Effects of caffeine on muscle strength: Influence of genetics

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Abstract: Research into whether caffeine is ergogenic for muscle strength is uncertain at present. Furthermore, recent research has emerged suggesting that single nucleotide polymorphisms (SNPs) may influence caffeine response variation, thus influencing performance. Further research on these topics is required to conclude whether caffeine is ergogenic for muscle strength, and whether genotype influences the ergogenicity of caffeine. **PURPOSE**: To investigate the effects of caffeine (5 $mg \cdot kg^{-1}$) on muscle strength, muscle activity and neuromuscular function. To also investigate the effects of genotypes (AA or AC/CC) in the CYP1A2 gene on the aforementioned measures. **METHODS**: Using a double-blind, randomised, repeated crossover design, 16 participants (11 male, 5 female) completed four experimental trials; two caffeine, two placebo, in which muscle strength, muscle activity and neuromuscular function was assessed. Participants were also categorised according to their genotype (AA or AC/CC). **RESULTS**: Caffeine significantly increased hand grip strength ($P = 0.004$), voluntary activation (VA) ($P = 0.002$) and peak surface electromyography (sEMG) for the vastus medialis (VM) ($P = 0.02$). However, caffeine had no significant effect on knee extension strength ($P \geq 0.05$) or peak sEMG for the vastus lateralis (VL) ($P \geq 0.05$). **CONCLUSION:** The results of the present study indicate that a caffeine dose of 5 $mg \cdot kg^{-1}$ consumed 45-minutes prior to exercise is ergogenic for hand grip strength, VA and muscle activity for the VM. However, since no effect on knee extension strength and muscle activity for the VL was observed, further research into these parameters is required to understand the relationship between caffeine and muscle strength. Lastly, the present study found that genotypes in the CYP1A2 gene had no effect on caffeine response variation. However, no participants in the study were homozygous for the C allele, suggesting that future research should look to include a sample size consisting of all three genotypes.

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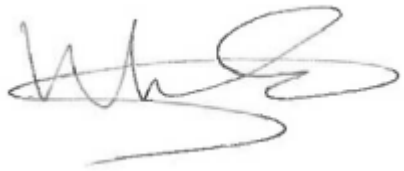
Declaration

“No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.”

Signed: 

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Definitions

Abbreviations	*Meaning*
SNP	Single nucleotide polymorphism
cAMP	Cyclic adenosine monophosphate
eNOS	Endothelial nitric oxide synthase
GI	Gastrointestinal
CMR	Caffeine mouth rinse
VA	Voluntary activation
MVC	Maximal voluntary contraction
1RM	One-repetition maximum
ES	Effect size
DNA	Deoxyribonucleic acid
VM	Vastus medialis
VL	Vastus lateralis
sEMG	Surface electromyography
M-wave	Compound muscle activation potential
LH	Luteinizing hormone

Chapter 1: Introduction

Caffeine (1,3,7 – trimethylxanthine), is one of the most widely used substances in the world. Found in coffee, teas, medications and many other products, caffeine is mostly utilized for its ability to stimulate the central nervous system, helping with alertness and preventing tiredness and fatigue (Graham, 2001). Used by both athletes and non-athletes alike, caffeine's influence on exercise performance is well-documented, with research dating back over 100 years (Rivers & Webber, 1907). Research investigating the impact of caffeine on performance covers a broad range of exercise modalities, including aerobic endurance, muscle strength, anaerobic power and muscle endurance (Grgic et al. 2020).

Caffeine has long been accepted to enhance performance in both aerobic and muscular endurance (Grgic et al. 2020). However, although the research regarding caffeine's effects on muscle strength shows promise for ergogenicity, the conclusion is still somewhat uncertain (Grgic et al. 2018). Caffeine is thought to enhance performance through multiple mechanisms: the antagonism of adenosine receptors, thus alleviating some perceptions of pain and fatigue; enhanced motor unit recruitment and firing rate and increased release of calcium from sarcoplasmic reticulum (Davis and Green, 2009; Gaspardone et al. 1995; Goldstein et al. 2010). Whilst the antagonism of adenosine receptors remains the most popular mechanism of action, research into the effects of genetics on caffeine response variation has emerged as an interesting and possible mediator for the effects of caffeine (Loy et al. 2015).

Research into how genetics may influence caffeine response variation has grown in popularity over recent years,especially with regards to its effects on caffeine supplementation for muscle strength performance. Single Nucleotide Polymorphisms

(SNPs) in the CYP1A2 gene influence how the body metabolises caffeine. The CYP1A2 gene is responsible for encoding the P450 1A2 enzyme, which is responsible for 95% of caffeine metabolism. The rs762551 SNP in the CYP1A2 gene can be used to classify individuals as either "fast" metabolisers (AA homozygotes) or "slow" metabolisers (C allele carriers) (Nehlig, 2018). However, the research on this topic is unclear at present. Rahimi (2019) found that individuals with the AA genotype experienced greater ergogenic effects from caffeine during muscle strength exercise when compared to their AC/CC counterparts. Whereas more recently, both Grgic et al. (2020) and Wong et al. (2021) both reported no significant effects of genotype on muscle strength performance.

This project aims to examine whether a caffeine dose of 5 $mg \cdot kg^{-1}$ is ergogenic for muscle strength, muscle activity and neuromuscular function. This aims to help further understand the presently unclear relationship between caffeine, muscle strength and neuromuscular activity. Moreover, a secondary aim of the study is to determine whether the rs762551 SNP in the CYP1A2 gene influences the response to caffeine for muscle strength, muscle activity and neuromuscular function. The first part of the literature review includes an overview of caffeine, including its mechanisms of action, side effects and response variation amongst individuals. The second part of the literature review provides a brief overview of the literature regarding caffeine's effects on exercise performance in general. The third part of the literature review details the relationship between caffeine and muscle strength, including an overview of muscle strength, the impact of caffeine on muscle strength performance, and the impact of caffeine response variation on muscle strength, with a section concluding the literature review at the end. The final section of the literature review contains the aims and hypotheses for the study.

Chapter 2: Literature Review

2.1 Caffeine

With research dating back to 1907 (Rivers and Webber, 1907), caffeine has long been established as an ergogenic aid in sport and is considered the most popular psychoactive substance in the world (Lee et al. 2020), utilized by both athletes and non-athletes alike. Caffeine is found naturally in fruit, leaves and beans; however it is also typically found in products such as soft drinks and energy bars (Reyes and Cornelis, 2018). The average daily caffeine intake for adults is 2.4 $mg \cdot kg^{-1}$, however daily doses of up to 400mg are considered safe for the general population, though doses of up to 12 $mg \cdot kg^{-1}$ have been used in exercise research (Jones 2017; Filip-Stachnik et al. 2021). The dose at which unfavourable side effects such as tachycardia, arrythmia and seizures is estimated to be 1.2 grams, whilst doses in the range of 10 to 14 grams and above are considered potentially life-threatening (Magdalan et al. 2017). 'Mild' side effects such as anxiety, insomnia and restlessness can occur at a much lower dose, which differs between individuals (Lara 2010).

Between 1984 and 2004, caffeine was on the World Anti-Doping Agency's (WADA) list of banned substances and in 1987, a urinary threshold of 12 μg/mL was implemented. However, caffeine use outside of competition was still common and once the substance was removed from WADA's named substance list in 2004, use in competition saw significant increases (Del Coso et al. 2011; Aguilar-Navarro et al. 2019).

Due to its extensive use, research interest in caffeine has increased over time to where it is now considered one of the most well-documented performance-enhancing aids (Graham, 2001; Grgic 2018; Grgic and Pickering, 2019). A number of meta-analyses

have been conducted to assess caffeine's ergogenicity on several exercise modalities . These include vertical jump height (Grgic et al. 2018), anaerobic power (Grgic 2018) aerobic endurance (Conger et al. 2011), muscle endurance (Polito et al. 2016), muscle strength (Grgic and Pickering, 2019), exercise speed (Christensen et al. 2017) and short-term high-intensity exercise (exercise between 100-150% of VO_{2max}) (Doherty and Smith, 2004).

Adding to the already rich literature on caffeine, several studies have also been conducted to examine caffeine's ergogenic potential in sport-specific tests. These sports include long-distance cycling (Ivy et al. 1979), rowing time trials (Bruce et al. 2000), resistance training (Davis and Green, 2009) and shot put throwing power (Bellar et al. 2012). Whilst the literature on caffeine covers a broad array of tests, there is significantly more research on aerobic endurance when compared to other types of exercise. This suggests that the future of caffeine research in sport should aim to bolster the literature on other forms of exercise where the effect of caffeine is uncertain.

2.1.1 Caffeine: Mechanisms of action

The effects of caffeine are thought to be facilitated through several different mechanisms: adenosine receptor antagonism, benzodiazepine receptor antagonism, phosphodiesterase inhibition and the release of calcium from intracellular stores (Myers et al. 1999). However, the dose of caffeine may influence what mechanisms take place.

Adenosine receptor antagonism

There are four documented G-protein-coupled adenosine receptors: A_1, A_{2A}, A_{2B} and A_3. Though the density and populations of these receptors may differ between individuals, these receptors are up regulated as caffeine intake increases (Martin et al. 2006). Due to their similar structures, caffeine is a strong antagonist of adenosine. The micromolar concentrations of caffeine obtained through low to moderate caffeine doses are known to block the A_1 and A_{2A} receptors (Rodak et al. 2021).

Adenosine is known to reduce the release of certain neurotransmitters in the central nervous system (CNS) and in animal studies, adenosine A_1 receptors are documented to inhibit the release of serotonin (Okada et al. 1999), acetylcholine (Rainnie et al. 1994), noradrenaline (Fredholm 1979) and glutamate (Yang et al. 2013). Both adenosine level and A_1 receptors have been shown to increase during periods sleep deprivation, this degrades the functioning of the cortex-basal ganglia-thalamus-cortex circuit and weakens both voluntary and involuntary attention, degrades the processing of sensory information, and impairs motor responses. Antagonism of these receptors thus alleviates the effects of daytime sleepiness and hypokinesia (Sil'kis 2014). This is potentially due to adenosine receptor antagonists, such as caffeine, theophylline and theobromine, reducing inhibitory signals to striatopallidal neurons whilst promoting the excitation of striatonigral neuronal projections (Rivera-Oliver and Díaz-Ríos, 2014; Jiang et al. 2019). Both of which are likely to contribute to the enhanced arousal and psychomotor activity accompanied with caffeine ingestion. As a result, the antagonism of adenosine receptors is thought to affect functions such as sleep, cognitive performance, memory and perceptions of fatigue (McLellan et al. 2016).

Lastly, a reduction in pain perception may be present due to adenosine receptor antagonism. When an individual produces a skeletal muscle contraction, adenosine

levels rise. This is because, during both maximal and submaximal muscle contractions, a state of muscle vasodilation occurs, of which adenosine is responsible for 20-40% of said vasodilation. Adenosine then proceeds to bind with A_1 receptors, which is the action that stimulates pain (Gaspardone et al. 1995). Therefore, once consumed, caffeine binds to A_1 and A_{2a} receptors which in turn limits the stimulation of pain, thus providing the user with analgesic effects (McLellan et al. 2016).

Benzodiazepine receptor antagonism

Caffeine has been shown to act as an antagonist to benzodiazepine receptors, thus influencing the effects of benzodiazepines throughout the body (Matilla et al. 1992). The effects of benzodiazepines are mostly sedative, as they act as a CNS depressant. Like adenosine, the antagonism of these receptors can influence factors such as sleep, cognitive function and perceptions of fatigue (Savage and Weilbo, 2005). However, research into animal models suggests that a very high dose of caffeine (20 $mg \cdot kg^{-1}$ +) is required for these effects to take place, and therefore, it is unlikely that any meaningful effect would take place in an *in vivo* human population (Nehlig et al. 1987).

Phosphodiesterase enzyme inhibitor

Research shows that caffeine acts, albeit weakly, as a phosphodiesterase enzyme inhibitor, which increases intracellular concentrations of cyclic adenosine monophosphate (cAMP) (Kalmar, 2005). cAMP has multiple functions, including the activation of protein kinase A, which phosphorylates enzymes used in glucose and lipid metabolism. cAMP also activates the hormone-sensitive lipase (HSL), influencing the

adrenaline cascade (Cappelletti et al. 2015). The role of caffeine and other phosphodiesterase inhibitors is to promote smooth muscle relaxation and vasodilation, thus improving blood flow (Boswell-Smith et al. 2006). However, it should be noted that the animal studies showing caffeine's ability to act as a phosphodiesterase inhibitor were using a supraphysiological dose (20 $mg \cdot kg^{-1}$) that would not be tolerable for humans (Tarnopolsky, 1994). Therefore, similarly to caffeine's role as a benzodiazepine receptor antagonist, it is unlikely that any meaningful effects will take place due to the inhibition of phosphodiesterase enzymes in an in vivo human population.

Calcium release from intracellular stores

Caffeine has also been used to increase calcium (Ca^{2+}) release from the sarcoplasmic reticulum, which may lead to a caffeine-generated muscle contraction with greater calcium concentrations (Supinski et al. 1984). Intracellular calcium is known to determine the activation of endothelial nitric oxide synthase (eNOS), thus producing higher amounts of nitric oxide. However, the increase in Ca^{2+} has been observed to be dose-dependent, with only doses greater than 8 $mg \cdot kg^{-1}$ increasing release and sensitivity to Ca^{2+}, making it unlikely that these effects will be present in an *in vivo* population (Ferreira et al. 2022; Klein et al. 1990; Daly, 2007).

2.1.2 Caffeine: Side effects

Caffeine is reported to have a number of side effects, including anxiety, tachycardia, heart palpitations, gastrointestinal (GI) distress, headaches, sleep disturbances and insomnia (Ramos-Campo et al. 2019; Pallarés et al. 2013). These side effects have the

potential to impact exercise performance both directly (occurrence during competition) or indirectly (impacting training, sleep deprivation etc.). Athletes should consider the side effects prior to consuming caffeine, for example, many athletes may already feel anxious prior to competition and caffeine ingestion could enhance that anxiety. Side effects may particularly affect athletes competing in a skill-based sport. This is because side effects such as caffeine-induced 'jitters' may hinder performance in sports such as tennis and golf (Pallarés et al. 2013). On the other hand, athletes that rely less on fine movements and more on physical capabilities, such as rugby players, may be able to utilise caffeine 'jitters' to their advantage (Pallarés et al. 2013; Graham and Spriet, 1995).

Mora-Rodríguez et al. (2015) suggest that whilst caffeine holds the potential for sleep disruption, the magnitude of this is determined by the timing of caffeine ingestion. The study found that caffeine ingestion during the morning only affected the sleep later in the day of one participant out of thirteen. The study accredits this to caffeine's half-life, which is generally around 4 to 6 hours. However, this varies between individuals, partly due to the aforementioned genetic polymorphisms influencing caffeine sensitivity and metabolism. Whilst caffeine may disturb sleep, it can also be used to mask the effects of sleep deprivation. A number of studies have assessed this, concluding that following sleep deprivation, caffeine has been shown to enhance vigilance, reaction time and physical performance (Kamimori et al. 2015; Bodenmann et al. 2012).

Spriet (2014) highlights that a key determinant in the prevalence of caffeine's side effects is the dose administered. Pallarés et al. (2013) noted that as caffeine dose is increased, both the likelihood of occurrence and magnitude of side effects may increase linearly . Therefore, although side effects can be serious, the likelihood of them

occurring can be minimised through using smaller doses, which have still shown to have ergogenic potential (Muñoz et al. 2020; Grgic et al. 2020; Yavuz, 2018). In fact, Spriet (2014) suggests that athletes should consider utilising smaller doses (~200mg) as they still appear to provide similar ergogenic effects to the moderate to higher doses, yet minimise the risk of side effects occurring.

