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CYP1A2 Genotype Modifies the Effects of Caffeine Compared With Placebo on Muscle Strength in Competitive Male Athletes

Oriana Wong, Keiko Marshall, Marc Sicova, Nanci S. Guest, Bibiana García-Bailo, and Ahmed El-Sohemy
University of Toronto

Caffeine is commonly used to improve athletic performance across a variety of sports. Previously, the *CYP1A2* gene has been shown to modify the effects of caffeine on endurance performance. The effect of caffeine on strength and power activities is unclear and may differ depending on an individual's *CYP1A2* genotype. A randomized controlled trial was used to determine whether caffeine impacts strength and power, determined by the handgrip and vertical jump tests, respectively, and whether *CYP1A2* genotype modifies any effects. Competitive male athletes (age = 25 ± 4 years) completed vertical jump ($n = 97$), and handgrip tests ($n = 102$) under three conditions: 0 (placebo), 2, or 4 mg of caffeine per kilogram of body mass (in milligrams per kilogram). *CYP1A2* (rs762551) genotype was determined from saliva samples. No differences between caffeine doses and placebo were observed for strength or power; however, significant Caffeine × Gene interactions were observed for all exercise tests. Individuals with the CC genotype experienced a 12.8% decrease in handgrip strength with 4 mg/kg of caffeine compared with placebo (53 ± 11 kg vs. 61 ± 17 kg, $p = .02$). No differences were observed in those with the AC or AA genotypes. Despite observing a significant Caffeine × Gene interaction for vertical jump performance, no differences were observed between caffeine doses and placebo for all genotypes. In summary, caffeine (4 mg/kg) worsened handgrip strength performance in those with the CC genotype, but no differences were observed in those with the AC or AA genotypes. Athletes may want to consider their *CYP1A2* genotype prior to using caffeine to improve muscle strength.

Keywords: ergogenic, genetic variation, handgrip, nutrigenomics, vertical jump

Caffeine is one of the most commonly used supplements to enhance athletic performance (Heckman et al., 2010), particularly by endurance athletes (Aguilar-Navarro et al., 2019). Although caffeine has consistently been shown to improve endurance performance when consumed at a dose of 3–6 mg/kg body mass, ~60 min prior to exercise, it is not uncommon to observe negligible or no benefit (Guest et al., 2021). Furthermore, previous studies have demonstrated that doses of caffeine, as low as 2 mg/kg (Graham & Spriet, 1995; Talanian & Spriet, 2016), can also be ergogenic, with lower risk of adverse effects (Guest et al., 2018; Spriet, 2014), such as sleep disturbances. Excessive caffeine use may impair sleep quality and duration (Bonnet & Arand, 1992), which can negatively impact physiological and psychological recovery from training (Nédélec et al., 2015).

Despite the widespread use of caffeine among athletes, its effects on strength and power performance remain inconsistent (Duncan et al., 2013; Grgic et al., 2020; Sabol et al., 2019). For example, 5 mg/kg of caffeine had no effect on handgrip strength in 14 male judo athletes (da Silva Athayde et al., 2018), but improved squat jump (SQJ) and countermovement jump (CMJ) in 25 Division I collegiate athletes (Bloms et al., 2016). However, 3 mg/kg of caffeine improved handgrip strength in 14 male jiu-jitsu athletes (Diaz-Lara et al., 2016), and 6 mg/kg of caffeine had no effect on CMJ height in 10 females who competed in recreational or international team sports (Ali et al., 2016). These inconsistent results may be due, in part, to interindividual differences in the rate of caffeine metabolism.

The *CYP1A2* gene determines the rate of caffeine metabolism between individuals (Sachse et al., 1999). The -163A > C single-nucleotide polymorphism in the *CYP1A2* gene (rs762551) alters CYP1A2 enzyme inducibility and activity. Individuals with the AA genotype possess the greatest enzymatic activity and are considered fast metabolizers of caffeine. Those with the AC and CC genotypes are classified as slow and ultraslow metabolizers, respectively (Guest et al., 2018).

