compared to PLA_{LAST} (2086.6 ± 547.0 Nm·s vs. 1913.6 ± 569.4 Nm·s;

p = 0.001), CAF_{MID} when compared to PLA_{LAST} (2069.0 ± 525.5 Nm·s vs. 1913.4 ± 569.4 Nm·s; p = 0.003) and PLA_{FIRST} when compared to PLA_{LAST} (1985.0 ± 584.6 Nm·s vs. 1913.6 ± 569.4 Nm·s; p = 0.002). No other significant interaction or main effects were found (p = >0.05).

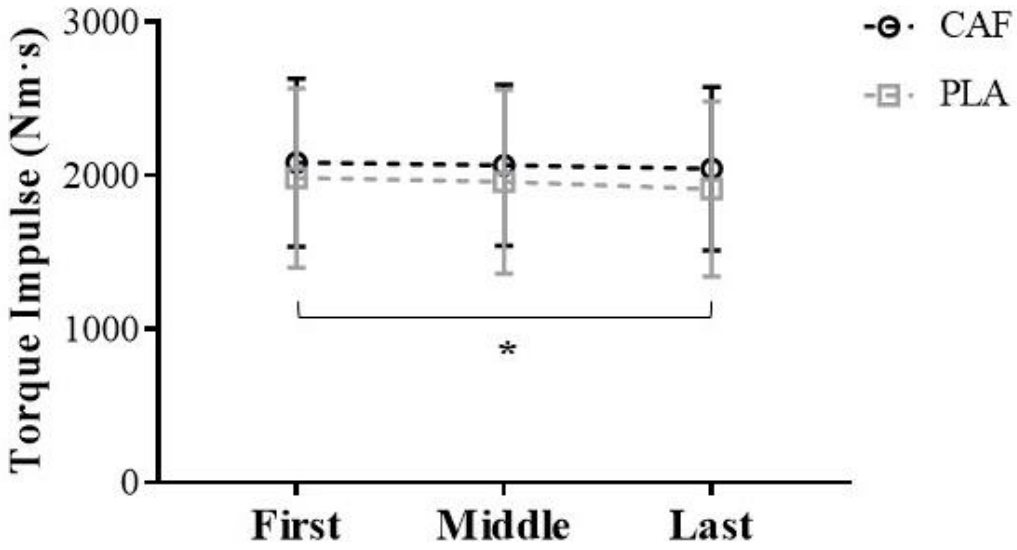

Figure 11. Visual representative of the mean torque impulse (TQ_{IMP}) ± SD at the first, middle, and last repetition of the fatigue protocols in the caffeine (CAF) and placebo (PLA) conditions.
*Indicates significantly greater TQ_{IMP} at PLA_{FIRST} when compared to PLA_{LAST}

4.4.5 VL Integrated EMG (VL_{iEMG})

There was a significant main effect for Time ($F_{1.1,27.7}$ = 57.72; p = <0.001; η^2 = 0.689) for VL_{iEMG}. Post-hoc paired samples t-tests indicated that VL_{iEMG} was significantly lower at FIRST when compared to MID (1.44 ± 0.58 µV vs. MID: 1.74 ± 0.68 µV; p = <0.001), at FIRST when compared to LAST (1.44 ± 0.58 µV vs. 2.11 ± 0.90 µV; p = <0.001), and at MID when compared to LAST (1.74 ± 0.68 µV vs. 2.11 ± 0.90 µV; p =

<0.001), when collapsed across condition. No other significant interaction or main effects were found (p = >0.05).

