The Effects of *CYP1A2* Gene Polymorphisms on Caffeine Pharmacokinetics and Exercise Performance

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A thesis presented in the partial fulfilment of the requirements for the degree of Master of Science in Nutrition and Dietetics

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Abstract

Background: Caffeine is one of the most popular psychoactive stimulants consumed globally. The *CYP1A2* gene encodes the cytochrome P450 1A2 enzyme, found in the liver, which is predominantly responsible (~95%) for caffeine metabolism in the body. A single nucleotide polymorphism (SNP) in the non-coding region of the *CYP1A2* gene (*CYP1A2*; rs762551) induces different expression levels of the enzyme, influencing the clearance rate of caffeine from the body. There are equivocal results as to whether the *CYP1A2* genotype is a determinant of exercise performance following supplementation with caffeine. This lack of consensus may be due to differences in trial designs, including mixed exercise modes, and solely male, or mixed sex participant cohorts with most studies using a caffeine dose of between 3-6 $mg \cdot kg^{-1}$, and commencing exercise 60-min following caffeine ingestion.

Purpose: This research aimed to determine if 6 $mg \cdot kg^{-1}$ caffeine ingested 60-min prior to commencing exercise impacts performance, and to identify if the *CYP1A2* gene, and consequent caffeine metabolism rates, has a role in improving exercise performance following caffeine consumption.

Methods: Thirty-eight healthy, recreationally active, male athletes were recruited for this study. All participants were classified as moderate caffeine users. Participants attended one familiarisation session, where their body composition was measured, practiced a 1-km run or 40-km cycle, and provided saliva samples for genotyping to identify their specific *CYP1A2* SNP. Two follow up sessions were undertaken one week apart, with participants completing either a 10-km run or 40-km cycle following the ingestion of an anhydrous caffeine capsule (6 $mg \cdot kg^{-1}$) or placebo (maltodextrin) following randomised, placebo-controlled double-blind protocols. Blood sampling was undertaken before, during and following exercise in the two exercise trials to measure plasma caffeine, paraxanthine and theophylline concentrations.

Results: Caffeine supplementation improved exercise performance by 1.8% ($p=0.05$; $\eta p^2=0.12$), with greater improvements in performance seen in the second half of exercise (2.4%; $p=0.02$; $\eta p^2=0.16$) in comparison with the first half (1.2%). Twenty-four of the 34 participants whose data

were used to analyse time to completion, showed an improvement in exercise performance with caffeine ingestion. Heart rate was higher in participants following caffeine ingestion compared to placebo ($p=0.02$; $\eta p^2=0.15$). Genotyping showed 50% of participants were homozygous AA allele carriers and 50% heterozygous AC allele carriers. No participants carried the CC allele polymorphism. Plasma caffeine concentrations were higher in AA allele carriers than AC allele carriers ($p=0.05$; $\eta p^2=0.207$). No gene-treatment interaction effects were observed in time to completion, heart rate (HR) or plasma concentrations of paraxanthine or theophylline. A significantly higher total sum of plasma caffeine was observed in the area under the concentration-time curve (AUC) in AA allele carriers compared with AC allele carriers ($p=0.01$).

Conclusion: Ingesting a dose of 6 $mg \cdot kg^{-1}$ caffeine 60 min prior to exercise is likely to improve performance in endurance activities in recreationally trained males. Plasma caffeine concentrations were significantly higher in AA allele carriers compared to AC allele carriers, though no gene-caffeine interaction was observed in time to completion, therefore the role of *CYP1A2* gene polymorphisms and caffeine consumption in determining enhancements in exercise performance remains unclear.

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Abbreviations

AA homozygotes- carrier of the AA variation of *CYP1A2* SNP; “fast” caffeine metaboliser

AC heterozygotes- carrier of the AC variation of *CYP1A2* SNP; “slow” caffeine metaboliser

ADP- adenosine diphosphate

AMP- adenosine monophosphate

ANOVA- analysis of variance

ATP- adenosine triphosphate

AUC- area under the concentration-time curve

CAF- caffeine

CC homozygotes- carrier of the CC variation of *CYP1A2* SNP; “ultra-slow” caffeine metaboliser

CNS- central nervous system

CYP1A2 gene- the gene responsible for ~95% caffeine metabolism

FFA- free fatty acid

HPLC- high performance liquid chromatography

HR- heart rate

PLA- placebo

SD- standard deviation

SNP- single nucleotide polymorphism

SPSS- statistical package for the social sciences

T1- timepoint 1 in exercise trial; pre-supplementation

T2- timepoint 2 in exercise trial; 60-min post supplementation and the commencement of exercise

T3- timepoint 3 in exercise trial; halfway through exercise

T4- timepoint 4 in exercise trial; the completion of the exercise trial

WADA- world anti-doping agency

Chapter 1: Introduction

1.1 Background

Caffeine (1,3,7-Trimethylxanthine) is consumed by up to 75% of elite athletes globally (Del Coso et al., 2011). With its widespread use, caffeine has engendered significant research interest over the past two decades in a bid to understand its ergogenic effects, and the interindividual variations that affect its influence in enhancing sports performance.

Caffeine has been long established as providing physical benefits. The consumption of caffeine in sports is motivated by ergogenic effects including; reduction in pain, cognitive enhancement, mental clarity, and increased time to fatigue (McLellan et al., 2016). Approximately 30% of participants are not responsive to caffeine and do not experience ergogenic effects (Doherty et al. 2002; Meyers et al., 2005), which may be due to endogenous and/or exogenous factors affecting caffeine metabolism. Research to determine caffeine's effect on physical capability was instigated in a 1907 study (Rivers et al., 1907), which examined the effect of caffeine on exercise performance. Research continued in the 1940s (Asmussen et al., 1948, Haldi et al., 1946), and into the 1970s when the ergogenic effects of caffeine became of mainstream interest (Costill et al., 1978; Perkins et al., 1975). Further studies have determined ergogenic benefits occur in those who respond to caffeine at a dose of 3-6 $mg \cdot kg^{-1}$ of caffeine consumed 60-min prior to exercise (Glaister et al., 2021; Graham, 2001; Grgic et al., 2021; Guest et al., 2021; Rahimi, 2019). However, limitations in this consensus include mixed sports modalities, gender, habituation, and training status of exercisers within studies.

Caffeine supplements are easy to obtain, cost effective, and available in many forms including powders, gels, and capsules. The performance-enhancing benefits of caffeine, and their frequent use among athletes, led to caffeine being temporarily banned by the World Anti-Doping Agency (WADA) between 1984 and 2004. Due to the prevalence of caffeine in everyday food and beverage products and medications, the ban was revoked and its use in professional sport allowed but limited to a urinary concentration of 12 $mg \cdot L^{-1}$ (Alonso et al., 2020; McLellan et al., 2016). To reach this threshold is rare with general caffeine consumption and a high supplementation is required to meet or exceed this limit. The training status of athletes is thought to affect caffeine's ergogenic effects as heavy exercise has been found to increase the expression of the *CYP1A2* enzyme and

increase the release of catecholamines, which in turn may increase the ergogenic benefits of caffeine, and thus exercise performance in comparison with less well-trained exercisers (Zouhal et al., 2008).

Once ingested, caffeine is absorbed through the oesophageal, stomach and intestinal epithelium from where it is transported throughout the body in the bloodstream. Caffeine, an amphiphilic molecule, is able to cross through lipid bilayers and cell membranes. With no first-pass metabolism of caffeine, and its amphiphilic properties, it is distributed non-selectively into all tissues and organs. As an adenosine antagonist, caffeine binds to adenosine receptors found throughout the body (Barreto et al., 2021). The cardiac and respiratory systems, the brain, and skeletal muscles contain the A1, A2A, A2B, and A3 adenosine receptors primarily targeted by caffeine and its metabolites (Graham, 2001; Varani et al., 2000). Adenosine binding to these receptors elicits fatigue and pain. With caffeine acting as an adenosine antagonist, the time to fatigue extends allowing for prolonged exertion during exercise, and decreased sensations of physical pain (Doherty et al., 2005; Sawynok, 1998). As a central nervous system (CNS) stimulant, caffeine provides cognitive and mental effects which can increase mood, alertness and mental clarity. Metabolism of caffeine occurs in the liver where it is demethylated into its three main metabolites: paraxanthine (84%), theobromine (4%), and theophylline (12%) (Barcelos et al., 2020). These caffeine metabolites are also adenosine receptor antagonists and independently exert similar physical and cognitive effects in the body to caffeine.

While the ability of caffeine to enhance physical and mental performance is well established, the mechanism by which it provides these benefits, and the interindividual variations that lead to differences in caffeine's effects are still being established. The impact of genetics, specifically the *CYP1A2* gene, is one such variable that may influence caffeine's effects on exercise performance. The *CYP1A2* gene encodes the cytochrome P450 1A2 enzyme in the liver, which metabolises many drugs including caffeine (Cappelletti et al., 2015; Nehlig, 2018; Sachse et al., 1999). The rate at which this enzyme metabolises caffeine is dependent on a single nucleotide polymorphism (SNP) (*CYP1A2;* rs762551) at intron 1 in the *CYP1A2* gene where a C→ A change in one or two alleles is found in up to 54% of the population (Sachse et al., 1999). Those carrying the AA allele variant of the gene are 'fast' metabolisers, those with the AC allele 'slow' metabolisers, and CC allele carriers are 'ultra-slow' metabolisers of caffeine (Barreto et al., 2021). The significance of the rate of

metabolism of caffeine in individuals is the length of time caffeine remains in the system. The half-life of caffeine, approximately 3-5 h (Seepika et al., 2022), is significantly longer in AC/CC allele carriers than AA allele carriers, which may influence the ergogenic effects of caffeine in exercise performance (Sachse et al., 1999). Caffeine half-life is also determined by dosage, clearance rates as well as other factors and therefore is not solely dependent on the *CYP1A2* gene variant in the individual.

The *CYP1A2* genotype may affect exercise performance post caffeine ingestion, though results are equivocal. Guest et al. (2018) showed AA allele carriers had decreased time to completion in a 10-km cycling trial after ingesting a caffeine dose of 4 $mg \cdot kg^{-1}$ (compared with placebo). CC allele carriers experienced an increased time to completion post caffeine ingestion (compared with placebo), and AC allele carriers showed no variation in results between caffeine and placebo groups (Guest et al., 2018). Rahimi (2019) showed homozygous A allele carriers were able to complete more repetitions of bench press, leg press, shoulder press, and seated cable row after 6 $mg \cdot kg^{-1}$ caffeine ingestion compared with C allele carriers (Rahimi, 2019). Countering this, one study showed aerobic performance and muscular endurance improved following caffeine ingestion in adolescent athletes compared with placebo but independent of *CYP1A2* genotype (Spineli et al., 2020). Likewise, Salinero et al. (2017) showed no significant difference in exercise performance between *CYP1A2* AA allele carriers and C allele carriers, though peak power was enhanced during Wingate testing after ingesting caffeine compared with placebo (Salinero et al., 2017).
The *CYP1A2* gene has been studied with no consensus reached as to its effect on caffeine metabolism and exercise performance and if ergogenic effects are differentially enhanced in AA allele carriers, or in AC/CC allele carriers. The variation in results may be due to differences in study design, with further research required to determine genetic influence on caffeine's mechanism of action and the resulting effects on sports performance. Further, variations in results may be due to differences in exercise type and duration between studies and illustrate the need for further research to identify the effects of the *CYP1A2* gene on caffeine metabolism and subsequent exercise performance.

1.2 Aims and objectives

This study aimed to identify the influence the *CYP1A2* genotypes and resulting caffeine metabolism rates have on exercise performance in recreationally trained male exercisers post caffeine

ingestion, and the effect of a dose of 6 mg·kg^{-1} caffeine consumed 60-min prior to exercise on exercise performance. The ergogenic effects of caffeine evaluated in this thesis to determine if caffeine has an ergogenic effect on exercise performance are aerobic and muscular endurance, and will be assessed by comparing time to completion following placebo and caffeine intake during running and cycling trials.

1.3 Hypotheses

1. Caffeine ingestion (6 mg·kg^{-1}) will decrease time to completion relative to placebo in recreational athletes.
2. Exercise performance will be differentially enhanced between athletes with different *CYP1A2* genotypes following the ingestion of (6 mg·kg^{-1}) caffeine.

1.4 Overview of thesis

This thesis has been organised into four chapters; **Chapter 1** is an introduction to the thesis topic of the effect of the *CYP1A2* gene on caffeine pharmacokinetics and exercise performance. **Chapter 2** is a comprehensive literature review examining pertinent research on caffeine pharmacokinetics, the *CYP1A2* gene and exercise performance in endurance cycling and running. **Chapter 3** is a research manuscript prepared for the requirements of publication in the academic journal 'Nutrients'. This research manuscript includes an abstract, introduction, aims and objectives of the study, and an in-depth documentation of the research process including methods, results, and discussion of findings. **Chapter 4** is a discussion of the findings of the research and includes strengths and limitations of the study and recommendations for future directions.

1.5 Researcher contributions

Research team	*Contributions to this thesis*
Chloe Masters Master of Science Nutrition and Dietetics candidate	Main author of this thesis including statistical analysis, interpretation, conclusions, and editing.
Assoc Prof Kay Rutherfurd-Markwick Primary academic supervisor	Provided guidance, review and feedback on the thesis design, direction, and writing and editing of this thesis.
Prof Ajmol Ali Dr Claire Badenhorst Dr Martin Dickens Co-supervisors	Provided guidance, review and feedback on the thesis design, direction, and writing and editing of this thesis.

Dr Hajar Mazahery	Assistance with data analysis and interpretation. Review and feedback of the thesis' results section.
Kyle Southward, PhD student	Primarily responsible for all participant recruitment, exercise trials and data collection. Completed analysis of blood and saliva samples.

Chapter 2: Literature Review

2.1 Introduction

Caffeine is a stimulant widely used among athletes due to its potential ergogenic effects. Caffeine has been shown to exert physiological effects in the body, reducing fatigue, perceived exertion, and improving performance time in some athletes (Ágoston et al., 2018; Barcelos et al., 2020; Barreto et al., 2021). The ergogenic effects of caffeine vary between individuals, with differences attributed to environmental and genetic influences (Graham, 2001), although little research is available on how *CYP1A2* genotype influences caffeine's ergogenic effects.

Caffeine is found naturally in many products including cocoa beans, coffee beans, tea leaves and kola nuts (Nehlig, 2018), and is included in medications such as over-the-counter pain relief (Graham, 2001). The variability in caffeine content within and between dietary products means it can be difficult to determine the amount of caffeine being consumed. For example, the caffeine content of coffee can range from 60 to 100 mg per cup depending on the method of preparation, coffee type, drink size, and environmental conditions in which the coffee beans were grown (Ágoston et al., 2018). Energy drinks can contain in excess of 280 mg of caffeine per serve (McLellan et al., 2016). With caffeine included in a wide range of everyday food and beverages, it can be difficult for athletes to accurately determine their caffeine consumption. Since consuming caffeine at a suboptimal dose and time may affect exercise performance, athletes often choose to use supplements containing known amounts of caffeine, enabling them to regulate the dose and consumption time relative to exercise (Alonso et al., 2020).

This narrative review focuses on the *CYP1A2* gene, and specifically the SNP rs762551 within this gene, to determine if the gene's influence on caffeine metabolism rates affect caffeine pharmacokinetics, and subsequently exercise performance in recreational exercisers. Caffeine metabolism and mechanisms of action will be explored, and pertinent literature will be examined regarding caffeine's ergogenic effects on running and cycling.

2.2 Reference collection

The research studies identified and included in this literature review were collected manually using the following search terms:

Caffeine OR ergogenic AND genetics OR *CYP1A2* AND exercise performance OR sport* OR exercise OR endurance exercise OR physical activity OR athelt*
Data bases searched include Massey Discover and Google Scholar and included articles from all journals.

2.3 Caffeine use in sports

As an ergogenic aid, caffeine is readily available in sports supplements such as pre-workout supplements, energy drinks, mouth rinses, tablets, and sports gels (Fig 2.1). These are marketed to recreational and professional athletes as they are proven to enhance the user's energy, performance, and focus (Alonso et al., 2020; Guest et al., 2021). Caffeine supplements are convenient to ingest, and their use normalised within sporting communities (Alonso et al., 2020). Caffeine supplements often contain additional compounds (creatine, electrolytes, and carbohydrates such as glucose), vitamins and minerals that are also known to aid exercise performance (Graham, 2001). Therefore, it can be difficult to know if it is caffeine or another compound in the supplement improving exercise performance. Kreutzer et al. (2022) found 85% of endurance athletes (n=254) consumed caffeine daily, and 24% of respondents consumed caffeine supplements (Fig 2.1) specifically for sport performance (Kreutzer et al., 2022).

