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The Effect of *CYP1A2* Gene Polymorphisms on Caffeine Pharmacokinetics and Exercise Performance in Male Recreational Athletes

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ABSTRACT

This study examined the effects of caffeine consumption on endurance exercise performance, and the influence of *CYP1A2* gene polymorphisms in caffeine pharmacokinetics and exercise performance. The data sets of two randomised, double blind, placebo-controlled crossover study design experiments have been merged. 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 of 60-min following a single dose of 6 mg·kg^{-1} caffeine (CAF) or placebo (maltodextrin; PLA) throughout which heart rate (HR) and time to completion (TTC) were measured. Caffeine ingestion improved TTC 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 TTC, 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 ($p = 0.01$). Ingesting a dose of 6 mg·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 main effects were observed in TTC; so, the role of *CYP1A2* gene polymorphisms in determining enhancements in exercise performance remains unclear.

1 | 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 et al. 2018), making caffeine a popular ergogenic aid among athletes. Caffeine's appeal has increased due to the perceptual, cognitive and 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, body composition and medications influencing the ergogenic effects on exercise performance (Cappelletti et al. 2015; Nehlig 2018; Domaszewski et al. 2025).

Physiological responses to caffeine consumption differ between individuals, with some finding caffeine enhances mental and physical performance, and others experiencing ergolytic effects (Yang et al. 2010). In some individuals, caffeine intake leads to sensations of nervousness and anxiety, restlessness, insomnia, headaches and tachycardia (Wikoff et al. 2017). Caffeine

Highlights

- A dose of 6 mg·kg^{-1} caffeine may improve exercise performance in both long-distance cycling and running in male recreational athletes.
- Participants *CYP1A2* genotype does not influence caffeine's benefits in sports performance in male recreational exercisers completing a 10-km run or 40-km cycle.
- Exercise performance was improved in the second half of exercise, supporting athletes' timing of caffeine intake 60-min before starting long distance exercise.

sensitivity and the likelihood of experiencing adverse side effects to caffeine have been attributed to polymorphisms in the *ADORA2A* gene where individuals with the *ADORA2A* TT phenotype have higher caffeine sensitivity than those with CT/CC polymorphisms (Rauf et al. 2025; Wikoff et al. 2017). The enzyme cytochrome P450 is a monooxygenase that metabolises drugs such as caffeine, which may indirectly influence exercise performance by modulating the ergogenic effects of ingested 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, contributing to differences in 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). In a recent systematic review, 4 of 17 studies found the AA allele polymorphism influenced the ergogenic effects of caffeine, with improvement in exercise performance in these athletes compared to athletes with AC or CC genotypes (Grgic et al. 2021). However, the remaining 13 studies showed no influence of the *CYP1A2* gene enhancing 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 (Zawieja et al. 2023; Minaei et al. 2022). One study found handgrip strength decreased in CC allele carriers following consumption of 5 mg·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 et al. 2011; Glaister et al. 2021; Ryan et al. 2013). 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. In tensiomyography testing of muscle fibres directly, Pakosz et al. (2024) found the optimal timing of caffeine ingestion to be 30-min, after which the muscle response to caffeine peaked. In consideration of *CYP1A2* genetic influence in caffeine pharmacokinetics, recent meta-analysis has identified no main effect in standardised mean differences between timing of caffeine ingestion and exercise performance outcomes between AA and AC phenotypes; however exercise performance in CC allele carriers were shown to improve as the timing between supplementation and exercise increases (Barreto et al. 2024).

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.

This study combines data from two separate but related experiments: a 10-km running trial and a 40-km cycling trial to increase the sample size, and therefore statistical power. A minimum sample size of 24 participants was needed to give statistical power of 0.8 with an effect size of $f = 0.25$ using ANOVA repeated measures, within–between interactions. G*Power software was used to calculate statistical power. Both experiments 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 takes approximately 1-h to complete and are average endurance distances many professional and recreational athletes complete during training and events (Cushman et al. 2014; Ransdell et al. 2009). Combining data from two different exercise modalities may be useful for building a further understanding of how recreational athletes can utilise caffeine as a tool for enhancing exercise performance as this population generally is not tied to one specific sport or exercise. Recreational athletes may choose to participate in any one or more of multiple differing exercise modalities on a given day, such as resistance exercise at a gym, or running in outside terrain. This may build a clearer picture of how individualised caffeine recommendations can be made for athletes; if a general recommendation of dosage and timing can be made for an individual, or if one needs to adapt their caffeine dose and timing based on fixed and variable endogenous and exogenous factors such as genetic phenotype, exercise type and duration chosen and fasted state. Those participating in duathlons/triathlons may also benefit from further insight due to the combination of running and cycling completed during these events.

This study aimed to determine the effect of 6 mg·kg^{-1} of caffeine intake on ~1-h time trial performance in male recreational exercisers. 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.

2 | Methods

2.1 | Participants

All participants recruited were male and identified as Tier 1: Recreationally Active (McKay et al. 2022). Participants were categorised as recreationally active by meeting the World Health Organisations guidelines for minimum activity of 150–300 min of moderate intensity activity or 75–150 min of vigorous activity per week, plus muscle strengthening activities 2 or more times per week (World Health Organisation 2020). The participants recruited from the two studies were 38 nonsmoking, recreationally active males (combined mean and SD: age = 31.9 ± 10.0 years; weight = 77.5 ± 8.0 kg).

2.2 | Study Design

The 10-km running trial and 40-km cycling trial were completed independently. Data were combined for this study to increase the sample size and statistical power. 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; Cycling trial- Southern A, Application SOA 18/44). Participants were screened for health issues and caffeine use and excluded from the study if caffeine intake was avoided, or above 4 standard cups of coffee (or equivalent) were typically consumed per day. Following the provision of an information sheet explaining the requirements, advantages and risks of the study, written consent was obtained from all participants.

Participants were required to abstain from caffeine consumption during the study, a period that spanned from the familiarisation session until 48-h following the final trial. Participants kept a food diary for 48-h prior to each trial and were asked to replicate their diet prior to each trial.

