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ORIGINAL ARTICLE

Caffeine, genetic variation and anaerobic performance in male athletes: a randomized controlled trial

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Abstract

Purpose The effect of caffeine on anaerobic performance is unclear and may differ depending on an individual's genetics. The goal of this study was to determine whether caffeine influences anaerobic performance in a 30 s Wingate test, and if 14 single nucleotide polymorphisms (SNPs) in nine genes, associated with caffeine metabolism or response, modify caffeine's effects.

Methods Competitive male athletes ($N = 100$; 25 ± 4 years) completed the Wingate under three conditions: 0, 2, or 4 mg of caffeine per kg of body mass (mg kg^{-1}), using a double-blinded, placebo-controlled design. Using saliva samples, participants were genotyped for the 14 SNPs. The outcomes were peak power (Watts [W]), average power (Watts [W]), and fatigue index (%).

Results There was no main effect of caffeine on Wingate outcomes. One significant caffeine–gene interaction was observed for *CYP1A2* (rs762551, $p = 0.004$) on average power. However, post hoc analysis showed no difference in caffeine's effects within *CYP1A2* genotypes for average power performance. No significant caffeine–gene interactions were observed for the remaining SNPs on peak power, average power and fatigue index.

Conclusion Caffeine had no effect on anaerobic performance and variations in several genes did not modify any effects of caffeine.

Trial registration This study was registered with clinicaltrials.gov (NCT02109783).

Keywords Caffeine · Genetics · Anaerobic · Exercise · Nutrigenomics · Wingate

Abbreviations

ANOVA	Analysis of variance
ADRβ2	β2 Adrenergic
COMT	Catechol-O-methyltransferase
CNS	Central nervous system
CYP1A2	Cytochrome P450 1A2
VO_{2peak}	Maximal aerobic capacity
rpm	Revolutions per minute
SNPs	Single nucleotide polymorphisms
VSM	Vascular smooth muscle
W	Watts
WAnT	Wingate anaerobic test

Introduction

Caffeine is one of the most commonly consumed supplements by athletes seeking to improve their athletic performance (Aguilar-Navarro et al. 2019; Burke 2008). The current recommendation for the use of caffeine across most sports is a dose of 3–6 mg kg^{-1}, approximately 60 min prior to exertion (Burke 2008; Guest et al. 2021). However, numerous studies have shown that the effect of caffeine as an ergogenic aid varies between individuals and across sport disciplines (Guest et al. 2018; Guest et al. 2021; Paton et al. 2015; Woolf et al. 2008). For example, only 13 of 20 cyclists improved their performance with ~ 3–4 mg kg^{-1} of caffeine, while the remaining participants experienced no change or worsened their performance with caffeine use (Paton et al.

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2015). Similarly, 5 mg kg^{-1} of caffeine improved overall peak power performance in 14 (78%) of the participants, on the Wingate anaerobic test (WAnT) in athletes engaging in ≥ 12 h per week of programmed physical activity (Woolf et al. 2008). Conversely, there was no overall improvement in average power or fatigue index on the WAnT, despite 13 (72%) and 9 (50%) of the participants, respectively, improving their performance under caffeine conditions. Inter-individual differences in caffeine metabolism or response might explain the inconsistent effects of caffeine across individuals engaging in anaerobic exercise. Previously, it has been reported that variability in the *CYP1A2* (rs762551) gene modifies endurance performance in competitive male athletes (Guest et al. 2018; Womack et al. 2012). Caffeine at both a 2 and 4 mg kg^{-1} dose improved performance by 4.8% and 6.8%, respectively, compared to placebo, in a 10 km cycling time trial, but only in those with the *CYP1A2* AA genotype (fast caffeine metabolizers) (Guest et al. 2018). Caffeine did not modify performance in those with the AC genotype (slow caffeine metabolizers) and worsened performance by 13.7% at the 4 mg kg^{-1} dose, compared to placebo, in those with the CC genotype (ultraslow metabolizers). The results of studies assessing the effect of caffeine on anaerobic performance have been inconsistent (Collomp et al. 1991; Grgic et al. 2020; Miyagi et al. 2018; San Juan et al. 2019; Skinner et al. 2010; Woolf et al. 2008) and could also be partly due to individual genetic differences (Guest et al. 2018; Womack et al. 2012; Woolf et al. 2008). For example, 6 mg kg^{-1} of caffeine improved peak and average power by 6.8% and 5.2%, respectively, compared to placebo, in eight male athletes who were members of the Spanish National Olympic Team (San Juan et al. 2019). Conversely, 6 mg kg^{-1} of caffeine did not affect performance on an anaerobic capacity test in 14 male cyclists (Miyagi et al. 2018). Similar to studies reporting on caffeine and endurance performance, the variable effects of caffeine on anaerobic performance might also be due to variability in *CYP1A2* (Guest et al. 2018; Womack et al. 2012), and other genes that have been associated with caffeine metabolism or response (Grgic et al. 2020; Guest et al. 2020).

In addition to *CYP1A2*, other genetic variants have been reported to modify caffeine's effects on athletic performance, such as *ADORA2A* (rs5751876) (Grgic et al. 2020), which encodes for adenosine 2a (A2a) receptors (Nardin et al. 2020), and *HTR2A* (rs6313) (Guest et al. 2020), which encodes for serotonin 2A (5-HT2A) receptors (Zhang and Stackman 2015). Those with the CC genotype of *ADORA2A* (rs5751876) improved WAnT performance with caffeine compared to placebo (Grgic et al. 2020). However, the study's sample size was small ($N = 20$) and not designed to determine differences in performance between genotypes after caffeine ingestion. The present study has a larger sample size ($N = 100$) and possesses all possible genotypes in *ADORA2A* (rs5751876). The effect of caffeine by *CYP1A2* genotype has been shown to vary across athletic disciplines (Guest et al. 2018; Salinero et al. 2017; Smith et al. 2019; Womack et al. 2012). Individuals with the AA genotype improved their endurance performance in response to caffeine in both 40 km (Womack et al. 2012) and 10 km (Guest et al. 2018) cycling time trials. Caffeine did not modify WAnT performance in those with the AA genotype of *CYP1A2* (rs762551), but improved performance in C-allele carriers (Salinero et al. 2017). However, this study grouped C-allele carriers together, which may have masked potential differences, as the two genotypes display differences in the rate of caffeine metabolism (Djordjevic et al. 2008; Ghotbi et al. 2007). The present study did not combine those with the AC and CC genotype so that any differences in performance between these groups could be determined. In addition to *ADORA2A* and *CYP1A2*, there are other genes that might influence anaerobic performance in response to caffeine.