The performance-enhancing effects of caffeine are likely due to adenosine antagonism, which occurs in the central nervous system (Doherty & Smith, 2005). Adenosine concentrations increase rapidly during exercise (Daly, 1982; Latini & Pedata, 2001), which may result in increased feelings of fatigue and pain when binding to its receptors in the central nervous system (Dunwiddie, 1985). Caffeine is commonly consumed by athletes to reduce fatigue and maintain prolonged exercise at a higher intensity (Doherty & Smith, 2005). However, binding of adenosine to its receptors on vascular smooth muscle (VSM) cells in the cardiovascular system has been shown to increase VSM relaxation, subsequently leading to vasodilation of coronary arteries (Van Dijk et al., 2018). Caffeine can decrease VSM relaxation, subsequently leading to vasoconstriction by binding to adenosine receptors on VSM cells (Higgins & Babu, 2013). Caffeine's effects on vasoconstriction may be more pronounced in those with the C-allele of *CYP1A2*, due to slower caffeine metabolism.

The objective of this study was to determine whether variability in *CYP1A2* (rs762551) modifies the effects of low (2 mg/kg) or moderate (4 mg/kg) doses of caffeine on strength and power performance. We hypothesized that higher doses of caffeine would

The authors are with the Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada. El-Sohemy (a.el.sohemy@utoronto.ca) is corresponding author.

improve performance in vertical jump and handgrip tests in individuals with the AA genotype only.

Methods

Participants and Recruitment

Recruitment methods and consent have been described previously (Guest et al., 2018). The University of Toronto Institutional Review Board approved the study and 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. The randomized, double-blinded placebo-controlled study recruited 113 competitive male athletes from the University of Toronto, Ryerson University, York University, the Canadian Sports Institute of Ontario, and local running clubs and gyms. Eleven participants in the handgrip analyses, and 16 participants in the vertical jump tests had to terminate their involvement in the study due to a sports-related injury, an inability to abstain from caffeine for the duration of the study, relocation or school scheduling conflicts, or exclusion due to incomplete data. Participants were, on average, 25 ± 4 years of age, with a body mass of 81.1 ± 13.1 kg. Their sport backgrounds were classified as endurance (e.g., cycling, cross-country skiing, marathon running); power (e.g., volleyball, powerlifting, dragon-boat); or mixed (e.g., basketball, swimming, rugby). A subanalysis was performed with the athletes' specialization (endurance, power, or strength) as a covariate. Study inclusion criteria specified that participants train or compete for at least 8 hr/week for nine out of 12 months of the year in their primary sport; they must have competed in their sport for at least 3 years.

Data Collection

The participants completed four 90–120 min visits, approximately 1 week apart, at the Goldring Centre for High Performance Sport at the University of Toronto. On the first visit, anthropometric data and a DNA sample were collected. Participants then completed a maximal aerobic capacity test (VO_2peak) and a standardized questionnaire on general health, caffeine intake, and sports history. Genotyping of the rs762551 single-nucleotide polymorphism in the *CYP1A2* gene was conducted using the Sequenom MassARRAY platform, as previously described (Josse et al., 2012).

Participants were instructed to maintain typical dietary and sleeping habits, abstain from caffeine throughout the duration of the study (4 weeks), and to avoid strenuous activity 48 hr prior to each visit. For the duration of the study, participants were asked to eat the identical meal(s) that they had consumed prior to the first visit. To ensure compliance, one day prior to all treatment visits, participants were reminded through e-mail or text message to replicate this "first" meal. On Visits 2–4, participants were randomly assigned to ingest capsules that contained 2 or 4 mg/kg of anhydrous caffeine, or placebo capsules containing dextrose that were similar in taste, volume, and color (A&C American Chemicals Ltd., Saint-Laurent, Canada). Participants were blinded to treatment allocation and were asked if they believed they had consumed caffeine following each of their treatment sessions. Balanced permutations blocked by time of entry was used for randomization of caffeine doses across treatment visits (randomization.com). After capsule ingestion, participants sat quietly for 25 min and completed questionnaires or used e-devices, and then began a standardized 7-min warm-up which consisted of light cycling and dynamic muscle activation. Blood pressure and heart rate (Polar Electro Inc., Bethpage, NY) were measured 3 min after capsule ingestion and prior to commencing their warm-up routine. Athletes completed four exercise tests in the following order: (a) vertical jump, (b) handgrip, (c) Wingate, and (d) 10-km cycling time trial. This protocol was repeated three times; once for each visit (0, 2, or 4 mg/kg). Only results from the handgrip and vertical jump tests are reported in the present study.

