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ORIGINAL ARTICLE

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Caffeine improves various aspects of athletic performance in adolescents independent of their 163 C > A *CYP1A2* genotypes

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Purpose: The purpose of this study was to investigate whether variations in 163 C > A *CYP1A2* genotypes (rs 762 551) (AA, AC, and CC) modify the ergogenic effects of caffeine (CAF) on strength, power, muscular endurance, agility, and endurance in adolescent athletes.

Methods: One hundred adolescents (age = 15 ± 2 years) were recruited. Participants ingested CAF (6 mg.kg^{-1}) or placebo (PLA, 300 mg of cellulose) 1 hour before performing a sequence of physical tests: handgrip strength, vertical jumps, agility test, sit-ups, push-ups, and the Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1).

Results: Compared to PLA, CAF enhanced ($P < .05$) sit-up (CAF = 37 ± 9; PLA = 35 ± 8 repetitions) and push-up repetitions (CAF = 26 ± 11; PLA = 24 ± 11 repetitions), and increased distance covered in Yo-Yo IR1 test (CAF = 1010.4 ± 378.9; PLA = 903.2 ± 325.7 m). There was no influence of CAF on handgrip strength (CAF = 35.1 ± 8.9; PLA = 33.7 ± 8.7 kgf), countermovement jump height (CAF = 49.3 ± 12.6; PLA = 47.9 ± 13.8 cm), spike jump height (CAF = 54.2 ± 13.6; PLA = 52.9 ± 14.5 cm), and time in agility test (CAF = 15.8 ± 1.1; PLA = 15.9 ± 1.3 s, $P > .05$). When present, the ergogenic effect of CAF was not dependent of genotype.

Conclusion: CAF improves muscular endurance and aerobic performance in adolescent athletes, regardless of their 163 C > A *CYP1A2* genotype.

KEYWORDS
caffeine, CYP, ergogenic effects, exercise, genetic polymorphism, teenagers

1 | INTRODUCTION

Caffeine (CAF) is one of the most consumed ergogenic aids around the world.[1] CAF acts on the central nervous system as an antagonist of the adenosine receptors, mainly A1 and A2a subtypes,[1,2] increasing the release of neurotransmitters such as dopamine, noradrenaline, and glutamate.[1] These central effects are associated with a reduced perception of pain and rating of perceived exertion (RPE), which delay the onset of fatigue.[1-5] Moreover, recent findings from isolated muscles suggest that CAF in a micromolar concentration (ie, maximal non-toxic blood plasma concentration attainable in humans) might increase calcium release from the sarcoplasmic reticulum, resulting in increased muscle force.[6] In vitro studies also suggest that CAF may inhibit phosphodiesterase, leading to phosphorylation of some enzymes involved in the glucose and lipid metabolism; these effects, however, have been demonstrated only with millimolar concentrations of CAF, which would be toxic in humans.[1,2,7,8]

The ergogenic effect of CAF has consistently been established in endurance-based tasks,[9-11] while conflicting results have been reported in tasks involving strength, power, agility, and muscular endurance.[10,12,13] A review demonstrated that ~ 40% of the studies in the literature found no significant improvements in team sports exercise, power-based sports, or resistance training exercises with CAF ingestion.[5] It is noteworthy that the magnitude of gain in performance with CAF appears to have an elevated interindividual variability, which may explain part of these conflicting results across studies. This interindividual variability in the ergogenic effect of CAF might be partially due to a polymorphism in the cytochrome P450 1A2 (*CYP1A2*) enzyme.[14-16] The *CYP1A2* is a hepatic enzyme involved in CAF metabolism.[15-17] An A→C substitution at position 734 (rs762551) of intron 1 in the CYP1A2 gene decreases the inducibility and activity of this enzyme, which provokes a considerable variation in time to CAF peaks at plasma (15-120 minutes)[1,2] and its half-life (2.5-4.5 hours).[2] Individuals with the homozygous AA genotype might have enhanced CAF metabolism and have been classified as "fast metabolizers"; C allele carriers (AC and CC), on the other hand, might have a reduced CAF metabolism, being classified as "slow metabolizers".[15,16] Consequently, CAF responsiveness may be influenced by the 163 C > A *CYP1A2* polymorphism.[14,17] On one hand, AC and CC carriers might be benefited by CAF ingestion during endurance exercise as the effects of CAF would be longer lasting.[18] On the other hand, the AA homozygote might have some advantages in relation to AC and CC during short-term, high-intensity exercise, as CAF may peak faster and in greater amount in plasma.[14]