Variability in *ADRβ2* may modify caffeine's psychological or physiological effects (Pickering and Grgic 2019). The *ADRβ2* gene encodes for β2 adrenergic (ADRβ2) receptors (Reihsaus et al. 1993) that respond to catecholamines such as epinephrine (Insel 1996), which are increased in the plasma following caffeine intake (Robertson et al. 1978; Yamada et al. 1989). Activation of ADRβ2 receptors results in increased cardiac output and bronchodilation (Dishy et al. 2001; Insel 1996; Snyder et al. 2008; Wilkins et al. 2008). This response leads to increased transport of oxygen and nutrients to exercising muscle, and waste products such as carbon dioxide, away from exercising muscle (Burton et al. 2004; Duncker and Bache 2008; Snyder et al. 2008). Additionally, compared to those with the GG genotype, individuals who are A-allele carriers of *ADRβ2* (rs1042713) are more likely to experience increased feelings of anxiety after caffeine ingestion (Day Tasevski et al. 2009), which is associated with impaired athletic performance (Englert and Bertrams 2012). In addition to feelings of anxiety, increased caffeinated coffee consumption has been associated with increased blood pressure in those who are T-allele carriers of *CYP1A1* (rs2470893), a gene that encodes for the cytochrome P450 1A1 (CYP1A1) enzyme (Amin et al. 2012), compared to those with the CC genotype (Amin et al. 2012). Caffeine also increases the concentration of plasma catecholamines, such as dopamine and epinephrine (Robertson et al. 1978), and an individual's response to catecholamines may vary depending on genetic variants that influence catecholamine metabolism or response (Gellekink et al. 2007; Muszkat et al. 2010). For example, individuals with the CC genotype in *ADRA2β* (rs4907299), a gene that encodes for α2B adrenergic (ADRA2B) receptors (Muszkat et al. 2010), are more likely to experience vasoconstriction compared to A-allele carriers in response to catecholamines

binding to α2 adrenergic receptors (Muszkat et al. 2010). ADRA2B receptors are a class of adrenergic receptors (Bylund et al. 1992), which are stimulated by epinephrine, norepinephrine and other catecholamines (Giovannitti et al. 2015), resulting in reduced coronary blood flow (Baumgart et al. 1999). Specifically, ADRA2B receptors possess greater affinity for epinephrine (Snyder et al. 2008). Additionally, catecholamine metabolism can vary based on an individual's genotype (Gellekink et al. 2007). The catechol-O-methyltransferase (COMT) enzyme, which is encoded by the *COMT* gene, is responsible for the breakdown of catecholamines (Kamal and Lappin 2019).Those who are A-allele carriers of *COMT* (rs4680) possess decreased enzymatic activity of COMT, leading to increased circulating catecholamines (Gellekink et al. 2007). Therefore, genetic variation associated with caffeine metabolism or response varies and may modify the effect of caffeine on anaerobic performance.

The purpose of this study was to determine the effects of 2 mg kg^{-1} and 4 mg kg^{-1} of caffeine on anaerobic performance as measured by the WAnT, and if 14 single nucleotide polymorphisms (SNPs) in nine genes associated with caffeine metabolism or response to caffeine modify caffeine's effects on the WAnT. We hypothesized that genetic variability in caffeine metabolism or individual response to caffeine would modify caffeine's effects on the WAnT.

Methods

Participants and recruitment

Recruitment and consent methods have been previously described in a study that examined the effect of caffeine and *CYP1A2* genotype on endurance performance in the same population (Guest et al. 2018). This trial was approved by the University of Toronto Institutional Review Board and registered with clinicaltrials.gov (NCT02109783). This study recruited 113 competitive male athletes from endurance (e.g., triathlon and cross-country skiing), power (e.g., boxing and powerlifting), and mixed (e.g., soccer and basketball) sports. Athletes were required to be training for ≥ 8 h per week, 9 out of 12 months of the year, for at least 3 years in their primary sport. Participants were excluded if they possessed a medical condition that required them to avoid caffeine, were currently injured, or were unable to abstain from caffeine for the duration of the study (4 weeks). Participants were instructed to abstain from caffeine for this duration to ensure that any effects are not due to a reversal of withdrawal symptoms, which would be the case if they abstained for only 24–48 h. Three athletes dropped out due to a sports-related injury, two due to school/work demands, two due to objection to abstaining from caffeine, and one due to relocation. Four participants were excluded due to incomplete data. One participant was excluded from the peak power analysis due to an implausible performance. This participant achieved a peak power of 2100 W, which was 26 Watts per kilogram of their body mass (W/kg) for their relative peak power. Previous reports have indicated that athletes who achieve a relative peak power > 13 W/kg are in the 90th percentile of this performance outcome (Zupan et al. 2009). Furthermore, a relative peak power of 21 W/kg is common in world class sprint cyclists, and the maximum value that has been reported is 24.8 W/kg (Gardner et al. 2005). Since this participant exceeded the maximum reported relative peak power, and their peak power performances on the other visits differed by over 1000 W and 13 W/kg, this participant was excluded from the peak power analysis. The remaining 100 athletes had a mean ± SD age of 25 ± 4 years and weighed 81 ± 12.4 kg.

Experimental design

A randomized, double-blinded, placebo-controlled design was implemented. Participants completed four visits (~ 90–120 min each), approximately 1 week apart, in the exercise laboratory at the Goldring Centre for High Performance Sport at the University of Toronto. The first visit consisted of obtaining anthropometric measurements, completing a maximal aerobic capacity test (VO_{2peak}), and answering a general health, caffeine, and sports history questionnaire. Saliva samples were collected for DNA isolation, and samples were subsequently genotyped for the 14 SNPs in nine genes associated with caffeine metabolism or response. Athletes were asked to maintain their regular sleeping and eating habits, avoid strenuous activity 48 h before each visit, and abstain from caffeine 1 week prior to the initial visit and for the remainder of the study (4 weeks total). The participants were instructed to replicate the meal they consumed prior to the first visit as closely as possible for subsequent visits, to maintain dietary consistency. On visits 2–4, participants were randomly assigned to ingest capsules that contained either 0, 2, or 4 mg kg^{-1} of caffeine (A&C American Chemicals Ltd., Saint-Laurent, Quebec, Canada). A laboratory member, not associated with the study, created the randomization protocol through randomization.com and was solely responsible for all documentation and "capsule envelope" preparation. All ledgers and documentation were stored securely and only accessible by this one individual. The envelopes for treatment visits were labeled Visit 1, 2 or 3 and the contents were unknown to all parties. The envelopes were prepared and picked up twice weekly from the location of preparation on an ongoing basis as participants signed up for the study. Envelopes were kept sealed and securely locked until opened by the participant at the time of ingestion. Each envelope contained three identical capsules equal to 2 or 4 mg kg^{-1} of caffeine or placebo (dextrose).

The athletes sat quietly for 25 min after capsule ingestion, completing their questionnaires, or using e-devices (smart phones, laptops or tablets). Blood pressure and heart rate (Polar Electro Inc., Bethpage, NY) were measured 3 and 20 min after capsule ingestion, as well as prior to commencing the warm-up routine as previously described (Guest et al. 2018). This was repeated three times, once for each treatment (0, 2, or 4 mg kg^{-1} of caffeine).