Handgrip Strength Test

Handgrip strength is considered to be a robust surrogate measure of overall body strength and is frequently used as a measure of strength in athletic testing (Cronin et al., 2017). In the present study, a hydraulic hand dynamometer was used to measure handgrip strength three times, with 30-s rest intervals between measurements. Hydraulic hand dynamometers have previously been observed to demonstrate high test reliability and validity (España-Romero et al., 2010). The mean of the two highest scores at each visit was used for analyses.

Vertical Jump Test

Vertical jump height tests are widely used as indicators of lower body power production (Watkins et al., 2017). Force plates are the gold standard for vertical jump height testing, and are used to assess the accuracy of other devices that measure vertical jump height, as they provide standardized and highly valid measurements (Buckthorpe et al., 2012). This study used the Dual-Top ACCUSWAY force plate (AMTI; Watertown, MA) and the AMTI NetForce program (AMTI) on a laptop to determine power output through vertical jump height using two different vertical jump exercises: SQJ and CMJ. Participants first completed the SQJ three times with 20-s rest intervals between attempts. The CMJ was performed next with 20-s rest intervals between attempts. The mean height of the two best jump attempts for the SQJ and CMJ was used for analyses.

Statistical Analyses

R (version 3.3.3) and RStudio (version 1.1.463) statistical packages were used for statistical analyses. All p values were two-sided with the threshold for significance set at $p < .05$. Descriptive characteristics were compared across *CYP1A2* genotypes using analysis of variance for the continuous variables: height, body mass, age, body fat, VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$), and dietary caffeine or caffeine used as an ergogenic aid. The categorical descriptive characteristic, distribution of sport type across *CYP1A2* genotypes, was assessed using chi-square tests.

The primary outcomes in the present study were handgrip strength (in kilograms), SQJ height (in centimeters), and CMJ height (in centimeters). A linear mixed-effects model adjusted for visit number and performance with the placebo dose (0 mg/kg dose) was used to determine the main effect of caffeine on handgrip strength, SQJ, and CMJ.

Athletes were then stratified by their *CYP1A2* genotype for each of the three outcome variables. This secondary analysis included four predictor variables: caffeine dose (categorical with three levels, 0, 2, and 4 mg/kg of caffeine); *CYP1A2* genotype (categorical with three levels, AA, AC, and CC genotypes); visit number (categorical with three levels, Visit 1, Visit 2, and Visit 3); and performance with the placebo dose, along with two 2-factor interactions Caffeine × *CYP1A2* and Caffeine × Visit (to account

for the randomization of caffeine doses across the visits) interactions. *CYP1A2* genotype was allocated as a fixed effect, as individuals either possess the AA, AC, or CC genotypes; all genotype groups are included in this study. Participant ID was allocated as a random effect, as each participant completed three treatment sessions. This approach prevented pseudoreplication from occurring within our statistical models. The analysis was performed with a linear mixed-effects model and was carried out for each of the three outcome variables; handgrip strength, SQJ, and CMJ. After identifying a significant Caffeine × Gene interaction, Dunnett's Multiple Comparisons post hoc test was performed within genotype groups across caffeine doses using lsmeans.

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 drop-out rate of 10%. This sample size calculation was based on three treatment doses and three genotype groups.

Results

Participant Characteristics

Descriptive characteristics across genotypes for the 102 participants who completed the handgrip test are shown in Table 1. Some participants ($n = 5$) were excluded from vertical jump analysis due to missing data. The genotype distributions were similar to previous reports (Cornelis et al., 2006; Ghotbi et al., 2007). In strength tests, those with the AA, AC, and CC genotypes accounted for 49% ($n = 50$), 43% ($n = 44$), and 8% ($n = 8$) of 102 participants, respectively. The genotype distribution across the 97 participants who completed the vertical jump tests was 51% AA ($n = 49$), 41% AC ($n = 40$), and 8% CC ($n = 8$). Descriptive characteristics did not differ across genotypes except for body mass. However, adjustment for body mass in the caffeine and caffeine–*CYP1A2* models did not alter any of the results.

Caffeine and Performance Outcomes

Average handgrip strength (in kilograms), SQJ (in centimeters), and CMJ (in centimeters) performance across caffeine doses (0, 2, 4 mg/kg body mass) are shown in Table 2. There was no effect of 2 mg/kg (55 ± 12 kg, $p = .55$, ES = –0.084) and 4 mg/kg (56 ± 11 kg, $p = .55$, ES = 0) compared with placebo (56 ± 12 kg) on handgrip strength. Similarly, there was no effect of 2 mg/kg (34 ± 6 cm, $p = .15$, ES = 0.28, and 42 ± 8 cm, $p = .50$, ES = 0.11, for SQJ and CMJ, respectively) and 4 mg/kg (33 ± 8 cm, $p = .15$, ES = 0.13, and 41 ± 10 cm, $p = .50$, ES = 0, for SQJ and CMJ, respectively) compared with placebo (32 ± 8 cm, and 41 ± 10 cm, for SQJ and CMJ, respectively) on vertical jump performance.