Studies investigating CAF ergogenicity and *CYP1A2* genotypes have produced contradictory results. For example, CAF intake (6 $mg.kg^{-1}$) improved 40-km cycling time trial performance to a greater extent in AA homozygotes (n = 16) than in C allele carriers (n = 19).[15] In another study, only cyclists carrying the AC genotype (n = 17) performed a 3-km cycling time trial better after CAF ingestion (6 $mg.kg^{-1}$), without any ergogenic effect of CAF in AA homozygotes (n = 21).[3] However, another study showed that CAF similarly improved performance during a 30-s Wingate test in both AA homozygotes (n = 5) and C allele carriers (n = 16).[19] Regarding muscular endurance and power, some reports have shown that CAF increased the number of repetitions during dynamic contractions performed at 85% of one-repetition maximum in AA homozygotes (n = 14) but not in C allele carriers (n = 16),[14] while jump height increased with CAF in AA homozygotes (n = 10) but not in C allele carriers (n = 9).[17]

Part of these inconsistencies may be due to the small sample size (almost all studies with <40 subjects). Another concern is that studies have clustered AC and CC carriers within the same group, probably due to the rarity of CC genotype. According to the Hardy-Weinberg equilibrium, ~40% of the general population carry the AA genotype, 50% the AC genotype, and only 10% the CC genotype.[16,18,20] Thus, to include a suitable number of CC genotype, the recruitment of a larger sample size is mandatory. Only one study, using a large sample size (n = 101), was able to split out C allele carriers in AC and CC groups, and found that CAF improved 10-km cycling time trial performance in participants with the AA genotype (n = 49), had no effect on the AC genotype (n = 44), and reduced performance in the CC genotype (n = 8).[20] Thus, studies with a large sample size are needed to determine the influence of the three CYP1A2 163 C > A genotypes (ie, AA, AC, and CC) with respect to the ergogenic effect of CAF on other physical capacities, such as strength, power, muscular endurance, agility, and endurance.

The purpose of the present study was to investigate whether variations in *CYP1A2* genotypes (AA, AC, and CC carriers) would affect CAF-induced improvements in strength, power, muscular endurance, agility, and aerobic performance. Although one study has suggested similar ergogenic effects of CAF on adolescents and adults, the ergogenic effect of CAF has been less explored with adolescents.[4] Thus, as team sports demanding strength, power, muscular endurance, agility, and endurance are largely practiced by adolescents, we opted to investigate the impact of CAF ingestion and *CYP1A2* genotypes on these physical capacities in this population. We hypothesized that individuals with the AA genotype would have greater CAF-induced increases in strength, power, muscular endurance, agility, and aerobic performance than those with the AC and CC genotypes.

2 | MATERIALS AND METHODS

2.1 | Participants

The sample size was calculated a priori using the following input parameters for the ANOVA test: (a) effect size (=0.40); (b) α error probability (=0.05); (c) power (1-β error probability = 0.95); and (d) number of groups (=3, ie, AA, AC and CC). It was estimated that 102 participants would be necessary. In an effort to overcome dropout rate, we recruited 109 subjects, but nine subjects dropped out before completing all tests. Thus, 100 adolescents engaged in competitive sports (volleyball, athletics, or soccer) volunteered for this study (age: 15 ± 2 years; height: 1.69 ± 0.10 m; body mass: 58.8 ± 11.9 kg; VO_2max: 44.0 ± 2.7 ml.kg^{-1}.min^{-1}). Participants were divided into three groups according to their genotype of the *CYP1A2*: AA (n = 49 participants), AC (n = 42 participants), and CC (n = 9 participants). As reported previously,[16,20,21] this distribution reached the Hardy-Weinberg equilibrium. Participants and their parents were informed about the experimental risks and signed an informed consent form before the tests. The experimental procedures were conducted in accordance with the Declaration of Helsinki (2008) and approved by the Ethics Committee of the Federal University of Alagoas (protocol number 1.541.599 in 05/2016).

2.2 | Experimental design

The study was conducted using a randomized, crossover, and double-blind design. Participants visited the laboratory three times and completed the three visits at the same time of day (either 9:00 AM or 2:00 PM) to minimize any influence of circadian rhythm on exercise performance. A minimum of a 72-hour interval was adopted between visits.

