nutraceuticals

MDPI

Article

The Effect of Caffeine Supplementation on Resistance and Jumping Exercise: The Interaction with *CYP1A2* and *ADORA2A* Genotypes

Emilia Zawieja [1,2], Agata Chmurzynska [1], Jacek Anioła [1], Bogna Zawieja [3] and Jason Cholewa [2,*]

[1] Department of Human Nutrition and Dietetics, Poznan University of Life Sciences, 60-637 Poznan, Poland; emilia.zawieja@up.poznan.pl (E.Z.); agata.chmurzynska@up.poznan.pl (A.C.); jacek.aniola@up.poznan.pl (J.A.)
[2] Department of Kinesiology, Coastal Carolina University, Conway, SC 29528, USA
[3] Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, 60-637 Poznan, Poland; bogna.zawieja@up.poznan.pl
* Correspondence: jason.m.cholewa@gmail.com; Tel.: +1-860-884-5998

Abstract: Purpose: To evaluate the association of *CYP1A2* and *ADORA2A* gene polymorphisms, paraxanthine concentrations, and habitual caffeine (CAF) intake with respect to muscular performance after acute CAF supplementation. Methods: A total of 27 resistance-trained males participating in the study ingested either 5 mg/kg of CAF or PL 45 min before a battery of exercise tests in a cross-over design. DNA was tested for the rs5751876 and rs762551 polymorphisms. Results: CAF improved performance in jumping average power, average velocity, max velocity, bench press in the first set, and peak power in the second set. For the *CYP1A2* genotype, C allele carriers improved in jumping average velocity (CAF: 1.77 ± 0.14 m/s, PL: 1.71 ± 0.16 m/s, $p < 0.001$), and AA homozygotes improved set 1 bench press (CAF: 9.7 ± 1.7 reps, PL: 8.9 ± 1.8 reps, $p = 0.046$). For the *ADORA2A* genotype, CC (CAF: 1.70 ± 0.20 m/s, PL: 1.67 ± 0.19 m/s, $p = 0.005$) and CT (CAF: 1.79 ± 0.09 m/s, PL: 1.74 ± 0.11 m/s, $p < 0.001$) improved in jumping average velocity and CT also improved in bench press set 2 peak power (CAF: 363 ± 76 W, PL: 323 ± 59 W, $p = 0.021$). For CAF habituation, CAF improved jumping average power ($p = 0.007$) and jumping average velocity ($p < 0.001$) in high users but not in low users ($p > 0.05$). Conclusions: CAF may improve jumping and bench press performance, irrespective of genotypes, but the associations with the genotypes in *CYP1A2* and *ADORA2A* genes, as well as habitual CAF intake, are not clear and require further investigation.

Keywords: caffeine; sport; single nucleotide polymorphism; athletes; nutrigenomics

Citation: Zawieja, E.; Chmurzynska, A.; Anioła, J.; Zawieja, B.; Cholewa, J. The Effect of Caffeine Supplementation on Resistance and Jumping Exercise: The Interaction with *CYP1A2* and *ADORA2A* Genotypes. *Nutraceuticals* **2023**, *3*, 274–289. https://doi.org/10.3390/ nutraceuticals3020022

Academic Editor: Ronan Lordan

Received: 1 May 2023
Revised: 1 June 2023
Accepted: 15 June 2023
Published: 19 June 2023

1. Introduction

Caffeine (CAF) (1,3,7-trimethylxanthine), a mild stimulant and widely-consumed sport supplement, is naturally present in a variety of foods but is most commonly consumed in the human diet from coffee, tea, and chocolate. The benefits of acute CAF intake in resistance exercise may result from stimulation of the central nervous system, thereby delaying fatigue [1]. However, even though CAF supplementation has been shown to significantly increase muscular strength, large inter-individual variations in response to CAF are observed [1]. This variability may be connected with genetic variations, habituation to CAF, the placebo (PL) effect, or training status.

Heterogeneity in individual responses to CAF may be partially explained by genetic variations in two genes, cytochrome P450 family 1 subfamily A member 2 (CYP1A2) rs762551 and adenosine A_{2A} receptor (ADORA2A) rs5751876. CYP1A2 is an enzyme responsible for >90% of CAF metabolism to paraxanthine (84% of metabolites), theophylline (12% of metabolites), and theobromine (4% of metabolites) [2]. Variations in the gene encoding for the CYP1A2 enzyme can influence CAF metabolism. A single nucleotide

polymorphism (SNP) (rs762551, 163 C>A) has been identified as the major source of inducibility of CYP1A2 [3]. Individuals with the homozygous AA alleles show enhanced CAF metabolism and have been classified as "fast metabolizers", whereas C allele carriers (AC and CC) have a reduced CAF metabolism and are known as "slow metabolizers" [3]. Relative to the amount of research on CAF and performance per se, there is limited information on the interactions between *CYP1A2* genotypes and the ergogenics of CAF. Some studies showed improvement with CAF in AA fast metabolizers in cycling trials [4,5]. However, studies investigating *CYP1A2* interaction with CAF on resistance exercise are scarce.

The relationship between *CYP1A2* AA homozygotes and greater ergogenic response to CAF ingestion may seem paradoxical. That is, a faster CAF clearance rate yields greater performance. This finding may be explained by examining the physiological activities of CAF's metabolites, specifically paraxanthine. Paraxanthine has been shown to cause similar physiological effects to CAF, such as catecholamine release, increased blood pressure, and increased lipolysis, despite smaller plasma concentrations than CAF [6]. These similar effects at lower concentrations may be because paraxanthine has a slightly higher affinity for adenosine receptor A_{2A} and a significantly higher affinity for A_{1A} receptors, whereby A_{1A} receptor antagonism seems most responsible for the acute effects of CAF ingestion [7–9]. In humans, two recent studies found that 100 to 200 mg of paraxanthine ingestion resulted in improved cognition, short-term memory, attention, and reaction time compared to PL [3,10]. With respect to resistance exercise, Jäger et al. [11] reported that 4 weeks of paraxanthine treatment in mice led to greater grip strength and forelimb hypertrophy compared to control or alpha-GPC supplementation. To our knowledge, the relationship between paraxanthine and resistance exercise performance has not yet been studied in humans. Based on the aforementioned clues, we hypothesize that *CYP1A2* AA homozygotes generate greater physiologically active plasma paraxanthine values during exercise, leading to a synergistic effect with CAF that does not occur to the same magnitude in *CYP1A2* C allele carriers.

The *ADORA2A* gene encodes for the adenosine receptor A_{2A}, found predominantly in the brain. The binding of adenosine to its brain receptors causes the sensation of fatigue [12]. Due to similarities in structure, CAF also has an affinity to adenosine receptors but acts as an inhibitor [12,13]. The *ADORA2A* genotype rs5751876 (1976 C>T) may affect the response to CAF ingestion and habitual CAF intake [14]. It appears that TT genotype carriers report greater CAF-induced anxiety compared to CC genotype carriers [15]. Studies investigating the influence of *ADORA2A* polymorphism on performance are scarce. One small study showed that *ADORA2A* TT female athletes improved their 10 min cycling performance after CAF supplementation, while C allele carriers did not [16]. Later, Grgic et al. [17] observed that C allele carriers might also benefit from CAF use since 21 out of 25 performance outcomes were improved with 3 mg/kg in C allele carriers. Other studies showed that *ADORA2A* polymorphism was not associated with a response to 3 mg/kg of CAF [18–20]. There are no studies investigating the relationship between CAF supplementation, *ADORA2A* TT polymorphisms, and resistance exercise.

In addition to *CYP1A2* and *ADORA2A* gene polymorphisms, habitual CAF intake is purported to be another factor contributing to the observed inter-individual variations in response to CAF supplementation. In rats, one week of CAF treatment increased the number of adenosine A_{1A} receptors, which could, in theory, decrease the anti-fatigue action of the same dose of CAF ingested [19]. Another study showed that chronic ingestion of CAF by mice caused a marked reduction in exploratory locomotor activity, and at least a 4-day withdrawal period was necessary to restore the activity to normal levels [21]. However, studies in humans are not consistent regarding the effect of habituation on the ergogenic response to acute CAF supplementation [22–28].

There are relatively few studies investigating the effects of the *CYP1A2* and *ADORA2A* genotypes and CAF habituation on the ergogenics of CAF. More research is needed to enable practitioners and coaches to tailor individualized CAF supplementation regimes for athletes to achieve the maximum possible ergogenic effect in their specific sport. For this reason, the aim of our study was to evaluate the effect of *CYP1A2* (rs762551) and *ADORA2A*

(rs5751876) gene polymorphisms, paraxanthine concentrations, and habitual CAF intake on muscular strength, power, and endurance after acute CAF supplementation.

2. Materials and Methods

2.1. Experimental Approach to the Problem

To investigate the effects of CAF intake on strength and power performance, a double-blind, counter-balanced, PL-controlled, cross-over design was used. Body composition, saliva collection for genetic analysis, and habitual CAF consumption were measured during the first session. Additionally, 1RM bench press testing was conducted during the first session. Familiarization with the isometric mid-thigh pull (IMTP), the modified Bosco jump test, and bench press muscular endurance tests were conducted during the first and second sessions. IMTP, jumping performance, and bench press performance were tested in two separate trials, one trial with CAF and the other with PL, in random order, each separated by 5–7 days of rest. Saliva samples were collected three times during each testing session, before drinking the testing beverage (pre-ingestion), 45 min post-ingestion, and after the exercise session (~120 min post-ingestion). All experimental procedures were approved by the Coastal Carolina University Institutional Review Board (IRB # 2020.03) prior to data collection. All experimental procedures were explained to the subjects, and written informed consent was collected prior to study participation.

