The acute effects of caffeine supplementation on muscle strength, power and endurance

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Abstract

Caffeine is a highly popular ergogenic aid, often consumed by athletes and non-athletes alike. The aim of this thesis was to explore: (a) the effects of caffeine on different exercise tasks; (b) the effects of varying doses of caffeine on resistance exercise performance; and (c) the effects of *ADORA2A* and *CYP1A2* genotype variations on the individual response to caffeine ingestion. This thesis is comprised of eight published studies – four reviews and four primary studies. The first study was an umbrella review of 21 published meta-analyses on the ergogenic effects of caffeine on exercise performance. This review showed that caffeine ingestion was ergogenic for aerobic endurance, muscle strength, muscle endurance, power, jumping performance, and exercise speed. The ergogenic effects of caffeine on muscle endurance, muscle strength, anaerobic power, and aerobic endurance were substantiated by moderate-quality evidence from moderate-to-high quality systematic reviews. The evidence for other outcomes was of low or very low quality and it was based on moderate-quality reviews. The second study was a narrative review that critically evaluated the evidence on the topic of caffeine supplementation when performing resistance exercise. This study provided a comprehensive overview of caffeine's effects on resistance exercise performance and its influence on the associated physiological responses. The third study was a meta-analysis that explored the effects of caffeine on maximum strength (one repetition maximum) and vertical jump height. This analysis found that caffeine ingestion provides an ergogenic effect on both outcomes. The fourth study was a meta-analysis that explored the acute effects of caffeine on Wingate (all-out, 30-s cycle sprint) test performance, showing ergogenic effects of caffeine on mean and peak power in this test. Based on these reviews of literature, it was identified that more research is needed to explore the effects of caffeine supplementation in trained individuals, the optimal dose of caffeine for improving anaerobic exercise performance, and the influence of genotype variations on the responses to caffeine ingestion.

To fill the evidence gap, the fifth study explored the acute effects of caffeine ingestion (6 mg/kg) on strength, power, muscular endurance, rating of perceived exertion (RPE), and pain perception in resistance-trained men. This study demonstrated that caffeine ingestion acutely reduced RPE and enhanced upper-body power and lower-body strength. Given that quite a high dose was used in the fifth study, and that several reviews suggested there may be a caffeine dose effect, the sixth study explored the acute effects of three different doses of caffeine (2 mg/kg, 4 mg/kg, and 6 mg/kg) on upper- and lower-body muscular strength and endurance. While caffeine ingestion enhanced lower-body strength and muscular endurance, this study

found no clear association between the caffeine dose and the magnitude of ergogenic effects. However, a relatively large individual variation in responses to caffeine was noted. The final two studies were, therefore, conducted to explore possible genetic determinants of individual responses to caffeine supplementation. The seventh study explored the influence of caffeine ingestion on movement velocity, muscular endurance, jumping, and sprinting performance in a sample of 20 *ADORA2A* (rs5751876) C allele carriers (CC/CT genotype). In contrast to previous findings on this topic, this study showed that C allele carriers exhibited ergogenic responses to caffeine in the majority of exercise outcomes. The eighth study explored the influence of variation in *CYP1A2* (rs762551) genotype in a sample of 22 men (AA homozygotes $n = 13$; C allele carriers $n = 9$) on the acute effects of caffeine ingestion on exercise performance, including velocity, power, and muscle endurance. Compared to placebo, caffeine ingestion improved exercise performance in most outcomes, but there was no significant genotype × caffeine interaction. Overall, the main findings of this thesis are that: (a) caffeine ingestion acutely enhances performance in various exercise tasks; (b) lower doses of caffeine may produce ergogenic effects comparable to those of higher doses of caffeine; and (c) the individual responses to caffeine ingestion may not be moderated by *ADORA2A* and *CYP1A2* genotype variation. The findings on ergogenic effects of different doses of caffeine and the influence of genotype on individual responses to caffeine need to be confirmed in future studies with larger sample sizes. These findings may be useful to athletes, coaches, and sports nutritionists in making evidence-based decisions about caffeine supplementation.

Student declaration

"I, Jozo Grgić, declare that the PhD thesis by publication entitled "The acute effects of caffeine supplementation on muscle strength, power and endurance" is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature: 

Date: 17/09/2020

Details of included papers: thesis by publication

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PART A:

DETAILS OF INCLUDED PAPERS: THESIS BY PUBLICATION

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Item/ Chapter No.	Paper Title	Publication Status (e.g. published, accepted for publication, to be revised and resubmitted, currently under review, unsubmitted but proposed to be submitted)	Publication Title and Details (e.g. date published, impact factor etc.)
4.	Wake Up and Smell the Coffee: Caffeine Supplementation and Exercise Performance—An Umbrella Review of 21 Published Meta-Analyses	Published	Published in June of 2020 in the British Journal of Sports Medicine (Impact factor: 12.022)
5.	The Influence of Caffeine Supplementation on Resistance Exercise: A Review	Published	Published in January of 2019 in Sports Medicine (Impact factor: 8.551)
6.	Effects of Caffeine Intake on Muscle Strength and Power: A Systematic Review and Meta-Analysis	Published	Published in March of 2018 in the Journal of the International Society of Sports Nutrition (Impact factor: 5.068)
7.	Caffeine Ingestion Enhances Wingate Performance: A Meta-Analysis	Published	Published in March of 2018 in the European Journal of Sport Science (Impact factor: 2.781)
8.	Caffeine Ingestion Acutely Enhances Muscular Strength and Power but Not Muscular Endurance in Resistance-Trained Men	Published	Published in May of 2017 in the European Journal of Sport Science (Impact factor: 2.781)
9.	What Dose of Caffeine to Use: Acute Effects of 3 Doses of Caffeine on Muscle Endurance and Strength	Published	Published in March of 2020 in the International Journal of Sports Physiology and Performance (Impact factor: 3.528)

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PART A:

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10.	ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation	Published	Published in March of 2020 in Nutrients (Impact factor: 4.546)
11.	CYP1A2 Genotype and Acute Effects of Caffeine on Resistance Exercise, Jumping, and Sprinting Performance	Published	Published in April of 2020 in the Journal of the International Society of Sports Nutrition (Impact factor: 5.068)

Declaration by [candidate name]: Signature: Date:

Jozo Grgic [redacted] 01/07/2020

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List of abbreviations

1RM: One repetition maximum

AMSTAR: Assessing the Methodological Quality of Systematic Reviews

ANOVA: Analysis of variance

BBS: Barbell back squat

BP: Bench press

CI: Confidence interval

CMA: Comprehensive Meta-Analysis

CMJ: Countermovement jump

CV: Coefficient of variation

DNA: Deoxyribonucleic acid

DOMS: Delayed onset muscle soreness

ES: Effect size

FFQ: Food frequency questionnaire

GRADE: Grading of Recommendations Assessment, Development and Evaluation

LP: Leg press

LPD: Lat pulldown

MBS: Machine-based squat

mTORC1: Mammalian mechanistic target of rapamycin complex 1

MVC: Maximum voluntary contraction

PAR-Q: Physical Activity Readiness Questionnaire

PCR: Polymerase chain reaction

PEDro: Physiotherapy Evidence Database

PI: Prediction interval

PICO: Participant-Intervention-Comparison-Outcome

PP: Pain perception

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RDB: Randomised double-blind study

RPE: Rating of perceived exertion

RSB: Randomised single-blind study

SD: Standard deviation

SJ: Squat jump

SMD: Standardised mean difference

ST: Sargent test

UK: United Kingdom

USA: United States of America

VUHREC: Victoria University Human Research Ethics Committee

WADA: World Anti-Doping Agency

1. General introduction

Caffeine is among the most commonly used psychoactive stimulants in the world (Graham, 2001). Data presented by the National Health and Nutrition Examination Survey indicated a high prevalence of caffeine consumption among Americans, with 89% of the participants indicating some caffeine intake, and with the average daily consumption of 211 ± 3 mg (Fulgoni, Keast, & Lieberman, 2015). Caffeine is also widely consumed in the sports and exercise settings, with research demonstrating that 74% of the tested anti-doping samples contained measurable levels of caffeine (Del Coso, Muñoz, & Muñoz-Guerra, 2011). The research investigating the effects of caffeine supplementation on sport and exercise performance initially focused on aerobic-type, endurance activities (Pasman, van Baak, Jeukendrup, & de Haan, 1995; Wiles, Bird, Hopkins, & Riley, 1992). It is well-established that caffeine supplementation can have a significant performance-enhancing effect on aerobic performance (Graham, 2001). Currently, however, there is growing interest in investigating the effects of caffeine ingestion on performance in high-intensity, anaerobic-type exercise (Davis & Green, 2009). However, for certain abilities, such as muscular strength, muscular endurance, and power, the evidence is still scarce or inconclusive (Davis & Green, 2009). Additionally, the inter-individual variation in responses to caffeine ingestion is commonly acknowledged in studies that plot individual responses (Jenkins, Trilk, Singhal, O'Connor, & Cureton, 2010; Pickering & Kiely, 2018). The differences in responses have been recently associated with genotype variations in *ADORA2A* and *CYP1A2*. However, the evidence on this topic is limited and conflicting (Pickering & Kiely, 2018). Therefore, this PhD research project investigated the acute effects of caffeine supplementation on muscular strength, muscular endurance, muscular power, movement velocity, jumping performance, perceived exertion, and pain perception, and whether these effects are influenced by the *ADORA2A* or *CYP1A2* genotype.

The eight key research questions of this PhD research project were:

1. What is the current state of evidence on the ergogenic effects of caffeine supplementation on exercise performance?
2. What is the current state of evidence on the effect of caffeine ingestion on resistance exercise performance and associated physiological responses?

3. What is the current state of evidence on the effect of caffeine ingestion on maximum effort, very short (up to 5-10 seconds), high-intensity exercise such as maximum strength testing and jumping?
4. What is the current state of evidence on the effect of caffeine ingestion on power output assessed by the Wingate test?
5. What is the effect of caffeine ingestion on muscular strength, muscular endurance, and power performance in resistance-trained men?
6. Is there a dose-response relationship between the amount of caffeine ingested and muscular strength and endurance?
7. Do *ADORA2A* C allele carriers exhibit ergogenic responses to caffeine ingestion on muscle strength, power, and endurance?
8. Is the *CPY1A2* genotype associated with the inter-individual variation in responses to caffeine ingestion in the context of muscle strength, power, and endurance exercise performance?

The above-mentioned questions are answered by conducting eight studies with the following aims:

1. The aim of the first study was to perform an umbrella review of meta-analyses that explored the acute effects of caffeine ingestion on exercise performance.
2. This aim of the second study was to critically evaluate and thoroughly discuss the evidence on the topic of caffeine supplementation in resistance exercise, as well as to provide practical guidelines for the application of caffeine supplementation in resistance exercise.
3. The aim of the third study was to perform systematic review and meta-analysis of studies that investigated the acute effects of caffeine ingestion on maximum dynamic muscular strength and jumping performance.
4. The aim of the fourth study was to perform a meta-analysis of studies that investigated the effect of caffeine ingestion on mean and peak power output in the Wingate test.

5. The aim of the fifth study was to examine the acute effects of caffeine supplementation (6 mg/kg) on muscular strength, muscular endurance, power, rating of perceived exertion, and pain perception in a sample of resistance-trained men.
6. The aim of the sixth study was to investigate the dose-response relationship between the amount of caffeine ingested and muscular strength and endurance.
7. The aim of the seventh study was to explore the acute effects of caffeine ingestion on movement velocity, muscular endurance, jumping and sprinting performance among *ADORA2A* C allele carriers.
8. The aim of the eight study was to explore the influence of *CYP1A2* genotype on the acute effects of caffeine ingestion on movement velocity, muscular endurance, jumping and sprinting performance.

The publication status of journal articles from these eight studies is presented in Table 1.

Table 1. The publication status of journal articles from the studies included in this thesis

Study 1 and chapter 4	**Title:** Wake Up and Smell the Coffee: Caffeine Supplementation and Exercise Performance—An Umbrella Review of 21 Published Meta-Analyses
	Status: Published in June 2020 in the British Journal of Sports Medicine (Impact factor: 12.022)
Study 2 and chapter 5	**Title:** The Influence of Caffeine Supplementation on Resistance Exercise: A Review
	Status: Published in January 2019 in Sports Medicine (Impact factor: 8.551)
Study 3 and chapter 6	**Title:** Effects of Caffeine Intake on Muscle Strength and Power: A Systematic Review and Meta-Analysis
	Status: Published in March 2018 in the Journal of the International Society of Sports Nutrition (Impact factor: 5.068)

Study 4 and chapter 7	**Title:** Caffeine Ingestion Enhances Wingate Performance: A Meta-Analysis
	Status: Published in March 2018 in the European Journal of Sport Science (Impact factor: 2.781)
Study 5 and chapter 8	**Title:** Caffeine Ingestion Acutely Enhances Muscular Strength and Power but Not Muscular Endurance in Resistance-Trained Men
	Status: Published in May 2017 in the European Journal of Sport Science (Impact factor: 2.781)
Study 6 and chapter 9	**Title:** What Dose of Caffeine to Use: Acute Effects of 3 Doses of Caffeine on Muscle Endurance and Strength
	Status: Published in March 2020 in the International Journal of Sports Physiology and Performance (Impact factor: 3.528)
Study 7 and chapter 10	**Title:** ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation
	Status: Published in March 2020 in Nutrients (Impact factor: 4.546)
Study 8 and chapter 11	**Title:** CYP1A2 Genotype and Acute Effects of Caffeine on Resistance Exercise, Jumping, and Sprinting Performance
	Status: Published in April 2020 in the Journal of the International Society of Sports Nutrition (Impact factor: 5.068)

1.1. Contribution to knowledge and statement of significance

This PhD project contributes to the current body of knowledge in four ways. Firstly, the four published reviews (studies 1 to 4) summarised the equivocal evidence presented in the literature regarding the acute effects of caffeine ingestion on resistance exercise, dynamic strength, as well as jumping and sprinting performance, and provided sound conclusions on the topics. These findings may be useful to athletes, coaches, and sports nutritionists in making evidence-based decisions about caffeine supplementation, and may inform future research in this area. Secondly, Study 5 expands the knowledge on the effects of caffeine ingestion on strength, muscular endurance, and power in resistance-trained men. The results of this study are of

interest to athletes competing in events in which strength, endurance, and power are important performance-related factors, such as powerlifting and weightlifting. Thirdly, Study 6 provided new insights into the effects of different doses of caffeine on muscular strength and endurance, contributing to the limited body of evidence on this topic. Fourthly, Studies 7 and 8 expanded the limited knowledge on the influence of genotype variations on the acute effects of caffeine ingestion on muscle strength, power, and endurance. Overall, the findings of the eight studies provided new evidence on the ergogenic effects of caffeine supplementation on predominantly anaerobic exercise performance that may facilitate the development of future evidence-based recommendations in this area.

1.2. Structure of the thesis

The present thesis is divided into 13 chapters. Chapter 1 provides a general introduction. Chapter 2 contains a review of the literature. Chapter 3 includes a brief overview of the research methodologies used in the eight studies. Chapters 4 to 11 include transcripts of the published journal articles. Chapter 12 includes an overall discussion coupled with suggestions for future research. Chapter 13 includes a conclusion.

2. Literature review

The ergogenic potential of caffeine for aerobic endurance performance has been extensively studied in the sports science literature, with research dating back to 1907 (Rivers & Webber, 1907). Currently, there is an abundance of evidence showing that caffeine can have an ergogenic effect on aerobic performance (Davis & Green, 2009). However, the research focused on high-intensity exercise performance has been much less represented in the academic literature, and, thus, this topic remains to be further explored. As highlighted by Davis and Green (2009), the impact of caffeine ingestion on maximum dynamic strength, muscular endurance, and power, is unclear. Further research in this area is warranted, as these fitness qualities may play an important role in many sports. For instance, muscular strength has been shown to positively influence the rate of force development and is highly correlated with jumping height/distance, short sprint performance, and some sport-specific skills (Suchomel, Nimphius, & Stone, 2016). Muscular endurance may be of importance in sports such as rowing (Lawton, Cronin, & McGuigan, 2013) while power (both immediate, such as jumping, and mean power, as recorded during a 30-second Wingate test) is correlated with results in several other performance tests (Bar-Or, 1987; Vescovi & McGuigan, 2008). If caffeine intake enhances strength, muscular endurance, and/or power, it might also improve performance in sport-specific situations. Furthermore, if effective, caffeine supplementation could be used to amplify the training stimulus.

2.1. Mechanisms of action

Some of the initially proposed mechanisms for the ergogenic effect of caffeine on exercise performance were enhanced fat oxidation and glycogen sparing (Costill, Dalsky & Fink, 1978). However, this hypothesis has received little support in the literature (Graham, 2001). Currently, it seems that a more likely mechanism is the antagonistic effect of caffeine on adenosine receptors (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). Adenosine binds to the A_1 and A_{2a} G protein-coupled receptors (McLellan, Caldwell, & Lieberman, 2016). The binding of adenosine to these receptors inhibits neurotransmitter release. Caffeine is structurally similar to adenosine, and, therefore, when ingested, it blocks the binding of adenosine to A_1 and A_{2a} receptors. When caffeine is ingested, it promotes the release of various neurotransmitters, such as acetylcholine and dopamine (McLellan et al., 2016), exerts central nervous system effects, and alters arousal, which can lead to improvements in performance (Green & Davis, 2009). Caffeine may also increase calcium release from the sarcoplasmic reticulum (Tarnopolsky,

2008). The increase in calcium release may result in a more forceful muscular contraction (Tarnopolsky, 2008), which might explain some of the ergogenic effects of caffeine on exercise performance.

Caffeine ingestion results in a wide array of physiological and psychological responses and, thus, it is difficult to isolate the key mechanism underpinning its ergogenic effect, especially from studies done in humans (Tallis, Duncan, & James, 2015). There is growing interest in exploring the effect of caffeine using single fibre animal models. In such studies, the muscle is isolated, and its activity is explored using an external electrical stimulus (Tallis et al., 2015). Work done using these mouse models shows that a greater force production of both predominantly fast, type II fibre muscle groups (in this example, mouse extensor digitorum longus) and predominantly slow, type I fibre muscle groups (in this example, mouse soleus in this case) is increased under the influence of caffeine, with improvements ranging from 3% to 6% (Tallis, James, Cox, & Duncan, 2012).

2.2. Effects of caffeine supplementation on muscular strength

A meta-analysis by Warren, Park, Maresca, McKibans, and Millard-Stafford (2010) showed that caffeine ingestion may have a significant ergogenic effect on maximal voluntary contraction (MVC) as assessed via isometric actions and on isokinetic apparatuses. However, compared to dynamic exercises that include both concentric and eccentric muscle actions, isometric actions have a lower practical application in training regimes of both athletes and fitness enthusiasts (Fleck & Kraemer, 2014). Additionally, the findings obtained using isometric muscle actions should not necessarily be generalised to dynamic muscle actions (Baker & Carlyon, 1994). Astorino, Rohmann, and Firth (2008) conducted a seminal study on the effects of caffeine on maximum dynamic strength (1RM). The authors reported no significant strength-enhancing effect of caffeine ingestion in a group of resistance-trained men. Williams, Cribb, Cooke, and Hayes (2008) obtained similar findings in a subsequent study among trained men. Goldstein, Jacobs, Whitehurst, Penhollow, and Antonio (2010) reported a significant increase in upper body strength following caffeine ingestion. The inconsistent findings of the studies prevent drawing sound conclusions about the ergogenic potential of caffeine for maximal dynamic strength outcomes. There is, therefore, an evident need for more research in this area.

2.3. Effects of caffeine supplementation on muscular power

Among the most commonly used tests of anaerobic capacity and power output is the Wingate test (Bar-Or, 1987). The available literature suggests that caffeine has a minimal ergogenic effect on Wingate test performance (Davis & Green, 2009). A commonly cited study supporting this is the hallmark work by Collomp, Ahmaidi, Audran, Chanal, and Prefaut (1991), which reported no significant increases in the peak power and mean power output in the Wingate test following the ingestion of caffeine. However, Collomp et al. (1991) included only six participants in their trials, which likely lead to issues with statistical power. Thus, despite the common belief that caffeine ingestion does not enhance Wingate test performance, this topic needs future studies.

Jumping tasks are also often used for the assessment of muscular power. Foskett, Ali, and Gant (2009) were the first to investigate the effects of caffeine ingestion on jumping performance. The authors reported a significant increase (3%) in jump height following ingestion of caffeine. The findings of Bloms, Fitzgerald, Short, and Whitehead (2016) also indicated that caffeine is an effective ergogenic aid for achieving acute improvements in countermovement jump height. However, Ali, O'Donnell, Foskett, and Rutherfurd-Markwick (2016) found no significant effect on countermovement jump height following caffeine ingestion. Given the inconsistent evidence and high importance of jumping abilities for many sports, it would be of both scientific and practical significance to further investigate the potential performance-enhancing impact of caffeine ingestion on jumping tasks.

2.4. Effects of caffeine supplementation on muscular endurance

Tarnopolsky (2008) suggested that caffeine intake should have a considerable positive, acute effect on endurance. Warren et al. (2010) confirmed that caffeine ingestion can enhance isometric and isokinetic muscular endurance, but this seems to be true primarily when assessed using open endpoint tests (unlike when using fixed endpoint tests). A meta-regression analysis from the same study showed that for every 1 mg/kg increase in caffeine dose the effect size (ES; standardised mean difference) for muscular endurance increased by 0.1. While a subsequent meta-analysis performed by Polito, Souza, Casonatto, and Farinatti (2016)

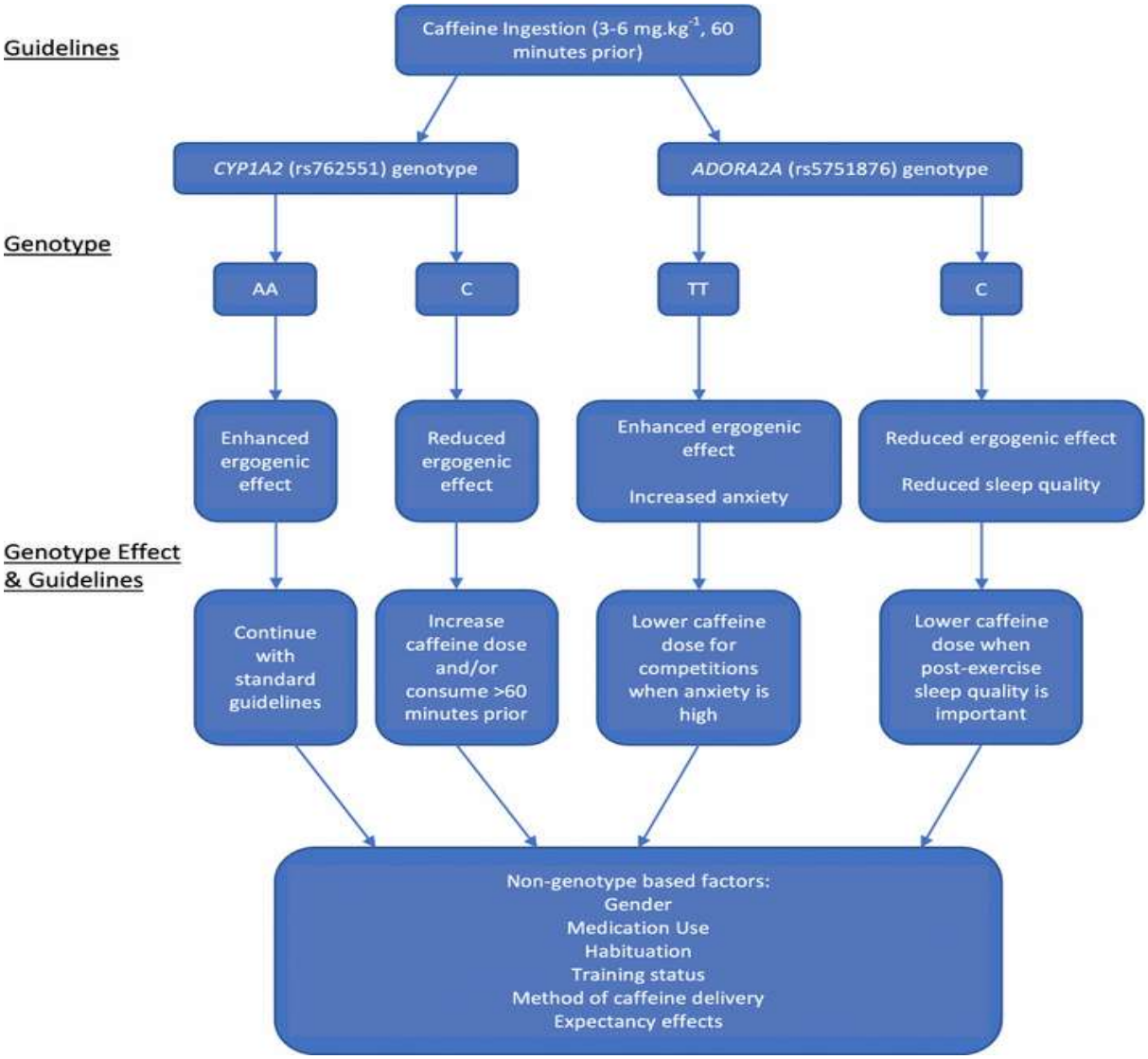
confirmed that caffeine ingestion may also enhance dynamic muscular endurance, none of the studies included in the review examined the dose-response relationship. It has often been thought that an increase in the dose of caffeine fails to elicit a further response. However, only a small number of studies have examined the dose-response effects of caffeine on human performance, indicating a need for future research (Tallis et al., 2015). Given that the responses to caffeine supplementation may vary substantially between individuals (Pickering & Kiely, 2018), it would be important to investigate if there is indeed a dose-response relationship between caffeine intake and muscular endurance in the same group of participants rather than pooling data from various studies, which differed in a range of methodological characteristics (unrelated to the caffeine dose) that may affect the ESs.