On the first visit, participants completed a questionnaire that included questions related to the habitual consumption of dietary products rich in CAF. This questionnaire contained a list of foods and beverages containing CAF. Participants indicated the amount and frequency that they consumed each food and beverage on the list. To calculate daily CAF consumption,[22] it was assumed that 150 mL of pure coffee = 100 mg of CAF, 28 g milk chocolate = 6 mg CAF, 250 mL of energy drink = 80 mg of CAF, 350 mL of cola = 46 mg of CAF, 150 mL of tea = 30 mg of CAF, 150 mL of coffee with milk = 33 mg of CAF, and 350 mL of guarana soda = 2 mg of CAF. After completing the questionnaire, anthropometric measurements and blood samples were collected. Blood samples (4 mL) were dispensed in vacuum tubes containing EDTA (BD Vacutainer®) and stored at −20°C until DNA extraction. After blood sample collection, a familiarization with the experimental tests was performed. A 5-minute warm-up (similar to the warm-up performed in their training routine) preceded the tests, which were performed in the same sequence as in the experimental sessions: handgrip force, agility, countermovement jump (CMJ), spike jump (SJ), sit-up, push-up, and Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1).

In the second and third visits, participants performed the battery of tests one hour after ingesting a capsule containing CAF (6 mg.kg^{-1}) or placebo (PLA, 300 mg of cellulose). After a 5-minute warm-up, the following tests were performed: handgrip force, agility, CMJ, SJ, sit-up, push-up, and Yo-Yo IR1. After the Yo-Yo IR1, the rating of perceived exertion (RPE) was measured using the 15-point Borg scale[23] (scores range from six to 20). All tests were conducted by experienced evaluators blinded for supplements and genotypes. Participants were instructed to maintain their regular food intake and to avoid exhaustive exercise, alcohol, or nutritional supplements 24 hours prior to the experimental trials. In addition, participants were instructed not to consume any food or beverage containing CAF during the 24 hours prior the experimental sessions, a time considered sufficient to fully clear CAF from the blood.[24]

2.3 | Supplementation protocol

Anhydrous powder caffeine (CAF, 6 mg.kg^{-1}) and cellulose (PLA, 300 mg) were encapsulated by a local pharmaceutical establishment (Ao Pharmaceutico®) and stored under ambient conditions, as recommended by the manufacturer. The CAF and PLA capsules were ingested with 250 mL of water. The CAF and PLA capsules had the same size (size 0), volume, color, and shape.

2.4 | Handgrip strength test

Handgrip strength was measured using a hydraulic Jamar® dynamometer (Sammons Preston Rolyan, 4, Sammons Court, Bolingbrook, IL, 60440). Participants were seated with their trunk in the upright position holding the dynamometer in their right hand, with their elbow positioned at the right side of their body. They were instructed to not use their legs to support the dynamometer. The dynamometer position was adjusted with its base resting on the first metacarpal and the handle resting on the middle of the four fingers. The maximum isometric force was maintained for 3 seconds. The participants were strongly encouraged to exert their maximal force. Three attempts separated by 15 seconds of recovery were performed, and the best score was used in further analysis.

2.5 | Vertical jump tests

Two types of vertical jumps were performed. In the CMJ, the participant started in a standing position, moved to a squat position, and quickly jumped as high as possible. The knee flexion and arm movement were free. For the SJ, the procedures were similar to those of the CMJ, except that the takeoff was preceded by a run-up approach with three or four acceleration steps, as described previously.[25] The height reached in both jumps was measured using a vertical jump measuring device (VERTEC® Sports Imports). Both types of jumps were performed three times, and the highest height reached for each type of jump was used in further analysis.

2.6 | Agility test

The agility test was developed in our laboratory (Figure S1). The subject ran 38-m as quickly as possible, with point of changes in the direction marked by 11 cones (1-2 = 2 m forward and turn right 90°; 2-3 = 10 m forward and turn right 135°; 3-4 = 2 m diagonal/forward; 4-10 = slalom each 2 m diagonal/forward; 10-11 = 12 m forward). The participants were asked to complete the entire route as quickly as possible, with the final time recorded by the same evaluator. They ran the route twice, separated by 5 minutes of recovery, with the best score used in further analysis.