2.2. Subjects

Twenty-eight recreationally resistance-trained males between 18 and 35 years of age were recruited for this study. One subject dropped out due to an injury. Twenty-seven subjects completed the study. Inclusion criteria included participation in resistance training, including the squat and bench press exercises, for a minimum of 3 days/week for at least 1 year. Exclusion criteria included consumption of anabolic agents known to influence muscular performance, existing musculoskeletal disorders, known cardiovascular disorders, and known adverse effects to CAF consumption. All subjects completed a health history questionnaire, and only subjects classified as "Low Risk", according to the American College of Sports Medicine, were included [29]. Subjects were asked to maintain their current level of training throughout the study but to avoid any strenuous activity for 24 h prior to each trial. Subjects were also instructed to abstain from alcohol and tobacco and not alter their habitual CAF intake during the study period, but to abstain from CAF ingestion 24 h prior to testing sessions.

Subjects were divided into groups based on *CYP1A2* and *ADORA2A* gene polymorphisms and habitual CAF intake post hoc. Descriptive characteristics are given in Table 1. There were no significant differences in baseline characteristics between subgroups, except for significant differences in CAF intake between the high- and low-intake groups ($p < 0.001$).

2.3. Procedures

Subjects reported to the laboratory on four separate occasions, separated by 5–7 days. All subjects were instructed to refrain from consuming food and caloric beverages at least 3 h prior to testing and were instructed to cease CAF ingestion at least 24 h prior to each trial. In the first session, anthropometric measures were conducted, saliva samples were collected for genotyping, the CAF intake questionnaire was administered, the 1RM bench press was tested according to National Strength and Conditioning Association (NSCA) procedures, and subjects were familiarized with the performance testing procedures [30]. The second session was a familiarization session, and subjects performed all performance tests with maximum effort. Sessions three and four consisted of performance testing. Either the PL or CAF was administered 45 min prior to exercise in a randomized fashion. Following a standardized dynamic warm-up, subjects performed IMTP, a vertical jump test, and four sets of bench presses. Two research assistants, including one NSCA Certified Strength and

Conditioning Specialist (NSCA-CSCS), were present during all testing sessions to ensure proper safety and execution of the exercises.

Table 1. Descriptive characteristics of subjects.

		CYP1A2		*ADORA2A*			Habitual CAF Intake	
	All	**AA**	**C allele Carriers**	**CC**	**CT**	**TT**	**Low**	**High**
Age (years)	22.6 ± 4.1	22.4 ± 3.6	22.9 ± 4.8	23.2 ± 4.8	22.7 ± 4.2	21.0 ± 1.8	22.1 ± 4.5	23.2 ± 3.9
Height (cm)	177.8 ± 7.2	178.6 ± 6.4	176.6 ± 8.2	174.9 ± 6.7	180.4 ± 6.8	177.9 ± 8.5	176.1 ± 7.6	179.6 ± 6.6
Weight (kg)	83.5 ± 17.4	83.4 ± 9.7	83.8 ± 25.3	80.8 ± 10.4	83.0 ± 15.2	92.5 ± 35.9	78.4 ± 16.0	89.1 ± 17.8
Body fat (%)	17.3 ± 7.2	16.2 ± 6.9	19.1 ± 7.4	18.6 ± 7.2	15.6 ± 5.9	19.1 ± 11.3	16.1 ± 4.6	18.6 ± 9.3
RT experience (years)	4.3 ± 2.2	4.2 ± 2.2	4.5 ± 2.3	4.1 ± 2.7	4.5 ± 2.9	4.5 ± 1.7	4.0 ± 2.0	4.7 ± 2.5
1RM Bench press (kg)	100 ± 21	105 ± 17	93 ± 23	100 ± 19	98 ± 16	111 ± 37	93 ± 17	108 ± 22
CAF intake (mg/d)	145 ± 137	171 ± 158	107 ± 93	152 ± 145	117 ± 135	212 ± 125	35 ± 31	264 ± 100
CYP1A2 (AA/C allele)	n = 16/n = 11	NA	NA	n = 7/n = 4	n = 8/n = 4	n = 1/n = 3	n = 7/n = 7	n = 9/n = 4
ADORA2A (CC/CT/TT)	n = 11/n = 12/ n = 4	n = 7/n = 8/ n = 1	n = 4/n = 4/ n = 3	NA	NA	NA	n = 6/n = 4/ n = 3	n = 5/n = 8/ n = 1

1RM, one repetition maximum; CAF, caffeine; RT, resistance training.

2.4. Anthropometrics

Height was measured to the nearest 0.1 cm without shoes using a stadiometer. Weight was measured to the nearest 0.1 kg using an electronic scale (Cosmed, Concord, CA, USA), with subjects wearing light-weight athletic clothing, no shoes, and having removed all metal and jewelry. Body composition was measured with whole-body densitometry using air displacement plethysmography (Bod Pod®, Cosmed, Concord, CA, USA). All testing was performed in accordance with the manufacturer's instructions, and subjects were tested while wearing only tight-fitting compression shorts and a lycra swim cap. Thoracic gas volume was estimated for all subjects using a predictive equation integral to the Bod Pod® software (OMNIA 2.1). The calculated value for body density was the Siri equation to estimate body composition. Data from the Bod Pod® included body weight, percent body fat, fat-free mass, and fat mass. Based on a small pilot study (n = 6), the intraclass correlation coefficient (ICC) and standard error of measurement (SEM) for the percent body fat from our lab are 0.998 and 0.56%, respectively.

2.5. One Repetition Maximum Testing

Repetition maximum testing was consistent with recognized guidelines as established by the National Strength and Conditioning Association [31]. Subjects performed a specific warm-up set of 5 repetitions performed at ~50% of their estimated 1RM followed by 1 to 2 sets of 2–3 repetitions at a load corresponding to ~60–80% 1RM. Then, subjects performed sets of 1 repetition of increasing weight for 1RM determination. A 3 to 5 min rest was provided between each successive attempt. All 1RM determinations were made within 5 attempts. Subjects were required to touch the bar to their lower chest without bouncing in the 1RM bench press to be considered valid. Using 1RM testing has been shown to be a valid (r = 0.88) [32] and reliable (ICC = 0.96) [33] measure to assess changes in muscle strength following resistance training in a trained population. Based on the results of a small pilot study (n = 6), the test–retest from our lab for bench press 1RM revealed an ICC and SEM of 0.980 and 1.00 kg, respectively.

2.6. Exercise Testing

Approximately 45 to 50 min after consuming the beverage (CAF or PL), subjects performed a standardized exercise session. The small difference in the pre-ingestion period is due to individual differences in salivation rate (saliva collection). First, subjects performed a 5 min aerobic warm-up consisting of incline walking on a motorized treadmill. Then, subjects performed 5 min of self-directed dynamic stretches that were recorded and repeated each session. Finally, subjects performed a specific warm-up for each exercise,

and exercises were performed in the following order: IMTP, jumping, and bench press. IMTP and jumping were separated by a 5 min rest. Jumping and the bench press-specific warm-up were separated by a 2 min rest.

2.7. Isometric Mid-Thigh Pull

Lower body muscular force was assessed with the isometric mid-thigh pull (IMTP), which is a reliable and well-validated measure of strength independent of training status [34], and has shown an ergogenic response to CAF supplementation [35]. A wooden platform with a 4400-newton capacity load cell (iLoad Pro, Loadstar Sensors, Fremont, CA, USA) mounted to the center and attached to a steel bar with an adjustable chain was constructed, similarly to that described by James et al. [36]. Accompanying software (iLoad Pro Digital USB, Loadstar Sensors, Fremont, CA, USA) sampling at 150 HZ was used to measure peak force. The portable IMTP device using a single axial load cell has demonstrated very high reliability (coefficient of variation (CV) = 3.10 N, 90% confidence intervals (CI): 2.4–4.6%; ICC = 0.96, 90% CI: 0.90–0.98) and acceptable validity (CV = 9.2 N, 90% CI: 7–14%; ICC = 0.88, 90% CI: 0.71–0.95) for measuring peak force when compared to the gold standard force plate measurement [35].

During the first familiarization session, subjects were positioned shoeless on the platform holding the bar such that their torsos were upright, their knees were flexed to 120–130 degrees, and their arms were straight. The number of chain links connecting the bar to the load cell was recorded and maintained for all testing conditions. Lifting straps were used to account for grip strength limitations. Subjects were instructed to remove any slack from the chain and to drive straight up as forcefully as possible. A researcher visually inspected the chain to ensure vertical alignment and that the subject was not leaning back. Subjects first performed an IMTP at 50% effort, and then successive pulls were performed at maximal effort for 5 s, separated by 2 min of rest. During the familiarization sessions, subjects performed 4 maximal effort pulls, or until the peak force no longer increased from attempt to attempt. During the testing sessions, subjects performed three IMTPs, and a fourth attempt was only performed if the third attempt was at least 60 newtons greater than the first two.