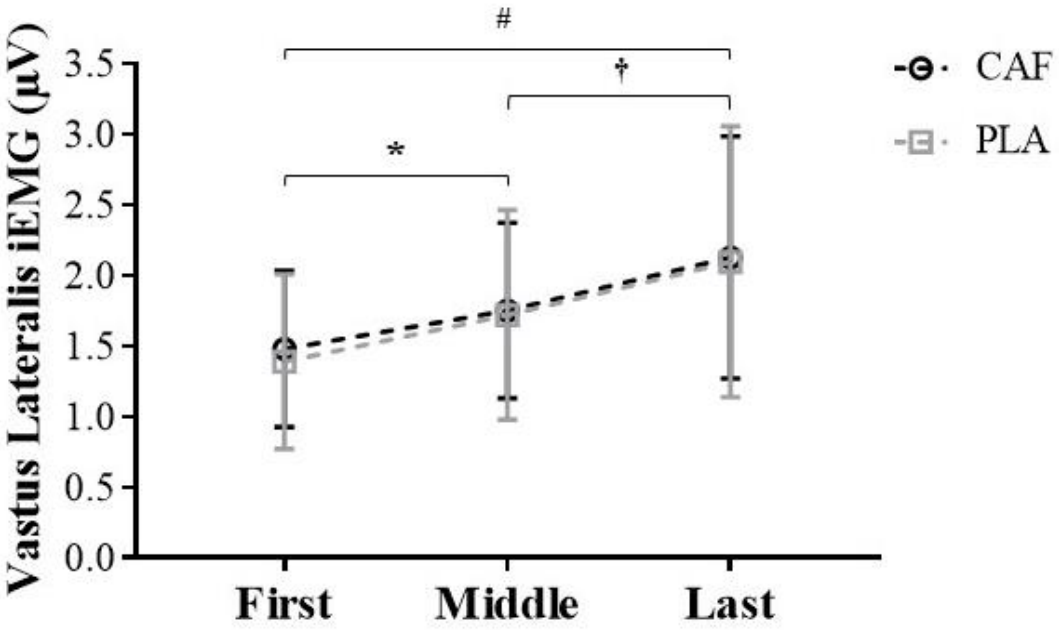

Figure 12. Visual representative of the mean *vastus lateralis* integrated electromyography (VL_{iEMG}) ± SD at the first, middle, and last repetition of the fatigue protocols in the caffeine (CAF) and placebo (PLA) conditions.
*Indicates significantly lower VL_{iEMG} at FIRST when compared to MID, when collapsed across condition.
†Indicates significantly lower VL_{iEMG} at MID when compared to LAST, when collapsed across condition.
#Indicates significantly lower VL_{iEMG} at FIRST when compared to LAST, when collapsed across condition.

4.4.6 VL Median Power Frequency (VL_{MDF})

There was a significant main effect for Time ($F_{1.2,31.7} = 41.936$; p = <0.001; η^2 = 0.617) for VL_{MDF}. Post-hoc paired samples t-tests found significantly higher VL_{MDF} values at FIRST when compared to MID (80.5 ± 17.5 Hz vs. 76.6 ± 16.4 Hz; p = <0.001), at FIRST when compared to LAST (80.5 ± 17.5 Hz vs. 69.8 ± 16.8 Hz; p = <0.001), and at MID when compared to LAST (76.6 ± 16.4 Hz vs. 69.8 ± 16.8 Hz; p = <0.001), when

collapsed across condition. No other significant interaction or main effects were found (p = >0.05).

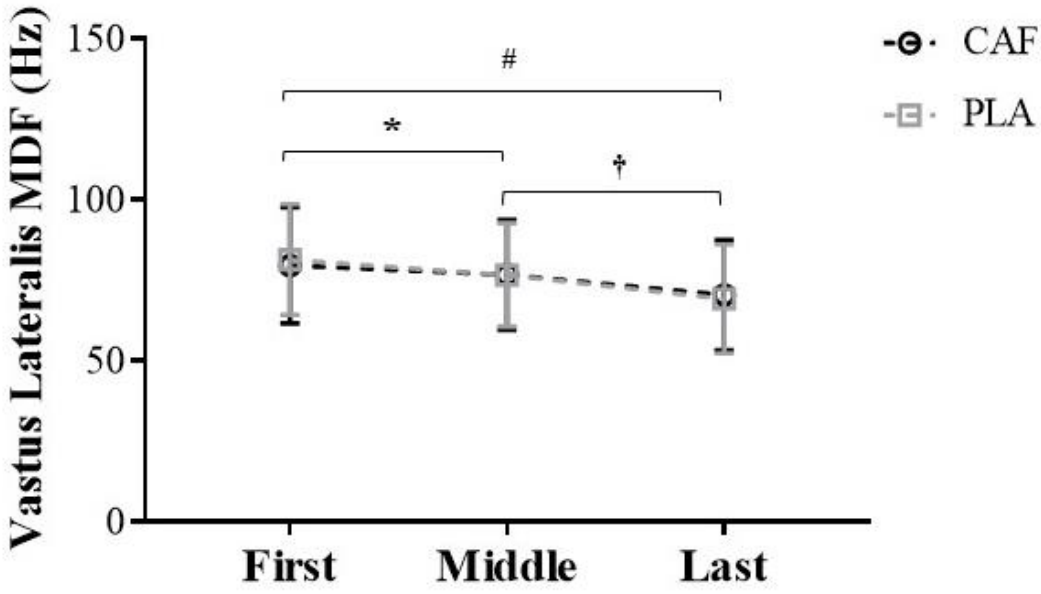

Figure 13. Visual representative of the mean *vastus lateralis* median power frequency (VL_{MDF}) ± SD at the first, middle, and last repetition of the fatigue protocols in the caffeine (CAF) and placebo (PLA) conditions.
*Indicates significantly greater VL_{MDF} at FIRST when compared to MID, when collapsed across condition.
†Indicates significantly greater VL_{MDF} at MID when compared to LAST, when collapsed across condition.
#Indicates significantly greater VL_{MDF} at FIRST when compared to LAST, when collapsed across condition.