2.1.3 Caffeine: Response variation

The effects of caffeine on exercise performance are well-documented. However, there is considerable variability in inter-individual response to caffeine. Factors such as genetics, source of caffeine and dose, have all been shown to impact the physiological responses associated with ingestion of caffeine.

Genetics

Some of this variation appears to be partly due to genetics, which can affect how caffeine is metabolised and therefore, how quickly it can take effect. Although single-nucleotide polymorphisms (SNPs) within the dopaminergic (DRD2 and COMT [Childs et al. 2008]), adenosine (AMPD1 [De Caterina and El-Sohemy, 2016]) and adrenergic (ADRA1A, ADRA2B etc. [De Caterina and El-Sohemy, 2016]) systems are thought to potentially affect response variation to caffeine ingestion, the ADORA2A and CYP1A2 are most commongenes used in caffeine research. The two are associated with caffeine sensitivity and metabolism, respectively.

The ADORA2A gene encodes for the adenosine A_{2A} receptor, which is associated with the regulation of myocardial oxygen demand and increased coronary circulation via

vasodilation (Higgins and Babu, 2013). Moreover, the receptor has been shown to influence dopamine release and regulate glutamate in the brain, which in turn may affect perceptions of pain (Fried et al. 2017). Previous research has identified that the A_{2A} subtypes of adenosine receptors represent the primary target of caffeine in the central nervous system (CNS), thus allowing for genetic variations in this gene to impact responses to acute caffeine ingestion (Cornelis et al. 2007). The rs5751876 SNP in the ADORA2A gene can be used to categorise individuals as either 'high' sensitivity (TT homozygotes) or 'low' sensitivity (C allele carriers). Interestingly, C allele carriers have been shown to have higher levels of habitual caffeine intake, suggesting that these individuals may need a higher caffeine dose to achieve the same physiological effect as those homozygous for the TT genotype (Cornelis et al. 2007). However, this is not certain as another reason for TT genotypes having a lower habitual intake could be due to a higher prevalence of side-effects/adverse responses due to their higher sensitivity, leading some to avoid caffeine (Alsene et al. 2003; Cornelis et al. 2007).

The first study investigating the influence of the ADORA2A genotype on caffeine ergogenicity was conducted by Loy et al. (2015). This study found that caffeine enhanced aerobic endurance for the TT genotype but not for the CC/CT genotype, concluding that C allele carriers should be identified as 'non-responders' to caffeine. However, this claim may only be relevant to aerobic endurance exercise as it is contested by Grgic et al. (2020) whoassessed the effect of caffeine supplementation on resistance exercise performance in C allele carriers. This study found that caffeine was indeed ergogenic for CC and CT heterozygotes, which challenges the belief that C allele carriers are 'non-responders'. However, alongside the aforementioned limitation of the differing types of exercise (endurance vs resistance), there were also no TT homozygotes included in Grgic et al. (2020)'s sample. To alleviate this limitation, Muñoz

et al. (2020) recruited a population of both TT homozygotes and C allele carriers in a study assessing the effect of caffeine on handgrip strength. However, this study found no between-genotype differences.

The CYP1A2 gene encodes for the P450 1A2 enzyme, which accounts for 95% of caffeine metabolism This process is responsible for the demethylation of caffeine into its metabolites paraxanthine, theobromine and theophylline (Begas et al. 2007). The rs762551 single nucleotide polymorphism (SNP) in this gene is used to categorise individuals as either 'fast' metabolisers (AA homozygotes) or 'slow' metabolisers (C allele carriers). It has been shown that 'slow' metabolisers have an increased risk of myocardial infarction and hypertension (Cornelis et al. 2006; Soares et al. 2018).

Guest et al. (2018) conducted a study which investigated the effects of two caffeine doses (2 and 4 $mg \cdot kg^{-1}$) and CYP1A2 genotype on cycling performance. This study reported that those carrying the C allele (slow metabolisers) were in fact negatively affected by the 4 $mg \cdot kg^{-1}$ dose, observed by a diminished cycling time trial performance , whilst receiving no significant effect from the lower dose. This is in contrast to the results found in those homozygous for the AA genotype (fast metabolisers) in which both ' caffeine doses enhanced performance. A number of exercise studies have also found that CYP1A2 genotype had no effect on performance (Puente et al. 2018; Salinero et al. 2017). However, such results can be found in studies utilising small samples sizes and/or missing an entire genotype categorisation (Pataky et al. 2016; Salinero et al. 2017. Due to the slower metabolism of caffeine in the C allele carriers, it is thought that the extended duration of vasoconstriction, which may harm performance. Alongside this, AA genotypes could experience a further advantagevia the downstream metabolites of caffeine, such as paraxanthine and theobromine, which may also have

ergogenic effects, and thus faster metabolisers will experience these effects sooner (Pickering and Grgic, 2019). With both the CYP1A2 and ADORA2A genes, it is unclear whether genotype variations influence caffeine's ergogenicity and thus requires further research, with emphasis on studies involving all genotypes and a variety of exercise modalities, to provide greater understanding of the ergogenic relationship between genotype and caffeine.

Habitual caffeine intake

Habitual caffeine intake is defined as the amount of caffeine an individual consumes in their regular day-to-day diet. Caffeine habitual intake can be hard to measure at times, this is because many commercially available products (coffee, pre-workout supplements etc…) have a large variability of caffeine content. For example, coffee products sold in retail shops have been shown to have differences of up to 8 to 9 times greater caffeine content than others sold in similar shops (Desbrow et al. 2012). For this reason, self-reporting caffeine intake can be unreliable. Habitual caffeine intake is also likely related to an individual's experience with side effects, such as feeling "jittery" and the incidence of sleep disturbances.

It is generally accepted that habitual intake has no significant effect on the ergogenicity of caffeine, as supported by a recent systematic review (Carvalho et al. 2022). A study by Gonçalves et al. (2017) employed a double-blind, crossover, counterbalanced design whereby 40 trained male cyclists were categorised into three groups by their habitual caffeine intake: low (58 ± 28mg), moderate (143 ± 25mg) or high (351 ± 139mg). The results of this study found that caffeine enhanced cycling time-trial performance for all three groups, yet habitual intake had no impact on caffeine's ergogenicity. However,

with a larger sample size for each category, a significant group interaction could have been observed, as 'low' habitual intake was around 60 seconds faster for the TT genotype when compared to the placebo, the 'moderate' group was around 90 seconds faster, but the 'high' intake group only saw an increase of around 20 seconds. Beaumont et al. (2017) found that in a population of "low" habitual caffeine consumers, caffeine tolerance was increased following four weeks of caffeine supplementation and diminished the ergogenic effect of caffeine on exercise The results of this study conflict with results reported by Lara et al. (2019) which found that caffeine's ergogenicity was maintained throughout twenty days of supplementation.

A limitation to be considered is the time of caffeine abstinence prior to trials. James (2014) suggests that in studies utilising a short abstinence period (12-48h), the results of the study may not reflect the impact of caffeine supplementation and effects of caffeine habituation on performance. Rather, the results may be influenced by the reversal of caffeine withdrawal effects. Therefore, future research may benefit from the inclusion of an abstinence period prior to trials and utilising a habitual caffeine intake criterion (e.g. 'moderate' intake) which could reduce the amount of variation in withdrawal symptoms. In contrast to this, there is also an argument to suggest avoiding a caffeine abstinence period. This is because it deviates from normal 'habitual' behaviour which could influence findings , suggesting that to reflect 'real life', participants should continue to follow their regular 'habitual' diet.

Caffeine dose timing

The most common timing of caffeine ingestion, in which plasma levels are thought to be at a peak, is 60 minutes prior to exercise (Graham, 2001). However, dose-timing is sport

dependent. This is because in sports where there is a build-up of fatigue, it may be beneficial to consume caffeine at later time, thus masking these effects of fatigue (Paton et al. 2010). However, ingesting caffeine 60 minutes before exercise is supported for muscle strength in a study by Harty et al. (2020). This study assessed muscle strength via isometric/isokinetic knee extensor testing, following caffeine ingestion at 120 minutes, 60 minutes and 30 minutes prior to exercise. The study found that caffeine ingestion at 60 minutes prior to exercise yielded the greatest results, likely due to plasma levels peaking around that time. However, the timing of caffeine ingestion depends on the caffeine form administered. Alternate sources of caffeine such as caffeine chewing gums have been shown to absorb faster than caffeine in capsule form, thus reaching peak plasma levels sooner (Ryan et al. 2013). The faster absorption in the buccal cavity when compared to the gut may be due to the extensive vascularisation in the region (Wickham and Spriet, 2018). First pass metabolism in the gut may also reduce the speed of absorption and overall effects of caffeine, meaning the avoidance of this process via absorption in the bucal cavity may also be beneficial to the efficacy of caffeine (Kamimori et al. 2002). However, a consequence of faster absorption could be the effects of caffeine finishing sooner than other forms. Additionally, with less research on caffeine chewing gums than other conventional forms such as caffeine anhydrous powder, further research is required to understand the optimal dose timings of caffeine chewing gums.

Caffeine source

The two most commonly consumed sources of caffeine are coffee and caffeine anhydrous powder (capsule form) (Trexler et al. 2016). However, alternate sources

such as chewing gums, mouth rinses, aerosols, inspired powders, energy bars, energy drinks and gels have grown in popularity (Trexler et al. 2016; Laizure et al. 2017; Guest et al. 2019). Laizure et al. (2017) highlights that the effects of caffeine may differ when consumed in unconventional forms such as inspired powders. This is due to the varying methods of absorption (buccal, gut etc.), which in turn influence the speed of metabolism and time to maximum plasma concentration.. Kamimori et al. (2002) assessed the rate of caffeine absorption and relative bioavailability for caffeinated chewing gums, in comparison to caffeine in capsule form. The results of the study found that caffeine in chewing gum form is absorbed significantly faster than caffeine in capsule form, however, both forms offer similar peak plasma levels. As previously mentioned, Kamimori and colleagues accredited the faster absorption to the substantial vascularisation in the buccal cavity (Kamimori et al. 2002).

Caffeinated gels are a popular supplement used by athletes, with its use prevalent in endurance events such as cycling due to its ease of application and digestability. Although the amount of research regarding caffeinated gels is less than that of other forms such as caffeine anhydrous powder, a few studies have been conducted to assess the effects of exercise performance following ingestion of the gels. Venier et al (2019) reported that 300 mg caffeine in gel form, ingested 10 minutes pre-exercise significantly increased muscle strength and anaerobic power. This is in contrast to other studies which found no effect of caffeinated gels (100 mg) on 2000 m rowing time trial performance when consumed 60 minutes prior to testing (Cooper et al. 2014; Scott et al. 2015). This suggests that although caffeinated gels may hold some ergogenic potential, timing of ingestion is a crucial factor to be considered. Since caffeine gels are likely to be absorbed primarily in the gut, the effects may take longer to occur (Wickham and Spriet, 2018). This means that whilst they are easy to use during exercise, in shorter exercise,

maximal plasma concentration may occur once the exercise has finished. Therefore, future research is needed to identify optimal timing of caffeine gel ingestion.

Caffeine mouth rinsing (CMR) has been highlighted as a promising method of caffeine ingestion. Bitter taste sensory receptors exist in the mouth, which are notably sensitive to caffeine. Poole and Tordoff (2017) propose that upon activation of these receptors, neural pathways in the brain associated with information processing and reward may be activated. Moreover, Wilson (2016) suggests that CMR may also reduce the aforementioned GI distress that could be caused by ingestion of other caffeine forms. Although Beaven et al. (2013) found that CMR with a 1.2% caffeine solution enhanced anaerobic power output in a repeated sprints test with cyclists, the findings of this are refuted by Clarke et al. (2015), which found that CMR had no influence on maximal strength in resistance-trained males. The uncertainty in CMR ergogenicity is likely due to CMR not increasing caffeine plasma levels, which Doering et al. (2014) suggests is needed to elicit an ergogenic effect. However, if the mechanism by which CMR works is only through the bitter taste sensory receptors, then anything with a bitter taste could elicit the same effect, meaning caffeine is not necessarily needed.

Similarly to caffeinated gels, caffeinated nasal sprays are gaining interest due to their ease of use. Arria et al. (2017) proposes that one mechanism of action for caffeinated nasal sprays could be due to the nasal mucosa being permeable, meaning that a small molecular compound such as caffeine may use the nasal cavity as a means for local and systemic application. Another proposed mechanism suggests that when ingested nasally, caffeine may be transported directly to the CNS via intracellular transport through the olfactory and trigeminal neural pathways (Phukan et al. 2016). Lastly, the nasal cavity possesses bitter taste receptors, similar to those of the oral cavity (Finger et

al. 2003). Therefore, the aforementioned activation of neural pathways in the brain associated with information processing and reward may be present with nasal sprays too. The ergogenicity of caffeine nasal sprays have been assessed in both aerobic and anaerobic events (De Pauw et al. 2017a; De Pauw et al. 2017b). Both of these studies reported that caffeinated nasal sprays had no significant effect on either aerobic or anaerobic performance.

Little research exists for caffeinated bars. However, absorption and delivery are expected to be similar to that of caffeine anhydrous capsules, gels or coffee (i.e. in the gut). Hogervorst et al. (2008) found that in a study investigating the effect of a carbohydrate bar with a caffeine content of 100mg on cycling performance, the bar significantly increased time to exhaustion. However, the study also found that the caffeinated bar had no effect on perceived exertion, average heart rate or perceived intensity. Nevertheless, although this research is promising, more research is required to fully understand the efficacy of caffeinated bars, with emphasis on doses and timing of doses.

Environmental factors

Environmental factors such as heat have been shown to influence caffeine's ergogenicity. This is because when used in elevated-heat environments, caffeine use is suggested to hold the potential to increase the risk of various dangers associated with exercising in hot temperatures concerning both internal temperatures and hydration (Nichols, 2014). Suvi et al. (2017) reported that when consumed in an environment consisting of 42°C heat and 20% humidity, caffeine ingestion increased both blood lactate and heart rate during constant-load walking exercise. In comparison, caffeine

has no significant physiological responses (RER, changes in blood lactate etc.) when ingested in non-hot conditions (Guest et al. 2021). However, the same study also found that caffeine had no effect on endurance capacity and thermoregulation. Therefore, the heat-elevated environment may have negated the ergogenic effect of caffeine. Moreover, Ely et al. (2011) observed that a high dose (9 $mg \cdot kg^{-1}$) of caffeine had no effect on body temperature during 30 min cycle ergometer exercise in 40°C heat.

Altitude is another environmental factor that could potentially alter caffeine's effects, due to added physiological challenges such as cardiovascular strain and thermoregulation (Guest et al. 2021). Whilst it is widely accepted that caffeine is ergogenic for exercise performance at sea level (Graham, 2001), minimal literature exists for caffeine's ergogenicity whilst in hypoxic conditions. The literature that does exist reports that in conditions ranging from 2000m to 4300m above sea level, moderate caffeine doses (4-6 $mg \cdot kg^{-1}$) significantly improved both time trial performances and time to exhaustion (Berglund and Hemmingsson, 1982; Fulco et al. 1994; Stadheim et al. 2015; Smirmaul et al. 2017).

2.1.3.1 Addressing interindividual variability

Betts and Gonzalez (2018) highlight that in exercise science, a common limitation in studies is a small sample size. Whilst it is also common to see a crossover design utilised to boost the scientific quality and value of an experiment, common methods of illustrating data such as the "dynamite plunger plot", may not accurately show the variability of individual responses (Drummond and Vowler, 2011). A method to discern true interindividual variability in responses can be to for participants to repeat treatment and control trials (repeated crossover design) (Senn et al. 2011). This was

proven by Goltz et al. (2018), who not only validated and proved the reproducibility of a previous study (King et al. 2017), but observed that interindividual variability was present in the results. As previously mentioned (see 2.1.3), genetics is a large factor in interindividual variability in responses and therefore, methods to address this not only boost the scientific quality of the study, but also provide further understanding to the underlying mechanisms behind responses. Betts and Gonzalez (2018) note that other methods to boost scientific quality may be limited by available equipment or cost, whereas whilst utilising a repeated crossover design may be more time-consuming, it does not require additional equipment or expertise.