Both the running and the cycling trials used a randomised, double blind, placebo-controlled crossover study design. Participants attended three sessions, 1 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 (running study) or cycled 40-km (cycle trial) to familiarise themselves with the treadmill and ergometer, respectively. The second two sessions were either a 10-km running trial or a 40-km cycling trial. Blood samples were taken from participants in a rested state immediately prior to consumption of 6 $mg \cdot kg^{-1}$ caffeine (Fluka Sigma-Aldrich, MO, USA) or placebo (maltodextrin) in a gelatine capsule (Vegie capsules, Biobalance, New Zealand). Participants then rested for 60-min before commencing exercise. Before the commencement of each cycling trial, participants were provided with a standardised meal (5× 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 were recorded electronically on the ergometer and manually at the completion of exercise. A bolus of water measuring 2-$mL \cdot kg^{-1}$ body mass was ingested by each participant at four time intervals throughout the exercise trials: initially with the caffeine or placebo supplement, second, at 60-min post caffeine or placebo consumption, then halfway through the exercise trial and lastly at the completion of the exercise trial. Once the participants had completed the exercise trial, blood samples were repeated immediately. In total, four blood samples were taken from each participant.

2.3 | Anthropometric and Physiological Measurements

Height and body mass 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 backboard. Body mass was measured using scales accurate to 0.1 kg (AND Weighing Hv 200-KGL, Australia) without shoes or excess clothing worn. Each participant's body mass 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 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 1-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, 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 distance. Participants were able to adjust the gears during the cycling trial but were not aware of the speed at which they were cycling. Velotron data captured each participant's time, distance and speed throughout the trial.

2.4 | Blood Sampling and Analysis

Blood samples were collected by a trained phlebotomist 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. The blood samples were centrifuged (MF-50, Hanil Science Industrial, Korea) for 10-min at 1330 × *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 × *g* for 10-min, and 400-μL supernatant placed in a glass HPLC vial for analysis. Reversed phase HPLC was used to measure caffeine and metabolite concentrations in each sample.

2.5 | Saliva Sampling for DNA Analysis

A saliva sample was obtained from each participant during the familiarisation session for DNA analysis. The bud method was used (Rutherfurd-Markwick et al. 2020) with two large cotton buds (Jumbo cotton applicators 18 cm, Livingston) 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 × *g*. A 1.5-mL sample of saliva was sent to the laboratory at Massey University for DNA extraction. These DNA samples were then sent for genotyping at the Liggins Institute (Auckland University, New Zealand).

2.6 | Statistical Analysis

A Levene's test was carried out to test for homogeneity of variance of the raw data. A two-way repeated measure 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 one-way ANOVA was used to compare plasma caffeine concentrations over time between genotypes during exercise. 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: $\eta_p^2 = 0.01$, $d = 0.2$; medium effect size: $\eta_p^2 = 0.09$, $d = 0.5$; large effect size: $\eta_p^2 = 0.25$, $d = 0.8$). 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 | Results

3.1 | Study Population Demographics

Half of the 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.

3.2 | Impact of Caffeine on Time Trial Performance

The data from 34 out of 38 participants were used to conduct a paired samples *t*-test to analyse TTC in this study, with 4 outliers removed due to incomplete data collected. A main effect was seen in TTC, with a 1-min (1.8%) time improvement in performance 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 1). Mean TTC in the first half of exercise was nonsignificantly 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. Results were consistent between exercise modalities with an improvement in TTC in CAF trials compared to PLA in both cycling (CAF 70.7 ± 3.9 min vs. PLA 72.4 ± 3.0 min; $p < 0.01$; $\eta_p^2 = 0.7$) and running (CAF 50.7 ± 6.8 min vs. PLA 52.2 ± 7.6 min; $p < 0.01$; $\eta_p^2 = 0.7$). No trial order effect was detected ($p = 0.88$; $\eta_p^2 = 0.001$).

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}; $p = 0.02$; $\eta_p^2 = 0.15$).

3.3 | Caffeine and Metabolites

The difference in caffeine, paraxanthine and theophylline plasma concentrations between the CAF and PLA trials are shown in Figures 2–4.

A small but significant difference was noted between plasma caffeine concentrations at the beginning of both trials (CAF 0.20 ± 0.38 μg·ml^{-1} vs. PLA 0.13 ± 0.36 μg·ml^{-1}; $p = 0.05$). Plasma caffeine concentrations were higher for CAF treatment than PLA 60-min after ingestion and remained higher throughout the exercise trial ($p < 0.001$; $\eta_p^2 = 0.987$; Figure 2). No trial order effect was observed ($p = 0.20$; $\eta p^2 = 0.057$). Higher plasma caffeine concentrations were shown from the commencement of exercise to the end of the exercise trials in CAF trials compared to PLA ($p < 0.001$, $d = 0.79$–3.91).

Low plasma paraxanthine concentrations were observed at the beginning of both exercise trials (T1: CAF 0.30 ± 0.57 μg·ml^{-1} vs. PLA 0.26 ± 0.55 μg·ml^{-1}; $p = 0.77$). Nonsignificant 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 (Figure 3; $p = 0.002$–0.02 $d = 0.381$–0.551). There was no trial order effect ($p = 0.18$).

No difference in plasma theophylline concentrations was detected at the beginning of both trials (0.04 ± 0.03 μg·ml^{-1}; $p = 0.55$). Plasma theophylline concentrations increased in the CAF trial compared to PLA (treatment-time interaction effect

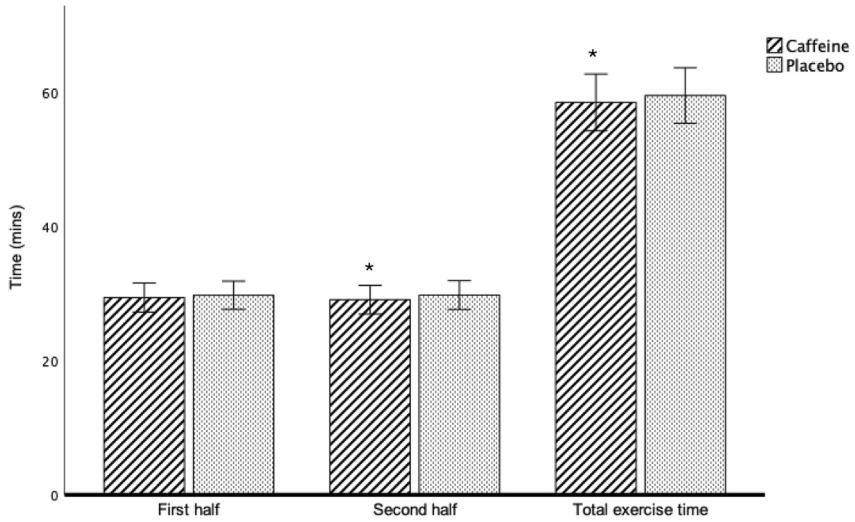

FIGURE 1 | Time to completion in CAF and PLA trials. First half indicates the mean time participants took to complete the first half of exercise. Second half indicates the mean time participants took to complete the second half of exercise. Total exercise time indicates the total mean time participants took to complete the full exercise trials. *Statistically significantly different to placebo trial at same time point ($p < 0.05$).