4.4.7 VL_{AMP}

There was a significant main effect for Condition ($F_{1,26} = 4.754$; $p = 0.038$; $\eta^2 = 0.155$) for VL_{AMP}. Post-hoc paired samples t-tests found significantly higher VL_{AMP} during CAF when compared PLA (0.074 ± 0.053 μVs^{-1} vs. 0.051 ± 0.032 μVs^{-1}; $p = 0.038$), when collapsed across time. There was also a significant main effect for Time ($F_{1.0,27.2} = 48.237$; $p = <0.001$; $\eta^2 = 0.650$) for VL_{AMP}. Follow-up paired samples t-test

found that VL_{AMP} was significantly lower at FIRST when compared to MID (0.050 ± 0.036 μVs^{-1} vs. 0.062 ± 0.044 μVs^{-1}; p = <0.001), FIRST when compared to LAST (0.050 ± 0.036 μVs^{-1} vs. 0.076 ± 0.055 μVs^{-1}; p = <0.001), and MID when compared to LAST (0.062 ± 0.044 μVs^{-1} vs. 0.076 ± 0.055 μVs^{-1}; p = <0.001), when collapsed across time. No other significant interaction or main effects were found (p = >0.05).

4.4.8 *RF iEMG (RF_{iEMG})*

There was a significant main effect for Time ($F_{1.1,28.1}$ = 41.478; p = <0.001; η^2 = 0.615) for RF_{iEMG}. Post-hoc paired samples t-tests indicated significantly lower RF_{iEMG} values at FIRST when compared to MID (1.26 ± 0.63 µV vs. 1.54 ± 0.74 µV; p = <0.001), FIRST when compared to LAST (1.26 ± 0.63 µV vs. 1.88 ± 0.99 µV; p = <0.001), and MID when compared to LAST (1.54 ± 0.74 µV vs. 1.88 ± 0.99 µV; p <0.001), when collapsed across condition. No other significant interaction or main effects were found (p = >0.05).

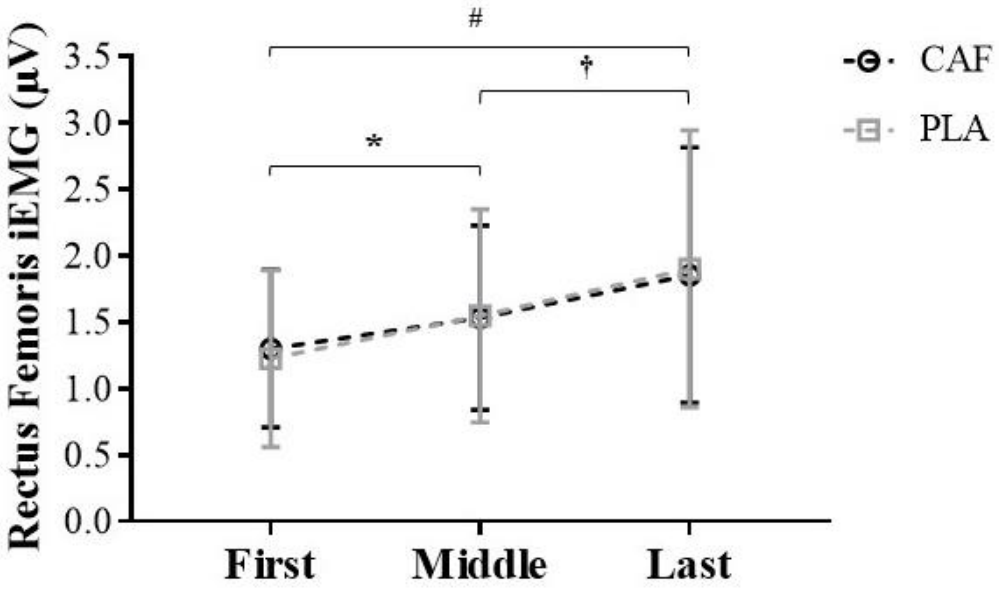

Figure 14. Visual representative of the mean *rectus femoris* integrated electromyography (RF_{iEMG}) ± SD at the first, middle, and last repetition of the fatigue protocols in the

caffeine (CAF) and placebo (PLA) conditions.
*Indicates significantly lower RF_{iEMG} at FIRST when compared to MID, when collapsed across condition.
†Indicates significantly lower RF_{iEMG} at MID when compared to LAST, when collapsed across condition.
#Indicates significantly lower RF_{iEMG} at FIRST when compared to LAST, when collapsed across condition.

