

THE EFFECT OF CYP1A2 GENE VARIANTS AND CAFFEINE
ON RATINGS OF PERCEIVED EXERTION

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CHAPTER I

INTRODUCTION

Background

An overall rating of perceived exertion (RPE) is the subjective sum or integration of multiple signals elicited from peripheral, cardiovascular, pulmonary and central nervous systems.¹Borg's 6-20 scale for measuring perceived exertion is more commonly used in exercise science research and applied physiology settings (e.g. cardiopulmonary rehabilitation), than the category-ratio 10 point scale. The Borg 6-20 RPE scale was constructed to increase linearly with exercise intensity and heart rate on a cycle ergometer.^{2,3} The scale is often used to rate an individual's subjective response during a graded exercise test.⁴The scale is also used as a prescriptive tool for administering exercise intensities in the clinical setting.^{3,4}Its widespread use in this setting demonstrates that the Borg 6-20 RPE scale has become a valuable tool in the clinical setting. Borg's 6-20 scale has previously been shown to be reliable in healthy men and women across a range of ages and intensities, with good between-trial correlations ($r \geq 0.92$).⁵

Previous work has shown RPE to be altered by regular exercise training.^{6,7}RPE has been shown to decrease at any given intensity following 6 weeks of interval and continuous exercise training.⁶RPE has also been found to be significantly ($p < 0.05$) different between training states (trained vs. untrained) at intensities between 50 and 80% of VO_{2max} .⁷These studies suggest that RPE, along with other parameters (aerobic capacity, work rate etc.), improve with exercise training. This reduced perception of effort at a given exercise intensity may provide ergogenic benefits.

Caffeine is one of the most readily available stimulants worldwide. However, while the International Olympic Committee has an allowable limit for athletes of 12 μ g of caffeine per ml of urine, the World Anti-Doping Agency does not consider caffeine to be a banned substance, but is monitoring its use.⁸The proposed mechanisms for caffeine's beneficial impact on exercise performance are not limited to the central or peripheral physiologic systems and include reduced RPE and pain perception, as well as enhanced CNS excitability and fat metabolism.⁸ However, while some research demonstrates performance enhancements with caffeine ingestion, other research finds no performance changes.⁹Furthermore, studies show contradictory effects of caffeine on RPE.^{10,11}One study found 5mg.kg⁻¹ of caffeine enabled young men to perform significantly more repetitions to failure ($p < 0.01$) while reporting significantly lower RPE levels ($p < 0.01$) at muscle failure, compared with a placebo.¹¹However, another study found that while caffeine ingestion (6mg.kg⁻¹) significantly improved 8.2km cycling time trials in women ($p < 0.05$), RPE was not altered by across the time-trial ($p > 0.05$).¹⁰However, the latter study did utilise the 0-10 category-ratio scale. An earlier study reported that caffeine increased plasma free fatty acid (FFA) levels prior to and during 90 minutes of treadmill running at 70% VO₂max, while not altering RPE, plasma glucose levels, epinephrine or norepinephrine, in varsity level athletes.¹²Nonetheless, the conflicting findings in the literature illustrate that the precise mechanism by which caffeine may alter RPE and enhance performance is unknown and not consistent across individuals.

The discrepancies in the findings may indicate a potential genetic factor as the underlying modulator of caffeine's effect on RPE. Caffeine is absorbed rapidly and wholly from the gastrointestinal tract and metabolised by cytochrome P-450 enzymes, which are the rate-limiting step for plasma clearance. The P-450 1A2 enzyme is coded for by the CYP1A2 gene, and is the primary enzyme which demethylates caffeine into dimethylxanthine metabolites.¹³ It has been estimated that variants of the CYP1A2 gene account for 90% of

the metabolism of caffeine in humans.¹⁴A particular A/C substitution within that gene has previously been shown to modulate caffeine metabolism as well as the influence of caffeine on exercise performance.¹⁵In short, carriers of the C allele metabolise caffeine more slowly than those with the A/A genotype, and would thus be more likely to be affected by caffeine supplementation. The influence of CYP1A2 gene polymorphisms on the ergogenic effects of caffeine has previously been studied in trained male cyclists (N=35).¹⁶The study revealed individuals homozygous for the A allele of the CYP1A2 polymorphism reduced their 40km time trial significantly more ($p<0.05$) following caffeine ingestion ($6\text{mg}\cdot\text{kg}^{-1}$) compared to C allele carriers.¹⁶

Statement of the Problem/ Purpose

Numerous studies have investigated the effect of caffeine on one's perception of effort during aerobic and resistance based exercises. However, the findings have been conflicting and it is apparent that caffeine does not alter each individual's RPE in the same way.^{10,11,17-21}The CYP1A2 gene has been identified as having an integral role in the metabolism of caffeine.¹³ Further, recent research has demonstrated significantly different ergogenic effects of caffeine dependent on a SNP along the CYP1A2 gene.¹⁶ Despite the disparity in findings pertaining to the effect of caffeine on RPE, and the findings linking the CYP1A2 gene to caffeine metabolism, there are currently no published investigations on the potential genetic influence on caffeine's impact on RPE. Therefore this study aimed to elucidate the relationship between a SNP at intron 1 of the CYP1A2 gene and caffeine's effect on RPE and exercise performance.

Hypotheses

It was hypothesised that subjects homozygous for the A allele would have significantly lower RPE at submaximal exercise intensities compared to C allele carriers following ingestion of $6\text{ mg}\cdot\text{kg}^{-1}$ of body weight of USP grade caffeine. It was also

hypothesised that caffeine ingestion would significantly reduce RPE versus placebo at submaximal intensities.

Importance of the Study

There is controversy surrounding the effect of caffeine on RPE during submaximal and maximal aerobic exercise performance. Understanding how caffeine affects RPE at specific exercise intensities has important ramifications for exercise physiologists working in both research and applied settings. However, the full ergogenic effects of caffeine are not yet understood, despite multiple postulations regarding the underlying physiologic mechanisms by which caffeine works. The results from this study may demonstrate novel and valuable findings to add to the scientific literature and yield a deeper understanding of individual variability in caffeine's effects on RPE and exercise performance.

Definitions

1. Single Nucleotide Polymorphism (SNP): A variation in a single base pair in a DNA sequence.
2. Genotype: The genetic make-up of an individual organism.
3. Allele: One member of a base pair occupying a specific spot on a chromosome.
4. Genetic Polymorphism: A place in the DNA where there is a variation (of two or more base pair nucleotide variants). Polymorphisms are larger in size than SNP's and involve long stretches of DNA. In other words a polymorphism is a change along a 'chunk' of DNA.

5. VO_2 max: The maximal volume of oxygen an individual can consume, typically averaged over one minute.

6. Polymerase Chain Reaction: This is a technique used in molecular biology to amplify a single or few copies of a piece of DNA, generating thousands to millions of copies of that piece of DNA. This allows for the analysis of a person's DNA.

7. Respiratory Exchange Ratio: This is the ratio between expired carbon dioxide and inspired oxygen.

CHAPTER II

REVIEW OF LITERATURE

Developing the RPE Scale

An individual's perception of a physical manifestation is a multi-faceted and complex phenomena. Most psychophysical studies have shown that the magnitude of stimulus-perception ratios are equal.^{22,23} This is why the Fechnerian log-function, which states subjective perceptions are proportional to the logarithm of the stimulus intensity, are postulated to yield an erroneous description of the general stimulus-response function.¹ It has been suggested that a power function should be used to describe the stimulus-response function.¹ Since perceptions can be variable, particularly when concerned with physical manifestations, a ratio-scaling method was used to propose an equation to handle the inter-individual variability:²⁴

$$R = a + c(S-b)^n,$$

Where R represents the intensity of the response to the stimulus (S), both a and b are constants regarding the starting point of the stimulus-response relationship, c is the proportionality constant, and n is the exponent.²⁴ However, a major drawback of the ratio-scaling method presented above is the inability to compare perceptions across individuals. For example, two subjects may rate the same 15lb weight as a 2 and 5 out of 10, respectively. Despite the clear disparity in perceptions of the weight, it cannot be interpreted that subject two perceives the weight as heavier than subject one.

To advance the field of psychophysiology and the inherent flaws with ratio-scaling methods, Gunnar Borg developed a category-ratio scale for the ratings of perceived exertion (RPE).²⁵ The scale was a 15-point scale with verbal anchors to aid the association of exertion

with the numerical ratings. From the scale one can determine if the exertion is more intense than another rating (i.e. 13 > 10); however zero intensity does not exist on the scale. The scale was constructed such that it increased linearly with intensity of exercise on a cycle ergometer.² Owing to the integrative nature of human physiology, it is convenient that oxygen consumption (VO_2) and heart rate also increase linearly with exercise intensity.¹ Category-ratio scales allow for direct inter-individual comparisons, unlike the previously constructed ratio-scaling methods.¹ The scale values range from 6 to 20 and are intended to correlate to heart rates spanning 60 to 200 beats per minute. Since the conception of the scale it has become the most widely used measure of perceived exertion within the field of exercise science.²⁶

Applications of the RPE Scale

In addition to the use of RPE within exercise science research, RPE is often used as a prescriptive tool by exercise physiologists in the clinical setting.^{3,4} In young men, RPE response from a graded exercise test can be used as a prescriptive tool to elicit a desired training heart rate within ~ 5 beats per minute.²⁷ Another study found the RPE scale to be valid for prescribing exercise between 50-70% of $\text{VO}_{2\text{max}}$ in young men.²⁸ However, the results of that study show RPE to be more accurate when exercise is completed on a cycle ergometer compared to a treadmill. Despite this, during the initial stages of an exercise regimen utilising the RPE may also help prevent individuals from exceeding their target heart rate.²⁹ Thus, the RPE scale is a useful and cost-effective tool for exercise prescriptions among sedentary, diseased, and unfit populations.