The most commonly used amount of caffeine in studies conducted in this area has been 6 mg/kg (Graham, 2001). However, there is a growing interest among researchers in investigating the ergogenic effects of caffeine at lower doses, such as 2 mg/kg, given the fact that significantly fewer side-effects occur at this dosage (Spriet, 2014). Therefore, future research should examine: (i) if there is a dose-response relationship between the amount of ingested caffeine and the magnitude of its ergogenic effect on muscular endurance; and (ii) whether the ergogenic effects commonly seen at moderate to high doses (i.e., 6 mg/kg) can also be observed with lower doses of caffeine such as 2 mg/kg and 4 mg/kg.

2.5. Associations of genotype with responses to caffeine ingestion

There is substantial inter-individual variability in responses to caffeine ingestion (Pickering, & Kiely, 2018). While some individuals experience enhanced performance, others show no improvement, and, in some cases, even decreases in performance (Pickering, & Kiely, 2018). One potential driver of the differences in individual responses is variation in *ADORA2A* and/or *CYP1A2* genotype (Pickering, & Kiely, 2018; Figure 1).

Figure 1. Genotype and non-genotype factors associated with the inter-individual variation in responses to caffeine ingestion (taken from Pickering & Kiely, 2018)



ADORA2A is the gene that encodes A_{2A} subtypes of adenosine receptors (Cornelis, El-Sohemy, & Campos, 2007). Previous research has suggested that this receptor represents the primary target of caffeine action in the central nervous system, and, thus, polymorphic variations in the *ADORA2A* gene may impact the acute responses to caffeine ingestion (Cornelis et al., 2007). The rs5751876 polymorphisms in the *ADORA2A* gene are comprised of a C-to-T substitution at nucleotide position 1083 (also known as 1976C>T) (Cornelis et al., 2007). Interestingly, as compared to TT homozygotes, *ADORA2A* C allele carriers have higher habitual caffeine

consumption, which may suggest that these individuals need higher doses of caffeine to obtain a pharmacological effect (Cornelis et al., 2007).

Only one study has explored the influence of variation in this gene—in this case, a common polymorphism (rs5751876)—on the ergogenic effects of caffeine on exercise performance (Loy, O'Connor, Lindheimer, & Covert, 2015). The study included 12 participants (6 TT homozygotes and 6 C allele carriers [i.e., CC/CT genotype]). These participants were untrained women who completed 20 min of cycling at 60% of VO_{2peak} followed by two 10-min cycling time trials. The exercise task was performed on two occasions, following the ingestion of 5 mg/kg of caffeine or placebo. Results indicated that caffeine ingestion was ergogenic for TT homozygotes but not for C allele carriers. Based on this study, C allele carriers were identified as “non-responders” to caffeine (Loy et al., 2015). To date, this is the only study that explored the influence of variations in *ADORA2A* on acute effects of caffeine ingestion on exercise performance, which highlights the need for future research.

The gene *CYP1A2* encodes cytochrome P450 1A2, an enzyme responsible for up to 95% of caffeine metabolism (Gu, Gonzalez, Kalow, & Tang, 1992). The speed of caffeine metabolism is affected by a single nucleotide polymorphism, rs762551, within this gene (Gu et al., 1992). Individuals with the AA genotype are commonly classified as "fast caffeine metabolisers", while C allele carriers (AC/CC genotypes) tend to have slower clearance of caffeine and are, therefore, commonly classified as "slow caffeine metabolisers" (Sachse, Brockmöller, Bauer, & Roots, 1999). Significantly greater ergogenic effects of caffeine on aerobic endurance have been reported for individuals with the AA genotype, compared with C allele carriers (Guest, Corey, Vescovi, & El-Sohemy, 2018; Womack et al., 2012). However, for high-intensity exercise tasks of a shorter duration, the evidence is less clear.

Guest et al. (2018) showed that male athletes with AA genotype had a 5% and 7% improvement in aerobic endurance with the ingestion of 2 mg/kg and 4 mg/kg of caffeine, respectively. However, individuals with the AC genotype did not improve performance, whereas the individuals with the CC genotype experienced decreases in performance after the ingestion of caffeine. Recently, Rahimi (2018) assessed the effects of caffeine ingestion on muscular

endurance using a resistance exercise protocol. The participants performed four exercises with a load corresponding to 85% of one-repetition maximum (1RM) to momentary muscular failure, following the ingestion of caffeine or placebo. Sixteen individuals were identified as C allele carries, while 14 participants were identified as AA homozygotes. A significant difference between the groups in the total number of performed repetitions following caffeine ingestion (AA = +13% vs. AC/CC = +1%) was found. This is the only study that examined this topic using a resistance exercise protocol, and it provides evidence in support of the importance of genotype in response to caffeine ingestion. Salinero et al. (2017) conducted a similar study and used the 30-second Wingate test for assessing performance. While improvements in peak and mean power output were seen with caffeine ingestion, no differences across the genotypes were found. Given the equivocal evidence presented in the literature, future work is needed to elucidate this research question.

3. Methodology and procedures

This PhD project includes eight papers: an umbrella review, a narrative review, two meta-analyses, and four randomised crossover trials. By applying the study designs, this project provides a thorough summary of the available literature and novel insights into the effects of caffeine ingestion on muscle strength, power, and endurance. The PhD project followed the conceptual framework proposed by Burke and Peeling (2018). In this framework, the focus of research on caffeine supplementation is set on controlling confounding factors that might affect the study results, such as the time of day when the testing is performed, environmental conditions and the acute nutritional status. Furthermore, to facilitate the translation of findings into practice, the performance tasks that are widely used in sports and exercise practice have been selected. Besides, the study quality was increased by selecting performance tasks with excellent measurement properties.

3.1. Study 1: Wake up and smell the coffee: caffeine supplementation and exercise performance—an umbrella review of 21 published meta-analyses

Grgic, J., Grgic, I., Pickering, C., Schoenfeld, B. J., Bishop, D. J., & Pedisic, Z. (2020). Wake up and smell the coffee: caffeine supplementation and exercise performance—an umbrella review of 21 published meta-analyses. *British Journal of Sports Medicine*, *54*(11), 681-688.

3.1.1. Methods

Data sources included the following databases: Academic Search Premier, AUSPORT, CINAHL, Cochrane Library, ERIC, Health Source: Nursing/Academic Edition, MasterFILE Premier, PsycINFO, PubMed/MEDLINE, Scopus, SPORTDiscus and Web of Science. Meta-analyses that examined the effects of caffeine ingestion on exercise performance were included in the review. The methodological quality of the included meta-analyses was assessed using the Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR) checklist. Quality of evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE). Prediction intervals were calculated for the pooled estimate from each of the included meta-analyses. The findings of included meta-analyses were summarised in a narrative fashion.

3.2. Study 2: The influence of caffeine supplementation on resistance exercise: a review

Grgic, J., Mikulic, P., Schoenfeld, B. J., Bishop, D. J., & Pedisic, Z. (2019). The influence of caffeine supplementation on resistance exercise: a review. *Sports Medicine*, *49*(1), 17-30.

3.2.1. Methods

To identify studies relevant for this review, comprehensive literature searches were performed using PubMed/MEDLINE, Scopus, and Google Scholar databases were undertaken. The search terms included: caffeine; habitual; 'pain perception'; 'rating of perceived exertion'; strength; endurance; power; ergogenic; 'resistance training'; and meta-analysis. Studies that investigated the application of caffeine supplementation in resistance exercise protocols were scrutinised. Given its broad scope, this review was conducted in a narrative fashion.

3.3. Study 3: Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis

Grgic, J., Trexler, E. T., Lazinica, B., & Pedisic, Z. (2018). Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis. *Journal of the International Society of Sports Nutrition*, *15*(11), 1-9.

3.3.1. Methods

The systematic literature search was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A search was performed through: PubMed/MEDLINE, Scopus, Cochrane Library, Web of Science (including Science Citation Index Expanded, Social Sciences Citation Index, and Arts & Humanities Citation Index), Google Scholar, Networked Digital Library of Theses and Dissertations, ProQuest Dissertation & Theses and Open Access Theses and Dissertations. Studies that explored the effects of caffeine ingestion on: (i) 1RM strength; and/or (ii) vertical jump height, were considered for this review. These outcomes were chosen as they are both characterised by maximal effort and very short duration. The 11-point Physiotherapy Evidence Database (PEDro) scale was used for the assessment of the methodological quality of studies. The search,

data extraction, and methodological quality appraisal were done independently by two authors of the paper, followed by a discussion about any differences in the assessments until an agreement has been reached. Meta-analyses of standardised mean differences between placebo and caffeine trials from individual studies were conducted using the random-effects model.

3.4. Study 4: Caffeine ingestion enhances Wingate performance: a meta-analysis

Grgic, J. (2018). Caffeine ingestion enhances Wingate performance: a meta-analysis. *European Journal of Sport Science*, *18*(2), 219-225.

3.4.1. Methods

Searches were performed through three databases, namely, PubMed/MEDLINE, Scopus and SPORTDiscus. The following syntax was used for the search: caffeine AND (Wingate OR anaerobic OR "peak power" OR "mean power"). To be included in the review, studies were required to meet the following criteria: (i) published in English; (ii) assessed the effects of caffeine ingestion on performance in the Wingate test; (iii) employed a crossover design; and (iv) included apparently healthy human participants. The PEDro scale was used for the assessment of the methodological quality of the included studies. A random-effects meta-analysis of standardised mean differences expressed as Hedge's g was performed to analyse the data.

3.5. Study 5: Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance-trained men.

Grgic, J., & Mikulic, P. (2017). Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance-trained men. *European Journal of Sport Science*, *17*(8), 1029-1036.

3.5.1. Participants

Ethical approval was obtained from the Committee for Scientific Research and Ethics of the Faculty of Kinesiology at the University of Zagreb. Twenty resistance-trained men satisfied the inclusion criteria and volunteered to participate in the study. Three participants failed to

complete all study protocols and therefore, the final number of participants included in the analysis was 17.

3.5.2. Experimental protocol

In this study, a randomised, double-blind, crossover design was used. A total of three sessions were completed. The first session was a familiarisation, during which, the participants were also introduced to the Borg scale for the estimation of the rating of perceived exertion, and to the pain perception scale. The participants were instructed to follow their general nutrition and exercise practices before the second and the third sessions, which involved ingestion of caffeine or placebo. They were instructed to keep track of their caloric and caffeine intakes using the "Myfitness pal" application (http://www.myfitnesspal.com). Caloric intake was tracked and replicated before the third session. In addition, the participants had to refrain from caffeine intake after 6 pm on the day prior to testing, as done in previous research (Duncan, Stanley, Parkhouse, Cook, & Smith, 2013). In the 24 hours preceding the testing, as well as on the testing days, the participants refrained from vigorous exercise.

3.5.3. Supplementation protocol

The prescribed amount (i.e., 6 mg/kg) of anhydrous caffeine was diluted in 250 ml of water and mixed with 20 grams of granulated orange-tasting beverage containing 65 calories (0 grams of protein, 16 grams of carbohydrates, and 0 grams of fat). Placebo was administrated in the same fashion without the anhydrous caffeine.

3.5.4. Testing procedures

The exercise tests of jumping, throwing, muscular strength and muscular endurance performance were performed. For the assessment of lower-body power, the vertical jump test was used (for a detailed description of the testing procedure, see Martinez, Campbell, Franek, Buchanan, & Colquhoun, 2016). The assessment of upper-body power was conducted using the seated medicine ball throw test, as described by Clemons, Campbell, and Jeansonne (2010). The 1RM barbell back squat test was used for the assessment of lower-body strength as described by Goldstein et al. (2010b). Upper-body strength was evaluated using the 1RM bench press test. Muscular endurance was evaluated by performing a single set to momentary

muscular failure in the back squat and bench press exercise tests with a load corresponding to 60% of 1RM. Within 5 seconds of the successful 1RM attempts and after the muscular endurance tests, the participants were asked to indicate their levels of perceived exertion and pain on the respective scales.

3.6. Study 6: What dose of caffeine to use: acute effects of 3 doses of caffeine on muscle endurance and strength

Grgic, J., Sabol, F., Venier, S., Mikulic, I., Bratkovic, N., Schoenfeld, B. J., Pickering, C., Bishop, D. J., Pedisic, Z., & Mikulic, P. (2020). What dose of caffeine to use: acute effects of 3 doses of caffeine on muscle endurance and strength. *International Journal of Sports Physiology and Performance*, *15*(4), 470-477.

3.6.1. Participants

The sample of participants in this study included 28 resistance-trained men. Only men with resistance training experience were included. Resistance-trained men were defined as having a minimum of 12 months of resistance training experience with a minimum weekly training frequency of two times on most weeks. The exclusion criteria were: (i) prior use of anabolic steroids; (ii) the use of caffeine supplementation (in any form) in the last six months; and (iii) existence of any health limitations. We decided to exclude individuals who consume caffeine supplements, as they may be able to differentiate between placebo and different doses of caffeine more correctly than those who do not consume caffeine supplements (Saunders et al., 2017).

3.6.2. Experimental protocol

This study was a double-blind, crossover trial. All participants attended a laboratory on six separate occasions, following a minimum 3-h fasting period. All trials were performed at the same time of the day for each participant to ensure that the results are not affected by circadian variation. The trials took place 5-7 days apart. The first session included familiarisation to the exercise protocol and responding to a Food Frequency Questionnaire (FFQ) to assess habitual caffeine intake. The questionnaire has previously been validated by Bühler, Lachenmeier, Schlegel, and Winkler (2014). After one familiarisation session, the five main sessions

(including three caffeine supplementation sessions, one placebo session, and one control session) were conducted in a randomised fashion. Twenty-four hours before the main trials, participants were required not to do any strenuous exercise. Additionally, the participants were required to refrain from caffeine ingestion 12 hours before the five experimental trials. Caffeine has a half-life of 4-6 hours and, thus, ceasing consumption for 12 hours prior to the exercise bout is sufficient for the removal of circulating concentrations of caffeine (Graham, 2001). Food intake was monitored during the 24-h period before all five experimental trials using an online food diary application (MyFitnessPal). Because the aim of this study was to investigate a dose-response relationship between caffeine intake and its ergogenic effects, the caffeine capsule was randomly administered on three different occasions with caffeine doses of 2 mg/kg, 4 mg/kg, and 6 mg/kg. Placebo was administered in the form of a capsule containing dextrose. Both in the caffeine trials and in the placebo trial, after the completion of the exercise session, the participants responded to the following question: "Which supplement do you think you have ingested?" The question had three possible responses: (a) caffeine; (b) placebo; and (c) do not know. They were also asked to state the reason for choosing the answer.

3.6.3. Testing procedures

As a part of the 1RM assessment, the participants first completed a set with 8-10 repetitions with 50% of their estimated 1RM. The second set was performed with approximately 75% of their estimated 1RM for three to five repetitions. Then, the participant completed the test using 95% of their estimated 1RM. The weight was then increased or decreased in the next attempts depending on whether the participant successfully lifted the load or not. All 1RMs were determined within three to five sets. Three to five minutes of rest were employed between the attempts. Muscular endurance was assessed with repetitions performed to momentary muscular failure with a load corresponding to 60% of 1RM. For the upper body, the bench press exercise was used, while for the lower-body, the barbell back squat exercise was used. Within five seconds of completing the exercise task, the participants were asked to indicate their levels of rating of perceived exertion (Borg, 1970) and perceived pain (Cook, O'connor, Oliver, & Lee, 1998).

3.7. Study 7: ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation

Grgic, J., Pickering, C., Bishop, D. J., Del Coso, J., Schoenfeld, B. J., Tinsley, G. M., & Pedisic, Z. (2020). ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation. *Nutrients*, *12*(741), 1-9.

3.7.1. Participants

The study was conducted in a sample of 22 resistance-trained men. Only men with resistance training experience were considered for inclusion. Only resistance-trained men were included in this study.

3.7.2. Experimental protocol

This study was a double-blind, crossover trial. Habitual caffeine intake was assessed using a FFQ. All participants attend a laboratory on four separate occasions following a minimum 3-h fasting period. All trials were performed at the same time of the day for each participant, to ensure that the results are not affected by circadian variation. The trials took place 4-7 days apart. The first two sessions included familiarisation to the exercise protocol. After the two familiarisation sessions, the two main sessions, including a caffeine supplementation session and a placebo session, were conducted in a randomised fashion. Twenty-four hours before the main trials participants were required not to do any strenuous exercise. The participants were also asked to track their food intake, and physical activity for 24-hours before the two main trials. Additionally, the participants were required to refrain from caffeine ingestion for 12 hours before the two experimental trials.

Caffeine was provided in a dose of 3 mg/kg. Placebo was administered in the form of a capsule containing dextrose. Both in the caffeine and in the placebo trials, after the completion of the exercise session, the participants responded to the following question: “Which supplement do you think you have ingested?” The question had three possible responses: (a) caffeine; (b) placebo; and (c) do not know. They were also asked to state the reason for choosing the answer. The morning after the trials the participants were required to respond to an eight-item

questionnaire for assessing side-effects (Salinero et al., 2017). Buccal swab samples were analysed to determine *ADORA2A* genotype.

3.7.3. Testing procedures

The assessment of 1RM was performed only in the first session. In all other sessions, the participants performed the bench press exercise with loads of 25%, 50%, 75%, and 90% of 1RM. With each load, the participants performed two sets of one repetition and were instructed to lift the load as fast as possible. A GymAware linear position transducer (GymAware Power Tool, Kinetic Performance Technologies, Canberra, Australia) was attached to the barbell and used to measure repetition velocity and power. Muscle endurance in the bench press exercise was evaluated by performing repetitions to momentary muscle failure with a load corresponding to 85% of 1RM. After the muscle endurance tests, the participants also performed a countermovement jump (CMJ) test and 30-second Wingate test.

3.8. Study 8: CYP1A2 genotype and acute effects of caffeine on resistance exercise, jumping, and sprinting performance

Grgic, J., Pickering, C., Bishop, D. J., Schoenfeld, B. J., Mikulic, P., & Pedisic, Z. (2020). CYP1A2 genotype and acute effects of caffeine on resistance exercise, jumping, and sprinting performance. *Journal of the International Society of Sports Nutrition*, *17*, 1-11.

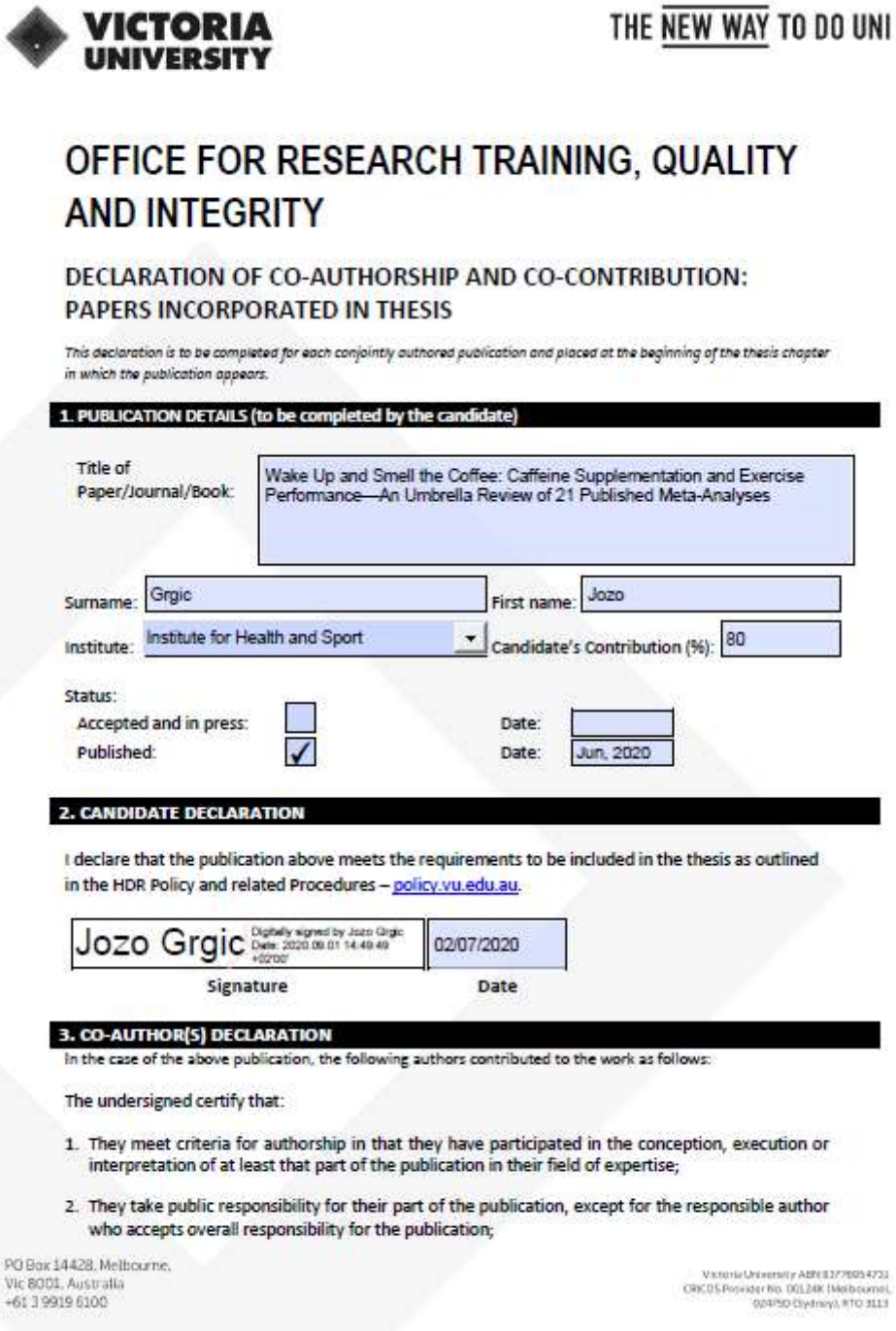
3.8.1. Methods

The same data as in Study 7 were analysed, with the exception that the analysis focused on *CYP1A2* genotype.

3.8.2. Ethics approval

No ethics clearance was needed for the four reviews, as these studies did not include any primary data collection. Ethics clearances for Study 5 and 6 were obtained from the Committee for Scientific Research and Ethics of the Faculty of Kinesiology at the University of Zagreb. For Study 7 and 8, the ethical approval was provided by the Victoria University Human Research Ethics Committee (VUHREC).

4. Wake up and smell the coffee: Caffeine supplementation and exercise performance—an umbrella review of 21 published meta-analyses



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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

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2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

Jozo Grgic Digitally signed by Jozo Grgic Date: 2020.09.01 14:49:49 +02'00' | 02/07/2020

Signature | Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

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3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

The original data has been electronically stored on VU R drive and the student's laptop for at least five years since June 2020.

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Ivana Grgic	5	Data collection and interpretation, and manuscript writing	[redacted]	02/07/2020
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Brad J. Schoenfeld	3	Data interpretation and manuscript writing		02/07/2020
David J. Bishop	3	Data interpretation and manuscript writing		02/07/2020
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Wake up and smell the coffee: Caffeine supplementation and exercise performance—an umbrella review of 21 published meta-analyses

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4.1. Abstract

Objective: To systematically review, summarise, and appraise findings of published meta-analyses that examined the effects of caffeine on exercise performance.

Design: Umbrella review.

Data sources: Twelve databases.

Eligibility criteria for selecting studies: Meta-analyses that examined the effects of caffeine ingestion on exercise performance.

Results: Eleven reviews (with a total of 21 meta-analyses) were included, all being of moderate or high methodological quality (assessed using the AMSTAR 2 checklist). In the meta-analyses, caffeine was ergogenic for aerobic endurance, muscle strength, muscle endurance, power, jumping performance, and exercise speed. However, not all analyses provided a definite direction for the effect of caffeine when considering the 95% prediction interval. Using the GRADE criteria the quality of evidence was generally categorised as moderate (with some low to very low quality of evidence). Most individual studies included in the published meta-analyses were conducted among young men.

Summary/Conclusion: Synthesis of the currently available meta-analyses suggest that caffeine ingestion improves exercise performance in a broad range of exercise tasks. Ergogenic effects of caffeine on muscle endurance, muscle strength, anaerobic power, and aerobic endurance were substantiated by moderate quality of evidence coming from moderate-to-high quality systematic reviews. For other outcomes, we found moderate quality reviews that presented evidence of very low or low quality. It seems that the magnitude of the effect of caffeine is generally greater for aerobic as compared with anaerobic exercise. More primary studies should be conducted among women, middle-aged and older adults to improve the generalisability of these findings.

4.2. Introduction

In 2018 the International Olympic Committee published a consensus statement regarding the effects of dietary supplements on exercise performance of athletes (Maughan et al., 2018). The consensus statement placed meta-analyses at the top of the evidence pyramid (Maughan et al., 2018). In sports nutrition research, meta-analyses provide a method of pooling available primary studies exploring the efficacy of a given supplement on a specific outcome (e.g., performance of an exercise test). As such, meta-analyses are used to support establishing evidence-based guidelines and decision-making for the effective prescription of nutritional supplements and ergogenic aids.