4.4.9 RF MDF (RF_{MDF})

There was a significant main effect for Time ($F_{1.2,32.1} = 29.139$; $p = <0.001$; $\eta^2 = 0.528$) for RF_{MPF}. Follow-up paired samples t-tests indicated significantly greater RF_{MPF} at FIRST when compared to MID (76.7 ± 15.7 Hz vs. 72.8 ± 13.6 Hz; p = 0.002), FIRST when compared to LAST (76.7 ± 15.3 Hz vs. 66.1 ± 14.3 Hz; p = <0.001), and MID when compared to LAST (72.8 ± 13.6 Hz vs. 66.1 ± 14.3 Hz; p = <0.001), when collapsed across condition. No other significant interaction or main effects were found (p = >0.05).

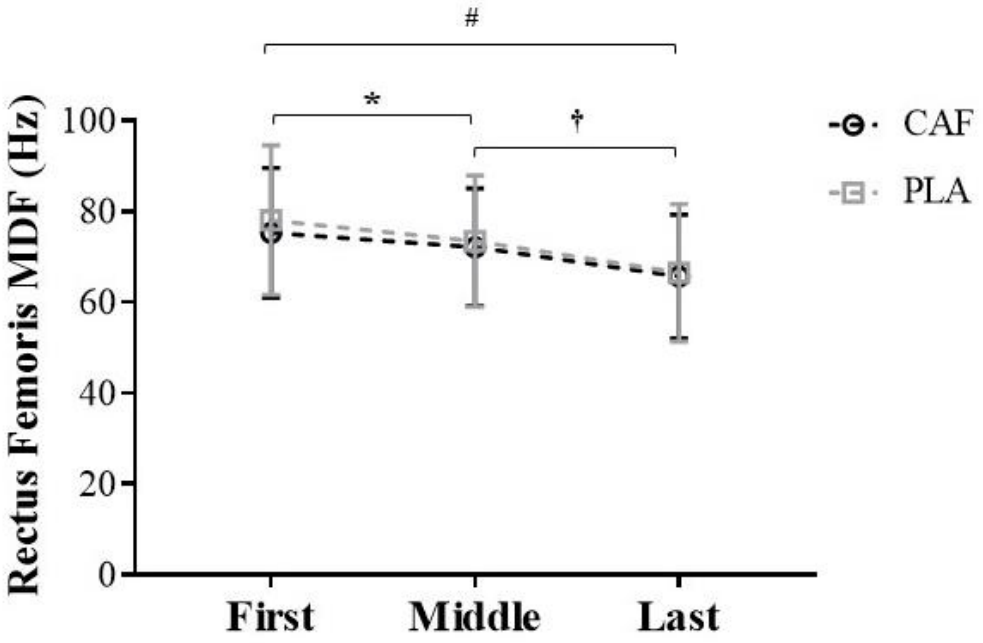

Figure 15. Visual representative of the mean *rectus femoris* median power frequency (RF_{MDF}) ± SD at the first, middle, and last repetition of the fatigue protocols in the caffeine (CAF) and placebo (PLA) conditions.
*Indicates significantly greater RF_{MDF} at FIRST when compared to MID, when collapsed across condition.
†Indicates significantly greater RF_{MDF} at MID when compared to LAST, when collapsed across condition.
#Indicates significantly greater RF_{MDF} at FIRST when compared to LAST, when collapsed across condition.

4.4.10 Normalized RF EMG AMP (RF_{AMP})

There was a significant main effect for Time ($F_{1.1,28.4} = 36.946$; $p = <0.001$; $\eta^2 = 0.587$) for RF_{AMP}. Post-hoc paired samples t-tests indicated significantly lower RF_{AMP} values at FIRST when compared to MID (0.041 ± 0.028 μVs^{-1} vs. 0.051 ± 0.034 μVs^{-1}; $p = <0.001$), FIRST when compared to LAST (0.041 ± 0.028 μVs^{-1} vs. 0.062 ± 0.045 μVs^{-1}; $p = <0.001$), and MID when compared to LAST (0.051 ± 0.035 vs. 0.062 ± 0.045; $p = <0.001$), when collapsed across condition. No other significant interaction or main effects were found ($p = >0.05$).

CHAPTER V

DISCUSSION

The purpose of this investigation was to examine whether genetic variation in CYP1A2 (-163A>C, rs762551) influences the effects of acute caffeine supplementation on neuromuscular function of the lower body at rest and in response to a fatiguing work bout. The main finding from this investigation was an overall lack of ergogenic effects of caffeine on neuromuscular function of the lower body musculature. However, the present data suggest that caffeine may augment the decline seen following rest in the placebo condition. These findings are fully discussed below at rest (section 5.1) and immediately following fatigue (section 5.2).