Participants began their 7-min warm-up routine 23 min after capsule ingestion. This routine consisted of light cycling for 5 min on a commercial spin bike followed by 2 min of dynamic stretching. On visits 2–4 all participants completed the same warm-up routine, followed by exercise testing in this order: (1) vertical jump, (2) handgrip, (3) Wingate anaerobic test and (4) 10 km cycling time trial. Only the results of the WAnT are reported here, which was completed by the participants ~ 50 min after caffeine ingestion.

Anthropometry

Height, body mass and total body fat percentage were measured, and details have been described previously (Guest et al. 2018). Height was measured with a Harpenden stadiometer (Holtain Ltd., Crymych, UK), and body mass was measured by an electronic floor scale (AND FW-150K, A&D Ltd., Tokyo, Japan). Total body fat percentage was measured by BC-558 IronMan Segmental Body Composition Monitor (Tanita Corporation of America, Inc., Arlington Heights, IL).

Maximal exercise test (VO_{2peak})

Athletes completed the maximal aerobic capacity (VO_{2peak}) test on a mechanically weighted and braked cycle ergometer (Monark Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden). Participants began the VO_{2peak} test at a work rate of 50 Watts (W), with 50 W work rate increases each minute for the first 2 min, and 25 W work rate increases each following minute until volitional fatigue (Guest et al. 2018).

Genotyping

Saliva samples were collected on visit one using the Oragene ON-500 kit (DNA Genotek, Ottawa, Ontario, Canada) for DNA isolation using standard procedures (Josse et al. 2012). Genotyping of the 14 SNPs in the nine genes associated with caffeine metabolism or response (Table 1) was conducted using the Sequenom MassArray platform as previously described (Josse et al. 2012).

Wingate anaerobic test

After completing the handgrip test, athletes performed light-intensity cycling (50–70 W) on a spin bike for 2–3 min as a repeated mini-warm-up (since the initial 7-min warm-up was 20 min prior) before maximal exertion on the WAnT bike (Monark Ergomedic 894E, Monark Exercise AB, Vansbro, Sweden) (Inbar et al. 1996). Athletes were weighed at the beginning of each treatment visit to ensure pedal resistance remained consistent for the duration of the study.

The Monark anaerobic test software (Bar-Or 1987) was used to record WAnT outcomes. The WAnT is a 30 s maximal cycling test, and the outcomes were peak power (W), average power (W) and fatigue index (%). Peak power is the greatest five second power output achieved during the 30 s effort, average power is the mean power output during the 30 s test, and fatigue index = [(peak power − lowest power output)/peak power]*100% (Zupan et al. 2009). The computer was connected to the bike to track the revolutions per minute (rpm). After reaching 140 rpm, a weighted basket containing weights equal to 7.5% of the athlete's body mass was programmed to automatically drop. Once the basket dropped, the 30 s all-out effort began. Participants were verbally encouraged throughout the 30 s WAnT to pedal as hard and as fast as they could.

Participants remained on the bike, spinning with very low resistance for exactly 2 min to allow accumulated muscle lactate to be released into the circulation. Blood lactate concentration was initially measured 2 min post-WAnT. Low-intensity exercise following maximal exertion is necessary to promote the circulation of lactate from muscle cells into the blood, which increases the accuracy of the measurement (Bangsbo et al. 1994; Overgaard et al. 2012). Blood lactate was measured with a finger prick test to draw blood and analyzed with Lactate Scout 4 (Lactate Scout 4 Lactate Analyzer, Lactate Scout, Elkhart, USA) 2 min post-WAnT. Lactate was measured again at 20 min post-WAnT, and every 5 min until blood lactate concertation reached < 2.5 mmol L^{-1} in preparation for the 10 km cycling time trial.

Statistical analysis

Descriptive data (height, body mass, age, body fat %, VO_{2peak} (mL × kg/min), dietary caffeine or caffeine used for sport, and sport type distribution) were compared between genotypes using a one-way ANOVA or, for sport type, a Chi-square test of independence. Descriptive data that differed significantly between genotypes did not alter any of the results when adjusted for in the caffeine–gene models. Data were assessed for normality prior to analysis.

The WAnT outcome variables were peak power (W), average power (W), and fatigue index (%). The primary

Table 1 Name and function of the 14 single nucleotide polymorphisms (SNPs) in nine genes associated with caffeine metabolism or individual response to caffeine

Gene (rs#)	Function
ADORA2A (rs5751876)	The *ADORA2A* gene encodes for adenosine 2a (A2a) receptors (Nardin et al. 2020). Previous reports have observed that caffeine improved Wingate performance compared to placebo in those with the CC genotype of *ADORA2A* (rs5751876) (Grgic et al. 2020)
ADRA2β (rs4907299)	The *ADRA2β* gene encodes for α2*β* adrenergic (ADRA2*β*) receptors (Muszkat et al. 2010). ADRA2*β* receptors are a class of adrenergic receptors (Bylund et al. 1992), which are stimulated by epinephrine, norepinephrine and other catecholamines (Giovannitti et al. 2015), resulting in reduced coronary blood flow (Baumgart et al. 1999). Specifically, ADRA2*β* receptors possess greater affinity for epinephrine (Snyder et al. 2008)
ADRβ2 (rs1042713, rs1042717, rs1801704)	The *ADRβ2* gene encodes for β2 adrenergic (ADRβ2) receptors (Reihsaus et al. 1993) which respond to catecholamines, such as epinephrine (Insel 1996), which are increased in the plasma following caffeine intake (Robertson et al. 1978; Yamada et al. 1989). Activation of ADRβ2 receptors results in increased cardiac output and bronchodilation (Dishy et al. 2001; Insel 1996; Snyder et al. 2008; Wilkins et al. 2008)
COMT (rs4680)	The catechol-O-methyltransferase (COMT) enzyme is encoded by the *COMT* gene, which is responsible for the breakdown of catecholamines (Kamal and Lappin 2019). Those who are A-allele carriers of *COMT* (rs4680) possess decreased enzymatic activity of COMT, leading to increased circulating catecholamines (Gellekink et al. 2007)
CYP1A1 (rs2470893)	The *CYP1A1* gene encodes for the cytochrome P450 1A1 (CYP1A1) enzyme (Amin et al. 2012). Increased caffeinated coffee consumption has been associated with increased blood pressure in those who are T-allele carriers of *CYP1A1* (rs2470893) compared to those with the CC genotype
CYP1A2 (rs2470890, rs762551)	The *CYP1A2* gene encodes the cytochrome P450 1A2 (CYP1A2) enzyme, which is responsible for > 95% of caffeine metabolism (Berthou et al. 1991). Although the rs2470890 SNP has not previously been associated with caffeine metabolism or response, it is an SNP in a gene that encodes for the cytochrome P450 class of enzymes which are responsible for the clearance and metabolism of many compounds, including caffeine (Lynch and Price 2007). Individuals who carry the C-allele of rs762551 have reduced inducibility and activity of the CYP1A2 enzyme (Djordjevic et al. 2008; Ghotbi et al. 2007)
DRD2 (rs6277)	The *DRD2* gene encodes for dopamine D2 receptors (Klimek et al. 2002), which are responsible for dopamine regulation in the CNS in addition to other receptors (Abi-Dargham et al. 2000). Dopamine D2 receptors are inhibitory, and activation of D2 receptors results in decreased production of dopamine (Bhatia et al. 2020). The rs6277 SNP in *DRD2* has been associated with dopamine D2 receptor density (Abi-Dargham et al. 2000; Hirvonen et al. 2005; Klimek et al. 2002)
HTR2A (rs6313)	Serotonin 5-HT_{2A} receptors are encoded by the *HTR2A* gene, and the rs6313 SNP in *HTR2A* may modify 5-HT_{2A} receptor binding activity (Khait et al. 2005; Zhang and Stackman 2015). 5-HT_{2A} receptors are located in the CNS, enteric nervous system, smooth skeletal muscle, and cardiovascular system (Berger et al. 2009). Increased binding activity of 5-HT_{2A} receptors has been associated with dopamine release in the CNS (Porras et al. 2002), which can impact motivation and reward (Wise 2005), thereby increasing work output during exercise (Andersen et al. 2013)
SLC6A4 (rs2020939, rs2066713, rs93003628)	The *SLC6A4* gene encodes for serotonin transporters (5-HTTs) (Lesch et al. 1996) which are responsible for the transport of serotonin from the synapse to presynaptic neurons within the central nervous system (CNS) (Grabe et al. 2009). Variability in the *SLC6A4* gene may alter the rate of serotonin uptake at the synapse (Lesch et al. 1996) and has previously been associated with motivation (Lesch et al. 1996) and psychiatric disorders (Grabe et al. 2009; Lesch et al. 1996)