One supplement with a long history of use for its ergogenic effects on performance is caffeine (Rivers & Webber, 1907). Caffeine ingestion is highly prevalent among athletes, especially since 2004 when it was removed from the World Anti-Doping Agency list of within-competition banned substances (Del Coso et al., 2011). For example, 74% of urine samples collected from 2004 to 2008 and analysed as a part of doping control contained caffeine (Del Coso et al., 2011). Given inconsistent evidence in the primary research that examined the effects of caffeine on exercise performance, several research groups explored this area using meta-analytical methods (Christensen, Shirai, Ritz, & Nordsborg, 2017; Conger, Warren, Hardy, & Millard-Stafford, 2011; Doherty & Smith, 2004; Doherty & Smith, 2005; Gonçalves Ribeiro et al., 2017; Grgic & Pickering, 2019; Grgic et al., 2018; Grgic, 2018; Polito et al., 2016; Shen, Brooks, Cincotta, & Manjourides, 2019; Southward, Rutherfurd-Markwick, & Ali, 2018; Warren et al., 2010). While these meta-analyses generally report ergogenic effects of caffeine on exercise performance, even adequately conducted meta-analyses tend to focus on the ergogenic effects of caffeine within just a single performance domain. As an illustration, Grgic and Pickering (2019) only examined the effects of caffeine ingestion on isokinetic peak torque.

Given that each meta-analysis is typically focused only on a specific aspect of exercise performance, it is challenging to: (1) compare the effects of caffeine ingestion on different performance domains; (2) comparatively assess the availability and strength of evidence for different performance domains; (3) establish comprehensive recommendations on the use of caffeine in sports and exercise; and (4) provide overall recommendations for future research on the ergogenic effects of caffeine on exercise performance. Such recommendations may increase

the uptake of evidence-based findings in the context of supplement prescription and guide future research in this area.

Consistency of meta-analytical findings is often lacking, as even meta-analyses that have examined the same outcome may produce conflicting findings. For instance, Gonçalves Ribeiro et al. (2017) did not observe significant effects of caffeine ingestion on power. In contrast, a subsequent meta-analysis by Grgic (2018) reported that caffeine ingestion is ergogenic for this outcome. Such conflicting findings hinder firm evidence-based conclusions from individual meta-analyses. Ultimately, the methods employed in a specific meta-analysis (e.g., the number of databases searched, the comprehensiveness of the search syntax, the methods used for analysing the data) determine the robustness of the pooled results. For example, a meta-analysis on the effects of caffeine supplementation on power conducted by Gonçalves Ribeiro et al. (2017) included only studies that were published between January 2010 and December 2015. Due to these restrictions, studies published before 2010 were excluded from consideration, and the authors provided no rationale for their approach. Only four studies that assessed power during Wingate tests were included in their review, and no significant pooled effects were found. Grgic (2018) conducted a similar meta-analysis without any restrictions regarding the year of publication; this analysis included 16 studies and reported significant improvements in both mean and peak power on the Wingate test with caffeine ingestion.

One proposed method to overcome some of the above, and other, potential limitations of meta-analyses is to perform umbrella reviews (Aromataris et al., 2015). Umbrella reviews (i.e., reviews that include the syntheses and appraisal of existing systematic reviews and meta-analyses) provide a comprehensive view of the evidence landscape on a given topic because they encompass larger scale of evidence (Aromataris et al., 2015). Such reviews help us to understand the current strengths and limitations of the entire body of evidence by comparing and contrasting findings from the entirety of the published data. Such a treatise on the effects of caffeine on exercise may be a useful resource for researchers, sports nutritionists, athletes, coaches, and others interested in the ergogenic effects of caffeine on acute exercise performance. To date, there are no published umbrella reviews focusing on the effects of caffeine on exercise performance.

The aim of the present paper is threefold: (1) to systematically review available meta-analytical evidence that has examined the effects of caffeine on exercise performance; (2) to addresses the quality, strengths, and limitations of the meta-analytical evidence; and (3) to identify current gaps in the literature and make key suggestions for future research.

4.3. Methods

4.3.1. Search strategy

This review followed the guidelines set forth by Aromataris et al. (2015). We systematically searched through 12 different databases, including: Academic Search Premier, AUSPORT, CINAHL, Cochrane Library, ERIC, Health Source: Nursing/Academic Edition, MasterFILE Premier, PsycINFO, PubMed/MEDLINE, Scopus, SPORTDiscus, and Web of Science. The databases were searched from the inception of indexing until 24th September 2018 using the following search syntax: caffeine AND (meta-an* OR "systematic review") AND (exercise OR training OR muscle OR "physical performance"). The search syntax for each database is provided in Table 2. Quotation marks and the wildcard symbol were used to narrow down the search. In each full-text that was read, we also screened the reference list as a part of a secondary search. The search was carried out independently by two authors (JG and IG) to prevent any selection bias. The authors independently examined the titles, abstracts, and when applicable, the full-texts of the identified publications. Upon examination, the authors compared their lists of included and excluded papers; any disagreements were resolved by discussion and agreement between the authors.

Table 2. Search syntax for all searched databases

Database	**Search syntax**
AUSPORT	caffeine AND (meta-an* OR "systematic review") AND (exercise OR training OR muscle OR "physical performance")
EBSCOHost Research Databases (including: Academic Search Premier, CINAHL, ERIC, Health Source: Nursing/Academic Edition, MasterFILE Premier, PsycINFO, SPORTDiscus)	caffeine AND (meta-an* OR "systematic review") AND (exercise OR training OR muscle OR "physical performance")
Cochrane Library	caffeine AND (meta-an* OR "systematic review") AND (exercise OR training OR muscle OR "physical performance")
PubMed/MEDLINE	caffeine[tw] AND (meta-an*[tw] OR "systematic review"[tw]) AND (exercise[tw] OR training[tw] OR muscle[tw] OR "physical performance"[tw])
Scopus	Title-abs-key(caffeine AND (meta-an* OR "systematic review") AND (exercise OR training OR muscle OR "physical performance"))
Web of Science (including Science Citation Index Expanded, Social Sciences Citation Index, and Arts & Humanities Citation Index)	TS=(caffeine AND (meta-an* OR "systematic review") AND (exercise OR training OR muscle OR "physical performance"))

4.3.2. Inclusion criteria

We included reviews coupled with a meta-analysis that examined the acute effects of caffeine ingestion on any exercise performance-related outcome. Both peer-reviewed and conference papers published in English or other languages were considered. Meta-analyses that included studies that combined caffeine with other ergogenic compounds, such as taurine, were excluded as they do not allow for the differentiation of the effects between the compounds. However, meta-analyses that included studies comparing caffeine and carbohydrate ingestion versus caffeine alone were included as long as the effect of caffeine could be isolated (i.e., two solutions were given to the participants, one with caffeine and one without). As reported by the Participant-Intervention-Comparison-Outcome (PICO) process, the following criteria were followed:

Participants

Apparently healthy individuals of both sexes and all ages.

Interventions

Any acute study examining the effects of caffeine ingestion on exercise performance.

Comparison group

Placebo (provided that the effects of caffeine could be isolated).

Outcome measures

Any form of exercise performance.

4.3.3. Data extraction

The following data were extracted from the included meta-analyses: (1) the list of authors and year of publication; (2) the number and type of studies included in the meta-analysis; (3) the pooled number of participants; (4) the type of exercise test that was evaluated; (5) the pooled ES with the 95% confidence interval (CI); (6) *p*-values; and (7) percent changes and I^2 values. The same two authors that carried out searches also conducted the data extraction process. All data were tabulated to a spreadsheet predefined for this review. After data extraction, the spreadsheets were cross-checked between the authors for accuracy.

4.3.4. Methodological quality evaluation

The methodological quality of the included meta-analyses was assessed using the validated Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR 2) checklist (Shea et al., 2017). Two reviewers (JG and IG) independently assessed the methodological quality of the included systematic reviews using the AMSTAR 2 checklist. This checklist contains 16 items that include questions regarding the use of the PICO description as a part of the inclusion criteria, the *a priori* registration of the review design, the comprehensiveness of the literature search, the number of authors that performed that search and data extraction, the description of included studies, the assessment of the quality of the included primary studies, reporting of sources of funding in the primary studies, the use of appropriate statistical methods, assessments of heterogeneity in the meta-analyses, and reporting of the potential conflicts of interest. Full details on the checklist can be found in the paper by Shea et al. (2017). Each item on this checklist is answered with a "yes", "no", "cannot answer", or "not applicable". Out of these possible answers, only the "yes" answer counts as a point in the total score for the assessed review. Based on the summary point scores, the meta-analyses were categorised as high quality (at least 80% of the items were satisfied); moderate quality (at least 40% of the items were satisfied); or low-quality (less than 40% of the items was satisfied), as performed previously (Johnson et al., 2014; Monasta et al., 2010).

4.3.5. Quality of evidence

To assess the quality of evidence we used the modified Grading of Recommendations Assessment, Development and Evaluation (GRADE) principles (Guyatt et al., 2011). For the purpose of this review, we examined the following GRADE aspects: (1) risk of bias (determined by the quality of the primary studies, as assessed in the original meta-analyses); (2) inconsistency (determined by variables such as the variation in the effects across the included studies and the overlap of the 95% CIs between the studies); (3) indirectness (determined by the generalisability of the findings while considering the study populations included in the primary research); (4) imprecision (determined by the total sample size in the analysis and the width of the 95% CI of the pooled effect size); and (5) publication bias (determined if the ES of the largest study in each analysis was smaller than the pooled estimate from the meta-analysis and by examining the asymmetry of the funnel plot). Based on these criteria, the meta-analytical evidence was classified as high, moderate, low, or very low. The GRADE assessment was

conducted independently by two authors (JG and IG), with discussion and agreement for any differences.

4.3.6. Prediction interval

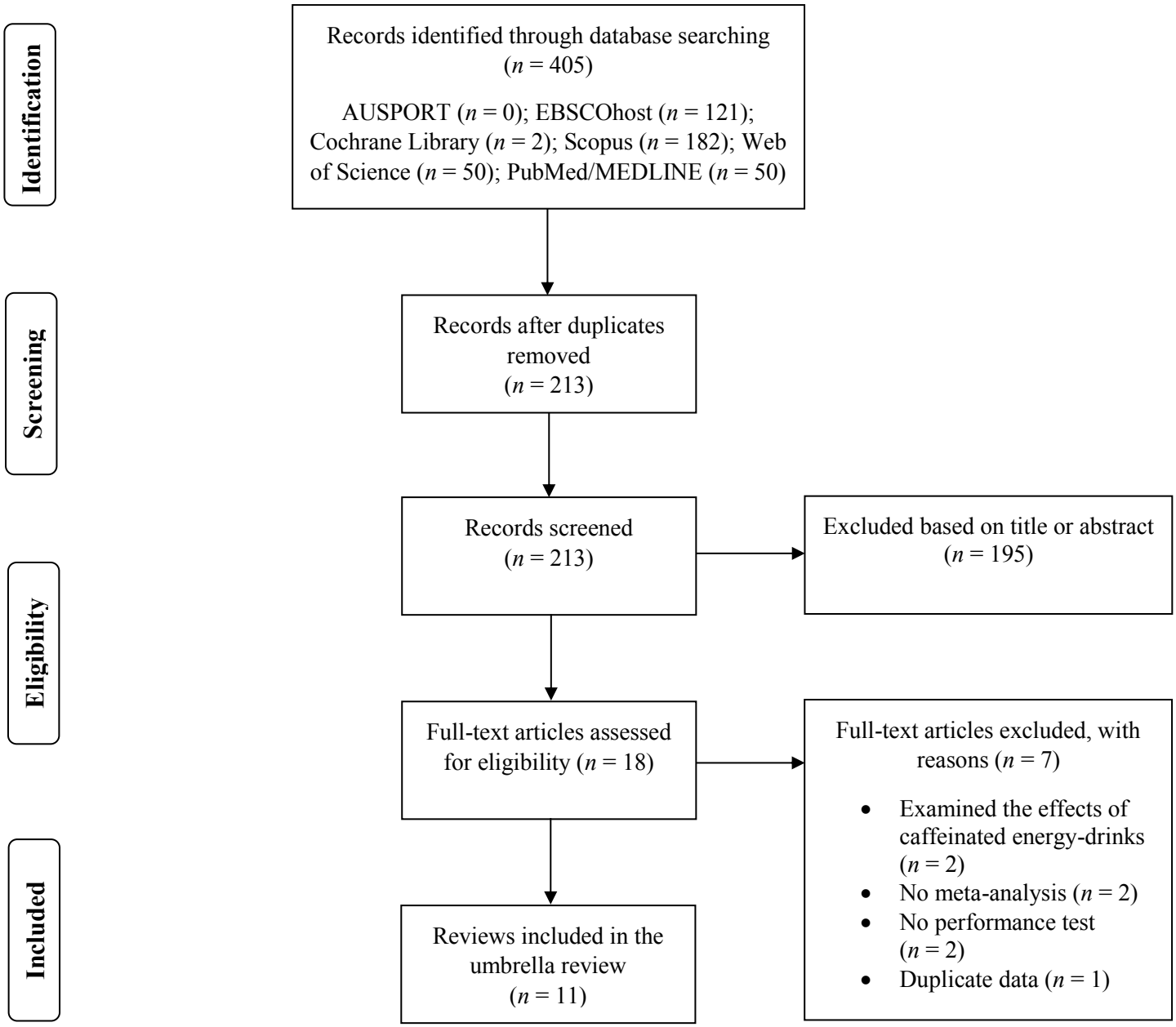
Using the number of included studies, the pooled standardised mean difference, the upper limit of the 95% CI and the tau-squared values (from each analysis), we calculated 95% prediction interval (PI) for all included meta-analyses (spreadsheet available at: https://www.meta-analysis.com/pages/prediction.php). The 95% PI represents the range in which the ES a future study conducted on the topic will most likely lie. If the tau-squared values were not provided in the meta-analysis, these data were either requested from the authors or re-calculated based on the data presented in the included studies.

4.4. Results

4.4.1. Search results

The initial literature search identified 405 search records. Out of that pool of search results, 18 full-texts were read. Seven reviews were excluded after reading the full-texts (Astorino & Roberson, 2010; Brown, Brown, & Foskett, 2013; Doherty & Smith, 2005; Ganio et al., 2009; Glaister & Gissane, 2018; Souza et al., 2017; Zhang et al., 2015). The reasons for their exclusion are provided in Table 3. Eleven reviews (with a total of 21 meta-analyses) were included in this umbrella review (Christensen et al., 2017; Conger et al., 2011; Doherty & Smith, 2004; Gonçalves Ribeiro et al., 2017; Grgic & Pickering, 2019; Grgic et al., 2018; Grgic, 2018; Polito et al., 2016; Shen et al., 2019; Southward et al., 2018; Warren et al., 2010). All included reviews were published in peer-reviewed journals. The flow diagram of the search process can be found in Figure 2.

Figure 2. Flow diagram of the search process



4.4.2. Characteristics of the meta-analyses

The included meta-analyses were published between the years 2004 and 2018. The median number of studies included per meta-analysis was 19 (range: 2 to 44). The prevalence of primary studies with male-only samples ranged from 72% to 100% across the meta-analyses. The assessed outcomes in the meta-analyses included: maximal speed during running, cycling or rowing (defined as the maximal achieved speed in exercise performance tests lasting from 45 seconds to 8 minutes that had either a fixed duration or a fixed distance), aerobic endurance (assessed by time-to-exhaustion, time-trial, and graded exercise tests), peak and mean power in the 30-second Wingate test, peak torque in an isokinetic strength assessment, strength in the one repetition maximum (1RM) test, height in a vertical jump test, muscular endurance (assessed both using isometric and dynamic tests), duration of time-trial or power during a time-trial, and maximal voluntary strength (assessed by pooling isometric, isokinetic, and 1RM tests). A summary of the included meta-analyses can be found in Table 4.

Table 3. Excluded reviews with the reasons for their exclusion

Reference	**Reasons for exclusion**
Astorino and Roberson (2010)	No meta-analysis performed.
Brown et al. (2013)	Examined the effects of energy drinks in which both caffeine and taurine was ingested.
Doherty and Smith (2005)	Conducted using the same search process as the initial analysis from this group of authors.
Ganio et al. (2009)	No meta-analysis performed.
Glaister and Gissane (2018)	Focused on physiological responses during exercise and not on exercise performance.
Souza et al. (2017)	Examined the effects of energy drinks in which both caffeine and taurine was ingested.
Zhang et al. (2015)	Focused on physiological responses during exercise and not on exercise performance.

Table 4. Summary of the meta-analyses included in the review

Reference	**Included studies**	**Number of included studies (sample size)**	**Performance test(s)**	**Effect size (95% CI) and *p*-value***	**95% PI**	**Percent change**	**I^2 (95% CI)**
Christensen et al. (2017)	Single or double-blind crossover study designs	9 studies (n = 97)	Speed during running, cycling or rowing**	0.41 (0.15, 0.68); p = 0.002	0.41 (0.09, 0.73)	~2%	0% (0%, 35%)
Conger et al. (2011)	Crossover study designs	Carbohydrate vs. caffeine + carbohydrate: 21 studies (n = 333) Caffeine vs. placebo: 36 studies (n = 352)	Any form of aerobic exercise if it was 10 minutes or longer in duration	Carbohydrate vs. caffeine + carbohydrate: 0.26 (0.15, 0.38); p < 0.001 Caffeine vs. placebo: 0.51 (0.41, 0.62); p < 0.001	Carbohydrate vs. caffeine + carbohydrate: 0.26 (-0.18, 0.70); Caffeine vs. placebo: 0.51 (-0.06, 1.08)	Carbohydrate vs. caffeine + carbohydrate: +6% Caffeine vs. placebo: +16%	Carbohydrate vs. caffeine + carbohydrate: 7% (0%, 42%) Caffeine vs. placebo: 24% (0%, 50%)
Doherty and Smith (2004)	Double-blind crossover study designs	24 studies (n = 217) for aerobic exercise, 6 studies for graded exercise tests (n = 62), and 12 studies for short-term high-	Exercise testing divided to aerobic exercise, graded exercise tests, and short-term high-intensity exercise	Aerobic exercise: 0.63 (0.50, 0.77) Graded exercise tests: 0.17 (-0.02, 0.36)	Aerobic exercise: 0.63 (0.06, 1.20) Graded exercise tests: 0.17 (-0.09, 0.44)	+12% across all exercise tests	Aerobic exercise: 4% (0%, 52%) Graded exercise tests: 0% (0%, 42%)

		intensity exercise (*n* = 127)		Short-term high-intensity exercise: 0.16 (0.01, 0.31)***	Short-term high-intensity exercise: 0.16 (-0.18, 0.50)		Short-term high-intensity exercise: 0% (0%, 24%)
Gonçalves Ribeiro et al. (2017)	Crossover study designs	7 studies (*n* = 91) for time-trial duration, 4 studies (*n* = 52) for power, and 2 studies (*n* = 31) for running distance	Time-trial duration, power, and running distance	Time-trial duration: 0.40 (0.11, 0.70); *p* = 0.007 Power: 0.18 (-0.21, 0.56); *p* = 0.366 Running distance: 0.38 (-0.13, 0.88); *p* = 0.142	Time-trial duration: 0.40 (0.01, 0.79) Power: 0.18 (-0.65, 1.01) Running distance: unable to determine	Time-trial duration: +2% Power: +4% Running distance: +11%	Time trial duration: 0% (0%, 19%) Power: 0% (0%, 43%) Running distance: 0% (unable to determine)
Grgic (2018)	Crossover study designs	16 studies (*n* = 246)	Peak and mean power in the 30-second Wingate test	Peak power: 0.27 (0.08, 0.47); *p* = 0.006 Mean power: 0.18 (0.05, 0.31); *p* = 0.005	Peak power: 0.27 (-0.35, 0.89) Mean power: 0.18 (0.04, 0.32)	Peak power: +4% Mean power: +3%	Peak power: 7% (0%, 44%) Mean power: 0% (0%, 28%)

Grgic and Pickering (2019)	Crossover study designs	10 studies (n = 133)	Peak torque in an isokinetic strength assessment	0.16 (0.06, 0.26); p = 0.003	0.16 (-0.17, 0.49)	+5%	15% (0%, 57%)
Grgic et al. (2018)	Single or double-blind crossover study designs	10 studies (n = 149) for strength and 10 studies (n = 145) for vertical jump	Strength in the 1RM test and height in a vertical jump test	1RM: 0.20 (0.03, 0.36); p = 0.023 Vertical jump: 0.17 (0.00, 0.34); p = 0.047	1RM: 0.20 (0.02, 0.39) Vertical jump: 0.17 (-0.03, 0.37)	1RM: +3% Vertical jump: +3%	1RM: 0% (0%, 37%) Vertical jump: 0% (0%, 47%)
Polito et al. (2016)	Double-blind crossover study designs	16 studies (n = 239) for muscular endurance and 3 studies (n = 46) for the 1RM test	Muscular endurance (assessed by repetitions to fatigue) and strength in the 1RM test	Muscular endurance: 0.38 (0.29, 0.48); p < 0.001 1RM: 0.09 (-0.07, 0.25); p = 0.25	Muscular endurance: 0.38 (0.02, 0.74) 1RM: 0.09 (-0.09, 0.27)	Muscular endurance: +6% 1RM: +2%	Muscular endurance: 24% (0, 56%) 1RM: 0% (0%, 43%)
Shen et al. (2018)	Crossover study designs	40 studies (n = 582)	Any form of aerobic exercise if it was 5 minutes or longer in duration	0.33 (0.21, 0.45)***	0.33 (0.21, 0.45)	+3%	0% (0%, 14%)

Southward et al. (2019)	Crossover study designs	44 studies (n = 639 for time trial duration and n = 350 for time-trial power)	Duration of the time trial or power during a time trial	Time trial duration: 0.28 (0.17, 0.40); $p < 0.0001$ Time-trial power: 0.22 (0.07, 0.37); $p = 0.004$	Time trial duration: 0.28 (0.17, 0.40) Time trial power: 0.22 (0.06, 0.38)	Time trial duration: +2% Time trial power: +3%	Time trial duration: 0% (0%, -56%) Time trial power: 0% (0%, 14%)
Warren et al. (2010)	Crossover study designs	27 studies (n = 576) for MVC and 23 studies (n = 388) for muscular endurance	MVC and muscular endurance	MVC: 0.19 (0.09, 0.29); $p < 0.001$ Muscular endurance: 0.28 (0.14, 0.42); $p < 0.001$	MVC: 0.19 (-0.18, 0.56) Muscular endurance: 0.28 (-0.29, 0.85)	MVC: +4% Muscular endurance: +14%	MVC: 44% (13%, 65%) Muscular endurance: 12% (0%, 46%)
* positive effect sizes and percentages show favouring of caffeine over placebo; ** defined as the maximal achieved speed in exercise performance tests lasting from 45 seconds to 8 minutes that had either a fixed duration or a fixed distance; *** p-values were not provided; *1RM*: one repetition maximum test; *MVC*: maximal voluntary contraction; *CI*: confidence interval; *PI*: prediction interval							

4.4.3. Effects of caffeine on exercise performance

The effects of caffeine ingestion on aerobic endurance were examined in five reviews with a total of eight meta-analyses; the majority reported ergogenic effects of caffeine (ES range: 0.22 to 0.61). The range of included primary studies was from two to 44 (average: 23 studies). Doherty and Smith (2004) did not report significant effects of caffeine on aerobic endurance performance when considering only graded exercise tests and including six studies. Gonçalves Ribeiro et al. (2017) did not report significant effects of caffeine on this outcome (analysed using maximum running distance tests) while including two studies. The 95% PIs for these analyses are reported in Table 4.

Four analyses examined the effects of caffeine on different measures of muscle strength. In three of these analyses, an ergogenic effect of caffeine was observed (ES range: 0.16 to 0.20). The range of included studies was from 3 to 27 (average: 13 studies). In the analysis by Grgic and Pickering (2019) the 95% PI was from -0.17 to 0.49. In the analysis by Grgic et al. (2018) the 95% PI was from 0.02 to 0.39, while in Warren et al.'s (2010) analysis the 95% PI was from -0.18 to 0.56. The 95% PI in the analysis by Polito et al. (2016) (this analysis did not report significant effects of caffeine on 1RM strength) was from -0.09 to 0.27.

Two analyses examined the effects of caffeine on muscular endurance. Both reported ergogenic effects of caffeine (ES range: 0.28 to 0.38). Polito et al. (2016) included 16, while Warren et al. (2010) included 23 studies. The 95% PI was from 0.02 to 0.74 and from -0.29 to 0.85 for the analyses by Polito et al. (2016) and Warren et al. (2010) respectively.

Anaerobic power was examined in two analyses. In a meta-analysis including four studies, Gonçalves Ribeiro et al. (2017) did not report significant ergogenic effects of caffeine on power. The 95% PI in this analysis was from -0.65 to 1.01. In an analysis including 16 studies, Grgic (2018) reported ergogenic effects of caffeine on both mean and peak power (ES range: 0.18 to 0.27). In the analysis for peak power, the 95% PI was from -0.35 to 0.89 while in the analysis for mean power, the 95% PI was from 0.04 to 0.32.

One meta-analysis, including 10 studies, examined the effects of caffeine on vertical jump height and reported an ergogenic effect of caffeine (effect size: 0.17) (Grgic et al., 2018). The 95% PI was from -0.03 to 0.37.

One meta-analysis, included nine studies, examined speed during running, cycling or rowing and reported ergogenic effects of caffeine (effect size: 0.41) (Christensen et al., 2017). The 95% PI was from 0.09 to 0.73.