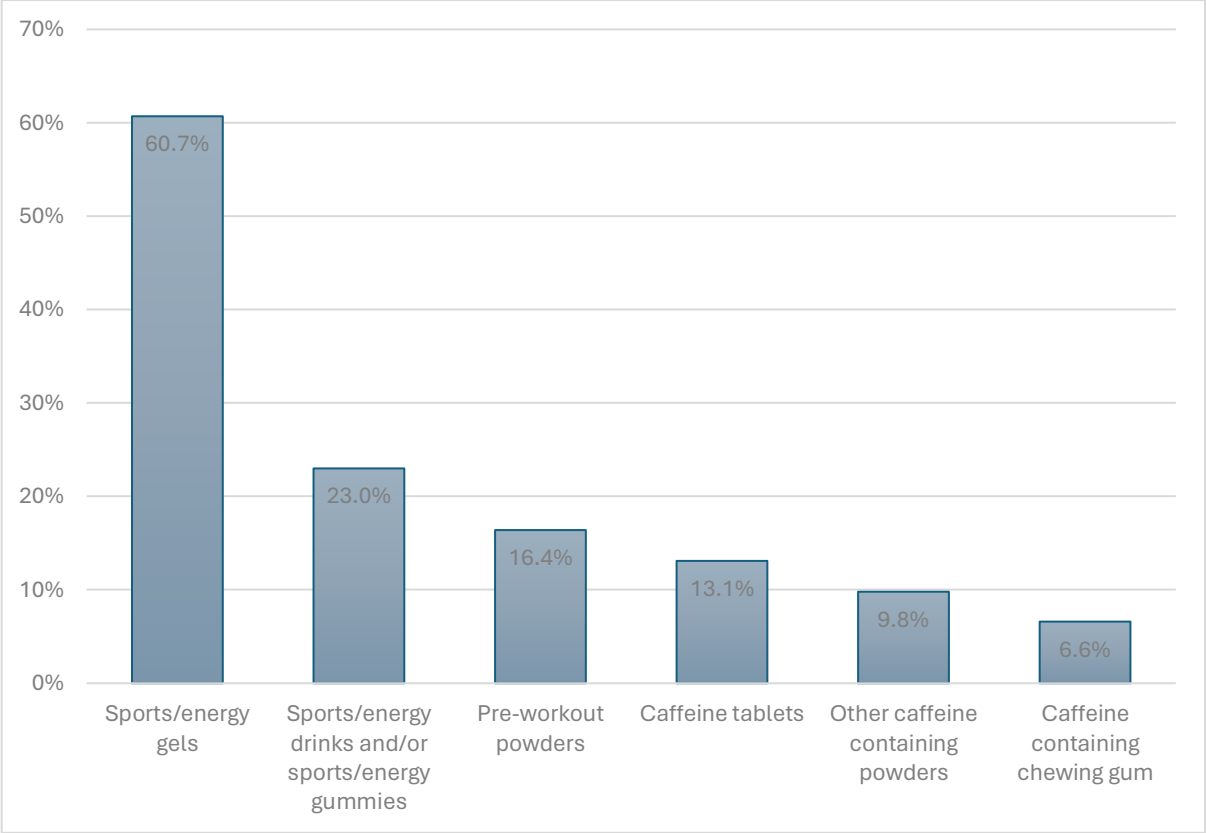

Figure 2.1 Percentage of endurance athletes (n=254) who report consuming different types of caffeine supplements prior to exercise.

Data sourced from (Kreutzer et al., 2022).

2.4 Caffeine metabolism

The rate of caffeine metabolism is determined by both endogenous factors (genetics and ethnicity), and exogenous factors (pregnancy, habituation, smoking status, and medications) (Ghotbi et al., 2007; Nehlig, 2018; Rasmussen et al., 2002). Upon ingestion, caffeine is absorbed through the epithelium in the intestinal tract into the bloodstream, where it is transported throughout the body (Wickham et al., 2018). As caffeine passes through the liver it is demethylated to give three metabolites; paraxanthine (84%), theophylline (4%), and theobromine (12%), which are further catabolised and non-selectively diffuse into organs throughout the body before being excreted, predominantly through urine via the kidneys (Barcelos et al., 2020; Barreto et al., 2021; Cappelletti et al., 2015; Nehlig, 2018). Caffeine plasma concentrations peak within 45-60-min of ingestion, with the half-life being dependent on clearance rates, dosage, and metabolism, but generally being between 3-5 h (Seepika et al., 2022).

2.4.1 Genetics

The *CYP1A2* gene encodes the primary enzyme responsible for 95% of caffeine metabolism; cytochrome P450 1A2 (Cappelletti et al., 2015; Nehlig, 2018). Single nucleotide polymorphisms (SNPs) in the *CYP1A2* gene (*CYP1A2;* rs762551) affect the rate of caffeine metabolism through different expression levels of the enzyme. This influences the half-life of caffeine, and consequently the length of time it remains in the body and therefore the time during which it can elicit effects. Those homozygous with the AA allele (*CYP1A2;* rs762551) are known to metabolise caffeine quickly; while those heterozygous with the AC allele and homozygous CC allele are classified as slow metabolisers (Barreto et al., 2021). The difference in the rate at which caffeine is metabolised means the time period during which caffeine's effects are experienced may differ (Salinero et al. 2017). This is because caffeine and its metabolites will remain in the system longer in AC and CC allele carriers due to their slower rate of caffeine metabolism (Djordjevic et al., 2010).

Physiological responses to caffeine consumption differ between individuals, with some finding caffeine enhances mental and physical performance, and other people experiencing ergolytic effects (Yang et al., 2010). People's response to caffeine is also influenced by dose, with some experiencing effects with a small dose, and others needing much more to bring about similar effects (Nehlig, 2018). In some individuals, caffeine intake leads to sensations of nervousness and anxiety, restlessness, insomnia, headaches, and tachycardia (Gonzalez de Mejia et al., 2014; Wikoff et al., 2017). Salinero et al. (2017) showed 31.3% of homozygous CC allele carriers experienced feelings of nervousness after ingesting caffeine (3 $mg \cdot kg^{-1}$ body weight), with no other genotypes reporting this effect (Salinero et al., 2017). However, the extent of the influence genetic variation has on instigating adverse effects from caffeine ingestion remains largely unclear. A meta-analysis (de Souza et al., 2022) suggested consuming a caffeine dose of ~3 $mg \cdot kg^{-1}$ body weight provides the user with ergogenic benefits while limiting the side effects associated with caffeine consumption. However, individual genetic variations were not considered in this recommendation. Further research may identify if there is a differentiation in side effects experienced in individuals with AA, AC, and CC polymorphisms at differing caffeine doses.

The inducibility of the *CYP1A2* gene has been shown to vary between populations. Population studies examining the frequency of *CYP1A2* polymorphisms show Egyptian, Caucasian, Turkish, and Chinese populations have a higher proportion of AA allele carriers (68%, 68%, 73%, 67%

respectively) compared with those of Ethiopian (60%), Japanese (61%) and Middle American (Mexican 54%, Costa Rican 45%) descent (Bilgen et al., 2008; Castorena-Torres et al., 2005; Cornelis et al., 2007).This implies that some interindividual variation in caffeine metabolism may be affected by heritability rather than environmental factors.

2.4.2 Other variables influencing caffeine metabolism

Cigarette smoking, pregnancy, and the oral contraceptive pill intake affects caffeine metabolism through modulating *CYP1A2* activity (Arnaud, 1993; Sachse et al., 1999). Exposure to compounds in cigarette smoke increases caffeine clearance rates in individuals with the AA allele of the *CYP1A2* gene, while AC/CC allele carriers do not experience the same increase (Cappelletti et al., 2015; Ghotbi et al., 2007; Rasmussen et al., 2002). Pregnancy and the ingestion of oral contraceptive pills increase the half-life of caffeine and its metabolites, which may lead to increased effects as clearance rate decreases and caffeine remains in the system longer than non-pregnant individuals and non-oral contraceptive users (Arnaud, 1993).

2.5 Caffeine's mechanisms of action

The process by which caffeine works in the body to produce physiological effects is thought to be primarily through blocking the effects of adenosine. Caffeine does this by competing for adenosine receptors throughout the body. This section will discuss what adenosine is, its physiological effects in the body, and the role of caffeine as an adenosine antagonist. It will also examine other mechanisms of action for caffeine such as through catecholamine and calcium stimulation, and the role of caffeine metabolites.

2.5.1 Adenosine receptor antagonism

Caffeine is an adenosine receptor antagonist. Adenosine elicits feelings of fatigue and pain when it binds to adenosine receptors and is an inhibitory neurotransmitter which reduces central nervous system (CNS) arousal by suppressing noradrenaline and dopamine release (Cappelletti et al., 2015; Sawynok, 1998). Adenosine is produced from the catabolic products of adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) (Costa et al., 2001; Fredholm, 2007). When AMP, ADP and ATP are used through physical activity, adenosine

concentrations increase and bind to adenosine receptors which promotes fatigue (Barreto et al., 2021; Duncan et al., 2013). Caffeine's chemical structure is similar to adenosine, which allows it to bind competitively and non-selectively to adenosine receptors, thus reducing the perceptions of exertion, fatigue, and pain mediated by adenosine (Barreto et al., 2021; Davis et al., 2003; Maridakis et al., 2007). Adenosine receptors A1, A2A, A2B, and A3, are found throughout the body including the brain, skeletal muscles, respiratory system, and cardiac muscles (Graham, 2001; Varani et al., 2000). The binding of caffeine, and its metabolites paraxanthine, theophylline and theobromine binding to these receptors (primarily A1 and A2A), blocks the effects of adenosine and allows caffeine to extend its effects to multiple organs and body systems simultaneously (Nikrandt et al., 2022; Varani et al., 2000).

2.5.2 Catecholamines

Caffeine indirectly stimulates the release of catecholamines (adrenaline, noradrenaline, and dopamine), increasing lipolysis, (Carrageta et al., 2018; Farias-Pereira et al., 2019; Wu et al., 2017), although the effects of fat metabolism on exercise performance remain unclear. One hypothesis suggests free fatty acids (FFA) and glycerol, which are released from adipocytes into plasma following caffeine ingestion are both used as a substrate during exercise, resulting in a slower rate to exhaustion (Apostolidis et al., 2019; Costill et al., 1978). However, other research has shown FFA release following caffeine ingestion is not ergogenic, as high concentrations of plasma FFA circulate in early exercise when they should be low as fat oxidation is used to provide energy (Graham et al. 2000). A meta-analysis has found increased lipolysis during exercise following caffeine ingestion of more than 3 $mg \cdot kg^{-1}$, with fat oxidation higher in untrained compared with trained exercisers (Collado-Mateo et al., 2020). This may explain a small portion of exercise performance improvement in endurance activities, where sustained prolonged exercise requires fat oxidation, followed by the use of glycogen as a fuel source. However, this does not explain the ergogenic effects reported in short duration anaerobic exercise such as the 30s Wingate test where glycogen is not required (Guest et al., 2021).

Adrenaline stimulates β-adrenergic receptors which have a multifaceted effect in the body; HR and cardiac output increase, and serum glucose and lactate concentrations increase, which in turn causes an increase in oxygen and substrate delivery to skeletal muscle, increasing the force and rate of skeletal muscle contractions (Zouhal et al., 2008). While caffeine's stimulus of

catecholamine release may explain a small portion of its ergogenicity, adenosine receptor antagonism and the resulting CNS stimulation is more likely to be the main mechanism of action.

2.5.3 Calcium

Caffeine's peripheral effects on skeletal muscle increase the release, and reuptake of calcium from the sarcoplasmic reticulum by mediating the opening of ryanodine receptors (Reggiani, 2021). This leads to increased force production in muscle contractions, positively influencing exercise performance by increasing power output with fewer muscle fibres recruited (Barcelos et al., 2020; Endo, 1977; Reggiani, 2021). This mechanism may allow for recruitment of some slow-twitch (type I) muscle fibres to be delayed, and recruited later in exercise, thus delaying fatigue in endurance exercise (Tallis et al., 2015).

Oxidative metabolism supplies energy to skeletal muscles (Ferrari et al., 1997), and is stimulated by calcium release from the sarcoplasmic reticulum (Smith et al., 2023). Further, calcium stimulates glycogenolysis and glycolysis as it mediates the breakdown of glycogen into glucose 1-phosphate and glucose, and glucose into pyruvate and ATP providing energy for skeletal muscle contraction (Gehlert et al., 2015). This makes calcium an integral component in the release of energy for skeletal muscle contraction, and thus, influencing exercise performance.

2.5.4 Caffeine metabolites

Caffeine, along with its metabolites paraxanthine, theophylline, and theobromine are all methylxanthines, which are thought to provide ergogenic benefits in sports performance (Daly et al., 1986), though to differing degrees. The methylxanthines have a similar chemical structure to adenosine, allowing them to bind to adenosine receptors. Caffeine has been shown to have the largest ergogenic effect, followed by paraxanthine as plasma concentrations accumulate with repeated caffeine ingestion. Theophylline has the highest affinity for adenosine receptors, however as with theobromine, the low plasma concentrations resulting from the metabolism of caffeine means they have little ergogenic effect (Mumford et al., 1996). Circulating serum paraxanthine concentrations are associated with a reduction in perceived exertion (Whalley et al., 2021). Paraxanthine increases calcium in skeletal muscle tissue and increases potassium (K^+) uptake into skeletal muscle via its effects on the sodium/potassium (Na^+/K^+) pump (Graham, 2001), and

stimulates lipolysis, thereby increasing FFA plasma concentrations (Hetzler et al., 1990). Furthermore, animal models have shown paraxanthine supplementation improves exercise performance in mice (Jäger et al., 2022). Therefore, as both caffeine and its metabolites, specifically paraxanthine, contribute to and enhance ergogenic effects in sports performance (Pickering et al., 2019) it may be difficult to determine the effect of *CYP1A2* genotypes on caffeine metabolism and exercise performance, as the final ergogenic effect is dependent on the concentration and potency of the total sum of caffeine and its metabolites.

2.6 Caffeine use in exercise

Research into the mechanism of caffeine's ergogenic actions has increased substantially in the past two decades, with an emphasis on the ideal caffeine dose to enhance athletic performance (Grgic et al., 2020; Grgic, 2021; Varani et al., 2000; Wang et al., 2020). Table 2.1 and 2.2 outline pertinent research investigating caffeine's effects on cycling and running performance respectively.

Table 2.1: Summary of studies exploring the effect of caffeine pharmacokinetics on exercise performance in cyclists (ordered by increasing dose).

Study	Participants	Caffeine dosage and timing	Exercise regime	Exercise performance
Desbrow et al. 2012	16 male cyclists	3 mg·kg^{-1} or 6 mg·kg^{-1}, 90-min	60-min of cycling at 75% peak power output	↑ 4.2%
Pitchford et al. 2014	9 male cyclists	3 mg·kg^{-1}, 90-min	Cycling to exhaustion in 35^{o}C heat and 25% relative heat (RH)	↑ 3%
Costill et al. 1978	8 competitive cyclists (2 female, 7 male)	330mg, 60-min	Participants cycled to exhaustion at 80% VO_2 max	↑ 19.5%
Wiles et al. 2007	8 trained male cyclists	5 mg·kg^{-1}, 60-min	1-km cycling time-trial	↑ 3.1%
Santos et al. 2013	8 male recreational cyclists	5 mg·kg^{-1}, 60-min	4-km cycling time-trial	↑ 2.3%
Tomazini et al. 2020	11 male recreational cyclists	5 mg·kg^{-1}, 60-min	4-km cycling time-trial	↑ 2%
Hunter et al. 2002	8 trained cyclists	6 mg·kg^{-1}, 60-min plus 0.33 mg·kg^{-1} per 15-min until time trial completion	Cycling time-trial 100-km distance	ND
Boyett et al. 2016	20 male cyclists, trained and untrained	6 mg·kg^{-1}, 60-min	3-km cycling time-trial	↑ Morning: 2.3%; evening: 1.8%
Miyagi et al. 2018	14 male cyclists	6 mg·kg^{-1}, 60-min	Graded cycling test to exhaustion ~8-12-min	↑ 11.8%
Ferreira Viana et al. 2020	9 male endurance trained cyclists	6 mg·kg^{-1}, 60-min	4-km cycling time-trial	↑ 8%

ND- no difference

↑- increase

Of the 18 studies included in Tables 2.1 and 2.2, 15 studies show an improvement in exercise performance following caffeine ingestion compared with placebo. Three studies show no difference in exercise performance between caffeine and placebo ingestion. The studies in Tables 2.1 and 2.2 have been ordered by caffeine dose size, from smallest (3 mg·kg^{-1}) to largest (6 mg·kg^{-1}).