Caffeine, *CYP1A2*, and Handgrip Strength

A significant Caffeine × *CYP1A2* interaction was observed in the handgrip strength test ($p = .012$). Therefore, we examined the association between caffeine and handgrip strength separately within each genotype across caffeine doses (Figure 1). In those with the AA genotype, there was no effect of 2 mg/kg (54 ± 12 kg,

Table 2 The *p* Values for Handgrip (in Kilograms), Squat Jump (in Centimeters), and Countermovement Jump (in Centimeters) Performance by Caffeine Dose

			Caffeine dose (mg/kg)		
Performance outcome	p^a	p^b	0[c]	2[c]	4[c]
Handgrip (kg)	.57	.55	56 ± 12	55 ± 12	56 ± 11
Squat jump (cm)	.16	.17	32 ± 8	34 ± 6	33 ± 8
Countermovement jump (cm)	.51	.53	41 ± 10	42 ± 8	41 ± 10

[a]The *p* values from a linear mixed-effects model adjusted for visit. [b]The *p* values from linear mixed-effects model adjusted for visit and performance with the placebo dose. [c]Average (mean ± *SD*) test performance.

Table 1 Descriptive Characteristics of Participants by *CYP1A2* (rs762551) Genotype

	CYP1A2 genotype (rs762551)			
Characteristics	AA ($n = 50$)	AC ($n = 44$)	CC ($n = 8$)	p^c
Height (cm), mean ± *SD*	178 ± 7	177 ± 6	181 ± 10	.17
Body mass (kg), mean ± *SD*	80.1 ± 12.1	79.3 ± 9.7	92.9 ± 24.9	**.019**
Age (years), mean ± *SD*	24 ± 4	25 ± 5	25 ± 5	.44
Body fat (%), mean ± *SD*	14.1 ± 4.4	13.8 ± 4.4	15.9 ± 6.0	.48
VO_2peak ($ml \cdot kg^{-1} min^{-1}$), mean ± *SD*	49 ± 8	47 ± 12	44 ± 12	.43
Caffeine, dietary[a] (mg/day), mean ± *SD* *(range of intakes)*	92 ± 133 (0–500)	80 ± 134 (0–430)	38 ± 69 (0–164)	.55
Caffeine, sport[b] (mg/day), mean ± *SD* (*range of intakes*)	60 ± 92 (0–300)	89 ± 110 (0–410)	80 ± 210 (0–600)	.45
Sport type (*n*)				.52
Endurance	20	20	2	
Power	20	20	4	
Mixed	10	4	2	

Note. Values are presented as mean ± *SD*. Bold values indicate $p < .05$.
[a]Average dietary caffeine intake excluding intake for sport. [b]Average caffeine intake specifically for sport performance (i.e., training, competition; includes coffee, energy drinks, gels, etc.). [c]The *p* values derived using analysis of variance, sport type using chi-square.

$p = .69$, ES = 0.09) or 4 mg/kg (55 ± 11 kg, $p = .42$, ES = 0.19) compared with placebo (53 ± 10 kg) on handgrip strength. Similarly, in those with the AC genotype, there was no effect of 2 mg/kg (56 ± 11 kg, $p = .67$, ES = –0.18) or 4 mg/kg (58 ± 11 kg, $p = .99$, ES = 0) compared with placebo (58 ± 12 kg). However, those with the CC genotype experienced a 12.8% (8 kg) decrease in handgrip strength with 4 mg/kg of caffeine (53 ± 11 kg, $p = .02$, ES = –0.60), compared with placebo (61 ± 17 kg). Figure 2 displays individual differences in handgrip strength with 4 mg/kg of caffeine compared with placebo by *CYP1A2* genotype. The individual differences in performance are consistent with the results of the post hoc analysis; the majority of participants with the CC genotype worsened their performance with 4 mg/kg of caffeine compared with placebo. No difference was observed between 2 mg/kg (54 ± 9 kg, $p = .10$, ES = –0.55) and placebo (61 ± 17 kg). Adjustment with an athlete's sport type in the handgrip strength model produced a significant Caffeine × *CYP1A2* interaction ($p = .011$). Similar to the original model, there was no difference in caffeine's effects in those with the AA or AC genotypes. However, those with the CC genotype worsened their performance with 2 ($p = .016$) and 4 mg/kg ($p = .001$) caffeine compared with placebo. No difference was observed between 2 and 4 mg/kg ($p = .92$) caffeine in this model. Despite observing a significant effect of sport type on handgrip strength performance ($p = .042$), the post hoc analysis revealed no differences in handgrip strength between athletes of different sport types.