2.8. Bosco Jump Test

To measure lower body anaerobic capacity, subjects performed a modified Bosco jump test [37]. Subjects stood with their feet hip-width apart and held a PVC dowel on their shoulders connected to a GymAware unit (Kinetic Performance, Canberra, Australia). The GymAware consists of a linear position transducer that attaches to the end of the barbell, which measures displacement and time and has shown good reliability for measuring displacement during countermovement jumps (ICC = 0.95; day-to-day mean difference = 0.47 $\pm$ 3.32 cm) [38]. To perform the test, subjects descended rapidly into a half squat and then immediately jumped straight up. Subjects were required to land with their knees and hips slightly flexed and return to a standing position prior to commencing the next countermovement vertical jump. A total of 15 countermovement vertical jumps were performed without any rest in between jumps. Peak and mean velocity and peak and mean power were provided by the GymAware software for each repetition, and the 15 repetitions were averaged to produce average mean power, average peak power, average mean velocity, and average peak velocity.

2.9. Bench Press Testing

Prior to testing, subjects performed 1 set of 10 repetitions at 50% of their 70% 1RM and 1 set of 5 repetitions at 75% of their 70% 1RM, separated by 2 min of rest. Subjects then performed 4 sets of bench press exercises with a load corresponding to 70% of the previously determined 1RM and a 3 min rest between sets. Subjects were instructed to perform as many repetitions as possible using the correct technique described above. For each repetition, subjects used a 2 s eccentric phase followed by a 1 s pause and a maximal

exertion concentric phase, cued by the instruction to "drive the bar off your chest as fast as possible". The rationale for using a maximum velocity concentric movement is that CAF has been shown to enhance high-velocity movements [39]. The first 3 sets were performed to 60% fatigue, measured by a 40% reduction in mean velocity via the GymAware. The fourth set was performed with a 2 s eccentric, no pause, and a 1–2 s concentric cadence until momentary muscular failure. The number of repetitions performed in each set was recorded.

Mean and peak power output and bar velocity during the bench press were measured for each repetition with a GymAware unit (Kinetic Performance, Canberra, Australia). High validity (r^2 = 0.91) and good reproducibility (typical error between sessions of 3–7% for displacement and velocity) have been reported in the bench press exercise measured with GymAware (version 2.6) [40]. Peak and mean power and mean velocity were recorded for each repetition. The number of repetitions performed at or above 90% peak and mean power was calculated and used to analyze the quality of repetitions, as previously described by Hoffman et al. [41].

2.10. Habitual CAF Consumption

Habitual CAF consumption was measured via a food frequency questionnaire (FFQ), adapted from a previously validated FFQ specifically developed to measure CAF consumption in young adults [42]. The FFQ was administered by a qualified nutritionist. Portions, in typically consumed measures, were assessed according to the following frequencies: greater than three times per day, two to three times per day, once per day, five to six times per week, two to four times per week, once per week, three times per month, and rarely or never. The list is composed of 10 dietary products with high CAF content: espresso drinks, brewed coffee, instant coffee, green tea, black tea, energy drinks, colas, dark and milk chocolates, sweet cocoa powder, and CAF supplements. Where possible, exact brands were identified, and subsequent CAF content was obtained. These logs were used to determine habitual CAF intake and stratify subjects into high- and low-intake groups via the median. Average CAF intake was 34.9 $\pm$ 30.6 mg/d (range: 0–89.1 mg/d) in the low users (<90 mg/d) group and 263.8 $\pm$ 100.3 mg/d (range: 135.6–432.6 mg/d) in the high users (>90 mg/d) group.

Subjects were also instructed to maintain the same dietary intakes, including CAF ingestion, throughout the approximate 1-month study period and to refrain from CAF intake for 24 h prior to each testing session. To ensure compliance, subjects were required to record their food intake 24 h prior to the first testing trial and were provided with a copy to replicate the same food intake prior to the second trial.

2.11. Supplements

In sessions three and four, subjects ingested either CAF or PL powder mixed with 10 ounces of water and artificial flavoring (Crystal Light, Kraft Foods Inc., Chicago, IL, USA) 45 min prior to the exercise warm-up (to allow substance absorption). This timing resulted in the treatments being consumed approximately an hour before the first performance test. Both testing beverages were identical in flavor and appearance. Study beverages were prepared by a third party not engaged in the study to ensure double-blinding. Anhydrous CAF was administered in a dose of 5 mg/kg of body weight (average 7.6 $\pm$ 87.1 mg per portion, range: 267.5–715.0 mg). PL and CAF supplements were provided by Aspire Train Perform (ATP Nutrition, Denver, CO, USA).

2.12. Genotyping

Saliva samples were collected by the primary investigator using the Oragene ON-575 kit (DNA Genotek, Ottawa, ON, Canada) according to manufacturer protocols. This protocol uses manual purification of DNA via a collection tube with 1 mL of suspension buffer containing proprietary reagents that stabilize the whole saliva sample prior to DNA extraction. Specifically, approximately 1 mL of saliva is mixed with the collection buffer

and 500 µL of this mixture is used for the DNA extraction. The final elution volume is 100 µL. This protocol provides consistent results in terms of the concentration and purity of DNA extracted from whole saliva [43].

Genomic DNA was isolated from saliva using a NucleoSpin® Tissue kit (Macherey-Nagel, Germany). Genotyping of the rs5751876 and rs762551 SNPs in the *ADORA2A* and *CYP1A2* genes, respectively, was performed via real-time polymerase chain reaction (PCR) using TaqMan probes (Single Tube Genotyping Assay, Thermo Fisher Scientific, Waltham, MA, USA). PCR was conducted using Light Cycler 480 (Hoffmann-La Roche, Basel, Switzerland). Because CAF metabolism is similar between *CYP1A2* heterozygotes and CC homozygotes, subjects were grouped as AA homozygotes or C allele carriers (AC, CC) for analysis. Genotype AA is known for fast CAF metabolism, and C allele carriers are slow metabolizers. Because subjects with TT, CT, and CC genotypes show different responses to CAF, for *ADORA2A* in our study, subjects were grouped as CC, CT, and TT.

2.13. Salivary Paraxanthine and CAF

Saliva samples were collected by the primary investigator via the passive saliva drool method into a 2 mL cryovial (Salimetrics, State College, PA, USA) according to manufacturer instructions. Samples were collected at baseline (pre-ingestion), 45 min post-ingestion, and post-exercise (~120 post-ingestion). Saliva samples were stored at −80 °C until analysis. Saliva CAF and paraxanthine concentrations were quantified following liquid–liquid extraction and high-pressure liquid chromatography as described by Perera et al. [44]. Salivary CAF and paraxanthine were measured using a Hitachi Lachom 7000 high-performance liquid chromatography (HPLC) analyzer (Merck, Darmstadt, Germany). A LiChroCART® 250-4 (LiChrospher®100, RP-18, 5 µm, Merck, Darmstadt, Germany) column was used. The mobile phase consisted of acetonitrile–acetic acid–H_2O (100:1:899). Paraxanthine, CAF, and the internal standard benzotriazole were quantitated with UV detection at 280 nm. Using an eluent flow rate of 1 mL/min, the retention times were 7 min (paraxanthine) and 14 min (CAF).

2.14. Statistical Analysis

A power analysis was performed using G*Power software (version 3.1.9.7; Germany, Dusseldorf) for repeated-measures analysis of variance (ANOVA) (within–between interaction), with an assumed true effect size f of 0.25, the alpha error level of 0.05, and the expected correlation between repeated measures of 0.75; the required sample size to achieve the statistical power of 90% for this study was 24 participants. The normality of the data was checked with the Shapiro–Wilk (Levene test version 3, 6, 2 package stats) test. The homogeneity of variance was checked with the Levene test with function leveneTest (from car packed version 3.1-1, in R). A 2 (treatment: CAF and PL) × 3 (time: pre, 45 min post, and ~120 min post-ingestion) general linear model (GLM) with repeated measures was used for CAF and paraxanthine concentrations analyses. A 2 (treatment: CAF and PL) × 3 (set: set 1, set 2, set 3) GLM with repeated measures was used for the number of bench press repetitions, the mean velocity, and the peak power in the bench press. A 2 (treatment: CAF and PL) × 2 (repetitions) GLM with repeated measures was used for IMTP. A 2 (treatment: CAF and PL) × 1 GLM with repeated measures was used for IMTP average, jumping variables, and bench press reps in set 4 and total. All analyses were completed using the gls procedure from nmle* (version 3.1-162) packed implemented in platform R, and an alpha level of <0.05 was set a priori.