Table 2.2: Summary of studies exploring the effect of caffeine pharmacokinetics on exercise performance in runners (ordered by increasing dose)

Study	Participants	Caffeine dosage and timing	Exercise regime	Exercise performance
Bridge et al. 2006	8 male distance runners	3 mg·kg^{-1}, 60-min	8-km outdoor run	↑ 1.2%
Khcharem et al. 2021	13 recreational runners	3 mg·kg^{-1}, 60-min	3-km outdoor run	↑ 1.1%
Whalley et al. 2020	14 recreational runners	3- 4.5 mg·kg^{-1}, 15-min	5-km outdoor run	↑ 1.4%
Stadheim et al. 2021	Twenty-three elite endurance-trained male athletes	4.5mg·kg^{-1}, 45-min	Time to exhaustion during an incremental test (running 10.5° incline, start speed 10.0·km·h^{-1}, and 0.5-km·h^{-1} increase in speed every 30 s)	↑ 5.5%
Al-Nawaiseh et al. 2022	11 runners (9 male; 2 female)	5 mg·kg^{-1}, 60-min	30-min downhill (-10^{o}) run to bring on muscle soreness. 5-km run 48 h later with caffeine or placebo	ND
Ramos-Campo et al. 2019	15 male middle distance runners	6 mg·kg^{-1}, 60-min	800 m outdoor run	ND
Seepika et al. 2022	8 male trained half-marathon runners	6 mg·kg^{-1}, 60-min +/- 3 mg·kg^{-1} 45-min after commencing exercise	Running to exhaustion	↑ 2.3%
Matsumura et al. 2023	13 male trained sprinters	6 mg·kg^{-1}, exercise commenced at peak plasma concentrations	100 m outdoor sprint	↑ 1.2%

ND- no difference

↑- increase

2.6.1 Caffeine dose

Following caffeine ingestion, performance times have been shown to improve for distances varying from short duration such as the Wingate test, to endurance exercise (30 s to ≥ 10-km), indicating the efficacy of caffeine as a tool for athletes to use in different exercise modes (Tables 2.1 and 2.2). Costill et al., (1978; Fig 2.1) showed time to exhaustion in a sample of cyclists increased by 19.5% after ingesting coffee containing 330mg (4-4.5 mg·kg^{-1}) caffeine compared with decaffeinated coffee. A similar cycling study showed that consumption of 3 mg·kg^{-1} of caffeine improved cycling

performance by 4.2% compared to placebo, though ingesting a dose higher than 6 mg·kg^{-1} of caffeine did not further improve exercise performance (Desbrow et al., 2012). Similarly, Pitchford et al. (2014) showed that ingestion of 3 mg·kg^{-1} of caffeine improved cycling time to completion in heated conditions (35^{o}C) compared to placebo (Pitchford et al., 2014).

A meta-analysis supports the ergogenic effects of caffeine in endurance running (Wang et al., 2022). In an analysis of 21 studies, Wang et al. (2022) showed caffeine supplementation (3-9 mg·kg^{-1}) improved time to exhaustion and reduced time to completion in recreational and trained runners. Another recent study not included in the meta-analysis also found a combined dose of 9 mg·kg^{-1} body weight of caffeine (delivered as an initial 6 mg·kg^{-1} dose before exercise followed by a 3 mg·kg^{-1} dose during exercise) increased time to exhaustion compared to placebo in half-marathon runners (Seepika et al., 2022).

In contrast to the above findings, a 100-km cycling time trial found no ergogenic benefits of caffeine (6 mg·kg^{-1}) ingestion in 8 trained cyclists (Hunter et al. 2002). Tarnopolsky et al. (1989) found no ergogenic influence from consumption of 6 mg·kg^{-1} body weight caffeine in endurance runners who normally consumed 200mg caffeine per day (Tarnopolsky et al., 1989). Similarly, a study by Al-Nawaiseh et al. (2022) showed a 5 mg·kg^{-1} dose of caffeine did not alter 5-km run times in athletes with muscle fatigue and pain (Al-Nawaiseh et al., 2022). Participants were required to complete a 30-min downhill treadmill run (-10% gradient) 48 h prior to completing the 5-km running trial to bring on delayed onset muscle soreness. The absence of ergogenic effect may be explained by the muscle fatigue and pain experienced by participants indicating caffeine may be effective in delaying fatigue acutely, though not improving performance in pre-fatigued muscle.

Discrepancies in findings of caffeine's ergogenic effects may be due to the training status of participants, as cyclists with the capacity to cycle 100-km may have a higher fitness capacity than exercisers participating in shorter duration trials. Additionally, trained athletes may be more consistent in performance than recreational athletes, therefore any differences in performance in trained athletes may be a more accurate reflection of caffeine's effects on exercise performance (Burke, 2008). Furthermore, differences in study design, specifically the differences in environment may explain the contrasting results. Laboratory conditions vs outside training and competition can vary in temperature, wind, ground gradient among other variables, and performing trials in an

outside setting reduces the amount of control that can be implemented compared with a laboratory setting (Burke, 2008).

2.6.2 Timing of caffeine ingestion and mode of delivery

As mentioned previously, caffeine supplements are generally consumed in research trials 60-min prior to exercise, as this allows for peak caffeine plasma concentrations to be reached during the exercise regime (Graham, 2001; Ryan et al., 2013). However, the time it takes for serum caffeine concentrations to peak has been found to vary between individuals (Matsumura et al., 2023), and ergogenic benefits begin prior to and continue after peak serum concentrations, as improvement in exercise performance has been shown in trials with caffeine administration ranging from 5-min to 120-min prior to exercise (Carswell et al., 2020; Costill et al., 1978; Desbrow et al., 2012; Matsumura et al., 2023; Ryan et al., 2013; Stadheim et al., 2021; Whalley et al., 2020). In a study of caffeine supplement behaviour in endurance athletes, 34.9% athletes who responded said they ingested caffeine less than 30-min before starting a competitive race, and 36.5% of respondents reported consuming caffeine 30-60-min before a race (Kreutzer et al., 2022). As previously mentioned, caffeine has been found to delay fatigue during exercise performance through CNS stimulation following its binding to adenosine receptors, thus the ideal timing of caffeine supplementation may be dependent on exercise duration. Therefore, caffeine's ergogenic effects may be optimised by educating athletes on timing strategies for ingestion prior to training or competition, and athletes may benefit by trying different timings to identify the time period from which they get the best results following caffeine ingestion.

The time it takes caffeine to be absorbed, enter the bloodstream and begin to elicit ergogenic effects is dependent upon its mode of delivery. Anhydrous caffeine, or pure caffeine powder, provided in capsules are used in many studies (Boyett et al., 2016; Bridge et al., 2006; Desbrow et al., 2012; Ferreira Viana et al., 2020; Hunter et al., 2002; Hurst et al., 2020; Khcharem et al., 2021; Matsumura et al., 2023; Miyagi et al., 2018; Pitchford et al., 2014; Santos et al., 2013; Tomazini et al., 2020) where caffeine is ingested orally and absorbed through the stomach and small intestine. Caffeinated chewing gum has been shown to have a faster absorption rate than capsules, and therefore may have faster onset of ergogenic effects, however total peak caffeine plasma levels remain the same between supplement types (Kamimori et al., 2002). This is due to caffeine being absorbed directly through the buccal cavity epithelium and into the blood stream though some caffeine may be absorbed through the stomach and small intestine by swallowing saliva containing

residual caffeine. Due to the difference in absorption times between delivery modes, athletes may also look to tailor the timing of their caffeine depending on their choice of supplement.

2.6.3 Habituation

Regular caffeine consumption was thought to reduce the ergogenic effects of caffeine compared with naïve caffeine users, due to both the increase in *CYP1A2* expression and upregulation of adenosine receptors in the body following regular caffeine intake (Graham, 2001). This means more caffeine is required to bind to the additional adenosine receptors to produce consistent physiological effects. Inconsistencies in the literature linking habitual caffeine intake with reduced ergogenic effects of caffeine was attributed to the withdrawal period differing between studies (Apostolidis et al., 2022; Filip-Stachnik et al., 2023; Grgic et al., 2021), and the interindividual variation in caffeine withdrawal symptoms (de Souza et al., 2022). Many studies require participants to abstain from caffeine consumption prior to exercise trials to accurately determine the effects of a set dose of caffeine. This may result in different conditions between research and in general exercise where athletes may not abstain from caffeine prior to consuming it for training or competition. Additionally, withdrawal symptoms experienced by some individuals such as headaches, fatigue, and anxiety, may negatively impact exercise performance (de Souza et al., 2022). However, a recent meta-analysis of 60 caffeine studies found no variation in the ergogenic effects of caffeine between habitual caffeine users and caffeine-naïve users across multiple exercise modes (Carvalho et al., 2022). Additionally, caffeine abstinence prior to research trials was not shown to affect exercise performance during the trial (Carvalho et al., 2022).

Determining the classification of high, moderate, and low caffeine users to define habitual caffeine users may contribute to the inconsistency in results. To the author's knowledge there is no definitive dose and frequency defining habitual caffeine consumers and caffeine naïve consumers. Therefore, the caffeine intake of those identified as habitual users and naïve users in the literature varies. Further, caffeine intake is determined using self-reported caffeine consumption. As previously discussed, the variation of caffeine content within and between food and beverage products can cause inconsistencies in estimating caffeine intake (Pickering et al., 2019b). Using blood samples to measure caffeine concentrations is a more accurate way to determine caffeine intake in regular users, however these tests can be expensive and invasive to perform, and

therefore impractical (Rothwell et al., 2014). Future research may focus on the use of standardised quantities, such as those proposed by Filip et al. (2020) to define an individual's caffeine use.

2.7 Effects of the *CYP1A2* SNP on caffeine metabolism and subsequent ergogenic effects during exercise performance

While there is much research analysing the ergogenic effects of caffeine on sports performance, there is comparably little known on how this is influenced by genetics, specifically the role of *CYP1A2* gene polymorphisms. The research that has been undertaken is inconclusive in determining the role that genetics plays in caffeine's ergogenic effects (Barreto et al., 2021; Grgic et al., 2021).

2.7.1 Influence of *CYP1A2* polymorphisms on caffeine's ergogenic effects during exercise

The relevant literature exploring the *CYP1A2* gene-caffeine interaction on exercise performance has been summarised in Table 2.3. Studies included cycling and running trials of varying duration. Caffeine dose ranged between 2-6 $mg \cdot kg^{-1}$ body weight with a common dose of 6 $mg \cdot kg^{-1}$ body weight ingested 60-min prior to exercise. Studies have been ordered by caffeine dose size in ascending order from 300 mg mouth rinse to 2 $mg \cdot kg^{-1}$ to 6 $mg \cdot kg^{-1}$.

Table 2.3: Summary of studies exploring the effect of polymorphisms within the *CYP1A2* gene and their influence on exercise performance following caffeine ingestion in differing exercise modalities (ordered by increasing dose)

Study	**Participants**	**Caffeine dosage and timing**	**Genotype (*CYP1A2*)**	**Exercise regime**	**Exercise performance**
Figueiredo et al. 2021	10 male athletes	300 mg, mouth rinsed for 10 seconds immediately prior to exercise	9 CC homozygotes; 1 AC heterozygote	10-km time trial run and vertical jump	ND
Guest et al. 2018	101 male athletes	2 or 4 mg·kg^{-1}, 25-min	49 AA homozygotes; 44 AC heterozygotes; 8 CC homozygotes	10-km cycling time trial	↑ AA homozygotes: 4.8% (2 mg·kg^{-1}); 6.8% (4 mg·kg^{-1}) ND AC heterozygotes. ↓ CC homozygotes: 13.7%
Salinero et al. 2017	21 active adults (14 male, 7 female)	3 mg·kg^{-1}, 60-min	5 AA homozygotes; 16 C- allele carriers	30s Wingate	ND
Carswell et al. 2020	18 adults (12 male, 6 female)	3 mg·kg^{-1}, 70-min	10 AA homozygotes; 8 C-allele carriers	15-min cycling time trial	ND
Grgic, Pickering, et al. 2020	22 male resistance exercisers	3 mg·kg^{-1}	13 AA homozygotes; 9 C allele carriers	Countermovement jump test, Wingate test, bench press	ND
Glaister et al. 2021	40 trained male cyclists	5 mg·kg^{-1}, 60-min	22 AA homozygotes; 18 C-allele carriers	30-min cycling time trial	ND
Pataky et al. 2016	38 cyclists (25 male and 13 female)	6 mg·kg^{-1}, 60-min +/- 25-ml 1.14% caffeine mouth rinse before exercise	21 AA homozygotes; 17 AC heterozygote	3-km cycle	↑ AA homozygotes and AC heterozygotes
Womack et al. 2021	35 trained male cyclists	6 mg·kg^{-1} 60-min	16 AA homozygotes; 19 C-allele carriers	40-km cycling time trial	↑ AA homozygotes: 4.9% ↑ AC/CC: 1.8%
Minaei et al. 2022	16 trained male athletes	6 mg·kg^{-1} 60-min	6 AA homozygotes; 10 AC heterozygote	Wingate test	↑ AA homozygotes: 5.6%

ND- No difference

AA homozygotes- participants carrying the AA *CYP1A2* SNP variation

AC heterozygotes- participants carrying the AC *CYP1A2* SNP variation

CC homozygotes- participants carrying the CC *CYP1A2* SNP variation

↑- increase

↓- decrease

In a 10-km cycling time trial, Guest et al. (2018) showed AA allele carriers' performance was improved following the ingestion of both 2 $mg \cdot kg^{-1}$ and 4 $mg \cdot kg^{-1}$ caffeine compared with placebo, with a decreased time to completion of 4.8% and 6.8%, respectively. This study (Guest et al., 2018) found homozygous CC allele carriers' performance worsened by 13.7% following caffeine supplementation compared to placebo. Participants with the AC allele had no difference in performance between caffeine and placebo in trials (Guest et al., 2018). Supporting this, Womack et al. (2012) showed AA allele carriers' exercise performance improved by 4.9% following 6 $mg \cdot kg^{-1}$ caffeine ingestion. In this study (Womack et al., 2012), AC allele carriers also experienced ergogenic effects following caffeine ingestion, though to a smaller extent than AA allele carrier's, with an improvement of 1.8%. Similarly, AA allele carriers experienced an improvement of 5.6% in peak power output during a Wingate test following a 6 $mg \cdot kg^{-1}$ dose of caffeine (Minaei et al., 2022). In comparison, while finding a gene-treatment interaction effect, Pataky et al. (2016) found AC allele carriers had a greater improvement in performance in a 3-km cycle than AA allele carriers with a decreased time to completion of 4.1% in AC allele carriers and 3.4% in AA allele carriers (Pataky et al., 2016).

In contrast to the findings discussed above, several studies have shown no difference in improvement in exercise performance between AA, AC, and CC allele carriers of the *CYP1A2* gene following caffeine ingestion. Carswell et al. (2020) showed that a 3 $mg \cdot kg^{-1}$ dose of caffeine ingested 70-min prior to a 15-min timed cycling trial improved exercise performance, though no differentiation in improvement was observed between homozygous AA allele carriers and AC and CC allele carriers (Carswell et al., 2020). Similarly, Glaister et al. (2021) showed no genetic effect on caffeine's ergogenicity in a 30-min cycling time trial (Glaister et al., 2021). Caffeine mouth rinsing immediately prior to a 10-km run did not elicit any differentiated benefits between those with AA, AC, and CC allele variations (Figueiredo et al., 2021)

The lack of consensus on which, if any, of the *CYP1A2* genotypes is more likely to have an improvement in exercise performance following caffeine ingestion could be due to differences in trial designs between studies. To date, most of the research exploring genetic contribution to caffeine's effect on exercise performance include mixed exercise modes (e.g., cycling, running, Wingate, bench press, jump height), participants that are either male, or mixed sex, with a caffeine dose of 3-6 $mg \cdot kg^{-1}$ ingested 60-min prior to exercise (Barreto et al., 2021; Carswell et al., 2020;

Ghotbi et al., 2007; Glaister et al., 2021; Grgic et al., 2021; Rasmussen et al., 2002; Salinero et al., 2017). Exercise duration between studies ranges between 30 s (Wingate test) and endurance distance (100-km cycling), with mixed exertion required (peak power output vs submaximal time trials) further differentiating study design. Therefore, the differences between study designs makes them difficult to compare.