Validity, Reliability and Correlates of the RPE Scale

Given that the scale was developed to increase as a function of exercise intensity, and with inter-individual comparisons in mind, the correct use of the scale is critical. Its correct use is even more pertinent considering that the physiological variables it is supposedly

integrating to create the perception may be affected by psychological factors.^{30,31} For example, one's exercise history, psychological affect, or mood could alter the relationship between RPE and physiological response to exercise intensity.³²⁻³⁴ It has also been shown that the treadmill protocol (e.g. Bruce or Balke) used during graded exercise testing results in significantly different ($p < 0.05$) RPE values in apparently healthy men and women.³⁵ In addition to these confounding variables, it is not clear whether time of day has an effect on RPE ratings during exercise or not.³⁶

Perception of effort has also been shown to correlate linearly with increases in core temperature and electroencephalogram (EEG) frequencies during exhaustive exercise in hot environments.³⁷ Further, during constant load cycling changes in subjects' perception of effort correlates with the changes in electrical muscular activity ($R^2 = 0.69$, $p < 0.01$).³⁸ Therefore, it can be deduced that RPE is a subjective sum integrating signals from cardiovascular (e.g. heart rate), peripheral (e.g. blood lactate) and central (e.g. EEG and EMG) physiologic systems. RPE has been proposed as a homeostasis-like measure that is inherently used by the subconscious to regulate exercise performance through afferent feedback and efferent command to prevent irreversible tissue damage.³⁹

In contrast to the studies highlighting the variability of the RPE scale across exercise protocols, psychological state, and time of day, several studies have found conflicting results, supporting the validity and reliability of the scale.²⁶ One study found RPE ratings from a graded exercise test were highly correlated with RPE ratings at exercise intensities assigned in a random order in both lean and obese subjects.⁴⁰ Noble and colleagues correlated effort ratings to blood and muscle lactate concentrations as well as heart rates during a progressive maximal exercise test on a cycle ergometer. The findings demonstrated that subject RPE values are positively correlated with heart rate and blood lactate concentrations.⁴¹ A meta-analysis assessing criterion-validity found RPE to be most valid under the following

conditions: 1) when men were asked to maximally exert themselves (while VO_2 and/or ventilation was measured), 2) when the exercise task was unusual [e.g. swimming] (while VO_2 was measured), and 3) when the 15-point RPE scale is used (while blood lactate concentration is measured).²⁶

RPE as a Training Adaptation

At any given workload, the energy demand is constant (i.e. it is not affected by training state).⁴² However, as aerobic capacity increases the relative percent of maximal work decreases at any given workload.⁴³ Given this it is logical to expect the perceived effort to be less at a fixed workload as aerobic capacity increases. Indeed, following an 11 week training programme RPE ratings were significantly ($p < 0.05$) lower at a fixed workload.⁴⁴ RPE was also found to be lower across submaximal exercise intensities following 6 weeks of rapid walking and stair stepping in subjects with rheumatoid arthritis.⁴⁵ RPE has previously been shown to be lower at any given intensity following 6 weeks of continuous and interval exercise training.⁶ RPE also differs significantly ($p < 0.05$) between training states (trained vs. untrained) at intensities between 50 and 80% of VO_2max .⁷ Typically, exercise physiologists consider physiological parameters (e.g. aerobic capacity, heart rate response etc.) to demonstrate training adaptations, but these studies suggest RPE may be another desired adaptation.

Pain Scale

From a psychophysical standpoint, exercise scientists and clinicians typically focus on an individual's RPE during graded exercise tests and rehabilitation programs. Anecdotally, muscle pain is commonly experienced during exercise, but it is often overlooked and may be unintentionally integrated into a person's perception of effort. To determine the validity and reliability of a 10-point scale for assessing pain, 7 men and 7 women completed three 1-minute cycling bouts at randomly ordered power outputs, and rated their muscle pain at each

power output. The results demonstrated high intra-class correlations ($R^2 = 0.88$ to 0.98) and thus good reliability in pain ratings.⁴⁶ It is important to note that the pain scale was developed to be distinctly different from Borg's RPE scale, insofar as it assesses the intensity of hurt felt in a specified muscle (usually quadriceps), and not the intensity of exertion from several physiologic systems.⁴⁶ Perception of muscle pain is not used to prescribe exercise intensities, although one could argue that the cliché of "no pain, no gain" is used by some to assess if the training intensity was appropriate.⁴⁷ It is possible that muscle pain is not used to prescribe exercise intensities, as to maintain a moderate-intensity of pain in the quadriceps during cycling, the power output must be reduced, thus the workload would not remain constant for a fixed duration.⁴⁷

Observed Effects of Caffeine

Caffeine is widely utilised by professional and amateur athletes to increase both mental and physical performance.⁴⁸ Amongst a sample of 15,716 American adults and children, 87% consumed an average of 193mg of caffeine per day.⁴⁹ Caffeine is likely so widely consumed in these small dosages due to its stimulatory properties, since larger doses can induce nausea and jitteriness.^{50,51} Although most studies utilising larger dosages (e.g. $5\text{mg}\cdot\text{kg}^{-1}$ of body weight) have shown caffeine ingestion results in ergogenic benefits,^{19,52-57} some studies have found little or no performance benefits following caffeine ingestion.^{58,59} Owing to the ergogenic nature of caffeine, the International Olympic Committee removes competitors if there is more than $12\mu\text{g}$ of caffeine in one ml of their urine.⁸ Caffeine has more than one physiological effect which results in beneficial performance changes, including enhanced central nervous system (CNS) excitability and fat metabolism as well reduced RPE and muscle pain.^{8,9,19}

Despite research demonstrating caffeine's ergogenic benefits, studies presenting individual data have shown some subjects to gain no performance enhancement following

caffeine ingestion.^{54,60} One of these studies found caffeine ingestion (at $5\text{mg}\cdot\text{kg}^{-1}$) significantly ($p<0.05$) increased the length of time subjects could run at 125% of $\text{VO}_{2\text{max}}$ in 11 of the 14 subjects they studied.⁵⁴ The other study reported caffeine ingestion (at $6\text{mg}\cdot\text{kg}^{-1}$) to increase ($p<0.05$) the length of time to fatigue during isometric contractions of the quadriceps in 9 of the 10 subjects they studied.⁶⁰

In addition to the studies demonstrating that some individuals do not gain any ergogenic benefits from caffeine ingestion, some studies have found caffeine does not reduce RPE both at fixed and at variable workloads. Caffeine supplementation of $6\text{mg}\cdot\text{kg}^{-1}$ of body weight did not reduce RPE ($p>0.05$), compared to placebo, during 3 sets of 10 maximal bench and leg press exercises in young men and women.²⁰ In another study, $5\text{mg}\cdot\text{kg}^{-1}$ of caffeine supplementation significantly improved 10km time trial performance versus placebo ($p<0.05$) in young men and women.¹⁷ However, in that study, subjects had higher heart rates in the caffeine trial compared to the placebo ($p<0.001$), but RPE was not significantly different between trials ($p>0.05$). Conversely, other studies have found caffeine ingestion reduces the perception of effort in both men and women. Caffeine supplementation ($6\text{mg}\cdot\text{kg}^{-1}$) was sufficient to reduce the perception of effort in men and women versus placebo ($p<0.05$) during 30 minutes of constant load cycling at $\sim 75\%$ $\text{VO}_{2\text{max}}$.²¹ Similarly, a commercially available 'Quick Energy' drink containing 179 mg of caffeine was shown to elicit several psychological effects during 60 minutes of submaximal cycling at $\sim 60\%$ $\text{VO}_{2\text{max}}$.⁶¹ Specifically, the authors of the latter study noted perceived exertion and muscle pain to be lower following caffeine ingestion compared to placebo ($p<0.001$), with no differences between men and women ($p>0.05$). These four studies all had similar sample sizes (N ranged from 9 to 17), subject ages (21 to 28 yrs). Additionally, all four studies were conducted in men and women. Although no differences between men and women in perception of effort or leg muscle pain ($p>0.05$) were reported in one of those studies, sex

differences in mechanisms of fatigue, as well as perception of pain and exertion have been reported elsewhere.^{62,63} Additionally, it is logical to assume some physiologic and perceptual changes would occur throughout a woman's menstrual cycle which is eliminated in men.

Given the potential for the menstrual cycle to influence physiologic systems and perceptions, it would follow that studying caffeine's effect on RPE in men only would elucidate the magnitude of the effect. However, the converse is true as studies examining the effect of caffeine on RPE are still conflicting when studied in men. In a study where the effect of 5mg.kg⁻¹ of caffeine on 10km cycling time trial performance in active (n=8) and endurance trained (n=8) men was examined, RPE was similar between caffeine and placebo trials (p>0.05).¹⁸ Similarly, 100mg of caffeine disguised in chewing gum did not alter the perception of effort (p>0.05) in college aged men completing a one minute push up test followed by a wingate test.⁶⁴ However, in the latter study, it is possible that the dose was not sufficient to elicit ergogenic benefits, as the dose administered would equal ~1.5mg.kg⁻¹ of caffeine for a 70kg man, and a previous review documents ergogenic benefits at doses between 3 and 6 mg.kg⁻¹.⁸ However, 5mg.kg⁻¹ of caffeine increased the number of bench press repetitions (p<0.01) and reduced both RPE (p<0.01) and muscle pain perception (p<0.05) in moderately trained men when compared to placebo.¹¹ Interestingly, in a study where subjects believed they were given caffeine (3mg.kg⁻¹) but were actually given a placebo, the subjects performed two extra leg extensions, and their RPE was lower, compared to what they believed to be a placebo (p<0.05).⁶⁵ The latter study indicates the need for the subject to be blinded to their treatment condition when comparing the effects of caffeine to a placebo. The above four studies further indicate that the effect of RPE does not only vary across sexes, but also across individuals and potentially the dose of caffeine consumed.

Myers et al. administered a low dose of caffeine (200mg) to adults and subsequently induced ischemia of their forearm by inflating a blood pressure cuff to 250mmHg. Subjects

then performed wrist curls with a 5g bar at a rate of 40 curls per minute. Pain was perceived lower in the caffeine trial after 15 and 30 seconds ($p < 0.02$ and $p < 0.05$, respectively), but not at 45 seconds ($p > 0.05$).⁶⁶ Additionally, a high dose of caffeine ($10 \text{ mg}\cdot\text{kg}^{-1}$ of body weight) significantly ($p < 0.05$) reduced perceptions of leg muscle pain during 30 minutes of moderate intensity cycling ($60\% \text{ VO}_{2\text{peak}}$) in college-aged men.⁶⁷ The authors of the latter study add that the observation of caffeine reducing naturally occurring muscle pain during exercise is likely explained by the hypoalgesic properties of caffeine. Using the same methods as that study, but with two doses of caffeine (5 and $10 \text{ mg}\cdot\text{kg}^{-1}$ of body weight), O'Connor et al. reported a dose-dependent effect of caffeine on leg muscle pain during exercise.⁶⁸ The results of that study elucidate that caffeine has a linear dose-dependent effect on leg muscle pain ($p < 0.0001$; $\eta^2 = 0.56$).⁶⁸ Although muscle pain ratings were lower with the higher dose, from a performance standpoint caffeine ingestion beyond $6 \text{ mg}\cdot\text{kg}^{-1}$ of body weight has no additional benefits. This would indicate that the reduced leg muscle pain does not significantly improve performance.