One meta-analysis examined various forms of "short-term high-intensity exercise" while pooling the effects of caffeine on: (1) time to exhaustion in various high-intensity short-term cycling and running efforts; (2) mean power, peak power output, and total work during high-intensity short-term cycling; and (3) time-trial time during 2000 m rowing (Doherty & Smith, 2004). This analysis included 16 studies and reported ergogenic effects of caffeine of 0.16; the 95% PI was -0.18 to 0.50.

Besides the main analysis (presented in Figure 3), several reviews also conducted additional subgroup analyses (e.g., for trained vs. untrained individuals, for upper vs. lower-body musculature) and these results are summarised in Table 5.

Figure 3. Summary of the effect sizes, 95% CIs (presented in the black lines), and 95% prediction intervals (95% PIs; presented in the grey lines) from the included meta-analyses. If there is no 95% PI presented, it was the same as the 95% CI

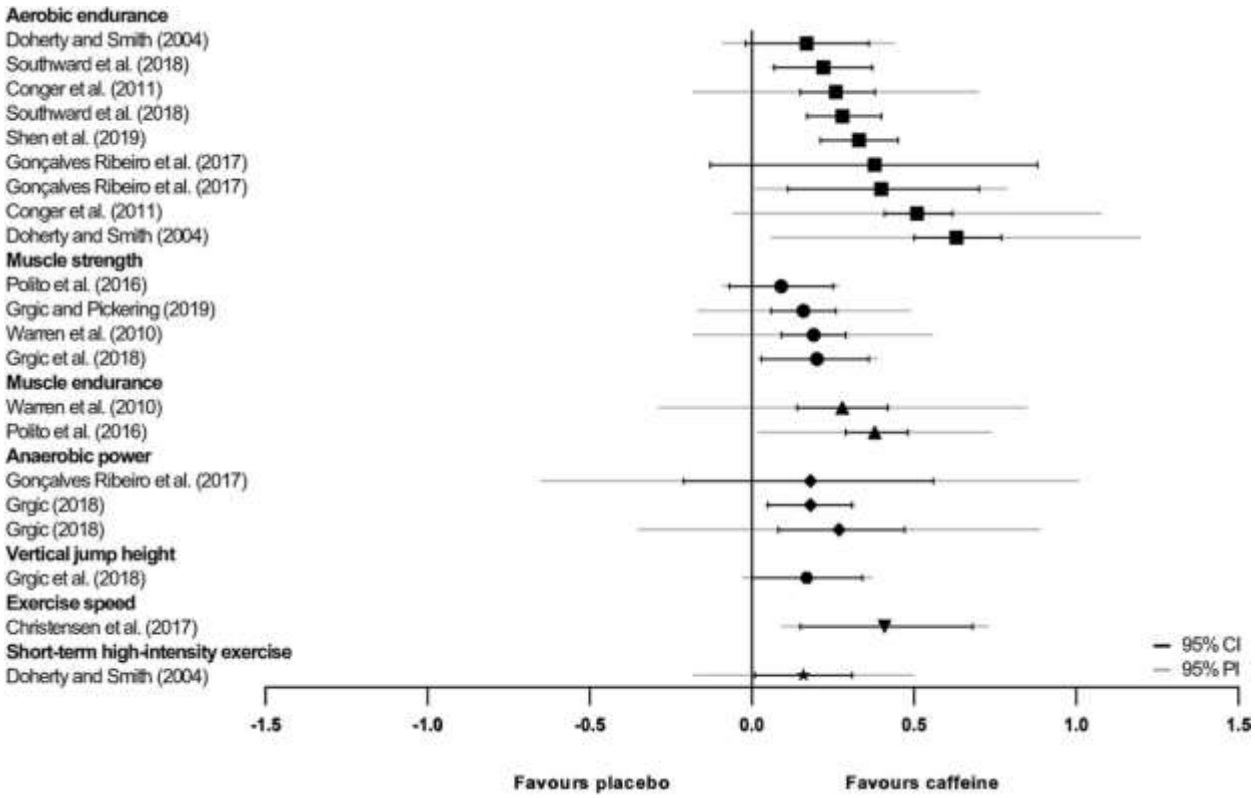


Table 5. Summary of subgroup analyses conducted in the included reviews

Reference	**Subgroup analyses focus**	**Subgroups analyses results***
Conger et al. (2011)	Timing of caffeine ingestion	Immediately before or during exercise: 0.26 (0.09, 0.42) – 9 studies ≥60 min before and during exercise: 0.16 (–0.11, 0.42) – 4 studies 30–90 min before exercise: 0.34 (0.16, 0.52) – 9 studies >90 min before exercise: 0.38 (–0.18, 0.95) – 1 study
	Exercise mode	Cycling: 0.30 (0.18, 0.42) – 18 studies Running: 0.08 (–0.15, 0.32) – 3 studies
	Performance test	Open endpoint: 0.40 (0.21, 0.60) – 7 studies Fixed endpoint: 0.20 (0.08, 0.33) – 14 studies
	Sustained submaximal exercise bout before performance task	No: 0.29 (0.13, 0.46) – 10 studies Yes: 0.24 (0.08, 0.40) – 11 studies
	Sex	Men: 0.23 (0.10, 0.37) – 16 studies Men and women: 0.33 (0.09, 0.58) – 4 studies Women: 0.50 (–0.11, 0.11) – 1 study

	Publication status	Unpublished studies: 0.13 (–0.08, 0.33) – 6 studies Published studies: 0.32 (0.19, 0.46) – 15 studies
Doherty and Smith (2004)	Exercise protocol	Time-to-exhaustion tests: 0.67 (0.52, 0.81) – 38 effect sizes Time trials: 0.13 (0.02, 0.25) – 27 effect sizes Graded exercise tests: 0.17 (-0.02, 0.36) – 11 effect sizes
	Training status	Trained: 0.15 (–0.08, 0.38) – 19 effect sizes Highly trained: 0.20 (0.09, 0.31) – 7 effect sizes
Grgic and Pickering (2019)	Muscle group	Knee extensors: 0.19 (0.10, 0.28) – 9 studies Other muscle groups: 0.10 (-0.02, 0.21) – 8 studies
	Angular velocity	Velocity of $30 \circ s^{-1}$: 0.16 (-0.08, 0.39) – 6 studies Velocity of $60 \circ s^{-1}$: 0.21 (0.07, 0.36) – 3 studies Velocity of $180 \circ s^{-1}$: 0.23 (0.07, 0.38) – 3 studies
Grgic et al. (2018) – muscular strength	Muscle group location	Upper body: 0.21 (0.02, 0.39) – 7 studies Lower body: 0.15 (−0.05, 0.34) – 8 studies
	Caffeine form	Capsule form of caffeine: 0.27 (0.04, 0.50) – 6 studies

		Liquid form of caffeine: 0.11 (−0.17, 0.39) – 3 studies
	Sex	Males: 0.21 (0.02, 0.41) – 8 studies Females: 0.15 (−0.13, 0.43) – 3 studies
	Training status	Trained participants: 0.18 (−0.02, 0.37) – 7 studies Untrained participants: 0.27 (−0.09, 0.63) – 4 studies
Grgic et al. (2018) – power	Caffeine form	Capsule form of caffeine: 0.14 (−0.06, 0.34) – 8 studies Liquid form of caffeine: 0.24 (−0.06, 0.54) – 3 studies
	Sex	Men: 0.16 (−0.02, 0,34) – 9 studies Women: 0.23 (−0.23, 0.69) – 3 studies
	Training status	Athletes: 0.23 (0.03, 0.42) – 8 studies Non-athletes: 0.03 (−0.33, 0.40) – 2 studies
	Exercise test	Countermovement jump: 0.14 (−0.04, 0.32) – 8 studies Sargent test: 0.31 (−0.09, 0.70) – 2 studies
Polito et al. (2016) – muscular strength	Muscle group location	Upper-body: 0.08 (−0.09, 0.25) – 4 effect sizes
	Muscle size	Large: 0.09 (−0.07, 0.25) – 5 effect sizes

	Sex	Men: 0.09 (−0.07, 0.26) – 4 effect sizes
	Caffeine form	Capsule: 0.09 (−0.07, 0.26) – 4 effect sizes
	Caffeine dose	≤ 4 mg/kg: 0.08 (–0.11, 0.28) – 2 effect sizes 6 mg/kg: 0.10 (–0.15, 0.36) – 3 effect sizes
	Timing of caffeine intake	45 min: 0.08 (–0.11, 0.28) – 2 effect sizes 60 min: 0.10 (–0.15, 0.36) – 3 effect sizes
Polito et al. (2016) – muscular endurance	Muscle group location	Upper-body: 0.32 (0.19, 0.44) – 24 effect sizes Lower-body: 0.42 (0.25, 0.58) – 14 effect sizes
	Muscle size	Large: 0.38 (0.28, 0.49) – 37 effect sizes Small: 0.40 (0.11, 0.68) – 5 effect sizes
	Sex	Men: 0.41 (0.31, 0.51) – 39 effect sizes
	Caffeine form	Capsule: 0.40 (0.29, 0.51) – 35 effect sizes Liquid: 0.32 (0.10, 0.56) – 7 effect sizes
	Caffeine dose	≤ 4 mg/kg: 0.43 (0.20, 0.65) – 11 effect sizes 5 mg/kg: 0.44 (0.20, 0.68) – 7 effect sizes

		6 mg/kg: 0.30 (0.14, 0.47) – 14 effect sizes > 6 mg/kg: 0.51 (0.28, 0.74) – 8 effect sizes
	Timing of caffeine intake	45 min: 0.23 (-0.04, 0.49) – 8 effect sizes 60 min: 0.42 (0.31, 0.53) – 32 effect sizes 90 min: 0.18 (-0.26, 0.63) – 2 effect sizes
Warren et al. (2010) – muscular strength**	Publication status	Published: 0.16 – 22 studies Unpublished: 0.31 – 5 studies
	Study design	Crossover: 0.20 – 25 studies Between-groups: 0.11 – 2 studies
	Sex	Men: 0.21 – 19 studies Men and women: 0.15 – 8 studies
	Training status	Trained: 0.13 – 6 studies Untrained: 0.21 – 21 studies
	Caffeine form	Solid: 0.25 – 18 studies Liquid: 0.05 – 8 studies

	Muscle action type	Isokinetic: 0.21 – 6 studies Isometric: 0.18 – 20 studies
	Muscle size	Large: 0.31 – 18 studies Small: 0.05 – 12 studies
	Muscle group location	Upper-body: 0.07 – 13 studies Lower-body: 0.29 – 18 studies
	Muscle group	Knee extensors: 0.40 – 15 studies Knee flexors: 0.04 – 4 studies Elbow flexors: 0.07 – studies
Warren et al. (2010) – muscular endurance**	Publication status	Published: 0.27 – 19 studies Unpublished: 0.31 – 4 studies
	Study design	Crossover: 0.26 – 20 studies Between-groups: 0.50 – studies
	Sex	Men: 0.21 – 15 studies Men and women: 0.43 – 7 studies

	Training status	Trained: 0.07 – 6 studies Untrained: 0.37 – 15 studies
	Caffeine form	Solid: 0.23 – 15 studies Liquid: 0.39 – 8 studies
	Muscle action type	Isokinetic: 0.20 – 6 studies Isometric: 0.36 – 12 studies Isotonic: 0.16 – 5 studies
	Exercise test	Open end point: 0.37 – 18 studies Fixed end point: -0.08 – 5 studies
	Type of load	Constant: 0.33 – 18 studies Variable: 0.09 – 5 studies
	Muscle size	Large: 0.23 – 17 studies Small: 0.40 – 8 studies
	Muscle group location	Upper-body: 0.37 – 12 studies Lower-body: 0.25 – 15 studies

	Muscle group	Knee extensors: 0.33 – 11 studies Knee flexors: -0.07 – 3 studies Elbow flexors: 0.31 – 4 studies Pectorals/shoulders/triceps: 0.31 – 4 studies Hip and knee extensors: 0.21 – 3 studies
* Presented as mean (95% confidence interval) ** Warren et al. (2010) did not present 95% confidence intervals		

4.4.4. Methodological quality evaluation

The methodological quality of the 11 included reviews is summarised in Table 6. The reviews scored from 44% to 88% of the maximum 16 points. Three reviews were classified as being of high-quality, while eight were classified as being of moderate methodological quality. None of the meta-analyses was considered to be of poor methodological quality. Several criteria on AMSTAR 2 checklist were under-reported in the analysed reviews: (1) none provided an *a priori* design (i.e., registration of the review methods in advance); (2) in four and five analyses the number of authors conducting the search and data extraction was not clear, respectively; (3) the list of excluded studies was not provided in any of the included reviews; and (4) sources of funding for the studies included in a given review were discussed only in three reviews.

Table 6. Results of the Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR 2) quality assessment

Reference	**AMSTAR items**																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Score**
Christensen et al. (2017)	Yes	No	Yes	Yes	Yes	Cannot answer	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	75% moderate
Conger et al. (2011)	Yes	No	Yes	Yes	Cannot answer	Cannot answer	No	Yes	No	No	Yes	Not applicable	Not applicable	Yes	Yes	Yes	50% moderate
Doherty and Smith (2004)	Yes	No	Yes	Yes	Cannot answer	Cannot answer	No	Yes	No	No	Yes	Not applicable	Not applicable	Yes	Yes	No	44% moderate
Gonçalves Ribeiro et al. (2017)	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	63% moderate
Grgic (2018)	Yes	No	Yes	Yes	No	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	69% moderate

Reference	AMSTAR items																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Score**
Grgic and Pickering (2019)	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	75% moderate
Grgic et al. (2018)	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	88% high
Polito et al. (2016)	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	81% high
Shen et al. (2019)	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	81% high
Southward et al. (2018)	Yes	No	Yes	Yes	Cannot answer	Cannot answer	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	69% Moderate
Warren et al. (2010)	Yes	No	Yes	Yes	Cannot answer	Cannot answer	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	75% moderate

4.4.5. Quality of the evidence

Based on the GRADE assessment, the included analyses were considered as providing very low (three meta-analyses), low (seven meta-analyses), or moderate quality of evidence (11 meta-analyses). For risk of bias, several reviews did not assess the quality of the included studies and thus were given "unclear" on this criterion. The meta-analyses were considered as not having serious inconsistency but were considered as having serious indirectness. The analyses were mostly considered as being "precise" on the imprecision GRADE item. Finally, three meta-analyses were considered as "strongly suspected" on the publication bias GRADE item. The results for each analysis are presented in Table 7.

Table 7. Results of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) assessment

Reference	**GRADE items**					**Quality of the evidence***
	Risk of bias	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	
Christensen et al. (2017)	Not serious	Not serious	Serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Not serious	Strongly suspected (asymmetry of the funnel plot was not explored and the ES of the largest study was smaller than the pooled estimate)	Low ⊕⊕OO
Conger et al. (2011)	Carbohydrate vs. caffeine + carbohydrate: unclear (no quality assessment performed)	Carbohydrate vs. caffeine + carbohydrate: not serious	Carbohydrate vs. caffeine + carbohydrate: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Carbohydrate vs. caffeine + carbohydrate: not serious	Carbohydrate vs. caffeine + carbohydrate: undetected	Carbohydrate vs. caffeine + carbohydrate: low ⊕⊕OO

	Caffeine vs. placebo: unclear (no quality assessment performed)	Caffeine vs. placebo: not serious	Caffeine vs. placebo: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Caffeine vs. placebo: not serious	Caffeine vs. placebo: undetected	Caffeine vs. placebo: low ⨁⨁OO
Doherty and Smith (2004)	Aerobic exercise: unclear (no quality assessment performed)	Aerobic exercise: not serious	Aerobic exercise: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Aerobic exercise: not serious	Aerobic exercise: undetected	Aerobic exercise: low ⨁⨁OO
	Graded exercise tests: unclear (no quality assessment performed)	Aerobic endurance as assessed by graded exercise tests: not serious	Aerobic endurance as assessed by graded exercise tests: serious indirectness (the majority of included studies were conducted in men and, therefore,	Aerobic endurance as assessed by graded exercise tests: serious limitation	Aerobic endurance as assessed by graded exercise tests: undetected	Aerobic endurance as assessed by graded exercise tests: very low ⨁OOO

			these results cannot be generalised to women)			
	Short-term high-intensity exercise: unclear (no quality assessment performed)	Short-term high-intensity exercise: not serious	Short-term high-intensity exercise: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Short-term high-intensity exercise: not serious	Short-term high-intensity exercise: undetected	Short-term high-intensity exercise: low ⨁⨁OO
Gonçalves Ribeiro et al. (2017)	Time-trial duration: serious limitation (the majority of included studies received "unclear risk of bias" on random sequence generation, allocation concealment, and on the blinding of outcome assessors)	Time-trial duration: not serious	Time-trial duration: serious indirectness (all of the included studies were conducted in men and, therefore, these results cannot be generalised to women)	Time-trial duration: not serious	Time-trial duration: undetected	Time-trial duration: low ⨁⨁OO

	Power: serious limitation (the majority of included studies received "unclear risk of bias" on random sequence generation, allocation concealment, and on the blinding of outcome assessors)	Power: not serious	Power: serious indirectness (all of the included studies were conducted in men and, therefore, these results cannot be generalised to women)	Power: serious limitation	Power: strongly suspected (asymmetry of the funnel plot was not explored and the ES of the largest study was smaller than the pooled estimate)	Power: very low ⨁OOO
	Running distance: serious limitation (the majority of included studies received "unclear risk of bias" on random sequence generation, allocation concealment, and on the blinding of outcome assessors)	Running distance: not serious	Running distance: serious indirectness (all of the included studies were conducted in men and, therefore, these results cannot be generalised to women)	Running distance: serious limitation	Running distance: strongly suspected (asymmetry of the funnel plot was not explored and the ES of the largest study was smaller than the pooled estimate)	Running distance: very low ⨁OOO
Grgic (2018)	Peak power: not serious	Peak power: not serious	Peak power: serious indirectness (the majority of included studies were conducted	Peak power: not serious	Peak power: undetected	Peak power: moderate ⨁⨁⨁O

			in men and, therefore, these results cannot be generalised to women)			
	Mean power: not serious	Mean power: not serious	Mean power: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Mean power: not serious	Mean power: undetected	Mean power: moderate ⨁⨁⨁O
Grgic and Pickering (2019)	Not serious	Not serious	Serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Not serious	Undetected	Moderate ⨁⨁⨁O
Grgic et al. (2018)	1RM: not serious	1RM: not serious	1RM: serious indirectness (the majority of included	1RM: not serious	1RM: undetected	1RM: moderate ⨁⨁⨁O

			studies were conducted in men and, therefore, these results cannot be generalised to women)			
	Vertical jump: not serious	Vertical jump: not serious	Vertical jump: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Vertical jump: not serious	Vertical jump: undetected	Vertical jump: moderate ⊕⊕⊕O
Polito et al. (2016)	1RM: not serious	1RM: not serious	1RM: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	1RM: serious limitation	1RM: undetected	1RM: low ⊕⊕OO

	Muscular endurance: not serious	Muscular endurance: not serious	Muscular endurance: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Muscular endurance: not serious	Muscular endurance: undetected	Muscular endurance: moderate ⊕⊕⊕O
Shen et al. (2019)	Not serious	Not serious	Serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Not serious	Undetected	Moderate ⊕⊕⊕O
Southward et al. (2018)	Time-trial time: not serious	Time-trial time: not serious	Time-trial duration: serious indirectness (the majority of included studies were conducted in men and, therefore,	Time-trial duration: not serious	Time-trial duration: undetected	Time-trial duration: moderate ⊕⊕⊕O

			these results cannot be generalised to women)			
	Time-trial power: not serious	Time-trial power: not serious	Time-trial power: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	Time-trial power: not serious	Time-trial power: undetected	Time-trial power: moderate ⊕⊕⊕O
Warren et al. (2010)	MVC: not serious	MVC: not serious	MVC: serious indirectness (the majority of included studies were conducted in men and, therefore, these results cannot be generalised to women)	MVC: not serious	MVC: undetected	MVC: moderate ⊕⊕⊕O
	Muscular endurance: not serious	Muscular endurance: not serious	Muscular endurance: serious indirectness (the majority of included studies were conducted	Muscular endurance: not serious	Muscular endurance: undetected	Muscular endurance: moderate

			in men and, therefore, these results cannot be generalised to women)			⊕⊕⊕O

1RM: one repetition maximum test; *MVC*: maximal voluntary contraction;

* classification based on the GRADE Handbook as:

⊕⊕⊕⊕ = high quality

⊕⊕⊕O = moderate quality

⊕⊕OO = low quality

⊕OOO = very low quality

4.5. Discussion

Based on the 11 included reviews it can be concluded that caffeine is ergogenic for different tests of exercise performance including aerobic endurance, muscle strength, muscle endurance, power, jumping performance, and exercise speed. Ergogenic effects of caffeine on muscle endurance, muscle strength, anaerobic power, and aerobic endurance were substantiated by moderate quality of evidence coming from moderate-to-high quality systematic reviews (Table 8). For other outcomes, we found moderate quality reviews that presented evidence of very low or low quality. In addition, not all analyses provided a definite direction for the effect of caffeine when considering the 95% PI. Several important aspects that refer to the generalisability of the meta-analytical findings as well as the spread of summary effects, need to be considered when interpreting these findings from a practical standpoint.

Table 8. Summary of the included meta-analyses based on the quality of the review, quality of evidence, and the 95% prediction interval categories

Quality of evidence and prediction interval (PI) categories	**Quality of the review**	
	Moderate	High
Quality of evidence: "very low" 95% PI includes zero	*14% of the included meta-analyses* - Aerobic endurance as assessed by graded exercise tests in Doherty and Smith (2004) - Aerobic endurance as assessed by running distance in Gonçalves Ribeiro et al. (2017) - Anaerobic power in Gonçalves Ribeiro et al. (2017)	/
Quality of evidence: "very low" 95% PI does not include zero	/	/
Quality of evidence: "low" 95% PI includes zero	*19% of the included meta-analyses* - Aerobic endurance in the carbohydrate vs. caffeine + carbohydrate comparison in Conger et al. (2011) - Aerobic endurance in the caffeine vs. placebo comparison in Conger et al. (2011) - Muscle strength in Polito et al. (2016) - Short-term high-intensity exercise in Doherty and Smith (2004)	/

Quality of evidence: "low" 95% PI does not include zero	*14% of the included meta-analyses* - Aerobic endurance as assessed by time trial duration in Gonçalves Ribeiro et al. (2017) - Aerobic endurance in Doherty and Smith (2004) - Exercise speed in Christensen et al. (2017)	/
Quality of evidence: "moderate" 95% PI includes zero	*24% of the included meta-analyses* - Muscle endurance in Warren et al. (2010) - Muscle strength in Grgic and Pickering (2019) - Muscle strength in Warren et al. (2010) - Peak anaerobic power in Grgic (2018) - Vertical jump in Grgic et al. (2018)	/
Quality of evidence: "moderate" 95% PI does not include zero	*14% of the included meta-analyses* - Time-trial time in Southward et al. (2018) - Time-trial power in Southward et al. (2018) - Mean anaerobic power in Grgic (2018)	*14% of the included meta-analyses* - Muscle strength in Grgic et al. (2018) - Muscle endurance in Polito et al. (2016) - Aerobic endurance in Shen et al. (2019)
Note: Quality of systematic review was assessed using the AMSTAR 2 checklist (none of the reviews were categorised as "low" quality); Quality of evidence was assessed using the GRADE criteria (none of the meta-analyses provided "high" quality of evidence)		

4.5.1. Generalisability of the results

Based on the GRADE assessment for directness of evidence, the included reviews were rated as having serious indirectness given that the evidence is not direct enough to apply to all populations. Scrutiny of the meta-analyses included in this umbrella review highlights that primary studies conducted among women are lacking. Specifically, in all of the included meta-analyses 72% to 100% of the pooled sample participants were men. Women may metabolise caffeine differently than men given that changes in circulating steroid hormones during phases of the menstrual cycle can impact caffeine elimination in women (Lane et al., 1992; Temple & Ziegler, 2011), which might also impact the ergogenic effect of caffeine on exercise performance in this population. When conducting studies in women, the differences in caffeine metabolism across the follicular and luteal phase of the menstrual cycle may increase the complexity of the study design, which might partially explain why studies in this population are lacking. While there are studies conducted in both sexes that suggest both men and women may experience similar acute effects of caffeine ingestion on exercise performance (Butts & Crowell, 1985; Sabblah et al., 2015), the generalisability of the meta-analytic findings is, however, limited mostly to men.

The majority of the primary studies were conducted in young individuals and, therefore, several meta-analyses are limited exclusively to young individuals (Christensen et al., 2017; Gonçalves Ribeiro et al., 2017; Grgic et al., 2018; Grgic, 2018; Polito et al., 2016; Shen et al., 2019; Southward et al., 2018). This may be relevant to highlight given that in animal models, with ageing there appears to be a reduced ergogenic effect of caffeine (Tallis, James, Cox, & Duncan, 2017). Caffeine has been shown to elicit positive effects on mood and cognitive performance in older adults (Tallis et al., 2013). If caffeine also increases exercise performance in older adults, it might also enhance performance during activities of daily living in these individuals. This is particularly important from a public health point of view, given that reduced physical functioning (e.g., in terms of reduced strength) may impact the quality of life in this population group (McPhee et al., 2016). Although some of the studies conducted in older adults shown an ergogenic effect of caffeine on exercise performance (Duncan, Clarke, Tallis, Guimaraes-Ferreira, & Wright, 2014; Norager, Jensen, Madsen, & Laurberg, 2005), additional studies that directly compare the effects of caffeine between young vs. older individuals are needed to explore if the effects of caffeine differ between age groups.