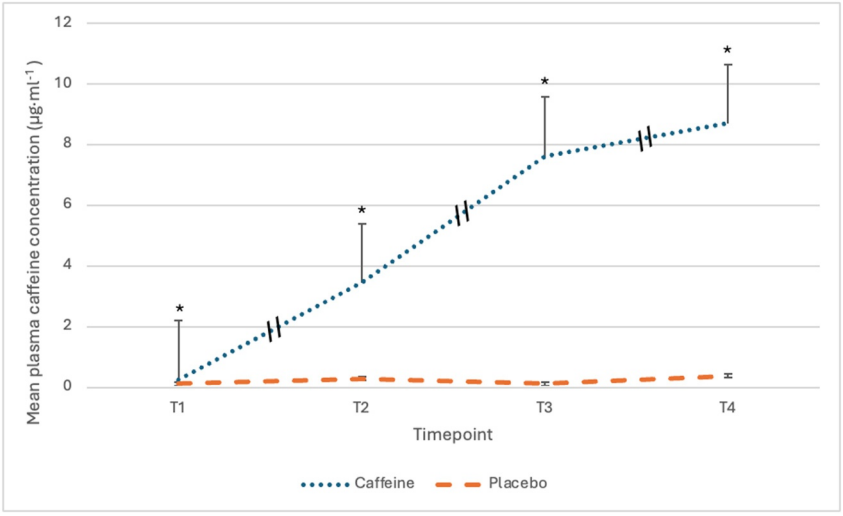

FIGURE 2 | Plasma caffeine concentrations during caffeine and placebo trials. T1 represents the time immediately prior to caffeine or placebo ingestion; T2 represents the time 60-min after caffeine or placebo ingestion and immediately before exercise; T3 represents 5-km of the running/20-km of the cycling time trial complete; T4 represents 10-km of the running/40-km of the cycling time trial complete. *Statistically significantly different to placebo trial at the same time point ($p < 0.05$).

$p < 0.001$; $\eta_p^2 = 0.78$). Timepoints 2–4 had significantly higher theophylline plasma concentrations in CAF trials compared to PLA (Figure 4; $p = 0.001$–0.006, $d = 0.481$–1.391). No treatment order effect was observed ($p = 0.14$).

3.4 | Caffeine-*CYP1A2* Gene Interaction Effects on Exercise Performance

A caffeine–gene interaction main effect was observed in plasma caffeine concentrations, with plasma caffeine concentrations higher in AA allele carriers compared with AC allele carriers ($p = 0.04$; $\eta_p^2 = 0.139$; Figure 5). A one-way ANOVA was then used to further analyse plasma caffeine concentrations in each genotype at each main time point during exercise. A main effect was observed at time point 2, the beginning of exercise, 60-min following caffeine ingestion, with significantly higher caffeine plasma concentrations in AA allele carriers compared to AC allele carriers (AA 4.73 ± 3.586 µg·ml^{-1}; vs. AC 1.564 ± 2.445 µg·ml^{-1}; $p = 0.007$; $\eta_p^2 = 0.221$; Table 1). No significant differences were observed at timepoints 1, 3 or 4.

No caffeine–gene main effects were shown in plasma paraxanthine and theophylline concentrations, or physical performance results in this study (Table 2). There was no difference in HR between genotypes ($p = 0.77$; $\eta_p^2 = 0.002$).

3.5 | Area Under the Concentration–Time Curve (AUC)

The total mean plasma caffeine concentration AUC was significantly higher in AA compared to AC allele carriers ($p = 0.01$; Figure 6), but no differences in total mean

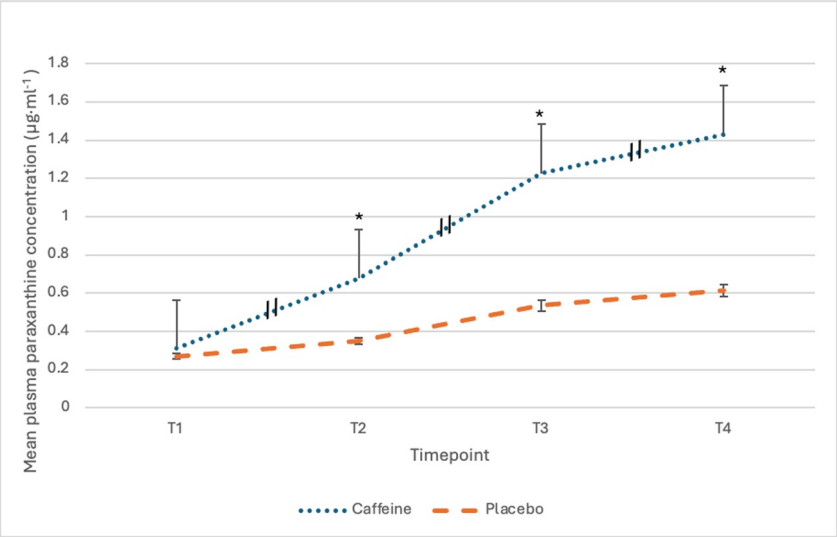

FIGURE 3 | Plasma paraxanthine concentrations during caffeine and placebo trials. T1 represents the time immediately prior to caffeine or placebo ingestion; T2 represents the time 60-min after caffeine or placebo ingestion and immediately before exercise; T3 represents 5-km of the running/20-km of the cycling time trial complete; T4 represents 10-km of the running/40-km of the cycling time trial complete. *Statistically significantly different to placebo trial at the same time point ($p < 0.05$).