Caffeine's Mechanisms of Action

The International Society of Sports Nutrition released a position stand in 2010 stating that anhydrous caffeine supplementation in low-to-moderate doses (e.g. $3\text{-}6 \text{ mg}\cdot\text{kg}^{-1}$ of body weight) enhances sport performance in trained athletes, but no additional benefits are seen at higher dosages (e.g. $\geq 9 \text{ mg}\cdot\text{kg}^{-1}$).⁸ The position stand also states caffeine supplementation to be ergogenic for sustained maximal endurance and high intensity intermittent activities.⁸ The anhydrous form of caffeine (at $4.45 \text{ mg}\cdot\text{kg}^{-1}$ of body weight) elevates plasma epinephrine levels more than placebo or coffee containing the same amount of caffeine ($p < 0.05$).⁶⁹ In their study, aerobically trained athletes were able to run 2-3km further at 85% of $\text{VO}_{2\text{max}}$ following anhydrous caffeine supplementation compared to four other treatment conditions (placebo, regular coffee, decaffeinated coffee, and decaffeinated coffee plus anhydrous

coffee).⁶⁹ The authors deduced that a component of coffee must act to moderate the mechanistic actions of caffeine. This is potentially due to the chlorogenic acids which are produced by the roasting of coffee beans, which in turn may reduce caffeine's ability to compete with adenosine at the adenosine receptor site.⁷⁰ However, it has been shown that consuming caffeinated coffee prior to consuming anhydrous caffeine does not interfere with its ergogenic effect.⁷¹ The conclusions could, therefore, be twofold, 1) anhydrous caffeine overpowers the chlorogenic acids found in coffee, or 2) not enough coffee was consumed relative to the dose of anhydrous caffeine to interfere with its ergogenic effect.

It is not surprising that caffeine has more than one mechanism of action, as it is thought caffeine inhibits adenosine from binding to adenosine A₁ and/or A_{2a} receptors, and these receptors are located at numerous sites throughout the human body.¹⁹ Plasma caffeine concentrations of 5-20 $\mu\text{mol.L}^{-1}$ can result in antagonistic binding with adenosine receptors.⁷² In rabbit muscle, these concentrations are sufficient to reverse the adenosine-mediated inhibition of phosphofructokinase (PFK), which is a rate limiting enzyme in anaerobic energy production.⁷³ Assuming the same cascade of events occurs in human skeletal muscle, this would give rise to a greater anaerobic energy production during exercise.⁷⁴ Silva-Cavalcante et al. studied 7 male cyclists in a placebo-controlled crossover study looking at the effects on caffeine and anaerobic energy production. The study found anaerobic energy production to be significantly ($p < 0.05$) greater following caffeine ingestion (5mg.kg^{-1} of body weight) during the first 3km of a 4km time trial.⁷⁵ The latter study supports previous findings,⁷³ and gives credence to the hypothesis that a moderate caffeine dose can prevent the adenosine-related inhibition of PFK.

Caffeine Metabolism

When ingested, caffeine is rapidly absorbed from the gastrointestinal (GI) tract.⁷⁶ Once absorbed into the GI tract, caffeine is metabolised by hepatic cytochrome P-450

enzymes, which are coded for by the CYP1A2 gene and are the rate-limiting step for plasma clearance.¹³ In humans, the CYP1A2 gene has only been detected in the liver where it appears to be controlled by gene expression and inducibility.⁷⁷ As caffeine is metabolised it undergoes demethylation into the dimethylxanthine metabolites paraxanthine, theobromine and theophylline.⁷⁸ These metabolites are then further demethylated into monomethylxanthines.⁷⁹ The clearance of caffeine into its metabolites and subsequently, into the blood stream can vary extremely both within and between individuals.^{80,81} The factors affecting caffeine's metabolism include medications, smoking (nicotine), pregnancy, ethnicity and genetics.¹³ As mentioned previously, the primary physiological effect of caffeine ingestion is the inhibition of adenosine receptors, specifically the A₁ and A_{2a} receptors which are primarily located within the CNS.^{13,19} Caffeine's antagonistic effect on A₁ receptors stimulates the release of the neurotransmitter acetylcholine and activation of A_{2a} receptors stimulates adenylyl cyclase and calcium channels.^{72,82}

Paraxanthine is the primary metabolite produced once caffeine undergoes demethylation.⁸³ Approximately 80% of the demethylated caffeine is bio-transformed into paraxanthine which similar to caffeine acts non-selectively on adenosine receptors, but with a higher affinity than caffeine.^{84,85} Additionally, the plasma clearance rates of paraxanthine are similar to those reported for caffeine (2.20 and 2.07 ml.min.kg⁻¹).⁷⁸ Further, the half-life of caffeine and paraxanthine are similar in humans (4.1 and 3.1 hrs. respectively).⁷⁸ Theophylline on the other hand is a bronchodilator which is commonly used to treat asthma at approximately two to five times the dose obtained by regular coffee drinkers.⁸³ Theophylline is cleared from plasma (0.93 ml.min.kg⁻¹) at a slower rate than caffeine or paraxanthine.⁷⁸ Although theophylline has a slower clearance rate relative to paraxanthine, theophylline exists in smaller absolute quantities than paraxanthine following the demethylation of caffeine. This explains why despite its slower clearance rate, the

bronchodilator effect of caffeine is less reported than the CNS stimulatory effects. Since theophylline is cleared from the plasma at a slower rate than paraxanthine, it is also no surprise that it has a longer half-life (6.2 hrs.).⁷⁸ The other metabolite from caffeine's demethylation is theobromine, which results in vasodilation and increased urine formation.⁸⁶ Theobromine has a similar clearance rate from plasma ($1.20 \text{ ml.min.kg}^{-1}$) and a similar half-life (7.2 hrs.) to theophylline.⁷⁸

In addition to working as an adenosine receptor antagonist, caffeine acts as a non-selective but weak phosphodiesterase (PDE) inhibitor.⁸⁷ The inhibition of PDE's is used clinically to treat an array of diseases including erectile dysfunction, sepsis, cardiovascular disease and pulmonary hypertension.⁸⁷ Inhibiting PDE's leads to the inactivation of intracellular second messengers such as cyclic adenosine monophosphate (cAMP).⁸⁷ When cAMP is activated at the cell membrane it binds to a G protein which catalyses the conversion of cytoplasmic adenosine triphosphate (ATP) into cAMP inside the cell, which in turn activates cAMP-dependent protein kinase.⁸⁸ These events lead to the cells response to the hormone which activated the cAMP. Once cAMP is formed within the cell it activates a cascade of enzymes meaning that few molecules of activated cAMP result in more of the next enzyme and so forth.⁸⁸ However, it is important to note that caffeine's main physiologic effect is to act as an adenosine receptor antagonist and not a PDE inhibitor.

Genetic Influence on Caffeine Metabolism

It has been estimated that variants of the CYP1A2 gene, which codes for the P-450 enzymes which demethylate caffeine, account for ~90-95% of caffeine metabolism in humans.^{14,89} The CYP1A2 gene is made up of seven exons and six introns, and is located on chromosome 15q24.1.⁸⁹ One study found a C/A single nucleotide polymorphism (SNP) located at intron 1 of the cytochrome P-450 gene to effect caffeine metabolism.¹⁵ The findings demonstrate that a C allele results in a slower caffeine metabolism following

caffeine ingestion in smokers, but not in non-smokers.¹⁵ Only one study was located that evaluated the influence of CYP1A2 polymorphisms on caffeine's ergogenic effects. In that study, men (N=35) completed a 40km time trial on a cycle ergometer in a placebo-controlled crossover style. Following caffeine ingestion (at 6mg.kg⁻¹), individuals homozygous for the A allele reduced their 40km time trial significantly (p<0.05) compared to C allele carriers.¹⁶ Given the slower metabolism of caffeine in C allele carriers one might expect the ergogenic effects of caffeine to be greater in C allele carriers, as caffeine would remain in the blood longer and potentially be bound to adenosine receptors for a longer period of time. Conversely, paraxanthine and theophylline have a higher affinity for adenosine receptors than caffeine does.⁸⁵ It has been suggested that a faster caffeine metabolism would lead to a more rapid production of paraxanthine and/or theophylline, facilitating a greater ergogenic effect in individuals homozygous for the A allele.¹⁶

Caffeine, the CYP1A2 Gene and Exercise

It is clear that inter-individual responses to caffeine's effect on perception of effort during exercise performance exist. It is also clear that the effects of caffeine are widespread due to it acting directly on adenosine receptors in the CNS. Recently published data indicate that a SNP of the CYP1A2 gene alters an individual's ergogenic benefit of caffeine ingestion.¹⁶ These recent data have made the link between the CYP1A2 gene and the variability in responders to caffeine ingestion. However, to date no published studies have examined the relationship between SNP's of the CYP1A2 gene and caffeine's effect on perception of effort during exercise performance. Theoretically speaking, individuals homozygous for the A allele should experience a greater reduction in perception of effort following moderate (~3-6mg.kg⁻¹ of body weight) caffeine ingestion than their C allele carrying counterparts.

CHAPTER III

METHODOLOGY

Subjects

Twelve apparently healthy men (age: 24 ± 1 yr., BMI: 23.9 ± 1.2 kg.m²) participated in the present study following approval from the Ball State University Institutional Review Board. Prior to engagement in the study protocol, all subjects provided informed written consent and completed a brief health history questionnaire to ensure they met the inclusion criteria of the study. Specifically, the inclusion criteria stated that subjects must be: 1) non-smokers (including smokeless tobacco users), 2) free from any known cardiovascular, pulmonary and/or metabolic disease, 3) weigh over 50 kg, 4) have no physical limitations that prevents completion of strenuous aerobic exercise, and 5) habituated caffeine users (consuming 50-320 mg of caffeine per day for the past 6 weeks). Definitions for known cardiovascular, pulmonary and/or metabolic diseases are the same as those outlined by the ACSM.⁹⁰

Recruitment

Subjects were recruited via mass email and word of mouth across Ball State University's campus. One subject did not return for the second visit and would not provide a reason for dropping out of the study. Other than the one subject there was no attrition to the study, and all enrolled subjects completed the study protocol.

Experimental Procedure

All data collection took place at the Human Performance Laboratory at Ball State University. Subjects reported to the laboratory at the same time of day on two separate occasions, with the total time commitment totalling approximately 3 hours. All visits to the

laboratory were conducted in the morning, prior to 9am. Subjects were asked to abstain from caffeine, nicotine, and alcohol consumption for 24 hours prior to each visit to the HPL, and have no food or drink other than water for 12 hours prior to their visit. Additionally, subjects were not tested if they had any flu or fever symptoms within the week prior to their exercise test, as exercise is contraindicated in individuals with these symptoms.⁹⁰ Finally, subjects were required to refrain from exercise for 24 hours prior to each visit to the laboratory. The latter requirement was precautionary, as muscular soreness from previous exercise may alter an individual's RPE response to exercise.

Upon arrival at the laboratory, the subject's height and weight were measured using a stadiometer and standard electronic weighing scales. Subjects then consumed a beverage containing either placebo (200ml of non-caloric, coloured and flavoured water) or USP grade caffeine (6mg.kg⁻¹ of body weight). A single-blind, randomised and crossover study design was utilised. Once the drink was consumed each subject remained seated for a period of one hour, after which a blood sample was taken from an antecubital vein.