2.2 Caffeine and exercise performance

As previously stated, the effects of caffeine on exercise performance are well recognised, with aerobic endurance being the most-documented type of exercise A number of reviews and meta-analyses have been conducted to examine the effects of caffeine on aerobic endurance, the majority of which report ergogenic effects of caffeine (Southward et al. 2018; Shen et al. 2019; Conger et al. 2011; Glaister and Moir, 2019). However, a meta-analysis by Gonçalves Ribeiro et al. (2017) reported no significant effect of caffeine on aerobic endurance, although this must be viewed with caution as only two studies in this meta-analysis reviewed aerobic endurance, both utilizing maximum distance running tests.

Caffeine's ergogenic potential for muscle endurance is also well-documented. Muscle endurance is defined as the ability of a muscle, or group of muscles to perform repeated contractions over a period of time sufficient to produce fatigue (Kojima et al. 2020). Both Polito et al. (2016) and Warren et al. (2010) conducted meta-analyses, including

16 and 23 studies respectively. Both meta-analyses reported that caffeine had a significant ergogenic effect on muscle endurance. A number of meta-analyses have also been conducted to identify caffeine's ergogenicity for anaerobic power, which is defined as the ability to generate force in relation to time (force × distance/time) (Ramos et al. 2013). Grgic (2018) found that caffeine ingestion enhanced both mean and peak power output, whilst including 16 studies in the analysis. However, Gonçalves Ribeiro et al. (2017) stated that caffeine had no significant effect on anaerobic power, in an analysis consisting of four studies. This is supported by Glaister et al. (2012), who reported that irrespective of dosage used, caffeine has no significant effect on short-duration sprint cycling performance.

The structure of caffeine is similar to that of many endogenous metabolites in the body. Since it is water- and lipid-soluble, it is able to be transported around the body in intracellular fluids, allowing it to cross the blood-brain barrier (Arnaud et al. 1987). For this reason, caffeine is able to influence many sites in the body, such as the CNS, skeletal muscle, smooth muscle and cardiovascular system (Thompson, 2002). Due to these physiological capabilities of caffeine, the relationship between caffeine and neuromuscular fatigue is a popular topic of research, which aims to determine caffeine's ability to prevent a deterioration in exercise performance. Central fatigue is defined by a decrease in voluntary activation (VA), and the relationship between caffeine and VA is well-documented, which suggests that caffeine ingestion significantly increases VA, thus helping prevent the effects of central fatigue (Sun et al. 2022). As found in many recent studies, VA is significantly decreased after endurance exercise.However, caffeine ingestion has been shown to slow the rate of decline in time-to-exhaustion exercise (Dittrich et al. 2021; Mariano et al. 2019). In contrast to this, Mesquita et al. (2020) reported that caffeine had no effect in slowing the decline in VA during single-limb

MVCs of the triceps surae. Behrens et al. (2015) suggests that caffeine may affect voluntary activation due to enhanced neural drive to the knee extensors, thus generating greater explosive voluntary strength. However, to further understand the relationship between caffeine and voluntary activation, further research is required. Moreover, the research should look to clarify the mechanisms causing these effects.

2.3 Caffeine and muscle strength

Whilst the literature on caffeine supplementation for muscle strength performance is growing, the conclusion as to caffeine's ergogenicity is uncertain. Caffeine is thought to affect muscle strength via mechanisms such as adenosine receptor antagonism However, other factors such as SNPs in genes may also impact caffeine's ergogenicity for muscle strength performance.

2.3.1 Muscle strength

Jones and Comfort (2020) defines muscle strength as "the ability to exert force on an external resistance". Numerous studies have proposed that the primary factor in producing a successful movement of a body or external force for an athlete is muscle strength (Styles et al. 2016; Seitz et al. 2014; Nimphius et al. 2010). Since many sporting scenarios require an athlete to manoeuvre their body or an external object against gravity, the relationship between muscle strength and performance is well-researched. This research suggests that muscle strength can influence both rate of force development and power output, which are considered two of the most significant factors in an athlete's performance (Stone et al. 2002). Common approaches used to

quantify muscle strength include MVCs completed via dynamometry, or one-repetition maximum (1RM) tests.

Muscle strength is determined by a number of neural, anatomical, muscular and technical factors. Strength is largely influenced by the amount of contractile proteins available. ACSM (2009) describes this as an individual's muscle size and architecture, suggesting that greater strength production will increase linearly with greater amounts of contractile proteins (larger muscles). Next, motor-unit recruitment and firing rates have been identified as key determinants of muscle strength by Rønnestad et al. (2010). Motor-unit recruitment describes the amount of the muscle being activated during a contraction and firing rate is defined as how frequently the motor-units are triggered. Thus, a greater firing rate will lead to greater force production. Rønnestad et al. (2010)highlights how these three mechanisms may work together simultaneously, as greater muscle size will allow for greater motor-unit recruitment, which in turn can be trained to reach a greater motor-unit firing rate. Motor control has also been shown to be a determinant of muscle strength. Ramos-Campo et al. (2021) describes this as an individual's ability to coordinate their muscles, suggesting that optimal strength requires the control of antagonistic muscles to minimise activity.

2.3.2 Impact of caffeine on muscle strength performance

In a systematic review by Grgic et al. (2020), three out of the four meta-analyses reviewed found that caffeine had an ergogenic effect on muscle strength. A meta-analysis by Warren et al. (2010), which consisted of 34 studies (27 of which investigated caffeine's effects on MVCs) found that caffeine enhanced muscle strength performance. Furthermore, it was found that the effects of caffeine on smaller muscle

groups such as the forearms were less prevalent than the effects produced on larger muscle groups such as knee extensors. This could potentially be due to differences in muscle activation. During an MVC, smaller muscle groups such as the ankle plantarflexors typically elicit voluntary activation levels of 90-99%, compared to larger muscle groups (e.g., the knee extensors) which usually elicit voluntary activation levels of 85-95% (Shield & Zhou, 2004). Since it appears that knee extensors are less 'readily activated' than the plantar flexors, there seems to be greater scope for caffeine to have an ergogenic effect in the knee extensors than the smaller groups. This is supported by Black et al. (2015), who reported a baseline voluntary activation of 97% in the elbow flexors and 83% in the quadriceps femoris. The study then found a significant increase in quadriceps femoris voluntary activation following caffeine ingestion; however, no effect was found on elbow flexors. Therefore, Warren et al. (2010) suggests that increased motor unit recruitment and enhanced central excitability may be exhibited primarily in the knee extensors.

A subgroup meta-analysis by Warren et al. (2010) comparing caffeine's effects on small and larger muscle groups has found that muscle group size and location had a significant effect on MVC strength. The analysis reported that the effect sizes in studies examining large (ES = 0.31) or lower-body (ES = 0.29) muscle groups was significantly greater than those reported in studies utilising small (ES = 0.05) or upper-body (0.03) muscle groups. These findings are also supported by Timmins and Saunders (2014), which found that an increase in isokinetic peak torque following caffeine ingestion increased linearly with muscle group size. However, future research should look to study this further by assessing a variety of muscle group sizes. Alongside enhanced motor-unit recruitment, Grgic and Pickering (2019) suggest that caffeine's mechanism of adenosine receptor antagonism may benefit muscle strength. As aforementioned,

caffeine's role as an adenosine receptor antagonist may provide analgesic effects. Throughout this meta-analysis, only one study investigated the effects of caffeine on muscle strength whilst taking perceptions of pain into account. Tallis and Yavuz (2018) found that caffeine doses of 3 $mg \cdot kg^{-1}$ and 6 $mg \cdot kg^{-1}$ significantly increased maximal strength of the knee extensors However, the study also found that caffeine had no significant effect on pain perception. The results of this study indicate that the increased strength was due to a different mechanism of action. One such mechanism could be the enhanced intracellular calcium release. It is theorized that this increases cross-bridge attachment which in turn enhances force production (Herrmann-Frank et al. 1999). However, it is unlikely that this mechanism is in action due to the dosages used not being high enough. It should be noted that the literature covering this is limited, meaning no conclusion can be drawn until a greater body of evidence is obtained.

Grgic et al. (2018) analysed the difference between the ergogenic effects of caffeine for upper body and lower body strength. This meta-analysis found that caffeine significantly increased upper body strength, but not lower body. The results of this analysis contrast those found by Warren et al. (2010), which suggests that larger muscles, such as the quadriceps femoris, have a motor-unit recruitment potential than smaller muscles such as the elbow flexors. Alongside the aforementioned mechanisms of action, the effects of adenosine on neurotransmission and arousal are thought to be underlying mechanisms which drive caffeine's ergogenic effects (Davis and Green, 2009). In the meta-analysis by Grgic et al. (2018), lower body strength was measured using 1RM tests for the machine leg press and free-weight squat exercises. This differs to the studies analysed by Warren et al. (2010), which investigated lower body isometric strength. This suggests that caffeine has a greater effect on isometric strength than dynamic strength, however, further research is required to validate this.

Polito et al. (2016) conducted a meta-analysis which reports that caffeine had no significant effect on muscle strength, but significantly increased muscle endurance. Continuing with the previous mechanism mentioned above, Polito and colleagues recognize that caffeine may increase intracellular calcium release to the muscle fibres but suggest the possibility that slow-twitch fibres may be more sensitive to this mechanism than fast-twitch fibres. Whilst this theory is supported by Davis and Green (2009), a greater body of evidence exists to suggest that caffeine does in fact enhance muscle strength performance (Warren et al. 2010; Grgic and Pickering, 2019). It should be noted that Polito et al. (2016) exclusively analysed isotonic exercises, with a limited number of trials. This suggests that the results from Polito et al. (2016) may differ to the aforementioned meta-analyses due to the lack of exercise test variety used in the analysis. Whilst this is not certain, an argument exists to suggest that if the exercise tests used across studies are not consistent, results may be influenced by the varying requirements of skill across tests. Moreover, all studies analysed by Polito et al. (2016) were very similar in their inclusion criteria. All participants were for the most part, young men with experience in resistance training, which prevents the analysis of a number of potential moderators. The effect of training status, sex and age cannot be identified in these results.

Each of these factors have shown to influence muscle strength, and therefore must be accounted for (Benton et al. 2013; Jones et al. 2021; Keller and Engelhardt, 2013). Temple and Ziegler (2011) found that women are less sensitive to the ergogenic effects of caffeine than men due to changes in circulating steroid hormones. However, due to the lack of female participants used in the studies, Polito et al. (2016) were not able to analyse this. Moreover, it has been found that training status can significantly affect caffeine's ergogenicity for other exercise modalities, as shown in swimming, with

greater results seen in trained individuals when compared to those untrained (Collomp et al. 1992). Due to the influence of these factors, future research should look to incorporate these measures as a control.

2.3.3 Impact of caffeine response variation on muscle strength performance

As previously mentioned, the CYP1A2 and ADORA2A genes may play a role in caffeine's ergogenicity for muscle strength performance. Grgic et al. (2020) found that both CC and CT genotypes for the ADORA2A gene elicited significantly greater results in resistance exercise following caffeine ingestion (3 $mg \cdot kg^{-1}$) in comparison to a placebo. Furthermore, Muñoz et al. (2020) found that caffeine ingestion of the same dose (3 $mg \cdot kg^{-1}$ also elicited greater hand grip strength in comparison to a placebo but found no between-genotype differences for TT homozygotes and C allele carriers. This suggests that although caffeine may be ergogenic for all genotypes in the ADORA2A gene, it is unclear whether a specific genotype experiences greater ergogenic effects than others following caffeine ingestion. Similarly, Grgic et al. (2020) also investigated the effect of the CYP1A2 genotype on bench press 1RM performance following 3 $mg \cdot kg^{-1}$ caffeine ingestion. This study also found that caffeine enhanced 1RM, but no between-genotype differences were observed for AA heterozygotes and C allele carriers.

2.3.4 Conclusion

To conclude, research suggests that caffeine may be ergogenic for muscle strength performance, but also for enhancing neuromuscular function, in the form of voluntary activation (Pickering and Grgic, 2019). However, due to some conflicting results on these topics, further research is needed to provide a greater understanding for the

relationship between caffeine, muscle strength and neuromuscular function. Furthermore, caffeine's uncertain ergogenic potential for muscle strength could be theorised due to the variations in caffeine responses. The factors that could mediate inter-individual differences in caffeine response include environmental factors, menstrual cycles and genetics (Pickering and Kiely, 2019; Guest et al. 2021). Therefore, further research into these factors too could play a vital role in understanding caffeine role in enhancing muscle strength performance.

2.4 Aims and hypotheses

The present study has two aims:

Primary aim:

To identify whether ingestion of 5 $mg \cdot kg^{-1}$ caffeine enhances muscle strength.

Secondary aims:

To identify whether ingestion of 5 $mg \cdot kg^{-1}$ caffeine enhances muscle activity and neuromuscular function.

To identify whether the effects of caffeine on muscle strength, muscle activity and neuromuscular function are influenced by genotypes in the rs762551 SNP within the CYP1A2 gene.

Hypotheses: It is hypothesised that ingestion of 5 $mg \cdot kg^{-1}$ caffeine 45 minutes prior to exercise, will significantly increase muscle strength performance, muscle activity and neuromuscular function.

It is also hypothesised that AA genotypes within the rs762551 SNP will experience significantly greater effects from caffeine than C allele carriers due to their faster metabolism of the supplement.

Chapter 3: Methodology

3.1 Participants

The present study recruited sixteen participants (11 male, 5 female); participant demographics are presented in table 5.1. Of the sixteen participants, nine (7 male, 2 female) completed all tests, whilst the remaining seven only completed hand grip tests. All participants were physically active and experienced with resistance training. All participants were free from any diseases (cardiovascular, metabolic etc.), illnesses or injuries, and we're deemed 'low risk', as determined by a pre-screening health questionnaire. Prior to testing, all participants were given a participant information sheet detailing all procedures demonstrated in the study and provided their written informed consent. All procedures demonstrated in this study were carried out in accordance with the Declaration of Helsinki and were approved by the University of Kent Research Ethics Advisory Group.

3.2 Study design

The study consisted of hand grip strength tests and MVCs of the knee extensors performed throughout five visits (1 familiarisation, 4 experimental trials). The study employed a double-blind, placebo-controlled repeated crossover design, with the aim to investigate the effects of caffeine on muscle strength, muscle activity and neuromuscular function, whilst also identifying the influence of genotypes on the effects of caffeine. Experimental trials began a minimum of three days after completion of the familiarisation visit and all trials were separated by a minimum of two days. All caffeine and placebo trials were completed in a randomised order. Using the saliva samples

obtained, DNA was extracted to be used for the analysis of the rs762551 SNP in the CYP1A2 gene. Participants were then categorised by their genotype (AA homozygotes; C allele carriers), so that between-genotype differences could be identified.

3.3 Preliminary measures and familiarisation

Firstly, participant's anthropometric data was recorded. Height was measured to the nearest 0.01 m and body mass was recorded to the nearest 0.1 kg. Following this, muscle strength was recorded through isometric knee extension MVCs performed on an isokinetic dynamometer (Cybex; HUMAC Norm; CSMi, Massachusetts, USA) and muscle activity of the vastus medialis (VM) and vastus lateralis (VL) was recorded using surface electromyography (sEMG). Initially, participants were verbally informed of the procedures to follow, and the correct dynamometer seating position was established and recorded. Then, using their right leg, participants completed a standardised warm up on the dynamometer, consisting of ten contractions, three seconds on and three seconds off, at 50% of perceived maximum intensity. Following this, participants performed a series of five MVCs with 60 s rest between contractions, measuring the maximal force output of their knee extensors. Each MVC lasted 5 s and at the peak torque of the final two contractions, a superimposed doublet was delivered to the femoral nerve, thus generating a supramaximal muscle contraction. Following each stimulated contraction, once the participant was fully at rest, another doublet was administered to the femoral nerve. Throughout the test, participants were given standardised verbal encouragement (e.g. "one, two, three, push, push, push"). Following all MVCs, the mean of the two greatest MVCs was used to establish the baseline results

for MVC torque. In the visits performing both tests, participants were given a five-minute rest period between tests.