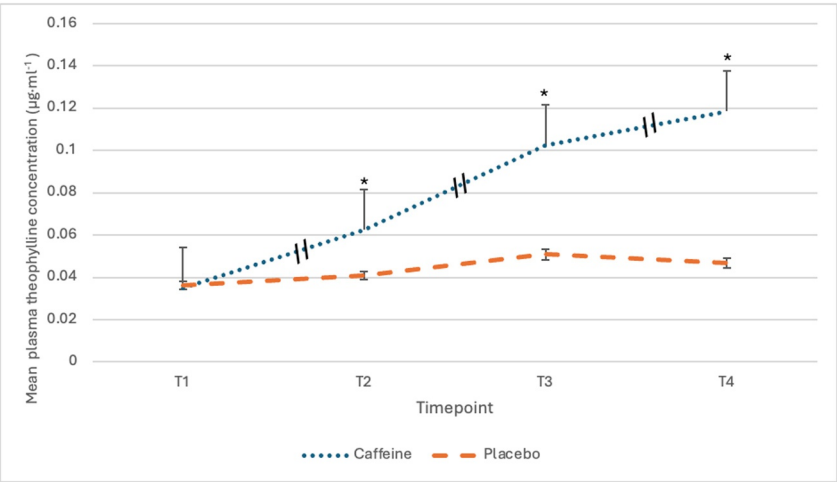

FIGURE 4 | Plasma theophylline concentrations during caffeine and placebo trials. T1 represents the time immediately prior to caffeine or placebo ingestion; T2 represents the time 60-min after caffeine or placebo ingestion and immediately before exercise; T3 represents 5-km of the running/20-km of the cycling time trial complete; T4 represents 10-km of the running/40-km of the cycling time trial complete. *Statistically significantly different to placebo trial at the same time point ($p < 0.05$).

paraxanthine ($p = 0.40$) and theophylline ($p = 0.62$) plasma concentrations were identified between genotype variants.

4 | Discussion

The first 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. The second aim was to determine if polymorphisms in the *CYP1A2* gene have an indirect effect on exercise performance by modulating caffeine metabolism. Findings of this study confirmed caffeine improved exercise performance and decreased TTC by 1.8% in ~1-h time trial. This level of improvement is within the range reported by several meta-analyses (Schubert and Astorino 2013; Southward, Rutherfurd-Markwick, Badenhorst, et al. 2018; Wang et al. 2022), adding to the evidence base for caffeine's ergogenic effects at a dose of 6 mg·kg^{-1}. Each participant's TTC 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. TTC 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 et al. 1978). A nonsignificant improvement in TTC of 1.2% was found in the first half of exercise in the CAF trial compared with PLA. However, there was an 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

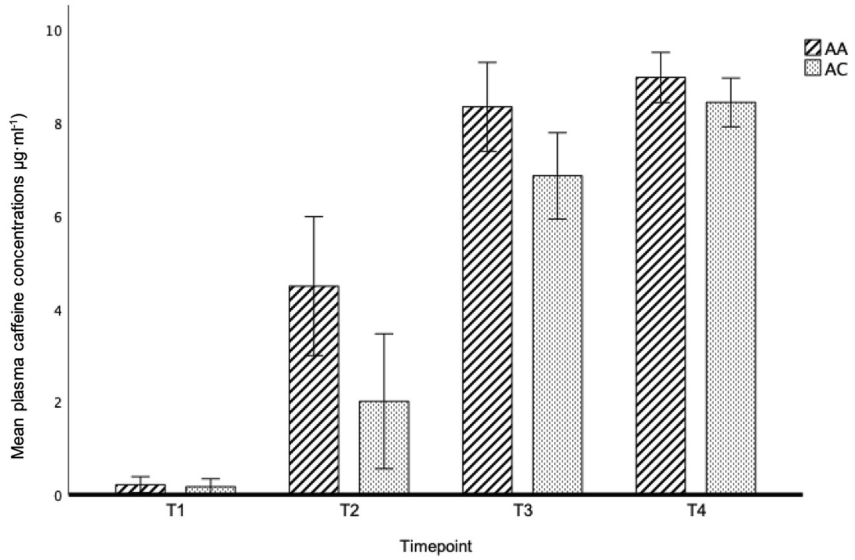

FIGURE 5 | Mean caffeine plasma concentrations in *CYP1A2* AA ('fast' metabolisers) compared with AC ('slow' metabolisers) allele carriers at different timepoints. T1 represents the time immediately prior to CAF/PLA ingestion; T2 represents the time 60-min after CAF/PLA ingestion and immediately before 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 difference between genotypes at the same timepoint ($p < 0.05$).

TABLE 1 | Caffeine plasma concentrations at time intervals by genotype.

		Mean ($\mu g \cdot ml^{-1}$)	SD	Caffeine*genotype *p*-value	Effect size (η_p^2)
T1	AA	0.239	0.308	0.756	0.003
	AC	0.196	0.448		
T2	AA	4.73	3.586	0.007	0.221
	AC	1.564	2.445		
T3	AA	7.494	2.324	0.073	0.103
	AC	5.961	2.352		
T4	AA	8.549	1.753	0.168	0.062
	AC	7.468	2.511		

Note: 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.

TABLE 2 | Interaction effect of *CYP1A2* genotype on caffeine treatment.

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

*Significant at $p < 0.05$.

et al. 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 et al. 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 et al. 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 2–4 (0.16–0.20; Table 3) during the exercise trial following caffeine ingestion, whereas increases in theophylline are small, theophylline has the highest affinity to adenosine receptors between caffeine and its metabolites (Mumford et al. 1996). This

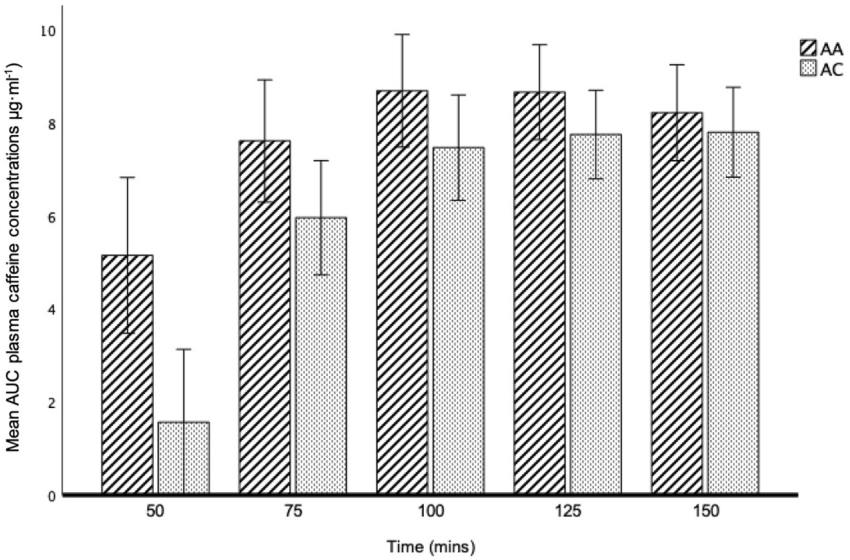

FIGURE 6 | AUC- total cumulative caffeine plasma concentrations 50–150-min following caffeine ingestion between AA ('fast' metabolisers) and AC ('slow' metabolisers) *CYP1A2* genotypes.

TABLE 3 | Metabolic ratio of paraxanthine and caffeine [paraxanthine/caffeine].