3. Results

3.1. Salivary CAF and Paraxanthine Concentrations

For salivary CAF concentrations, the following interactions were significant: treatment × time ($p < 0.001$, $\eta^2 = 0.77$) and treatment × time × habituation ($p < 0.001$, $\eta^2 = 0.03$). There was also a significant main effect of treatment ($p < 0.001$, $\eta^2 = 0.95$). Salivary CAF concentration increased 30-fold after CAF ingestion ($p < 0.001$) and was

20 times greater than during the PL trial ($p < 0.001$) at 45 min post-ingestion ($p < 0.001$) (Figure 1). Compared to 45 min post-ingestion, salivary CAF concentration decreased post-exercise (~120 min post-ingestion) (−27%, $p = 0.0616$) but was still greater than pre-ingestion (22 times higher, $p < 0.001$) and greater than PL ($p < 0.001$). Post hoc analysis for treatment × time × habituation interaction showed that in high CAF users, CAF concentrations increased from pre-ingestion to 45 min post-ingestion ($p < 0.001$) and then significantly decreased ~120 post-ingestion compared to 45 min post-ingestion ($p = 0.020$). However, in low CAF users, CAF concentrations increased from pre-ingestion to 45 min post-ingestion ($p < 0.001$) and did not significantly change from 45 min to ~120 min post-ingestion ($p = 0.987$). CAF concentration at 45 min post-ingestion in high CAF users was 36% higher than in low CAF users.

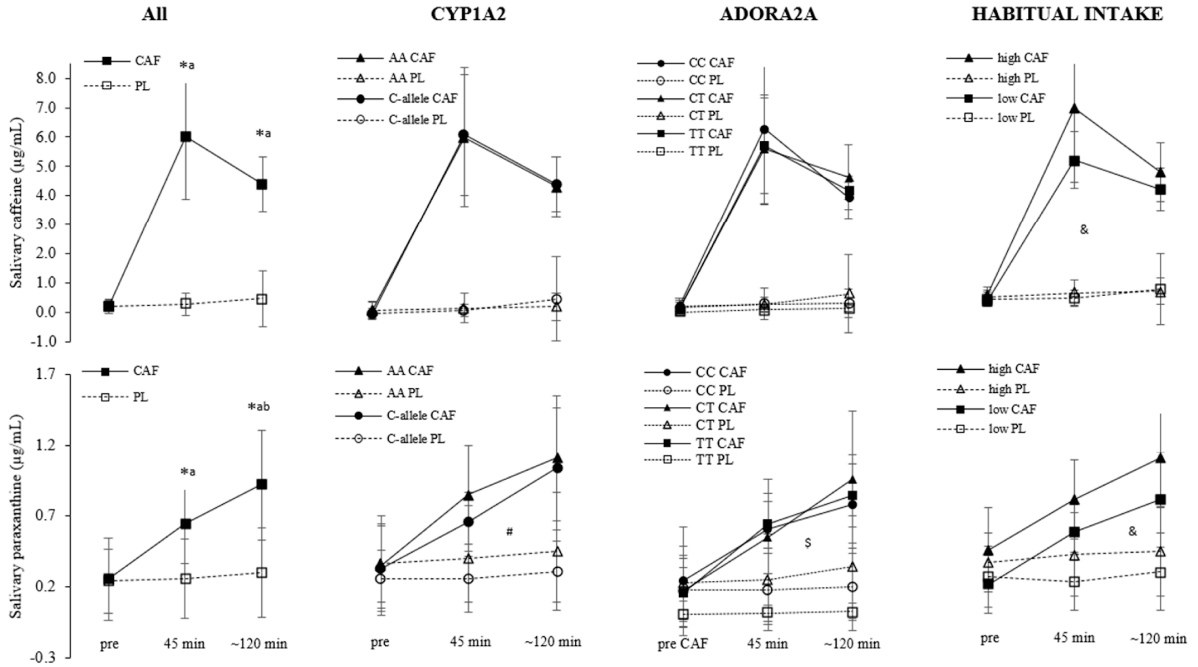

Figure 1. Salivary CAF and paraxanthine concentrations. CAF, caffeine; PL, placebo. * CAF significantly different from PL, # significant treatment × time × *CYP1A2* interaction $ significant treatment × time × *ADORA2A* interaction, & significant treatment × time × habituation interaction, [a] significantly different than pre-ingestion, [b] significantly different than 45 min post-ingestion.

For salivary PXN concentrations the following interactions were significant: treatment × time ($p < 0.001$, $\eta^2 = 0.62$), treatment × time × *CYP1A2* ($p < 0.001$, $\eta^2 = 0.05$), treatment × time × *ADORA2A* ($p < 0.001$, $\eta^2 = 0.08$), and treatment × time × habituation ($p = 0.0142$, $\eta^2 = 0.07$). There was also a significant main effect of treatment ($p < 0.001$, $\eta^2 = 0.61$). After CAF intake, paraxanthine concentrations increased 2.5 times 45 min post-ingestion ($p < 0.001$) and then increased further ~120 min post-ingestion compared to baseline ($p < 0.001$). Paraxanthine concentrations after CAF ingestion were higher ~120 min post-ingestion compared to 45 min post-ingestion ($p = 0.006$). Paraxanthine concentrations after CAF were higher than after PL at 45 min ($p = 0.001$) and at ~120 min post-ingestion ($p < 0.001$). Post hoc analysis for treatment × time × *CYP1A2* interaction showed that in C allele slow metabolizers, paraxanthine concentration increased from pre-ingestion to 45 min post ($p < 0.001$) and then from 45 min to ~120 min post-ingestion ($p < 0.001$). At ~120 min post-ingestion, paraxanthine concentrations were higher after CAF than after PL ($p < 0.001$). However, compared to PL paraxanthine concentrations, after CAF intake were not significantly different at 45 min post-ingestion ($p = 0.105$). On the other hand, in AA fast metabolizers, paraxanthine concentrations increased significantly from pre-ingestion to 45 min post-ingestion ($p < 0.001$) but did not increase significantly from 45 min to ~120 min

post-ingestion ($p = 0.952$). At 45 min post-ingestion, paraxanthine concentrations were higher compared to PL ($p = 0.045$) but not at ~120 min ($p = 0.082$). Post hoc analysis for treatment × time × *ADORA2A* interaction revealed that in CC, paraxanthine concentrations increased from pre- to 45 min post-ingestion ($p < 0.001$), but it did not significantly increase further from 45 min to ~120 min post-ingestion ($p = 0.726$). At both 45 min and ~120 min post-ingestion, paraxanthine levels were higher after CAF compared to PL ($p = 0.029$ and $p = 0.004$, respectively). A similar situation was reported for TT, paraxanthine concentrations increased from pre- to 45 min post-ingestion ($p < 0.001$), but it did not significantly increase further from 45 min to ~120 min post-ingestion ($p = 1.000$). However, paraxanthine levels after CAF were not different than after PL at both 45 min and ~120 min post-ingestion ($p = 0.561$ and $p = 0.739$, respectively). For CT, paraxanthine concentrations increased first from pre- to 45 min post-ingestion ($p < 0.001$) and then increased further from 45 min to ~120 post-ingestion ($p < 0.001$). At ~120 min post-ingestion, paraxanthine levels were higher after CAF than after PL ($p = 0.001$), but not at 45 min post-ingestion ($p = 0.551$).

3.2. IMTP

When analyzing two IMTP measurements, there were no significant interactions. There were also no significant differences in IMTP when analyzing the IMTP average (Table 2).

Table 2. The effect of caffeine supplementation on isometric mid-thigh pull and jump performance: interaction with *CYP1A2* and *ADORA2A* genes and habitual caffeine intake.

		All Subjects	*CYP1A2* rs762551		*ADORA2A* rs5751876			Habitual Caffeine Intake	
			AA	C-Allele Carriers	CC	CT	TT	High	Low
1 IMTP force (N)	CAF	2805 ± 525	2970 ± 543	2580 ± 424	2775 ± 548	2780 ± 526	2954 ± 586	2921 ± 620	2706 ± 426
	PL	2804 ± 564	2894 ± 650	2680 ± 420	2898 ± 661	2674 ± 501	2958 ± 542	2851 ± 658	2763 ± 493
2 IMTP force (N)	CAF	2794 ± 574	2977 ± 582	2544 ± 480	2768 ± 660	2777 ± 535	2911 ± 606	2944 ± 694	2665 ± 432
	PL	2757 ± 541	2874 ± 552	2596 ± 506	2764 ± 608	2690 ± 483	2941 ± 643	2866 ± 616	2664 ± 471
Av IMTP force (N)	CAF	2800 ± 544	2974 ± 555	2562 ± 449	2772 ± 597	2778 ± 527	2933 ± 590	2932 ± 650	2686 ± 426
	PL	2780 ± 543	2884 ± 602	2638 ± 330	2831 ± 630	2682 ± 478	2949 ± 588	2858 ± 624	2713 ± 477
Jump peak power (W)	CAF	2139 ± 395	2169 ± 370	2096 ± 444	2018 ± 394	2178 ± 276	2355 ± 661	2237 ± 363	2048 ± 415
	PL	2063 ± 486	2027 ± 526	2050 ± 377	1926 ± 376	2092 ± 399	2353 ± 897	2137 ± 525	1994 ± 455
Jump av power (W)	CAF	1846 ± 344 *	1852 ± 322	1836 ± 389	1722 ± 302	1901 ± 285	2022 ± 559	1941 ± 295 *	1758 ± 372
	PL	1792 ± 352	1779 ± 364	1767 ± 314	1682 ± 294	1834 ± 316	1970 ± 573	1852 ± 355	1736 ± 371
Jump velocity max (m/s)	CAF	1.87 ± 0.15 *	1.87 ± 0.16	1.87 ± 0.15	1.82 ± 0.19	1.91 ± 0.10	1.89 ± 0.14	1.83 ± 0.13	1.91 ± 0.16
	PL	1.83 ± 0.15	1.82 ± 0.14	1.82 ± 0.18	1.78 ± 0.19	1.85 ± −0.12	1.89 ± 0.11	1.78 ± 0.15	1.87 ± 0.15
Jump velocity av (m/s)	CAF	1.75 ± 0.15 *	1.74 ± 0.16	1.77 ± 0.14 *	1.70 ± 0.20 *	1.79 ± 0.09 *	1.77 ± 0.12	1.71 ± 0.13 *	1.78 ± 0.16
	PL	1.72 ± 0.15	1.71 ± 0.15	1.71 ± 0.16	1.67 ± 0.19	1.74 ± 0.11	1.75 ± 0.13	1.66 ± 0.14	1.76 ± 0.15

CAF, caffeine trial; PL, placebo trial; IMTP, isometric mid-thigh pull. * CAF significantly different from PL ($p < 0.05$).