Hand grip strength was measured using an MIE Digital Pinch/Grip Analyser (MIE Medical Research Ltd, Leeds, UK) to perform maximal hand grip MVCs. Once participants had been verbally instructed, a standardised procedure was followed using the protocol demonstrated by Sunderland et al. (1989) (see below). Following this, similarly to the previous test, participants performed a series of MVCs to measure grip strength. Participants completed three MVCs lasting five seconds each, with 60 s rest between attempts. Similar standardised verbal encouragement was used (e.g. "one, two, three, squeeze, squeeze, squeeze"). The mean of the two greatest MVCs was used to establish the baseline maximal hand grip strength measurement. Completing both tests as minimum of three times as to familiarise the participants with the test. At the end of this visit, participants then provided a stimulated saliva sample of approximately 1 mL (see 'Saliva collection' section below).

3.4 Experimental trials

Experimental trials consisted of four laboratory visits: two caffeine trials and two placebo trials, in a randomised order. Prior to all experimental trials, participants were required to abstain from caffeinated or alcohol foods, drinks and supplements for 24 hours and avoid strenuous physical activity for the 48 hours before each trial. Participants were given food and drink diaries to record their diets during the 24 hours prior to the first experimental trial and were then asked to replicate that diet for all subsequent trials. All trials took place roughly around the same time of day for each participant (morning [9-11am] or afternoon [12-4pm]), although all trials were

separated by a minimum of two days. Initially upon arrival to the laboratory, participants performed the same series of submaximal and MVCs demonstrated in the familiarisation trial (warm-up, unstimulated and stimulated). Following this, participants consumed a capsule containing either 5 $mg \cdot kg^{-1}$ body mass of caffeine anhydrous powder (Food Grade; Sigma-Aldrich, Missouri, USA) or 5 $mg \cdot kg^{-1}$ body mass of microcrystalline cellulose (Life Lab Ltd.; Lancashire; UK) , depending on whether they were assigned caffeine or placebo on that trial, respectively. Capsule ingestion was accompanied by 3 $mL \cdot kg^{-1}$ body mass of water. Following this, participants remained seated, resting for 45 minutes. They were permitted to consume as much water as they would like, though any water consumption in the first trial was replicated on all following trials. No other food or beverages were permitted during this period of seated rest.

Participants then repeated the series of MVCs performed both prior to capsule ingestion and in the familiarisation trial. Participants also taking part in the hand grip strength test then rested for 5 minutes before commencing with the second test. Following this, participants were verbally instructed on the procedures to follow again, and performed a series of three hand grip MVCs, as performed previously.

3.5 Saliva collection

Upon completion of the familiarisation trial, participants provided a stimulated saliva sample, approximately 1mL collected in a Sterilin 7mL Polystyrene Bijou Container (ThermoFisher Scientific, Waltham, Massachusetts, USA) and aliquoted into 1.5mL Eppendorf tubes (Eppendorf, Hamburg, Germany). These tubes were then spun in a centrifuge at 13,000 RPM for 5 minutes. Following this, the supernatant obtained was

aliquoted into Eppendorf tubes and frozen at -80°C for later analysis. The cellular component obtained from the samples was then discarded.

3.6 CYP1A2 genotyping

Using the supernatant obtained from the saliva samples, DNA was extracted using the Zymo Research Quick-DNA Miniprep Kit (Zymo Research; Irvine; California; USA). the extracted DNA was then analysed for genotyping of the rs762551 SNP in the CYP1A2 gene. Genotyping was performed using rhAmp assays (Integrated DNA Technologies, Coralville, Iowa, USA) on a LightCycler 96 real-time PCR system (Roche; Basel; Switzerland). Using the analysis from calculated by the LightCyler 96 Application Software, participants were categorised into two groups: 'Fast' metabolisers (AA homozygotes) and 'Slow' metabolisers (C allele carriers).

3.7 Isokinetic dynamometry

Throughout all visits, an isokinetic dynamometer (Cybex; HUMAC Norm; CSMi, Massachusetts, USA) was used and calibrated according to the manufacturer's instructions. To ensure no superfluous muscle activation occurs, participants were secured in the dynamometer with their torso and waist secured. Then, the the right leg was attached to the lever arm, making sure that the axis of rotation of the lever arm was in line with the lateral epicondyle of the femur. Hip and knee angels were set at 85° and 90° respectively, and full extension was set at 0°. Using the padded Velcro strap, the lower right leg was secured to the lever arm, positioned above the malleoli. The dynamometer seated position and chair measurements were then recorded for each participant individually, to then be replicated on all subsequent trials.

3.8 Hand grip dynamometry

For all data collection, an MIE Digital Pinch/Grip Analyser (MIE Medical Research Ltd, Leeds, UK) was used. The standardised procedure by Sunderland et al. (1989) required participants to be seated with their right hand resting on their right leg. The analyser was then placed in the participants' hand, with a marked point on the bar touching the web of skin between the participant's index finger and thumb. This marked location remained the same for everyone. The dynamometer was positioned so that the participant could not see the digital display. Before the test began, the digital display was reset to 0, in case of any involuntary flexion of the fingers caused by the positioning of the hand. The participants were then instructed to squeeze their hand as hard as they could for 5 s, during which the peak torque was recorded. The procedure and position were followed strictly.

3.8 Surface electromyography (sEMG)

The study used 37.5mm × 37.5mm Ag/AgC1 electrodes (Whitesensor 4821Q, Ambu Ltd, Denmark) at an inner-electrode distance of 37.5mm to perform bipolar surface electromyography, measuring the activity of the VL and VM. Electrodes were placed on the VL and VM muscle bellies proximal to the knee and parallel to the fibres of the muscles, following the SENIAM guidelines. The electrode locations were shaved, abraded and cleaned prior to application to reduce interference and the sites used were recorded and marked for replication in following trials. All data was recorded at a frequency of 2.5 kHz and amplified (gain 1000 for both VL and VM) using a signal amplifier (EMG2-R, Biopac Systems, California, USA and EMG100c, Biopac Systems,

California, USA) prior to being band pass-filtered (10-500 Hz) and recorded with Spike2 software (Spike2 Version 7; Cambridge Electronic Design). Quantitative data was extracted from Spike2 and peak sEMG values were manually selected.

3.9 Peripheral nerve stimulation

Peripheral nerve stimulated was performed using an electrical stimulator (DS7r, Digitimer, Hertfordshire, UK) (maximum voltage = 40 v) capable of producing a single square wave pulse. An adhesive electrode (100 mm × 50 mm; Phoenix Healthcare Produces Ltd, Nottingham, UK) was used as the anode, applied to the gluteal fold, and a motor point pen (Motor Point Pen; Compex, DJO Global, Guildford, UK) was used as the cathode, placed on the femoral nerve. The motor point pen was used to test muscle sites in the femoral triangle, to determine the location capable of producing the greatest twitch force and compound muscle activation potential (M-wave) peak-to-peak amplitude. Once located, a 37.5 mm × 37.5 mm electrode (Whitesensor 4821Q, Ambu Ltd, Denmark) was applied to the site, to ensure all stimulation occurred on the same location. The electrode location was marked and standardised for replication on all following visits. The intensity needed for a supramaximal contraction was calculated by stimulating the site, starting at 100 mA. This was followed by increments of 20 mA in stimulation intensity, until a plateau in twitch force and M-wave was observed. The resulting intensity was then increased by 30% to ensure supramaximal stimulation (M_{max}), this was the intensity used when delivering superimposed and resting doublets. Voluntary activation was calculated using the interpolated twitch technique, calculated as: $Voluntary\ activation\ (\%) = 1 - \left(\frac{superimposed\ doublet}{potentiated\ doublet}\right) \times 100$.

3.10 Menstrual cycle

All female participants who experienced 'natural' menstrual cycles were required to monitor two menstrual cycles via a logbook prior to taking part in the study. Ovulation tests (One Step Ovulation Test; AI DE Diagnostics, China) were also provided to the participants to record surges in luteinizing hormone (LH). Participants were instructed to use these ovulation tests 2-3 days before their predicted date of ovulation, in accordance with the manufacturer's instructions. 12:00 midday was used as the time point to determine which day the cycle starts/ends (see Table 1.) Using the recorded dates of menstrual cycles and LH surges, all experimental trials were scheduled in an attempt to coincide with the follicular phase of the third menstrual cycle.

Table 1. Definition of start/end of menstruation.

Onset of menses	
If bleeding starts before 12:00 midday	It is classified as day 1 of menstruation
If bleeding starts after 12:00 midday	The following day is classified as day 1 of menstruation
End of menses	
If bleeding ends before 12:00 midday	It is classified as the end of menstruation
If bleeding ends after 12:00 midday	The following day is classified as end of menstruation

3.11 Statistical analysis

Firstly, a Shapiro-Wilk test was performed on all data, to test for normality. Mean values for the pre-to-post capsule changes in CAF and PLA trials were compared using paired t-tests and Wilcoxon signed ranks tests, for normally and non-normally distributed data, respectively. Next, three-way ANOVAs (repeated measures for time [2 levels: CAF 1 & PLA 1 vs CAF 2 & PLA 2], supplement [2 levels: CAF vs PLA], and between groups for genotype [2 levels: AA vs AC/CC]) were used. The common data transformations were not possible for non-normally distributed data due to the negative values in the

variables. However, the data distribution was close to normal, and the three-way ANOVA is considered robust to moderate violations of this assumption and still provides valid results. To further examine the main effects of trials, Bonferroni corrected pairwise comparisons (paired t-tests) were performed. Day-to-day reliability, within-day variability of repeated measures and test-retest reliability were all assessed using Intraclass Correlation Coefficients (see 2.1.3.1). Reliability thresholds from Portney and Watkins (2000) were used: < 0.05 = 'poor' reliability; 0.5 to 0.75 = 'moderate' reliability; 0.75 to 0.9 = 'good' reliability; > 0,9 = 'excellent' reliability.

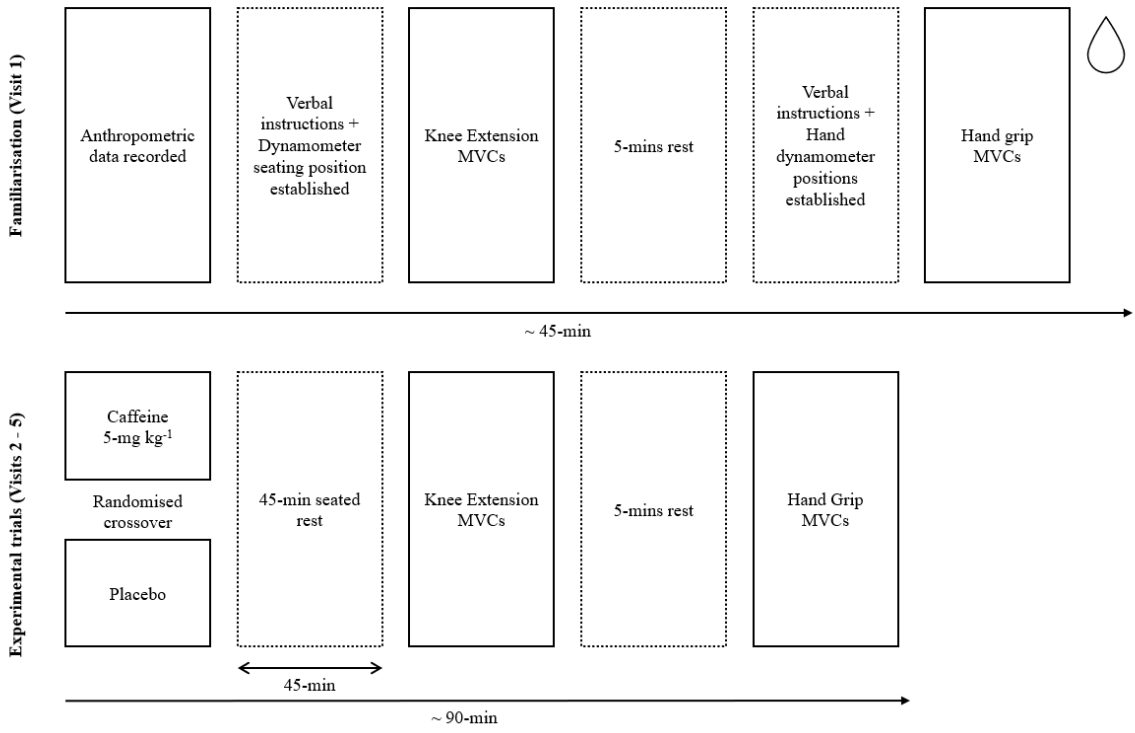

Figure 1. Schematic of study design. Droplet icon represents saliva sample.

Chapter 4: Results

4.1 CYP1A2 genotype

For the rs762551 SNP in the CYP1A2 gene. 7 individuals were homozygous for the A allele (AA, i.e. 'fast' metaboliser) and 9 were homozygous for the C allele (CC, i.e. 'slow' metaboliser), none were heterozygous (AC, i.e. 'slow' metaboliser). Participant characteristics were not different between genotypes ($P \geq 0.05$, Table *2*).

Table 2. Participant characteristics. Participants were categorised according to CYP1A2 ('fast' or 'slow' metaboliser)

	CYP1A2	
	'Fast'	**'Slow'**
Male	6	5
Female	1	4
Age (years)	22.3 ± 3.4	23.8 ± 5.3
Height (cm)	177.1 ± 10.8	171.1 ± 13.5
Body mass (kg)	77.6 ± 15.9	74.4 ± 15.2

4.2 Hand grip

A significant main effect of supplement was observed ($F_{1,15} = 11.735$, $P = 0.004$). However, no significant time ($F_{1,15} = 0.772$, $P \geq 0.05$), time × genotype ($F_{1,15} = 0.003$, $P \geq 0.05$), supplement × genotype ($F_{1,15} = 1.416$, $P \geq 0.05$), time × supplement ($F_{1,15} = 0.971$, $P \geq 0.05$) or time × supplement × genotype ($F_{1,15} = 1.701$, $P \geq 0.05$) interactions were observed.

Day-to-day reliability of all pre-capsule values was indicative of excellent reliability ($ICC_{3.1} = 0.956$), with 95% confidence intervals from 0.908 to 0.982, both excellent.

Within-day variability of repeated measures for all placebo pre- and post- capsule values was indicative of excellent reliability ($ICC_{3,1}$ = 0.987), with 95% confidence intervals from 0.973 to 0.995, both excellent. Test-retest repeatability for the % increase in hand grip performance in CAF 1 and CAF 2 trials indicates poor reliability ($ICC_{3,1}$ = 0.174), with 95% confidence intervals from poor (-0.337) to moderate (0.605).