5.1 ***Neuromuscular Function: Pre vs. Post Supplementation***

Caffeine has been widely studied for its proposed ergogenic effects, especially prior to exercise. While several proposed mechanisms exist, caffeine's primary mechanism of action is thought to be through adenosine receptor antagonism. As adenosine receptors are located in a variety of tissues throughout the human body (Reppert et al. 1991), it has been hypothesized that caffeine may impact skeletal muscle through peripheral, spinal, and/or supraspinal mechanisms (Fimland et al. 2010; Kalmar 2005). Therefore, a variety of measurements were employed in the present investigation to elucidate the potential physiological mechanisms associated with caffeine supplementation.

In terms of exercise, caffeine is commonly used as an ergogenic aid and has been purported to increase muscular function, especially prior to resistance exercise (see review by (Grgic et al. 2018). However, to date, much of the data has been conflicting, especially in terms of muscular strength. For example, several investigations have reported no change in maximal strength (Behrens et al. 2015b; Fimland et al. 2010) following caffeine supplementation, with one investigator even reporting a decrease in maximal strength (Bond et al. 1986). On the contrary, a meta-analysis by Warren et al. (2010) reported a moderate effect (ES = 0.19) for maximal strength, which equated to roughly a 4% improvement over placebo. Interestingly, the authors performed a separate muscle-specific analysis, which indicated that caffeine may have a more potent effect on maximal strength in the quadriceps musculature (ES = 0.37), with an estimated 7% increase in maximal strength when compared to placebo (Warren et al. 2010). However, in the present investigation, we failed to show a significant improvement in quadriceps MVIC strength following caffeine administration. Caffeine did, however, offset the decline in MVIC seen in the placebo condition (Figure 1). While the present data exhibits "responders" and "non-responders" similar to the previous work of Meyers and Cafarelli (2005), the vast majority of subjects in the present study would be classified as non-responders to caffeine supplementation, as 24 out of 35 subjects analyzed had a decreased MVT following from CAF_{PRE} to CAF_{POST}. Thus, caffeine may have a potential utility in maintaining muscle function in settings of prolonged rest, such as prolonged traveling to events, reserve athletes in sporting events, etc. However, taken as a whole, the results of the present investigation do not support the efficacy of caffeine administration for improved muscular strength, contrary to much of the previous literature.

A large portion of the previous literature has suggested that augmented CNS activity is the most plausible explanation for the ergogenic effects reported by previous authors (Behrens et al. 2015a; Meyers and Cafarelli 2005; Plaskett and Cafarelli 2001; Tarnopolsky and Cupido 2000). More specifically, a number of authors have suggested this may be due to an increase in MU recruitment and/or increased MU firing rates (Bazzucchi et al. 2011; Kalmar et al. 2006). As the MU is the final pathway of the central nervous system and directly integrates with skeletal muscle, it is logical to hypothesize the improvements in skeletal muscle function may be facilitated through augmented MU behavior. However, our results indicate an overall lack of change in MU firing behavior following caffeine administration, when compared to pre-supplement or placebo conditions. Although the previous work of Walton and colleagues (2002) found a significant increase in self-sustaining firing rates of MUs in the tibialis anterior following caffeine ingestion, our results align with those of Meyers and Cafarelli (2005) and Kalmar and Cafarelli (1999), who reported no alteration in MU firing behavior following caffeine supplementation. Interestingly, each of these previous investigations used the same caffeine dosage as the present investigation (i.e. 6 mg/kg/bw). It is worth noting the potential for muscle specific alterations in MU behavior, as Walton et al. (2002) examined the tibialis anterior, while our work and that of Kalmar and Cafarelli (1999) and Meyers and Cafarelli (2005) examined MU behavior of the quadriceps musculature. However, it is premature to draw any specific conclusion until more data is available. Finally, it is important to acknowledge that in the present investigation, MVT was greater at CAF_{POST} compared to PLA_{POST}. Therefore, it appears there may be a reduced neuromuscular efficiency following PLA, as the same level of excitation to the

motorneuron pool, as evidenced by no significant changes in global EMG or MU firing properties, was required for a lower absolute force output.