Prior to beginning the exercise test, subjects were fitted with a telemetric heart rate monitor (Polar Electro, Kempele, Finland). This was utilised to measure resting and exercising heart rate. At the end of the one hour period of rest, each subject's resting blood pressure was measured by a trained technician using a standard auscultatory method and aneroid sphygmomanometer. Subjects returned to the lab for their second visit 7 days after their initial visit and received the alternative treatment (either placebo or treatment).

Instrumentation

Once habituated to the cycle, subjects were fitted with breathing apparatus enabling the measurement of their metabolic responses to exercise. Expired air was collected using a 2-way non-rebreathing valve, and analysed with a metabolic measurement system (Parvo Medics, Sandy, Utah), for the utilisation of O₂ (VO₂), production of CO₂ (VCO₂), respiratory

rate, respiratory exchange ratio (RER), and ventilation (V_E). The system was calibrated at the start of each testing session, and every two tests if multiple tests were being performed. The system was calibrated using a 3 litre syringe and standard gases with known concentrations. Metabolic values were averaged over 30 seconds, and the peak value was used for statistical analyses. Total test time and peak power output (Watts) were recorded from the metabolic cart and cycle ergometer, respectively.

Maximal Exercise Testing

Following the one hour period of seated rest, the subject positioned themselves on an electromagnetically braked cycle ergometer (Corival, Lode, Netherlands) with the seat and handlebars adjusted by the subject to feel most comfortable. Subjects were given a short period to habituate themselves to the cycle and breathing apparatus before the exercise test commenced. Subjects were required to maintain a minimum cadence of 50 revolutions per minute (rpm) throughout the duration of the exercise test. The cycle protocol began at 25 Watts and increased by 25 Watts per minute until volitional exhaustion or a point where the subject's pedal rate fell below 50 rpm. All subjects were given verbal encouragement to continue for as long as possible. A maximal effort was determined by at least two of the following, previously used criteria: 1) respiratory exchange ratio ≥ 1.1 , 2) reaching $\geq 85\%$ of age-predicted maximal heart rate, and 3) RPE ≥ 17 .⁹¹

Ratings of Perceived Exertion

During the final ten minutes of the subject's one hour period of seated rest, a standardised script was used to provide subjects with verbal instructions on how to use the RPE scale.⁹¹ Specifically, subjects were told that 1) the 6-20 scale will be used to determine their perceived intensity of effort, and 2) the verbal descriptors of the scale ("very light", "light", "somewhat hard", and "hard") may be used to aid their association of effort with the numbers on the scale. Additionally, subjects were given examples of the extremes of effort

(i.e. 6 and 20). Specifically subjects were told that “a 6 on this scale represents the effort given whilst lying down” (e.g. resting) and that “a 20 on this scale represents the most effort imaginable (e.g. running to safety from an attacker)”. After this script was read to subjects they were asked if they had any questions regarding how to use the RPE scale. RPE were collected during the final ten seconds of every third minute. Perception of effort was only assessed every third minute due to the small increments of the cycle protocol (25 Watts/minute). It was assumed that subject’s with a higher VO₂max may report an increase in RPE each minute, due to knowledge of an increased workload, opposed to responding to the scale as intended.

Genotyping

Investigators were blinded to genotype until the subject completed the study protocol. Additionally, genotyping was completed by an investigator not involved with the exercise testing. Blood samples were obtained from the antecubital region of the subjects arm after the 1 hour period of seated rest but prior to the exercise test. Whole blood samples were frozen at -20°C and shipped to a separate laboratory at James Madison University, VA, for genotyping. Genomic DNA was isolated from whole blood samples using previously described methods.¹⁶ The CYP1A2 genotype was determined using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) and allele-specific primers using previously described methods.⁹² All genetic analyses were completed by Dr. Chris Womack at James Madison University.

Statistical Analyses

Because the AC and CC genotypes have previously been shown to have similar effects on caffeine metabolism,¹⁶ all C allele carriers were grouped together for the statistical analysis. All statistical analyses were conducted using SPSS for Windows (version 20.0.1; SPSS, Chicago, Illinois). Subject characteristics are presented using descriptive statistics

(mean \pm standard deviation). Paired samples t-tests were used to compare subject characteristics, resting heart rate, blood pressure, test time, heart rate at peak exercise and peak power output. RPE responses were analysed at 75, 150, 225, and 300 Watts using two-way repeated measures ANOVA, with genotype (AA or AC/CC) as the between-subjects variable, and treatment condition (caffeine or placebo) as a within-subjects variable. RER at peak exercise, as well as absolute and relative VO_2max were also compared using a two-way repeated measure ANOVA. Greenhouse-Geisser adjustments for degrees of freedom were employed when Mauchly's test of sphericity was significant ($p < 0.05$). Significant statistical analyses were followed up using the bonferroni post-hoc method. Statistical significance for all analyses was set at $\alpha \leq 0.05$.

CHAPTER IV
RESEARCH MANUSCRIPT

Journal Format: Medicine & Science in Sports & Exercise

Title: The Effect of CYP1A2 Gene Variants and Caffeine on Ratings of Perceived Exertion.

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Conflict of Interest: None.

Abstract

Purpose:The purpose of the present study was to elucidate if caffeine ingestion reduced perception of effort at submaximal intensities during a maximal exercise test. A secondary purpose of this study was to examine the role of a single nucleotide polymorphism (SNP) at intron 1 of cytochrome P-450 gene in modulating caffeine's influence on ratings of perceived exertion (RPE) at the same submaximal exercise intensities. **Methods:**Twelve healthy men (age: 24 ± 1 yr., BMI: 23.9 ± 1.2 kg.m²) volunteered to participate in the present study. Subjects consumed 6 mg.kg⁻¹ of USP grade caffeine in 200ml of non-caloric, coloured and flavoured water, or a placebo-matched drink in a single-blind, randomised and crossover style design. Subjects remained seated for 1 hour after consuming the assigned drink, and subsequently completed an incremental maximal exercise test on a bicycle ergometer, which started at 0 Watts for 1 minute and increased by 25 Watts per minute until volitional exhaustion. RPE was reported every third minute during the test. DNA was obtained from whole blood samples and genotypes were determined using previously described methods. Similar to previous studies looking at this SNP, subjects were categorised into groups of AA homozygotes and C allele carriers for statistical analyses between genotypes. Two-way repeated measures ANOVA's were performed (Treatment \times Genotype) for RPE responses at submaximal workloads up to 300 Watts. Significant results were followed up using the bonferroni post-hoc method. **Results:**There were no significant differences between individuals homozygous for the A variant and C allele carriers for age, height, weight, body mass index (BMI), and VO₂max. A significant Time \times Treatment interaction was observed ($F=5.804$, $p<0.05$) for the rate of increase in RPE between trials. A significant Treatment \times Genotype interaction was also found ($F=5.714$, $p<0.05$), by which C allele carriers exhibited greater reductions in RPE during the caffeine trial compared to AA homozygotes.**Conclusion:** The findings of the present study indicate that perception of effort

is reduced in individuals who metabolise caffeine at a slower rate (i.e. in C allele carriers). It is postulated that AA homozygotes do not experience reductions in RPE due to a greater cardiovascular workload and enhanced CNS excitability following caffeine ingestion.

Introduction

In the 1960's, a Swedish psychologist by the name of Gunnar Borg presented a scale for measuring one's perception of effort during physical tasks.¹ The 15-point scale was developed with verbal anchors to assist an individual in associating their exertion to the numbers on the scale such that each number was more effortful than the previous (i.e. 12>11).² The rating given is the subjective integration of signals from peripheral, cardiovascular, pulmonary and central nervous systems.² Since its conception, the Borg 6-20 RPE scale has proven to be a valid and reliable tool for measuring perceived effort during exercise, particularly when used with male subjects.³ RPE is now commonly used to rate subjective response during a graded exercise test (GXT).⁴ The scale is also used as a prescriptive tool for exercise intensity in the clinical setting (e.g. cardiopulmonary rehabilitation).⁵

Caffeine is a widely consumed stimulant which is reported to elicit ergogenic benefits when consumed in moderate doses (e.g. 3-6mg.kg⁻¹ of body weight).⁶ Caffeine has been shown to reduce RPE and pain perception, and increase fat metabolism and central nervous system excitability.⁶ However, the effects of caffeine on perception of effort during maximal and submaximal exercise are not consistent across all individuals.⁷⁻¹³ Moreover, in two studies where individual data were reported, some, but not all individuals showed an ergogenic benefit following caffeine ingestion.^{14,15} In contrast to the conflicting findings on RPE, caffeine's effect on pain perception is more consistent, potentially due to the analgesic effect of caffeine.¹⁶⁻¹⁸

Caffeine is primarily metabolised by hepatic cytochrome P-450 enzymes, which are coded for by the CYP1A2 gene.¹⁹ Once ingested, caffeine is rapidly and wholly absorbed from the gastrointestinal tract.²⁰ As caffeine is metabolised it undergoes demethylation into three primary dimethylxanthines; paraxanthine, theophylline and theobromine.²¹ These are

then metabolised into monomethylxanthines.²² Once metabolised, the primary physiologic effect of caffeine is as an adenosine receptor antagonist.²³ The primary adenosine receptor targets are located within the Central Nervous System (CNS). Antagonistic action at these receptors stimulates the release of acetylcholine and adenylyl cyclase.^{24,25} The metabolites of caffeine; paraxanthine, theophylline and theobromine serve to act as a non-selective adenosine receptor antagonist, a bronchodilator, and a vasodilator, respectively.²⁶⁻²⁹ Caffeine is also a weak, but non-selective phosphodiesterase (PDE) inhibitor, which acts to inactivate intracellular second messengers such as cyclic adenosine monophosphate (cAMP).²⁷

An (A/C) single nucleotide polymorphism (SNP) of the cytochrome P-450 CYP1A2 gene at intron 1 effects the inducibility of the P-450 enzyme with the presence of a C allele influencing blood markers of caffeine metabolism.³⁰ When individuals homozygous for the A allele consumed caffeine at $6\text{mg}\cdot\text{kg}^{-1}$ of body weight, they improved their 40km time trial performance significantly ($p<0.05$) more than their C allele counterparts.³¹ It is postulated that a faster caffeine metabolism results in a more rapid appearance of paraxanthine and theophylline, which have a higher affinity for adenosine receptors than caffeine.^{31,32}

A deeper understanding of the manner in which caffeine may alter one's perceptions has important application to both research and applied settings. Given the conflicting findings regarding caffeine's effect on RPE, and the link between a CYP1A2 polymorphism and caffeine's ergogenicity, the purpose of this study was twofold: 1) to elucidate if caffeine's effect on RPE differs among SNP's of the CYP1A2 gene at submaximal intensities, and 2) to clarify if caffeine's effects on RPE at submaximal intensities are present irrespective of the CYP1A2 SNP. It was hypothesised that individuals homozygous for the A allele would have lower RPE at all submaximal intensities. It was also hypothesised that when pooled together, no main effect of caffeine on RPE would be found.