2.7 | Sit-up test

The sit-up test was performed as previously described.[26] Briefly, participants were in a dorsal decubitus position, with their knees flexed at 90 degrees and hands positioned behind their head. The evaluator fixed the participant's feet to the ground and, at the evaluator's signal, the participant initiated bending movements of the trunk until it touched the thighs, returning to the initial position with the back touching the floor. The number of repetitions within one minute was recorded.

2.8 | Push-up test

The push-up test was set up as previously suggested.[26,27] The hands were positioned approximately 10 to 20 cm away from the shoulder joint. Participants flexed their elbows until the chest touched the evaluator's fist positioned on the floor and returned to the initial position. Participants were continually asked to maintain a straight body position.[26] Incorrect repetitions were disregarded, and the number of correct repetitions within one minute was recorded and used in further analysis.

2.9 | Yo-Yo IR1

Following the protocol of Bangsbo, Iaia and Krustrup,[28] participants ran (20 m) back and forth between markers (20 m). The Yo-Yo IR1 test started with four running cycles at a pace between 10 and 13 km.h^{-1} (0-160 m), followed by seven running cycles at a pace between 13.5 and 14 km.h^{-1} (160-440 m) and further increments of 0.5 km.h^{-1} every eight cycles until exhaustion. A 10-s recovery interval was adopted between each cycle. The running velocity was controlled by sound signal. Exhaustion was assumed when participants were unable to reach the markers within three consecutive sound signals or by voluntary disengagement. The total distance covered was considered an indicator of endurance performance and was used in further analysis. The VO_2max was calculated using the following equation:

$$VO_2max(\mathrm{ml}\cdot\mathrm{kg}^{-1}\cdot min^{-1}) = \mathrm{distance}\times 0.0084 + 36.4$$

where distance is the maximum distance in meters attained in Yo-Yo IR1 test.

2.10 | Genotyping

2.10.1 | DNA extraction

DNA was extracted from 300 μL of blood using a FlexiGene DNA Kit (Qiagen) according to the manufacturer's recommendations. All genotyping was performed by an investigator not involved in the experimental protocol. Investigators were also blinded to the participants' genotype until all tests had been concluded.

2.10.2 | CYP1A2

Genotyping of the rs 762551 polymorphism, located in intron 1 of the *CYP1A2* gene, was performed through the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) protocol, as described by Cornelis, El-Sohemy and Campos.[21] For amplification of the 920 bp fragment, the primers 5′-CAACCCTGCCAATCTCAAGCAC-3′ and 5′-AGAAGCTCTGTGGCCGAGAAGG-3′ were used. The amplification conditions consisted of initial denaturation at 95°C for 5 minutes, followed by 39 cycles at 94°C for 15 seconds, 61°C for 1 minute and 72°C for 1 minute and one final extension step at 72°C for 10 minutes. Restriction products were evaluated by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under UV light. The presence of a 920 bp fragment after ApaI digestion characterizes the AA genotype, while the presence of the 709 bp and

211 bp fragments characterizes the CC genotype, as briefly described by Womack et al.[15]

2.10.3 | Statistical analysis

Statistical analyses were performed using a statistical package (Statistica® version 10.0, StataSoft). Data distribution was checked by the Kolmogorov-Smirnov test. Once normal distribution was confirmed, one-way ANOVA was used to compare participant characteristics among AA, AC, and CC genotypes. A two-way repeated measure ANOVA was used to verify genotypes (AA vs AC vs CC) and influence of the supplements (CAF vs PLA) on dependent variables. When a main effect or interaction was detected by ANOVA, a paired comparison using Bonferroni correction was used to locate the differences. Cohen's effect size (ES) was calculated for paired post-hoc comparisons and interpreted using Cohen's scale as follows[29]: small (0.2), moderate (0.5), and large (0.8). The level of significance was set at $P < .05$.

3 | RESULTS

Participants reported no side effects during the experiment or during the 24 hours after the test. The main characteristics of the participants are shown in Table 1. There was a difference among the AA, AC, and CC for body mass ($P = .038$) and VO_2max ($P = .018$). The body mass was higher in the AA than in the CC ($P = .036$), but there was no difference between the AA and AC ($P = .999$) or between the AC and CC ($P = .060$). VO_2max was higher in the AC than in the CC ($P = .030$), but there was no difference between the AA and AC ($P = .152$) or between the AA and CC ($P = .404$).