analysis used a linear mixed effects model adjusted for visit to account for a trial order effect and baseline performance (i.e., performance at the 0 mg kg^{-1} dose) to determine the main effect of caffeine on each outcome of the WAnT. Athletes were then stratified by their genotype for each of the 14 SNPs analyzed. This analysis included four predictor variables, caffeine dose, gene, visit, and baseline performance, along with two two-factor interactions; caffeine–gene and caffeine–visit interactions to account for the randomization of caffeine doses across the visits. These analyses were also performed with a linear mixed effects model and were carried out for each of the 14 SNPs analyzed. A linear mixed effects model was used for the analyses as each participant repeated three treatment sessions, and the genotype groups were unbalanced because of an unequal number of participants in each of the genotypes. A Bonferroni adjustment

was applied to α (which reduces the likelihood of Type I error) to account for family-wise comparisons, as 14 linear mixed effects models were performed for each of the three outcome variables. The Bonferroni corrected p value $= \alpha/N$ was determined where $\alpha = 0.05$ is the original p value and $N = 14$ to reflect the number of statistical tests applied to each outcome. All p values are two tailed, and $p \leq 0.004$ was used as the threshold for significance. After identifying a significant caffeine–gene interaction ($p \leq 0.004$), Dunnett's multiple comparisons post hoc analysis was performed within genotype groups across caffeine doses using lsmeans. Data were analyzed using the R (version 3.3.3) and RStudio (version 1.1.463) statistical packages.

Table 2 Peak power (W), average power (W), and fatigue index (%) performance across caffeine doses ($N = 99$)

Performance outcome	p^a	p^b	Caffeine dose (mg/kg)		
			0[c]	2[c]	4[c]
Peak power	0.80	0.79	951 ± 184	959 ± 197	954 ± 178
Average power	1.0	1.0	704 ± 122	704 ± 128	703 ± 117
Fatigue index	0.57	0.54	52 ± 9	54 ± 12	53 ± 10

[a]p values for the main effect of caffeine on performance

[b]p values from linear mixed effects models adjusted for visit and baseline performance

[c]Average (mean ± SD) test performance

Table 3 p values of all caffeine–gene interactions for peak power, average power, and fatigue index

Gene (rs#)	Peak power	Average power	Fatigue index
ADORA2A (rs5751876)	0.791	0.811	0.508
ADRA2β (rs4907299)	0.920	0.471	0.174
ADRβ2 (rs1042713)	0.075	0.482	0.872
ADRβ2 (rs1042717)	0.620	0.945	0.985
ADRβ2 (rs1801704)	0.018	0.312	0.098
COMT (rs4680)	0.824	0.993	0.975
CYP1A1 (rs2470893)	0.862	0.459	0.591
CYP1A2 (rs2470890)	0.094	0.175	0.228
CYP1A2 (rs762551)	0.117	0.004*	0.226
DRD2 (rs6277)	0.893	0.697	0.669
HTR2A (rs6313)	0.685	0.823	0.771
SLC6A4 (rs2020939)	0.863	0.236	0.720
SLC6A4 (rs2066713)	0.740	0.942	0.338
SLC6A4 (rs9303628)	0.475	0.710	0.500

All caffeine–gene p interaction values are derived from a linear mixed effects model with the following covariates: visit number, baseline performance, genotype and caffeine dose. Only caffeine–gene interactions are reported in this table

*Indicates significance ($p \leq 0.004$)

Sample size calculations have been previously described (Guest et al. 2018). Effect sizes are reported as standardized differences between caffeine treatments for all participants and individual genotypes using Cohen's $d = (M_2 - M_1)/SD_{pooled}$, with $SD_{pooled} = (SD_1^2 + SD_2^2)^{1/2}$ (Cohen 1992). The sample size was determined with an effect size (ES) based on the 10 km cycling time trial. A medium ES of 0.5 min was used, and power was set to 0.8. A sample size of 110 was determined to provide sufficient power for the analysis and account for a potential participant dropout rate of 10%. This sample size calculation was based on three treatment doses and three genotype groups.

Results

Main effect of caffeine across all participants

There was no effect of 2 mg kg^{-1} (959 ± 197 W, $p = 0.81$, 95% CI (− 29, 45), ES = 0.04) or 4 mg kg^{-1} (954 ± 178 W, $p = 1.0$, 95% CI (− 32, 38), ES = 0.02) compared to placebo (951 ± 184 W) on peak power output (Table 2). No effect was observed for 2 mg kg^{-1} (704 ± 128 W, $p = 1.0$, 95% CI

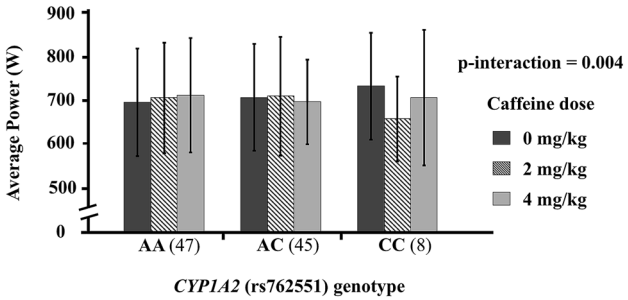

Fig. 1 Mean (SD) average power by caffeine dose and *CYP1A2* (rs762551) (n) genotype. p interaction values (Caffeine–*CYP1A2*) were generated from an adjusted model