	Mean plasma concentration ($\mu g \cdot ml^{-1}$)		
Timepoint	**Caffeine**	**Paraxanthine**	**Metabolic ratio**
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

Note: 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.

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.

With a main effect observed in TTC in the second half of exercise (timepoints T3–T4), with TTC in CAF trials 2.4% faster than PLA trials, no correlating main effect in caffeine plasma concentrations was seen between AA and AC genotypes in the second half of exercise, although AA allele carriers had higher plasma caffeine levels (T3; AA CAF 7.494 ± 2.324 $\mu g \cdot ml^{-1}$ vs. AC CAF 5.961 ± 2.352 $\mu g \cdot ml^{-1}$; $p = 0.073$; $\eta_p^2 = 0.103$; Table 1). In contrast, there was no main effect in TTC in the first half of exercise, however AA allele carriers had significantly higher caffeine plasma concentrations compared to AC allele carriers (T2; AA 4.73 ± 3.586 $\mu g \cdot ml^{-1}$; vs. AC 1.564 ± 2.445 $\mu g \cdot ml^{-1}$; $p = 0.007$; $\eta_p^2 = 0.221$; Table 1), though this did not translate into improvement in exercise performance. This may be in part due to the ergogenic effects of caffeine in AA allele carriers being insufficient to provide a significant difference in exercise performance in conjunction with AC allele carriers having lower caffeine plasma concentrations and thereby potentially not yet experiencing ergogenic benefits. Additionally, lower adenosine availability in the first half of exercise (T2–T3) prior to the onset of fatigue may attenuate caffeine's ergogenic effects with lower numbers of adenosine receptors available.

Caffeine plasma concentrations were low (< 0.2 $\mu g \cdot ml^{-1}$) at timepoint 1 (prior to ingestion of supplement) in both the CAF and PLA trials. Participants were asked to abstain from caffeine-containing products for the duration of the study, from the familiarisation session to 48-h post final exercise trial. As a small volume of caffeine was detected in plasma concentrations at timepoint 1, this indicates a noncompliance to caffeine abstention or unintentional caffeine ingestion in one or more participants. 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 directly after the running and cycling trial were completed (Caffeine 8.69 ± 1.14 $\mu g \cdot ml^{-1}$; ~110-min post caffeine ingestion). In similar studies, caffeine was consumed 60-min prior to exercise to allow for its absorption and to reach maximum plasma concentrations (Graham 2001; N. S. 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 that caffeine plasma concentrations peaked 30-min post ingestion in individuals consuming anhydrous caffeine. 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 anhydrous and within liquid forms (Kamimori et al. 2002; Mumford et al. 1996; N. S. Guest et al. 2021), with all factors contributing to high inter-individual variability in caffeine plasma concentrations. A recent study by Domaszewski et al. (2025) shows that higher

percentages of body fat in individuals correlates with higher serum caffeine levels and slower metabolism of caffeine, which further contributes to interindividual variability in caffeine metabolism.

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. Although 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 prior to caffeine ingestion and 125–150-min following caffeine ingestion. Individuals with a higher paraxanthine/caffeine ratio have been reported to have a reduced perception of exertion during exercise (Whalley et al. 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 et al. 2000).

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 because they did not apply 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 TTC 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; N. 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 (Zawieja et al. 2023; Pataky et al. 2016). A recent systematic review of 19 studies found in exercise trials where a caffeine–gene main effect was seen, AA and AC allele carriers experienced ergogenic benefits in exercise following caffeine ingestion where CC allele carriers had either no improvements or adverse effects in exercise performance (Messenburger et al. 2025). Similarly, a systematic review and meta-analysis by Barreto et al. (2024) found that caffeine improved exercise performance in athletes with AA and AC polymorphisms though performance decreased in CC allele carriers. However, athletes with CC genotypes were found to benefit from higher caffeine doses compared with AA and AC genotypes and had improved exercise performance with longer timing between caffeine ingestion and commencing exercise. 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 TTC 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, heavy exercise and consuming cruciferous vegetables. Additionally, differences in study design (caffeine dose and exercise modality) may influence trial outcomes.

Mean plasma caffeine concentrations were higher at each timepoint (T2–T4) in those with AA alleles than those with the AC polymorphism ($p = 0.04$; $\eta_p^2 = 0.139$; Figure 6). Further, analysis of the AUC of mean plasma caffeine concentrations showed a significantly higher concentration in AA allele carriers compared with AC allele carriers ($p = 0.01$). However, 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 (N. S. 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; N. S. 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 (N. S. Guest et al. 2021).

4.1 | Research Limitations and Future Directions

The pooling of running and cycling data pose a limitation in this study as using two different exercise modalities may obscure the ergogenic effects of caffeine and the influence of genotype polymorphisms. Running and cycling may not be physiologically comparable in effort and intensity. As caffeine is found to delay the onset of fatigue, there may be differences in timing of the onset of fatigue in participants between exercise types due to physical exertion, which may influence results.

Male recreationally trained exercisers were the primary focus in this study, and further research may look to include well-trained/professional athletes, or female participants as the use of oral contraceptives and physiological differences between the sexes such as hormonal fluctuations during the four phases of the menstrual cycle have been shown to modify *CYP1A2* activity and metabolic rate (Nehlig 2018; Ruiz-Moreno et al. 2020). 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. To determine the extent of the *CYP1A2* genes influence in caffeine pharmacokinetics and subsequent impact on exercise performance, it will be beneficial to compare data from

individuals representing all three polymorphisms to determine any differences between and within individuals with differing polymorphisms.

Future studies may also look to compare the *ADORA2A* 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.

A further limitation is the method of screening habitual caffeine ingestion in participants by excluding those who drink more than 4 cups per day or the equivalent. The caffeine content of coffee varies considerably, with between 51 and 322 mg of caffeine found in one cup depending on type of coffee bean and how it is processed (Severini et al. 2017). Therefore, there may be considerable variation in participants' usual caffeine intake.

Caffeine metabolism is affected by many external factors such as medications, diet, recent food ingestion and body composition, all for which were not controlled for in participants in this study. Further studies may look to capture this data in participant screening or apply controls to increase data validity. The cycling exercise trials in this study included providing study participants with a small portion of food prior to exercise, whereas no food was provided in the running trial. Difference in the fasted state of participants may influence caffeine metabolism and therefore results obtained between studies.

Inclusion of VO_2peak in future work would enhance participant characterisation and comparability across studies.

5 | 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 athletes. 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. The effects of *ADORA2A* gene polymorphisms may exert influence on caffeine pharmacodynamics alongside effects from *CYP1A2* gene polymorphisms and warrant further research.