4.5.2. Methodological quality

While the meta-analyses included in the present umbrella review show that caffeine ingestion may indeed be ergogenic across a large range of exercise tasks, some additional considerations may help to improve future meta-analyses on this topic. Several of the included meta-analyses did not adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, which currently represent a widely-accepted standard for reporting meta-analyses. It should be taken into account that the PRISMA guidelines were published in 2009, which is five years after the review by Doherty and Smith (2004). Nonetheless, several meta-analyses that did not follow the guidelines were published following the release of the PRISMA statement (Christensen et al., 2017; Conger et al., 2011; Gonçalves Ribeiro et al., 2017; Warren et al., 2010).

None of the 11 meta-analyses registered their protocol for a review and thus did not receive a point on item 2 of the AMSTAR 2 checklist. Protocols of systematic reviews can be registered in the PROSPERO database. If registered, such protocols can help reduce the risk of wasteful duplication of reviews by independent research groups. However, the PROSPERO database is primarily focused on health outcomes and not exercise performance. As stated on their website, "PROSPERO includes protocol details for systematic reviews relevant to health and social care, welfare, public health, education, crime, justice, and international development, where there is a health related outcome." The authors are not aware of any registries that focus on the publishing of protocols for systematic reviews in the sport and exercise field. Given that the number of published systematic reviews has increased over the last years, the formation of such a register for this line of research appears warranted.

Publication bias, as highlighted by Borenstein, Hedges, Higgins, and Rothstein (2011) can occur because studies that report higher (and significant) ESs are more likely to be published than those with low or non-significant ESs (i.e., the file drawer problem). Therefore, the inclusion of only published studies in a given meta-analysis can lead to publication bias and may be a concern for the validity of the results. Four meta-analyses included in this umbrella review also examined unpublished literature in the form of master's theses and doctoral dissertations (Conger et al., 2011; Grgic et al., 2018; Polito et al., 2016; Warren et al., 2010).

In the meta-analysis by Conger et al. (2011) the ES of the unpublished studies was 0.13 (95% CI: –0.08, 0.33), while the ES of the published studies was 0.32 (95% CI: 0.19, 0.46). These results might indeed suggest that studies with smaller ESs tend to remain unpublished and to avoid publication bias future meta-analyses should consider including unpublished results as well. The reviews that included unpublished literature highlight that, in many cases, such unpublished documents may be of equal or even greater methodological quality as those that found in peer-reviewed journals. The influence of unpublished results can be examined by conducting a sensitivity analysis in which the pooled results are inspected after the exclusion of these studies. In this context, journal editors and reviewers are also encouraged to facilitate greater acceptance and publication of studies with results that would appear to be "less favourable" (or statistically non-significant) to truly progress this area of work.

4.5.3. The spread of summary effects

Based on the GRADE assessment of inconsistency, the reviews were classified as not possessing serious inconsistencies. Indeed, the ESs across individual studies indicate that the studies rarely show a negative effect of caffeine supplementation on exercise performance. The effects in the primary studies were either positive or around the null value. In addition, the 95% CI from the primary studies largely overlap.

One interesting aspect refers to the spread of summary effects. Historically, caffeine ingestion has been suggested to predominantly provide a performance-enhancing effect on aerobic exercise performance (Tarnopolsky, 1994). As shown both here and by others (Davis & Green, 2009; Grgic & Mikulic, 2017), it is evident that caffeine ingestion enhances performance in anaerobic exercise tasks as well. However, it is possible that the magnitude of the effect of caffeine is greater for aerobic as compared with anaerobic exercise. The ESs for meta-analyses that focused on aerobic tests of performance are generally higher than those that used anaerobic tests of performance (Figure 3). Future studies may consider investigating the effects of caffeine ingestion on both aerobic and anaerobic tests of performance in the same sample to further explore whether the ES differs between tasks that rely on predominantly oxidative or predominantly non-oxidative energy pathways.

4.5.4. The optimal dose of caffeine

While the included meta-analyses report that caffeine ingestion may be ergogenic across a broad range of exercise activities, the "optimal" dose of caffeine remains elusive. If we observe the dosage used in the primary studies (across all of the included meta-analyses), it is clear that most of the studies used a single dose of caffeine (most commonly 6 mg/kg). Warren et al. (2010) examined the dose-response effects between the amount of caffeine ingested and its ergogenic effect on muscular endurance. This analysis found that for an increase in caffeine dose by 1 mg/kg, the relative ES for muscular endurance increased by 0.10. However, these results should be interpreted with caution given that the dosage explained only 16% of the between-study variance. To explore the optimal doses of caffeine for exercise performance future dose-response studies are needed. The optimal doses may differ based on the source of caffeine (Wickham & Spriet, 2018) exercise test (Grgic et al., 2019b; Pallarés et al., 2013; Sabol, Grgic, & Mikulic, 2019), muscle action type (Tallis & Yavuz, 2018) and between individuals (Jenkins et al., 2008; Pickering & Kiely, 2018), which needs to be taken into account when prescribing caffeine supplementation.

4.5.6. Is coffee a good way to consume caffeine?

Whilst the results of this umbrella review suggest that caffeine is ergogenic in the majority of exercise situations, it is important to keep in mind that the majority of studies utilise caffeine anhydrous as the caffeine source, with a smaller group of studies utilising caffeine-containing supplements such as energy drinks, bars, and gels. Coffee, whilst a widely used method of caffeine ingestion globally, is relatively under-explored as a pre-exercise performance enhancer. Recently, Hodgson, Randell, and Jeukendrup (2013) reported that caffeine anhydrous and coffee, standardised to deliver a caffeine dose of 5 mg/kg, were similarly effective in enhancing aerobic endurance performance. Similar results have been reported for resistance and sprint exercise (Richardson & Clarke, 2016; Trexler et al., 2016). As a result, coffee is likely an effective ergogenic aid; the main issue here is a practical one. To be ergogenic, the caffeine dose from coffee likely has to fall within the 3-6 mg/kg range. The caffeine dose received from coffee depends on many factors, including bean type, preparation method, and size of the cup, with large differences in caffeine concentrations between different coffee brands and flavours, and within the same brand across time (Desbrow, Hall, & Irwin, 2019; Desbrow, Henry, & Scheelings, 2012; Desbrow, Hughes, Leveritt, & Scheelings, 2007). As a result, whilst the "average" cup of coffee contains around 100 mg of caffeine—meaning that two cups

would deliver ~200 mg, representing ~3 mg/kg for a 70kg individual—this amount is hard to quantify in the specific cup of coffee that person is drinking (Desbrow et al., 2007). Nevertheless, as a broad rule of thumb, two cups of coffee, consumed around 60 minutes before exercise, should exert an ergogenic effect in most individuals.

4.5.7. Suggestions for future research

Subgroup analyses conducted in the included meta-analyses in most cases are based on a low number of included studies (or effect sizes), which limits any definitive conclusions. Many areas remain unclear when it comes to caffeine supplementation. Some of these areas include:

1. The effects of caffeine habituation – does habituation to caffeine reduce (or eliminate) its ergogenic effect following caffeine supplementation? The included meta-analyses could not explore the differences in effects between low and high habitual caffeine users as currently there is a lack of primary studies investigating this topic. The body of research is limited and equivocal, with some studies suggesting that low habitual caffeine users experience greater ergogenic effects than the high habitual users while others report similar acute responses to caffeine ingestion in terms of exercise performance regardless of habituation (Bell & McLellan, 2002; Gonçalves et al., 2017). Pickering and Kiely (2019) suggested the possibility that the response may be dose-dependent, which may be an interesting aspect to explore in future studies.

2. Optimal timing of caffeine ingestion – most studies provided caffeine supplementation 60 minutes per exercise; therefore, it remains unclear if smaller/greater effects of caffeine would be observed with shorter/longer wait time from ingestion to exercise. This area needs further exploration and there is potential that different timing may be required for different doses or genotypes (Pickering, 2019; Talanian & Spriet, 2016).

3. Effects of different sources of caffeine – most of the included studies in the meta-analyses used the capsule form of caffeine. It remains unclear if comparable results can also be seen with alternate sources of caffeine, such as caffeine mouth rinsing, caffeine gels, and chewing gums. A detailed review on the topic of alternate forms of caffeine can be found elsewhere (Wickham & Spriet, 2018).

4. Effects of caffeine among trained vs. untrained individuals – while it has been suggested that trained individuals might respond better to caffeine ingestion, the current evidence on this topic is scarce and conflicting (Astorino et al., 2012; Brooks et al., 2015;

Collomp et al., 1992). The meta-analyses that have tried to explore this matter were commonly performed on a limited number of studies. For example, Grgic et al. (2018) only included seven and four studies for their subgroup analysis of the effects of caffeine among trained and untrained individuals, respectively. The majority of the studies pooled in the mentioned subgroup analysis only examined the effects of caffeine on strength performance in either trained or untrained individuals. The only study included in the review by Grgic et al (2018) that directly compared the effects of caffeine between trained and untrained individuals reported ergogenic effects of caffeine in untrained but not in trained individuals (Brooks et al., 2015). These results are in contrast to the common belief about greater responsiveness to caffeine in trained individuals. Future work is needed on this topic (for additional discussion on this topic see the reviews by Burke, 2008 and Tallis et al. 2015).

5. Chronic effects of caffeine on exercise adaptations – while many studies have examined the acute effects of caffeine supplementation on exercise performance it remains unclear whether these acute increases in performance also impact chronic adaptations to training, and in which way. Ultimately, individuals interested in the acute performance-enhancing effects of caffeine are likely candidates to continue to use caffeine supplementation over the long-term. Aspects of long-term supplementation that refer to habituation and to the attenuation of caffeine's effects, as well as the effects of chronic caffeine supplementation on training adaptations, need to be further investigated.

We hope that highlighting some of these areas will help catalyse future high-quality research.

4.6. Conclusions

Caffeine ingestion may be ergogenic for a broad range of exercise tasks. The performance-enhancing effects of caffeine on: (a) muscle endurance; (b) muscle strength; (c) anaerobic power; and (d) aerobic endurance, were supported by moderate-to-high quality reviews and moderate quality of evidence. For other outcomes, even though the reviews were of moderate quality, the presented evidence was of very low or low quality. It seems that the magnitude of the effect of caffeine is generally greater for aerobic as compared with anaerobic exercise. The quality of the evidence from some meta-analyses was considered to be low which highlights the need for future high-quality studies. More primary studies should be conducted among women and older adults to improve the generalisability of these findings.

What is already known?

►Currently, there are several meta-analyses examining the effects of caffeine ingestion on exercise performance.

►Given the often narrow scope (i.e., focus on only one test of performance) of a meta-analysis, the credibility of this type of evidence for the effects of caffeine on exercise performance across the totality of the evidence is unclear.

►Caffeine has been shown to be ergogenic for exercise performance; however, it remains unclear if the effect of caffeine differs between various exercise tests/tasks.

What are the new findings?

►Of the 11 included reviews, all report significant improvements in at least one component of exercise performance following caffeine ingestion with the ES magnitude ranging from trivial to moderate.

►The effect sizes for meta-analyses that focused on aerobic tests of performance are generally higher than those that used anaerobic tests of performance.

►The generalisability of the meta-analytic findings is limited mostly to men and young individuals.

Contributors

JG and ZP conceived the idea for the review. JG and IG conducted the study selection the data extraction and quality assessment. ZP contributed to data extraction and conceptualisation of quality assessment. JG drafted the initial manuscript. CP, ZP, IG, BJS, and DJB contributed to writing the manuscript.

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5. The influence of caffeine supplementation on resistance exercise: a review

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2. CANDIDATE DECLARATION

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3. CO-AUTHOR(S) DECLARATION

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The influence of caffeine supplementation on resistance exercise: a review

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5.1. Abstract

This paper aims to critically evaluate and thoroughly discuss the evidence on the topic of caffeine supplementation when performing resistance exercise, as well as provide practical guidelines for the ingestion of caffeine prior to resistance exercise. Based on the current evidence, it seems that caffeine increases both maximal strength and muscular endurance. Furthermore, power appears to be enhanced with caffeine supplementation, although this effect might, to a certain extent, be caffeine dose- and external load-dependent. A reduction in rating of perceived exertion (RPE) might contribute to the performance-enhancing effects of caffeine supplementation, as some studies have observed decreases in RPE coupled with increases in performance following caffeine ingestion. However, the same does not seem to be the case for pain perception, as there is evidence showing acute increases in resistance exercise performance without any significant effects of caffeine ingestion on pain perception. Some studies have reported that caffeine ingestion did not affect exercise-induced muscle damage but that it might reduce perceived resistance exercise-induced delayed onset muscle soreness; however, this needs to be explored further. There is some evidence that caffeine ingestion, compared to a placebo, may lead to greater increases in the production of testosterone and cortisol following resistance exercise. However, given that the acute changes in hormone levels seem to be weakly correlated with hallmark adaptations to resistance exercise, such as hypertrophy and increased muscular strength, these findings are likely of questionable practical significance. Although not without contrasting findings, the available evidence suggests that caffeine ingestion can lead to acute increases in blood pressure (primarily systolic), and, thus, caution is needed regarding caffeine supplementation among individuals with high blood pressure. In the vast majority of studies, caffeine was administered in capsule or powder forms, and therefore, the effects of alternative forms of caffeine such as chewing gums or mouth rinses on resistance exercise performance remain unclear. The emerging evidence suggests that coffee might be at least equally ergogenic as caffeine alone when the caffeine dose is matched. Doses in the range of 3 to 9 mg/kg seem to be adequate for eliciting an ergogenic effect when administered 60 min pre-exercise. In general, caffeine seems to be safe when taken in the recommended doses. However, at doses as high as 9 mg/kg or higher, side-effects such as insomnia might be more pronounced. It remains unclear whether habituation reduces the ergogenic benefits of caffeine on resistance exercise, as no evidence exists for this type of exercise. Caution is needed when extrapolating these conclusions to females as the vast majority of studies involved only male participants.

Key points

- Caffeine supplementation may acutely enhance muscular endurance, maximal strength, and power in resistance exercise.
- Doses in the range 3 to 9 mg/kg seem to be adequate for eliciting ergogenic effects. Caffeine seems to be generally safe when taken in these doses. However, at doses as high as 9 mg/kg or higher, side effects might be more pronounced.
- Blood pressure may be increased following caffeine ingestion, and, therefore, caution is needed regarding caffeine supplementation among individuals with high blood pressure.
- The mechanism by which caffeine intake affects resistance exercise performance is likely multifactorial.

5.2. Introduction

Caffeine is one of the most commonly consumed drugs in the world (Graham, 2001), and a national survey indicated that 89% of American adults ingest caffeine with an average daily consumption (mean ± standard deviation) of 211 ± 472 mg (Fulgoni et al., 2015). This amount of caffeine is contained in approximately two cups of brewed coffee. Because of the ergogenic effects of caffeine on exercise performance, its use is also very prevalent among athletes (Van Thuyne, Roels, & Delbeke, 2005). Although several previous reviews have focused on the ergogenic benefits of caffeine on exercise performance (Astorino & Roberson, 2010; Burke, 2008; Davis & Green, 2009; Ganio, Klau, Casa, Armstrong, & Maresh, 2009; Goldstein et al., 2010a; Graham, 2001; Spriet, 1995; Spriet, 2014; Wickham & Spriet, 2018), none of them explicitly focused on resistance exercise. Therefore, there remains ambiguity regarding the effects of caffeine supplementation on resistance exercise.

Several muscular qualities are important when discussing resistance exercise, including muscular strength, muscular endurance, and muscular power. Muscular strength is 'the capacity to exert force under a particular set of biomechanical conditions' (Carroll, Riek, & Carson, 2001). The following forms of muscular strength are usually assessed in research studies: dynamic strength (concentric actions coupled with eccentric actions), isometric strength (a muscle action in which the muscle-tendon complex does not change its length), and reactive strength (an ability to change quickly from eccentric to concentric muscle actions) (Suchomel et al., 2016). A commonly used field-based test for assessing dynamic strength is the one-repetition maximum (1RM) test, while in laboratory settings dynamic strength is commonly assessed using isokinetic dynamometers (Levinger et al., 2009). Several neural factors such as motor unit recruitment, motor unit synchronisation, rate coding, and neuromuscular inhibition underpin strength (a more detailed discussion of these factors can be found elsewhere (Suchomel, Nimphius, Bellon, & Stone, 2018)). Muscular endurance can be defined as 'the ability of a muscle or muscle group to perform repeated contractions against a load for an extended period' (Kell, Bell, & Quinney, 2001). Muscular endurance is commonly assessed by performing repetitions of a given task to momentary muscular failure with a load corresponding to, for example, 50-60% of 1RM, or by measuring the time that a person is able to maintain force production at a given percentage of the force that corresponds to their maximal voluntary contraction (MVC). Muscular power denotes the rate of muscular work and, in resistance

exercise, it is commonly assessed by using linear position transducer(s) or a force plate (Cormie, McBride, & McCaulley, 2007).

There is a growing number of studies investigating the effects of caffeine supplementation on pain perception, ratings of perceived exertion (RPE), muscular qualities (e.g., maximal strength, muscular endurance and power), muscle damage and cardiovascular and hormonal responses to resistance exercise. However, given their mixed results, this paper aims to critically evaluate and thoroughly discuss the evidence on the topic and to provide practical guidelines for the application of caffeine supplementation when performing resistance exercise.

5.3. Possible mechanisms for the ergogenic effect of caffeine on exercise performance

Some of the initially-proposed mechanisms for the ergogenic effect of caffeine on exercise performance were enhanced fat oxidation and subsequent glycogen sparing (Costill et al., 1978). However, these proposed mechanisms received little support in the literature, given that caffeine ingestion has been observed to be beneficial even in shorter duration exercise protocols (e.g., <30 minutes) in which glycogen levels do not appear to be a limiting factor (Graham, 2001). These mechanisms also could not explain the observed ergogenic effects of caffeine on high-intensity, short-duration, anaerobic exercise performance (Davis & Green, 2009). Currently accepted mechanism(s) relate to the antagonistic effect of caffeine on adenosine receptors (McLellan et al., 2016). The binding of adenosine to A_1 and A_{2a} G protein-coupled receptors (McLellan et al., 2016) inhibits the release of various neurotransmitters (such as acetylcholine and dopamine). Caffeine is structurally similar to adenosine, and, therefore, when ingested it blocks the binding of adenosine to the A_1 and A_{2a} receptors and promotes the release of these neurotransmitters (McLellan et al., 2016). Thus, caffeine exerts central nervous system effects and alters arousal, which may lead to improvements in performance (Davis & Green, 2009). Caffeine also increases calcium release from the sarcoplasmic reticulum and motor unit recruitment, which may result in a more forceful muscular contraction and help explain some of the ergogenic effects of caffeine on resistance exercise performance (Bazzucchi, Felici, Montini, Figura, & Sacchetti, 2011; Tarnopolsky, 2008). Furthermore, studies conducted in both animals and humans suggest that caffeine may have a direct effect on the skeletal muscle tissue, which may, at least partially, explain the ergogenic effect of caffeine (Mohr, Van Soeren, Graham, & Kjaer, 1998; Tallis et al., 2012; Tallis et al., 2015).

5.3.1. Effects of caffeine on ratings of perceived exertion

RPE is commonly assessed using the Borg 0-10, or the 6-20 point scale (Borg, 1982). Caffeine may reduce RPE, which might allow an individual to perform more work with reduced subjective strain (Tarnopolsky, 2008). When assessed in an aerobic exercise setting, the reductions in RPE explain up to 29% of the ergogenic effect of caffeine on submaximal aerobic exercise performance (Doherty & Smith, 2005), suggesting that a reduced RPE is a relevant factor in performance-increasing mechanisms.

Several studies observing a positive effect of caffeine on performance (e.g., acute increases in strength and muscular endurance) have also reported a reduction in RPE. For instance, Grgic and Mikulic (2017) showed a 3% increase in 1RM barbell back squat performance and a corresponding 7% reduction in RPE (using the 6-20 point scale) with caffeine ingestion in a sample of resistance-trained individuals. Using a protocol that focused on muscular endurance, Duncan and Oxford (2012) also reported a 13% decrease in RPE (using the 0-10 point scale) and an ergogenic effect of caffeine on muscular endurance. A subsequent study by Duncan et al. (2013) confirmed these findings. However, the majority of the remaining studies have observed no significant effect of caffeine ingestion on RPE. For instance, Astorino, Terzi, Roberson, and Burnett (2010) did not find a reduction in RPE at doses of 2 and 5 mg/kg of caffeine even though improvements in strength were evident with the 5 mg/kg dose. Similarly, Duncan and Oxford (2011) did not find a significant reduction in RPE ($p = 0.082$) when using a dose of 5 mg/kg administered one hour before performing repetitions to momentary muscular failure with 60% 1RM on the bench press. Similar results have also been observed in other related studies (Da Silva et al., 2015; Green et al., 2007; Hudson, Green, Bishop, & Richardson, 2008; Woolf, Bidwell, & Carlson, 2008; Woolf, Bidwell, & Carlson, 2009). While Arazi, Hoseinihaji, and Eghbali (2016) found that a dose of 2 mg/kg is sufficient to achieve an RPE-reducing effect, this reduction in RPE was not accompanied by any increases in muscular strength or muscular endurance.

It can be hypothesised that exercise selection may determine the RPE response, given that complex, multi-joint exercises activate more muscle groups and, thus, require greater exertion. Two studies that did not observe a reduction in RPE used single-joint exercises, such as knee

extensions and arm curls, which are less demanding than multi-joint exercises (Hudson et al., 2008; Hurley, Hatfield, & Riebe, 2013). While exercise selection might play a role in determining this effect, this hypothesis remains speculative as some studies using single-joint exercises reported a reduction in RPE following caffeine ingestion (Hurley et al., 2013) and others using the bench press exercise (i.e., a multi-joint upper-body exercise) did not show significant reductions in RPE following caffeine ingestion (Da Silva et al., 2015; Woolf et al., 2008; Woolf et al., 2009). Doherty and Smith (2005) reported that RPE is lowered during prolonged aerobic exercise, but that it remains unaltered when assessed at exercise termination. Due to the nature of resistance exercise, RPE is evaluated almost exclusively at exercise termination, which might be a reason why studies have often reported no differences in RPE following caffeine ingestion. While a reduction in rating of perceived exertion might contribute to the performance-enhancing effects of caffeine, a firm conclusion cannot be made on this topic due to the inconsistent evidence presented in the literature.

5.3.2. Effects of caffeine on pain perception

Due to its blockade of adenosine receptors, caffeine is a common ingredient of over-the-counter medications for pain relief (Laska et al., 1984). Resistance exercise may lead to significant acute increases in pain perception (Cook, O'Connor PJ, & Ray, 2000), which raises the possibility that a reduction in pain perception might contribute to the ergogenic effects of caffeine. Some studies have reported that caffeine ingestion decreases pain perception but without any significant effects on performance (Arazi et al., 2016b; Grgic & Mikulic, 2017). Tallis and Yavuz (2018) and Sabblah, Dixon, and Bottoms (2015) did not observe any significant reductions in pain perception, although caffeine ingestion increased muscular strength, suggesting that factors other than the reduced perception of pain contributed to the ergogenic effect. Although two studies reported that improvements in performance were accompanied by a decrease in pain perception (Duncan & Oxford, 2012; Duncan et al., 2013), there was also a decrease in RPE that made it difficult to determine exactly what contributed to the ergogenic effect. Based on the current evidence, it seems that mechanism(s) other than reductions in pain perception contribute to the enhanced resistance exercise performance with caffeine ingestion.

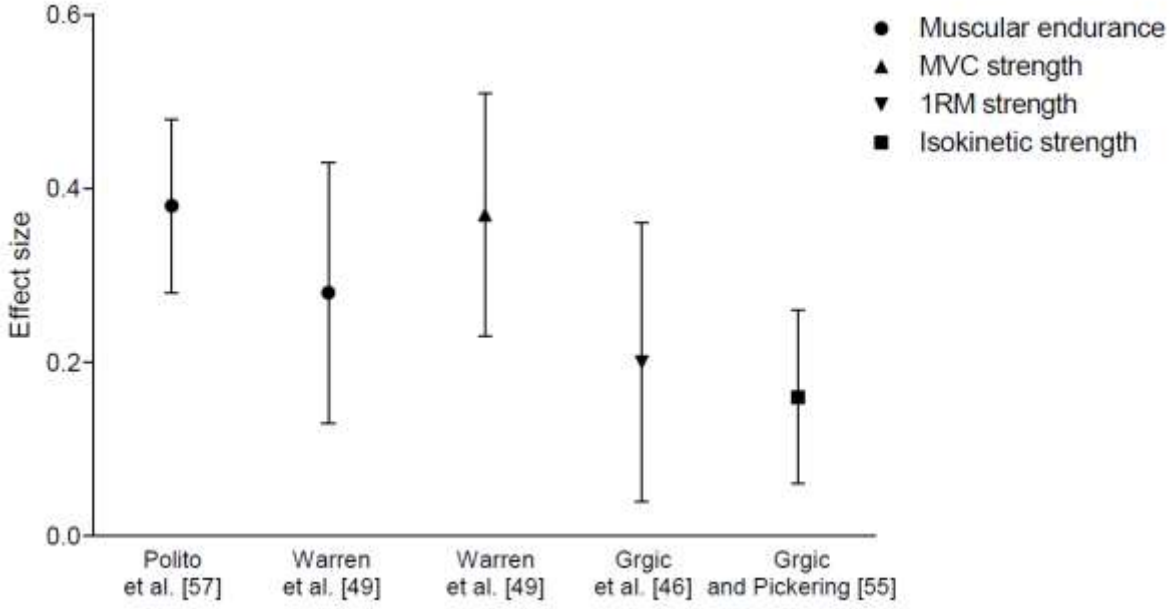
5.4. Effects of caffeine on strength

5.4.1. 1RM strength

Some of the initial studies that investigated the effects of caffeine on 1RM dynamic strength did not show a significant ergogenic effect. For instance, Astorino et al. (2008) did not find any performance-enhancing effects of caffeine ingestion on 1RM strength in the bench press and leg press exercises among resistance-trained men. However, a study by Goldstein et al. (2010b), involving resistance-trained women, showed that caffeine ingestion may significantly improve upper-body 1RM as assessed by the bench press exercise.