Since its appearance in the literature by Merton (1954), the interpolated twitch technique (ITT) has become a common tool to assess muscle activation, or in simpler terms, the percentage of muscle subjects are able to voluntary activate (%VA; (Behm et al. 1996). Previous research has reported conflicting results, with several authors (Kalmar and Cafarelli 2004; Meyers and Cafarelli 2005; Tarnopolsky and Cupido 2000) reporting no change in %VA, and others reporting significant increases (Behrens et al. 2015a; Kalmar and Cafarelli 1999; Plaskett and Cafarelli 2001), including a meta-analysis by Warren et al. (2010) reporting a large (ES = 0.67) effect for caffeine on quadriceps %VA. However, similar to the previously discussed MVIC data, we failed to see any significant changes in %VA following either CAF or PLA in the present investigation. While not all of the subjects in the present investigation were resistance-trained, the population was at least recreationally active and exhibited a high degree of %VA at baseline (95.0 ± 4.0% when collapsed across genotype; (Herda et al. 2011). Thus, the potential for an increase in %VA following caffeine administration may have been limited. Thus, we failed to reported any increases in excitation following caffeine supplementation. Our data also indicate that there was no increase in spinal excitability following caffeine. Traditionally used as a surrogate for spinal excitability, the H-Reflex reflects the activation of the alpha-motorneurons by the Ia afferent pathways (Schieppati 1987). Previous literature has shown mixed effects for caffeine on H-reflex amplitude, with several investigations finding increased spinal excitability (Eke-Okoro 1982; Walton et al. 2003) and numerous reporting equivocal results (Behrens et al. 2015a; Behrens et al. 2015b; Kalmar and

Cafarelli 1999). Our results are in line with the later, in which we found no significant change in Soleus H-Reflex (when normalized to M-Wave) at any time point. It is important to note that no significant change in SOL M-Wave were found from pre- to post-testing in any condition, which indicates that the probe placement was not different during the SOL M-Wave measurement and a valid M-Wave measurement was achieved (Walton et al. 2003). In line with these findings, no significant change in RF M-Wave, VL M-Wave or Normalized EMG AMP were found from pre- to post-testing in either condition, which is consistent with the findings of (Kalmar and Cafarelli 2006). Thus, our data does not support the previous reports of increased neural drive or corticospinal excitability following caffeine supplementation.

The lack of change in spinal and supraspinal properties in the present investigation points towards peripheral mechanisms as the logical explanation for the maintained MVT following caffeine supplementation. Indeed, several previous investigations have reported enhanced skeletal muscle twitch properties following caffeine supplementation (Bazzucchi et al. 2011; Kalmar and Cafarelli 2006). However, it is crucial to acknowledge the decrease in nearly all evoked twitch properties 1-hour post ingestion of PLA. Indeed, the work of Bazzucchi et al. (2011), who utilized a very similar experimental design, also reported decreased twitch properties in the post-placebo condition. Additionally, while values for twitch properties were not reported, Kalmar and Cafarelli (1999) reported a decrease in MVC strength in the placebo condition, which may have been attributed to declining twitch properties. Although neither of these authors speculated on potential mechanisms for this decreased contractile function, we speculate this may be due to a decrease in muscle temperature following the 1-hour rest period, as

muscle decreased muscle temperature has been shown to alter muscle contractile properties (Davies and Young 1983; Holewijn and Heus 1992). Indeed, this hypothesis has been proposed previously by Fowles and coworkers (2000), who reported significant decreases in twitch torque following not only maximal passive stretching of the plantar flexors, but in the control condition as well. The authors speculated that the decrease in evoked twitch torque in the control condition was most likely due to a reduced muscle temperature (Fowles et al. 2000). However, muscle temperature was not recorded during the present investigation, and therefore, this hypothesis remains speculative. It is also important to acknowledge that caffeine supplementation maintained MVIC and contractile properties near baseline values, which is consistent with previous work in the quadriceps musculature by Behrens et al. (2015a). Thus, our data suggest that while caffeine did not significantly improve skeletal muscle function, caffeine clearly exhibited an ergogenic effect to offset the decline seen in the placebo condition. This has significant physiological implications, as alterations in calcium handling have been the most commonly proposed peripheral mechanism of caffeine and has been supported by numerous *in vitro* investigations. For example, *in vitro* evidence has suggested that there is an increase intracellular calcium from the sarcoplasmic reticulum and/or increased sensitivity of the muscle fibers with caffeine intake (Allen et al. 2008), potentially due to the interaction of caffeine with the ryanodine receptors of the sarcoplasmic reticulum (Penner et al. 1989). While we did not measure intracellular in the present investigation, our data provide non-invasive evidence of potential alterations in calcium handling at the contractile level, as evidenced by lack of decline in contractile function following CAF seen in the PLA condition. This is in contrast to a recent investigation, where authors not