Results of research into the effect of the *CYP1A2* gene on caffeine pharmacokinetics and exercise performance (Table 2.3) may also differ due to differences in statistical power between each study. Participant numbers vary considerably between studies (<20 participants to 100+ participants). The high number of participants included in some studies, such as Guest et al., (2018) with 101 participants, allows the grouping of genotypes into the three variations (AA homozygotes, AC heterozygotes and CC homozygotes). As previously discussed, homozygous CC allele carriers make up only 10 % of the population (Sachse et al., 1999), with many studies grouping them with heterozygous AC allele carriers due to low sample numbers, which may contribute to differences in results between studies. Due to the different rate of caffeine metabolism in AC and CC allele carriers (AC carriers: 'slow' metabolisers; CC allele carriers: 'ultra-slow' metabolisers), combining the two gene variations into one group may not provide accurate results of caffeine's effect on exercise performance.

2.8 Summary

The ergogenic effects of caffeine on exercise performance (resistance/power, speed, cardiorespiratory endurance) have been widely reported in the literature, in various exercise types, and of varying duration and intensities, though the exact mechanism of action is unclear. It is likely that caffeine binding to adenosine receptors and stimulating the CNS is the main action resulting in improved exercise performance.

The ideal dose and timing of caffeine ingestion is widely accepted as 3-6 $mg \cdot kg^{-1}$ body weight, 60-min prior to exercise, with higher doses (~9 $mg \cdot kg^{-1}$) shown to provide no additional benefits to performance (Sökmen et al., 2008). However, variation in responses to caffeine are wide, with some experiencing improved performance, and others having no response. This may be due to interindividual factors such as genetics, caffeine habituation, and training status of exercisers. Additionally, the difference between laboratory conditions compared with exercise in real life

(Khcharem et al., 2021) may make it more difficult to accurately determine the ergogenic effects of caffeine.

Studies designed to determine the influence of polymorphisms in the *CYP1A2* gene at intron 1 with the substitution of a C→ A allele, on caffeine pharmacokinetics and exercise performance have yielded mixed conclusions, likely due to varying trial designs and low statistical power. With the rise of sport supplement use by athletes, specifically caffeine, it may be beneficial to continue research into the influence of *CYP1A2* gene polymorphisms on caffeine pharmacokinetics to maximise the ergogenic benefits experienced by athletes from caffeine ingestion.

Chapter 3: The effect of *CYP1A2* gene polymorphisms on caffeine pharmacokinetics and exercise performance in male recreational athletes

This research manuscript has been prepared for the academic journal 'Nutrients'. References have been included in Chicago style to meet journal requirements.

3.1 Abstract

This study examined the effects of caffeine consumption on cardiorespiratory endurance exercise performance, and the role of *CYP1A2* gene polymorphisms in the modulation of caffeine pharmacokinetics and thereby exercise performance.

Thirty-eight recreationally active male participants provided saliva samples for *CYP1A2* genotyping (AA homozygotes n=19; AC heterozygotes n=19) and completed either a 10-km run or 40-km cycling time trial 60-min following a single dose of 6 mg·kg^{-1} caffeine or placebo (maltodextrin) after which time to completion and heart rate (HR) were measured.

Caffeine ingestion improved time to completion by 1.8% ($p=0.05$; $\eta p^2=0.12$). HR was higher in CAF trials compared to PLA ($p=0.02$; $\eta p^2=0.15$). Plasma caffeine concentrations were higher in AA allele carriers compared with AC allele carriers ($p=0.04$; $\eta p^2=0.139$). No caffeine-gene interaction effects were observed in time to completion, HR, or plasma concentrations of paraxanthine and theophylline. Total caffeine plasma concentrations in the area under the concentration-time curve (AUC) were significantly higher in AA allele carriers compared with AC allele carriers ($p=0.01$).

In conclusion, exercise performance is significantly improved in recreationally active men following a caffeine dose of 6 mg·kg^{-1}, independent of *CYP1A2* genotype.

Keywords: *Genetics, sport, endurance exercise, physical activity, athlete*

3.2 Introduction

Caffeine is a methylxanthine that has been shown to elicit ergogenic effects in those who respond to caffeine's effects during exercise, improving strength, speed, and power (Grgic et al., 2018; Southward, Rutherfurd-Markwick, and Ali 2018), making caffeine a popular ergogenic aid among athletes. Caffeine's appeal has increased due to the perceptual and cognitive physiological effects it can exert such as reducing fatigue and perceived exertion and improving mood and cognition (Ágoston et al., 2018; Barcelos et al., 2020; Barreto et al., 2021). However, interindividual variation in response to caffeine is broad, with many factors, such as genetics, gender, and medications influencing the ergogenic effects on exercise performance (Cappelletti et al., 2015; Nehlig 2018).

The enzyme cytochrome P450 is a monooxygenase that metabolises drugs such as caffeine and is thought to influence exercise performance following the ingestion of caffeine. This may be due to polymorphisms in the *CYP1A2* gene which encodes the P450 enzyme resulting in variations in allele expression, and subsequently the rate of enzymatic activity (Nelson et al., 2004). Those with the AA allele variation are thought to be 'fast' metabolisers of caffeine, those with the AC allele are 'slow' metabolisers, and the CC allele carriers 'ultra-slow' metabolisers (Nehlig 2018; Sachse et al., 1999). Therefore, the rate of caffeine metabolism may differ between individuals depending on their *CYP1A2* genotype, resulting in different impacts on exercise performance.

The effect of the *CYP1A2* gene on exercise performance following caffeine ingestion has been of increasing interest in the last decade (Grgic et al., 2021). A recent systematic review of 17 studies found variations in the *CYP1A2* gene improved exercise performance in four studies, with caffeine improving exercise performance in AA allele genotypes over AC or CC genotypes (Grgic et al., 2021). However, the remaining 13 studies showed no influence from the *CYP1A2* gene on improvement in exercise performance. Subsequent research has shown differing results from anaerobic and aerobic exercise, with some studies finding no caffeine-gene interactions (Glaister et al., 2021; Sicova et al., 2021), and others finding an improvement in performance in AA allele carriers (Emilia et al., 2023; Minaei et al., 2022). One study found handgrip strength decreased in CC allele carriers following consumption of 5 $mg \cdot kg^{-1}$ caffeine (Wong et al., 2021), but no difference in performance in AA or AC genotypes. The differing results between studies may be due to small sample sizes, grouping AC and CC allele carriers together, and differing exercise modalities between studies (Grgic et al., 2021; Sicova et al., 2021).

The consensus on optimal dosage and timing of caffeine ingestion for ergogenic benefits in exercise performance is widely accepted as 3-6 mg·kg^{-1} caffeine 60-min prior to exercise (Del Coso, Muñoz, and Muñoz-Guerra 2011; Glaister et al., 2021; Ryan et al., 2013). A low-to-moderate dose of caffeine (3-6 mg·kg^{-1}) has been found to provide similar improvements in exercise performance. Consuming a higher dose of caffeine (9 mg·kg^{-1}) prior to exercise has yielded mixed results, with some studies reporting ergolytic effects at higher doses due to an onset of nervousness, jitters, sleep disruptions, and anxiety in athletes (Nawrot et al., 2003). Other studies report some ergogenic effects following a high caffeine dose (relative to placebo), but no further improvements in performance compared with a dose of 6 mg·kg^{-1} (Graham and Spriet 1995; Pickering and Kiely 2019a; Salinero et al., 2017; Wang et al., 2020). The timing of caffeine supplementation 60-min prior to exercise in research trials is generally used as it allows caffeine to reach peak serum concentrations during the exercise trial (Ryan et al., 2013). However, as caffeine has been found to improve exercise performance when fatigue sets in (Davis et al., 2003), the optimal time to consume caffeine supplements to maximise the ergogenic benefits may vary depending on different exercise modes, caffeine metabolism, and mode of delivery.

Caffeine is a universally popular ergogenic aid, and understanding the factors that affect intra- and interindividual variation in response to caffeine may assist in using caffeine more effectively. As the research behind the *CYP1A2* gene's influence on caffeine pharmacokinetics and exercise performance is unclear, further research in this area may provide clarity on dosing and timing strategies for use in enhancing exercise performance. Additionally, it can be translated into use by individuals using caffeine for non-exercise related activities such as study and work.

This study combines data from two separate research trials: a 10-km running trial and a 40-km cycling trial to increase the sample size, and therefore statistical power. Both research trials recruited recreationally active males, who consumed a dose of 6 mg·kg^{-1} caffeine or placebo (maltodextrin) 60-min prior to exercise. The distances of 10-km running, and 40-km cycling were chosen for this study as each take approximately 1-h to complete and are average endurance distances many professional and recreational athletes complete during training and events (Cushman, Markert, and Rho 2014; Ransdell, Vener, and Huberty 2009). This study aimed to determine the effect a dose of 6 mg·kg^{-1} of caffeine consumed 60-min before commencing either a

10-km running, or 40-km cycling time trial had on exercise performance by analysing caffeine's ergogenic effects on aerobic and muscular endurance through comparing time to completion in exercise trials. Further, it aimed to determine the impact of *CYP1A2* gene polymorphisms on the rate of caffeine metabolism and subsequently exercise performance following caffeine ingestion in male recreational exercisers.

3.3 Methods

Participants

A 10-km running trial and a 40-km cycling trial were completed independently. Data were combined for this study to increase the sample size and statistical power. The participants recruited from the two studies were 38 non-smoking, recreationally active males (combined mean and standard deviation: age= 31.94 ± 9.98 years; weight= 77.47 ± 7.98 kg). Data from 14 participants was collected during the 40-km cycling trial, and data from 24 participants was collected from the 10-km running trial. Two separate ethics applications were approved by the Massey University Human Ethics Committee (Running trial- Southern A, Application 15/12, Appendix 1; Cycling trial- Southern A, Application SOA 18/44). Following the provision of an information sheet (Appendix 2) explaining the requirements, advantages, and risks of the study, written consent (Appendix 3) was obtained from all participants. Participants were screened for health issues and caffeine use (Appendix 4; Appendix 5) and excluded from the study if caffeine intake was avoided, or above 4 standard cups of coffee (or equivalent) were typically consumed per day.

Participants were required to abstain from caffeine consumption during the study, a time period which occurred from the familiarisation session until 48 h following the final trial. Participants kept a food diary (Appendix 6) for 48 h prior to each trial and were asked to replicate their diet prior to each trial.

Study Design

Both the running and the cycling trials used a randomised, double-blind, placebo-controlled cross-over design study. Participants attended 3 sessions, one week apart. The initial visit was a familiarisation session where blood test procedures, and the food diary were explained to participants. Participants ran 1-km (run study) or cycled 40-km (cycle trial) to familiarise themselves with the treadmill or ergometer. The second two sessions were exercise trials. Blood samples taken

immediately prior to consumption of a caffeine or placebo capsule and participants then rested for 60-min before commencing exercise. They were instructed to consume a light meal approximately 2 h prior to the trial commencing. Before the commencement of each cycling trial, participants were provided with a standardised meal (5x Tom and Luke Snackaballs, salted caramel flavour), but this was not provided to participants in the running trials.

Run times were recorded at 5-km and 10-km distances and cycling times recorded electronically on the ergometer, and manually at the completion of exercise. Water (2-ml·kg^{-1} body mass) was ingested with the caffeine or placebo supplement, at 60-min post caffeine or placebo consumption, halfway through the exercise trial, and at the completion of the exercise trial (Fig 3.1). Once the participants had completed the exercise trial, blood samples were repeated. In total four blood samples were taken from each participant.

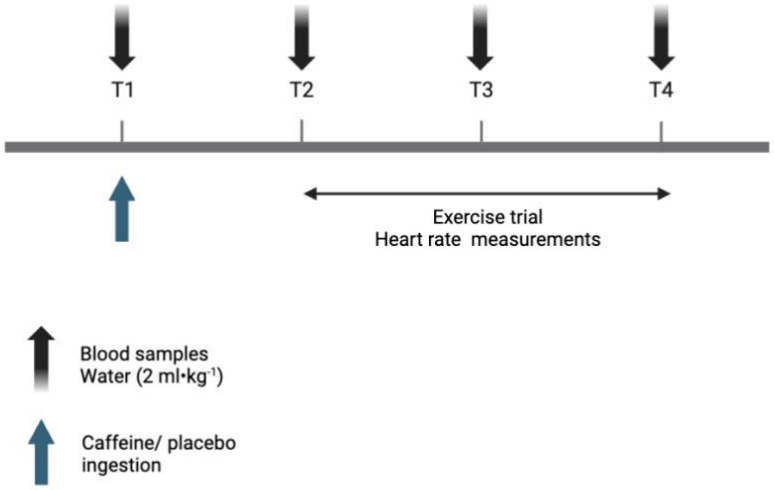

Figure 3.1 Schematic diagram of physical exercise trials.

T1 represents the time point 60-min prior to the exercise trial; T2 represents the timepoint the exercise time trial commenced; T3 represents the halfway point of the exercise time trial; T4 represents the completion of the exercise time trial.

Caffeine and placebo treatments

Participants consumed (6 mg·kg^{-1}) caffeine (Fluka Sigma-Aldrich, MO, USA) or placebo (maltodextrin) in a gelatin capsule (Vegie capsules, Biobalance, New Zealand).

Anthropometric and physiological measurements

Height and weight were measured during the familiarisation session. Height was measured with a stadiometer (Seca portable stadiometer, Amtech, New Zealand). Participants were instructed to stand with their head angled in the Frankfurt plane and bare heels against the stadiometer's back board. Weight was measured using scales accurate to 0.1 kg (AND Weighing Hv 200-KGL, Australia) without shoes or excess clothing worn. Each participant's weight measured during the familiarisation session was used to calculate their dose of caffeine provided.

Heart rate was measured using a short-range telemetry chest strap and watch (T31 Polar heart rate monitor, Kempele, Finland), and recorded at 1-km intervals.

The 10-km running time trial was completed indoors on a treadmill (ELG70, Woodway, Waukesha, Wisconsin, USA). To simulate running outside on a flat terrain, a 1% incline was set on the treadmill. Participants were instructed to complete the 10-km as quickly as possible, and were advised when they had completed each km. No further information or encouragement was given. Participants were able to adjust the speed on the treadmill, but were not aware of the speed they were running at. Each participant's time, distance and speed were recorded throughout the trial.

The 40-km cycling time trial was completed on a cycle ergometer (Velotron Racemate^{TM}, Quarq, USA). Participants provided their own seats and pedals for comfort during the familiarisation session and cycling trials. Cyclists were encouraged to cycle as quickly as possible, and advised when they had completed each 10-km. Participants were able to adjust the gears during the cycling trial, but were not aware of the speed at which they were cycling at. Velotron data captured each participants time, distance and speed throughout the trial.

Blood sampling and analysis

Blood samples were collected to measure caffeine, paraxanthine, and theophylline plasma concentrations before, during and after exercise. Blood samples were taken via a cannula inserted into one of the basilic, cephalic, or median veins of the antecubital area. The cannula was secured to the participant's arm using surgical tape and a bandage. An extension kit was connected to the cannula with blood samples then drawn using a syringe. Blood samples (12-ml) were taken prior to caffeine ingestion and 50-min post caffeine ingestion. In the running and cycling trials blood samples were collected halfway through exercise, and at the completion of exercise. Each sample

was aliquoted into one 6-ml EDTA vacutainer and one 6-ml lithium heparin vacutainer tube. After mixing, the blood samples were centrifuged (MF-50, Hanil Science Industrial, Korea) for 10-min at 1330 x *g*, with the plasma from each sample then dispensed into three 1.5-ml Eppendorf tubes. Samples were frozen and stored at -80°C until concentration measurements for caffeine and its metabolites' (paraxanthine and theophylline) were undertaken.

Plasma caffeine, paraxanthine and theophylline concentrations were analysed by high-performance liquid chromatography (HPLC). In preparation for analysis, each thawed plasma sample was deproteinised by combining a 400 µl aliquot of plasma with 400 µl of 0.8 M perchloric acid and vortexing for 10 s. Samples were then centrifuged at 9900 x *g* for 10-min, and 400 µl supernatant placed in a glass HPLC vial for analysis. Reversed-phase HPLC (Appendix 7) was used to measure caffeine and metabolite concentrations in each sample.