3.3. Jump Performance

There were no significant interactions for jumping max power. There was a significant effect of treatment ($p = 0.002$, $\eta^2 = 0.25$) and treatment × habituation interaction ($p = 0.017$, $\eta^2 = 0.25$) in jumping average power. Jumping average power was higher after CAF than after PL in all participants (Table 2). Post hoc for the treatment × habituation interaction showed significant differences between CAF and PL in high CAF users ($p = 0.007$) and not in low CAF users ($p = 1.000$). There was also no difference between low and high CAF users. There was a significant effect of treatment ($p < 0.001$, $\eta^2 = 0.22$) and treatment × *ADORA2A* interaction ($p = 0.004$, $\eta^2 = 0.19$) in jumping max velocity. Jumping max velocity was higher after CAF than after PL in all participants (Table 2). Post hoc for the treatment × *ADORA2A* interaction showed significant differences between CAF and PL in CT ($p < 0.001$) and not in CC and TT. There was a significant effect of treatment ($p < 0.001$, $\eta^2 = 0.49$), treatment × *CYP1A2* ($p < 0.001$, $\eta^2 = 0.43$), treatment × *ADORA2A* ($p = 0.005$, $\eta^2 = 0.30$), and treatment × habituation ($p < 0.001$, $\eta^2 = 0.40$) interactions in jumping average velocity. Jumping average velocity was higher after CAF than after PL in all participants (Table 2). Post hoc for the treatment × *CYP1A2* interaction showed significant

differences between CAF and PL only in C allele slow metabolizers ($p < 0.001$). Post hoc for the treatment × *ADORA2A* interaction showed significant differences between CAF and PL in CC ($p = 0.005$) and CT ($p < 0.001$), and not in TT. Post hoc for the treatment × habituation interaction showed significant differences between CAF and PL only in high CAF users ($p < 0.001$) and not in low CAF users.

3.4. Bench Press

For bench press repetitions (three sets), there was a significant main effect of treatment ($p < 0.001$, $\eta^2 = 0.21$) regardless of set. There were the following significant interactions: treatment × set ($p < 0.001$, $\eta^2 = 0.04$), treatment × set × *CYP1A2* ($p < 0.001$, $\eta^2 = 0.18$), and treatment × set × *ADORA2A* ($p = 0.027$, $\eta^2 = 0.10$). Post hoc analysis for the treatment × set interaction showed that in set 1, CAF differed from PL ($p = 0.053$) (Table 3). Post hoc analysis for the treatment × set × *CYP1A2* interaction showed that in set 1, only AA fast metabolizers performed better after CAF compared to PL ($p = 0.046$) and not C allele slow metabolizers. Post hoc analysis for the treatment × set × *ADORA2A* interaction did not show differences in CC, CT, and TT in response to CAF. No significant interactions were found for bench press reps in set 4 to exhaustion and the total number of reps in all sets.

Table 3. The effect of caffeine supplementation on bench press repetitions: interaction with *CYP1A2* and *ADORA2A* genes and habitual caffeine intake.

		All Subjects	*CYP1A2* rs762551		*ADORA2A* rs5751876			Habitual Caffeine Intake	
			AA	C-Allele Carriers	CC	CT	TT	Low	High
Set 1 (reps to 60% fatigue)	CAF	9.4 ± 1.8 $^{\$}$	9.7 ± 1.7 *	9.0 ± 1.9	9.1 ± 2.1	9.6 ± 1.3	9.8 ± 2.8	9.1 ± 1.8	9.7 ± 1.9
	PL	8.9 ± 1.8	8.9 ± 1.8	8.9 ± 1.9	9.0 ± 2.0	8.8 ± 1.7	9.0 ± 2.0	8.9 ± 1.5	8.9 ± 2.1
Set 2 (reps to 60% fatigue)	CAF	7.8 ± 1.5 ^{a}	8.1 ± 1.5 ^{a}	7.5 ± 1.4 ^{a}	7.5 ± 1.6 ^{a}	8.1 ± 1.2 ^{a}	8.0 ± 2.0	7.5 ± 1.6	8.1 ± 1.3
	PL	7.9 ± 1.4 ^{a}	7.6 ± 1.3	8.3 ± 1.5	7.9 ± 1.6	7.9 ± 1.2	7.8 ± 1.5	7.8 ± 1.0	8.0 ± 1.7
Set 3 (reps to 60% fatigue)	CAF	7.2 ± 1.5 ^{a}	7.3 ± 1.4 ^{a}	7.2 ± 1.5 ^{a}	7.5 ± 1.4 ^{a}	7.1 ± 1.6 ^{a}	7.0 ± 1.4 ^{a}	7.4 ± 1.0	7.0 ± 1.8
	PL	7.1 ± 1.5 ^{a}	7.3 ± 1.6	6.8 ± 1.2 ^{ab}	7.3 ± 1.7	6.8 ± 1.2 ^{a}	7.5 ± 1.7	7.4 ± 1.6	6.9 ± 1.4
Set 4 (reps to exhaustion)	CAF	12.0 ± 3.0	12.3 ± 2.8	11.6 ± 3.4	11.2 ± 3.7	12.4 ± 2.2	13.3 ± 3.3	12.8 ± 3.1	11.4 ± 28
	PL	12.1 ± 3.7	12.4 ± 3.8	11.6 ± 3.6	11.4 ± 3.8	11.9 ± 3.0	14.8 ± 5.1	12.8 ± 3.8	11.4 ± 3.7
Total reps (four sets)	CAF	36.5 ± 5.9	37.3 ± 5.7	35.3 ± 6.3	35.2 ± 6.6	37.2 ± 4.5	38.0 ± 8.6	36.8 ± 5.5	36.2 ± 6.5
	PL	36.0 ± 6.3	36.3 ± 6.8	35.6 ± 5.9	35.6 ± 7.5	35.4 ± 4.8	39.0 ± 8.0	36.9 ± 5.7	35.1 ± 6.9

CAF, caffeine trial; PL, placebo trial. * CAF significantly different from PL ($p < 0.05$), $^{\$}$ CAF tended to be higher than PL ($p = 0.053$), ^{a} significantly different from set 1, ^{b} significantly different from set 2.

For peak power in the bench press, the following interactions were significant: treatment × set ($p < 0.001$, $\eta^2 = 0.33$), treatment × set × *CYP1A2* ($p = 0.025$, $\eta^2 = 0.01$), and treatment × set × *ADORA2A* ($p < 0.001$, $\eta^2 = 0.21$) (Table 4). The main effect of treatment approached significance ($p = 0.066$, $\eta^2 = 0.01$). Post hoc analysis for the treatment × set interaction showed that in set 2, the difference between CAF and PL approached significance ($p = 0.068$). Post hoc analysis for the treatment × set × *CYP1A2* interaction showed that there were no differences between CAF and PL relative to the *CYP1A2* genotype. Post hoc analysis for the treatment × set × *ADORA2A* showed that there was a significant difference between CAF and PL in CT in set 2 ($p = 0.021$). For bench press mean velocity, only the treatment × set ($p < 0.001$, $\eta^2 = 0.22$) interaction was significant, and the treatment × set × habituation interaction approached significance ($p = 0.064$, $\eta^2 = 0.03$) (Table 4). There was also a significant main effect of treatment ($p < 0.001$, $\eta^2 = 0.11$). Post hoc analysis for the treatment × set interaction did not reveal any significant differences between CAF and PL.

Table 4. The effect of caffeine supplementation on bench press peak power: interaction with *CYP1A2* and *ADORA2A* genes and habitual caffeine intake.