Author Contributions

C.M.: Principal researcher, research study proposal, data cleaning and analysis, results formulation and subsequent discussion, manuscript preparation. K.S.: Study design, ethics applications, volunteer recruitment, facilitation of exercise trials, data collection. K.R.-M., A.A., C.B., and M.D.: Academic supervisors who provided supervision for the research study including the design and conduct of the study and manuscript preparation.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All relevant data is presented in this manuscript.

References

Ágoston, C., R. Urbán, O. Király, M. D. Griffiths, P. J. Rogers, and Z. Demetrovics. 2018. "Why Do You Drink Caffeine? The Development of the Motives for Caffeine Consumption Questionnaire (MCCQ) and Its Relationship With Gender, Age and the Types of Caffeinated Beverages." *International Journal of Mental Health and Addiction* 16, no. 4: 981–999. https://doi.org/10.1007/s11469-017-9822-3.

Banks, N. F., P. M. Tomko, R. J. Colquhoun, T. W. D. Muddle, S. R. Emerson, and N. D. M. Jenkins. 2019. "Genetic Polymorphisms in ADORA2A and CYP1A2 Influence Caffeine's Effect on Postprandial Glycaemia." *Scientific Reports* 9, no. 1: 10532. https://doi.org/10.1038/s41598-019-46931-0.

Barcelos, R. P., F. D. Lima, N. R. Carvalho, G. Bresciani, and L. F. F. Royes. 2020. "Caffeine Effects on Systemic Metabolism, Oxidative-Inflammatory Pathways, and Exercise Performance." *Nutrition Research* 80: 1–17. https://doi.org/10.1016/j.nutres.2020.05.005.

Barreto, G., G. P. Esteves, F. Marticorena, T. N. Oliveira, J. Grgic, and B. Saunders. 2024. "Caffeine, CYP1A2 Genotype, and Exercise Performance: A Systematic Review and Meta-Analysis." *Medicine & Science in Sports & Exercise* 56, no. 2: 328–339. https://doi.org/10.1249/mss.0000000000003313.

Barreto, G., B. Grecco, P. Merola, C. E. G. Reis, B. Gualano, and B. Saunders. 2021. "Novel Insights on Caffeine Supplementation, CYP1A2 Genotype, Physiological Responses and Exercise Performance." *European Journal of Applied Physiology* 121, no. 3: 749–769. https://doi.org/10.1007/s00421-020-04571-7.

Cappelletti, S., D. Piacentino, G. Sani, and M. Aromatario. 2015. "Caffeine: Cognitive and Physical Performance Enhancer or Psychoactive Drug?" *Current Neuropharmacology* 13, no. 1: 71–88. https://doi.org/10.2174/1570159x13666141210215655.

Carswell, A. T., K. Howland, B. Martinez-Gonzalez, P. Baron, and G. Davison. 2020. "The Effect of Caffeine on Cognitive Performance Is Influenced by CYP1A2 But Not ADORA2A Genotype, Yet Neither Genotype Affects Exercise Performance in Healthy Adults." *European Journal of Applied Physiology* 120, no. 7: 1495–1508. https://doi.org/10.1007/s00421-020-04384-8.

Costill, D. L., G. P. Dalsky, and W. J. Fink. 1978. "Effects of Caffeine Ingestion on Metabolism and Exercise Performance." *Medicine & Science in Sports* 10, no. 3: 155–158. https://pubmed.ncbi.nlm.nih.gov/723503/.

Cushman, D. M., M. Markert, and M. Rho. 2014. "Performance Trends in Large 10-km Road Running Races in the United States." *Journal of Strength & Conditioning Research* 28, no. 4: 892–901. https://doi.org/10.1519/jsc.0000000000000249.

Daly, J. W. 1982. "Adenosine Receptors: Targets for Future Drugs." *Journal of Medicinal Chemistry* 25, no. 3: 197–207. https://doi.org/10.1021/jm00345a001.

Davis, J. M., Z. Zhao, H. S. Stock, K. A. Mehl, J. Buggy, and G. A. Hand. 2003. "Central Nervous System Effects of Caffeine and Adenosine on Fatigue." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 284, no. 2: R399–R404. https://doi.org/10.1152/ajpregu.00386.2002.

Del Coso, J., G. Muñoz, and J. Muñoz-Guerra. 2011. "Prevalence of Caffeine Use in Elite Athletes Following Its Removal From the World Anti-Doping Agency List of Banned Substances." *Applied Physiology Nutrition and Metabolism* 36, no. 4: 555–561. https://doi.org/10.1139/h11-052.

Domaszewski, P., M. Konieczny, P. Pakosz, J. Matuska, E. Skorupska, and M. M. Santafé. 2025. "Obesity as an Influencing Factor for the Occurrence of Caffeine-Induced Effects in Women." *Nutrition, Metabolism, and Cardiovascular Diseases: Nutrition, Metabolism, and Cardiovascular Diseases* 35, no. 4: 103836. https://doi.org/10.1016/j.numecd.2024.103836.

Figueiredo, N., M. Queiroz, F. P. Felício, et al. 2021. "Acute Caffeine Mouth Rinsing Does Not Improve 10-km Running Performance in CYP1A2 C-Allele Carriers, Clinical Nutrition ESPEN, Acute Caffeine Mouth Rinsing Does Not Improve 10-km Running Performance in CYP1A2 C-Allele Carriers." *Clinical Nutrition ESPEN* 42: 93–97. https://doi.org/10.1016/j.clnesp.2021.02.012.

Furge, L. L., and K. J. Fletke. 2007. "HPLC Determination of Caffeine and Paraxanthine in Urine: An Assay for Cytochrome P450 1A2 Activity." *Biochemistry and Molecular Biology Education* 35, no. 2: 138–144. https://doi.org/10.1002/bmb.28.

Glaister, M., K. Chopra, A. L. Pereira De Sena, C. Sternbach, L. Morina, and Y. Mavrommatis. 2021. "Caffeine, Exercise Physiology, and Time-Trial Performance: No Effect of *ADORA2A* or *CYP1A2* Genotypes." *Applied Physiology Nutrition and Metabolism* 46, no. 6: 541–551. https://doi.org/10.1139/apnm-2020-0551.

Graham, T. E. 2001. "Caffeine and Exercise: Metabolism, Endurance and Performance." *Sports Medicine* 31, no. 11: 785–807. https://doi.org/10.2165/00007256-200131110-00002.