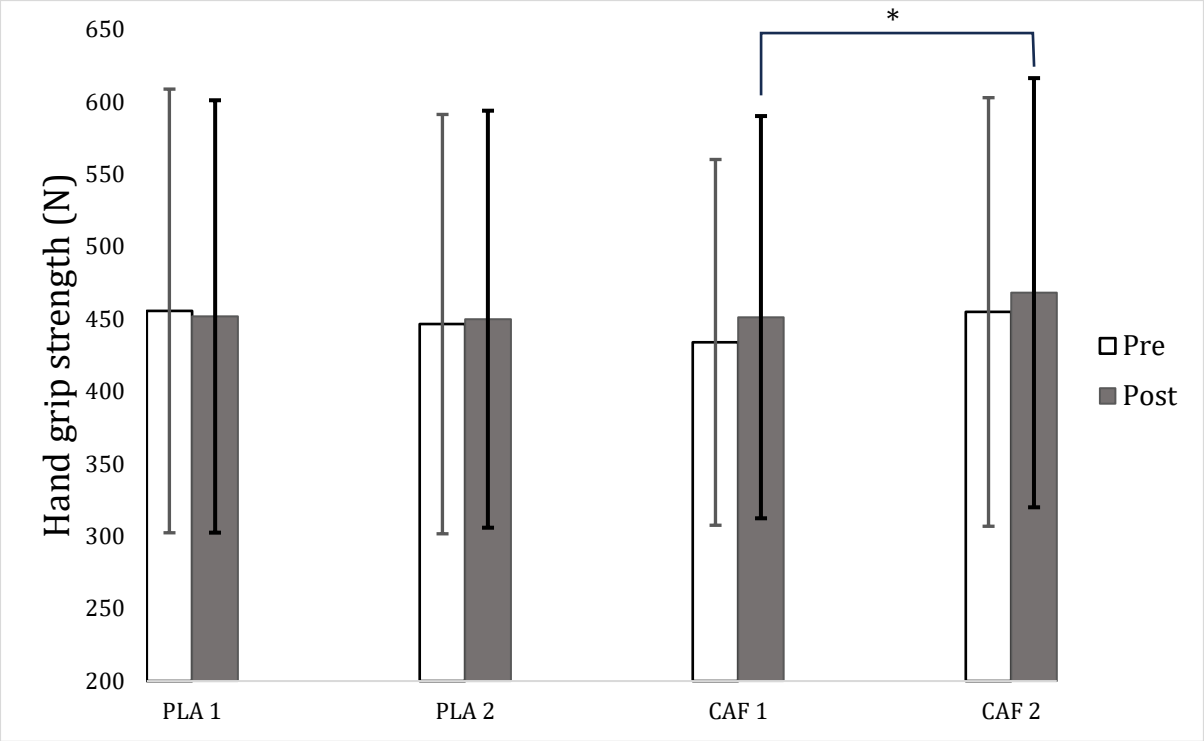

Figure 2. Mean hand grip strength. * signifies increase in hand grip strength compared to the placebo (*P* = 004). PLA 1 & 2: Placebo trials 1 and 2. CAF 1 & 2: Caffeine trials 1 and 2.

4.3 Knee extension

No significant time ($F_{1,15}$ = 0.319, $P \geq 0.05$), time × genotype ($F_{1,15}$ = 1.351, $P \geq 0.05$), supplement ($F_{1,15}$ = 1.532, $P \geq 0.05$), supplement × genotype ($F_{1,15}$ = 0.731, $P \geq 0.05$),

time × supplement ($F_{1,15}$ = 0.053, $P \geq 0.05$) or time × supplement × genotype ($F_{1,15}$ = 0.248, $P \geq 0.05$) interactions were observed.

Day-to-day reliability of all pre-capsule values was indicative of excellent reliability ($ICC_{3,1}$ = 0.917), with 95% confidence intervals ranging from good (0.791) to excellent (0.978). Within-day variability of repeated measures for all placebo pre- and post-capsule values indicated excellent reliability ($ICC_{3,1}$ = 0.939), with 95% confidence intervals ranging from good (0.844) to excellent (0.984). Test-retest repeatability on % increase in knee extension strength in CAF 1 and CAF 2 indicated good reliability ($ICC_{3,1}$ = 0.819), with 95% confidence intervals ranging from poor (0.388) to excellent (0.956).

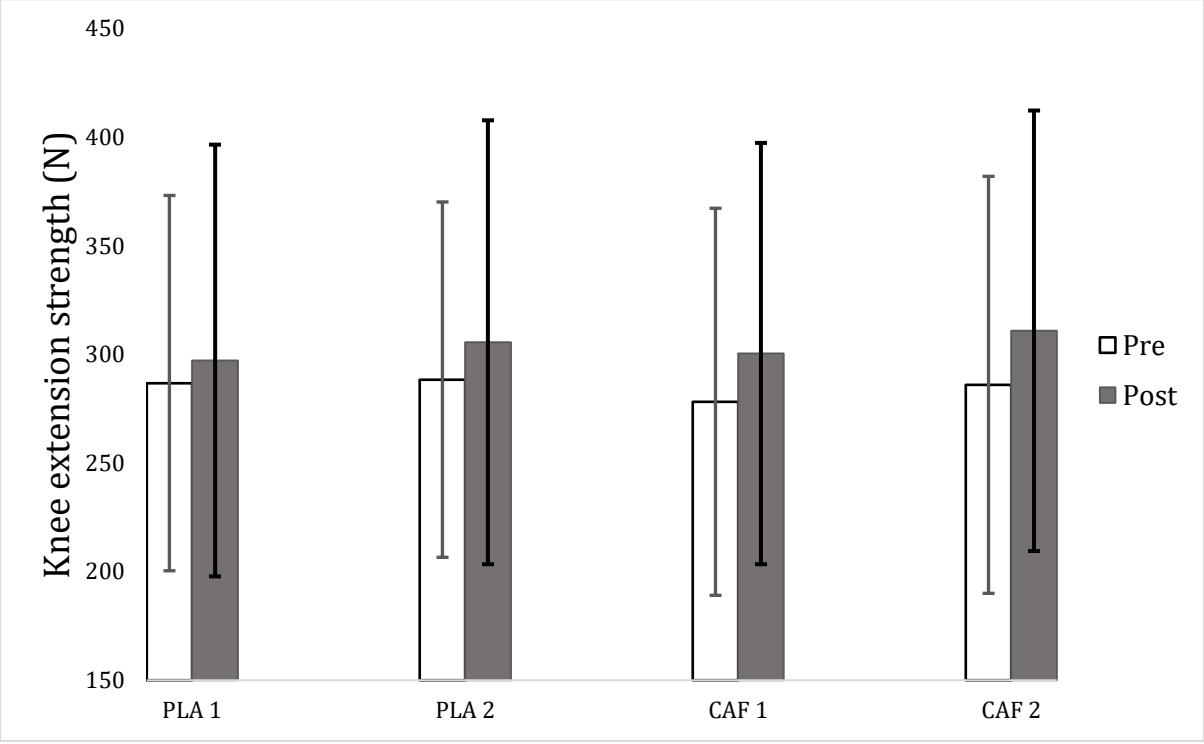

Figure 3. Mean knee extension strength. No significant effects of caffeine on knee extension MVC strength ($P > 0.05$). PLA 1 & 2: Placebo trials 1 and 2. CAF 1 & 2: Caffeine trials 1 and 2.

4.4 Voluntary activation (VA)

A significant main effect of supplement was observed ($F_{1,15}$ = 21.153, P = 0.002). However, no significant time ($F_{1,15}$ = 0.002, $P \geq 0.05$), time × genotype ($F_{1,15}$ = 0.298, $P \geq 0.05$), supplement × genotype ($F_{1,15}$ = 0.013, $P \geq 0.05$), time × supplement ($F_{1,15}$ = 2.381, $P \geq 0.05$) or time × supplement × genotype ($F_{1,15}$ = 0.372, $P \geq 0.05$) interactions were observed.

Day-to-day reliability of all pre-capsule values indicated good reliability ($ICC_{3,1}$ = 0.819), with 95% confidence intervals from moderate (0.595) to excellent (0.949). Within-day variability of repeated measures of the placebo pre- and post- capsule values indicated moderate reliability ($ICC_{3,1}$ = 0.710), with 95% confidence intervals from poor (0.419) to excellent (0.912). Test-retest repeatability on theincrease in VA in CAF 1 and CAF 2 trials was indicative of poor reliability ($ICC_{3,1}$ = 0.115), with 95% confidence intervals from poor (-0.557) to moderate (0.696).

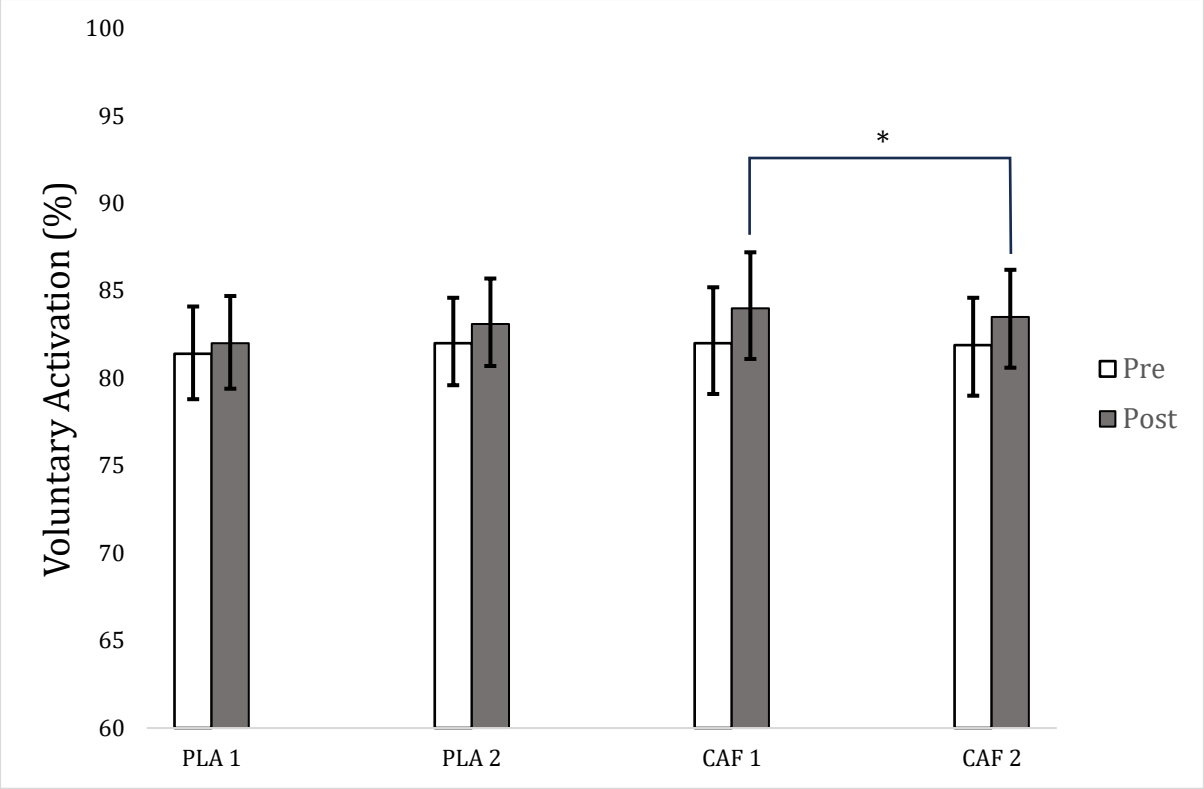

Figure 4. Mean VA. * signifies increase in VA when compared to the placebo (P = 0.002). PLA 1 & 2: Placebo trials 1 and 2. CAF 1 & 2: Caffeine trials 1 and 2.

4.5 Peak sEMG (Vastus lateralis)

No significant time ($F_{1,15}$ = 0.323, $P \geq 0.05$), time × genotype ($F_{1,15}$ = 0.561, $P \geq 0.05$), supplement ($F_{1,15}$ = 0.983, $P \geq 0.05$), supplement × genotype ($F_{1,15}$ = 0.169, $P \geq 0.05$), time × supplement ($F_{1,15}$ = 0.180, $P \geq 0.05$) or time × supplement × genotype ($F_{1,15}$ = 0.040, $P \geq 0.05$) interactions were observed.

Day-to-day reliability of all pre-capsule values indicated moderate reliability ($ICC_{3,1}$ = 0.738), with 95% confidence intervals from poor (0.379) to excellent (0.951). Within-day variability of repeated measures for all placebo pre- and post- capsule values indicated good reliability ($ICC_{3,1}$ = 0.783), with 95% confidence intervals from poor (0.453) to excellent (0.961). Test-retest repeatability on the increase in peak VL sEMG

in CAF 1 and CAF 2 trials indicated poor reliability ($ICC_{3,1}$ = -0.103), with 95% confidence intervals from poor (-0.796) to moderate (0.706).

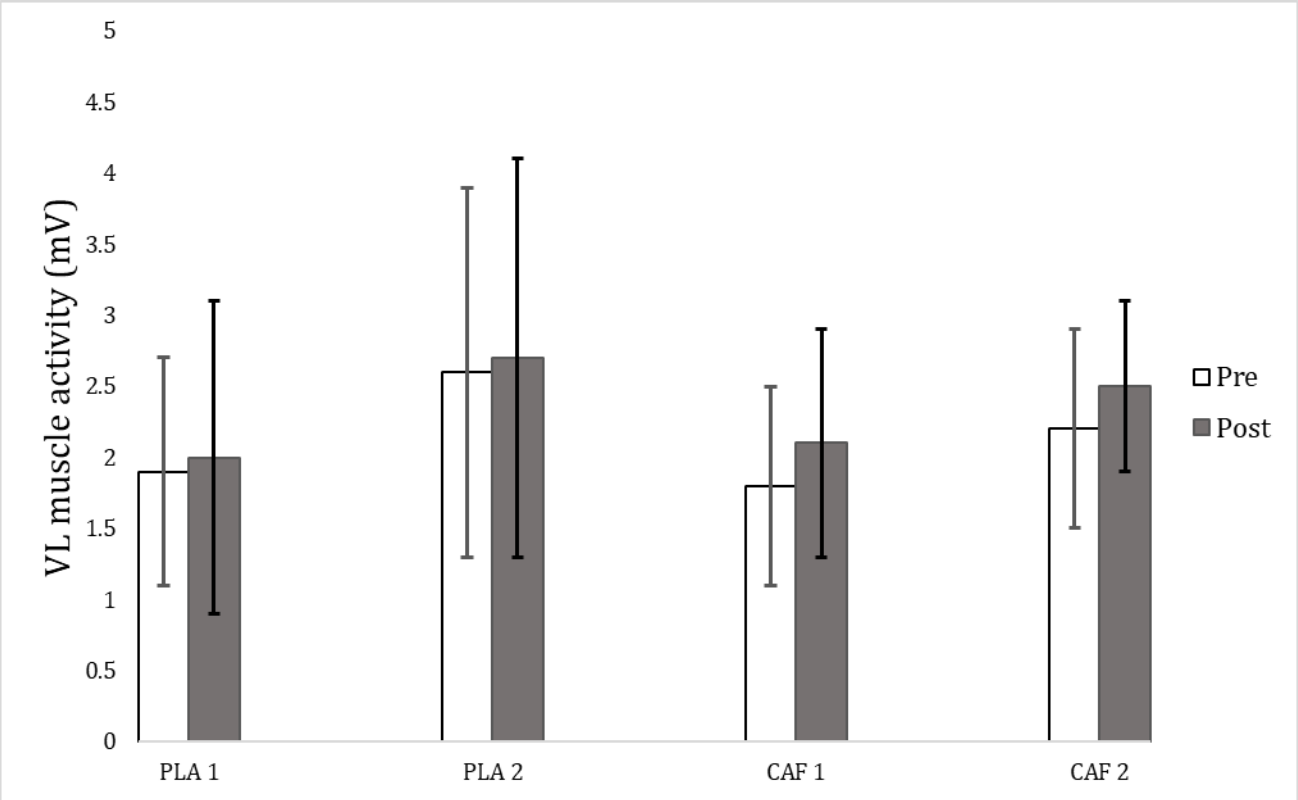

Figure 5. Mean muscle activation of the VL. No significant effect of caffeine on peak sEMG of the VL ($P > 0.05$). PLA 1 & 2: Placebo trials 1 and 2. CAF 1 & 2: Caffeine trials 1 and 2.