	All Subjects		*CYP1A2* rs762551		*ADORA2A* rs5751876			Habitual Caffeine Intake	
			AA	C-Allele Carriers	CC	CT	TT	High	Low
Set 1 peak power (W)	CAF	372 ± 79	381 ± 84	357 ± 74	364 ± 51	371 ± 95	394 ± 107	375 ± 72	368 ± 88
	PL	373 ± 81	383 ± 72	359 ± 94	374 ± 71	359 ± 64	413 ± 148	376 ± 89	371 ± 75
Set 2 peak power (W)	CAF	367 ± 78 *	369 ± 73	364 ± 87	356 ± 60	363 ± 76 *	410 ± 126	373 ± 85	361 ± 73
	PL	346 ± 77 [a]	348 ± 67 [a]	342 ± 91	361 ± 61	323 ± 59	372 ± 145	352 ± 88	340 ± 69
Set 3 peak power (W)	CAF	353 ± 73 [a]	358 ± 66 [a]	346 ± 85	344 ± 53	349 ± 75	388 ± 121	360 ± 75	346 ± 74
	PL	340 ± 87 [a]	343 ± 69 [a]	334 ± 115	342 ± 77	318 ± 58	398 ± 164	348 ± 104	333 ± 73
Set 1 mean velocity (m/s)	CAF	0.54 ± 0.08	0.52 ± 0.08	0.56 ± 0.07	0.54 ± 0.06	0.54 ± 0.11	0.52 ± 0.03	0.50 ± 0.04	0.57 ± 0.10
	PL	0.54 ± 0.08	0.52 ± 0.07	0.56 ± 0.08	0.54 ± 0.08	0.54 ± 0.08	0.53 ± 0.08	0.49 ± 0.05	0.58 ± 0.08
Set 2 mean velocity (m/s)	CAF	0.53 ± 0.08	0.50 ± 0.08	0.57 ± 0.07	0.52 ± 0.05	0.54 ± 0.10	0.53 ± 0.09	0.50 ± 0.05	0.56 ± 0.09
	PL	0.50 ± 0.08 [a]	0.48 ± 0.07	0.53 ± 0.08	0.52 ± 0.07	0.48 ± 0.09	048 ± 0.07	0.47 ± 0.05	0.52 ± 0.09
Set 3 mean velocity (m/s)	CAF	0.51 ± 0.09	0.49 ± 0.09	0.54 ± 0.08	0.50 ± 0.07	0.52 ± 0.10	0.51 ± 0.09	0.48 ± 0.06	0.54 ± 0.10
	PL	0.49 ± 0.09 [a]	0.47 ± 0.08	0.52 ± 0.09	0.49 ± 0.08	0.48 ± 0.10	0.51 ± 0.07	0.46 ± 0.06	0.52 ± 0.10

CAF, caffeine trial; PL, placebo trial. * CAF significantly different from PL ($p < 0.05$), [a] significantly different from set 1 ($p < 0.05$).

4. Discussion

The purpose of this study was to investigate the interactions between *CYP1A2* (rs762551) and *ADORA2A* (rs5751876) gene polymorphisms, paraxanthine concentrations, and habitual CAF intake on muscular strength, power, and endurance after acute CAF supplementation. Our results suggest that, overall, CAF supplementation improved several performance outcomes, especially jumping performance and maximum power in the bench press, but had little or no effect on other outcomes, that is, bench press endurance and maximal force in IMTP. The response to CAF supplementation may, in some cases, depend on *CYP1A2* and *ADORA2A* genotypes and habitual CAF intake.

4.1. Strength and Power Performance

In our study, mean power averaged across 15 jumps increased with CAF by 3% compared to PL, while average velocity and maximum velocity increased by 2% each. The influence of CAF on vertical jump performance (single jump or repeated jumps) has been measured previously in many studies. Specifically, two meta-analyses concluded that CAF might improve muscle power measured by jump performance with effect sizes ranging from 0.17 to 0.22 (2 to 4%), which is in agreement with our results [45,46].

When it comes to muscular strength, the main effect of treatment (CAF vs. PL) was present for bench press repetitions and mean velocity in the three rounds favoring CAF. CAF in our study increased peak power in set 2 by 6% and tended to increase the number of repetitions in set 1 of the bench press. However, there were no differences between CAF and PL in bench press repetitions in round 4 to failure, the total number of repetitions done in all four rounds, nor in the maximal strength measured in IMTP. It might seem, hence, that CAF in our study had only a small effect on strength, specifically upper-body strength. CAF's effect on strength has been summarized in several available meta-analyses. One of them found a significant increase in isokinetic strength with CAF [46]. The second one showed improved upper-body maximal strength, but the effect was small [45], which is in agreement with our results of bench press performance. Similarly, in a recent randomized trial, Filip–Stachnik et al. [47] showed that CAF increased mean power output and mean bar velocity during a multiple-set bench press exercise. Regarding maximal force production in IMTP, Harty et al. [35] showed that peak and mean IMTP force were significantly enhanced relative to PL for males, which was in contrast to our results. Generally, the majority of data suggest benefits from CAF on strength performance, but the effect is small, ranging from 0.16 to 0.20 (percent change: 2 to 7%) [45,46].

4.2. CYP1A2 and ADORA2A Genotypes

It was previously suggested that the response to CAF supplementation might depend on CAF metabolism regulated by genetic differences. The most frequently considered genes are *CYP1A2* rs762551 and *ADORA2A* rs5751876. In our study, SNPs in those genes were significant factors in some performance outcomes. *CYP1A2* AA homozygotes, called fast metabolizers, improved the number of bench press repetitions in set 1. On the other hand, *CYP1A2* C allele carriers, called slow metabolizers, improved average jump velocity on CAF. Generally, our study suggests no clear influence of the *CYP1A2* genotype on performance. Some previous studies showed no influence of the *CYP1A2* genotype on exercise performance [18,20,48], while others showed improved resistance performance in AA fast metabolizers and not in C allele carriers [5].

We also hypothesized that the mechanism of benefits in AA homozygotes could be explained by faster CAF metabolism to its metabolites (i.e., paraxanthine, theophylline, and theobromine), which have higher affinity to adenosine receptors [49]. Approximately 70–80% of CAF is metabolized to paraxanthine, which has been shown in mice to promote greater psychomotor stimulation and locomotor activity than CAF [50]. This would result in greater attenuation of fatigue and, thus, increased strength and endurance. We can neither confirm nor reject this hypothesis. Interestingly, a recent study found that subjects who metabolized a higher proportion of CAF to paraxanthine recorded a lower perceived exertion, but there was no connection with the 5 km running trial [51]. Further research is warranted to explore the relationship between CAF metabolism, paraxanthine, and exercise performance.

The *ADORA2A* (C>T) gene encodes for the adenosine receptor A_{2A} found predominantly in the brain and has a role in the down-regulation of dopamine and glutamate release [52]. Individuals with the TT genotype reported the highest anxiety, and CC individuals reported the least anxiety after 150 mg of caffeine [15]. High sensitivity may impair the response to CAF, which can be even ergolytic for TT homozygotes. In our study, TT homozygotes did not improve in any outcome measure after CAF ingestion. On the other hand, *ADORA2A* CC and CT improved jumping average velocity, and CT heterozygotes also improved peak power in set 2 of the bench press after CAF compared to PL. Even though our results suggest benefits from CAF ingestion in *ADORA2A* C allele carriers and no differences in TT homozygotes, it is important to realize that allele distribution in our population was very uneven. Among our participants (n = 27), only 4 were TT homozygous.

To our knowledge, our study is the first to evaluate the associations of *ADORA2A* polymorphism and the response to CAF in resistance exercise. One previous study assessed the effect of variation in this gene on the ergogenic effects of CAF on exercise performance in untrained females [16]. The study included six TT homozygotes and six C allele carriers and used 20 min of cycling at a work rate eliciting 60% of VO_2peak followed by two 10 min cycling time trials after both 5 mg/kg of CAF and PL. The results were opposite to ours, with TT homozygotes improving after CAF. They called TT homozygotes 'CAF responders' and C allele carriers 'non-responders'. However, there are several differences between those two studies. First, different participants' characteristics (resistant-trained males vs. untrained females). Second, different exercise protocols (IMTP, jumping, and bench press vs. cycling time trial). The other study by Grgic et al. [17] included only C allele carriers. The participants in that study were resistance-trained males (CC and CT genotypes). Performance was measured after 3 mg/kg CAF and PL with movement velocity, power output, and muscle endurance during the bench press exercise, countermovement jump height, and power output during a Wingate test. A total of 21 of 25 measured outcomes improved with CAF compared to PL, showing that C allele carriers are not real CAF non-responders. Others did not see any difference between C allele carriers and TT homozygotes in handball players (a countermovement jump, a sprint test, an agility test, an isometric handgrip test, and several ball throws) with 3 mg/kg CAF in healthy adults in a 15 min cycling trial, also with 3 mg/kg CAF, and in male cyclists in a 30 min time trial with mg/kg

CAF [18,20,48]. The results of the effect of *ADORA2A* gene polymorphism on exercise response to CAF are ambiguous and require further investigation. Our study and the study by Grgic et al. [17] are the only ones including strength and power exercises showing the C allele to be beneficial.

4.3. Habitual CAF Intake

Habituation to CAF and its influence in response to acute CAF supplementation is controversial. In our study, participants were divided into low and high CAF users by median. The range of daily CAF intake in the low-intake group was between 0.0 and 89.1 mg, and in the high-intake group, between 135.6 and 432.6 mg. Interestingly, only high habitual CAF users improved jumping average power and velocity with CAF compared to PL. Our results do not seem to confirm the negative influence of CAF habituation on exercise performance.