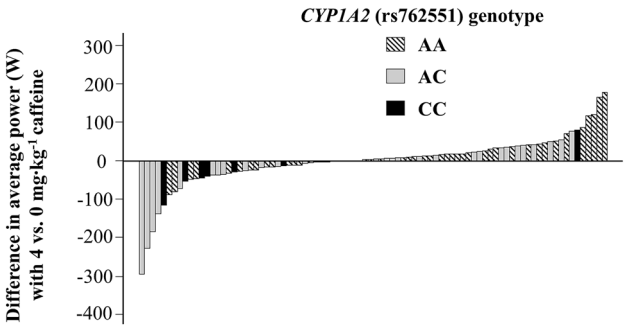

Fig. 2 Individual differences in average power with the 4 mg kg^{-1} dose of caffeine compared to placebo by *CYP1A2* (rs762551) genotype

Table 4 Average power performance (W) and caffeine dose across caffeine–genotypes

Gene (*N*)	Caffeine dose (mg/kg)			p^{4-0}	p^{2-0}	p^{4-2}
	0	2	4			
ADORA2A (rs5751876)						
CC (29)	709 ± 106	705 ± 106	699 ± 107	0.79	0.99	0.95
CT (46)	719 ± 139	722 ± 147	721 ± 124	1.0	1.0	1.0
TT (24)	666 ± 99	666 ± 110	675 ± 114	0.99	1.0	1.0
ADRA2B (rs4907299)						
GG (50)	707 ± 127	715 ± 140	716 ± 124	0.95	0.92	1.0
GT (40)	701 ± 124	690 ± 123	685 ± 117	0.50	0.92	0.94
TT (9)	695 ± 93	701 ± 76	715 ± 74	0.91	1.0	0.92
ADRB2 (rs1042713)						
AA (24)	683 ± 95	685 ± 92	696 ± 100	0.98	0.99	1.0
AG (44)	684 ± 98	673 ± 105	679 ± 104	0.98	0.78	0.98
GG (31)	747 ± 158	762 ± 162	743 ± 139	1.0	0.82	0.69
ADRB2 (rs1042717)						
AA (4)	670 ± 154	657 ± 161	679 ± 138	0.99	1.0	0.96
AG (35)	720 ± 141	721 ± 148	718 ± 130	1.0	1.0	1.0
GG (60)	696 ± 108	697 ± 114	697 ± 109	0.99	1.0	0.95
ADRB2 (rs1801704)						
CC (12)	727 ± 98	746 ± 109	724 ± 82	1.0	0.91	0.90
CT (38)	721 ± 155	723 ± 162	711 ± 147	0.69	1.0	0.63
TT (49)	684 ± 94	678 ± 96	693 ± 98	0.93	0.99	0.73
COMT (rs4680)						
AA (30)	697 ± 111	702 ± 110	703 ± 111	1.0	1.0	1.0
GA (39)	705 ± 116	704 ± 125	697 ± 115	0.98	1.0	1.0
GG (30)	708 ± 142	705 ± 152	712 ± 130	1.0	1.0	1.0
CYP1A1 (rs2470893)						
AA (4)	680 ± 50	733 ± 86	711 ± 72	0.81	0.62	1.0
GA (28)	739 ± 135	751 ± 125	744 ± 136	1.0	0.94	0.99
GG (67)	690 ± 117	682 ± 127	686 ± 108	0.93	0.93	1.0
CYP1A2 (rs2470890)						
CC (26)	701 ± 162	681 ± 169	683 ± 142	0.70	0.49	1.0
CT (45)	706 ± 108	705 ± 119	705 ± 109	0.99	1.0	0.99
TT (28)	702 ± 103	722 ± 97	720 ± 106	0.63	0.45	1.0
CYP1A2 (rs762551)						
CC (8)	733 ± 122	658 ± 97	707 ± 155	0.88	0.01	0.12
AC (45)	707 ± 122	710 ± 135	697 ± 97	0.72	1.0	0.66
AA (46)	696 ± 124	705 ± 127	709 ± 131	0.71	0.64	1.0
DRD2 (rs6277)						
CC (34)	698 ± 140	691 ± 160	687 ± 122	0.77	0.99	0.95
TC (46)	713 ± 110	727 ± 114	728 ± 121	0.68	0.74	1.0
TT (18)	687 ± 124	670 ± 87	672 ± 94	0.98	0.82	0.98
HTR2A (rs6313)						
CC (34)	700 ± 123	703 ± 134	704 ± 122	1.0	1.0	0.99
CT (48)	687 ± 111	689 ± 116	692 ± 121	0.99	1.0	1.0
TT (17)	756 ± 140	747 ± 148	735 ± 96	0.77	0.97	0.98
SLC6A4 (rs2020939)						
CC (28)	703 ± 118	695 ± 138	704 ± 135	1.0	0.84	0.87
TC (41)	717 ± 121	716 ± 111	724 ± 119	0.98	1.0	0.98
TT (30)	685 ± 128	694 ± 143	674 ± 93	0.83	0.83	0.23
SLC6A4 (rs2066713)						

Table 4 (continued)

Gene (*N*)	Caffeine dose (mg/kg)			p^{4-0}	p^{2-0}	p^{4-2}
	0	2	4			
CC (55)	707 ± 137	703 ± 142	701 ± 125	0.91	1.0	0.93
CT (33)	719 ± 101	722 ± 105	723 ± 107	0.99	1.0	0.98
TT (11)	639 ± 77	653 ± 115	653 ± 95	0.99	0.98	1.0
SLC6A4 (rs9303628)						
CC (36)	698 ± 137	698 ± 150	690 ± 126	0.92	1.0	0.82
CT (43)	707 ± 113	708 ± 111	706 ± 105	1.0	1.0	1.0
TT (20)	706 ± 118	704 ± 126	722 ± 131	0.92	1.0	0.79

Values reported as mean ± SD

p^{4-0}: *p* value for the difference in performance between 4 mg/kg vs 0 mg/kg

p^{2-0}: *p* value for the difference in performance between 2 mg/kg vs 0 mg/kg

p^{4-2}: *p* value for the difference in performance between 4 mg/kg vs 2 mg/kg

All *p* values are derived from a linear mixed effects model with the following covariates: visit number, baseline performance, genotype and caffeine dose

*Indicates $p \leq 0.004$

(− 25, 25), ES = 0) or 4 mg kg^{-1} (703 ± 117 W, $p = 0.99$, 95% CI (− 24, 22), ES = − 0.01) compared to placebo (704 ± 122 W) on average power output (Table 2). Similarly, there was no effect of 2 mg kg^{-1} (54 ± 12%, $p = 0.67$, 95% CI (− 1, 4), ES = 0.19) or 4 mg kg^{-1} (53 ± 10%, $p = 0.99$, 95% CI (− 1, 3), ES = 0.11) compared to placebo (52 ± 9%) on fatigue index (Table 2). A trial order effect was observed for peak ($p = 0.0001$) and average ($p = 0.001$) power, but not for fatigue index ($p = 0.74$). However, adjustment with the visit variable, which was done to account for a trial order effect, did not alter any of the results (Table 2).