4.6 Peak sEMG (Vastus medialis)

A significant main effect of supplement was observed ($F_{1,15}$ = 14.181, P = 0.02). No further significant time ($F_{1,15}$ = 1.024, $P \geq 0.05$), time × genotype ($F_{1,15}$ = 0.542, $P \geq 0.05$), supplement × genotype ($F_{1,15}$ = 3.559, $P \geq 0.05$), time × supplement ($F_{1,15}$ = 0.135, $P \geq 0.05$) or time × supplement × genotype ($F_{1,15}$ = 0.072, $P \geq 0.05$) interactions were observed.

Day-to-day reliability of all pre-capsule values indicated moderate reliability ($ICC_{3,1}$ = 0.537), with 95% confidence intervals from poor (0.126) to good (0.898). Within-day

variability of repeated measures for all placebo pre- and post- capsule values indicated good reliability ($ICC_{3,1}$ = 0.782), with 95% confidence intervals from poor (0.451) to excellent (0.961). Test-retest repeatability on the increase in peak VM sEMG in CAF 1 and CAF 2 trials indicated poor reliability ($ICC_{3,1}$ = -0.279), with 95% confidence intervals from poor (-0.854) to moderate (0.602).

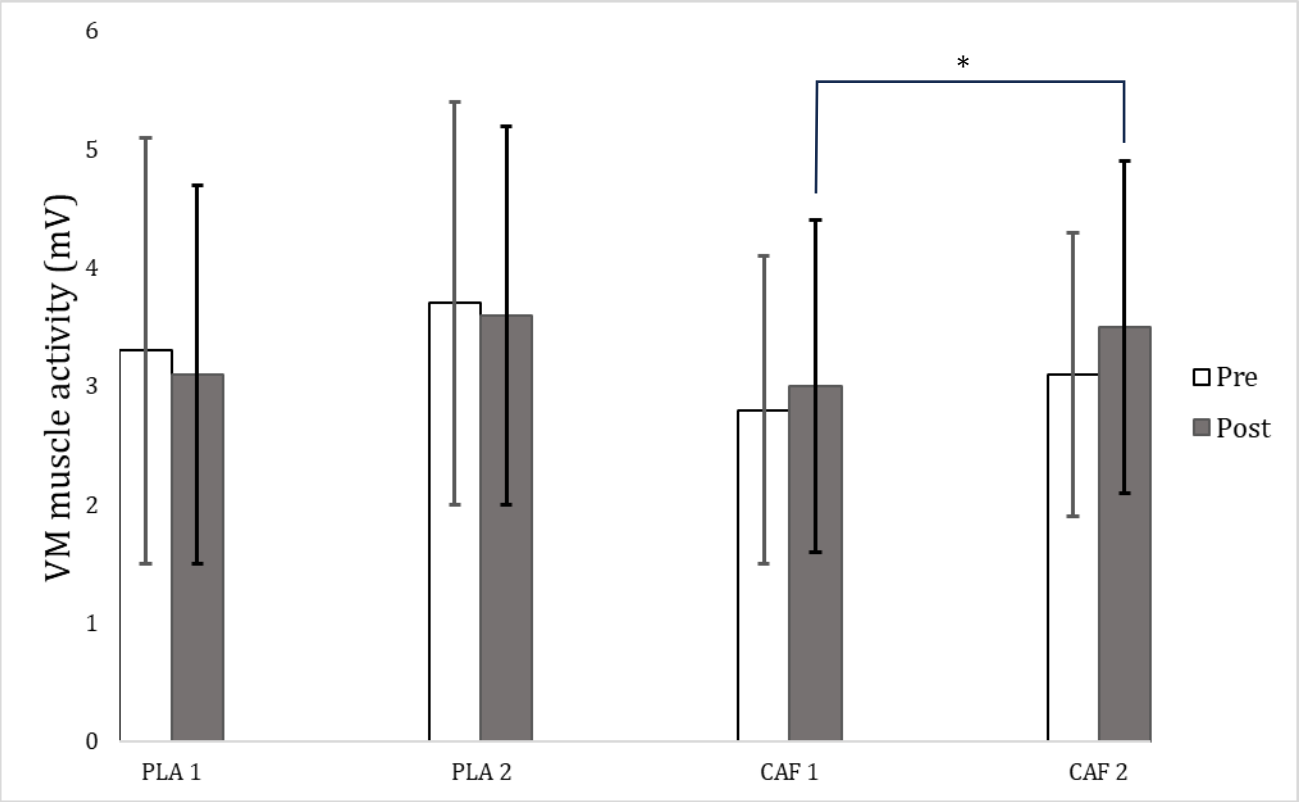

Figure 6. Mean muscle activation of the VM. * signifies increase in peak sEMG of the VM when compared to the placebo (P = 0.02). PLA 1 & 2: Placebo trials 1 and 2. CAF 1 & 2: Caffeine trials 1 and 2.

Chapter 5: Discussion

The purpose of this study was to determine whether a caffeine dose of 5 $mg \cdot kg^{-1}$ enhanced muscle strength, muscle activity and neuromuscular function. A secondary aim of the study was to determine whether variations in the rs762551 SNP in the CYP1A2 gene had any effect on caffeine's ergogenicity for the parameters listed above. In a randomised, double-blind manner, participants completed four experimental trials: two caffeine and two placebo, in which they completed a series of knee extension and hand grip MVCs to assess muscle strength. Alongside the knee extension MVCs, sEMG was performed on the VM and VL to measure muscle activity, and neuromuscular function was assessed using interpolated twitch technique. Using DNA extracted from saliva samples, participants were categorised as either 'fast' metabolisers (AA homozygotes) or 'slow' metabolisers (C allele carriers), followed by a comparison of the results for both groups. The present study found that caffeine ingestion significantly improved hand grip strength, VA and peak sEMG for the VL. However, caffeine had no significant effect on knee extension muscle strength or peak sEMG for the VM. Moreover, the results found no significant differences between genetic groups (AA vs AC/CC) for any of the parameters tested, including participant characteristics.

5.1 Muscle strength

The present study found that caffeine significantly improved hand grip strength when compared to the placebo. These results are in line with much of the current research, which too suggests that caffeine is ergogenic for hand grip strength (Muñoz et al. 2020; Del Coso et al, 2014; Spinelli et al. 2020). In contrast to these results, Kammerer et al.

(2014) reported that caffeine had no significant effect on hand grip strength. However, a notable limitation of this study is that it used a fixed dose of 80 mg caffeine for all participants, in energy drink form. Therefore, all participants received the same dose, regardless of body mass. A more reliable method would be to use a dose calculated via body mass, as used in the present study. Due to the conflictive results in this field, there is no consensus on whether caffeine is certainly ergogenic for muscle strength. Grgic et al. (2020) suggests that the use of different hand-grip dynamometer models and caffeine doses could be reasons behind the variation of results in this area, suggesting that future research may wish to investigate the dose-response relationship on muscle strength for a variety of caffeine doses. The current study also found that caffeine had no significant effect on muscle strength performance in the knee extension movement. Whilst this aligns with research from Brooks et al. (2015) and Sabblah et al. (2015), which both reported that caffeine had no significant effect on lower body maximal strength, the results from the present study conflict with much of the existing literature (Grgic et al. 2018; Warren et al. 2010). However, Warren et al. (2010) highlighted that methods of measuring muscle strength vary from isometric MVCs to free-weight 1RM tests. This suggests that the inconsistency in results in this field could be due to the variation of testing methods. This is supported by Warneke et al. (2023), who found that measurements of strength such as free-weight 1RM and isometric MVCs produce different estimations of maximal strength capacity for an individual, suggesting that one should not be substituted for the other. The authors accredited this difference to a number of factors, such as the complexity of dynamic movements, differing muscle lengths in isometric tests and the ease of familiarisation to the testing conditions.

The present study found that caffeine was ergogenic for small muscle groups (hand grip) but not for larger muscle groups (knee extensors). This contrasts with Warren et

al. (2010) which suggested that larger muscle groups are not as 'readily activated', with muscle activation levels of 85-95%, in comparison to smaller muscle groups which typically see activation levels of 95-99%. It is theorised that larger muscle groups may experience greater results from caffeine ingestion due to being further away from their activation 'limit'. However, in contrast to these findings, and correlating with the findings from the present study, a meta-analysis by Grgic et al. (2018) found that caffeine was effective in enhancing upper-body strength, but not lower body. However, it should be noted that none of the studies in this analysis reported on the reliability of their strength assessments, which indicates a limitation of these studies. Alongside the inconclusive results in this field, the results of the present study indicate that further research into the relationship between caffeine's ergogenicity for larger and smaller muscle groups in required, to address whether either experiences a greater effect of caffeine than the other.

As stated, in the present study, caffeine was significantly enhanced hand grip strength. The current research suggests that the mechanisms of enhanced motor-unit recruitment is likely to be the primary mechanism behind caffeine's ergogenicity for muscle strength performance (Grgic and Pickering, 2019). Black et al. (2015) claims that the increased motor-unit recruitment may be explained by its ability to increase both cortical and spinal neuron excitability, thus allowing for greater force generation. It is proposed that with an increased caffeine dose, the performance enhancing properties will increase linearly. Whilst this theory would support the findings of Pallarés et al. (2013), Pickering and Grgic (2019) highlight that this is merely a hypothesis and is yet to be explored in research.

McLellan et al. (2016) argues that adenosine receptor antagonism is a key mechanism for caffeine's ability to enhance exercise performance. Caffeine binds to A_1 and A_{2A} receptors, which prevents adenosine from binding to them. Adenosine binding to A_1 - receptors is the mechanism that stimulates pain, and therefore, with caffeine binding to these receptors, the stimulation of pain will be reduced, providing the individual with analgesic effects (Gaspardone et al. 1995). Furthermore, caffeine reverses the tonic inhibitory influence of adenosine in the CNS, which may prevent the adenosine-induced decrease in excitatory neurotransmitter release and firing rate of central neurons. Moreover, adenosine A_{2A} receptor agonists have been shown to decrease cerebral cortical neuron firing rates, which leads to hypoactivity, depression of locomotor activity and impairment of coordination (Phillis, 2001).

However, whilst adenosine receptor antagonism is a widely accepted mechanism of caffeine, it's relevance to muscle strength performance is questionable. Black et al. (2015) suggested that the antagonism of adenosine receptors was thought to provide an ergogenic effect for endurance athletes, due to the shrouding of the effects of fatigue. However, whether this mechanism influences muscle strength is unclear and therefore may not be relevant to the present study. It may be useful for future muscle strength research to measure perceptions of pain, in case an analgesic effect enhances performance.

Herrmann-Frank et al. (1999) proposed that enhanced force development following caffeine ingestion may be due to enhanced intracellular calcium release to the muscle fibres, which is theorised to enhance cross-bridge attachment. However, much of the research on this mechanism relies on in-vitro studies, using toxic doses (Magkos and Kavouras, 2005), and whilst there is some research to suggest that this mechanism is

present and effective *in* vivo, further 'clinically relevant' research is required to validate this claim (Tallis et al. 2012).

The present study reported that day-to-day reliability of pre-capsule values was indicative of 'excellent' reliability, for both hand grip and knee extension dynamometry. It was observed that within-day variability of repeated measures was again indicative of 'excellent' reliability for both hand grip and knee extension dynamometry. Lastly, it was reported that test-retest repeatability for the increase in hand grip and knee extension strength was indicative of 'poor' and 'good' reliability, respectively. This suggests that although caffeine significantly improved hand grip strength, the increase in strength for CAF 1 and CAF 2 varied significantly. This may question the validity of the hand grip test results as the results were not reliably replicated, suggesting that any future research should aim to ensure that the measurement tools used reproduce the same results in repeated measures visits.

5.2 Voluntary activation

The present study found that caffeine significantly increased VA when compared to the placebo. These results align with previous literature from Behrens et al. (2015), which found that a caffeine dose of 8 $mg \cdot kg^{-1}$ significantly increased VA of the knee extensors during isometric, concentric and eccentric MVCs. However, Behrens at al. (2015) accredited their strength 'gains' in maximal knee extension torque to the caffeine-induced increase in VA, but the present study observed no such improvement in torque readings. Moreover, caffeine has been observed to increase motor-evoked potentials and cortically evoked twitches of the VL during isometric MVCs (Kalmar and Cafarelli, 2006). Whilst the research from Behrens et al. (2015) maintains the most relevance to

the present study, due to the similarity in methodology and intervention, Mesquita et al. (2020) conducted a study investigating the effects of caffeine on the decline in VA during single-limb MVCs using the triceps surae. This study found that caffeine had no effect on slowing the decline of VA during the MVCs following a fatigue protocol. However, as previously mentioned, the present study did not investigate fatigue, and therefore, the relevance of this study to the present study is limited. Similar to the mechanisms associated with caffeine's effects on muscle strength, Behrens et al. (2015) also suggested that the aforementioned caffeine-related increases in CNS function may also enhance VA.

As for the reliability of testing methods for VA in the present study, the day-to-day reliability of all pre-capsule values across all trials indicated 'good' reliability, the within-day variability of repeated measures for all pre- and post- placebo values indicated 'moderate' reliability, and the test-retest repeatability for increase in VA indicated 'poor' reliability. This suggests that the increase in VA following CAF ingestion in both CAF 1 and CAF 2 trials had a large variation. Tamilio et al. (2022) highlights that if there is variability in the ergogenicity of caffeine following standardised experimental trials, it is likely to be related to the mood, motivation, diet or prior activity of the participant. The present study monitored diet and exercise prior to trials, but mood, motivation and sleep were not accounted for, which have been shown to influence the neuromuscular function (Duncan and Oxford, 2011; Knowles et al. 2018). Moreover, the 'moderate' reliability for within-day variability of repeated measures suggests that there was still some variation in pre- and post- capsule values, even though there was no caffeine ingested in PLA trials. Knaier et al. (2019) reported that strength performance variance could be due to the time-of-day at which the test was performed. Therefore, although the present study standardised trials to take place roughly at the

same time (morning or afternoon), future research may which to standardise the time to the hour. It is also possible that mood, motivation and sleep also influenced these results, and as previously mentioned, they were not accounted for.

5.3 Muscle activation

The present study also found conflicting results for muscle activation. It was observed that when compared to the placebo, caffeine significantly increased peak sEMG for the VM, but not the VL. Bazzucchi et al. (2011) observed that a dose of 6 $mg \cdot kg^{-1}$ caffeine significantly increased EMG of the biceps. The study also suggested that caffeine may increase neuromuscular efficiency, due to caffeine-induced increase in motor unit firing rate. Furthermore, Sun et al. (2022) conducted a meta-analysis which reported that caffeine significantly increases EMG when compared to a placebo, which supports the results for VM activation in the present study but not for VL activation. However, Fimland et al. (2010) conducted a study that investigated the effects of caffeine (6 $mg \cdot kg^{-1}$) on EMG activity for the soleus and gastrocnemius medialis muscles. The results of this study found that caffeine had no significant effect on EMG activity, which aligns with the results for VL activation in the present study. Since the majority of the research in this field suggests that caffeine does increase muscle activity (Sun et al. 2022), the results of Fimland et al. (2010) and the VL activation results in the present study may be explained by methodological issues, such as that plasma concentrations of caffeine were not measured in either study. This means that although the timing of dosages was estimated to be sufficient to reach peak plasma concentrations, it remained theoretical.