Methodology

Subjects

Subjects were recruited via mass email and word of mouth from students and staff at Ball State University, IN. Twelve apparently healthy men (age: 23.9 ± 1.2 yrs; BMI: 23.9 ± 1.2 kg.m²) participated in the present study, which was approved by the Ball State University Institutional Review Board. All subjects provided informed written consent prior to their engagement in the study. All subjects completed a self-report health history questionnaire to screen for the inclusion and exclusion criteria of the study. Specifically, subjects were only enrolled if they were: 1) non-smokers (including smokeless tobacco users), 2) free of known cardiovascular and/or metabolic disease, 3) weigh over 50 kg, 4) free of physical limitations that prevents completion of strenuous aerobic exercise, and 5) habituated caffeine users (consuming 50-320 mg of caffeine per day for the past 6 weeks). One subject voluntarily refused to return after the first testing session. The remaining twelve subjects completed both treatment conditions.

Experimental Procedure

Maximal exercise tests were conducted at the Human Performance Laboratory at Ball State University. Subjects reported to the laboratory at the same time of day on two separate occasions, with the total time commitment totalling approximately 3 hours. All visits to the laboratory were conducted in the morning, prior to 9am. Subjects were asked to abstain from caffeine, nicotine, and alcohol consumption for 24 hours prior to each visit to the laboratory. Subjects were also instructed to consume no food or drink other than water for 12 hours prior to their visit. Additionally subjects were required to refrain from exercise for 24 hours prior to each visit to the laboratory. Lastly, subjects were not tested if they had any flu or fever symptoms within the previous week.

After arriving at the laboratory, each subject's height and weight were measured using a stadiometer and standard electronic weighing scales. Subjects then consumed a beverage containing either placebo (200ml of non-caloric, coloured and flavoured water) or USP grade caffeine (at $6\text{mg}\cdot\text{kg}^{-1}$ of body weight). A single-blind, randomised and crossover study design was utilised. Once the drink was consumed each subject remained seated for a period of one hour, after which a blood sample was taken from an antecubital vein.

Prior to beginning the exercise test, subjects were fitted with a telemetric heart rate monitor (Polar Electro, Kempele, Finland). This was utilised to measure resting and exercising heart rate. Further, each subject's resting blood pressure was measured by a trained technician using a standard auscultatory method and aneroid sphygmomanometer. A maximal exercise test on a cycle ergometer was then completed. Subjects returned to the lab for their second visit 7 days after their initial visit and received the alternative treatment.

Instrumentation

Once habituated to the cycle, subjects were fitted with breathing apparatus (e.g. mouthpiece and nose-clip) enabling the measurement of their metabolic responses to exercise. Expired air was collected using a 2-way non-rebreathing valve, and analysed with a metabolic measurement system (Parvo Medics, Sandy, Utah), for the utilisation of O_2 (VO_2), production of CO_2 (VCO_2), respiratory rate, respiratory exchange ratio (RER), and ventilation (VE). The system was calibrated at the start of each testing session, and every two tests if multiple tests were being performed. The system was calibrated using a 3 litre syringe and standard gases with known concentrations. Metabolic values were averaged over 30 seconds, and the peak value was used for statistical analyses. Total test time and peak power output (Watts) were recorded from the metabolic cart and cycle ergometer, respectively.

Maximal Exercise Testing

After the subject's blood was drawn, the subject was given a brief period (~2 minutes) to habituate themselves to an electromagnetically braked bicycle ergometer (Corival, Lode, Netherlands). This included adjusting the seat height and handlebar position. Subjects were required to maintain a minimum cadence of 50 revolutions per minute (rpm) throughout the duration of the exercise test. The protocol for the graded exercise test began at 25 Watts and increased by 25 Watts per minute until volitional exhaustion or a point when the subject's cadence fell below 50 rpm. All subjects were given verbal encouragement throughout the exercise test. A maximal effort was determined by at least two of the following, previously used criteria: 1) respiratory exchange ratio ≥ 1.1 , 2) reaching $\geq 85\%$ of age-predicted maximal heart rate, and 3) RPE ≥ 17 .³³

Ratings of Perceived Exertion

During the final ten minutes of the subject's one hour period of seated rest, a standardised script was used to provide subjects with verbal instructions on how to use the RPE scale.³³ Specifically, subjects were told that 1) the 6-20 scale will be used to determine their perceived intensity of effort, and 2) the verbal descriptors of the scale ("very light", "light", "somewhat hard", and "hard") may be used to aid their association of effort with the numbers on the scale. Additionally, subjects were given examples of the extremes of effort (i.e. 6 and 20). Specifically, subjects were told that "a 6 on this scale represents the effort given whilst lying down" (e.g. resting) and that "a 20 on this scale represents the most effort imaginable (e.g. running to safety from an attacker)". After this script was read to subjects they were asked if they had any questions regarding how to use the RPE scale. RPE were collected during the final ten seconds of every third minute during the graded exercise test. Perception of effort was only assessed every third minute due to the small increments of the cycle protocol (25 Watts/minute). It was assumed that subject's with a higher VO₂max may

report an increase in RPE each minute, due to knowledge of an increased workload, opposed to responding to the scale as intended.

Genotyping

Investigators were blinded to the subject's genotype until the subject completed all visits of the study. Additionally, genotyping was completed by an investigator not involved with the exercise testing. Whole blood was taken from the blood samples obtained prior to the exercise test, frozen at -20°C, and shipped to a separate laboratory at James Madison University, VA, for genotyping. Genomic DNA was isolated from whole blood samples using previously described methods.³¹ The CYP1A2 genotype was determined using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) and allele-specific primers using previously described methods.³⁴

Statistical Analyses

Because the AC and CC genotypes have previously been shown to have similar effects on caffeine metabolism,³¹ all C allele carriers were grouped together for the statistical analysis. All statistical analyses were conducted using SPSS for Windows (version 20.0.1; SPSS, Chicago, Illinois). Subject characteristics are presented using descriptive statistics (mean \pm standard deviation). Paired samples t-tests were used to compare subject characteristics, resting heart rate, blood pressure, test time, heart rate at peak exercise and peak power output. RPE responses were analysed at 75, 150, 225, and 300 Watts using two-way repeated measures ANOVA, with genotype (AA or AC/CC) as the between-subjects variable, and treatment condition (caffeine or placebo) as a within-subjects variable. RER at peak exercise, as well as absolute and relative VO₂max were also compared using a two-way repeated measure ANOVA. Greenhouse-Geisser adjustments for degrees of freedom were employed when Mauchly's test of sphericity was significant ($p < 0.05$). Significant statistical

analyses were followed up using the bonferroni post-hoc method. Statistical significance for all analyses was set at $\alpha \leq 0.05$.

Results

Subject Characteristics

Of the 12 subjects analysed for this study, 6 subjects (50%) were C allele carriers, which follows a similar genotype distribution to previous studies looking at the CYP1A2 gene using larger sample sizes.³¹ Demographic information for each genotype group (AA or C allele carriers) are presented in Table 1. There were no significant differences ($p > 0.05$) between AA homozygotes and C allele carriers for age, height, weight or BMI. Subjects consumed 458 ± 43 mg of caffeine, and the maximum and minimum doses consumed were 522 and 391.8 mg, respectively.

Effect of Caffeine on Resting Heart Rate and Blood Pressure

When data were pooled, resting heart rate was not significantly different between caffeine and placebo trials ($p > 0.05$). Additionally, resting diastolic blood pressure was not significantly different between trials ($p > 0.05$). However, resting systolic blood pressure was significantly ($p < 0.05$) higher in the caffeine (121.8 ± 9.5 mmHg) versus placebo trial (111.7 ± 9.2 mmHg).

Effect of Caffeine on Exercise Performance

Test time, heart rate at peak exercise and peak power output were not significantly different between trials. However, AA homozygotes did have a significantly higher heart rate at peak exercise during the caffeine trial (192.7 ± 5.8 bpm) compared to C allele carriers (187.2 ± 5.7 bpm). Figure 1 illustrates the relative VO₂max for AA homozygotes and C allele carriers between caffeine and placebo trials. There were no significant differences in relative VO₂max between trials or genotype groups. Similarly, when VO₂max was expressed in litres per minute (absolute values), no significant differences were found between trials or

genotype groups. Although there was no significant difference between caffeine and placebo trials for RER at peak exercise (1.19 ± 0.09 vs. 1.21 ± 0.09 , respectively), there was a significant difference in RER at peak exercise between genotype groups, such that C allele carriers had a lower RER at peak exercise in the caffeine compared to placebo trial (1.14 ± 0.06 vs. 1.18 ± 0.06 , respectively).

Caffeine's impact on RPE

When all 12 subjects were analysed as one group, the data revealed a significant Time \times Treatment interaction for the RPE response during caffeine and placebo trials. Figure 2 suggests this is driven by the differences in RPE responses between caffeine and placebo trials (17.0 ± 1.5 vs. 17.9 ± 1.6 , respectively) at an absolute workload of 300 Watts. No main effect of Treatment on RPE responses was observed when all 12 subjects were analysed together.

Genotype effects on RPE

When the data were grouped into AA homozygotes and C allele carriers of the CYP1A2 gene, there was no significant effect for Treatment \times Time \times Genotype. However, there was significant interaction effect for Treatment \times Genotype. As Table 2 shows, this interaction appears to be driven by differences at 300 Watts. Following caffeine ingestion, C allele carriers had a reduction in RPE at 300 Watts compared to placebo (16.5 ± 0.8 vs. 18.2 ± 1.6 , respectively) which tended towards significance ($p < 0.10$). AA homozygotes did not have a reduction in RPE at 300 Watts following caffeine ingestion (17.5 ± 2.0 vs. 17.7 ± 1.8 , respectively).

Discussion

The major finding of the present study was that caffeine alters the RPE/workload relationship compared to placebo, regardless of genotype. As figure 2 illustrates, this interaction appears to be driven by lower (17.0 ± 1.5 vs. 17.9 ± 1.6) RPE responses at 300 Watts

under the caffeine condition. For the subjects in the present study, this intensity (300 Watts) was approximately 80% VO₂max. Another major finding is the significant Treatment × Genotype interaction, which appears to be due to reduced RPE ratings at 300 Watts (see Table 2). Although these reductions in RPE appear small, in the context of a 15-point scale a mere 1 point reduction represents an approximate 7% change. Previous studies that examined caffeine's effect on RPE using various exercise modes and bouts report conflicting findings.⁷⁻¹³ Moreover, studies presenting individual data have revealed caffeine 'responders' and 'non-responders'.^{14,15} However, the authors believe that this is the first study to demonstrate different RPE responses based on a specific polymorphism of the CYP1A2 gene.