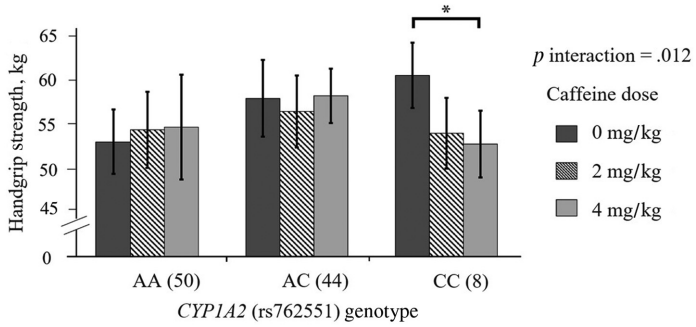

Figure 1 — Average (mean ± SEM) handgrip strength by caffeine dose and *CYP1A2* genotype. The *p* interaction values were generated from an adjusted model. *Within the CC genotype, the 4 mg/kg dose of caffeine differed significantly from placebo ($p = .022$).

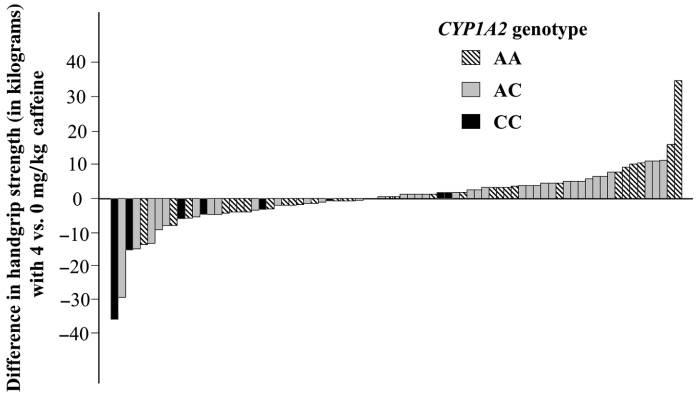

Figure 2 — Individual differences in handgrip strength with the 4 mg/kg dose of caffeine compared with placebo by *CYP1A2* genotype.

Caffeine, *CYP1A2*, and SQJ Performance

A significant Caffeine × *CYP1A2* interaction was also observed for SQJ performance ($p = .038$). However, when examining the association between caffeine and SQJ performance within each genotype, no significant differences in caffeine's effects were observed (Figure 3). In those with the AA genotype, there was no effect of 2 mg/kg (35 ± 6 cm, $p = .15$, ES = 0.51) and 4 mg/kg (33 ± 8 cm, $p = .99$, ES = 0.14) compared with placebo (32 ± 6 cm). Similarly, in those with the AC genotype, there was no effect of 2 mg/kg (33 ± 7 cm, $p = .82$, ES = 0.12) and 4 mg/kg (34 ± 9 cm, $p = .28$, ES = 0.21) compared with placebo (32 ± 10 cm). No effect was observed with 2 mg/kg (31 ± 5 cm, $p = .56$, ES = –0.70) and 4 mg/kg (31 ± 5 cm, $p = .73$, ES = –0.70) compared with placebo (35 ± 7 cm) in those with the CC genotype. Adjustment with an athlete's sport type in the SQJ model produced a significant Caffeine × *CYP1A2* interaction ($p = .04$). Similar to the original model, there was no difference in caffeine's effects in those with the AA, AC, or CC genotypes. Despite observing a significant effect of sport type on SQJ performance ($p < .0001$), the post hoc analysis revealed no differences in SQJ performance between athletes of different sport types.