Saliva sampling for DNA analysis

A saliva sample was obtained from each participant during the familiarisation session for DNA analysis (Appendix 8). The bud method was used (Rutherfurd-Markwick et al. 2020) with two large cotton buds (Jumbo cotton applicators 18 cm, Livingston; Appendix 9) inserted into the mouth and left for 3-min, one inside the cheek and one under the tongue. The cotton buds were removed after the 3-min period and placed into a test tube where saliva was extracted by centrifuging (MF-50, Hanil Science Industrial, Korea) for 2-min at 1330 x *g*. A 1.5-ml sample of saliva was sent to the laboratory at Massey University for DNA extraction (Appendix 8). These DNA samples were then sent for genotyping at the Liggins Institute (Auckland University, New Zealand).

Statistical analysis

A two-way repeated measures analysis of variance (ANOVA) was used to calculate differences between caffeine and placebo for plasma caffeine, paraxanthine and theophylline concentrations, time to completion, cardiac output, *CYP1A2* genotype effects, and trial order effects. A paired t-test with Hedge's correction factor was used in post hoc analysis to identify differences between specific time points and/or treatments. A Mann Whitney- U test was performed in analysis of the AUC. Eta squared and Cohen's d were used to calculate effect sizes (small effect size: ηp^2=0.01, d=0.2; medium effect size: ηp^2=0.09, d=0.5; large effect size: ηp^2=0.25, d=0.8). Data are presented

as mean ± standard deviation (SD). Statistical significance was set at $p<0.05$. Statistical Package for the Social Sciences (SPSS, Chicago, IL) Version 29.0 was used to analyse the data.

3.4 Results

3.4.1 Study population demographics

Half of participants (n=19) were homozygous AA allele carriers (fast metabolisers) and half (n=19) were AC allele carriers (slow metabolisers). No participants were homozygous CC allele carriers, or 'ultra-slow' metabolisers. All participants recruited were male and identified as Tier 1: Recreationally Active (McKay et al. 2022).

3.4.2 Impact of caffeine on time trial performance

The data from 34 out of 38 participants were used to analyse time to completion in this study, with 4 outliers removed due to incomplete data collected. Overall, a 1.8% improvement in performance was observed in CAF trials compared to PLA (CAF 58.5 ± 12.2 min vs PLA 59.5 ± 11.9 min; $p=0.05$; $\eta p^2=0.12$; Figure 3.2). Mean time to completion in the first half of exercise was non-significantly 1.2% lower in CAF trials compared to PLA (CAF 29.4 ± 6.2 min vs PLA 29.7 ± 6.0 min; $p=0.36$; $\eta p^2=0.03$), but 2.4% significantly faster in the second half of exercise in CAF trials compared to PLA (CAF 29.1 ± 6.1 min vs PLA 29.8 ± 6.1 min; $p=0.02$; $\eta p^2=0.16$). Of the 34 participants, 24 had faster times to completion in CAF trials compared to PLA. No trial order effect was detected ($p=0.88$; $\eta p^2=0.001$).

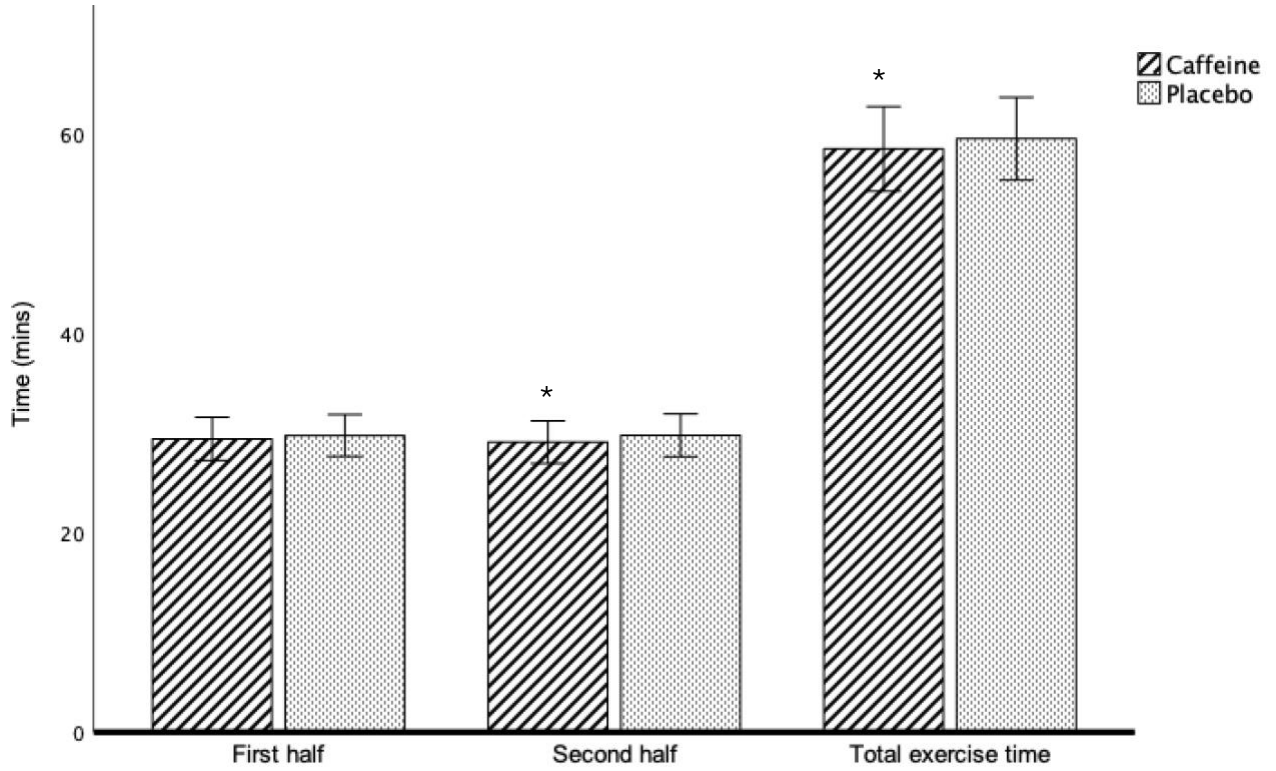

Figure 3.2 Time to completion in CAF and PLA trials.

First half indicates the mean time participants took to complete the first half of exercise. Second half indicates the mean time participants took to complete the second half of exercise. Total exercise time indicates the total mean time participants took to complete the full exercise trials.* statistically significantly difference to placebo trial at same time point ($p<0.05$).

3.4.3 Impact of caffeine consumption on cardiac output

The data from 38 participants was used to analyse HR. Mean HR was 2.4% higher during the CAF trial compared to PLA (CAF 164.5 ± 14.5 beats·min^{-1} vs PLA 160.6 ± 15.8 beats·min^{-1}; $p=0.02$; $\eta p^2= 0.15$).

3.4.4 Caffeine and metabolites

The difference in caffeine, paraxanthine and theophylline plasma concentrations between the CAF and PLA trials are shown in Figures 3.4- 3.6.

Plasma Caffeine Concentrations

A small but significant difference was noted between plasma caffeine concentrations at the beginning of both trials (CAF 0.20 ± 0.38 mg·ml^{-1} vs PLA 0.13 ± 0.36 mg·ml^{-1}; $p=0.05$). Plasma

caffeine concentrations were higher for CAF than PLA 60-min after ingestion and remained higher throughout the exercise trial ($p<0.001$; $\eta p^2=0.987$; Figure 3.3). No trial order effect was observed ($p=0.20$; $\eta p^2=0.057$). Timepoints 2-4 were found to have higher plasma caffeine concentrations in CAF trials compared to PLA ($p<0.001$, $d=0.79$- 3.91).

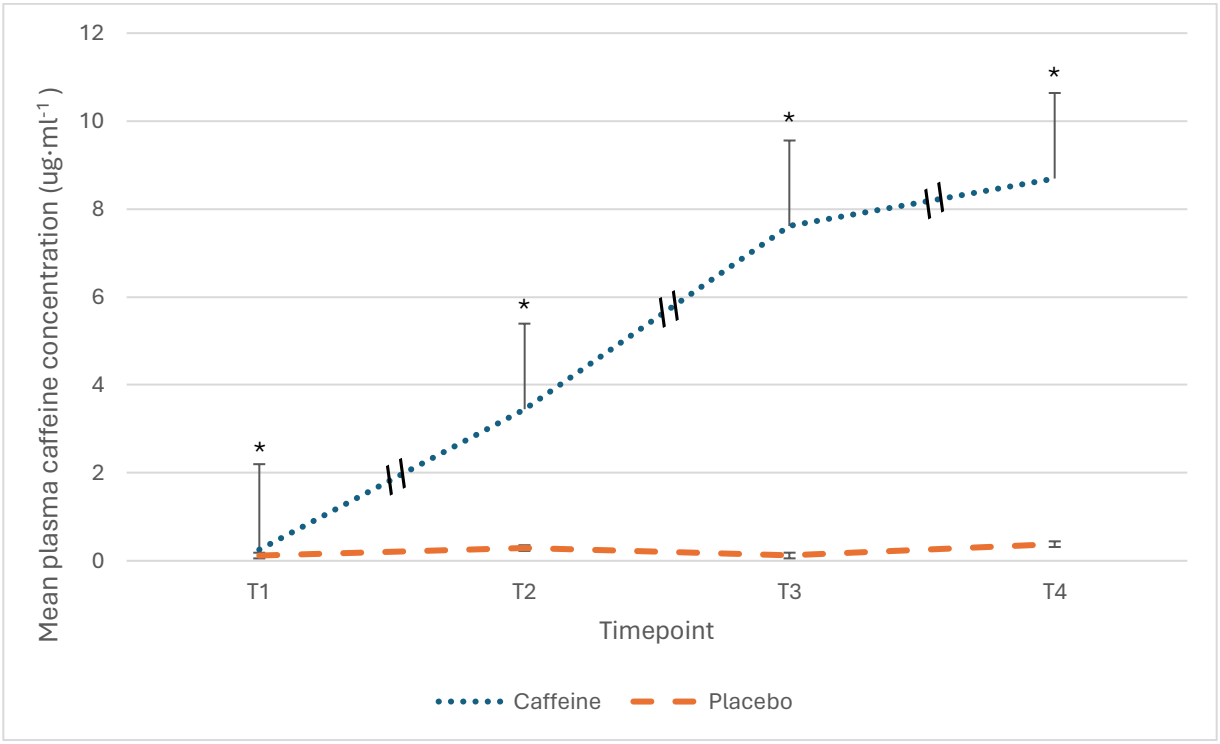

Figure 3.3 Plasma caffeine concentrations during CAF and PLA trials.

T1 represents the time immediately prior to CAF/PLA ingestion; T2 represents the time 60-min after CAF/PLA ingestion and immediately before exercise; T3 represents 5-km of the running/ 20-km of the cycling time trial complete; T4 represents 10-km of the running/ 40-km of the cycling time trial complete. * statistically significantly different to PLA trial at same time point ($p<0.05$).

Plasma Paraxanthine Concentrations

Low plasma paraxanthine concentrations were observed at the beginning of both exercise trials (T1: CAF 0.30 ± 0.57 $mg \cdot ml^{-1}$ vs PLA 0.26 ± 0.55 $mg \cdot ml^{-1}$; $p=0.77$). Non-significant increases in plasma paraxanthine concentrations were observed in both CAF and PLA trials following caffeine ingestion (treatment-time interaction effect $p=0.16$; $\eta p^2=0.165$). Mean paraxanthine plasma concentrations were significantly higher in CAF trials compared to PLA at timepoints 2-4 (Fig 3.4; $p=0.002$- 0.02 $d=0.381$- 0.551). There was no trial order effect ($p=0.18$).

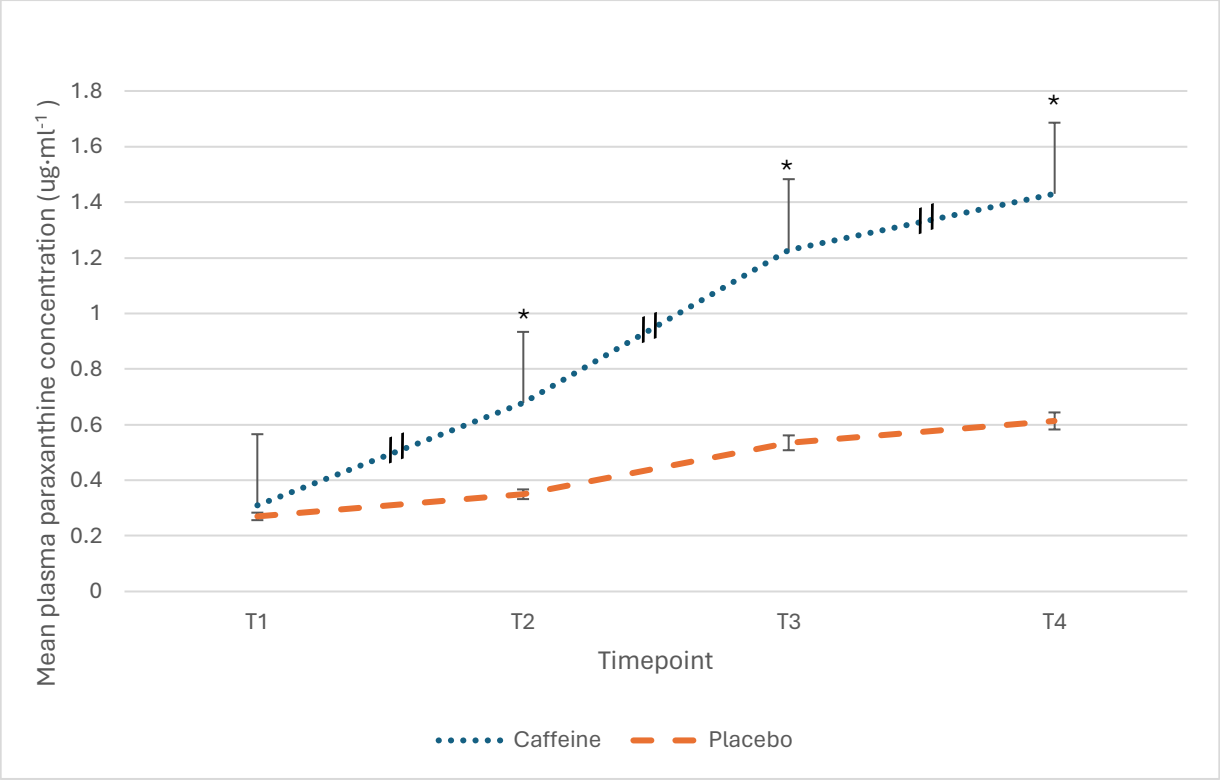

Figure 3.4 Plasma paraxanthine concentrations during CAF and PLA trials.

T1 represents the time immediately prior to CAF/PLA ingestion; T2 represents the time 60-min after CAF/PLA ingestion and immediately before exercise; T3 represents 5-km of the running/ 20-km of the cycling time trial complete; T4 represents 10-km of the running/ 40-km of the cycling time trial complete. * statistically significantly different to PLA trial at same time point ($p<0.05$).

Plasma Theophylline Concentrations

No difference in plasma theophylline concentrations were detected at the beginning of both trials (0.04 ± 0.03 mg·ml^{-1}; $p=0.55$). Plasma theophylline concentrations increased in the CAF trial compared to PLA (treatment-time interaction effect $p<0.001$; $\eta p^2= 0.78$). Timepoints 2-4 had significantly higher theophylline plasma concentrations in CAF trials compared to PLA (Fig 3.5; $p=0.001$- 0.006, $d=0.481$- 1.391). No treatment order effect was observed ($p=0.14$).