3.1 | Handgrip strength test

There was no main effect of supplement ($P = .058$), genotype ($P = .096$), or genotype vs supplement interaction ($P = .210$) for handgrip strength (Figure S2A and Table 2).

3.2 | Vertical jump tests

There was no main effect of supplement ($P = .105$), genotype ($P = .753$), or genotype vs supplement interaction ($P = .935$) for CMJ (Figure S2B and Table 2). Similarly, there was no main effect of supplement ($P = .306$), genotype ($P = .832$), or genotype vs supplement interaction ($P = .571$) for SJ (Figure S2C and Table 2).

3.3 | Agility test

There was no main effect of supplement ($P = .736$) for the agility test (Figure S3 and Table 2). However, there was a significant effect of genotype ($P = .005$), with higher values (worse performance) in AC than in AA ($P = .037$) and CC ($P = .018$), without differences between AA and CC ($P = .530$). There was no genotype vs supplement interaction ($P = .417$) for the agility test (Figure S3 and Table 2).

3.4 | Sit-up and push-up tests

Compared to the PLA, CAF improved performance in the sit-up (main effect of supplement, $P = .001$, ES = 0.263; Figure S4A and Table 2) and the push-up tests (main effect of supplement, $P = .004$, ES = 0.177; Figure S4B and Table 2). There was no main effect of genotype ($P = .122$ and 0.964, respectively) or genotype vs supplement interaction ($P = .733$ and $P = .138$, respectively) for sit-up and push-up tests (Figure S4A,B and Table 2).

3.5 | Yo-Yo IR1

Compared to PLA, the CAF increased the total distance covered during the Yo-Yo IR1 (main effect of supplement, $P = .019$, ES = 0.303; Figure S5 and Table 2). There was also a significant main effect of genotype ($P = .038$), with post-hoc analysis detecting a slight tendency toward lower values (worse performance) in AC than in CC ($P = .068$), without differences between AA and AC ($P = .207$) or AA and CC ($P = .614$). There was no genotype vs supplement interaction for distance ($P = .783$, Figure S5 and Table 2). There was no main effect of supplement ($P = .331$), genotype ($P = .502$), or genotype vs supplement interaction ($P = .466$) for RPE (Table 2).

4 | DISCUSSION

To the best of our knowledge, this is the first study investigating the influence of the CYP1A2 - 163 C > A polymorphism (rs 762551) on the ergogenic effect of CAF on strength, power, muscular endurance, agility, and endurance in a large sample of adolescent athletes. Compared to the PLA, CAF ingestion enhanced the number of repetitions in the sit-up and push-up tests, and the distance covered during a Yo-Yo IR1 test. No influence of the CYP1A2 genotype on the ergogenic effect of CAF, however, was noted for any physical capacity investigated.

It has been reported that CAF ingestion improves handgrip force by ~4.0% in male and female adolescent tennis

TABLE 1 Main characteristics of the participants

Characteristics	Pooled (n = 100)	AA (n = 49)	AC (n = 42)	CC (n = 9)	*P*
Age (y)	15.5 ± 2.0	15.3 ± 2.0	15.5 ± 1.9	16.3 ± 1.6	.381
Height (m)	1.69 ± 0.10	1.68 ± 0.09	1.69 ± 0.11	1.76 ± 0.07	.054
Body mass (kg)	58.8 ± 11.9	57.5 ± 10.4*	58.2 ± 12.9	68.2 ± 10.9	.038
VO_2max ($ml.kg^{-1}.min^{-1}$)	44.0 ± 2.7	44.3 ± 2.7	43.2 ± 2.4*	45.8 ± 3.5	.017
Habitual caffeine intake (mg/d)	48.8 ± 41.7	42.3 ± 39.8	58.6 ± 44.9	32.7 ± 23.9	.191
Sports type (%)					
Volleyball	23	10	11	2	
Football	61	32	25	4	
Track and field	16	7	6	3	

Note: Data are mean ± SD.