A prevalent issue among individual studies examining the effects of caffeine supplementation on resistance exercise performance is the use of small sample sizes (Williams et al., 2008), which may result in low statistical power. To better understand the equivocal evidence reported in the literature, Grgic et al. (2018) recently conducted a meta-analysis of studies assessing the impact of caffeine on 1RM muscular strength. The findings of this review suggested that caffeine ingestion enhances 1RM muscular strength compared to placebo (Figure 4). Subgroup analyses revealed that caffeine ingestion increased upper- but not lower-body strength. The raw difference between the mean effects of placebo and caffeine in the subgroup analysis equated to 3.5 kg (95% confidence interval [CI]: 1.5, 4.8 kg) and 1.7 kg (95% CI: -1.7, 5.0 kg) of lifted weight for the upper-body and the lower-body, respectively. From a physiological perspective, there appears to be no rationale as to why caffeine would increase upper- but not lower body strength. In fact, as we discuss below (section 3.2), due to the differences between the upper- and lower-body in the amount of muscle mass involved, the opposite results might be expected. That said, the subgroup analyses for lower- and upper-body strength were limited as they included only seven and eight studies, respectively. While the meta-analysis provided some evidence that caffeine increases 1RM strength, given the relatively small number of studies investigating this topic, future research is warranted.

Figure 4. Summary of meta-analytic findings on the effects of caffeine on muscular endurance and muscular strength, as shown by Polito et al. (2016), Warren et al. (2010), Grgic et al. (2018), and Grgic and Pickering (2019). Effect sizes are expressed as Cohen's *d*. The range represents 95% confidence intervals. All effects were significant. *MVC* maximal voluntary contraction, *1RM* one-repetition maximum



5.4.2. Isometric and isokinetic strength

Using a model focused on the dorsiflexor muscles, Tarnopolsky and Cupido (2000) reported no significant effect of caffeine ingestion on enhancing MVC. However, in an experiment performed by Park et al. (2008) that focused on the knee extensor muscles, caffeine led to significant increases (+10%) in MVC compared to a placebo. Some of these findings can possibly be attributed to differences in the activation of smaller versus larger muscle groups. Indeed, a meta-analytic review by Warren et al. (2010), which pooled MVC tests (with the majority of studies using isometric tests of strength), reported that caffeine ingestion may significantly increase MVC by ~4%. However, this effect seemed to be evident primarily in the knee extensor muscles (+7%) and not in smaller muscle groups, such as the dorsiflexors.

During a MVC, the activation of the knee extensor muscles is usually lower when compared with other muscle groups (Shield & Zhou, 2004; Warren et al., 2010). For instance, smaller muscles such as the tibialis anterior can be activated up to 99% of their maximum during a MVC and, hence, the activation of these muscles is already at near-maximal level (Connelly, Rice, Roos, & Vandervoort, 1999; Gandevia & McKenzie, 1988). However, knee extensor activation is usually 85 to 95% of its maximal activation and, therefore, Warren et al.'s (2010)

hypothesis was that with caffeine ingestion, the muscle activation in this muscle group can be enhanced, which in turn can augment the MVC. Caffeine ingestion has been reported to increase cortical and spinal neuron excitability (Kalmar & Cafarelli, 2006), which might increase muscle activation through an increase in motor unit recruitment. Indeed, Black, Waddell, and Gonglach (2015) demonstrated that caffeine ingestion enhances MVC and motor unit recruitment in the knee extensors but not in the elbow flexors, supporting the hypothesis by Warren et al. (2010).

Recently, Tallis and Yavuz (2018) reported that caffeine ingestion enhanced isokinetic strength in the knee extensors but not in the elbow flexors, adding to the evidence showing that benefits of supplementation might be related to the different activation of smaller versus larger muscle groups. The results by Tallis and Yavuz (2018) for isokinetic strength were confirmed in a recent meta-analysis (Grgic & Pickering, 2019), whereby the pooled relative ES from ten included studies was 0.16 (+6%), suggesting that caffeine ingestion enhances isokinetic strength. However, again, this effect was not observed in smaller muscle groups such as the elbow flexors and was predominately manifested in the knee extensors.

In summary, the current evidence suggests that caffeine ingestion may have an ergogenic effect on muscular strength across all muscle action types (Behrens et al., 2015). As such, these findings are likely to have the highest application in sports such as powerlifting and weightlifting. However, studies conducted specifically among competitive powerlifters and weightlifters are needed, given that most of the previous studies included untrained or recreationally trained individuals. More evidence is needed to examine the differences between small and large muscle groups, as well as between the upper- and lower-body musculature. Although it seems that caffeine enhances MVC, isometric actions and isokinetic apparatuses are used to a lesser degree in traditional resistance exercise routines, which somewhat limits the practical application of these findings.

5.5. Effects of caffeine on muscular endurance

Several individual studies (Da Silva et al., 2015; Duncan & Oxford, 2012; Duncan et al., 2013) and meta-analytic reviews (Polito et al., 2016; Warren et al., 2010) show that caffeine (most commonly administered in a dose of 5 to 6 mg/kg) can have an ergogenic effect on muscular

endurance, with improvements found for both the upper-body and the lower-body musculature (Duncan & Oxford, 2012; Duncan et al., 2013). Forest plots in the reviews conducted by Polito et al. (2016) and Warren et al. (2010) indicate that studies almost never show that caffeine produces an ergolytic effect on muscular endurance performance. Specifically, in the work by Warren et al. (2010), out of the 23 studies included in the meta-analysis, sample ESs for only four studies (Bond, Gresham, McRae, & Tearney, 1986; Jacobs, Pasternak, & Bell, 2003; Kalmar & Cafarelli, 2006; Kalmar, Del Balso, & Cafarelli, 2006) favoured the placebo group. The ESs in these four studies ranged from -0.32 to -0.03, but none were statistically significant. In the review by Polito et al. (2016), none of the studies favoured placebo. The pooled ESs in these reviews ranged from 0.28 to 0.38, that is, +6% to +7%. The raw difference between mean effects of placebo and caffeine for the number of completed repetitions in the studies included in the Polito et al. (2016) review ranged from -0.3 to +6 repetitions. In the studies identified by Warren et al. (2010), the time to maintain an isometric contraction at a given percentage of MVC (a test used to assess muscular endurance) with caffeine ingestion ranged from 8 to 32 s. Future long-term studies are needed to explore if these small acute increases in performance also impact long-term adaptations to resistance exercise.

Limited evidence also shows an ergogenic effect of caffeine on muscular endurance in a sleep-deprived condition (6 hours of sleep or less) (Cook, Beaven, Kilduff, & Drawer, 2012). Several studies that carried out muscular endurance assessments following maximum strength testing did not observe a significant ergogenic effect of caffeine on muscular endurance (Astorino et al., 2008; Goldstein et al., 2010b; Grgic & Mikulic, 2007), suggesting that caffeine supplementation may not be as effective on muscular endurance as fatigue develops. These results seem surprising given that caffeine ingestion has been shown to slow down the fatigue-induced loss of force production (Pethick, Winter, & Burnley, 2017). Caffeine ingestion should, therefore, theoretically be ergogenic even in the presence of fatigue and the exact reasons for the lack of an ergogenic effect of caffeine in the referenced studies remain unclear. Studies that investigated the effects of caffeine supplementation on muscular endurance among females also did not show a significant performance-enhancing effect (Arazi et al., 2016b; Goldstein et al., 2010b; Sabblah et al., 2015) albeit, with sample sizes of 10, 15, and 8 participants, respectively. Phases of the menstrual cycle might play an important role in studies involving women given that caffeine clearance is slower in the luteal phase of the cycle (Lane, Steege, Rupp, & Kuhn, 1992). Furthermore, the use of oral contraceptives may alter caffeine metabolism (Nehlig,

2018), which also needs to be considered when conducting studies among women. This topic seems to be under-investigated in this population and requires further attention. In summary, it seems that caffeine can acutely enhance muscular endurance, but details such as fatigue-related and sex-specific responses require future study to better determine its effectiveness.

5.6. Effects of caffeine on power

Most of the studies on power outcomes focused on variations of jump performance (Grgic et al., 2018), power recorded during the Wingate 30-s test (Grgic, 2018), or repeated and intermittent-sprints performance (Glaister, Muniz-Pumares, Patterson, Foley, & McInnes, 2015; Schneiker, Bishop, Dawson, & Hackett, 2006). Caffeine may acutely enhance these components of power (Glaister et al., 2015; Grgic et al., 2018; Grgic, 2018; Schneiker et al., 2006), but there is limited research on the effects of caffeine on power expression measured as contraction velocity during traditional dynamic resistance exercises. In a study by Mora-Rodríguez, Pallarés, López-Samanes, Ortega, and Fernández-Elías (2012) 12 trained men performed three exercise trials: (i) a morning training session (10:00 a.m.) after the ingestion of 3 mg/kg of caffeine, (ii) a morning training session after ingesting a placebo, and (iii) an afternoon session (18:00 p.m.) following the ingestion of a placebo. Bar displacement velocity was measured during the squat and bench press exercises with loads that elicited a bar velocity of 1 m/s and with a load corresponding to 75% of 1RM. Results showed that power increased with all loads with caffeine ingestion, except for the bench press velocity at 1 m/s (p = 0.06, Cohen's d = 0.68). Using the same dose of caffeine in a group of 14 Brazilian jiu-jitsu athletes, Diaz-Lara et al. (2016) confirmed that caffeine may be ergogenic for power, showing an increase in maximal power and mean power in the bench press exercise.

Pallarés et al. (2013) sought to investigate contraction velocity at three different doses of caffeine (i.e., 3, 6, and 9 mg/kg) and across four different loading schemes, namely, 25%, 50%, 75%, and 90% of 1RM performed using the bench press and barbell back squat exercises. When measured at loads of 25% and 50% of 1RM, all doses of caffeine resulted in increased power in both exercises. At higher loads, higher doses seem to be needed to augment power, both in the bench press and in the squat exercises. These results suggest that greater doses of caffeine might be warranted for a performance-enhancing effect when exercising with higher loads. Such large doses of caffeine also seem to generate more side effects (Pallarés et al., 2013),

which also needs to be considered. In the same sample, caffeine has been shown to have a more pronounced effect on power when administered in the morning versus in the afternoon hours (Mora-Rodríguez et al., 2015). Such results could be due to the reduced capacity to activate/recruit the musculature in the morning hours (Mora-Rodríguez et al., 2015). Therefore, when administered in the morning, caffeine may augment the ability to activate/recruit the musculature (Mora-Rodríguez et al., 2015). Also, side effects such as insomnia may be even more prevalent when supplementing with caffeine in the afternoon hours (Mora-Rodríguez et al., 2015), which does highlight that time-of-day is an important variable to consider when prescribing caffeine supplementation.

It seems that caffeine may enhance contraction velocity, although this finding is based only on the results from a few studies. Given some of the mixed evidence presented for maximal strength, this might indicate that caffeine has a more pronounced effect on contraction velocity than on maximal force production. Future studies should consider examining changes in both 1RM strength and contraction velocity (with lower loads) in the same group of participants to investigate if this is indeed the case. The limited research to date suggests that caffeine ingestion may acutely increase muscle power in resistance exercise and, therefore, athletes competing in events in which power is a significant performance-related variable might consider using caffeine supplementation pre-exercise.

5.7. Effects of caffeine on muscle damage and delayed onset muscle soreness

5.7.1. Delayed onset muscle soreness

Resistance exercise may lead to exercise-induced muscle damage and delayed onset muscle soreness (DOMS) (Vierck et al., 2000). Exercise-induced muscle damage commonly brings about DOMS, which can be defined as the pain felt upon palpation or movement of the affected tissue (Clarkson, Nosaka, & Braun, 1992). DOMS appears within a few hours post-workout, peaks 1 to 3 days following the exercise session, and can last up to 10 days (Isner-Horobeti et al., 2013). Because caffeine is an adenosine antagonist, its consumption might increase the response of the sympathetic nervous system, and, thus, decrease the perception of muscle soreness (Astorino, Cottrell, Lozano, Aburto-Pratt, & Duhon, 2012).

Two of the initial studies (Hurley et al., 2013; Maridakis, O'Connor, Dudley, & McCully, 2007) that investigated the effects of caffeine ingestion on DOMS following resistance exercise observed that caffeine might indeed reduce DOMS. Hurley et al. (2013) employed a training protocol that consisted of five sets of biceps curls exercise performed with a load corresponding to 75% of 1RM. On days 1 to 5, the participants were required to assess their levels of soreness on three different scales: overall soreness, overall fatigue, and soreness on a palpation scale. Administration of caffeine (5 mg/kg) allowed the participants to perform a significantly greater number of repetitions during the fifth set of bicep curls. However, despite greater total work performed following caffeine ingestion, the overall perception of soreness was significantly lower on day 2 and day 3 with caffeine ingestion as compared to placebo. Because soreness peaks 1 to 3 days following exercise, the results of this study indicate that caffeine can significantly reduce the perception of soreness following resistance exercise. Hurley et al. (2013) also assessed creatine kinase levels and, consistent with the results of Machado et al. (2010) (see section "Muscle damage"), they reported that caffeine ingestion did not significantly affect creatine kinase levels.

In the Maridakis et al. (2007) study, during the first visit (no supplement ingestion), the participants underwent an electrically-stimulated eccentric exercise of the quadriceps that consisted of 64 eccentric actions; a protocol known to bring about DOMS (Dudley et al., 1997). Twenty-four and 48 hours following the protocol, the participants consumed either a placebo or caffeine (5 mg/kg) in a counterbalanced fashion and expressed their perceived levels of soreness after performing an MVC and a submaximal eccentric test. The results showed that with the ingestion of caffeine there was a significant reduction in DOMS with a greater effect observed during the MVC as compared to submaximal eccentric movements. In a recent study, Green, Martin, and Corona (2018) showed that caffeine increased peak torque but did not impact the perception of soreness in a group of 16 participants using a caffeine dose of 6 mg/kg. While Maridakis et al. (2007) used a protocol that involved maximal and submaximal eccentric movements, the protocol in this study for assessing DOMS involved expressing subjective levels of soreness after stepping down from a box (Green et al., 2010), which might explain the differences in results between the studies. The use of different methods for assessing DOMS somewhat limits the comparison of results between the studies.

In summary, there is some preliminary evidence to suggest that caffeine ingestion may indeed reduce DOMS, which is not surprising given that caffeine can have a hypoalgesic effect. That said, given the small number of studies, further research exploring this topic is warranted. The studies that have been conducted so far mostly administered caffeine only pre-exercise. However, Caldwell et al. (2017) recently explored the effects of ingesting caffeine on perceived soreness in the days following exercise (i.e., a 164 km endurance cycling event). Given that the authors reported positive effects of caffeine on relieving feelings of soreness during the three days of recovery post-exercise, this is an area that could be further explored in resistance exercise as well.

5.7.2. Muscle damage

Machado et al. (2010) investigated the effects of caffeine ingestion on blood markers of muscle damage, including creatine kinase, lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase. Fifteen participants took part in a resistance exercise protocol consisting of six exercises performed in three sets of ten repetitions. The caffeine dose was 4.5 mg/kg. All the abovementioned markers of muscle damage increased after the resistance exercise session with no significant differences found between the caffeine and placebo conditions. In this study, researchers equated the total work (calculated as load × sets × repetitions) between the caffeine and placebo sessions. However, given that caffeine may enhance acute exercise performance, this might consequently lead to greater increases in markers of muscle damage. This hypothesis could be explored in future studies that do not equate the total work between the caffeine and placebo trials.

5.8. Effects of caffeine on hormonal responses

Acute increases in hormones such as testosterone (a primary anabolic hormone), cortisol (a systemic catabolic marker), and growth hormone (a hormone associated with reproduction and stimulation of cellular growth) following resistance exercise have received considerable attention in the literature (Kraemer & Ratamess, 2005). It has been suggested that acute changes in these hormones influence resistance training adaptations such as muscular hypertrophy and increases in strength (Kraemer & Ratamess, 2005). However, others recently found that the acute changes in hormones are weakly correlated with long-term adaptations to resistance training (West & Phillips, 2012). Thus, although some studies (Beaven et al., 2008; Woolf et

al., 2008; Wu & Lin, 2010; Wu, 2015) reported that caffeine ingestion, as compared to placebo, may lead to greater increases in the production of testosterone and cortisol following resistance exercise (even when the workload is matched between the conditions), the practical applicability of these findings remains unclear.

5.9. Effects of caffeine on muscle protein synthesis and anabolic signalling

One of the hallmark adaptations to resistance exercise is muscular hypertrophy. In general, it is accepted that the anabolic mammalian mechanistic target of rapamycin complex 1 (mTORC1) signalling cascade mediates muscular hypertrophy which is a cumulative result of acute increases in protein synthesis above protein degradation (i.e., net protein accretion) (Bodine et al., 2001; Damas, Phillips, Vechin, & Ugrinowitsch, 2015). Some of the studies conducted in cultured cells have observed that caffeine inhibited mTOR activity (McMahon, Yue, Santen, & Lawrence, 2005; Miwa et al., 2012), albeit, such effects were seen at supra-physiological concentrations of caffeine. A recent study by Moore et al. (2017) conducted in mice (with physiological concentrations of caffeine that would be observed in humans following moderate caffeine intake), showed that caffeine did not negatively affect mTOR activity or muscle protein synthesis after a bout of electrically-stimulated contractions. Moreover, caffeine even enhanced the phosphorylation of ribosomal protein S6 suggesting a positive effect of caffeine on anabolic signalling. Furthermore, work on rats in the same study showed that caffeine did not affect plantaris muscle hypertrophy (Moore et al., 2017). While cell culture and animal models may provide some interesting findings, they also may have limited relevance to humans. Currently, there are no published studies examining the effects of caffeine on muscle protein synthesis and anabolic signalling in response to resistance exercise in humans. While there are some unpublished observations involving resistance-trained men in whom caffeine ingestion did not negatively affect muscle protein synthesis responses following resistance exercise (Bui, 2015), these results remain to be published. Therefore, this is an interesting area that could be explored in future research.

5.10. Effects of caffeine on cardiovascular responses

5.10.1. Blood pressure

Even under resting conditions, caffeine ingestion of 250 mg has been shown to increase blood pressure (Mosqueda-Garcia, Tseng, Biaggioni, Robertson, & Robertson, 1990). Also, resistance exercise may lead to significant acute increases in systolic and diastolic blood pressure (de Freitas Brito, de Oliveira, do Socorro Brasileiro-Santos, & da Cruz Santos, 2014). Therefore, it is possible that the combination of this type of exercise with caffeine ingestion might augment acute blood pressure responses.

Only a few studies to date have focused on the effects of caffeine on the cardiovascular system in resistance exercise. Jacobs et al. (2003) initially reported that the ingestion of caffeine did not increase systolic blood pressure more than the ingestion of placebo during a resistance exercise session consisting of three supersets (leg press exercise followed by the bench press exercise). Following caffeine ingestion, Astorino, Rohmann, Firth, and Kelly (2007) reported increases in systolic but not diastolic blood pressure. In a study including normotensive and hypertensive men, Astorino, Martin, Schachtsiek, and Wong (2013), confirmed their initial findings by showing that caffeine ingestion increases resting, exercise, and recovery systolic blood pressure. The same effect on blood pressure was observed in a study by Goldstein et al. (2010b), in which the ingestion of caffeine led to an increase in systolic blood pressure by 4 mmHg. Comparable results were observed by others as well (Woolf et al., 2008). When ingested before physical activity, caffeine may reduce myocardial blood flow during exercise (Higgins & Babu, 2013). This reduction in blood flow likely explains the augmented increases in blood pressure that may occur with the ingestion of caffeine in resistance exercise (Higgins & Babu, 2013).

Passmore, Kondowe, and Johnston (1987) have reported that caffeine doses of 45, 90, 180, and 360 mg increase blood pressure in a dose-response fashion (i.e., greater increases with higher doses). Therefore, the discrepancy in findings between studies of subjects participating in resistance exercise might be explained by the caffeine dose, as Jacobs et al. (2003) used a dose of 4.5 mg/kg, while Astorino et al. (2007) and, subsequently, Goldstein et al. (2010b), used a dose of 6 mg/kg. Although variations in dosage might help explain these findings, it is important to highlight that a caffeine dose of 4 mg/kg was reported to increase blood pressure (Souza,

Casonatto, Poton, Willardson, & Polito, 2014). Furthermore, in some studies, a dose of 5 mg/kg did not result in greater increases in blood pressure over placebo alone, highlighting the equivocal nature of research done in this area (Woolf et al., 2009). Factors such as participants' posture, arm support, arm position, left or right-hand side, cuff, and empty/full bladder are all known to influence blood pressure estimates (Beevers, Lip, & O'Brien, 2001). However, most of the studies only reported the timing of measurement and posture, making the between-study comparison of the results difficult. Due to the effects of caffeine on blood pressure, this supplement might not be recommendable for individuals with high blood pressure, as it may result in excessive cardiovascular demands (Pincomb et al., 1985). Therefore, caution is needed when considering caffeine supplementation in these populations.

5.10.2. Heart rate

Besides blood pressure, heart rate is another important cardiovascular variable that needs to be considered. Astorino et al. (2007) also evaluated heart rate responses in a cohort of resistance-trained men performing 1RM and muscular endurance tests on both the bench press and leg press exercises. They observed that heart rate before starting the exercise bout and pre- bench-press increased by ten beats per min with the ingestion of caffeine. While some studies observed similar effects of caffeine on this variable (Green et al., 2007; Hudson et al., 2008: Richardson & Clarke, 2016), others have reported no differences in heart rate responses between the caffeine and placebo conditions (Astorino et al., 2013; Da Silva et al., 2015; Duncan & Oxford, 2012; Souza et al., 2014; Woolf et al., 2008; Woolf et al., 2009). Some discrepancies between the studies might be related to the habitual caffeine intake of participants. Specifically, there is evidence to suggest that increases in heart rate with caffeine ingestion are exacerbated in individuals who habitually consume lower amounts of caffeine as compared to high habitual users (Dodd, Brooks, Powers, & Tulley, 1991; Temple et al., 2017). However, while some studies did not assess habitual caffeine intake (Da Silva et al., 2015; Duncan & Oxford, 2012), the participants in others reported a wide range of habitual caffeine intake varying from 30 to 600 mg (Astorino et al., 2007). Given these limitations, future studies should consider exploring potential differences in the effects of caffeine ingestion on heart rate responses in resistance exercise between low and high habitual caffeine users. Future work is warranted on the effects of caffeine on heart rate variability (time differences between consecutive heartbeats) in resistance exercise, as there is evidence (in other forms of exercise) that caffeine ingestion may negatively impact this outcome (Bunsawat, White, Kappus, & Baynard, 2015).

5.11. Caffeine form

The most common forms of caffeine administration for supplementation purposes are capsules and powder mixed with liquid. Currently, there is a growing interest in investigating the effects of caffeine administered in alternative forms such as chewing gums, bars, gels, mouth rinses, energy drinks, and aerosols (Wickham & Spriet, 2018). Some of these forms of caffeine may have a faster absorption rate, which might be of interest in many sporting situations (Wickham & Spriet, 2018). For instance, Kamimori et al. (2002) observed that the time to reach maximal caffeine concentration in the blood was 44 to 80 min with caffeine administered in chewing gum, while in the capsule trials this time amounted to 84 to 120 min. Pharmacokinetics of different forms of caffeine are discussed in more detail in a recent paper by Wickham and Spriet (2018). For resistance exercise protocols only three studies have been conducted with alternative forms of caffeine. One study explored the effect of caffeine mouth rinse on muscular endurance and reported no significant increases in volume load with caffeine ingestion (Clarke, Kornilios, & Richardson, 2015). This can probably be explained by the observation that caffeine administered in this form does not increase blood caffeine concentration (Doering, Fell, Leveritt, Desbrow, & Shing, 2014). Another study investigated the effects of a sugar-free drink containing a fixed dose of 160 mg of caffeine and a placebo beverage on 1RM bench press performance and upper-body muscular endurance (Eckerson et al. 2013). No significant increases in either strength or muscular endurance were found following caffeine ingestion. Some unpublished observations suggest that consumption of caffeinated chewing gum (fixed dose of 75 mg of caffeine) can increase 1RM squat performance (Martin, 2015). However, the study has yet to be published, which precludes its scrutinisation. This area of research is currently in its infancy and needs further exploration.