Caffeine–gene interactions

Two *p* values for caffeine–gene interactions (*ADRB2* (rs1801704), *CYP1A2* (rs762551)) reached significance with $\alpha = 0.05$ (Table 3). After correcting the original alpha of 0.05 with a Bonferroni adjustment, one caffeine–gene interaction remained significant ($p \leq 0.004$) for *CYP1A2* (rs762551, $p = 0.004$) on WAnT average power performance.

Average power performance by *CYP1A2* (rs762551) genotype

A significant caffeine–*CYP1A2* (rs762551) interaction was observed for WAnT average power performance ($p = 0.004$, Fig. 1). However, when examining the association between caffeine and average power within each genotype, no significant differences were observed (Figs. 1, 2). In those with the AA genotype, there was no effect of 2 mg kg^{-1} (705 ± 127 W, $p = 0.64$, 95% CI (− 27, 45), ES = 0.07) or 4 mg kg^{-1} (709 ± 131 W, $p = 0.71$, 95% CI: (− 25, 47), ES = 0.09) compared to placebo (696 ± 124 W). Similarly, in those with the AC genotype, there was no effect of 2 mg kg^{-1} (710 ± 135 W, $p = 1.0$, 95% CI (− 34, 40), ES = 0.02) or 4 mg kg^{-1} (697 ± 97 W, $p = 0.72$, 95% CI (− 42, 22), ES = − 0.09) compared to placebo (707 ± 122 W). No effect was observed with 2 mg kg^{-1} (658 ± 97 W, $p = 0.01$, 95% CI (− 146, − 4), ES = − 0.72) or 4 mg kg^{-1} (707 ± 155 W, $p = 0.88$, 95% CI (− 116, 64), ES = − 0.20) compared to placebo (733 ± 122 W) in those with the CC genotype. No significant caffeine–gene interactions were observed for the remaining 13 SNPs on average power performance (Tables 3, 4). No significant caffeine–gene interactions were observed for any of the 14 SNPs on peak power (Tables 3, 5) and fatigue index (Tables 3, 6).

Discussion

The current study examined whether 14 SNPs in nine genes associated with caffeine metabolism or response, modified the effect of caffeine on anaerobic performance as measured by the WAnT, in competitive male athletes. Our results show that in the total study population, caffeine was not ergogenic for anaerobic performance as measured by the WAnT test, which is consistent with previous studies using similar doses (Collomp et al. 1991; Duncan et al. 2019; Greer et al. 2006), but in contrast to other reports showing benefits of caffeine (Grgic and Mikulic 2020; San Juan et al. 2019). Despite observing a significant caffeine–*CYP1A2* interaction with average power, post hoc analysis showed no significant differences between caffeine doses within *CYP1A2* genotypes.

The effect of caffeine on anaerobic performance has been shown to vary among both trained (Collomp et al. 1992; San Juan et al. 2019) and untrained (Collomp et al. 1991; Greer et al. 2006) individuals. For example, ~ 4 mg kg^{-1} of caffeine improved performance in 2 × 100 m maximal speed

Table 5 Peak power performance (W) and caffeine dose across caffeine–genotypes

Gene (*N*)	Caffeine dose (mg/kg)			p^{4-0}	p^{2-0}	p^{4-2}
	0	2	4			
ADORA2A (rs5751876)						
CC (29)	953 ± 150	964 ± 206	943 ± 154	0.95	0.99	0.79
CT (46)	977 ± 218	992 ± 205	987 ± 201	0.97	0.93	1.0
TT (24)	900 ± 141	889 ± 154	904 ± 151	1.0	1.0	1.0
ADRA2B (rs4907299)						
GG (50)	947 ± 200	965 ± 224	961 ± 196	0.98	0.85	0.99
GT (40)	955 ± 180	946 ± 178	943 ± 171	0.96	1.0	0.99
TT (19)	956 ± 107	984 ± 105	966 ± 96	1.0	0.99	1.0
ADRB2 (rs1042713)						
AA (24)	940 ± 174	928 ± 161	945 ± 176	1.0	1.0	1.0
AG (44)	916 ± 140	898 ± 150	912 ± 147	1.0	0.84	0.93
GG (31)	1009 ± 232	1069 ± 237	1020 ± 205	0.97	0.07	0.25
ADRB2 (rs1042717)						
AA (4)	964 ± 198	938 ± 209	979 ± 171	0.99	1.0	0.97
AG (35)	973 ± 215	970 ± 197	976 ± 204	1.0	0.99	0.95
GG (60)	937 ± 165	954 ± 199	940 ± 164	1.0	0.74	0.54
ADRB2 (rs1801704)						
CC (12)	967 ± 122	1090 ± 226	972 ± 92	1.0	0.028	0.050
CT (38)	963 ± 235	968 ± 229	968 ± 223	1.0	1.0	1.0
TT (49)	938 ± 151	919 ± 145	939 ± 156	1.0	0.87	0.89
COMT (rs4680)						
AA (30)	945 ± 174	973 ± 217	956 ± 167	1.0	0.84	0.89
GA (39)	944 ± 169	947 ± 187	930 ± 176	0.99	1.0	0.98
GG (30)	967 ± 216	960 ± 194	983 ± 193	0.98	1.0	0.99
CYP1A1 (rs2470893)						
AA (4)	907 ± 81	968 ± 176	949 ± 152	0.91	0.92	1.0
GA (28)	997 ± 202	1017 ± 185	1012 ± 206	0.97	0.98	1.0
GG (67)	935 ± 179	934 ± 200	930 ± 164	0.98	1.0	0.93
CYP1A2 (rs2470890)						
CC (26)	967 ± 244	936 ± 218	945 ± 215	0.90	0.60	0.98
CT (45)	939 ± 163	941 ± 182	946 ± 168	1.0	1.0	1.0
TT (28)	955 ± 155	1009 ± 198	975 ± 162	0.87	0.07	0.46
CYP1A2 (rs762551)						
CC (8)	994 ± 191	917 ± 135	966 ± 201	0.99	0.29	0.56
AC (45)	955 ± 197	955 ± 181	947 ± 158	0.96	1.0	0.99
AA (46)	940 ± 172	970 ± 221	959 ± 196	0.88	0.22	0.77
DRD2 (rs6277)						
CC (34)	951 ± 201	939 ± 209	941 ± 174	0.99	1.0	1.0
TC (46)	971 ± 174	998 ± 205	990 ± 192	0.97	0.87	1.0
TT (18)	899 ± 181	900 ± 134	887 ± 135	0.72	1.0	0.83
HTR2A (rs6313)						
CC (34)	939 ± 188	936 ± 199	941 ± 186	1.0	1.0	1.0
CT (48)	931 ± 162	954 ± 205	944 ± 180	0.93	0.62	0.97
TT (17)	1034 ± 222	1017 ± 166	1008 ± 156	0.95	0.97	1.0
SLC6A4 (rs2020939)						
CC (28)	946 ± 196	947 ± 212	955 ± 209	1.0	0.99	0.98
TC (41)	977 ± 164	993 ± 194	977 ± 170	1.0	0.85	0.82
TT (30)	920 ± 200	923 ± 185	921 ± 158	1.0	0.99	0.99
SLC6A4 (rs2066713)						