The aforementioned mechanism of caffeine, adenosine receptor antagonism, is thought to influence muscle activation through both spinal and supraspinal pathways; with

caffeine increasing both motorneuron excitability and self-sustained motor-unit firing rates (Kalmar and Cafarelli, 2006). Furthermore, the research that has shown caffeine to significantly increase VA and muscle strength supports the effects of caffeine on central drive (Sun et al. 2022). Whilst there are some isolated studies that do not support this and suggest that caffeine doesn't influence recruitment and/or coding of motor neurons (Fimland et al. 2010), the majority of the research suggests that caffeine does enhance these functions (Sun et al. 2022). Fimland

Day-to-day reliability of all pre-capsule values was indicative of 'moderate' reliability for peak sEMG for both VM and VL. Within-day variability of repeated measures indicated 'good' reliability for both VM and VL peak sEMG. Lastly, test-retest repeatability for the increase in peak sEMG for both VM and VL indicated 'poor' reliability for both. The 'poor' test-retest reliability suggests that the increase in peak sEMG in CAF 1 and CAF 2 trials were inconsistent, showing large variation. Such variations could be explained by a number of causes, such as sleep, motivation and stress, which as previously mentioned, were not measured. Moreover, the present study only stabilised the right leg. Previous research has shown that stabilising the hips and resting leg with Velcro straps may help increase the accuracy and reliability of isometric knee extension measurements (Tsaopoulos et al. 2011). This lack of stabilisation could potentially allow for slight variation in form, which may cause the variation in sEMG results.

5.4 Genotype

The present study observed that genotypes in the rs762551 SNP within the CYP1A2 gene had no significant effect on the effect of caffeine on muscle strength, VA or muscle

activation. These findings are supported by Grgic et al. (2020) which found no significant trial × genotype effects for the CYP1A2 gene. It is possible that the effects of caffeine ingestion are similar across CYP1A2 genotypes and therefore, do not report a significant difference. Grgic et al. (2020) utilised a battery of testing methods, including resistance exercise, jumping and sprinting, all of which reported no effect of genotype. Furthermore, Wong et al. (2021) found that a caffeine dose of 4 $mg \cdot kg^{-1}$ had no significant effects on hand grip strength for AA and AC genotypes, but decreased hand grip strength for those with the CC genotype. Wong et al. (2021) notes that caffeine has been observed to increase endurance by reducing flow-mediated vasodilation and myocardial blood flow, which can impair oxygen flow to the heart and other muscles. Therefore, is it possible that since CC genotypes are considered 'slow' metabolisers, these mechanisms may take longer to occur, meaning the impaired oxygen flow may be alleviated later than that experienced by AA homozygotes. However, it remains unclear as to why a reduction in performance was seen in only CC homozygotes and not AC heterozygotes.

Rahimi (2019) reported that caffeine was only ergogenic for those homozygous for the A allele. The study found that on average, AA genotypes were able to complete one more rep at 85% of 1RM for four different resistance exercises when compared to a placebo. In comparison, AC/CC cgenotypes completed the same number of repetitions in both CAF and PLA trials. It is noteworthy that Grgic et al. (2020) used a dose of 3 $mg \cdot kg^{-1}$, whereas Rahimi (2019) used a dose of 6 $mg \cdot kg^{-1}$. In fact, Grgic et al. (2020) accredits the difference in results between these two studies to the difference of doses, suggesting that perhaps differences in responses to caffeine between genotypes may only become apparent at higher doses. This is contested by the results of the present study, which utilised a dose of 5 $mg \cdot kg^{-1}$, a smaller dose to that used in Rahimi (2019), yet also found

different results. Grgic et al. (2020) also suggested that the methods used by Rahimi (2019) may explain the results found. The primary outcome measure used by Rahimi (2019) was the number of repetitions performed at 85% of 1RM for the bench press, leg press, seated cable row and shoulder press. It is highlighted by Grgic et al. (2020) that this method can be considered as a 'crude' test of muscle strength and that results may differ when more accurate measurements of strength are used.

It is thought that the effects of the CYP1A2 genotype on caffeine response variation may be due to the cytochrome P450 1A2 enzyme. The CYP1A2 gene encodes for this enzyme, which is responsible for ~95% of caffeine metabolism, responsible for the demethylation of caffeine into metabolites such as paraxanthine and theobromine (Begas et al. 2007). Therefore, if there are inter-genotype variations in this enzyme, it is possible that the time course of the effects of caffeine may be influenced. Differences in performance, such as those seen from Rahimi (2019) could be explained by this difference in time course of effects. It is plausible that C allele carriers ('slow' metabolisers) may benefit from an earlier timing of dosage, allowing for plasma caffeine levels to reach peak concentrations following a slower metabolism. However, this remains theoretical and may be an area for future research.

5.5 Limitations

Firstly, the present study did not consider that side effects could influence the performance outcomes. Ramos-Campo et al. (2019) found that when compared to the placebo, caffeine trials had a significantly greater occurrence of side effects. As previously mentioned, these can include GI distress, anxiety and headaches.(Pallarés et al. 2013). Effects such as these may influence performance by altering motivation,

concentration and exercise tolerance and must be accounted for in future studies (Ford et al. 2017; Martins et al. 2020). Side effects could be measured using a self-report questionnaire given to all participants during experimental trials. Next, plasma levels of caffeine were not measured at any point during this study. The time to reach peak plasma levels for caffeine is thought to be between 30 and 120 minutes, depending on individual metabolism speeds (Guest et al: 2021).As previously mentioned, it is possible that inter-genotype variations in the cytochrome P450 enzyme could influence the metabolism of caffeine and therefore, affect the time to reach peak plasma concentrations. If the timing of dosages were not optimised for genotypes, this his could provide insight as to why no significant caffeine × genotype effects were observed in the present study. It could be theorised that the 'slow' metabolisers needed more time to allow for caffeine to reach peak plasma levels. Whilst the present study did not include this in the methodology due to financial restrictions, future caffeine research should look to monitor caffeine plasma levels to ensure that it can be included in the analysis and comparison of results.

Leading on, the present study utilised a 45-minute 'absorption' period following capsule ingestion. As previously mentioned, for some individuals, caffeine may take up to 120 minutes to reach maximum plasma concentrations (Mumford et al. 1996). Therefore, similar to the previous limitation, it is possible that the 45-minute 'absorption' period was not enough for some participants. For this reason, future research may wish to assess a series of absorption times, to determine whether certain genotypes receive greater ergogenic effects are different timepoints.

Lastly, the present study had no participants with the CC genotype. However, this is not unexpected since the number of individuals with the CC genotype in the population is

thought to be less than 10% (Sachse et al. 1999). Therefore, in order to recruit ten participants with the CC genotype, a larger sample size of around 100 would be needed to achieve this. It cannot be assumed that the results of CC genotypes would be uniform with those of the AC genotypes, meaning that a sample size of all three genotypes (AA/AC/CC) in the rs721551 SNP would be needed to fully elucidate whether the CYP1A2 gene has a significant effect on caffeine response variation. Therefore, it is possible that the results of the present study may have differed had there been a larger sample size and differentiation between AC and CC genotypes.

5.6 Conclusion

In conclusion, the present research demonstrated that a caffeine dosage of 5 $mg \cdot kg^{-1}$ consumed 45-minutes prior to exercise enhanced hand grip strength, voluntary activation and peak sEMG for the VM in comparison to a placebo. These may be a result of the effects caffeine exhibits on the CNS and the antagonism of adenosine receptors. However, caffeine had no significant effect on muscle strength for the knee extensors or peak sEMG for the VL. Lastly, genotypes (AA vs AC/CC) for the rs762551 SNP in the CYP1A2 gene had no effect on the effect of caffeine on muscle strength, voluntary activation and peak sEMG for the VM and VL.

Whilst day-to-day reliability of pre-capsule values and within-day variability of placebo pre- and post-capsule values both indicated moderate to excellent reliability, test-retest repeatability for the caffeine trials was often indicative of poor reliability. Whilst genetics may be a contributor to interindividual variability in exercise responses, the lack of a significant interaction of genotype in the present study suggests that the SNP measured (rs761551) was not responsible for the variation in test-retest repeatability.

Therefore, future research should look to identify and evaluate the influence of other SNPs on exercise responses, to further understand the impact that genetics has on interindividual variability in exercise responses.

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Chapter 7: Appendices

7.1 Trial randomisation order

ID		ID	
WC-01-	CPCP	WCF-01-	PCPC
WC-03-	CPPC	WCF-03-	CPCP
		WCF-04-	PCCP
WC-05-	PCPC	WCF-05-	PCCP
WC-06-	CPCP	WCF-06-	PPCC
WC-08-	CPPC		
WC-09-	CCPP		
WC-10-	PCPC		
WC-12-	PCCP		
WC-14-	PPCC		
WC-16-	PCPC		
WC-17-	CCPP		

Figure 7. Trial randomisation order.

7.2 Food and drink diary

Time of day	**Description of Food or Drink**	**Weight, amount or estimated portion**

Figure 8. Self-report food and drink diary.

7.3 Caffeinated food and drink sheet

Caffeinated Foods and Drinks

Below is a list of caffeinated foods and drinks, none of the following should be consumed within the 24-hour period prior to experimental trials.

Filtered coffee

Espresso

Soluble instant coffee

Tea (leaves or bag)

Iced Tea

Green tea

Hot chocolate

Caffeinated soft drinks

Sugar-free caffeinated soft drinks

Energy drinks

Dark chocolate

Chocolate

Medications containing caffeine (e.g. Excedrin, Diurex, Panadol, Lemsip etc.)

Any supplements containing caffeine

Pre-workout supplements etc

This is not an exhaustive list. Please avoid any food or beverage that you know to contain caffeine, in any amount.

Figure 9. Caffeinated food and drink list.

7.4 LH test protocol

One Step Ovulation Test

Introduction

The menstrual cycle is separated into two main phases (Follicular and Luteal phases). When a woman is about to ovulate, her body releases a large about of a hormone known as LH (Luteinising

Hormone), this marks the beginning of the Luteal phase. LH is always present in your urine, but the levels increase (surge) just before ovulation. Throughout the different stages of your menstrual cycle, factors such as hormones and metabolism change. Therefore, to ensure that the menstrual effects (metabolism of caffeine etc.) are always the same during the study, I aim to schedule all visits within the Follicular phase. This will be done by monitoring the LH surge for 2 months, then using these dates to predict when the Follicular phase *should* be. This test will be repeated before each visit as well, to ensure the LH surge has not begun.

Specimen collection

- Do not use the first morning urine sample (LH is synthesized in your body in the early morning and will not show up in your urine until later in the day).
- The best time for collection is between 10am-8pm, so choose a time that suits you.
- Aim to collect urine at the same time each day. If you can, reduce liquid intake around 2 hours before testing to avoid urine dilution.

Before you begin

- Do not open pouch until you are ready to begin test.
- Make sure you have a watch, clock, or timer ready.
- Allow urine samples and test kit to reach room temperature (approx. 20mins).

Test procedure

- Determine the day to begin testing (see other side).
- Collect urine sample in a clean, dry container.
- To begin testing, open the sealed pouch and remove the strip. Do not remove the strip until you are ready to begin testing.
- With the arrows pointing downwards towards the urine, place the test strip vertically (straight) into the urine sample, for 5-10 seconds. DO NOT allow the urine to go above the MARK level line.
- Remove the strip from the urine and place on a clean, dry surface.
- Wait for coloured bands to appear. Depending on the concentration of LH in the urine specimen, positive results may be observed within 1 minute. However, to confirm negative results, the complete reaction time of 10 minutes is required. Reults obtained after 30 minutes may be considered invalid.

Interpretation of results

- After each test, you must decide if you are having a LH surge.
- To determine your result, you must compare the colour intensity of the test band to the control band. The control band is used to compare the test band against and also confirms that you have completed the test correctly.

See other side of page.

Positive for LH surge

- If two colour bands are visible and the test band **is of almost equal or greater colour intensity (darker)** than the control band, this is a positive result and a good indication that the LH surge is occurring. You should ovulate within the next 24-36 hours.

Negative for LH surge

- If two bands are visible but the test band is of a less intense colour (paler) than the control band or cannot be seen, this means the LH level is at or near its normal level and that the surge is not in progress. You should continue with daily testing.

Invalid result

- If no control band appears within 5 minutes, the result is invalid and should be ignored. A visible control line is needed in all cases to confirm a proper test result. Repeat test with a new test kit.

Your cycle length	Day to start test on
21 days	Day 6
22 days	Day 6
23 days	Day 7
24 days	Day 7
25 days	Day 8
26 days	Day 9
27 days	Day 10
28 days	Day 11
29 days	Day 12
30 days	Day 13
31 days	Day 14
32 days	Day 15
33 days	Day 16
34 days	Day 17
35 days	Day 18
36 days	Day 19
37 days	Day 20
38 days	Day 21
39 days	Day 22
40 days	Day 23

Figure 10. LH surge test start dates.

7.5 Menstrual cycle logbook non-OC users

Menstrual Cycle Logbook

(Non-Oral Contraceptive users)

Use the following logbook to track your menstrual phase (period).

Using the table below, place an '**S**' and '**F**' on the start (**S**) and finish (**F) dates** of your period.

- The first day of your period is determined by 12:00 midday
  - Spotting does not count.
  - If your period starts before 12:00 midday, that is the day your period started.
  - If your period starts after 12:00 midday, the next day is classed as the start of your period.
- The last day of your period is also determined by 12:00 midday

- If your period ends before 12:00 midday, that is the day your period ends.
- If your period finishes after 12:00 midday, the next day is the classed as the day your period ends.

Follow the template below if you do not understand.

	Day																												
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

	Days in the month																														
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
January			S							F																					
February					S						F																				
March				S					F																						

Figure 11. Self-report menstrual cycle log (Non-OC).

7.6 Menstrual cycle logbook (OC users)

Menstrual Cycle Logbook

(Oral Contraceptive users)

Use the following logbook to track your menstrual phase (period).

Using the table below, place an '**S**' and '**F**' on the start (**S**) and finish (**F) dates** of your period.

- The first day of your period is determined by 12:00 midday
  - Spotting does not count.
  - If your period starts before 12:00 midday, that is the day your period started.
  - If your period starts after 12:00 midday, the next day is classed as the start of your period.
- The last day of your period is also determined by 12:00 midday

- If your period ends before 12:00 midday, that is the day your period ends.
- If your period finishes after 12:00 midday, the next day is the classed as the day your period ends.

- Place an '**X**' on the day you **stop** taking your oral contraceptive.
- Place an '**O**' on the day you **start** taking your oral contraceptive again.

Follow the template below if you do not understand.

Day																														
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

Days in the month																															
Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
January		X		S				F	O																						
February		X			S			F	O																						
March		X		S				F	O																						

Figure 12. Self-report menstrual cycle logbook (OC).

7.7 Pre-screening health questionnaire

HEALTH QUESTIONNAIRE

Participant ID…… Age……

Please answer these questions truthfully and completely. The sole purpose of this questionnaire is to ensure that you are in a fit and healthy state to complete the exercise test.

ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL.

SECTION 1: GENERAL HEALTH QUESTIONS

Please read the 12 questions below carefully and answer each one honestly: check YES or NO.

	YES	NO
➢ Has your doctor ever said that you have a heart condition or high blood pressure?	☐	☐
➢ Do you feel pain in your chest at rest, during your daily activities of living, or when you do physical activity?	☐	☐
➢ Do you lose balance because of dizziness or have you lost consciousness in the last 12 months? (Please answer NO if your dizziness was associated with over-breathing including vigorous exercise).	☐	☐
➢ Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
If yes, please list condition(s) here:		
➢ Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
If yes, please list condition(s) and medications here:		
➢ Do you currently have (or have you had within the past 12 months) a bone, joint or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past but it *does not limit your ability* to be physically active.	☐	☐
If yes, please list condition(s) here:		
➢ Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐
➢ Have you had a viral infection in the last 2 weeks (cough, cold, sore throat, etc.)?		
If YES please provide details below:	☐	☐

- Do you have an allergy or intolerance to any foods or food components? □ □

If YES, please provide details here:

10. Please provide brief details of your current weekly levels of physical activity (sport, physical fitness or conditioning activities):

	Activity	Duration (mins.)
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Saturday		
Sunday		

11. Are there any reasons why you would not be able to consume caffeine? □ □

If YES, please provide details here:

Please sign the declaration on the consent form. You do not need to complete section 2.

If you answered YES to one or more of the questions in Section 1 - PLEASE GO TO SECTION 2.