Previous studies utilising 30-60 minutes of cycling at 60-75% VO₂max have found low-to-moderate doses of caffeine to reduce perception of effort in both male and female subjects, by approximately 1-2 arbitrary units.^{10,13} The findings of the present study support these observations and further implicate a SNP at intron 1 of the CYP1A2 gene as one potential moderator of caffeine's influence on RPE at high intensities (e.g. 300 Watts). On the contrary, other studies have yielded contradictory findings in young males and females during 8-10km cycling time trials.^{7,8,35} The studies that have failed to find a reduction in RPE following moderate caffeine ingestion (5-6mg.kg⁻¹) have used trained subjects. Nonetheless, one study found 179mg of caffeine in the form of a 'Quick Energy' drink to reduce perception of effort during 60 minutes submaximal cycling in trained males and females.¹⁰ Therefore, it appears that the novel finding from the present study is the difference in RPE reductions between individuals with different SNP's at intron 1 of the CYP1A2 gene, following caffeine ingestion.

In the present study, no significant differences were found between trials for test time and VO₂max, indicating that our findings were not driven by differences in subject effort between trials. Although VO₂max was unaffected by caffeine ingestion, AA homozygotes

had a higher heart rate at peak exercise compared to placebo (192.7 ± 5.8 vs. 187.2 ± 5.7 bpm, respectively). This finding is similar to previous studies in young men and women who completed endurance based aerobic exercise (e.g. 10km time trial).^{7,10} However, in young men and women who completed 3 sets of 10 maximal repetitions (bench and leg press), peak heart rate was not significantly increased by caffeine consumption ($6\text{mg}\cdot\text{kg}^{-1}$).¹²

Since increases in heart rate up to ~ 100 beats per minute are driven by the removal of vagal tone, and increases in heart rate above 100 beats per minute are largely driven by sympathetic stimulation,³⁶ it is theorised that caffeine elicits an effect on peak heart rate and not resting heart rate. This is logical because caffeine's postulated mechanism of action is adenosine receptor antagonism which results in greater CNS excitability.^{6,23,37} One of the many actions of adenosine is to reduce the activity of excitable tissues (e.g. the heart).³⁸ Since caffeine actively competes for adenosine receptors, not only is there an increase in heart rate due to caffeine's excitatory effect on the CNS, there is also an increase in heart rate due to a reduction in adenosine binding to adenosine receptor sites. The finding that peak heart rate is higher in AA homozygotes following caffeine ingestion supports the theory that individuals homozygous for the A variant of the gene under study metabolise caffeine at a quicker rate than C allele carriers.

Caffeine ingestion has previously been demonstrated to be ergogenic in a 40km cycling time trial for AA homozygotes but not for C allele carriers of the same gene.³¹ This study hypothesised that caffeine ingestion would reduce perception of effort in AA homozygotes, but not in C allele carriers. However, the finding of a significant interaction between Treatment \times Genotype, indicates C allele carriers reported lower RPE at high intensities. Given the integrative nature of the RPE scale (i.e. subjective sum of peripheral, cardiovascular, pulmonary and central nervous systems), it is postulated that a faster caffeine metabolism among those homozygous for the A allele relative to C allele carriers, results in

increased RPE.³⁰ A faster caffeine metabolism has been suggested to result in a more rapid production of paraxanthine and/or theophylline, which have a higher affinity than caffeine for adenosine receptors.³² Furthermore, previous work has shown that a single C/A SNP at intron 1 of the cytochrome p-450 gene to result in a slower caffeine metabolism among smokers, but not non-smokers.³⁰ However, a pertinent limitation of that study is the differential methods utilised to study caffeine metabolism between smokers and non-smokers (i.e. blood vs. urine markers). An interesting avenue for future research is to measure plasma caffeine concentrations across these genotypes to elucidate whether one genotype group metabolises caffeine quicker than the other, in smokers and non-smokers. Nonetheless, the findings of the present study are in agreement with those of Womack et al. such that individuals homozygous for the A variant of the CYP1A2 gene have a different ergogenic response to caffeine than C allele carriers.³¹

The data from the present study suggest that caffeine's effect on the RPE/workload relationship is only evident at higher intensities. This is encouraging given that a recent review paper cites caffeine to be ergogenic for sustained maximal endurance exercise and time-trial performance.⁶ However, it must be noted that aside from a few individuals (e.g. highly trained athletes) 80% VO₂max is beyond the onset of blood lactate accumulation and therefore not sustainable.³⁹ Given the relationship between RPE, heart rate and blood lactate concentrations,^{3,40} it is assumed that the results of the present study indicate caffeine changes the heart rate and blood lactate response to exercise in individuals homozygous for the A variant.

Although these results do provide evidence for an interaction between the identified SNP and caffeine's ability to reduce perception of effort, the results are not without flaw. For example, in the present study, RPE was measured during a maximal exercise test, and not a performance trial (e.g. time trial). During a maximal exercise test with an incremental

protocol, such as the protocol in the present study, physiological ‘steady state’ is not achieved due to the workload increasing each minute. Physiological ‘steady state’ is typically achieved by using 2-3 minute stages at a fixed intensity and cadence (e.g. YMCA protocol).⁴¹ Since physiological ‘steady state’ was not achieved during the protocol of the present study, it cannot be deduced that RPE was reduced at a given metabolic cost. Further, it is unlikely that a reduction in RPE during a 1 minute stage at any given intensity would translate to reduced perception of effort during prolonged exercise at the same intensity. Future studies are encouraged to elucidate the genetic influence on caffeine’s ability to reduce perception of effort during a time-trial or specific exercise task performance rather than a maximal exercise test.

Future studies are also encouraged to measure the plasma caffeine concentrations across genotypes to establish if AA homozygotes do metabolise caffeine faster than C allele carriers in non-smoking subjects. Although peak concentrations are evident in the blood within 1 hour, elevated levels can appear as early as 15-45 minutes post caffeine ingestion.^{42,43} It is possible that elevated levels of caffeine appear in the blood faster in individuals homozygous for the A variant compared to C allele carriers, however this is purely speculative. Obtaining knowledge of the interplay between the SNP and time differences in elevated plasma caffeine concentrations may enable coaches to identify which athletes may and may not respond to caffeine supplementation as well as providing insight into the timing of caffeine administration across genotypes for optimal athletic performance.

Furthermore, it is not known if greater doses are required to elicit a similar reduction in RPE in AA homozygotes versus C allele carriers. The International Society of Sports Nutrition states that caffeine is effective for improving athletic performance in athletes when consumed at 3-6 mg.kg⁻¹.⁶ This is supported by Desbrow et al. who state caffeine doses of 3 mg.kg⁻¹ improve cycling performance in trained athletes, but 6 mg.kg⁻¹ confers no additional

benefit.⁴⁴ It is possible that a higher dose is needed to elicit a commensurate ergogenic benefit in AA homozygotes versus C allele carriers. However, this is speculative and future research should elucidate the dose-response relationship across these genotypes.

Although the results of the present study indicate a significant interaction between caffeine and the genotype under study, caution must be urged when extrapolating the results to other populations. The participants in the present study had a large range of cardiorespiratory fitness levels (36.5 to 74.1 ml.kg.min⁻¹) and fairly small age range (22.4 to 25.5 yrs.). Therefore, it is not known if this genetic polymorphism alters the RPE/workload relationship following caffeine ingestion in women, older adults, smokers, or persons diagnosed with chronic diseases. Given the findings of the present study, future studies should examine the interaction between this SNP, as well as other suitable polymorphisms, and caffeine's ability to blunt RPE response during exercise in these other populations.

Conclusion

The novel finding of the present study is that a SNP at intron 1 of the CYP1A2 gene appears to modulate caffeine's ability to reduce perception of effort at high intensity exercise. Although it is important to note that reductions in RPE may not occur across all exercise intensities. It is postulated that a faster caffeine metabolism among individuals homozygous for the A variant of the selected CYP1A2 polymorphism serves to enhance perception of effort due to a greater cardiovascular workload and enhanced CNS excitability. However, other factors including dose, other genetic polymorphisms, and time-of-day may also influence caffeine's ergogenic effect on exercise performance. In conclusion, caffeine only appears to reduce perception of effort at high intensities, and is therefore more likely to be of ergogenic benefit during time-trials and endurance performance in trained athletes than during exercise training.

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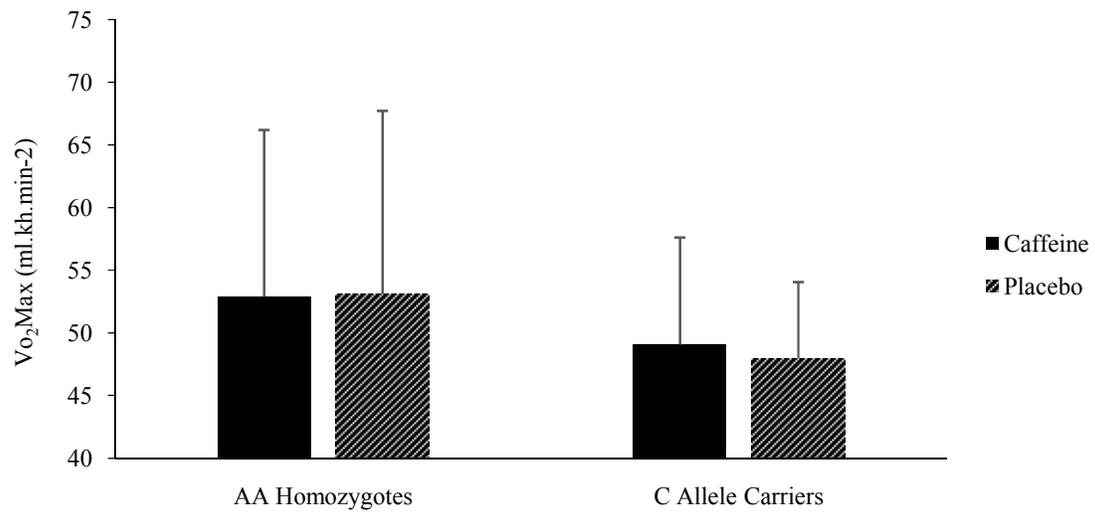