*Significantly lower than CC group ($P < .05$).

players (ES = 0.180)[30] and by ~3.5% in adult male swimmers (ES = 0.368).[31] In the present study, CAF did not significantly improve handgrip strength, but it is noteworthy that the p value for the main effect of supplement approximated significance ($P = .058$) and the percentage of improvement with CAF was ~4.1%, similar to previously reported.[30,31] The main mechanism by which CAF would increase maximal isometric force could be attributed to an increase in muscle recruitment and/or in calcium release from sarcoplasmic reticulum.[1,7] This last is still debated as studies have reported that millimolar concentrations of CAF (considered toxic in humans) would be necessary to increase tetanic force and calcium release.[1,2,7,8] However, it is intriguing that studies with isolated muscles using a micromolar concentration of CAF, which could be reached with common ergogenic CAF dosages (ie, up to 9 $mg.kg^{-1}$ body mass), have reported an increase in isolated skeletal muscle contractility.[6] Regarding the *CYP1A2* genotype, it is difficult to compare our results with others because no previous study has investigated whether CAF-induced increases in handgrip strength are dependent on the *CYP1A2* genotype. Our findings suggest, however, that CAF might produce a marginal effect on handgrip force, regardless of *CYP1A2* genotype.

In the present study, CAF failed to increase CMJ and SJ performance, corroborating previous studies showing no improvement in CMJ after CAF intake in male adult soccer players[32] and in female team-sport players.[33] Other studies, however, have reported an ergogenic effect of CAF on jump performance in male adolescent basketball players[34] (ES = 0.181) and male adult volleyball players[12] (ES = 0.399). It is difficult to explain the reason for these contradictory findings, but an improvement in jump performance with CAF ingestion, when present, would be associated with a better neuromuscular function, mainly due to the higher conduction speed and/or the greater recruitment of muscle fibers.[11,13,35] Regarding the influence of *CYP1A2* genotypes, we found no influence of polymorphisms on the responsiveness to CAF in jump performance. This finding differs from a previous study that reported increased jump height in basketball players after CAF ingestion only in AA genotype.[17] A notable aspect is that the C allele carriers (AC and CC) in the mentioned study included only 9 individuals, which may explain why the 2.3% improvement with CAF in C allele carriers did not reach statistical significance.[17] Interestingly, a recent study found that CAF improves jump performance in resistance-trained men, but this improvement was independent of *CYP1A2* genotypes.[36] Together, our findings and those in the reviewed literature indicate that CAF may be ergogenic for jump performance in some circumstances, but it seems to be independent of *CYP1A2* genotype.

While in the present study CAF was not ergogenic in strength- and power-based tests, CAF consistently increased muscular endurance. The number of repetitions in the sit-up test was increased by 6.3% with CAF ingestion. Similarly, CAF ingestion increased 8.2% the number of repetitions in the push-up test. These findings are in accordance with recent meta-analyses showing that caffeine increases muscular endurance.[11,37] Improved muscular endurance with CAF ingestion has been attributed to an increased central motor drive and corticospinal excitability, with a concomitant reduction in perception of effort mediated by the antagonism of CAF in the adenosine receptors.[7] In relation to genotypes, we did not find an influence of the *CYP1A2* polymorphism on responsiveness to CAF. Contradictory findings in relation to the influence of *CYP1A2* genotypes on CAF-induced improvements in muscular endurance have been reported, with one study showing an increased number of repetitions (85% of 1RM performed until failure) after CAF ingestion only in AA homozygotes (n = 14).[14] In another study, CAF improved

TABLE 2 Performance during the battery of tests after caffeine and placebo ingestion in groups with different 163 C > A *CYP1A2* genotypes

	AA genotype		AC genotype		CC genotype	
	CAF	PLA	CAF	PLA	CAF	PLA
Handgrip strength test (kgf)	35.4 ± 8.4	33.2 ± 8.3	33.6 ± 9.3	33.0 ± 9.1	40.3 ± 8.3	39.8 ± 7.7
CMJ test (cm)	48.6 ± 12.3	47.2 ± 13.3	49.6 ± 12.0	48.0 ± 13.2	51.8 ± 17.5	51.0 ± 19.6
SJ test (cm)	53.8 ± 13.3	51.9 ± 13.8	54.3 ± 13.8	53.6 ± 15.3	55.7 ± 16.2	55.6 ± 16.1
Agility test (s)[#]	15.6 ± 1.0	15.7 ± 1.0	16.2 ± 1.2	16.3 ± 1.5	15.2 ± 0.8	15.0 ± 1.0
Sit-up test (repetitions)[*]	38.0 ± 8.3	36.1 ± 7.4	35.8 ± 8.5	33.1 ± 8.6	40.8 ± 9.4	38.3 ± 8.6
Push-up test (repetitions)[*]	25.8 ± 11.0	24.9 ± 9.6	26.4 ± 13.4	23.2 ± 13.1	26.7 ± 8.1	24.0 ± 6.8
Yo-Yo IR1 test (m)[*, ##]	1046.5 ± 356.5	942.8 ± 320.1	935.2 ± 394.8	811.4 ± 286.4	1164.4 ± 388.0	1115.6 ± 415.9
RPE_{end}	18.1 ± 3.2	17.4 ± 3.2	17.5 ± 3.5	16.5 ± 4.0	17.3 ± 3.8	17.8 ± 2.5