SECTION 2: CHRONIC MEDICAL CONDITIONS

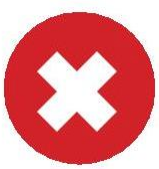
Please read the questions below carefully and answer each one honestly: check YES or NO.

		YES	NO
1.	**Do you have arthritis, osteoporosis, or back problems?** If YES answer questions 1a-1c. If NO go to Question 2.	□	□
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebrae (e.g. spondylolisthesis), and/or spondyloysis/pars defect (a crack in the bony ring on the back of the spinal column)?	□	□
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	□	□
2.	**Do you have cancer of any kind?** If YES answer questions 2a-2b. If NO, go to Question 3.	□	□
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head and neck?	□	□
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	□	□
3.	**Do you have heart disease or cardiovascular disease? This includes coronary artery disease, high blood pressure, heart failure, diagnosed abnormality or heart rhythm.** If YES answer questions 3a-3e. If NO go to Question 4.	□	□
3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
3b.	Do you have an irregular heartbeat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	□	□
3c.	Do you have chronic heart failure?	□	□
3d.	Do you have a resting blood pressure equal to or greater than 160/90mmHg with or without medication? Answer YES if you do not know your resting blood pressure.	□	□
3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	□	□

		YES	NO
4.	**Do you have any metabolic conditions? This includes Type 1 Diabetes, Type 2 Diabetes and Pre-Diabetes.** If YES answer questions 4a-4c. If NO, go to Question 5.	□	□
4a.	Is your blood sugar often above 13mmol/L? (Answer YES if you are not sure).	□	□
4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, OR the sensation in your toes and feet?	□	□
4c.	Do you have other metabolic conditions (such as thyroid disorders, current pregnancy related diabetes, chronic kidney disease, or liver problems)?	□	□
5.	**Do you have any mental health problems or learning difficulties?** This includes Alzheimer's, dementia, depression, anxiety disorder, eating disorder, psychotic disorder, intellectual disability and down syndrome. If YES answer questions 5a-5b. If NO go to Question 6.	□	□
5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
5b.	Do you also have back problems affecting nerves or muscles?	□	□
6.	**Do you have a respiratory disease?** This includes chronic obstructive pulmonary disease, asthma, pulmonary high blood pressure. If YES answer questions 6a-6d. If NO, go to Question 7.	□	□
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
6b.	Has your doctor ever said you blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	□	□
6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	□	□
6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	□	□
7.	**Do you have a spinal cord injury?** This includes tetraplegia and paraplegia. If YES answer questions 7a-7c. If NO, go to Question 8.	□	□
7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	□	□
7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as autonomic dysreflexia)?	□	□

		YES	NO
8.	**Have you had a stroke?** This includes transient ischemic attack (TIA) or cerebrovascular event. If YES answer questions 8a-8c. If NO go to Question 9.	□	□
8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
8b.	Do you have any impairment in walking or mobility?	□	□
8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	□	□
9.	**Do you have any other medical condition which is not listed above or do you have two or more medical conditions?** If you have other medical conditions, answer questions 9a-9c. If NO go to Question 10.	□	□
9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	□	□
9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, and kidney problems)?	□	□
9c.	Do you currently live with two or more medical conditions? Please list your medical condition(s) and any related medications here:	□	□
10.	**Have you had a viral infection in the last 2 weeks (cough, cold, sore throat, etc.)?** If YES please provide details below:	□	□
11.	**Is there any other reason why you cannot take part in this exercise test?** If YES please provide details below:	□	□

If you answered NO to all of the follow-up questions about your medical condition, you are cleared to take part in the exercise test.



If you answered YES to one or more of the follow-up questions about your medical condition it is strongly advised that you should seek further advice from a medical professional before taking part in the exercise test.

Figure 13. Pre-screening health questionnaire.

7.8 Participant information sheet

Effects of caffeine on muscle strength: Influence of genetics

I would like to invite you to take part in my research study. However, before you decide, I want you to understand why the research is being done, and what I will ask you to do. It is a good idea to talk to your friends or family about whether you should take part.

What is the purpose of the research?

The aim of the study is to investigate the effects of caffeine ingestion on muscle strength performance. While caffeine is one of the most well researched substances, there is noticeably less literature for its effects on muscle strength. The study also hopes to look at how an individual's genetics may cause the effects of caffeine to differ person-to-person. This is because the relationship between genetics and caffeine is a relatively new field, meaning there are several interesting areas to research in this field.

This research is funded by the University of Kent and has been approved by the ethics committee of the School of Sport and Exercise Sciences. Please see below for further details about this study.

Why have I been invited to take part in this study?

I am looking for both males and females (18 to 45 years old) who meet the inclusion criteria. If you agree I will also ask some further questions/provide a questionnaire to further determine your suitability to take part in the study.

Inclusion criteria:

Healthy, active individuals. familiarised with resistance training.

Exclusion criteria:

You should not take part if you are:

Currently injured, on long term medication, have any other medical conditions that may be aggravated by use of caffeine, currently using any medication (other than contraceptives) and currently taking part in another study that may compromise the results of this study.

How long am I expected to be part of the study?

Females:

3 months: 2 months of self-monitoring your menstrual cycle; 1 month of visits to the Chipperfield Building, University of Kent (5 in total: 1 familiarisation and 4 experimental trials).

Males:

1 month: 5 visits (1 familiarisation, 4 experimental trials) to the Chipperfield Building at the University of Kent.

The familiarisation session will last approximately 45 minutes and each trial will last 1 hour and 30 minutes.

Do I have to take part in the study?

No, taking part in the study is entirely voluntary. If you agree and want to take part, I will ask you to sign a form giving your written consent, you will also receive a copy of this form. If at any point you want to withdraw from the study, you are free to do so and are not required to give a reason. There will be no penalty if you do not wish to take part or if you wish to withdraw. If you do withdraw mid-study, all data collected from your participation can be destroyed if you wish.

What will I be asked to do if I agree to take part?

Firstly, I will ensure that you understand what will happen in the study and what you will be required to do. I will then ask you to sign the written consent form and fill out a short health questionnaire (PAR-Q) to make sure you meet the inclusion criteria for the study.

Female participants:

You will be required to self-monitor your menstrual cycle for the 2 months prior to testing. For the 2-3 days before your estimated Luteinizing Hormone (LH) surge (which is approximately the point of ovulation- so samples would start approx. day 6-10 of cycle), you will be required to use urine dipsticks daily to identify the date of LH surge. As well as this, you will be asked to complete a logbook, dating the start and finish of menstruation. This is to help us identify the point of ovulation in your cycle because this marks the point between the 2 main phases of the cycle (follicular phase and luteal phase). We need to know this because we hope to undertake all of your testing in the same phase since menstrual cycle phase may influence caffeine's metabolism and other effects in the body. So, we will use this information so that experimental trials can be scheduled to coincide within the follicular phase.

All participants:

Familiarisation trial (Visit 1)

Upon arrival, your height and weight will be recorded. You will need to be prepared to exercise at maximum intensity. Next, the location capable of generating the largest twitch force (muscle activation in response to stimulation of a nerve) and muscle action potential (earliest muscle response to stimulation of a nerve) in the femoral triangle will be determined via sending electrical impulses through a motor point pen, this is a non-painful technique that elicits a short involuntary muscle contraction (this may feel weird however it is not painful). Once located, an electrode will be placed on the site for all subsequent impulses. Following this, the intensity needed for testing will be calculated via small increments (20mA) of electrical stimulation intensity until a plateau in twitch force is observed. The resulting intensity will then be increased by a further 30% to ensure a supramaximal (greater than maximal) stimulation intensity. You will then be required to complete a warm-up, performing 10 knee extension contractions at a perceived effort of 5 (out of 10). Each contraction will last 3 seconds with 3 seconds rest between contractions. Following this, you will be required to complete knee extension maximal voluntary contractions (MVCs), where you must 'push'/contract as hard as you can. You will be required to repeat this 5 times and in the final two contractions, your femoral nerve will be stimulated. This stimulation will consist of the predetermined intensity of electrical impulse (see start of paragraph) being delivered to the electrode on the femoral nerve, generating a supramaximal muscle contraction. Stimulation will occur both mid-contraction at the point of peak torque and at rest a few seconds after the contraction. These contractions will be performed whilst seated and strapped into a device called an "Isokinetic Dynamometer", which is a bit like a chair (not too dissimilar to a leg extension machine, see below). Once sufficient results have been obtained from the MVCs,

you will take a 5-minute rest period prior to performing a hand-grip strength test (using a hand-grip strength measuring device). This test will require no warm-up and similarly to the previous test, will require to you to complete MVCs until 3 consecutive results are within 5% of each other.

Following testing, you will be required to rinse your mouth with water ready for a saliva sample. Following this and 10 minutes after rinsing your mouth, you will give a saliva sample. You will be required to spit a small saliva sample into a sterile plastic tube.

Experimental trials (Visits 2-5)

Upon arrival, you will perform the same series of series of maximal and submaximal muscle contractions performed in visit 1 (warm-up, unstimulated and stimulated). Following this, you will consume a capsule containing either caffeine or a placebo (microcrystalline cellulose – which is a common inert 'filler' ingredient found in many tablets and capsules) and wait for 45 mins before commencing with testing (you must stay in the lab throughout this rest period, you may wish to bring a book or computer to use during this time). Throughout the trials, neither you or the researcher will know what capsule (caffeine or placebo) has been administered and the researcher shall not know this until the study has concluded. After each trial, you will be asked to guess whether you consumed the caffeine or placebo capsule.

45 minutes after the capsule has been consumed, you will perform the same series of MVCs previously demonstrated (warm-up, unstimulated and stimulated). There will be a minimum of 2 days separating visits to allow for sufficient recovery.

Samples

As previously mentioned, at the end of the familiarisation visit, you will provide a saliva sample. The reason for this is so that DNA can be extracted from your saliva, so that later analysis can identify which genetic 'category' you fall into (e.g. caffeine fast or slow metaboliser; caffeine high or low sensitivity genotypes).

When the visit has concluded, the saliva sample collected in the plastic container will be spun in a centrifuge to remove any cells. Following this, the sample will be stored and frozen at -80°C for later analysis. The cellular component separated from the sample will be discarded. At a later date, the sample will be analysed by extracting DNA to measure genes that allow us to know if you are a fast/slow metaboliser and a high/low caffeine sensitive genetic type). Your saliva sample will not be used for any other purpose and will be discarded after the study has concluded.

Standardisation of procedures

In the 24-hours prior to experimental trials, you will be asked to abstain from consuming any caffeinated or alcoholic foods or beverages. You will also be asked to avoid any strenuous physical activity for the 48 hours prior to the trials. This will be recorded using a physical activity log, which will then be replicated prior to subsequent visits.

You will be given a food and drink diary to record your diet during the 24 hours prior to the first experimental trial. You will then be required to replicate this diet identically in the 24 hours prior to all subsequent trials.

All of the above will be verbally confirmed prior to each visit.

Are there any risks of taking part?

The study will require to you exercise at a maximal intensity. Maximal tests can sometimes leave you nauseous, dizzy or short of breath. However, these effects are temporary and once the exercise has finished, should disappear soon after. It should be noted that maximal exercise always holds a risk of cardiac complications, even if the participant is 'healthy'. However, before testing you will be asked to complete a health questionnaire/physical activity readiness questionnaire (PAR-Q) to ensure you meet inclusion criteria. Immediate first aid treatment will be available and provided by qualified first aiders in the unlikely event of an injury or illness.

A small site on the right thigh will be shaved prior to placement of electronic devices. This holds the potential for skin irritation. Prior to testing, you will be asked whether you have any allergies to the procedures and products used in testing. However, in the event of an allergic reaction, testing will immediately stop.

Caffeine can cause some side effects in some individuals (e.g. headache, nervousness, nausea). These side effects are rare and if they do occur are generally very mild. If you know you are likely to experience any adverse reactions to caffeine, you should not take part.

What are the possible benefits from taking part?

The results of the study will help to advance our understanding of how caffeine affects the body (especially in females, which is a currently under-studied area). We can also provide

you with a copy of your personal results, if you wish, to help you understand how caffeine influences your performance.

Will my taking part be confidential?

Yes. The consent form you sign will be stored securely within the School of Sport and Exercise Sciences' premises in accordance with the Data Protection Act 2018 and the University's own data protection procedures. You can access the University of Kent's privacy notice related to research here:

https://cdn-researchkent.pressidium.com/ris-operations/wp-content/uploads/sites/2308/2020/06/GDPR-Privacy-Notice-Research.pdf

No data will be passed on to any third party. The results may be published in a student dissertation or possibly submitted to scientific journals and/or conferences but all data will be anonymous (i.e. you will be represented only by a code and the results will only present overall group summaries of all data combined). You can receive a copy of your personal results and/or a summary of the overall findings if you wish.

The consent form includes space for an anonymous code that will be written against your test scores and the questionnaires you complete. So, no one will be able to see what your test scores and questionnaire answers are except for the research team (see end of document) who have the consent form with both your code and your name. The consent forms are kept for up to 12 months after testing so that we can show our ethics committee that you gave written informed consent and are then securely destroyed. Anonymous data such as your test scores and questionnaires will be stored for up to five years after the study.

What will happen to the results of the research?

The data collected during the sessions will be used to write a master's thesis. The study may also be presented at academic conferences and will be submitted for publication in scientific journals. All data presentation will be anonymous and will not allow identification of any participants.

Who is organising and funding the study?

The study is organised and funded by the School of Sport and Exercise Sciences at the University of Kent.

Who has reviewed this study?

The School of Sport and Exercise Sciences Research Ethics and Advisory Group (REAG) at the University of Kent has approved this study.

Who can I contact if I need to ask more questions about the study?

You can contact the researcher at any time using the contact details below.

Who can I contact if I want to complain about the study?

If you have any concerns or wish to complain about any aspect of the way you have been approached or treated during the course of this study, you may contact the SSES Director of Research and Innovation, Lex Mauger by email (L.Mauger@kent.ac.uk) or the chair of the SSES Research Ethics and Advisory Group, Dr Karen Hambly (K.Hambly@kent.ac.uk).

Covid-19, Safe Travel and Social Distancing

You will be required to follow the latest Government guidance on safe travel and social distancing when travelling to our facilities for testing, including any recommendations for non-symptomatic COVID-19 test on the day (e.g. a lateral flow self-test). Within our buildings, we may have a one-way system and social distancing procedures in place if the current guidance requires it (and all who are able should wear a face covering). We will call and/or e-mail you in advance of each visit to run through some symptom checks and provide specific instructions for when you arrive. Researchers will also be wearing appropriate PPE (masks and/or visors) and will also have completed a non-symptomatic COVID-19 test on the day, and will only come into the lab once the negative test result is received. If not, you would be informed as soon as possible and advised not to attend for your lab visit.

What should I do now?

If you are happy to participate in the research, then please contact the researcher using the contact details below.

Will Searle – ws215@kent.ac.uk Research Team: Will Searle (PGR Student); Professor Glen Davison; Dr Sam Smith

Postal address for all researchers:

School of Sport and Exercise Sciences
The University of Kent
Chipperfield Building
Canterbury
Kent
CT2 7PE

Website: http://www.kent.ac.uk/sport-sciences

Figure 14. Participant information sheet.

7.9 Consent form

CONSENT FORM

Title of project: Effects of caffeine on muscle strength: Influence of genetics

Name of investigators: Will Searle

Participant Identification Number for this project:

	Please initial box
1. I confirm I have read and understand the information sheet, dated __/___/2022 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. If I wish to withdraw, I may contact Will Searle (ws215@kent.ac.uk).	☐
3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised data.	☐
4. I understand that I must read the health questionnaire carefully and answer the questions to the best of my ability, and that the researchers will use my answers to this questionnaire to assess my suitability for participation.	☐
5. I agree to take part in the above research project.	☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent *(if different from lead researcher)*	Date	Signature

Figure 15. Informed consent form.