In a previous study, Filip–Stachnik et al. [47] showed that an acute dose of 6 mg/kg CAF before resistance exercise increased mean power output and mean bar velocity during a multiple-set bench press exercise protocol among mild CAF users. Participants in that study ingested, on average, 115 ± 41 mg of CAF per day, which is two-fold less than our participants in high CAF users (264 ± 100 mg/d) and more than our low CAF consumers (35 ± 31 mg/d). In another study, there were no differences in response to acute 3 mg/kg CAF in performance outcomes between low (65 ± 46 mg/d) and high (235 ± 82 mg/d) CAF habitual users [3]. The results of CAF habituation are still inconclusive. In contrast to previous studies, our results showed more benefits from acute CAF supplementation in high habitual users, but only in 2 out of 18 outcomes.

4.4. Limitations

Our study is not without limitations. First, CAF habitual intake was measured via previously described FFQ, which may not give entirely reliable results [42]. This is because of the large variability in the CAF content of commonly consumed beverages. New biomarkers of coffee consumption may be more useful in identifying habitual coffee users, but their availability is still limited [53]. Secondly, the interpretation of the interaction of *ADORA2A* genotype and response to CAF is limited due to the very low number of participants in the TT group (n = 4, ~15%). This genotype distribution is in agreement with previous studies showing TT genotype frequency at the level of 14–19% [19,54]. In our study, we genotyped our participants a posteriori, but in the future, it would be advisable to determine the *ADORA2A* genotypes before participant recruitment so that the analyzed subgroups are more even in number.

5. Conclusions

In conclusion, acute 5 mg/kg CAF supplementation may improve exercise performance, but the associations with the genotypes in *CYP1A2* and *ADORA2A* genes, as well as habitual CAF intake, are not clear. For *CYP1A2,* ergogenic properties of CAF were observed in one outcome in AA fast metabolizers and in another outcome in C allele slow metabolizers. For *ADORA2A,* two outcomes were improved in the CT genotype, one in the CC genotype, and none in TT. Regarding CAF habitual intake, only high CAF users improved in two outcomes after CAF compared to PL.

Author Contributions: J.C. and E.Z. had input into the study conception, design, and conduct of the study. J.A. performed HPLC measurements. E.Z. and A.C. performed DNA extraction and genotyping. E.Z. and B.Z. analyzed the data and performed statistical analysis. E.Z. and J.C. wrote the manuscript. A.C. provided expert input and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the internal sources (statutory funds) of the Poznan University of Life Sciences. The scholarship for E.Z.'s visit to Coastal Carolina University was funded by the Polish-US Fulbright Commission (Junior Research Award, IIE grantee ID number: PS00281163).

Institutional Review Board Statement: All experimental procedures were approved by the Coastal Carolina University Institutional Review Board (IRB # 2020.03) prior to data collection. The study was conducted in accordance with the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Guest, N.S.; VanDusseldorp, T.A.; Nelson, M.T.; Grgic, J.; Schoenfeld, B.J.; Jenkins, N.D.M.; Arent, S.M.; Antonio, J.; Stout, J.R.; Trexler, E.T.; et al. International society of sports nutrition position stand: Caffeine and exercise performance. *J. Int. Soc. Sport. Nutr.* **2021**, *18*, 1. [CrossRef]
2. Gu, L.; Gonzalez, F.J.; Kalow, W.; Tang, B.K. Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics* **1992**, *2*, 73–77. [CrossRef]
3. Xing, D.; Yoo, C.; Gonzalez, D.; Nottingham, K.; Dickerson, B.; Leonard, M.; Ko, J.; Faries, M.; Kephart, W.; Purpura, M.; et al. Dose-Response of Paraxanthine on Cognitive Function: A Double Blind, Placebo Controlled, Crossover Trial. *Nutrients* **2021**, *13*, 4478. [CrossRef]
4. Guest, N.; Corey, P.; Vescovi, J.; El-Sohemy, A. Caffeine, *CYP1A2* Genotype, and Endurance Performance in Athletes. *Med. Sci. Sport. Exerc.* **2018**, *50*, 1570–1578. [CrossRef] [PubMed]
5. Womack, C.J.; Saunders, M.J.; Bechtel, M.K.; Bolton, D.J.; Martin, M.; Luden, N.D.; Dunham, W.; Hancock, M. The influence of a CYP1A2 polymorphism on the ergogenic effects of caffeine. *J. Int. Soc. Sport. Nutr.* **2012**, *9*, 7. [CrossRef]
6. Benowitz, N.L.; Jacob, P., 3rd; Mayan, H.; Denaro, C. Sympathomimetic effects of paraxanthine and caffeine in humans. *Clin. Pharmacol. Ther.* **1995**, *58*, 684–691. [CrossRef]
7. Chou, C.C.; Vickroy, T.W. Antagonism of adenosine receptors by caffeine and caffeine metabolites in equine forebrain tissues. *Am. J. Vet. Res.* **2003**, *64*, 216–224. [CrossRef]
8. Karcz-Kubicha, M.; Antoniou, K.; Terasmaa, A.; Quarta, D.; Solinas, M.; Justinova, Z.; Pezzola, A.; Reggio, R.; Müller, C.E.; Fuxe, K.; et al. Involvement of adenosine A1 and A2A receptors in the motor effects of caffeine after its acute and chronic administration. *Neuropsychopharmacology* **2003**, *28*, 1281–1291. [CrossRef] [PubMed]
9. Shi, D.; Daly, J.W. Chronic effects of xanthines on levels of central receptors in mice. *Cell. Mol. Neurobiol.* **1999**, *19*, 719–732. [CrossRef]
10. Yoo, C.; Xing, D.; Gonzalez, D.; Nottingham, K.; Dickerson, B.; Leonard, M.; Ko, J.; Faries, M.; Kephart, W.; Purpura, M.; et al. Acute Paraxanthine Ingestion Improves Cognition and Short-Term Memory and Helps Sustain Attention in a Double-Blind, Placebo-Controlled, Crossover Trial. *Nutrients* **2021**, *13*, 3980. [CrossRef] [PubMed]
11. Jäger, R.; Purpura, M.; Wells, S.D.; Liao, K.; Godavarthi, A. Paraxanthine Supplementation Increases Muscle Mass, Strength, and Endurance in Mice. *Nutrients* **2022**, *14*, 893. [CrossRef]
12. Davis, J.M.; Zhao, Z.; Stock, H.S.; Mehl, K.A.; Buggy, J.; Hand, G.A. Central nervous system effects of caffeine and adenosine on fatigue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284*, R399–R404. [CrossRef]
13. Fredholm, B.B.; Yang, J.; Wang, Y. Low, but not high, dose caffeine is a readily available probe for adenosine actions. *Mol. Asp. Med.* **2017**, *55*, 20–25. [CrossRef] [PubMed]
14. Cornelis, M.C.; El-Sohemy, A.; Campos, H. Genetic polymorphism of the adenosine A2A receptor is associated with habitual caffeine consumption. *Am. J. Clin. Nutr.* **2007**, *86*, 240–244. [CrossRef]
15. Childs, E.; Hohoff, C.; Deckert, J.; Xu, K.; Badner, J.; de Wit, H. Association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology* **2008**, *33*, 2791–2800. [CrossRef] [PubMed]
16. Loy, B.D.; O'Connor, P.J.; Lindheimer, J.B.; Covert, S.F. Caffeine is ergogenic for adenosine A2A receptor gene (ADORA2A) T allele homozygotes: A pilot study. *J. Caf. Res.* **2015**, *5*, 73–81. [CrossRef]
17. Grgic, J.; Pickering, C.; Bishop, D.J.; Del Coso, J.; Schoenfeld, B.J.; Tinsley, G.M.; Pedisic, Z. ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation. *Nutrients* **2020**, *12*, 741. [CrossRef]
18. Carswell, A.T.; Howland, K.; Martinez-Gonzalez, B.; Baron, P.; Davison, G. The effect of caffeine on cognitive performance is influenced by *CYP1A2* but not ADORA2A genotype, yet neither genotype affects exercise performance in healthy adults. *Eur. J. Appl. Physiol.* **2020**, *120*, 1495–1508. [CrossRef] [PubMed]
19. Fredholm, B.B. Adenosine actions and adenosine receptors after 1 week treatment with caffeine. *Acta Physiol. Scand.* **1982**, *115*, 283–286. [CrossRef]
20. Muñoz, A.; López-Samanes, Á.; Aguilar-Navarro, M.; Varillas-Delgado, D.; Rivilla-García, J.; Moreno-Pérez, V.; Del Coso, J. Effects of *CYP1A2* and *ADORA2A* Genotypes on the Ergogenic Response to Caffeine in Professional Handball Players. *Genes* **2020**, *11*, 933. [CrossRef]
21. Nikodijević, O.; Jacobson, K.A.; Daly, J.W. Locomotor activity in mice during chronic treatment with CAF and withdrawal. *Pharmacol. Biochem. Behav.* **1993**, *44*, 199–216. [CrossRef]