Caffeine, *CYP1A2*, and CMJ Performance

A significant Caffeine × *CYP1A2* interaction was also observed for CMJ performance ($p = .025$). However, no significant differences in caffeine's effects were observed (Figure 4). In those with the AA genotype, there was no effect of 2 mg/kg (43 ± 7 cm, $p = .25$, ES = 0.27) or 4 mg/kg (41 ± 10 cm, $p = 1.0$, ES = 0) compared with placebo (41 ± 8 cm). Similarly, in those with the AC genotype,

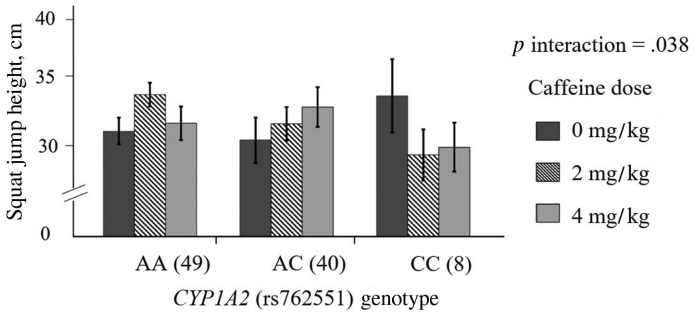

Figure 3 — Average (mean ± SEM) squat jump height by caffeine dose and *CYP1A2* genotype.

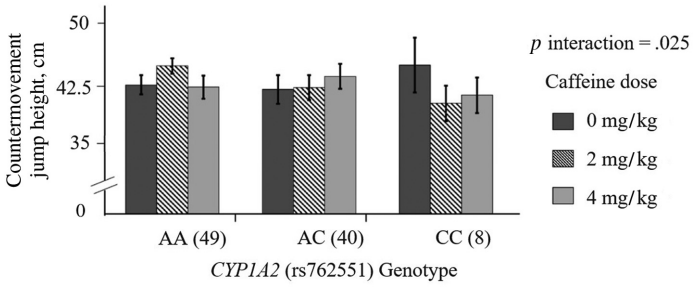

Figure 4 — Average (mean ± SEM) countermovement jump height by caffeine dose and *CYP1A2* genotype.

there was no effect of 2 mg/kg (41 ± 10 cm, $p = 1.0$, ES = 0.10) or 4 mg/kg (42 ± 10 cm, $p = .70$, ES = 0.19) compared with placebo (40 ± 11 cm). No effect was observed with 2 mg/kg (39 ± 6 cm, $p = .43$, ES = –0.52) and 4 mg/kg (40 ± 6 cm, $p = .73$, ES = –0.39) compared with placebo (43 ± 10 cm) in those with the CC genotype. Adjustment with an athlete's sport type in the CMJ model produced a significant Caffeine × *CYP1A2* interaction ($p = .03$). Similar to the original model, there was no difference in caffeine's effects in those with the AA, AC, or CC genotypes. Despite observing a significant effect of sport type on CMJ performance ($p < .0001$), the post hoc analysis revealed no differences in CMJ performance between athletes of different sport types.

Treatment Blinding

Following the 10-km cycling time trial, responses were collected from 86 participants who were asked whether they believed they had consumed caffeine. Out of 172 caffeine trials, 31% (54) were correctly identified as caffeine containing, 56% (96) reported "no caffeine" and 13% (22) reported "maybe caffeine." Only 3% (3) of participants correctly identified all three trials (i.e., two caffeine and one placebo).

Discussion

This study investigated the effects of acute caffeine consumption on handgrip strength and vertical jump performance and examined whether this relationship is modified by *CYP1A2* genotype. There was no main effect of caffeine on handgrip, SQJ, or CMJ performance. However, a significant Caffeine × *CYP1A2* interaction was observed for all three exercise tests. Among those with the CC genotype who are considered ultraslow metabolizers of caffeine, 4 mg/kg of caffeine impaired handgrip strength performance by ~13% compared with placebo. Handgrip strength measurements did not differ between caffeine doses and placebo in those with the AA or AC genotypes. Although a significant Caffeine × *CYP1A2* interaction was observed for both jump tests, further examination found no significant effect of caffeine within any of the genotypes.

A recent study found no effect of genetic variability in caffeine metabolism on the ergogenicity of caffeine on muscle strength and power (Spineli et al., 2020). In contrast, we found that *CYP1A2* genotype modified the effect of caffeine on handgrip strength. Notably, the mean ± *SD* age of participants was 15 ± 2 years, in contrast with 25 ± 4 years in the present study. Children have a limited ability to utilize fast-twitch motor units compared with adults, which may dampen performance in strength or power activities (Armatas et al., 2010). This might have masked any effects of caffeine and *CYP1A2* on strength and power performance, contributing to the apparent discrepancy between studies. In addition, caffeine use is not recommended in children as their rate of caffeine clearance might be slower (Bramstedt, 2007) and may reduce caffeine's effects on athletic performance.