Note: Data are reported as mean ± SD.

Abbreviations: CAF, caffeine; CMJ, counter movement Jump; PLA, placebo; RPE_{end}, rating of perceived effort at the end of Yo-Yo IR1 test; SJ, spike jump; Yo-Yo IR1, Yo-Yo intermittent recovery test level 1.

[#]Significant main effect of genotype, with higher values in the AC than in the AA and CC groups.

*Significant main effect of supplement, with higher values in the CAF than in the PLA condition ($P < .05$).

[##]Significant main effect of genotype ($P < .05$), with post-hoc detecting a tendency of lower values in the AC than in the CC groups ($P = .068$).

muscular endurance to a similar degree in both AA homozygotes and C allele carriers.[36] No prior study has investigated the effect of CAF ingestion separately in AC and CC genotype, so our findings expand our understanding by showing that CAF improves muscular endurance in all three variants of the *CYP1A2* polymorphism.

The distance covered in the Yo-Yo IR1 test was 11.9% higher in the CAF than in the PLA. A previous study showed an ergogenic effect of CAF ingestion on performance of adult males and females during a Yo-Yo IR2 test[9] (ES = 1.527), a test similar to the Yo-Yo IR1. However, Pettersen et al[38] found no improvements in Yo-Yo IR2 performance after CAF ingestion in young male football players (ES = 0.03). Abian-Vicen et al[34] also found no effect of CAF ingestion on performance during a Yo-Yo IR1 test with male adolescent basketball players (ES = 0.106). In addition, no study has investigated the influence of the *CYP1A2* genotypes on the ergogenicity of CAF in the Yo-Yo IR1 test. Some studies using other endurance-based tasks have produced conflicting results, with some of them showing improvements in endurance performance after CAF ingestion only in adult AA homozygotes,[15,20] while another study showed no effect of *CYP1A2* genotypes on endurance performance.[39] The reason for these contradictory results is unclear, and further studies will be necessary to clarify whether different CYP1A2 genotypes would influence responsiveness to CAF in different endurance tasks.

With the exception of Guest et al,[20] studies investigating the influence of the *CYP1A2* genotypes on CAF-induced improvement in exercise performance have been conducted with a small number of adult athletes[14,15,36] and non-athletes.[19,39] In addition, as the frequency of the CC genotype in the population is low (~10%), previous studies have clustered C allele carriers (AC and CC) within a single group.[3,15,17,19] It has recently been reported that CAF ingestion reduces endurance performance in CC homozygotes[20] but has no effect in AC heterozygotes, indicating that further studies should split AC from CC carriers when investigating the influence of *CYP1A2* genotypes on exercise performance. We recruited a large sample size for *CYP1A2* genotypes, which permitted us to investigate the effect of CAF ingestion in the three different genotypes.

The present study has some limitations that should be mentioned. We did not measure plasma CAF concentration; therefore, we cannot ascertain if CAF peaked similarly across genotypes or by which extension this may have influenced our results. In addition, we did not measure blood/muscle biochemical markers that could have helped to provide an explanation of the main mechanisms behind our findings.

In conclusion, the findings of the present study suggest that the ergogenic effect of CAF on muscular endurance and endurance performance may be independent of the 163 C > A *CYP1A2* genotype, at least in a population similar to that investigated in the present study.

5 | PERSPECTIVE

Our data suggest that the acute intake of 6 $mg.kg^{-1}$ of CAF 60 minutes before exercise can improve muscular endurance and endurance performance in adolescent athletes, regardless their CYP1A2 genotype.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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