Table 5 (continued)

Gene (*N*)	Caffeine dose (mg/kg)			p^{4-0}	p^{2-0}	p^{4-2}
	0	2	4			
CC (55)	954 ± 207	963 ± 220	952 ± 192	1.0	0.81	0.67
CT (33)	983 ± 146	979 ± 151	982 ± 156	1.0	0.96	0.97
TT (11)	844 ± 126	879 ± 191	882 ± 161	0.93	0.94	1.0
SLC6A4 (rs9303628)						
CC (36)	933 ± 210	936 ± 204	940 ± 196	1.0	0.99	1.0
CT (43)	971 ± 159	978 ± 196	952 ± 153	0.80	0.99	0.52
TT (20)	941 ± 190	957 ± 191	984 ± 201	0.71	1.0	0.86

Values reported as mean ± SD

p^{4-0}: *p* value for the difference in performance between 4 mg/kg vs 0 mg/kg

p^{2-0}: *p* value for the difference in performance between 2 mg/kg vs 0 mg/kg

p^{4-2}: *p* value for the difference in performance between 4 mg/kg vs 2 mg/kg

All *p* values are derived from a linear mixed effects model with the following covariates: visit number, baseline performance, genotype and caffeine dose

*Indicates $p \leq 0.004$

swimming efforts in highly trained individuals, but not in untrained participants (Collomp et al. 1992). Similarly, 6 mg kg^{-1} of caffeine improved both peak and average power on the WAnT in eight male Spanish National Olympic Team athletes (San Juan et al. 2019). Conversely, 5 mg kg^{-1} of caffeine did not improve WAnT performance in 18 recreationally trained males (Greer et al. 2006), and 6 recreationally active participants (Collomp et al. 1991). We hypothesized that genetic variability in caffeine metabolism or response could explain these effects, but did not observe this result in the present study.

A significant caffeine-*CYP1A2* interaction was observed for average power performance. However, in post hoc analyses we found no significant differences between caffeine doses within each of the *CYP1A2* genotypes. Genetic variation in caffeine metabolism, as determined by the *CYP1A2* (rs762551) gene, has been shown to vary the impact of caffeinated coffee on both myocardial infarction and hypertension (Cornelis et al. 2006; Palatini et al. 2009). The *CYP1A2* gene encodes the cytochrome P450 1A2 (CYP1A2) enzyme, which is responsible for > 95% of caffeine metabolism (Berthou et al. 1991). Individuals who carry the C-allele of rs762551 have reduced inducibility and activity of the CYP1A2 enzyme (Djordjevic et al. 2008; Ghotbi et al. 2007). The ability of *CYP1A2* genotype to modify the effects of caffeine on the WAnT differs between studies, with one study reporting an improvement in C-allele carriers (Salinero et al. 2017), and others reporting no modifying effects of *CYP1A2* in response to caffeine administration (Grgic and Mikulic 2020; Salinero et al. 2017). These inconsistent results may be explained by caffeine's mechanism of action.

Caffeine's performance-enhancing effects begin with adenosine antagonism, which occurs in the central nervous system (CNS). Adenosine is produced through adenine nucleotide breakdown of adenosine triphosphate (ATP) (Broberg and Sahlin 1989; Costa et al. 2001) and increases during exercise in skeletal and smooth muscle, the cardiovascular system and the brain (Daly 1982; Latini and Pedata 2001). In the CNS, adenosine has been shown to increase feelings of fatigue and pain when binding to its receptors (Dunwiddie 1985). Caffeine, which is similar in structure to adenosine, acts as an adenosine receptor antagonist, preventing the inhibitory effects of adenosine in the CNS. Owing to these effects, caffeine is commonly consumed by athletes to mitigate fatigue and to maintain prolonged exercise at a higher intensity (Doherty and Smith 2005). In contrast, potentially undesirable effects of adenosine antagonism via caffeine have been observed in the cardiovascular system (Higgins and Babu 2013; van Dijk et al. 2018). The binding of adenosine to its receptors on vascular smooth muscle (VSM) cells has been shown to increase VSM relaxation, subsequently leading to the vasodilation of coronary arteries (van Dijk et al. 2018). Acting as an adenosine antagonist, caffeine can decrease VSM relaxation, subsequently leading to vasoconstriction by binding to adenosine receptors present on VSM cells (Higgins and Babu 2013). Increased vasoconstriction can impair the transport of oxygen and nutrients to exercising muscle and may lead to worsened athletic performance (Burton et al. 2004).

Previously, caffeine has been shown to improve endurance performance in those with the AA genotype of *CYP1A2* (Guest et al. 2018; Womack et al. 2012). Endurance activities, such as a 10 km cycling time trial, elicit more fatigue compared to maximal tests, such as the WAnT, indicating the rise in adenosine may be greater during long-duration efforts compared to short-duration maximal efforts (Broberg and Sahlin 1989). Therefore, the modifying effects on caffeine by *CYP1A2* genotype might

Table 6 Fatigue index performance (%) and caffeine dose across caffeine–genotypes