Researchers have only recently begun to compare the effects of caffeine alone and caffeinated coffee using a resistance exercise protocol. The first study that examined this matter was conducted by Trexler, Smith-Ryan, Roelofs, Hirsch, and Mock (2016). The authors investigated the effects of: (i) caffeine administered in an absolute dose of 300 mg, (ii) coffee with a dose of 303 mg of caffeine, and (iii) a placebo. The effects of coffee on 1RM leg press exercise performance were greater than the effects of caffeine ingestion. The second study that investigated this topic in relation to resistance exercise is the work by Richardson and Clarke

(2016) who tested muscular endurance in the squat exercise. Results showed that both caffeinated coffee and decaffeinated coffee plus 5 mg/kg of anhydrous caffeine resulted in significantly better squat exercise performance compared to other conditions. Therefore, notwithstanding the lack of studies conducted in this area, based on the current evidence, it may be inferred that both coffee and caffeine anhydrous are suitable pre-workout options, while the choice would be a matter of personal preference.

5.12. Caffeine dose, timing, and habitual intake

The most commonly used dose of caffeine in studies examining the effects of caffeine on exercise performance is 6 mg/kg (Graham, 2001). This dose is relatively high, as, for an 85-kg individual, it equates to the amount of caffeine in approximately four to five cups of coffee. As discussed elsewhere (Spriet, 2014), there is a growing interest in investigating the effects of lower doses of caffeine (i.e., ≤3 mg/kg) on exercise performance as these doses may still lead to improvements in alertness and mood during exercise and are associated with few, if any, side effects (Spriet, 2014).

Astorino et al. (2011) reported that performance of the knee extension and flexion exercises was significantly improved with a 5 mg/kg dose of caffeine. However, no improvement in performance was observed with a 2 mg/kg dose. Using the same doses, Arazi et al. (2016b) observed that caffeine did not improve leg press strength and muscular endurance at either 2 or 5 mg/kg doses. Tallis and Yavuz (2018) observed that both 3 and 6 mg/kg caffeine doses were effective for increasing lower-body strength. Furthermore, as stated earlier when discussing power outcomes (section "Effects of caffeine on power"), three studies (Diaz-Lara et al., 2016; Mora-Rodríguez et al., 2012; Pallarés et al., 2013) have investigated the effects of 3 mg/kg of caffeine on resistance exercise performance and power and suggested that this dose can be ergogenic. However, at specific external loads, a higher dose was needed to achieve an increase in performance. A meta-regression by Warren et al. (2010) suggested that there is a dose-response relationship between the doses of caffeine and the magnitude of the effects on muscular endurance. Specifically, for an increase in caffeine dose of 1 mg/kg muscular endurance ES increased by 0.10. However, optimal doses of caffeine still need to be further explored in resistance exercise protocols and other sport and exercise settings (Tallis et al.,

2015). Starting with a lower dose (such as 3 mg/kg) may be a good initial option; the doses can be adjusted after that according to the individual responses.

As with the caffeine dose, the optimal timing of caffeine supplementation has been under-investigated. Caffeine has a half-life of 4 to 6 hours, and its plasma concentration reaches maximum approximately one hour after ingestion (although this can depend on the source of caffeine and can vary considerably between individuals) (Spriet, 2014; Magkos & Kavouras, 2005). Therefore, in most studies, the exercise session begins one hour after the supplement is ingested. Instead of the common 60-min waiting time, some studies have used a 45-min (Williams et al., 2008) or a 90-min (Jacobs et al., 2003) waiting time and did not show performance-enhancing effects of caffeine. However, it remains unclear if the waiting time was responsible for the lack of a significant effect. This might have been a consequence of other factors, such as small sample sizes, as the studies included 13 and 9 participants, respectively (Jacobs et al., 2003; Williams et al., 2008). Also, genetic differences in caffeine metabolism among the participants (as discussed in section "Genetic differences in responses to caffeine ingestion") may have contributed to the outcomes. Because of the lack of studies, the optimal timing of caffeine intake for resistance exercise remains unclear. Nevertheless, it is well-established that ergogenic effects can be seen one-hour post-ingestion when using capsule or powder forms of caffeine (Grgic & Pickering, 2019; Grgic et al., 2018; Polito et al., 2016; Warren et al., 2010). There is limited research regarding the influence of habitual caffeine intake and the acute effects of caffeine supplementation on exercise performance. Based on the available evidence, it does not seem that habitual caffeine ingestion reduces the ergogenic benefits of caffeine supplementation (Dodd et al., 1991; Glaister et al., 2008; Gonçalves et al., 2017; Motl, O'Connor, & Dishman, 2003; Tarnopolsky & Cupido, 2000; Wiles et al., 1992). However, there are some contrasting findings (Bell & McLellan, 2002; Evans et al., 2018) suggesting that non-habitual caffeine users experience a greater magnitude of the ergogenic effect with caffeine supplementation compared with habitual caffeine users. Some limitations of these studies include that Bell and McLellan (2002) did not report if the questionnaire they used for assessing habitual caffeine intake had previously been validated while Evans et al. (2018) used a dose of caffeine that was relatively small (on average, 2.5 mg/kg; ~200 mg vs. 3 to 6 mg/kg in most other studies). It might be that habitual consumers need more caffeine to achieve the same ergogenic effect as low habitual users.

Gonçalves et al. (2017) explored this topic in a large sample (n = 40) grouped into tertiles representing low, moderate, and high habitual caffeine users, where the habitual caffeine intake was assessed using a previously validated questionnaire. This study suggested that habitual caffeine intake does not cancel out the performance benefits of the acute supplementation with caffeine. However, this study used a 30-min cycling time trial test and given that there is no research done in this area using resistance exercise protocols, this remains an important avenue for future research.

Additional factors such as ingestion of caffeine in a fed vs. fasted state are important to consider given that the absorption of caffeine is slower in a fed state (McLellan et al., 2016). Indeed, a dose of 3 mg/kg of caffeine administered 60-90 min pre-exercise has been shown to be ergogenic in a fasted (McLellan & Bell, 2004) but not in a fed state (Desbrow, Barrett, Minahan, Grant, & Leveritt, 2009). Additionally, withdrawal is another variable to consider given that habitual caffeine users may experience headache and increased irritability after caffeine abstinence of 24 hours (Juliano & Griffiths, 2004). These symptoms may confound the study design, because the performance under the placebo condition may be impaired due to the withdrawal effects (McLellan et al., 2016).

5.13. Genetic differences in responses to caffeine ingestion

There is a substantial inter-individual variability in responses to caffeine ingestion (Pickering & Kiely, 2018). While some individuals experience enhanced performance, others show no improvement, and, in some cases, even performance decrements (Pickering & Kiely, 2018). Based on some recent evidence it seems that genotype might play an important role in the inter-individual variability in responses. The initial studies that explored the genetic differences in responses to caffeine ingestion while using an exercise protocol report mixed findings (Klein et al., 2012; Puente, Abián-Vicén, Del Coso, Lara, & Salinero, 2018; Womack et al., 2012). For instance, Womack et al. (2012) reported a greater effect of caffeine on exercise performance in AA than in C allele carriers while others found no significant effect of this polymorphism on caffeine's ergogenic effect (Puente et al., 2018). Most of these studies had small to moderate-sized samples (n = 16 to 35). However, in a large cohort of male athletes (n = 101), Guest et al. (2018) showed that the individuals with the AA genotype had a 5% and 7% improvement in

time trial performance with the ingestion of 2 mg/kg and 4 mg/kg of caffeine, respectively. Individuals with the AC genotype did not improve performance following caffeine supplementation, and those with the CC genotype experienced decreases in performance after the ingestion of caffeine. Recently, Rahimi (2019) assessed the effects of caffeine ingestion on muscular endurance using a resistance exercise protocol. A significant difference was observed between the groups for the total number of performed repetitions following caffeine ingestion (AA = +13% vs. AC/CC = +1%; p = 0.002). While this is the only study that examined this topic using a resistance exercise protocol, it does provide compelling evidence in support of the importance of considering genotype when assessing the response to caffeine ingestion.

5.14. Placebo effects of caffeine supplementation

Pollo, Carlino, and Benedetti (2008) investigated the placebo effect on leg extensions exercise performance and reported that the administration of a placebo, alongside the suggestion that it was caffeine, increased mean muscle work and decreased self-perceived muscle fatigue. Duncan, Lyons, and Hankey (2009) confirmed the findings by Pollo et al. (2008) as their results showed that the participants were able to perform two more repetitions under the perceived caffeine condition, and this was accompanied by a reduced RPE, thereby highlighting the power of a placebo for driving positive effects on exercise outcomes (Beedie & Foad, 2009).

In their proof-of-principle study, Saunders et al. (2017) reported that the participants who correctly identified placebo experienced possible harmful effects on performance. Furthermore, those who thought that they ingested caffeine while ingesting placebo also appeared to improve their performance. Therefore, to investigate if any performance-enhancing effects are undoubtedly related to caffeine ingestion or merely a placebo effect, it would be of importance to ask the participants to indicate which trial they perceived to be the caffeine trial. Unfortunately, this question was not asked in several studies examining the effects of caffeine on resistance exercise (Da Silva et al., 2015; Goldstein et al., 2010b; Grgic & Mikulic, 2017; Williams et al., 2008; Woolf et al., 2009), and the results of such studies therefore need to be interpreted with caution. Although not in all cases, some studies that investigated the effectiveness of the blinding indicated that blinding of the participants is effective, as only 29% to 60% of the participants correctly identified the caffeine trials (Astorino et al., 2008; Astorino, Martin, Schachtsiek, Wong, & Ng, 2011; Duncan et al., 2013). It is interesting that in the Bond

et al. (1986) study, there was no blinding of the participants or the investigators, yet, no effect of caffeine on performance was seen (the percent changes and ESs actually favoured the placebo trial). Furthermore, in the work by Tallis, Muhammad, Islam, and Duncan (2016) an equal improvement in peak concentric force was found in the trial in which the participants were told that they were given caffeine and did indeed receive a caffeine dose, and in the trial in which the participants were told that they were given placebo even though they received caffeine. These results seem encouraging as they reflect the true effect of caffeine supplementation on performance. Nonetheless, future research is necessary to differentiate between the actual effects of caffeine and placebo effects.

5.15. Conclusions

Current evidence suggests that caffeine ingestion increases maximal strength, as assessed by 1RM and MVC tests, and muscular endurance. Furthermore, studies show that power is enhanced by caffeine supplementation, although this effect might be caffeine dose- and external load-dependent. While a reduction in RPE potentially contributes to the performance-enhancing effects of caffeine supplementation, the same was not found for pain perception. Some studies have reported that caffeine ingestion did not affect exercise-induced muscle damage but that it might even reduce resistance exercise-induced DOMS. There is some evidence that caffeine ingestion, as compared to placebo, leads to greater increases in the production of testosterone and cortisol following resistance exercise. However, given that the acute changes in hormone levels are weakly correlated with long-term adaptations to resistance exercise, such as hypertrophy and increased muscular strength, these findings are likely of questionable practical significance.

Although not without contrasting findings, the available evidence suggests that caffeine ingestion can lead to acute increases in blood pressure (primarily systolic), and, thus, caution is needed regarding caffeine supplementation among individuals with high blood pressure. In the vast majority of studies, caffeine was administered in capsule or powder forms, and the effects of alternative forms such as chewing gums or mouth rinses on resistance exercise performance therefore remain unclear. The emerging evidence suggests that coffee is at least equally ergogenic as caffeine alone when the caffeine dose is matched. Nevertheless, more research is needed on this topic. Doses in the range 3-9 mg/kg seem to be adequate for eliciting an

ergogenic effect when administered 60 min pre-exercise. It remains unclear what the minimal effective doses are for different types of resistance exercise.

In general, caffeine was found to be safe when taken in the recommended doses. However, at doses as high as 9 mg/kg or higher, side-effects such as insomnia are more pronounced, which needs to be considered when prescribing caffeine supplementation. It remains unclear whether habituation cancels out the ergogenic benefits of caffeine on resistance exercise performance, as no evidence exists for this type of exercise. In some cases, administering placebo alone with the suggestion that it is caffeine has also been shown to enhance performance and reduce RPE. Therefore, the effectiveness of the blinding needs to be considered in future research. Caution is needed when extrapolating these conclusions to females as the vast majority of studies involved only male participants. Finally, most of the studies done in this area report small-to-moderate acute improvements in resistance exercise performance following caffeine ingestion. Therefore, future long-term intervention studies are needed to explore if such acute increases in performance with caffeine ingestion also impact long-term adaptations to resistance exercise.

Compliance with Ethical Standards

Conflict of interest

Jozo Grgic, Pavle Mikulic, Brad J. Schoenfeld, David J. Bishop and Zeljko Pedisic declare that they have no conflicts of interest relevant to the content of this review.

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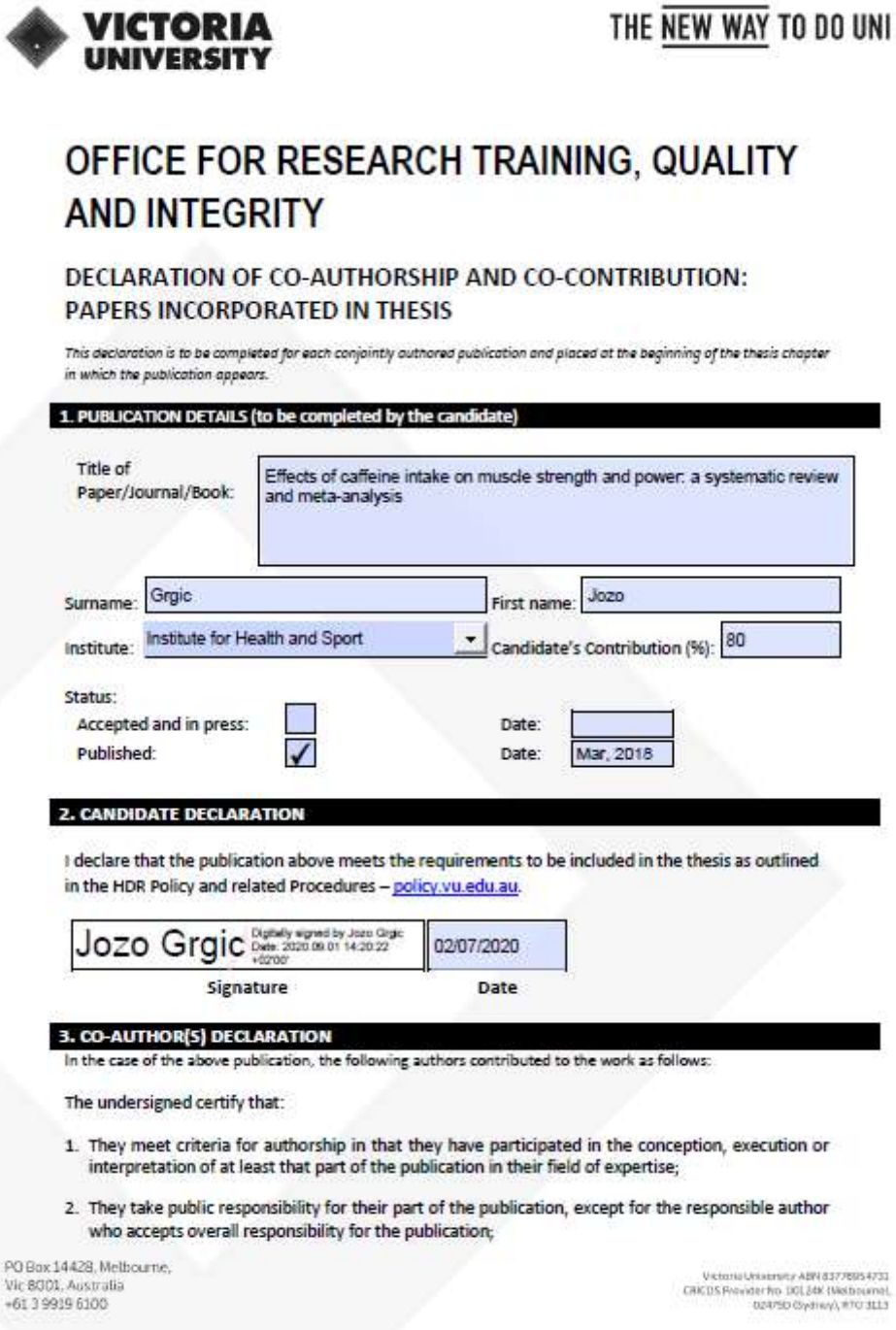
6. Effects of caffeine intake on muscle strength and power: A systematic review and meta-analysis



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3. CO-AUTHOR(S) DECLARATION

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Effects of caffeine intake on muscle strength and power: A systematic review and meta-analysis

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6.1. Abstract

Background: Caffeine is commonly used as an ergogenic aid. Literature about the effects of caffeine ingestion on muscle strength and power is equivocal. The aim of this systematic review and meta-analysis was to summarise results from individual studies on the effects of caffeine intake on muscle strength and power.

Methods: A search through eight databases was performed to find studies on the effects of caffeine on: (i) maximal muscle strength measured using 1 repetition maximum tests; and (ii) muscle power assessed by tests of vertical jump. Meta-analyses of standardised mean differences (SMD) between placebo and caffeine trials from individual studies were conducted using the random effects model.

Results: Ten studies on the strength outcome and ten studies on the power outcome met the inclusion criteria for the meta-analyses. Caffeine ingestion improved both strength (SMD = 0.20; 95% confidence interval [CI]: 0.03, 0.36; $p = 0.023$) and power (SMD = 0.17; 95% CI: 0.00, 0.34; $p = 0.047$). A subgroup analysis indicated that caffeine significantly improves upper (SMD = 0.21; 95% CI: 0.02, 0.39; $p = 0.026$) but not lower body strength (SMD = 0.15; 95% CI: -0.05, 0.34; $p = 0.147$).

Conclusion: The meta-analyses showed significant ergogenic effects of caffeine ingestion on maximal muscle strength of upper body and muscle power. Future studies should more rigorously control the effectiveness of blinding. Due to the paucity of evidence, additional findings are needed in the female population and using different forms of caffeine, such as gum and gel.

6.2. Introduction

Caffeine's ergogenic potential has been extensively studied in the sports science literature, with research dating back to 1907 (Rivers & Webber, 1907). From investigating caffeine's effects on aerobic exercise, in recent years the research focus has shifted to anaerobic exercise performance outcomes, such as muscular endurance, muscle strength, and jumping tasks that require muscle power. While caffeine has been found to significantly enhance muscular endurance (Polito et al., 2016), the effects of caffeine ingestion on maximal muscle strength (commonly operationalised as one repetition maximum [1RM]) and muscle power (commonly operationalised as vertical jump) remain unclear, and the practical utility of caffeine ingestion for enhancing performance in such physical tasks has not been fully elucidated.

The pioneering work on caffeine's effects on strength by Astorino et al. (2008) reported no significant strength-enhancing effects with caffeine ingestion in a group of resistance trained men. Recent work by Grgic and Mikulic (2017), however, found a significant 3% increase in lower body strength with caffeine ingestion using the barbell back squat 1RM as a measure of maximal strength. Goldstein et al. (2010b) reported a significant increase in upper body strength with caffeine ingestion, while Williams et al. (2008) reported no ergogenic effect. The inconsistent results of individual studies prevent drawing sound conclusions regarding the ergogenic potential of caffeine for maximal strength outcomes.

Equivocal findings have also been presented for the effects of caffeine intake on muscle power. A recent study by Ali et al. (2016) reported no effect on countermovement jump height with caffeine ingestion. However, the findings of Bloms et al. (2016) support conclusions about caffeine as an effective ergogenic aid for achieving acute improvements in countermovement jump height and peak force. Given the importance of jumping abilities for many common sports, it would be of both scientific and practical significance to determine a reasonably precise estimate regarding the potential performance-enhancing impact of caffeine ingestion on muscle power.

Several aspects that vary between studies, including the exercise used, participants' characteristics (e.g., age, sex, and training experience), and caffeine form, might be responsible

for the inconsistency of findings. Most importantly, small sample sizes often limited the statistical power to detect significant effects (Cohen, 1988). A meta-analysis of individual studies is needed to circumvent these issues and provide in-depth, evidence-based scrutiny of the current body of evidence. The first meta-analytic investigation on the topic of caffeine and strength was performed by Warren et al. (2010), who found a mean increase of approximately 7% in lower body maximal voluntary contraction with caffeine ingestion. A limitation of the meta-analysis that only two of the included studies tested the effects of caffeine ingestion on 1RM, which significantly restricted the findings to isometric and isokinetic strength outcomes.

The latest meta-analysis on the topic, done by Polito et al. (2016), found no significant effect of caffeine intake on performance in 1RM strength tests. However, only three studies met the inclusion criteria for the meta-analysis. The total number of pooled participants was relatively low (n = 46), potentially indicating issues with the statistical power of the analysis. Furthermore, the small number of included studies prevented subgroup analyses for possible moderators that may potentially impact the ergogenic potential of caffeine. Since the review by Polito et al. (2016), a number of experimental trials have been published (Arazi, Dehlavinejad, & Gholizadeh, 2016; Arazi et al., 2016b; Brooks, Wyld, & Chrismas, 2015; Diaz-Lara et al., 2016; Grgic & Mikulic, 2017; Martin, 2015; Sabblah et al., 2015), presenting novel findings for females (Sabblah et al., 2015), trained (Grgic & Mikulic, 2017; Martin, 2015), and untrained men (Arazi et al., 2016a; Brooks et al., 2015), athletes (Diaz-Lara et al., 2016), and adolescents (Arazi et al., 2016b); as such, an updated review appears to be warranted.

No previous meta-analyses have pooled the results of individual studies on the effects of caffeine on muscle power. The aim of this systematic review was, therefore, twofold: (a) to perform an updated meta-analysis of the acute effects of caffeine ingestion on maximal muscle strength; and (b) to conduct the first meta-analysis of acute effects of caffeine ingestion on muscle power assessed by vertical jump tests. The results may benefit athletes and practitioners in a variety of sports in which muscle strength and/or power are important determinants of performance.

6.3. Methods

6.3.1. Search strategy

The systematic literature search was performed following the PRISMA guidelines (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009). A search of the following databases was performed: PubMed/MEDLINE, Scopus, Cochrane Library, Web of Science (including Science Citation Index Expanded, Social Sciences Citation Index, and Arts & Humanities Citation Index), Google Scholar, Networked Digital Library of Theses and Dissertations, ProQuest Dissertation & Theses and Open Access Theses and Dissertations. The search for the studies on the effects of caffeine on strength was restricted to the documents published from 2015 onwards as the review by Polito et al. (2016), with a search performed in March 2015 was used as a reference point. The review by Polito et al. (2016) was assessed for rigor and deemed as of high-quality. Thus, the studies (Astorino et al., 2008; Goldstein et al., 2010b; Williams et al., 2008) included in the work by Polito et al. (2016) were also included in the present review. The following syntax was used for the primary search: caffeine AND ("muscle strength" OR "ergogenic aid" OR performance OR "resistance exercise" OR "resistance training" OR recovery OR "strength training").

A separate search was done for the studies on the effects of caffeine on power outcomes. The following syntax with no time restriction was used: caffeine AND ("vertical jump" OR "countermovement jump" OR "squat jump" OR plyometrics OR "height" OR "drop jump" OR "depth jump" OR "jump training").

The search results were downloaded and filtered in EndNote software (X8; Clarivate Analytics, New York, USA). A secondary search was performed by screening the reference lists of all selected studies, and by conducting forward citation tracking (using Google Scholar and Scopus) of studies found meeting the inclusion criteria. The search concluded on April 19th, 2017.

6.3.2. Inclusion criteria

To warrant inclusion in the current analysis potential studies were required to meet the following criteria:

(a) an experimental trial published in English in a peer-reviewed journal, or a doctoral or a master's thesis;

(b) assessed the effects of caffeine ingestion in the form of capsule, liquid, gum or gel on dynamic maximal muscle strength (i.e., the greatest amount of weight lifted in a single repetition – 1RM) using constant external resistance, and/or on muscle power assessed using a vertical jump test (both peak force and vertical jump height were considered);

(c) caffeine was not co-ingested with other drugs/substances or potentially ergogenic compounds;

(e) employed a single or double-blind, randomised crossover design;

(f) used human participants without known chronic disease or injury.

Studies were excluded from the analysis if any of the above criteria were violated. Caffeine ingestion via coffee was not considered as coffee has several other biologically active compounds that might moderate the impact of caffeine.

6.3.3. Study coding and data extraction

For all studies meeting the inclusion criteria, the following information was tabulated on a predefined coding sheet using Microsoft Excel software (Microsoft Corporation, WA, USA):

(a) author(s), title and year of publication;
(b) sample size, participants' sex, participants' age (categorised as: adolescents [10-18 years]; young adults [18-39 years]; middle-aged adults [40-64 years];and seniors [≥65 years], and participants' experience in resistance training (categorised as: untrained [less than 1 year of experience]; and trained [more than 1 year of experience]) for studies assessing strength outcomes, and experience in sport training using the same categories as above for studies assessing muscle power.
(c) caffeine form, dosage, and time of ingestion before the experimental session(s);
(d) the exercises used for assessing muscle strength and power with the accompanying mean ± standard deviation (SD) data for the placebo and caffeine trials;
(e) habitual caffeine intake by the participants;
(f) the number of participants indicating which trial they perceived to be the caffeine trial;
(g) reported side effects;

(h) reported funding for conducting the studies.

6.3.4. Methodological quality

The 11-point PEDro scale was used for the assessment of the methodological quality of studies (Maher, Sherrington, Herbert, Moseley, & Elkins, 2003). The first item concerns external validity and is not included in the total score; hence, the maximal score on the scale is 10. Studies were classified as in McCrary, Ackermann, and Halaki (2015). Two authors of the article (JG and BL) performed the search, coding, and appraisal of methodological quality independently, with discussion and consensus over any observed differences. Before correcting for observed differences, the overall agreement between the two independent data extractions was very high (Cohen's kappa = 0.94).