only reported a lack of improvement in skeletal muscle twitch properties, but also proposed that toxic dosages of caffeine are needed to increase skeletal muscle function based on an animal model used in the same investigation (Neyroud et al. 2018). However, in the present investigation a particularly interesting change is seen in $-dt/dt_D$, where our data suggests a -7.4% slower $-dt/dt_D$ at PLA_{POST}, when compared to PLA_{PRE}, and a 0.4% increase in $-dt/dt_D$ was found from CAF_{PRE} to CAF_{POST}. Further support for altered twitch behavior can be found in Figure 9, where significant relationships we found between the percent change in contractile function and the percent change in MVIC. However, it is clear that these relationships are negatively shifted on both the x and y axes in the PLA condition. Thus, it seems logical that alterations in Ca^{2+} handling at the cellular level are most likely responsible for the lack of decline in MVT seen in the CAF condition. Interestingly, Meyers and Cafarelli (2005) reported a similar hypothesis when they reported that caffeine offset the decline in contractile function during fatigue, thus increasing time to fatigue. The authors reported the evoked twitch amplitude and the maximal relaxation rate were significantly correlated in both caffeine and placebo conditions, suggesting that the increase in time of fatigue may be partially explained by caffeine's effects on calcium reuptake and twitch force(Meyers and Cafarelli 2005). Finally, another potential mechanism of caffeine proposed by previous authors is increased conduction velocity Bazzucchi et al. (2011), which could potentially be a byproduct of increased calcium sensitivity at the muscle level. Future research is needed to clearly elucidate the mechanism of action before definitive conclusions can be drawn.

5.2 ***Fatigue***

Fatigue, in terms of exercise, is defined as a reduction of maximal muscle force or power (Taylor et al. 2000). As skeletal muscle force is modulated through the interplay of MU recruitment and rate coding (i.e. firing rate), it is well established during a fatiguing exercise bout, larger, higher-threshold MUs are progressively recruited as the smaller, lower-threshold MUs fatigue (Contessa et al. 2016; Muddle et al. 2018). This process, known as the size the principle (Henneman 1957), sets the foundation for the maintenance of force production throughout fatiguing contractions. While there are both non-invasive and invasive measures of MU behavior, the surface EMG signal represents a global activation of the muscle (Farina et al. 2004; Jenkins et al. 2016). In submaximal fatiguing efforts, such as the protocol used in the present investigation, a progressive increase in the EMG amplitude and integrated EMG are hallmarks of the fatigue process (Cifrek et al. 2009; Jenkins et al. 2015a; Naeije and Zorn 1982). Indeed, the present data display significant increases in normalized RF_{AMP} and VL_{AMP}, as well as RF_{iEMG} and VL_{iEMG} over the course of the fatigue protocol, independent of condition. Our results appear to be consistent with the size principle, as evidenced by the significant, positive relationship between in the MUAP vs. RT relationship. Our data also indicate an increase in the A term, along with the subsequent decrease in b term, of the MUAP vs. RT relationship across the fatigue protocol, indicating the progressive recruitment of larger MUs over time. Additionally, no significant differences were seen between conditions, indicating that caffeine does not appear to alter this process, or increase the recruitment of high-threshold MUs, as previous hypothesized (Bazzucchi et al. 2011). As the A term represents the theoretical MUAP of the smallest recruited MU, and the b term represents

the MUAP growth per unit of increase in RT (Miller et al. 2019), our data suggest progressively larger MUs and a slower growth rate were required to maintain the required force production through the fatigue protocol (Figure 4). While only a few exceptions to the size principle have been noted in the literature (Bawa and Murnaghan 2009; Bawa et al. 2006; Westgaard and De Luca 1999), there is conflicting literature on alterations in firing rate behavior during fatigue. For example, previous investigations have reported both increases (Contessa et al. 2009; Contessa et al. 2016; Muddle et al. 2018) and decreases (McManus et al. 2015; Mottram et al. 2005) in the firing rates of active MUs with fatigue. The lack of change in the slope or y-intercept of the MFR vs. RT relationship, along with the previously reported increased MUAP amplitude, suggest that both MU firing rates and recruitment modulation were utilized to maintain force production. Thus, our data provide support for the hypothesis of several previous investigators, who suggested an increase in excitation to the MU pool, and thus, higher MU firing rates, to counteract the decreasing twitch forces seen with the development of fatigue (Contessa et al. 2016; De Luca and Contessa 2015).