Figure 1

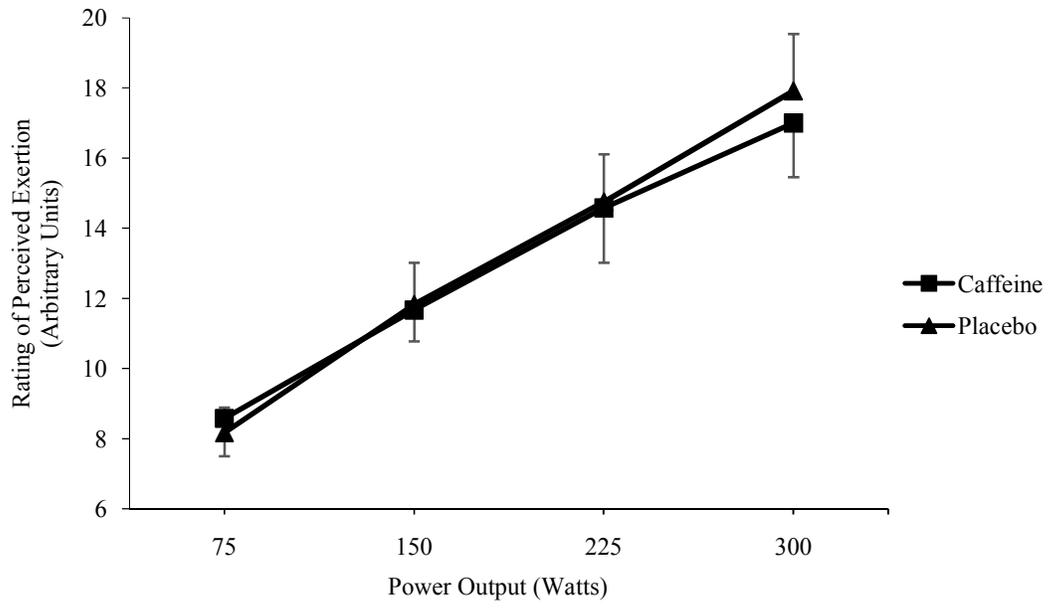


Figure 2

Tables

	A/A Genotype (n=6)	C Allele Carriers (n=6)
Age	23.9±1.2	23.9±1.5
Height (cm)	182.8±7.8	179.0±5.8
Weight (kg)	76.7±9.0	78.6±6.5
BMI (kg.m ²)	22.9±1.4	24.6±2.6

Table 1

Intensity (Watts)	AA Homozygotes		C Allele Carriers	
	CAFF	PLA	CAFF	PLA
75	8.8±1.2	8.0±0.6	8.3±1.0	8.3±0.8
150	11.7±0.8	11.5±1.2	11.7±1.0	12.2±1.2
225	14.3±1.5	14.3±1.2	14.8±1.7	15.2±1.5
300	17.5±2.0	17.7±1.8	16.5±0.8	18.2±1.6

Table 2

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study found that regardless of genotype, caffeine ingestion alters the RPE/workload relationship when compared to placebo. Caffeine appears to elicit this effect by lowering perception of effort at a workload of 300 Watts, which in the present study was associated with approximately 80% VO₂max. In general exercise prescription, 80% VO₂max is considered a vigorous exercise intensity.⁹⁰ Ratings of perceived exertion between 14 and 17 are associated with workloads corresponding to 64-91% VO₂max, and are considered to be a vigorous intensity of exercise.⁹³ Further, an RPE of ≥ 18 represents near-maximal to maximal workloads.⁹³ Our data show that under the placebo condition, subject's perceived exercise at ~80% VO₂max to be 17.9 \pm 1.6 versus 17.0 \pm 1.5 under the caffeine condition. This suggests that under the placebo subjects perceived vigorous intensity exercise to feel near-maximal exertion, and that caffeine ingestion enabled subjects to perceive the same workload as vigorous intensity exercise. Anecdotally, following the caffeine trial many subjects reported feeling that the exercise did not feel as effortful compared to the placebo trial.

Previous studies examining caffeine's effect on RPE using various exercise modes and bouts have found conflicting findings.^{11,17,18,20,21,61,64} Moreover, studies that have presented individual data have revealed caffeine to elicit an ergogenic effect in some individuals and retard performance in a small number of subjects.^{54,60} Only one study has examined the effect of a SNP at intron 1 of the P-450 gene on exercise performance, finding that individuals homozygous for the A variant produce a significant reduction in 40km time trial performance versus C allele carriers.¹⁶ The findings of these studies provided the rationale to examine the effect of the same SNP on caffeine's ability to reduce perception of

effort. The data from the present study reveal a significant Treatment \times Genotype interaction, indicating that the effects of caffeine on RPE were significantly different between the genotype groups. The effect tended toward significance at a workload of 300 Watts ($p < 0.10$), such that C allele carriers perceived the workload to be less effortful than individuals homozygous for the A variant. This absolute workload corresponds to approximately 80% $VO_2\text{max}$. Table 3 demonstrates that at 300 Watts, individuals homozygous for the A allele perceived effort to be similar between caffeine and placebo trials (17.5 ± 2.0 and 17.7 ± 1.8 , respectively). Conversely, at 300 Watts, C allele carriers perceived exercise to be less effortful following caffeine ingestion (16.5 ± 0.8 vs. 18.2 ± 1.6 , respectively). Although these reductions in RPE appear small, in the context of a 15-point scale a mere 1 point reduction represents an approximate 7% change. To our knowledge this is the first study to demonstrate different RPE responses based on a SNP at intron 1 of the P-450 gene.

Low-to-moderate doses of caffeine have previously been demonstrated to reduce perception of effort among males and females during 30-60 minutes of cycling at 60-75% $VO_2\text{max}$.^{21,61} Cycling at this intensity would correspond to an approximate RPE of 14, which is a lower intensity than where the RPE differences occur in the present study. However, it must not be ignored that other studies have found no effect of caffeine on perception of effort during 8-10km cycling time trials in young male and female subjects.^{10,17,18} Although the majority of studies reporting no reduction in RPE following moderate caffeine ingestion (5-6mg.kg⁻¹) have used trained subjects. Despite the allusion that caffeine may only reduce RPE in untrained individuals, it cannot be ignored that a previous study found a 'Quick Energy' drink containing 179mg of caffeine reduced perception of effort during 60 minutes submaximal cycling in trained males and females.⁶¹ The novel finding of the present study is therefore that caffeine alters perception of effort to a greater degree in C allele carriers than in AA homozygotes.

Although the precise mechanism(s) for caffeine's ergogenic effects are not known, it is known that caffeine acts as an adenosine antagonist by competing for adenosine receptor sites.^{72,87} Despite the knowledge that caffeine and one of its primary metabolites (paraxanthine) act through this mechanism,^{84,85} it is not clear why C allele carriers of the P-450 gene do not improve performance by as much as AA homozygotes during a 40km cycling time trial following caffeine ingestion.¹⁶ It is postulated that the differential effects in the two genotypes are due to a faster metabolism of caffeine in AA homozygotes compared to C allele carriers. Furthermore, previous work has shown that a single C/A SNP at intron 1 of the cytochrome p-450 gene to result in a slower caffeine metabolism among smokers, but not non-smokers.¹⁵ However, a pertinent limitation of that study is the differential methods utilised to study caffeine metabolism between smokers and non-smokers (i.e. blood vs. urine markers). Although the difference in metabolism is only proposed, it appears logical from a physiological standpoint, since a faster metabolism would result in more paraxanthine within the blood, and paraxanthine has a higher affinity for adenosine receptor sites than caffeine.^{84,85}

These previous findings formed the hypothesis that caffeine ingestion would reduce perception of effort in AA homozygotes, but not in C allele carriers. The finding of a significant Treatment \times Genotype interaction, which indicated the C allele carriers reported lower RPE at high intensities jettisons our hypothesis. It is theorised that greater adenosine antagonism due to a faster metabolism in AA homozygotes results in a greater cardiovascular workload and CNS excitability, as evidenced by a significantly higher maximal heart rate following caffeine ingestion in AA homozygotes. Although the findings jettison the hypothesis, they may still align with previous findings,¹⁶ as the present study found evidence for a faster caffeine metabolism in AA homozygotes. Thus it would be beneficial for future studies to measure the rate of appearance of caffeine and its metabolites to elucidate whether

there is indeed a faster caffeine metabolism in individuals homozygous for the A variant of the gene we studied. There is evidence that the time for caffeine to appear in plasma varies from 15-45 minutes, but peak plasma concentrations of caffeine typically appear at 1 hour post-ingestion.^{94,95} If indeed AA homozygotes metabolise caffeine quicker, it is possible that caffeine appears in their plasma sooner than in C allele carriers. If this is the case, and caffeine appears in the plasma of AA homozygotes after just 15 minutes, it is feasible to assume that caffeine and paraxanthine would have longer to compete for adenosine receptor sites than if it took 45 minutes to appear in the plasma. However, the latter is purely speculative and future studies are needed to elucidate the precise mechanism(s) by which caffeine elicits its ergogenic effects in AA homozygotes and C allele carriers.

Furthermore, it is possible that C allele carriers need to consume a larger dose of caffeine to elicit the same ergogenic effect that AA homozygotes gain from a lower dose. However, if the postulations made from the results of this study are true, a larger dose may reverse the ability of caffeine to reduce RPE in C allele carriers. When individual data have been presented not all individuals respond to the same extent as others with regard to the ability to increase time to fatigue.⁶⁰ The International Society of Sports Nutrition states that caffeine is effective for improving athletic performance in athletes when consumed at 3-6 mg.kg⁻¹.⁸ The fact that the effective dose for caffeine's ergogenic effect has a range of 3 mg.kg⁻¹ implies that a single dose is not effective for individuals of the same weight. However, this notion is speculative and future research is encouraged to elucidate the dose-response relationship across these genotypes.

Our data suggest that caffeine affects the relationship between RPE and workload, but only at higher intensities (~80% VO₂max). A recent review paper concluded that caffeine enhances performance during maximal endurance exercise and time-trial performances.⁸ It could be argued that during such events, exercise intensity would be high. It is theorised that

during maximal endurance performances, the brain limits exercise performance to maintain homeostasis.⁹⁶ It is feasible that the ability of caffeine to alter one's perception of effort may result in advantageous changes to the pacing strategy. However, it must be noted that aside from a few individuals (e.g. highly trained athletes) 80% VO₂max is beyond the onset of blood lactate accumulation and therefore not sustainable.⁹⁷ Therefore caffeine may provide the most benefit to highly trained individuals and other factors may be more important in lesser trained individuals. Given the relationship between RPE, heart rate and blood lactate concentrations,^{26,41} it is assumed that the findings of the present study indicate that caffeine changes the heart rate and blood lactate response during submaximal exercise in individuals homozygous for the A variant.

In the present study, no significant differences were found between trials for test time and maximal oxygen consumption, indicating that our findings were not driven by differences in subject effort between trials. Although our results demonstrate that RER was not significantly different between trials, there was a significant Genotype × Treatment interaction, such that C allele carriers had a lower RER at peak exercise under the caffeine trial compared to the placebo trial. A lower RER has previously been cited to indicate a greater rate of lipolysis in exercise to exhaustion at 80% VO₂max.⁵² This would suggest that C allele carriers have an increased rate of lipolysis following caffeine ingestion, whereas caffeine does not change the fuel substrates metabolised in AA homozygotes. However, the same dose of caffeine (6 mg.kg⁻¹) has also been shown to increase blood glucose levels during exercise in young men versus placebo,⁹⁸ so we cannot exclude the possibility that subjects with the AA polymorphism relied more heavily on glycolytic metabolism than C allele carriers. Although C allele carriers had a significantly lower mean RER during the caffeine trial, the relatively small difference (0.04) is likely not meaningful.