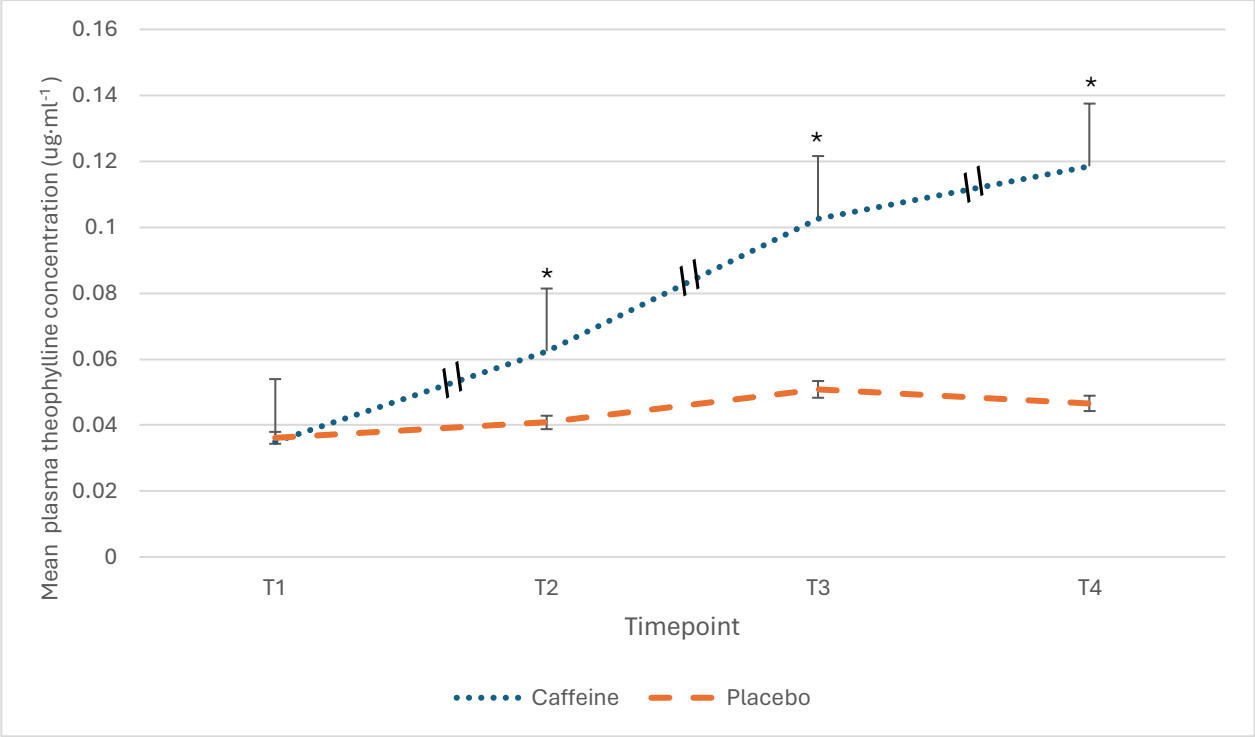

Figure 3.5 Plasma theophylline concentrations during CAF and PLA trials.

T1 represents the time immediately prior to CAF/PLA ingestion; T2 represents the time 60-min after CAF/PLA ingestion and immediately before exercise; T3 represents 5-km of the running/ 20-km of the cycling time trial complete; T4 represents 10-km of the running/ 40-km of the cycling time trial complete. * statistically significantly different to PLA trial at same time point ($p<0.05$).

3.4.5 Caffeine-*CYP1A2* gene interaction effects on exercise performance

A significant caffeine-gene interaction effect was observed in plasma caffeine concentrations, with plasma caffeine concentrations higher in AA allele carriers compared with AC allele carriers ($p=0.04$; $\eta p^2=0.139$; Fig 3.6). No caffeine-gene interactions were shown in plasma paraxanthine and theophylline concentrations, or physical performance results in this study (Table 3.1). There was no difference in HR between genotypes ($p=0.77$; $\eta p^2= 0.002$).

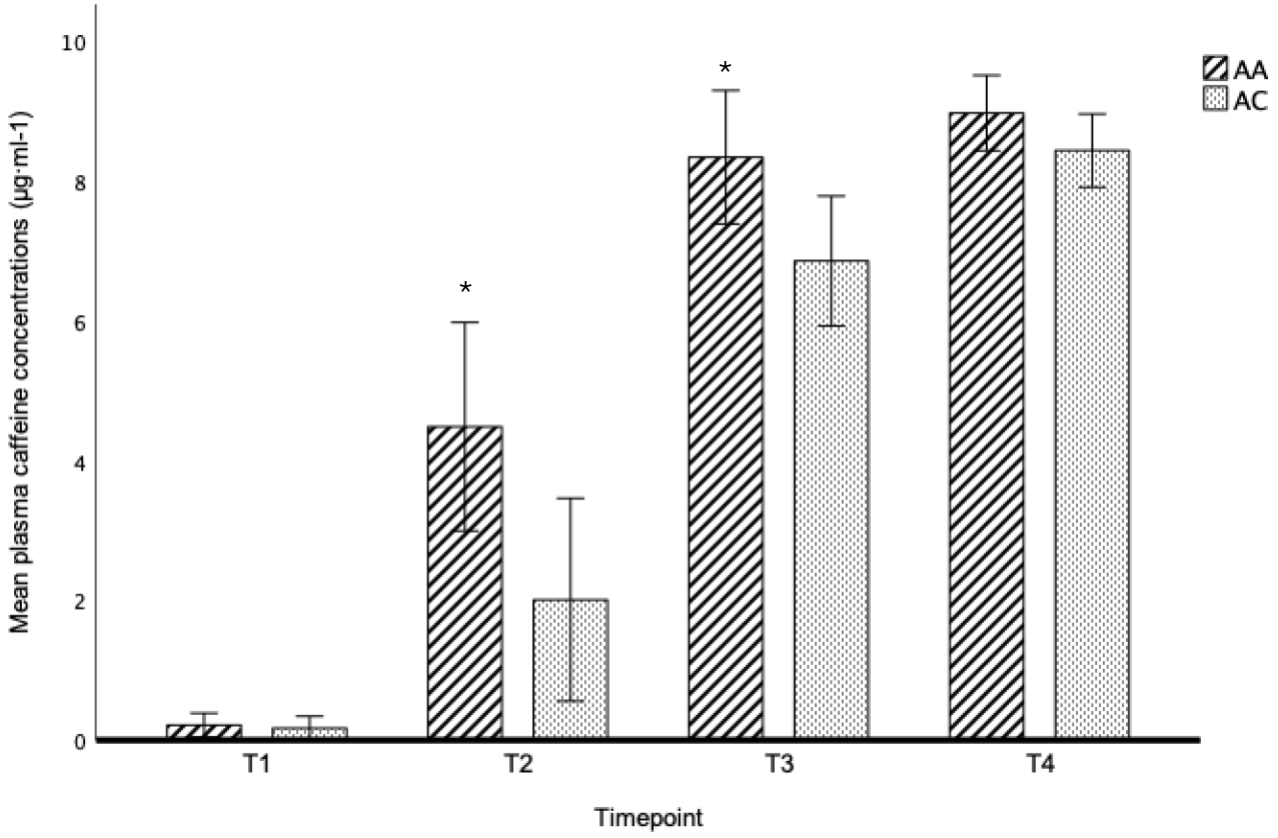

Figure 3.6 Mean caffeine plasma concentrations in *CYP1A2* AA ('fast' metabolisers) compared with AC ('slow' metabolisers) allele carriers at different timepoints.

T1 represents the time immediately prior to CAF/PLA ingestion; T2 represents the time 60-min after CAF/PLA ingestion and immediately before exercise; T3 represents 5-km of the running/ 20-km of the cycling time trial complete; T4 represents 10-km of the running/ 40-km of the cycling time trial complete. * statistically significant between genotypes at the same timepoint ($p<0.05$).

Table 3.1 Interaction effect of *CYP1A2* genotype on caffeine treatment

Measure	**Treatment-gene interaction effect p-value***	**Effect size (ηp^2)**
Caffeine ($mg \cdot ml^{-1}$)	0.04	0.139
Paraxanthine ($mg \cdot ml^{-1}$)	0.13	0.156
Theophylline ($mg \cdot ml^{-1}$)	0.78	0.032
Time (min)	0.89	0.001
Heart rate ($beats \cdot min^{-1}$)	0.77	0.002
*Significant at $p<0.05$		

3.4.6 Area under the concentration-time curve (AUC)

The total mean plasma caffeine concentration AUC was significantly higher in AA compared to AC allele carriers (p=0.01; Fig 3.7), indicating a faster clearance rate in homozygous AA allele carriers compared to AC individuals. In the AUC, no differences in total mean paraxanthine (p=0.40) and theophylline (p=0.62) plasma concentrations were identified between genotype variants.

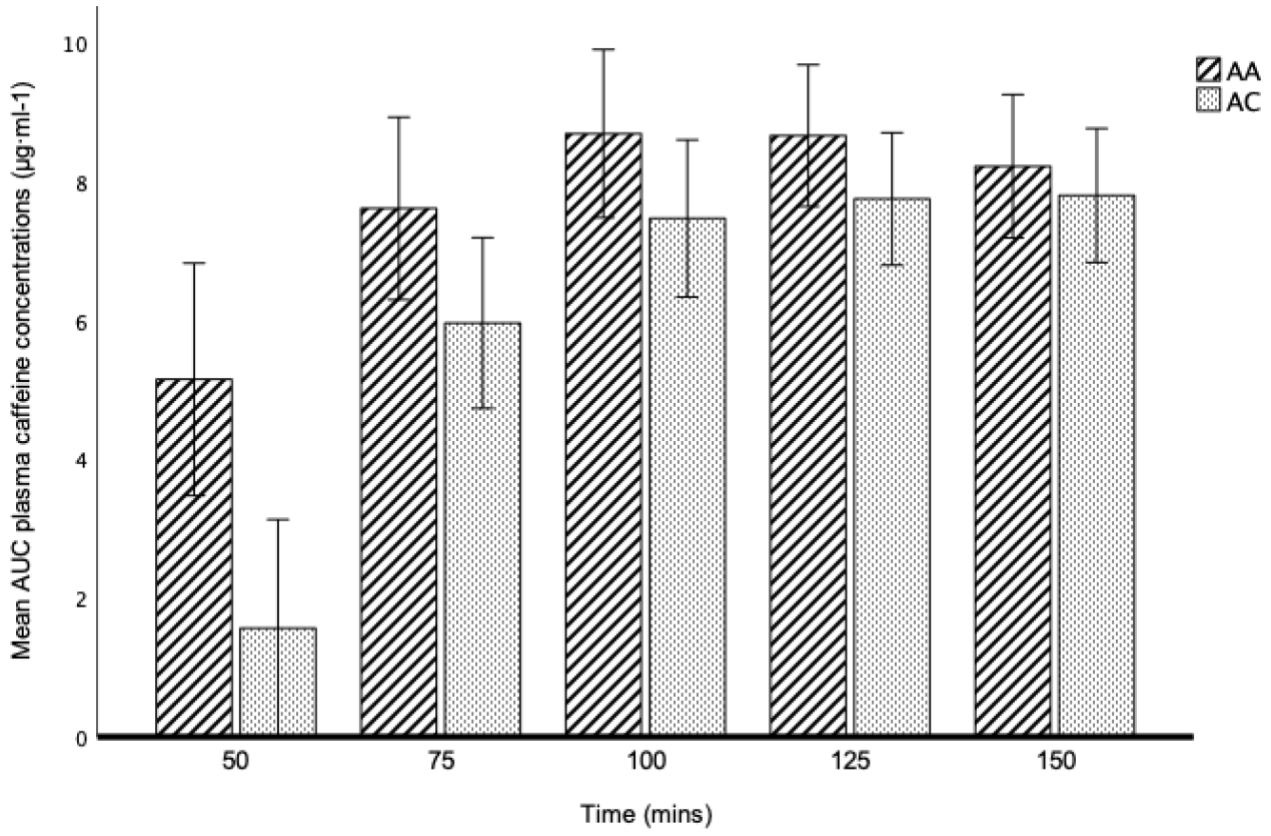

Figure 3.7 AUC- total cumulative caffeine plasma concentrations 50-150-min following caffeine ingestion between AA ('fast' metabolisers) and AC ('slow' metabolisers) *CYP1A2* genotypes

3.5 Discussion

The aim of this study was to determine if 6 mg·kg^{-1} of caffeine, ingested 60-min prior to exercise, would improve exercise performance in recreational male athletes, and if polymorphisms in the *CYP1A2* gene influenced caffeine pharmacokinetics, and in turn exercise performance. Overall, there was a 1.8% improvement in exercise performance following a 6 mg·kg^{-1} dose of caffeine ingested 60-min prior to exercise. However, improvements in exercise performance were not influenced by *CYP1A2* genotype.

A dose of 6 mg·kg^{-1} of caffeine improved exercise performance and decreased time to completion by 1.8%. This level of improvement is within the range reported by several meta-analyses (Schubert and Astorino 2013; Southward et al., 2018; Ziyu et al., 2022), adding to the evidence base for caffeine's ergogenic effects at a dose of 6 mg·kg^{-1}. Each participant's time to completion was compared between trials to determine if CAF influenced performance times compared with PLA, and if so, identify differences in performance between the first and second halves of exercise. Time to completion was also analysed to examine the effect of the timing of caffeine ingestion compared with exercise performance as caffeine is thought to be beneficial in endurance exercise as fatigue increases (Costill, Dalsky, and Fink 1978). A non-significant improvement in time to completion of 1.2% was found in the first half of exercise in the CAF trial compared with PLA. However, there was a significant improvement in time to complete the second half of exercise of 2.4% in the CAF trial compared to PLA. This supports the notion that caffeine's ergogenic benefits may be increased during periods of fatigue, such as later in exercise (Costill, Dalsky, and Fink 1978). There are several reasons that may explain the larger improvement in performance in the second half of exercise. Caffeine is known to bind to adenosine receptors, blocking adenosine from eliciting pain and fatigue during exercise (Costill, Dalsky, and Fink 1978). Therefore, the improvement in the second half of exercise may be due to caffeine's analgesic effects. The increased improvement in time performance in the second half of exercise may also be due to the metabolism of caffeine into paraxanthine and theophylline, which are adenosine antagonists (Daly 1982; Greer, Friars, and Graham 2000). The metabolic ratio of paraxanthine to caffeine [paraxanthine]/[caffeine] is a biomarker of *CYP1A2* enzyme activity as it shows the rate of caffeine's demethylation into paraxanthine. The paraxanthine/caffeine metabolic ratio is consistent between timepoints 2 to 4 (0.16-0.20) during the exercise trial following caffeine ingestion, while increases in theophylline are small, theophylline has the highest affinity to adenosine receptors between caffeine and its metabolites (Mumford et al., 1996). This may enhance caffeine's ergogenic effects in exercise performance by further competing with adenosine receptors along with caffeine to delay fatigue. However, smaller improvements in time performance in the first half of the trials compared with the second half may be due to caffeine's influence on perceived effort, or participants pacing themselves to extend time to fatigue.

Caffeine plasma concentrations were low (>0.2 mg·ml^{-1}) at timepoint 1 (prior to ingestion of supplement) in both the CAF and PLA trials due to participants abstaining from caffeine-containing products for three days prior to the trials. After caffeine ingestion plasma concentrations increased significantly over time. Mean caffeine plasma concentrations remained unchanged during the PLA trials, which was expected. Plasma caffeine concentrations in the CAF trial peaked at time point 4 (Caffeine 8.69 ± 1.14 mg·ml^{-1}; ~110-min post caffeine ingestion (directly after the running and cycling trial were completed). In similar studies, caffeine is consumed 60-min prior to exercise to allow for its absorption and to reach maximum plasma concentrations (Graham 2001; Guest et al., 2021; Seepika et al., 2022), however peak plasma levels were not reached within 60-min in this study, with peak caffeine plasma concentrations occurring 95-110-min post caffeine ingestion. Mumford et al., (1996) found caffeine plasma concentrations peaked 30-min post ingestion in individuals consuming caffeine in capsules (Mumford et al., 1996). Differences in timings of maximum caffeine plasma concentration may be due to factors influencing caffeine metabolism such as genotype polymorphisms, but also due to the method of caffeine administration such as capsules, including type of capsule used, and caffeine beverages, gels, mouth rinses or chewing gum (Kamimori et al., 2002; Mumford et al., 1996; Guest et al., 2021), with all factors contributing to high inter-individual variability in caffeine plasma concentrations.

Caffeine is demethylated into its metabolites; paraxanthine which makes up approximately 84%, theophylline 4% and theobromine 12% (Nehlig 2018). The metabolic ratio of caffeine shows *CYP1A2* activity through its accumulation in the system and subsequent metabolism into its metabolites (Tian et al., 2019). The distribution of paraxanthine concentration in this study is in line with the distribution found in the literature (Jodynis-Liebert et al., 2004; Lajin et al., 2021), with the metabolic ratio of paraxanthine [paraxanthine]/[caffeine] being between 0.16 and 1.20 (Table 3.2). While some studies (Furge and Fletke 2007; Shirley et al., 2003) observed a higher metabolic ratio for paraxanthine as plasma concentrations are measured over a longer time period (4-12 h), the metabolites in this study were only measured between -60-min and 125- 150-min. Individuals with a higher paraxanthine/caffeine ratio have been reported to have a reduced perception of exertion during exercise (Whalley, Paton, and Dearing 2021). Further, the presence of paraxanthine and theophylline in conjunction with caffeine in the body may increase physiological actions such as reducing fatigue and perceived exertion and therefore enhance exercise performance (Daly 1982; Greer, Friars, and Graham 2000).