22. Bangsbo, J.; Jacobsen, K.; Nordberg, N.; Christensen, N.J.; Graham, T. Acute and habitual caffeine ingestion and metabolic responses to steady-state exercise. *J. Appl. Physiol.* **1992**, *72*, 1297–1303. [CrossRef]
23. Karayigit, R.; Aras, D. One week of low or moderate doses of caffeinated coffee consumption does not induce tolerance to the acute effects of caffeine on sprint performance. *Eur. J. Hum. Mov.* **2021**, *47*, 49–60.
24. Grgic, J.; Mikulic, P. Acute effects of caffeine supplementation on resistance exercise, jumping, and Wingate performance: No influence of habitual caffeine intake. *Eur. J. Sport Sci.* **2020**, *2*, 1165–1175. [CrossRef]
25. Beaumont, R.; Cordery, P.; Funnell, M.; Mears, S.; James, L.; Watson, P. Chronic ingestion of a low dose of caffeine induces tolerance to the performance benefits of caffeine. *J. Sport. Sci.* **2017**, *35*, 1920–1927. [CrossRef]
26. Gonçalves, L.S.; Painelli, V.S.; Yamaguchi, G.; Oliveira, L.F.; Saunders, B.; da Silva, R.P.; Maciel, E.; Artioli, G.G.; Roschel, H.; Gualano, B. Dispelling the myth that habitual caffeine consumption influences the performance response to acute caffeine supplementation. *J. Appl. Physiol.* **2017**, *123*, 213–220. [CrossRef] [PubMed]
27. Irwin, C.; Desbrow, B.; Ellis, A.; O'Keeffe, B.; Grant, G.; Leveritt, M. Caffeine withdrawal and high-intensity endurance cycling performance. *J. Sport. Sci.* **2011**, *29*, 509–515. [CrossRef]
28. Lara, B.; Ruiz-Moreno, C.; Salinero, J.J.; Del Coso, J. Time course of tolerance to the performance benefits of caffeine. *PLoS ONE* **2019**, *14*, e0210275. [CrossRef] [PubMed]
29. Green, M.; FACSM. RISK STRATIFICATION: Effective Use of ACSM Guidelines and Integration of Professional Judgment. *ACSMs Health Fit. J.* **2020**, *14*, 22–28. [CrossRef]
30. Kraemer, W.J.; Ratamess, N.A.; Fry, A.C.; French, D.N. Strength training: Development and evaluation of methodology. In *Physiological Assessment of Human Fitness*; Maud, P.J., Foster, C., Eds.; Human Kinetics: Champaign, IL, USA, 2005.
31. Baechle, T.R.; Earle, R.W. *Essential of Strength and Conditioning*, 2nd ed.; Human Kinetics Publishers: Champaign, IL, USA, 2000.
32. Verdijk, L.B.; van Loon, L.; Meijer, K.; Savelberg, H.H. One-repetition maximum strength test represents a valid means to assess leg strength in vivo in humans. *J. Sport. Sci.* **2009**, *27*, 59–68. [CrossRef] [PubMed]
33. Ritti-Dias, R.M.; Avelar, A.; Salvador, E.P.; Cyrino, E.S. Influence of previous experience on resistance training on reliability of one-repetition maximum test. *J. Strength Cond. Res.* **2011**, *25*, 1418–1422. [CrossRef]
34. Drake, D.; Kennedy, R.; Wallace, E. The Validity and Responsiveness of Isometric Lower Body Multi-Joint Tests of Muscular Strength: A Systematic Review. *Sport. Med. Open* **2017**, *3*, 23. [CrossRef]
35. Harty, P.S.; Zabriskie, H.A.; Stecker, R.A.; Currier, B.S.; Tinsley, G.M.; Surowiec, K.; Jagim, A.R.; Richmond, S.R.; Kerksick, C.M. Caffeine Timing Improves Lower-Body Muscular Performance: A Randomized Trial. *Front. Nutr.* **2020**, *7*, 585900. [CrossRef] [PubMed]
36. James, L.P.; Roberts, L.A.; Haff, G.G.; Kelly, V.G.; Beckman, E.M. Validity and Reliability of a Portable Isometric Mid-Thigh Clean Pull. *J. Strength Cond. Res.* **2017**, *31*, 1378–1386. [CrossRef] [PubMed]
37. Kent, M. *The Oxford Dictionary of Sports Science & Medicine*, 3rd ed.; Oxford University Press: Oxford, UK, 2006.
38. Wadhi, T.; Rauch, J.T.; Tamulevicius, N.; Andersen, J.C.; De Souza, E.O. Validity and Reliability of the GymAware Linear Position Transducer for Squat Jump and Counter-Movement Jump Height. *Sports* **2018**, *6*, 177. [CrossRef]
39. Raya-González, J.; Rendo-Urteaga, T.; Domínguez, R.; Castillo, D.; Rodríguez-Fernández, A.; Grgic, J. Acute Effects of Caffeine Supplementation on Movement Velocity in Resistance Exercise: A Systematic Review and Meta-analysis. *Sport. Med.* **2020**, *50*, 717–729. [CrossRef] [PubMed]
40. Dorrell, H.F.; Moore, J.M.; Smith, M.F.; Gee, T.I. Validity and reliability of a linear positional transducer across commonly practised resistance training exercises. *J. Sport. Sci.* **2019**, *37*, 67–73. [CrossRef]
41. Hoffman, J.R.; Ratamess, N.A.; Kang, J.; Rashti, S.L.; Faigenbaum, A.D. Effect of betaine supplementation on power performance and fatigue. *J. Int. Soc. Sport. Nutr.* **2009**, *6*, 7. [CrossRef]
42. Bühler, E.; Lachenmeier, D.W.; Schlegel, K.; Winkler, G. Development of a tool to assess the caffeine intake among teenagers and young adults. *Ernahr. Umsch.* **2004**, *61*, 58–63.
43. Garbieri, T.F.; Brozoski, D.T.; Dionísio, T.J.; Santos, C.F.; Neves, L.T. Human DNA extraction from whole saliva that was fresh or stored for 3, 6 or 12 months using five different protocols. *J. Appl. Oral Sci.* **2017**, *25*, 147–158. [CrossRef]
44. Perera, V.; Gross, A.S.; McLachlan, A.J. Caffeine and paraxanthine HPLC assay for CYP1A2 phenotype assessment using saliva and plasma. *Biomed. Chromatogr.* **2010**, *24*, 1136–1144. [CrossRef]
45. Chou, J.; Trexler, E.T.; Lazinica, B.; Pedisic, Z. Effects of caffeine intake on muscle strength and power: A systematic review and meta-analysis. *J. Int. Soc. Sport. Nutr.* **2018**, *15*, 11.
46. Grgic, J.; Pickering, C. The effects of caffeine ingestion on isokinetic muscular strength: A meta-analysis. *J. Sci. Med. Sport* **2019**, *22*, 353–360. [CrossRef] [PubMed]
47. Filip-Stachnik, A.; Krzysztofik, M.; Kaszuba, M.; Leznicka, K.; Kostrzewa, M.; Del Coso, J.; Wilk, M. Effects of Acute Caffeine Intake on Power Output and Movement Velocity During a Multiple-Set Bench Press Exercise among Mild Caffeine Users. *J. Hum. Kinet.* **2021**, *78*, 219–228. [CrossRef] [PubMed]
48. Glaister, M.; Chopra, K.; Pereira De Sena, A.L.; Sternbach, C.; Morina, L.; Mavrommatis, Y. Caffeine, exercise physiology, and time-trial performance: No effect of *ADORA2A* or *CYP1A2* genotypes. *Appl. Physiol. Nutr. Metab.* **2021**, *46*, 541–551. [CrossRef]
49. Daly, J.W.; Butts-Lamb, P.; Padgett, W. Subclasses of adenosine receptors in the central nervous system: Interaction with caffeine and related methylxanthines. *Cell. Mol. Neurobiol.* **1983**, *3*, 69–80. [CrossRef] [PubMed]

50. Orrú, M.; Guitart, X.; Karcz-Kubicha, M.; Solinas, M.; Justinova, Z.; Barodia, S.K.; Zanoveli, J.; Cortes, A.; Lluis, C.; Casado, V.; et al. Psychostimulant pharmacological profile of paraxanthine, the main metabolite of caffeine in humans. *Neuropharmacology* **2013**, *67*, 476–484. [CrossRef]
51. Whalley, P.; Paton, C.; Dearing, C.G. Caffeine metabolites are associated with different forms of caffeine supplementation and with perceived exertion during endurance exercise. *Biol. Sport.* **2021**, *38*, 261–267. [CrossRef]
52. Meeusen, R.; Roelands, B.; Spriet, L.L. Caffeine, exercise and the brain. In *Limits of Human Endurance*; Karger Publishers: London, UK, 2013; Volume 76, pp. 1–12.
53. Rothwell, J.A.; Fillâtre, Y.; Martin, J.F.; Lyan, B.; Pujos-Guillot, E.; Fezeu, L.; Hercberg, S.; Comte, B.; Galan, P.; Touvier, M.; et al. New biomarkers of coffee consumption identified by the non-targeted metabolomic profiling of cohort study subjects. *PLoS ONE* **2014**, *9*, e93474. [CrossRef]
54. Dhaenens, C.M.; Burnouf, S.; Simonin, C.; Van Brussel, E.; Duhamel, A.; Defebvre, L.; Duru, C.; Vuillaume, I.; Cazeneuve, C.; Charles, P.; et al. Huntington French Speaking Network. A genetic variation in the ADORA2A gene modifies age at onset in Huntington's disease. *Neurobiol. Dis.* **2009**, *35*, 474–476. [CrossRef]