To further support this hypothesis, our data show significant decreases in twitch properties from pre- to post-fatigue, regardless of condition. For example, along with the significant reductions seen across time, we saw an approximate 56.9%, 52.4%, and 59.2% reduction in pTT_D, $+dT/dT_D$, and $–dT/dT_D$, respectively, when collapsed across conditions. As the doublet stimulations reflect the contractile ability of the muscle by removing the series elastic component (Herda et al. 2013), the present data represent clear declines in contractile function with fatigue (Dolmage and Cafarelli 1991). Consistent with previous fatigue literature, our data indicated a significant decrease in

VL_{MDF} and RF_{MDF} over the course of the fatiguing exercise protocol. Previous research has suggested that EMG median power frequency (MDF) is altered by fatigue, most likely through metabolite accumulation, in which conduction velocity is slowed (Beck et al. 2005; Bouissou et al. 1989; Brody et al. 1991; Hill et al. 2016). Thus, the significant decrease in EMG MDF seen in the present study supports the decline in skeletal muscle function following the fatiguing protocol. In concert with the decline in twitch properties, we reported a significant decrease in %VA following the fatigue protocol, independent of condition. This may lend support to central activation failure (Kent-Braun 1999) and/or locomotor fatigue (Amann and Dempsey 2008), as it appears both central and peripheral factors were present.

Previously literature has suggested that caffeine may increase time to exhaustion during submaximal exercise (Kalmar and Cafarelli 1999; Meyers and Cafarelli 2005; Pethick et al. 2018; Plaskett and Cafarelli 2001). However, the data in the present investigation does not support this, as evidenced by the lack of significant change in TQ_{IMP} between conditions. Despite TQ_{AVG} being significantly higher in the CAF condition, this did not manifest into increased time to fatigue or work performed, as measured through TQ_{IMP}. Thus, our findings are in agreement with Fimland et al. (2010), who found no ergogenic benefit during and recovery from a submaximal fatiguing exercise protocol. It is interesting to note that Pethick et al. (2018) reported a significantly slower loss of torque complexity in the caffeine condition, leading to an approximately 30% increase in time to fatigue. As TQ_{CV} can be considered a crude measure of complexity, it is interesting to note that TQ_{CV} was significantly lower in the CAF condition in the present investigation (Figure 10). However, this did not amount to an

increase in time to fatigue or total work performed, potentially due to decrements associated with central and/or peripheral fatigue mechanisms previously discussed.

5.3 CYP1A2 Genotype

The role of genetics in exercise and nutrition has recently become an area of interest for a number of investigations, with the lofty goal of providing a genetic link to the variable responses seen in human subjects research. Specific to caffeine, the CYP1A2 genotype has become the topic of numerous recent investigations. Interestingly, to date, the results of these investigations have been mixed, with some authors reporting genotype specific performance benefits ((Guest et al. 2018; Salinero et al. 2017; Womack et al. 2012), with others showing equivocal results (Algrain et al. 2016; Giersch et al. 2018; Puente et al. 2018). The results of the present investigation align with the later, as we reported no significant performance or neuromuscular differences between CYP1A2 genotypes. While %VA was significantly different between genotypes, this is most likely due to the more homogenous training background of the SLOW group, compared to the higher variability in the FAST group. Further, as differences in %VA were not attributable to caffeine consumption, there is no logical pathway by which the CYP1A2 genotype would alter %VA independent of caffeine consumption. Thus, the results of our investigation suggest that CYP1A2 genotype does not mediate neuromuscular function and fatigability of the quadriceps following caffeine consumption in college-aged males.

5.4 Conclusions

In contrast to much of the previous literature, the present investigation provides data that 6 mg/kg/bw of caffeine anhydrous provides limited ergogenic benefits to

exercise performance. However, our data suggests that caffeine alters peripheral skeletal muscle properties, as opposed to mediating muscle activation through spinal or supraspinal mechanisms as previously suggested. Based on these data, it can be hypothesized that caffeine may act through alterations in calcium handling and/or changes in calcium sensitivity at the muscle fiber level, as proposed by previous investigators. Additionally, the present data does not support the notion that the CYP1A2 genotype mediates the acute responses to caffeine in college-aged males. However, more data is needed before more definitive conclusions can be drawn.

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APPENDICES

Oklahoma State University Institutional Review Board

Date:	Thursday, August 10, 2017
IRB Application No	ED1788
Proposal Title:	Genetic Polymorphisms and the Effects of Caffeine on Neuromuscular Function
Reviewed and Processed as:	Expedited

Status Recommended by Reviewer(s): Approved **Protocol Expires: 8/9/2018**

Principal Investigator(s):

Nathaniel Jenkins
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Stillwater, OK 74078

Ryan J Colquhoun
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The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval. Protocol modifications requiring approval may include changes to the title, PI advisor, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2. Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of the research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Dawnett Watkins 219 Scott Hall (phone: 405-744-5700, dawnett.watkins@okstate.edu).

Sincerely,

Hugh Crethar, Chair Institutional Review Board

Approved: 07/26/2018
Expires: 07/25/2019
Protocol #: ED-18-97

VITA

RYAN JAMES COLQUHOUN

Candidate for the Degree of

Doctor of Philosophy

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