Table 3.2 Metabolic ratio of paraxanthine and caffeine [paraxanthine/caffeine].

Timepoint	Mean plasma concentration ($mg \cdot ml^{-1}$)		Metabolic ratio
	Caffeine	Paraxanthine	
T1	0.25	0.30	1.20
T2	3.45	0.68	0.20
T3	7.61	1.23	0.16
T4	8.70	1.43	0.16

T1 represents the time immediately prior to caffeine/placebo ingestion; T2 represents the time 60-min after CAF/PLA ingestion and immediately before exercise; T3 represents 5-km of the running/ 20-km of the cycling time trial complete; T4 represents 10-km of the running/ 40-km of the cycling time trial complete.

Although no participants in this study had the CC allele variant, the distribution of allele variations were similar to population distributions of the *CYP1A2* polymorphisms, where 46% of the population have the AA allele variation and 44% are AC carriers (Sachse et al., 1999). Those carriers of the CC *CYP1A2* SNP are thought to make up 10% of the population (Sachse et al., 1999), therefore it is likely that no CC allele carrier numbers were recruited in this trial due to small population numbers, or not applying to take part in the research trials due to the negative effects caffeine can cause in CC allele carriers.

No influence from the *CYP1A2* gene was observed in the time to completion and HR in this study. To date the literature examining the effect of *CYP1A2* polymorphisms on the ergogenic effects of caffeine in exercise performance are inconclusive (Carswell et al., 2020; Figueiredo et al., 2021; Glaister et al., 2021; Grgic et al., 2020; Guest et al., 2018; Minaei et al., 2022; Rahimi 2019; Salinero et al., 2017; Spineli et al., 2020; Wong et al., 2021), and there is no clear consensus as to which *CYP1A2* polymorphism (AA, AC, or CC) is more likely to modulate the ergogenic effects of ingested caffeine (Emilia et al., 2023; Pataky et al., 2016). Plasma caffeine concentrations were higher in AA allele carriers compared with AC allele carriers ($p=0.04$), and further research should be conducted with a larger sample size to determine if there is a relationship between the *CYP1A2* genotype, caffeine ingestion, and exercise performance. Though results between studies are equivocal, our findings of no caffeine-gene interaction in time to completion is in line with some current research using similar caffeine dosage and timing, and exercise modalities in trials (Carswell et al., 2020;

Figueiredo et al., 2021; Glaister et al., 2021; Grgic et al., 2020). The equivocal results between studies may be due to other factors affecting the inducibility of the *CYP1A2* enzyme, such as medications, cigarette smoking, heavy exercise, and consuming cruciferous vegetables. Additionally, differences in study design (caffeine dose and exercise modality) may influence trial outcomes.

Those carrying the AA polymorphism of the *CYP1A2* gene metabolise caffeine at a faster rate than those with AC and CC polymorphisms. This means that maximum plasma caffeine concentrations are reached faster in AA allele carriers. This has been observed in this study with mean plasma caffeine concentrations higher at each timepoint (2-4) in those with AA alleles than those with the AC polymorphism ($p=0.04$; $\eta p^2= 0.139$; Figure 3.7). Further, analysis of the AUC of mean plasma caffeine concentrations showed a significantly higher concentrations in AA allele carriers compared with AC allele carriers ($p=0.01$). Therefore, caffeine clearance, or its demethylation to its metabolites paraxanthine, theophylline and theobromine is faster in AA allele carriers compared with AC allele carriers. The higher plasma caffeine concentrations in AA allele carriers did not lead to an improvement in exercise performance in AA allele carriers which may be attenuated by variations in participants *ADORA2A* genotypes (CC, CT, and TT polymorphisms). The *ADORA2A* gene encodes the adenosine A2A receptor, in which adenosine and caffeine both compete for this binding site (Guest et al., 2021). *ADORA2A* affects the physiological responses to caffeine including cardiac output, catecholamine release, sleep quality and duration, and glutamic response (Banks et al., 2019; Guest et al., 2021). Additionally, the *ADORA2A* gene influences sensitivity in response to caffeine's effects, with those possessing the TT variation found to experience negative effects following caffeine ingestion such as anxiety and nervousness, which may attenuate caffeine's ergogenic effects in exercise performance (Guest et al., 2021).

Research limitations and future directions

Further research may look to replicate this trial with a larger total sample size of 46 participants, as calculated by G*Power, to accurately identify what, if any, effects *CYP1A2* polymorphisms exert on caffeine metabolism and exercise performance. This study focuses primarily on male recreationally trained exercisers, and further research may look to include female exercisers, or well-trained/ professional athletes. Future studies should include CC allele carriers, as differences in ergogenic effects of caffeine between AC and CC allele carriers may be significant enough to differentiate

performance outcomes. Future studies may also look to compare the *ADORA2* genotypes of participants along with *CYP1A2* genotypes to assess if caffeine sensitivity and caffeine uptake into cells influences *CYP1A2* activity and therefore exercise performance, though a much larger sample size would be required to include all genotype variations (*CYP1A2* AA, AC and CC and *ADORA2A* CC, CT, and TT) with sufficient statistical power.

3.6 Conclusion

The results of this study confirm a dose of 6 $mg \cdot kg^{-1}$ caffeine 60-min prior to exercise improves endurance exercise performance in male recreational exercisers by reducing time to completion and increasing HR in 10-km running and 40-km cycling time trials. Caffeine's ergogenic effects were not influenced by the *CYP1A2* gene in physical performance. However, mean plasma caffeine concentrations were shown to be higher in AA allele carriers compared to AC allele carriers.

Chapter 4: Conclusions and Recommendations

4.1 Study aim and objectives

This study had two main aims; firstly, to determine the effects of caffeine ingestion, 60-min prior to exercise, on exercise performance in male recreational exercisers. The second, to determine if genetic variations in the *CYP1A2* gene influence caffeine pharmacodynamics and consequently exercise performance. To do this we examined the effect of *CYP1A2* genotypes, and their resulting caffeine metabolism rates, on exercise performance post caffeine ingestion (6 $mg \cdot kg^{-1}$) in recreationally trained male exercisers completing either a 10-km run or 40-km cycling time trial. A dose of 6 $mg \cdot kg^{-1}$ caffeine ingested 60-min prior to exercise improved exercise performance through decreasing time to completion by 1.8% ($p=0.05$; $\eta p^2=0.12$) in caffeine trials compared to placebo with a significantly faster time to completion in the second half of exercise (2.4%; $p=0.02$; $\eta p^2=0.16$) compared to the first half (1.2%). No differences in exercise performance were observed between *CYP1A2* genotypes in time to completion of the trials.

4.2 Research impact

This study adds to the research base supporting caffeine's erogenicity, and benefits in sports performance, specifically in male recreational exercisers. A novel insight from this study is the use of the AUC to analyse caffeine metabolism rates and clearance in different *CYP1A2* genotypes. To the author's knowledge AUC has not been used to analyse the caffeine metabolic rate in individuals with different *CYP1A2* polymorphisms during endurance exercise (Grzegorzewski et al., 2022; McLean et al., 2000; McLean and Graham 2002; Skinner et al., 2013; Tian et al., 2019; Tuma et al., 2024).

4.3 Study strengths and limitations

A strength of the present study includes its use of randomised, placebo controlled, double blinded study protocols to conduct the exercise trials, minimising bias from researchers involved in the study. Further, no trial order effects were identified. The research protocols were reproducible and could be extended between different modes of exercise, meaning data from the two separate trials could be combined for a greater sample size.

A limitation of this study is that the trials were performed in the laboratory, the 10-km running trial on a treadmill, and the 40-km cycling trial completed on a cycle-ergometer in controlled settings. These settings may not represent exercise completed outside or under realistic conditions for exercisers and therefore, exercise performance may differ following caffeine ingestion outside of research trials. However, this may also be considered a strength as the controlled environment controls for variables such as temperature, gradient, and wind which may impact trial outcomes. While the pooling of two studies to increase the statistical power of the research has been identified as a strength of the current study presented here, it may be that an even larger sample of a total of 46 participants (calculated using G*Power) is needed, thus a larger statistical power, to identify differences in exercise performance between those with AA, AC and CC polymorphisms in the *CYP1A2* gene following caffeine ingestion. Additionally, no participants in the present study had the CC allele polymorphism, which is present in approximately 10% of the population (Sachse et al. 1999), therefore we could not determine any effects of the CC polymorphism on caffeine metabolism and exercise performance following caffeine consumption.

4.4 Future directions

As sports supplements are regularly used within the sporting community as a tool for improving exercise performance, understanding the ideal dose, and timing of caffeine consumption is important in supplement use to optimise performance. Further, caffeine dosage and timing specific for recreational and professional athletes may differ as frequent, high intensity/duration exercise induces the upregulation of adenosine receptors within the body. Thus, once adenosine receptor numbers increase, more caffeine is required to bind to adenosine receptors to achieve the same physiological effects. Additionally, frequent, high intensity/duration exercise long-term may increase expression of the *CYP1A2* gene, resulting in the rate of caffeine metabolism increasing. Therefore, well-trained/elite-level athletes may require additional or higher caffeine doses during endurance exercise to keep their plasma caffeine concentrations raised to utilise caffeine's ergogenic benefits throughout the duration of exercise, whereas less well-trained athletes may not need this. Given these limitations, future research may look to:

- Identify the frequency of caffeine consumption for ergogenic benefits in recreational and well-trained/elite-level athletes.

- Examine common modes of delivery of caffeine in well-trained and recreationally trained athletes to identify the optimal timing of ingestion of different types of caffeine supplements (e.g., capsule, gum, drink, mouth wash).
- Compare the effect of caffeine on exercise performance between recreational and well-trained athletes to identify if there are additional benefits obtained from multiple caffeine doses within one endurance exercise period in heavy exercisers compared with light exercisers. Further, this research may identify any effects heavy exercise has on the *CYP1A2* gene's inducibility between the two cohorts.

Enzymatic activity of the cytochrome P450 enzyme has been found to be lower in females compared with males (Ou-Yang et al., 2000). Furthermore, the rate of caffeine clearance has been found to slow during the luteal phase of the menstrual cycle (Lane et al., 1992). However, it is not known if eumenorrheic females may experience differing rates of enzyme activity within the different phases of the menstrual cycle (e.g. luteal, follicular, ovulation, menstruation). As such female athletes may have varying responses to caffeine throughout the menstrual cycle (if naturally menstruating and eumenorrheic), which may affect exercise performance, but this possibility remains to be investigated. To understand the effects of the menstrual cycle on caffeine pharmacokinetics as a tool for improvement in exercise performance in female athletes, and the effect of the menstrual cycle on the *CYP1A2* gene, future research in this field may look to perform trials with a mixed sex cohort of participants to include eumenorrheic females at each stage of the menstrual cycle over 2-3 menstrual cycles. Evaluation of caffeine, caffeine metabolites, and exercise performance at each cycle stage of the menstrual cycle would provide insight into intra- and interindividual reactions to caffeine, factors inducing the *CYP1A2* gene, and effective caffeine dosing and time strategies for female athletes aiming to enhance their performance.

Caffeine plasma concentrations were higher in AA allele carriers in CAF trials compared to AC allele carriers. Further, AA allele carriers had a significantly higher plasma caffeine concentration in the first half of exercise though no differentiation in exercise performance was observed between CAF and PLA trials. Also, there was no difference in plasma caffeine concentrations between AA and AC allele carriers in the second half of exercise, although a significant difference in exercise performance was shown over this time period in CAF trials compared to PLA trials. Therefore, further research should investigate the effects of providing AA carriers and AC carriers with caffeine doses at different times (e.g. 60 min and 90 min) prior to exercise to determine if providing caffeine

at different times based on *CYP1A2* genotype provides the most ergogenic benefits to exercise. . This may also provide insight as to whether plasma caffeine concentrations have a threshold that needs to be reached in order to observe ergogenic benefits during exercise performance. As caffeine and its metabolites paraxanthine, theophylline and theobromine are all adenosine receptor antagonists, and provide ergogenic benefits in varying magnitude during exercise performance, it may be difficult to identify the impact of the *CYP1A2* genotype on exercise performance. Due to the equivocal research to date on *CYP1A2's* influence on caffeine pharmacokinetics, and the results observed in this present study in caffeine plasma concentrations and effect on HR, further research should be undertaken including a cohort of CC allele carriers of the *CYP1A2* gene and with a larger sample size of 46 participants in total (as calculated in G*Power), and thus a larger statistical power to determine if any differences in exercise performance can be identified between AA, AC, and CC allele carriers.

4.5 Conclusion

This research study was successful in meeting its two main aims of determining the effect of the ingestion of 6 $mg \cdot kg^{-1}$ caffeine prior to exercise, identifying a 1.8% improvement in recreational male athletes' performance in endurance exercise. Further, this cohort of participants experienced no difference in exercise performance based on their *CYP1A2* genotype. However, as total caffeine plasma concentrations were significantly higher in AA allele carriers over AC allele carriers of the *CYP1A2* gene, further research is warranted to repeat these research procedures with a larger sample size to provide further insight into the effect polymorphisms of the *CYP1A2* gene have on caffeine metabolism rates, and exercise performance. Recommendations for athletes consuming caffeine supplements for ergogenic reasons include trialing caffeine ingestion at differing times prior to and during exercise to identify the ideal time to consume caffeine dependent on their sport, and mode of delivery. This may attenuate inter- and intraindividual variations that influence caffeine metabolism and the pharmacodynamics of caffeine supplements in exercise performance.

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Appendices

Appendix 1 Ethics approval running trial

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

COPY FOR YOUR INFORMATION

15 April 2015

Kyle Southward
10 Elsfield Place
Torbay
AUCKLAND 0630

Dear Kyle

Re: HEC: Southern A Application – 15/12
The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes

Thank you for your letter dated 14 April 2015.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Mr Jeremy Hubbard, Acting Chair
Massey University Human Ethics Committee: Southern A

cc Dr Ajmol Ali
School of Sport & Exercise
ALBANY

Dr Kay Rutherfurd-Markwick
School of Food & Nutrition
ALBANY

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Appendix 2 Participant information sheet

The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

INFORMATION SHEET

Invitation to participate in research study

I, Kyle Southward, am a postgraduate student currently undertaking a thesis to complete a Master's degree in Sport and Exercise Science at Massey University under the supervision of Dr Ajmol Ali from the School of Sport and Exercise and Dr Kay Rutherfurd-Markwick from the School of Food and Nutrition. Dr Austen Ganley and Dr Karyn O'Keeffe will conduct the genetic assays and analyse the sleep data, respectively. Megan Wraith, Deepna Nathu, Frances Martin and Angela Tyrrell from Auckland University of Technology will be assisting with data collection.

Caffeine is the most consumed psychoactive drug in the world and has been shown to have a performance-enhancing effect when taken in the correct doses. However, the effects of caffeine intake vary between individuals and have been attributed to factors such as age, gender and more recently genetics. A gene called CYP1A2 has been linked with caffeine metabolism. This gene can occur naturally in three variations which affects the rate of caffeine metabolism. These three variations can be grouped into fast, medium or slow metabolisers of caffeine. The differences in the rate of caffeine metabolism are likely to influence exercise performance. Little research has investigated the effects of genetics and caffeine ingestion on exercise performance. This study aims to investigate the effects of genotype and caffeine ingestion on exercise performance as well as mood, sleep and immune function.

As a participant in this study you will stand to benefit from gaining knowledge of your fitness levels as well as how caffeine ingestion affects performance – both positively and negatively. This will allow you to determine if caffeine can lead to an improved exercise performance and possibly could be incorporated into your future training sessions.

Participant recruitment and involvement

Approximately 36 participants will be recruited for this study to provide sufficient statistical

power. To participate in this study you must be:

- Male.
- Between the ages of 18 – 50 years old.
- Able to run 10-km comfortably without stopping.
- Able to consume caffeine. (if you don't consume any caffeine you cannot take part in this study)
- A non-smoker.
- Pass health screening questionnaire
-

Risks/discomforts as a result of this study may include:

- Physical discomfort or muscle soreness as a result of exercise.
- Mild soreness as a result of blood sampling procedures.
- Possible impact on sleep due to caffeine consumption

Before commencing this study you will be asked to complete a health screen questionnaire and a caffeine consumption questionnaire. If you have any medical condition listed on the health screen questionnaire then you

will have to be excluded from the study. All information collected on these questionnaires is strictly confidential and will only be used for the purpose of this study.