Although VO_2max was unaffected by caffeine ingestion, AA homozygotes had a higher heart rate at peak exercise compared to placebo. This finding is similar to previous studies in young men and women who completed endurance based aerobic exercise (e.g. 10km time trial).^{17,61} However, in young men and women who completed 3 sets of 10 maximal repetitions (bench and leg press), peak heart rate was not significantly increased by caffeine consumption ($6\text{mg}\cdot\text{kg}^{-1}$).²⁰ Since increases in heart rate up to ~ 100 beats per minute are driven by the removal of vagal tone, and increases in heart rate above 100 beats per minute are largely driven by sympathetic stimulation,⁹⁹ it is theorised that caffeine elicits an effect on peak heart rate and not resting heart rate. This is logical because caffeine's postulated mechanism of action is adenosine receptor antagonism which results in greater CNS excitability.^{8,9,19} One of the many actions of adenosine is to reduce the activity of excitable tissues (e.g. the heart).¹⁰⁰ Since caffeine actively competes for adenosine receptors, not only is there an increase in heart rate due to caffeine's excitatory effect on the CNS, there is also an increase in heart rate due to a reduction in adenosine binding to adenosine receptor sites. The finding that peak heart rate is higher in AA homozygotes following caffeine ingestion supports the theory that individuals homozygous for the A variant of the gene under study metabolise caffeine at a quicker rate than C allele carriers. It is theorised that the greater maximal heart rate observed in AA homozygotes following caffeine ingestion inflates the perception of effort to pre-caffeine (i.e. placebo) levels.

Our study was designed such that subjects completed a maximal exercise test and RPE was recorded every three minutes. Therefore we cannot be certain that if RPE were collected more often (e.g. every minute) the results would remain the same. Additionally, we cannot draw conclusions pertaining to caffeine's ability to improve performance and reduce perception of effort simultaneously, since RPE's were recorded during an incremental maximal exercise test and these data are not akin to an endurance bout at the same intensity.

Moreover due to the nature of an incremental protocol, physiological ‘steady state’ is not achieved due to the workload increasing each minute. Physiological ‘steady state’ requires approximately 2-3 minutes exercise at a constant workload (e.g. YMCA protocol).⁹⁰ It is not known if after completing each intensity for 2-3 minutes, perception of the effort required to sustain that workload would increase, decrease, or remain the same. Future studies should elucidate the genetic influence on caffeine’s ability to reduce perception of effort during a time-trial or exercise to exhaustion at a specific intensity rather than attempting to assess differences over a range of intensities through the completion of a maximal exercise test.

Although our results indicate a significant interaction between caffeine and the genotype under study, caution must be urged when interpreting these results. Indeed, the association between reductions in perception of effort and C allele carriers completing high intensity exercise on a cycle ergometer does not translate to causation. It is possible that in a study using a larger sample size, the same association may not be significant. Further, despite the large range of cardiorespiratory fitness levels (36.5 to 74.1 ml.kg.min⁻¹) among our cohort, subjects were of similar age (22.4 to 25.5 years). Therefore, it is not known if this genetic polymorphism alters the RPE/workload relationship following caffeine ingestion in women, older adults, smokers, or persons diagnosed with chronic diseases. Given the findings of the present study, future studies should examine the interaction between this SNP, as well as other suitable polymorphisms, and caffeine’s ability to alter the RPE/workload relationship during exercise in these other populations.

In conclusion, the present study demonstrated: 1) caffeine significantly alters the RPE/workload relationship, and 2) caffeine’s ability to reduce perception of effort is dependent upon a SNP located at intron 1 of the P-450 gene. These findings are presented with two caveats: 1) the difference in the RPE/workload relationship observed following caffeine ingestion appears to be driven by differences in perception of effort at high intensity

exercise (~80% VO₂max), and 2) reductions in RPE may not occur across all exercise intensities. The second key finding is presented with the caveat that the difference in reductions of effort perception observed between genotypes does not imply causation, but association. Caution is urged when generalising these results to populations other than those studied within this cohort (i.e. young, healthy males). The mechanism(s) by which this SNP appears to modulate caffeine's ergogenicity are yet to be establish. Nonetheless, it is postulated that a faster caffeine metabolism among individuals homozygous for the A variant of the selected polymorphism results in enhanced CNS excitability through caffeine and paraxanthine actively competing for adenosine receptor sites. It is theorised that this results in a greater RPE value as CNS excitability and greater cardiovascular workload are integrated into the perception of effort. However, other factors including dose, other genetic polymorphisms, and time-of-day may also affect caffeine's ergogenic effect on exercise performance.

APPENDICES

Appendix A: Informed Consent

Consent Form: The Effect of CYP1A2 Gene Variants and Caffeine on Ratings of Perceived Exertion.

The purpose of this study is to assess the effect of caffeine consumption on blood clotting activity at rest and during exercise, and the influence of genetics on these responses.

Most adverse cardiovascular events such as heart attack and stroke are caused by a blood clot that blocks an artery, preventing blood and oxygen from passing through. Risk of such an adverse event is elevated during exercise, likely because clotting activity increases in response to physical exertion. Caffeine, which has many known effects on the body including increased heart rate, blood pressure, feelings of alertness and dampened pain perception, may also impact the activity in the blood that causes clot formation. Furthermore, the use of caffeine before exercise, which is known to improve athletic performance, may influence the coagulation response to exertion. We are studying how caffeine consumption affects enzymes and measures of clotting time that reflect your ability to create a blood clot at rest and during exercise. We are also exploring how your DNA might affect these responses. These measures are known to be related to the risk of heart attack and stroke, and the results of this study may provide important information regarding the cardiovascular safety of the use of caffeine at rest and during exercise.

Healthy, non-smoking men who weigh at least 110 pounds and are at least 18 yrs of age may be eligible to participate in this study, which will be done in two separate visits to the Human Performance Laboratory. Participants must have no physical limitations that impact the ability to exercise, and should be taking no medications that may influence the results of the study. Your physician's approval to participate may be required, but no additional information from your medical file will be collected. You may be eligible if you consume approximately one caffeinated beverage (*e.g.* coffee, soda, energy drink) each day. Prior to each visit you will be asked to avoid caffeine for at least 24 hours. During one visit you will drink approximately one cup of a placebo, and the other visit you will drink a preparation that contains 6 mg of caffeine per kg of body weight. For an average adult male, this is approximately the amount of caffeine in two cups of coffee. You will not be told which drink you consumed until after the study is completed. After consuming the drink, you will rest for one hour. You will then be asked to complete a maximal exercise test on a stationary bicycle. The exercise test will start at a very low intensity and will become progressively more difficult until you are unable to keep up with the recommended pedal rate. During each test you'll wear a nose clip and mouthpiece to allow the assessment of your maximal aerobic capacity (VO₂max). The two exercise tests will be separated by a minimum of 7 days. During the exercise tests, your blood pressure and heart rate will be monitored. A 10-ml (<1 Tbsp) blood sample will be obtained from a vein in your arm before and immediately after each exercise test. These samples will be used to measure blood clotting activity as well as to extract your DNA. Each test is expected to last 8-15 minutes, and your total time commitment to this study is expected to be less than 3 hours.

As with any physical exercise, there is potential for muscle or joint soreness, and a slight risk of more serious muscular (*e.g.* muscle strain/sprain) or cardiovascular (*e.g.* heart attack, stroke) injury involved with this study. Investigators will take measures to prevent such injuries, including ensuring adequate warm-up and monitoring hemodynamic responses during exercise. The most common side effects of caffeine include elevated heart rate and blood pressure, irritability, restlessness and possibly stomach discomfort. Risks of blood drawing may include discomfort, bruising, and, in rare instances,

infection, lightheadedness, and fainting. We will use sterile procedures and trained personnel to ensure that there is minimal discomfort with obtaining the blood samples. Emergency medical treatment is available in the event of injury or serious adverse reaction to the caffeine. You will assume responsibility for the costs of medical care that is provided. In the unlikely event of injury or illness of any kind as a result of participation in this research project, Ball State University, its agents and employees will assume whatever responsibility is required by law.

You will be given any information collected as part of your participation in this project (*e.g.* VO₂max, heart rate, blood pressure, measures of coagulation activity). The results of this study may yield important information about cardiovascular risk related to caffeine use, and may enhance the ability of nutritionists and exercise professionals to deliver safe and effective recommendations for individuals who consume caffeine recreationally or to enhance athletic performance.

Your participation in this study is completely voluntary and you are free to withdraw from the study at any time for any reason without penalty or prejudice from the investigators. Please feel free to ask any questions of the investigator before signing this Informed Consent form and beginning the study, and at any time during the study. All study materials will be maintained as confidential. Your information will be stored for three years after completion of the study. Data will be stored in a locked filing cabinet in the researcher's office. Electronic files, which will be password protected, will be stored on the principal investigator's computer and preferred backup medium. If you are not eligible or choose not to participate in this study your information will not be retained, and will be destroyed by shredding paper files, permanently deleting electronic information, and destroying blood and DNA samples.

For one's rights as a research subject contact the Director, Office of Research Integrity, Ball State University, Muncie, IN, 765-285-5070 or irb@bsu.edu.

I, _____, agree to participate in this research project entitled, "Hemostatic Responses to Caffeine at Rest and During Exercise." I have had the study explained to me and my questions have been answered to my satisfaction. I have read the description of this project and give my consent to participate. I understand that I will receive a copy of this informed consent form to keep for future reference.

Participant's Signature

Date

Investigator's Signature

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Appendix B: Health History Questionnaire

THE EFFECT OF CYP1A2 GENE VARIANTS AND CAFFEINE ON RATINGS OF PERCEIVED EXERTION.

Please mark all *true* statements:

History

You have had:

- A heart attack
- Heart surgery
- Cardiac catheterization
- Coronary angioplasty (PTCA)
- Pacemaker/implantable cardiac
- Defibrillator/rhythm disturbance
- Heart valve disease
- Heart failure
- Heart transplantation
- Congenital heart disease

Symptoms

- You experience chest discomfort with exertion.
- You experience unreasonable breathlessness.
- You experience dizziness, fainting, or blackouts.
- You take heart medications.

Other Health Issues

- You have diabetes.
- You have asthma or other lung disease.
- You have burning or cramping sensation in your lower legs when walking short distances.
- You have musculoskeletal problems that limit your physical activity.
- You have concerns about the safety of exercise.
- You take prescription medication(s).
- You are pregnant.

Cardiovascular Risk Factors

- You are a man older than 45 years.
- You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal.
- You smoke, or quit smoking within the previous 6 months.
- Your blood pressure is $> 140/90$ mm Hg.
- You do not know your blood pressure.
- You take blood pressure medication.
- Your blood cholesterol level is >200 mg/dL.
- Your good cholesterol (HDL) is <40 mg/dl OR your bad cholesterol (LDL) is >130 mg/dl
- You do not know your cholesterol level.
- You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
- You are physically inactive (i.e., you get <30 minutes of physical activity on a least 3 days per week).
- You are >20 pounds overweight.
- None of the above is true.

Caffeine History: Briefly describe your typical caffeine use. Specify the type (*e.g.* coffee, energy drink) and number of caffeinated drinks (drinks per day, days per week) you usually consume:

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