Greer, F., D. Friars, and T. E. Graham. 2000. "Comparison of Caffeine and Theophylline Ingestion: Exercise Metabolism and Endurance." *Journal of Applied Physiology* 89, no. 5: 1837–1844. https://doi.org/10.1152/jappl.2000.89.5.1837.

Grgic, J., C. Pickering, D. J. Bishop, B. J. Schoenfeld, P. Mikulic, and Z. Pedisic. 2020. "CYP1A2 Genotype and Acute Effects of Caffeine on Resistance Exercise, Jumping, and Sprinting Performance." *Journal of the International Society of Sports Nutrition* 17, no. 1: 21. https://doi.org/10.1186/s12970-020-00349-6.

Grgic, J., C. Pickering, J. Del Coso, B. J. Schoenfeld, and P. Mikulic. 2021. "CYP1A2 Genotype and Acute Ergogenic Effects of Caffeine Intake on Exercise Performance: A Systematic Review." *European Journal of Nutrition* 60, no. 3: 1181–1195. https://doi.org/10.1007/s00394-020-02427-6.

Grgic, J., E. T. Trexler, B. Lazinica, and Z. Pedisic. 2018. "Effects of Caffeine Intake on Muscle Strength and Power: A Systematic Review and Meta-Analysis." *Journal of the International Society of Sports Nutrition* 15, no. 1: 11. https://doi.org/10.1186/s12970-018-0216-0.

Guest, N., P. Corey, J. Vescovi, and A. El-Sohemy. 2018. "Caffeine, CYP1A2 Genotype, and Endurance Performance in Athletes." *Medicine & Science in Sports & Exercise* 50, no. 8: 1570–1578. https://doi.org/10.1249/mss.0000000000001596.

Guest, N. S., T. A. Van Dusseldorp, M. T. Nelson, et al. 2021. "International Society of Sports Nutrition Position Stand: Caffeine and Exercise Performance." *Journal of the International Society of Sports Nutrition* 18, no. 1: 1. https://doi.org/10.1186/s12970-020-00383-4.

Jodynis-Liebert, J., J. Flieger, A. Matuszewska, and J. Juszczyk. 2004. "Serum Metabolite/Caffeine Ratios as a Test for Liver Function." *Journal of Clinical Pharmacology* 44, no. 4: 338–347. https://doi.org/10.1177/0091270004263468.

Kamimori, G. H., C. S. Karyekar, R. Otterstetter, et al. 2002. "The Rate of Absorption and Relative Bioavailability of Caffeine Administered in Chewing Gum Versus Capsules to Normal Healthy Volunteers." *International Journal of Pharmaceutics* 234, no. 1–2: 159–167. https://doi.org/10.1016/s0378-5173(01)00958-9.

Lajin, B., N. Schweighofer, W. Goessler, and B. Obermayer-Pietsch. 2021. "The Determination of the Paraxanthine/Caffeine Ratio as a Metabolic Biomarker for CYP1A2 Activity in Various Human Matrices by UHPLC-ESIMS/MS." *Talanta* 234: 122658. https://doi.org/10.1016/j.talanta.2021.122658.

McKay, A. K. A., T. Stellingwerff, E. S. Smith, et al. 2022. "Defining Training and Performance Caliber: A Participant Classification Framework." *International Journal of Sports Physiology and Performance* 17, no. 2: 317–331. https://doi.org/10.1123/ijspp.2021-0451.

Messenburger, G., F. Núñez, O. Porepp, et al. 2025. "Influence of the CYP1A2 Genotype on the Exercise Performance of Physically Active Individuals Under Caffeine Supplementation: A Systematic Review." *Nutrition Research* 144: 16–31. https://doi.org/10.1016/j.nutres.2025.10.005.

Minaei, S., M. R. Rahimi, H. Mohammadi, et al. 2022. "CYP1A2 Genotype Polymorphism Influences the Effect of Caffeine on Anaerobic Performance in Trained Males." *International Journal of Sport Nutrition and Exercise Metabolism* 32, no. 1: 16–21. https://doi.org/10.1123/ijsnem.2021-0090.

Mumford, G. K., N. L. Benowitz, S. M. Evans, et al. 1996. "Absorption Rate of Methylxanthines Following Capsules, Cola and Chocolate." *European Journal of Clinical Pharmacology* 51, no. 3–4: 319–325. https://doi.org/10.1007/s002280050205.

Nehlig, A. 2018. "Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption." *Pharmacological Reviews* 70, no. 2: 384–411. https://doi.org/10.1124/pr.117.014407.

Nelson, D. R., D. C. Zeldin, S. M. Hoffman, L. J. Maltais, H. M. Wain, and D. W. Nebert. 2004. "Comparison of Cytochrome P450 (CYP) Genes From the Mouse and Human Genomes, Including Nomenclature Recommendations for Genes, Pseudogenes and alternative-splice Variants." *Pharmacogenetics* 14, no. 1: 1–18. https://doi.org/10.1097/00008571-200401000-00001.

Pakosz, P., M. Konieczny, P. Domaszewski, et al. 2024. "Muscle Contraction Time After Caffeine Intake Is Faster After 30 Minutes Than After 60 Minutes." *Journal of the International Society of Sports Nutrition* 21, no. 1: 2306295. https://doi.org/10.1080/15502783.2024.2306295.

Pataky, M. W., C. J. Womack, M. J. Saunders, et al. 2016. "Caffeine and 3-km Cycling Performance: Effects of Mouth Rinsing, Genotype, and Time of Day." *Scandinavian Journal of Medicine & Science in Sports* 26, no. 6: 613–619. https://doi.org/10.1111/sms.12501.

Rahimi, R. 2019. "The Effect of CYP1A2 Genotype on the Ergogenic Properties of Caffeine During Resistance Exercise: A Randomized, Double-Blind, Placebo-Controlled, Crossover Study." *Irish Journal of Medical Science* 188, no. 1: 337–345. https://doi.org/10.1007/s11845-018-1780-7.

Ransdell, L. B., J. Vener, and J. Huberty. 2009. "Masters Athletes: An Analysis of Running, Swimming and Cycling Performance by Age and Gender." Supplement, *Journal of Exercise Science and Fitness* 7, no. 2: S61–S73. https://doi.org/10.1016/s1728-869x(09)60024-1.

Rauf, M. Q., L. Sharma, E. Essiet, et al. 2025. "Caffeine Consumption Patterns, Health Impacts, and Media Influence: A Narrative Review." *Cureus* 17, no. 6: e86215. https://doi.org/10.7759/cureus.86215.