Gene (*N*)	Caffeine dose (mg/kg)			p^{4-0}	p^{2-0}	p^{4-2}
	0	2	4			
ADORA2A (rs5751876)						
CC (29)	50 ± 9	53 ± 10	53 ± 9	0.58	0.68	1.0
CT (46)	53 ± 10	53 ± 12	52 ± 10	1.0	1.0	1.0
TT (24)	55 ± 9	57 ± 13	53 ± 13	0.90	0.99	0.65
ADRA2B (rs4907299)						
GG (50)	51 ± 7	52 ± 10	51 ± 10	1.0	0.99	0.97
GT (40)	53 ± 11	54 ± 13	55 ± 11	0.74	0.96	0.98
TT (9)	58 ± 11	61 ± 12	51 ± 6	0.43	0.97	0.13
ADRB2 (rs1042713)						
AA (24)	54 ± 8	56 ± 13	53 ± 12	1.0	0.86	0.85
AG (44)	52 ± 9	53 ± 11	52 ± 11	1.0	0.95	0.95
GG (31)	53 ± 11	53 ± 12	54 ± 8	1.0	1.0	1.0
ADRB2 (rs1042717)						
AA (4)	57 ± 10	58 ± 5	59 ± 6	1.0	1.0	1.0
AG (35)	52 ± 11	54 ± 13	52 ± 8	1.0	0.96	0.84
GG (60)	52 ± 9	53 ± 11	53 ± 12	0.97	1.0	1.0
ADRB2 (rs1801704)						
CC (12)	53 ± 8	56 ± 11	52 ± 9	1.0	0.95	0.83
CT (38)	51 ± 11	50 ± 11	53 ± 11	0.74	0.96	0.33
TT (49)	54 ± 8	56 ± 12	53 ± 10	0.96	0.51	0.16
COMT (rs4680)						
AA (30)	51 ± 8	53 ± 9	51 ± 8	1.0	0.96	1.0
GA (39)	52 ± 12	54 ± 14	52 ± 11	1.0	0.88	0.92
GG (30)	55 ± 7	55 ± 11	55 ± 12	1.0	1.0	1.0
CYP1A1 (rs2470893)						
AA (4)	47 ± 7	57 ± 12	52 ± 8	0.96	0.43	0.83
GA (28)	51 ± 11	52 ± 7	52 ± 8	0.97	1.0	0.99
GG (67)	53 ± 9	54 ± 13	53 ± 11	1.0	0.97	0.86
CYP1A2 (rs2470890)						
CC (26)	55 ± 8	59 ± 16	55 ± 12	1.0	0.35	0.31
CT (45)	51 ± 11	50 ± 10	52 ± 10	0.98	0.96	0.76
TT (28)	53 ± 8	54 ± 8	52 ± 9	1.0	0.92	0.78
CYP1A2 (rs762551)						
CC (8)	53 ± 11	63 ± 14	58 ± 17	0.79	0.10	0.65
AC (45)	52 ± 11	52 ± 13	52 ± 10	1.0	1.0	1.0
AA (46)	53 ± 8	53 ± 9	53 ± 10	0.99	1.0	0.99
DRD2 (rs6277)						
CC (34)	53 ± 9	57 ± 14	54 ± 11	0.90	0.25	0.78
TC (46)	53 ± 9	52 ± 10	53 ± 11	0.98	0.94	1.0
TT (18)	50 ± 12	51 ± 10	49 ± 8	1.0	0.97	0.99
HTR2A (rs6313)						
CC (34)	51 ± 10	50 ± 11	50 ± 11	1.0	0.99	1.0
CT (48)	54 ± 9	56 ± 13	54 ± 11	1.0	0.52	0.70
TT (17)	53 ± 9	53 ± 9	53 ± 7	1.0	1.0	1.0
SLC6A4 (rs2020939)						
CC (28)	52 ± 9	54 ± 13	51 ± 10	0.98	0.90	0.60
TC (41)	54 ± 10	53 ± 8	53 ± 12	1.0	1.0	1.0
TT (30)	51 ± 9	53 ± 15	53 ± 8	0.93	0.85	1.0
SLC6A4 (rs2066713)						

Table 6 (continued)

Gene (*N*)	Caffeine dose (mg/kg)			p^{4-0}	p^{2-0}	p^{4-2}
	0	2	4			
CC (55)	51 ± 10	54 ± 13	52 ± 11	0.92	0.28	0.78
CT (33)	55 ± 9	53 ± 9	54 ± 10	0.95	0.71	0.98
TT (11)	51 ± 6	53 ± 11	50 ± 8	1.0	0.97	0.94
SLC6A4 (rs9303628)						
CC (36)	50 ± 10	54 ± 15	52 ± 10	0.76	0.25	0.91
CT (43)	54 ± 10	53 ± 8	53 ± 11	0.98	0.98	1.0
TT (20)	53 ± 7	53 ± 11	52 ± 9	0.99	1.0	0.98

Values reported as mean ± SD

p^{4-0}: *p* value for the difference in performance between 4 mg/kg vs 0 mg/kg

p^{2-0}: *p* value for the difference in performance between 2 mg/kg vs 0 mg/kg

p^{4-2}: *p* value for the difference in performance between 4 mg/kg vs 2 mg/kg

All *p* values are derived from a linear mixed effects model with the following covariates: visit number, baseline performance, genotype and caffeine dose

*Indicates $p \leq 0.004$

only occur in longer duration sustained efforts, as adenosine antagonism may not be able to occur during short duration maximal efforts. The largest study to examine the effect of caffeine and *CYP1A2* genotype on endurance performance (Guest et al. 2018) observed that those with the CC genotype worsened their performance with caffeine, but AC genotype individuals did not modify their performance. Previous studies examining the effect of caffeine and *CYP1A2* on Wingate performance combined C-allele carriers together (Salinero et al. 2017); however, the present study did not combine those with the AC and CC genotype, so that any potential differences in performance between these groups could be determined. Future studies should examine the interaction between caffeine and *CYP1A2* genotype on repeated high-intensity exercise and anaerobic metabolism to further understand the role of caffeine and genetic variation in caffeine metabolism or response on short bursts of intense exercise.

The present study has some limitations. For example, only male athletes were included. However, sex differences may also confound the effects of caffeine by genotype (Denden et al. 2016). Furthermore, oral contraceptives are known to decrease the rate of caffeine clearance (Hukkanen et al. 2011) and Canadian females between the ages of 20–29 years consume oral contraceptives more than any other age group (Black et al. 2009). This study had a mean ± SD age of 25 ± 4 years; therefore female athletes were excluded to control for any potential effects of sex or the use of oral contraceptives on the rate of caffeine clearance. Although caffeine doses were randomized to reduce learning effects, another limitation is a lack of a familiarization trial to the WAnT, and improvements in performance may be due to a trial order effect. We did observe a trial order effect for peak and average power, but not fatigue index. To account for this, we included trial order number (the visit variable) as a co-variate in our statistical model to account for familiarization to the protocol. However, the inclusion of the visit variable in our statistical model did not alter any of the results. Although this is the largest study to determine the effect of caffeine on anaerobic performance, some sub-groups are small because of the low frequency of certain genotypes. Despite this, the study was designed to account for three caffeine does and three genotype groups.

In summary, we found that caffeine did not affect Wingate performance, and none of the effects were modified by 14 SNPs in nine genes associated with caffeine metabolism or response. These results suggest that caffeine might not improve anaerobic performance.

Author contributions MS wrote the first draft and contributed to the literature search, conducted the statistical analyses, and managed all aspects of manuscript preparation and submission. NG collected all of the initial data and NG and AE-S contributed to the design of the study, data screening and extraction, and contributed to the writing and editing of the manuscript. NG and AE-S secured funding. PT contributed to data analyses and editing of the manuscript. All authors approved the final manuscript.

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Availability of data and material This study was registered with clinicaltrials.gov on April 10, 2014 (NCT02109783). The datasets generated during and/or analyzed during the current study are not publicly available.

Code availability Data was analyzed using the R (version 3.3.3) and RStudio (version 1.1.463) statistical packages.

Declarations

Conflict of interest AE-S is the Founder and holds shares in Nutrigenomix Inc. NG is on the Science Advisory Board of Nutrigenomix Inc. MS and PT declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval This trial was approved by the University of Toronto Institutional Review Board.

Consent to participate Written informed consent was obtained from all participants. Participants were aware of the potential benefits and risks of the trial prior to signing consent forms and participating in the study.

Consent for publication Patients signed informed consent regarding publishing their data and photographs.

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