Project procedures

Familiarisation

Before the main trials begin you will be asked to complete a familiarisation session. This is a full familiarisation session and will accustom you to the main trials. During the familiarisation session a sterile cotton bud will be used to take a swab from the inside of your cheek; this will be used to determine your CYP1A2 genotype. Participants will be placed in three different groups for the main trials based on their genotype. Height and weight will also be measured during this initial session.

You will also be shown how to complete the two-day food record diary prior to each main trial. You will be asked to record your food and beverage intake for the day before and day of the main trial; you will need to replicate the intake for the second main trial. Please refrain from consuming caffeine-containing foods and beverages (e.g. tea, coffee, energy drinks, and chocolate) during this period. Also, we ask that you come in for the main trial after observing a two-hour fast (only water intake allowed).

Furthermore, we will show you how to use the sleep diary and how to wear the Actigraph sleep monitor. We will ask you to wear the Actigraph (like a watch on your wrist) and complete the sleep diary for three days leading up to the main trial as well as three days after the 10-km time trial.

For all trials you will be asked to wear comfortable running apparel such as trainers, shorts and a shirt. Please bring a towel as showering facilities will be available.

Main trials

The main trials will consist of 2 x 3-h sessions. Each session will consist of a 10-km time trial run on a treadmill. In one of the trials you will be given a caffeine capsule (6 mg per kg body mass) and in the other you will be given a placebo (flour capsule). Base measures will be taken at the beginning of each session, followed by caffeine or placebo ingestion and pre-exercise measures. Pre and post-exercise measures include a blood sample, saliva sample, urine sample and blood pressure. Before and approximately 45-min after caffeine ingestion leg strength and jump height will be measured using an isokinetic dynamometer and jump mat. One hour after caffeine or placebo ingestion, you will begin the 10-km time trial. Throughout the trial expired heart rate will be continuously measured. At 2.5-km, 5-km, 7.5-km and 10-km a number of perceptual measures including ratings of perceived exertion (RPE), Felt Arousal Scale (FAS) and Feeling Scale (FS) measures will be taken, along with another blood sample at 5-km. One hour after exercise has ended another set of measures will be taken.

At three stages during the session (before caffeine ingestion, approximately 1 hour after caffeine ingestion, and 1 hour after exercise), you will be asked to complete a profile of moods states (POMS) questionnaire and a set of cognitive tests at the beginning of each session. You will also be asked to return one and two days after the main session so that another set of measures can be taken (blood, urine, saliva, POMS, cognitive and perceptual tests).

Individuals trained in resuscitation (NZ Red Cross First Aid, Level 2) and the use of a defibrillator will be present for all exercise sessions. In addition, the researchers will be constantly monitoring physiological and perceptual variables that will aid in identifying any issues.

Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;

- withdraw from the study up until two weeks following the data collection;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Note: As a participant you can agree to receive your genetic information. Before agreeing to this you should be aware that under New Zealand law an insurance company could ask you to disclose such information should you apply for life or health related insurance – such as medical cover. You could be obliged to disclose it even if the insurer does not ask for it expressly. Not disclosing it could result in the insurer not having to pay out under the policy.

Data Management:

All data and materials collected will be used only for this study. Hard copies will be kept in a locked filing cabinet on the Massey University Albany Campus accessible only to the researchers. Soft copies will be stored on password-protected magnetic media accessible only to the researchers. All participants will be assigned a code that will be used when collecting and presenting data. The raw results data will be kept for 10 years under the control of the researchers. Dr Ajmol Ali or another relevant member of staff from School of Sport and Exercise, and will dispose of the collected biological samples after 5 years.

The samples (blood, urine and saliva) will be collected in 2015 and, due to the number of assays being conducted, will be stored in the -80°C freezer in the Sport and Exercise Science Laboratory (B60, Oteha Rohe Campus). Samples will be analysed over a 5-year period. Any remaining samples will be destroyed in 2020 (5 years after initial collection).

Project Contacts:

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Dr Ajmol Ali (School of Sport and Exercise, Massey University)
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Frances Martin (School of Sport and Recreation, AUT)
[redacted]; [redacted]

Megan Wraith (School of Sport and Recreation, AUT)
[redacted]; [redacted]

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/12. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63487, email humanethicsoutha@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

- If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim

Appendix 3 Participant consent form

The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

PARTICIPANT CONSENT FORM - INDIVIDUAL

- I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.
- I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions.
- I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project).
- I understand that any samples (urine, saliva, blood) collected from me will only be used for this study, and that samples may be analysed over the next five (5) years.
- I agree to submit genetic material to the researcher for use only in this study. (Genetic material will not be deposited into a gene data bank.)
- I understand that if my genetic information obtained by the researcher is disclosed to me, I may have to pass this information to an insurance company should I seek life or health-related insurance cover in the future. I understand that failure to disclose the information could invalidate my insurance policy
- I agree to participate in this study under the conditions set out in the Information Sheet.

Signature:_____ Date:_____

Full name (printed):

Date of Birth:_____

Appendix 4 Pre-exercise health screening questionnaire

Pre exercise health screening questionnaire

Name:_____

Address:_____

Phone:_____

Age:_____

Please read the following questions carefully. If you have any difficulty, please advise the medical practitioner, nurse or exercise specialist who is conducting the exercise test.

Please answer all the following questions by ticking only one box per question.

This questionnaire has been designed to identify the small number of persons (15-69 years of age) for whom physical activity might be inappropriate. The questions are based on the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept. of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Qu 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

Qu 2. Do you feel pain in your chest when you do physical activity?

Yes ☐ No ☐

Qu 3. In the past month have you had any chest pain or palpitations when you were not doing physical activity?

Yes ☐ No ☐

Qu 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

Qu 5. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Yes ☐ No ☐

Qu 6. Do you have a bone or joint problem that could be made worse through vigorous exercise?

Yes ☐ No ☐

Qu 7. Do you know of any other reason why you should not do physical activity?

Yes ☐ No ☐

Qu 8. Do you have any immediate family members that had heart problems prior to the age of 60?

Yes ☐ No ☐

Qu 9. Have you been hospitalized recently?

Yes ☐ No ☐

Qu 10. Do you have any infectious disease that may be transmitted in blood?

Yes ☐ No ☐

Qu 11. This test may include the taking of blood for testing of various markers. Do you have any objection to this?

Yes ☐ No ☐

Qu 12. Have you ever been diagnosed with or suffered from sleep disorders?

Yes ☐ No ☐

Qu 13. Have you ever had any adverse reactions from consuming caffeine?

Yes ☐ No ☐

Qu 14. Do you smoke, or have you ever smoked?

Yes ☐ No ☐

Qu 15. Do you use an inhaler or corticosteroid cream?

Yes ☐ No ☐

Qu 16. Do you have celiac disease or gluten intolerance?

Yes ☐ No ☐

You should be aware that even amongst healthy persons who undertake regular physical activity there is a risk of sudden death during exercise. Though extremely rare, such cases can occur in people with an undiagnosed heart condition. If you have any reason to suspect that you may have a heart condition that will put you at risk during exercise, you should seek advice from a medical practitioner before undertaking an exercise test.

I have read, understood and completed this questionnaire.

Signature:_____ Date:_____

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2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med SCI Sport Exerc* 28(4): 468-472.

Appendix 5 Participant screening questionnaire

The effects of genetics and caffeine ingestion on exercise performance, mood, sleep and immune function in male athletes.

Participant screening questionnaire.

Name:_____

Gender:_____

Age:_____

Address:_____

_____

Phone:_____

Email:_____

Qu 1. How often do you exercise per week?

Less than1h ☐ 1h – 3h ☐

3h - 6h ☐ more than 6h ☐

Qu 2. Are you able to run 10km at a comfortable pace without stopping?

Yes ☐ No ☐

Qu 3. How often do you drink tea? (times per week/day)

_____

Qu 4. How often do you drink coffee? (times per week/day)

_____

Qu 5. What type of tea or coffee do you most often consume? (Espresso, decaf, earl grey, etc…)

_____

Qu 6. How often do you consume energy drinks? (Red bull, Monster, etc…)

_____

Qu 7. How often do you consume soft drinks? (Coca-cola, pepsi etc…)

Qu 8. Describe how caffeinated products normally affect you.

Qu 9. Do you normally eat chocolate or drink coffee, tea, soft drinks or energy drinks after 8pm?

Appendix 6 Food diary

Caffeine Study Food Diary

What to do?

- Record all that you eat and drink on the following dates.

_____

_____

_____

_____

- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.
- Include all meals, snacks, and drinks, even tap water.
- Include anything you have added to foods such as sauces, gravies,spreads, dressings, etc.
- Write down any information that might indicate size or weight of the food to identify the portion size eaten.
- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.
- Use as many pages of the booklet as you need.

Describing Food and Drink

- Provide as much detail as possible about the type of food eaten. For example **brand names and varieties / types** of food.

General description	**Food record description**
Breakfast example – cereal, milk, sugar	1 cup Sanitarium Natural Muesli 1 cup Pam's whole milk 1 tsp Chelsea white sugar
Coffee	1 tsp Gregg's instant coffee 1 x 200ml cup of water 2 Tbsp Meadow fresh light green milk
Pasta	1 cup San Remo spirals whole grain pasta (boiled)

Pie	Big Ben Classic Mince and Cheese Pie (170g)

- Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled…

General description	**Food record description**
2 eggs	2 size 7 eggs fried in 2tsp canola oil 2 size 6 eggs (soft boiled)
Fish	100g salmon (no skin) poached in 1 cup of water for 10-min

- When using foods that are cooked (eg. pasta, rice, meat, vegetables, etc), please record the **cooked portion** of food.

General description	**Food record description**
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat	90g lean T-bone steak (fat and bone removed)
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

- Please specify the **actual amount of food eaten** (eg. for leftovers, foodswhere there is waste)

General description	**Food record description**
Apple	1 x 120g Granny Smith Apple (peeled, core not eaten – core equated to ¼ of the apple)
Fried chicken drumstick	100g chicken drumstick (100g includes skin and bone); fried in 3 Tbsp Fern leaf semi-soft butter

Record recipes of home prepared dishes where possible and the proportion of the dish you ate. There are blank pages for you to add recipes or additionalinformation.

Recording the amounts of food you eat

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons and tablespoons. Eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.
- By weight marked on the packages – eg. a 425g tin of baked beans, a 32g cereal bar, 600ml Coke.
- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat, fruits, vegetables and cheese.
- For bread – describe the size of the slices of bread (eg. sandwich, medium, toast) – also include brand and variety.
- Using comparisons – eg. Meat equal to the size of a pack of cards, a scoop of ice cream equal to the size of a hen's egg.
- Use the food record instructions provided to help describe portion sizes.

General description	**Food record description**
Cheese	1 heaped tablespoon of grated cheese 1 slice cheese (8.5 x 2.5 x 2mm) 1 cube cheese, match box size Grated cheese, size 10B

- If you go out for meals, describe the food eaten in as much detail as possible.

- ***Please eat as normally as possible - don't adjust what you would normally eat just because you are keeping a diet record and be honest! Your food record will be identified with a number rather than your name.***

Example day

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**
Example 7:55am	Sanitarium weetbix	2 weetbix
" "	Anchor Blue Top milk	150ml

" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275-ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Homemade pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 rashers bacon (fat removed), 1 Tbsp chopped spring onion, 3 Tbsp mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	6 x chocolate covered Girl Guide biscuits (standard size)
6.00pm	Lasagne	½ cup cooked mince, 1 cup cooked Budget lasagne shaped pasta , ½ cup Wattie's creamy mushroom and herb pasta sauce, ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp grated Edam cheese
6.30pm	Banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing

Date_____ **DAY 1**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Appendix 7 Plasma and urine caffeine and caffeine metabolite HPLC assay

Plasma and urine caffeine and caffeine metabolite HPLC assay

Preparation of samples

1. Dispense 400 µl of 0.8 M perchloric acid into labelled Eppendorf tubes.
2. Add 400 µl of well mixed plasma/urine sample to perchloric acid.
3. Vortex for approximately 10 sec to ensure samples is properly mixed.
4. Centrifuge for 10-min at 10000 rcf (espresso microcentrifuge, Thermo Sceintific IEC, US)
5. Remove 400 µl aliquot of supernatant, careful not to suck up any precipitate, and place into glass HPLC vial.

HPLC analysis

Mobile phase A: 0.1% trifluoroacetic acid ($C_2HF_3O_2$) in water (H_2O)

Mobile phase B: 0.1% trifluoroacetic acid ($C_2HF_3O_2$) in 40% acetonitrile (C_2H_3N) Stationary phase:

Phenomenex luna

Flow rate: 0.75ml·min^{-1}

Oven temperature: 22°C

Wavelength: 274 nm

Injection volume: 20 µl

Standards: 0.0625 µl·ml^{-1}, 0.125 µl·ml^{-1}, 0.25 µl·ml^{-1}, 0.5 µl·ml^{-1}, 1 µl·ml^{-1}, 2.5 µl·ml^{-1}, 5 µl·ml^{-1}, 10 µl·ml^{-1}, 25 µl·ml^{-1}, 50 µl·ml^{-1}

Gradient method:

Table 13.1. HPLC gradient method of mobile phase concentrations A and B.Time (min)	A Concentration (%)	B Concentration (%)
0	100	0
15	65	35
20	65	35
28	50	50
30	0	100
35	0	100
41	100	0

Appendix 8 DNA extraction method from saliva

DNA extraction method from saliva

DNA extraction

- Add preservation buffer to whole saliva in 1:1 ratio (can be stored up to 28 days)
- Add 250 µl of saliva/buffer solution to 1.5-ml centrifuge tube
- Vortex sample for 10-min
- Incubate at 95 °C for 10-min
- Add 0.68 volume of 5M KAc, mix and incubate on ice for 10-min
- Centrifuge at room temperature for 10-min at 15000 x g
- Pipette supernatant into a fresh centrifuge tube and discard the pellet
- Add 1.2 volumes of room temperature 95-100% ethanol and mix by inverting the tube
- Allow the sample to stand at room temperature for 10-min
- Centrifuge the tube at 15000 x g for 2-5-min
- Carefully remove the supernatant with a pipette without disturbing the DNA pellet
- Carefully add 300 µl of 70% ethanol and vortex to mix
- Centrifuge for a further 2-5-min at 15000 x g
- Remove ethanol from centrifuge tube without disturbing DNA pellet
- Incubate tube at 90 °C for 1-min
- Add 50-100 µl of 1 x TE solution to DNA pellet and vortex for 10 sec
- Rehydrate DNA pellet by incubating at room temperature overnight followed by vortexing
- DNA can be stored at 4 °C for up to 2 months or -20 °C for long term storage

Preservation buffer solution (20ml; pH 8.7)

- 0.3M Tris HCl	6ml	Buffer
- 0.67M urea	0.804g	Denaturing agent
- 0.6% SDS	1.2ml	Denaturing agent
- 20nM EDTA	4ml	Chelating agent
- 0.67 NaOAc	4.5ml	Denaturing agent
- 0.1M Ascorbate	0.35g	Reducing agent
- H_2O	4.3ml	

Appendix 9 Saliva collection method and SOPs

Saliva collection method and standard operating procedures

Equipment:

1 x 15-ml sterile, conical centrifuge tube 2 x

large, sterile cotton bud applicator

1 x 1.5-ml Eppendorf

Gloves

Facial tissues

Centrifuge (MF-50, Hanil Science Industrial, Korea)

Scissors

Stop watch

Procedure:

- Prepare centrifuge tube and Eppendorf before-hand by cutting the Eppendorf tip and lid off using scissors.
- Place the trimmed Eppendorf tube inside the centrifuge tube.
- Research should wash hands and put gloves on
- Give cotton bud applicator to participant and have them place the buds in the mouth. (One under the tongue and one on the inside of the cheek.
- Start timer and wait for three min to allow for the buds to absorb adequate saliva.
- Asking the participant to rotate the buds may encourage better saliva absorption.
- After three min ask the participant to remove one of the buds at a time and pass it to the researcher. Tissues should be available to the participant in case of drool.
- The researcher then places the buds on top of the Eppendorf inside the centrifuge tube.
- The stalks of the cotton applicators are then cut off to allow the centrifuge tube to be sealed with a lid.
- Place centrifuge tubes in centrifuge (MF-50, Hanil Science Industrial, Korea) and centrifuge for 3-min at 3500 rpm (1328.46xg).
- Once centrifuged, remove lid and using tweezers remove both the buds and the Eppendorf from the centrifuge tube. Discard Eppendorf and cotton buds in yellow biohazard bags.
- Pipette 3 aliquots of 500 µl of saliva into Eppendorf tubes to be stored and analysed.
- Discard centrifuge tube and gloves in yellow biohazard bags