Ruiz-Moreno, C., B. Lara, J. J. Salinero, D. Brito de Souza, J. M. Ordovás, and J. Del Coso. 2020. "Time Course of Tolerance to Adverse Effects

Associated With the Ingestion of a Moderate Dose of Caffeine." *European Journal of Nutrition* 59, no. 7: 3293–3302. https://doi.org/10.1007/s00394-019-02167-2.

Rutherfurd-Markwick, K., C. Starck, D. K. Dulsen, and A. Ali. 2020. "Comparison of Three Salivary Collection Methods to Assess Physiological Markers." *Science Repository*.

Ryan, E. J., C. H. Kim, E. J. Fickes, et al. 2013. "Caffeine Gum and Cycling Performance: A Timing Study." *Journal of Strength & Conditioning Research* 27, no. 1: 259–264. https://doi.org/10.1519/jsc.0b013e3182541d03.

Sachse, C., J. Brockmöller, S. Bauer, and I. Roots. 1999. "Functional Significance of a C→A Polymorphism in Intron 1 of the Cytochrome P450 CYP1A2 Gene Tested With Caffeine." *British Journal of Clinical Pharmacology* 47, no. 4: 445–449. https://doi.org/10.1046/j.1365-2125.1999.00898.x.

Salinero, J. J., B. Lara, D. Ruiz-Vicente, et al. 2017. "CYP1A2 Genotype Variations Do Not Modify the Benefits and Drawbacks of Caffeine During Exercise: A Pilot Study." *Nutrients* 9, no. 3: 269. https://doi.org/10.3390/nu9030269.

Schubert, M. M., and T. A. Astorino. 2013. "A Systematic Review of the Efficacy of Ergogenic Aids for Improving Running Performance." *Journal of Strength & Conditioning Research* 27, no. 6: 1699–1707. https://doi.org/10.1519/jsc.0b013e31826cad24.

Seepika, N., S. Chinapong, P. Nunchanok, et al. 2022. "The Effects of Different Dosing Strategies of Caffeine Ingestion During an Endurance Performance Event in Male Half Marathon Runners." *Journal of Physical Education & Sport* 22, no. 9: 2173–2181. https://doi.org/10.7752/jpes.2022.09277.

Severini, C., A. Derossi, I. Ricci, A. Fiore, and R. Caporizzi. 2017. "How Much Caffeine in Coffee Cup? Effects of Processing Operations." *Extraction Methods and Variables*. https://doi.org/10.5772/intechopen.69002.

Shirley, K. L., Y. Y. Hon, S. R. Penzak, Y. W. Lam, V. Spratlin, and M. W. Jann. 2003. "Correlation of Cytochrome P450 (CYP) 1A2 Activity Using Caffeine Phenotyping and Olanzapine Disposition in Healthy Volunteers." *Neuropsychopharmacology*28, no. 5: 961–966. https://doi.org/10.1038/sj.npp.1300123.

Sicova, M., N. S. Guest, P. N. Tyrrell, and A. El-Sohemy. 2021. "Caffeine, Genetic Variation and Anaerobic Performance in Male Athletes: A Randomized Controlled Trial." *European Journal of Applied Physiology* 121, no. 12: 3499–3513. https://doi.org/10.1007/s00421-021-04799-x.

Southward, K., K. Rutherfurd-Markwick, C. Badenhorst, and A. Ali. 2018. "The Role of Genetics in Moderating the Inter-Individual Differences in the Ergogenicity of Caffeine." *Nutrients* 10, no. 10: 1352. https://doi.org/10.3390/nu10101352.

Southward, K., K. J. Rutherfurd-Markwick, and A. Ali. 2018. "Correction to: The Effect of Acute Caffeine Ingestion on Endurance Performance: A Systematic Review and Meta-Analysis." *Sports Medicine* 48, no. 10: 2425–2441. https://doi.org/10.1007/s40279-018-0967-4.

Spineli, H., M. P. Pinto, B. P. Dos Santos, et al. 2020. "Caffeine Improves Various Aspects of Athletic Performance in Adolescents Independent of Their 163 C > A CYP1A2 Genotypes." *Scandinavian Journal of Medicine & Science in Sports* 30, no. 10: 1869–1877. https://doi.org/10.1111/sms.13749.

Tian, D. D., S. Natesan, J. R. White Jr, and M. F. Paine. 2019. "Effects of Common CYP1A2 Genotypes and Other Key Factors on Intraindividual Variation in the Caffeine Metabolic Ratio: An Exploratory Analysis." *Clinical and Translational Science* 12, no. 1: 39–46. https://doi.org/10.1111/cts.12598.

Wang, Z., B. Qiu, J. Gao, and J. Del Coso. 2022. "Effects of Caffeine Intake on Endurance Running Performance and Time to Exhaustion: A Systematic Review and Meta-Analysis." *Nutrients* 15, no. 1: 148. https://doi.org/10.3390/nu15010148.

Whalley, P., C. Paton, and C. G. Dearing. 2021. "Caffeine Metabolites Are Associated With Different Forms of Caffeine Supplementation and With Perceived Exertion During Endurance Exercise." *Biology of Sport* 38, no. 2: 261–267. https://doi.org/10.5114/biolsport.2020.98455.

Wikoff, D., B. T. Welsh, R. Henderson, et al. 2017. "Systematic Review of the Potential Adverse Effects of Caffeine Consumption in Healthy Adults, Pregnant Women, Adolescents, and Children." *Food and Chemical Toxicology*109, no. Pt 1: 585–648. https://doi.org/10.1016/j.fct.2017.04.002.

Wong, O., K. Marshall, M. Sicova, N. S. Guest, B. García-Bailo, and A. El-Sohemy. 2021. "CYP1A2 Genotype Modifies the Effects of Caffeine Compared With Placebo on Muscle Strength in Competitive Male Athletes." *International Journal of Sport Nutrition and Exercise Metabolism* 31, no. 5: 420–426. https://doi.org/10.1123/ijsnem.2020-0395.

World Health Organisation. 2020. *World Health Organisation Guidelines on Physical Activity and Sedentary Behaviour*. World Health Organisation.

Yang, A., A. A. Palmer, and H. de Wit. 2010. "Genetics of Caffeine Consumption and Responses to Caffeine." *Psychopharmacology* 211, no. 3: 245–257. https://doi.org/10.1007/s00213-010-1900-1.

Zawieja, E., A. Chmurzynska, J. Anioła, B. Zawieja, and J. Cholewa. 2023. "The Effect of Caffeine Supplementation on Resistance and Jumping Exercise: The Interaction With *CYP1A2* and *ADORA2A* Genotypes." *Nutraceuticals* 3, no. 2: 274–289. https://doi.org/10.3390/nutraceuticals3020022.