The rate of caffeine metabolism may differ between individuals with the AC and CC genotypes (Ghotbi et al., 2007; Guest et al., 2018). Yet, some studies on handgrip strength and vertical jump performance have grouped these individuals together for analysis, largely due to sample size constraints (Grgic et al., 2020; Muñoz et al., 2020). 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. We did not combine those with the AC and CC genotype so that any differences in performance between these groups could be determined. Sex differences could also confound the effects of caffeine by *CYP1A2* genotype (Denden et al., 2016); however, another study (Muñoz et al., 2020) included male and female participants. Oral contraceptives are known to decrease the rate of caffeine clearance (Hukkanen et al., 2011) and Canadian females between the ages of 20 and 29 years commonly consume oral contraceptives (Black et al., 2009). The present 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.

During exercise, caffeine in the circulation has been shown to reduce flow-mediated vasodilation and myocardial blood flow, which can impair oxygen transport to the heart and other tissues (Higgins & Babu, 2013; Tian et al., 2019). Caffeine's effects on vasoconstriction may be more pronounced in those with the CC genotype of *CYP1A2* due to slower caffeine metabolism, which could explain the impairment observed with endurance performance (Guest et al., 2018) and muscle strength with 4 mg/kg of caffeine compared with placebo. The same cohort of athletes was used in the present study; five of the eight participants with the CC genotype of *CYP1A2* impaired their performance in the handgrip and both jump tests, as well as their endurance performance reported previously (Guest et al., 2018). This suggests caffeine appears to worsen overall performance in these individuals. We did not observe an effect of caffeine and *CYP1A2* genotype on vertical jump performance which could be explained by the effect of blood flow restriction training on vertical jump height. Blood flow restriction training has been shown to alter muscle strength, but not vertical jump performance (Horiuchi et al., 2018).

Endurance activities, such as a 10-km cycling time trial, elicit more fatigue compared with maximal tests, such as the vertical jump or handgrip strength test. Therefore, the rise in adenosine may be greater during long-duration efforts compared with short-duration maximal efforts. This may explain why those with the AA genotype in this study do not improve their handgrip or vertical jump performance with caffeine supplementation as adenosine antagonism may not be able to occur during short-duration maximal efforts.

The present study included competitive male athletes with a specific set of criteria which reduced differences in age, sex, and exercise habits. Using objective tests, such as the handgrip and vertical jump, allow for an accurate measure of performance compared with measuring performance in competition, which can vary due to several factors, including the presence of overtraining syndrome and familiarity with the racing distances or sport.

The present study has some limitations. For example, timing of caffeine administration may impact its effects on performance. However, peak values of plasma caffeine occur over a wide time range (30–120 min) between individuals (Guest et al., 2021). In addition, 6 mg/kg of caffeine capsules administered 30 min prior to exercise improved 2,000-m rowing performance by 2% in trained rowers (Carr et al., 2011). Therefore, caffeine may be ergogenic for some individuals after 30 min. In the future, these findings should be replicated in female athletes and the general population, with other common performance measures (e.g., one repetition maximum bench press) to improve the understanding of the relationship between caffeine and genetic variability in caffeine metabolism on strength and power performance. Another limitation is the small number of individuals with the CC genotype, which is why most

studies group C-allele carriers together. Although caffeine doses were randomized to reduce learning effects, another limitation is a lack of a familiarization trial to the handgrip and vertical jump tests. Furthermore, four participants who worsened their performance with the CC genotype of *CYP1A2* consumed the 4 mg/kg caffeine dose on the first visit, and improvements in performance across the visits may be attributed to a learning effect.

In conclusion, we found that 4 mg/kg of caffeine negatively impacted handgrip strength in competitive male athletes with the CC genotype of *CYP1A2*. No differences in handgrip strength were observed between caffeine and placebo in those with the AA or AC genotype, and no effects of caffeine were observed with both jump tests in all genotypes. Awareness of *CYP1A2* genotype may be important for athletes considering caffeine supplementation, both as an ergogenic aid and as a general dietary substance.

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