6.3.5. Statistical analysis

The meta-analysis was performed using the Comprehensive Meta-analysis software, version 2 (Biostat Inc., Englewood, NJ, USA). Standardised mean differences (Hedge's g [SMD]) and 95% confidence intervals (CI) were calculated between the placebo and caffeine trials based on their means and standard deviations in 1RM (kg) and vertical jump (cm) tests, the correlations between the trials, and the number of participants. An analysis of peak force in the vertical jump test was not be performed as only two studies reported such outcomes (Bloms et al., 2016; Diaz-Lara et al., 2016). Since none of the studies reported correlation, a 0.5 correlation was assumed for all trials, as recommended by Follmann, Elliott, Suh, & Cutler (1992). When a study measured muscle strength and/or power under multiple conditions (e.g., used more than one caffeine dose, tested more than one muscle group), SMDs and variances were averaged across the different conditions. SMDs of ≤0.2, 0.2-0.5, 0.5-0.8, and >0.8 were considered to represent small, medium, large and very large effects, respectively (Cohen, 1988). The random effects model was used for analysis of both muscle strength and muscle power outcomes. The statistical significance threshold was set *a priori* at $p < 0.05$.

Subgroup analyses for the effects of caffeine on muscle strength were performed for the following study characteristics: (a) upper body strength; (b) lower body strength; (c) the capsule form of caffeine; (d) the liquid form of caffeine; (e) females; (f) males; (g) untrained; and (h)

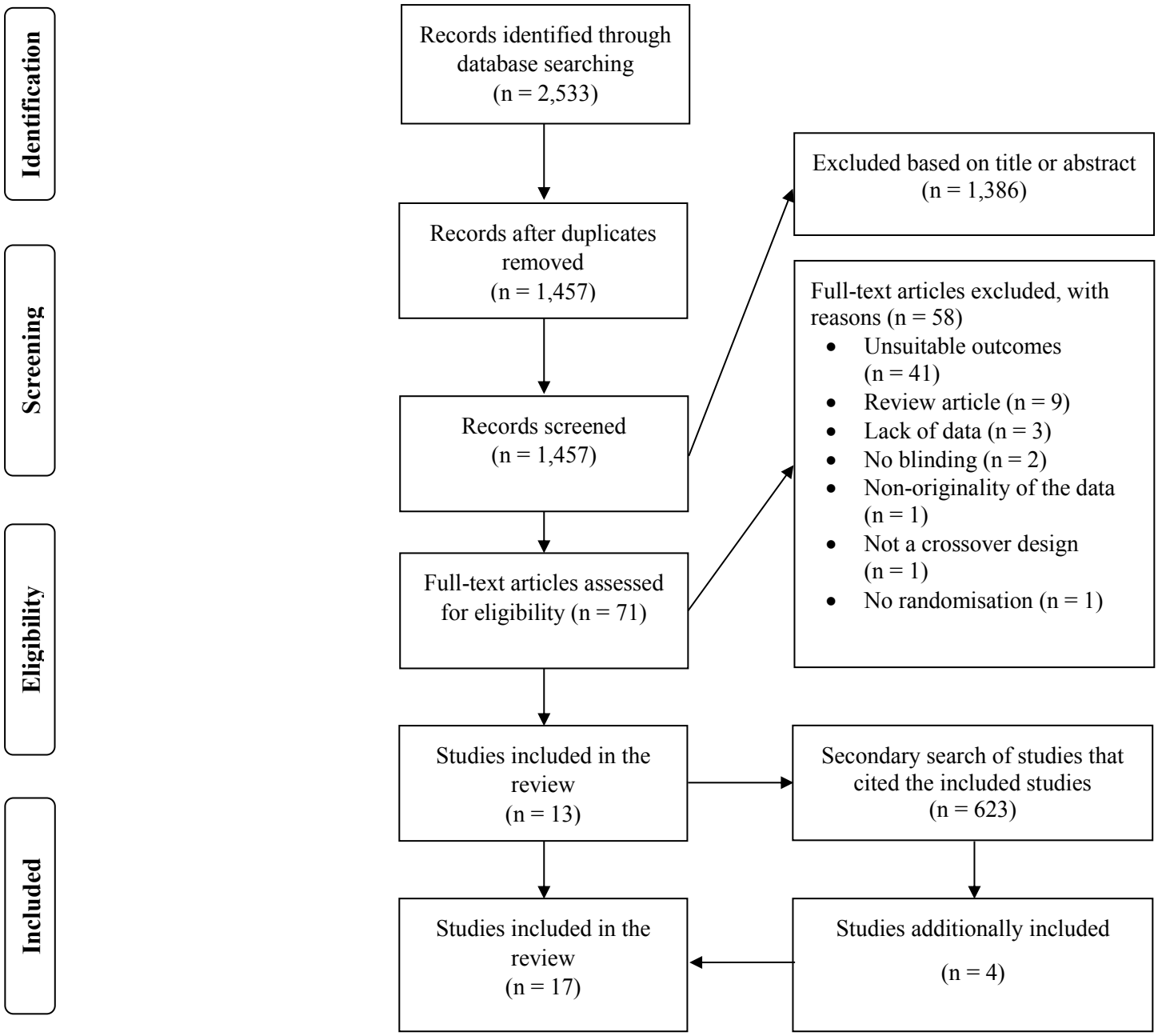
trained. Subgroup analyses for the effects of caffeine on muscle power were performed for the following characteristics: (a) the capsule form of caffeine; (b) the liquid form of caffeine; (c) females; (d) males; (e) athletes; (f) non- athletes; (g) countermovement and squat jump tests; and (h) Sargent jump tests.

The I^2 statistic was used to assess the degree of heterogeneity, with values from ≤50% indicating low heterogeneity, 50-75% moderate heterogeneity and >75% high level of heterogeneity. Funnel plots were constructed for both muscle strength and muscle power outcomes, plotting standard error against Hedge's g. Funnel plot asymmetry arising from potential publication bias was assessed using the Trim-and-Fill method (Duval & Tweedie, 2000).

6.4. Results

The literature search yielded a total of 2533 documents. After a preliminary screening of titles and abstracts, 71 full-text studies were scrutinised. In total, ten studies were found meeting the inclusion criteria for strength outcomes (Arazi et al., 2016a; Arazi et al., 2016b; Astorino et al., 2008; Brooks et al., 2016; Diaz-Lara et al., 2016; Goldstein et al., 2010b; Grgic & Mikulic, 2017; Martin, 2016; Sabblah et al., 2015; Williams et al., 2008; Table 9) with a total of 149 participants (males n = 116, females n = 33). Ten studies were found assessing muscle power outcomes (Ali et al., 2016; Andrade-Souza, Bertuzzi, de Araujo, Bishop, & Lima-Silva, 2015; Arazi et al., 2016b; Bloms et al., 2016; Clarke, Highton, Close, & Twist, 2019; Diaz-Lara et al., 2016; Foskett et al., 2009; Gant, Ali, & Foskett, 2010; Gauvin, 2016; Grgic & Mikulic, 2017) with a total of 145 participants (males n = 116, females n = 29). According to their age, all participants were classified as adolescents or young adults. Three studies (Arazi et al., 2016b; Diaz-Lara et al., 2016; Grgic & Mikulic, 2017) assessed both muscle strength and muscle power. The results of the search and study selection process are depicted in Figure 5.

Figure 5. Flow diagram of the search and study selection process



Fifteen studies were published in peer-reviewed journals, while two studies were master's theses (Gauvin, 2016; Martin, 2015). The median number of participants per study was 14. Most of the studies used a double-blind design (i.e., 15 studies), with two studies (Bloms et al., 2016; Sabblah et al., 2015) using a single-blind design. Caffeine dosage varied from 0.9 mg/kg to 7 mg/kg. Only one study administered caffeine in the form of gel (Martin, 2015), while the rest used capsule or liquid forms. Only nine studies reported habitual caffeine intake, with Astorino et al. (2008) and Goldstein et al. (2010b) reporting a large range of habitual caffeine intakes among the participants (0-600 mg/day). Only three studies (Andrade-Souza et al., 2015; Astorino et al., 2008; Foskett et al., 2009) reported assessing the effectiveness of the blinding, with 50%, 60%, and 33% of the participants correctly differentiating between the placebo and the caffeine trials, respectively. Individual characteristics of the included studies are reported in Table 9.

Results of the meta-analysis indicated a significant difference ($p = 0.023$) between the placebo and caffeine trials on measures of maximal strength (Figure 6). The pooled SMD for the effects of caffeine ingestion on muscle strength was 0.20 (95% CI: 0.03, 0.36). Results from all of the subgroup analysis may be found in Table 10 (Figures 7 and 8).

Figure 6. Forest plot showing differences between the effects of placebo and caffeine trials on measures of maximal muscular strength. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the x-axis denote the standardised mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

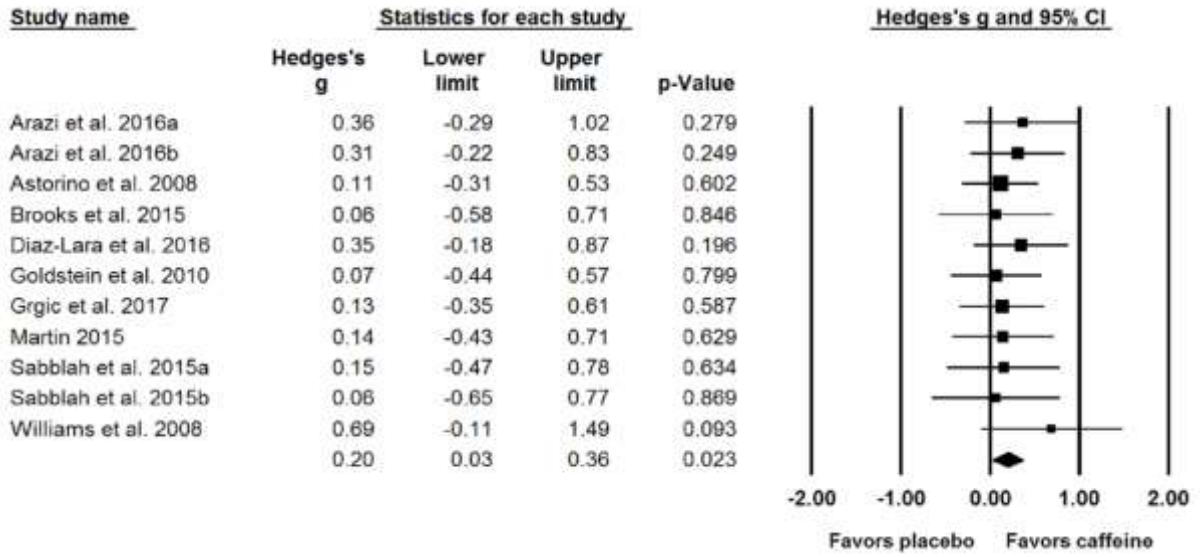


Figure 7. Forest plot showing differences between the effects of placebo and caffeine trials on measures of upper-body maximal muscle strength. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the x-axis denote the standardised mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

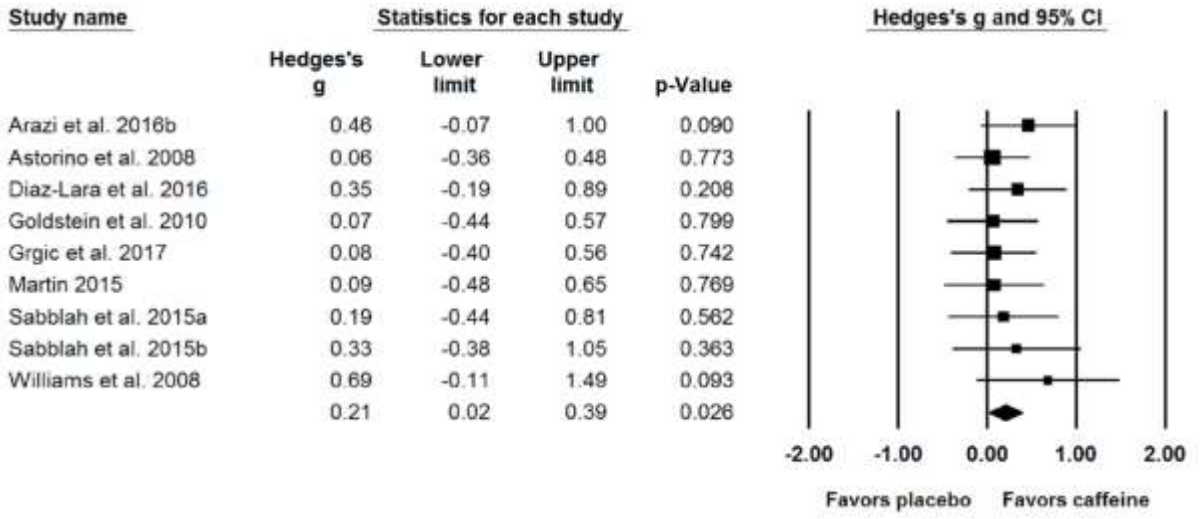


Figure 8. Forest plot showing differences between the effects of placebo and caffeine trials on measures of lower-body maximal muscle strength. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the x-axis denote the standardised mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

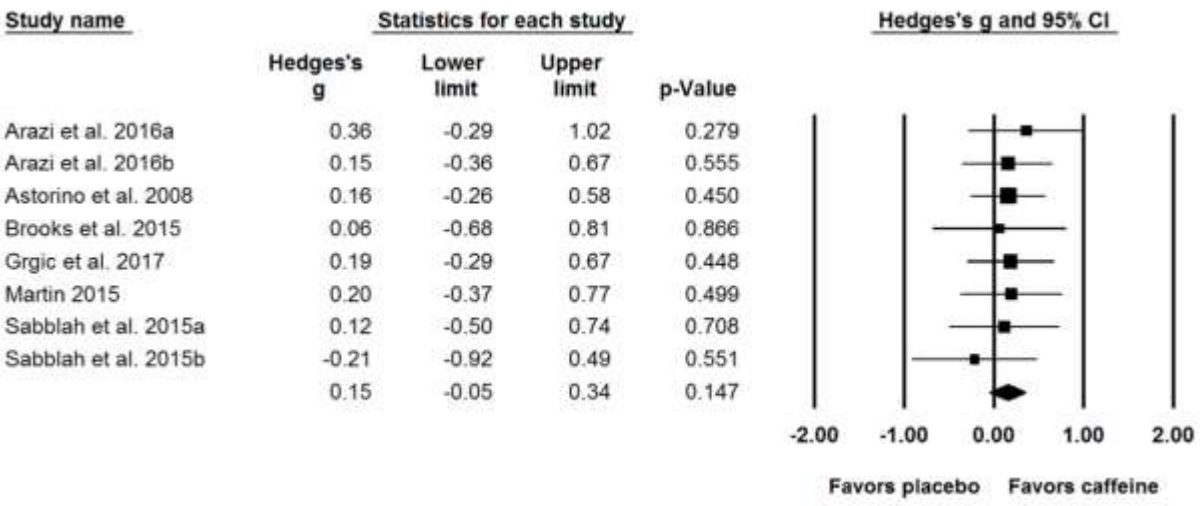


Table 9. Studies included in the analysis: summary of study designs

Study	Study design	Participants age (years)	Sample size and sex	Resistance/sport training experience	Habitual caffeine intake (mg/day)*	Caffeine form	Caffeine dosage (mg/kg)	Timing of caffeine ingestion before the experimental session(s) [minutes])	Exercise(s) used for the muscle strength/power assessment	PEDro score
Ali et al. (2016)	RDB	24 ± 4	10 females	Athletes	0-300	Capsule	6	60	CMJ	10
Andrade-Souza et al. (2015)	RDB	25 ± 3	11 males	Athletes	N/A	Capsule	6	60	CMJ	8
Arazi et al. (2016b)	RDB	17 ± 1	10 females	Untrained/Athletes	< 60	Capsule	2 and 5	60	LP and ST	10
Arazi et al. (2016a)	RDB	21 ± 4	15 males	Untrained	N/A	Capsule	6	60	BP and LP	10
Astorino et al. (2008)	RDB	23 ± 4	22 males	Trained	110 ± 152	Capsule	6	60	BP and LP	10
Bloms et al. (2016)	RSB	20 ± 1 21 ± 2	9 females 16 males	Athletes	N/A	Capsule	5	60	CMJ and SJ	8

Brooks et al. (2015)	RDB	21 ± 3	14 males	Untrained	N/A	Capsule	5	60	MBS	10
Clarke et al. (2016)	RDB	21 ± 2	8 males	Athletes	N/A	Capsule	3	60 and during the testing sessions	CMJ	10
Diaz-Lara et al. (2016)	RDB	29 ± 3	14 males	Trained/Athletes	< 60	Capsule	3	60	BP and CMJ	10
Foskett et al. (2009)	RDB	24 ± 5	12 males	Athletes	0-350	Liquid	6	60	CMJ	10
Gant et al. (2010)	RDB	21 ± 3	15 males	Athletes	N/A	Liquid	260 (fixed) 3.7 on average	60 and during the testing sessions	CMJ	10
Gauvin (2016)	RDB	22 ± 2	23 males	Untrained/Non-athletes	<200 per week	Capsule	7	60	CMJ	9
Goldstein et al. (2010b)	RDB	25 ± 7	15 females	Trained	< 250 (*n* = 8) > 250 (*n* = 7)	Liquid	6	60	BP	10

Grgic and Mikulic (2017)	RDB	26 ± 6	17 males	Trained/Non-athletes	58 ± 92	Liquid	6	60	BP, BBS and ST	9
Martin (2015)	RDB	20 ± 1	12 males	Trained	N/A	Gel	75 (fixed) - 0.9 on average	60	BP and BBS	10
Sabblah et al. (2015)	RSB	24 ± 3	7 females	Trained	N/A	Liquid	5	60	BP and MBS	8
		28 ± 6	10 males							
Williams et al. (2008)	RDB	26 ± 4	9 males	Trained	'Low' (no exact values)	Capsule	300 (fixed) - 3.6 on average	45	BP and LPD	10
*** intake per day unless stated otherwise; RDB: randomised double-blind study; RSB: randomised single-blind study; CMJ: countermovement jump; SJ: squat jump; LP: leg press; ST: Sargent test; BP: bench press; MBS: machine-based squat; LPD: lat pulldown; BBS: barbell back squat**										

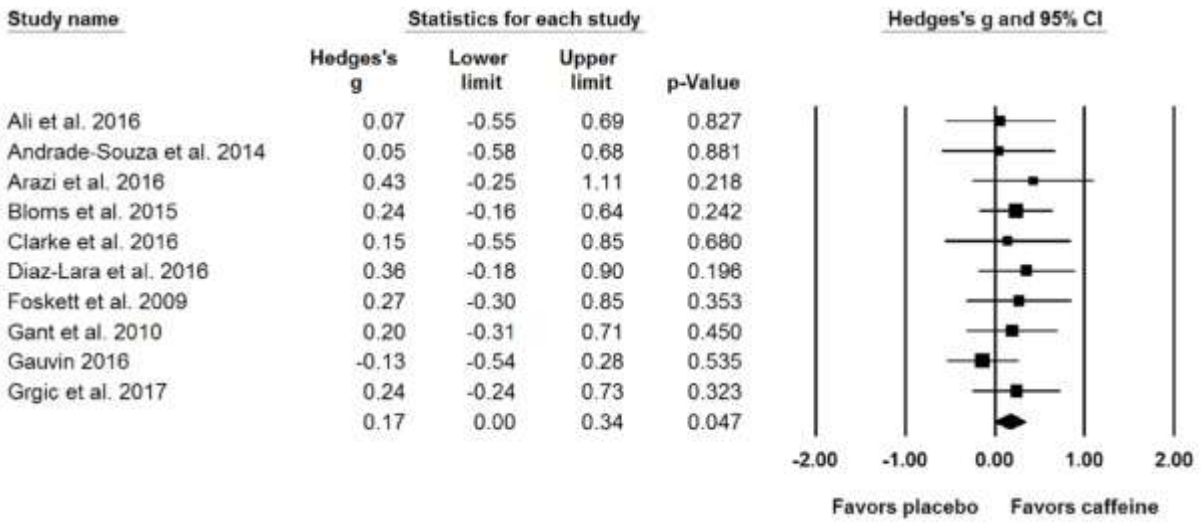
Table 10. Results from the subgroup meta-analyses

Subgroup analysis	**SMD [95% CI]**	**p-value**	**Mean caffeine dose (mg/kg [range])**
Strength outcomes			
Upper body strength	0.21 [0.02, 0.39]	0.026	4.7 [0.9-6]
Lower body strength	0.15 [-0.05, 0.34]	0.147	4.8 [0.9-6]
Capsule form of caffeine	0.27 [0.04, 0.50]	0.023	4.7 [2-6]
Liquid form of caffeine	0.11 [-0.17, 0.39]	0.462	6 [6]
Males	0.21 [0.02, 0.41]	0.034	4.7 [0.9-6]
Females	0.15 [-0.13, 0.43]	0.294	5 [2-6]
Trained participants	0.18 [-0.02, 0.37]	0.076	4.8 [0.9-6]
Untrained participants	0.27 [-0.09, 0.63]	0.144	4.8 [2-5]
Power outcomes			
Capsule form of caffeine	0.14 [-0.06, 0.34]	0.174	4.6 [2-7]
Liquid form of caffeine	0.24 [-0.06, 0.54]	0.124	5.2 [3.7-6]

Males	0.16 [-0.02, 0,34]	0.081	5.3 [3-7]
Females	0.23 [-0.23, 0.69]	0.323	4.8 [2-6]
Athletes	0.23 [0.03, 0.42]	0.025	4.4 [2-6]
Non athletes	0.03 [-0.33, 0.40]	0.854	6.5 [6-7]
Countermovement jump	0.14 [-0.04, 0.32]	0.138	5.0 [3.7-7]
Sargent test	0.31 [-0.09, 0.70]	0.129	4.3 [2-6]
SMD: standardised mean difference; CI: confidence interval			

The meta-analysis performed for muscle power indicated a significant difference (SMD = 0.17; 95% CI: 0.00, 0.34; $p = 0.047$) between the placebo and caffeine trials (Figure 9). Results from all of the subgroup analysis can be found in Table 10.

Figure 9. Forest plot showing differences between the effects of placebo and caffeine trials on measures of muscle power expressed as vertical jump height. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the x-axis denote the standardised mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)



The I^2 statistic showed low heterogeneity for the studies assessing muscle strength and muscle power ($I^2 = 0.0$; $p = 0.981$, and $I^2 = 0.0$; $p = 0.933$, respectively). The analysis of funnel plots did not reveal substantial asymmetry for muscle strength or muscle power outcomes. The Trim-and-Fill method changed the pooled SMD for muscle power from 0.17 (95% CI: 0.00, 0.34) to 0.12 (95% CI: -0.01, 0.26). The Trim-and-Fill method did not have an impact on the pooled ES for muscle strength outcomes.

The mean PEDro methodological quality score was 9.6, with the values for individual studies ranging from 8-10. Three studies (Andrade-Souza et al., 2015; Bloms et al., 2016; Sabblah et

al., 2015) were categorised as being of "good methodological quality" (PEDro score = 8), while all other studies were classified as being of "excellent quality".

6.5. Discussion

The results of the meta-analysis show that caffeine may be an effective ergogenic aid for muscle strength and power. The pooled effects of caffeine on performance were small to medium. It is important to note that even small improvements in performance in some sports may translate to meaningful differences in competitive outcomes (Le Meur, Hausswirth, & Mujika, 2012; Pyne, Mujika, & Reilly, 2009). A previous meta-analysis did not show a significant effect of caffeine supplementation on muscle strength (Polito et al., 2016), and the results of individual studies investigating caffeine's effects on muscle power have not been previously pooled in a meta-analysis. Our novel results showing that caffeine may induce practically meaningful improvements in muscle strength and power can, therefore, be used to inform athletes, coaches, and sports nutritionists, as well as future research endeavors in this area, about the ergogenic potential of caffeine.

6.5.1. Strength outcomes

6.5.1.1. Upper and lower body strength

The subgroup analysis indicated a significant increase in upper body, but not lower body strength, with caffeine ingestion. These results are somewhat unexpected, as Warren et al. (2010) suggested that larger muscles, such as those of the lower body, have a greater motor unit recruitment capability with caffeine intake than smaller muscles, such as those of the arm. Motor unit recruitment, in addition to the reduced rate of perceived exertion and the central effects of adenosine on neurotransmission, arousal, and pain perception, are considered to be underlying mechanisms by which caffeine can enhance performance, although the exact mechanisms remain to be fully elucidated (Davis & Green, 2009; Graham, 2001). Based on the current results, it may be surmised that caffeine is a useful ergogenic aid for achieving acute increases in maximal upper body strength. In the included studies, lower body maximal strength was evaluated using only leg press and squat (machine-based and free weight) tests. Two studies (Grgic & Mikulic, 2017; Martin, 2015) used a free weight exercise (barbell back squat), and both reported a significant increase in lower body strength. Warren et al. (2010) concluded

that caffeine ingestion might increase lower body isometric strength. Our findings do not indicate a strength increasing effect with caffeine ingestion for lower body dynamic strength. It is worth noting that in general, the included studies did not report on the reliability of their strength assessment, indicating potential reasons for the surprising findings for lower body strength. Further research is needed to examine the effects of caffeine on dynamic strength. Such studies may benefit from using a larger variety of dynamic lower body strength tests, as the current findings are mostly limited to a small selection of primarily machine-based tests.

6.5.1.2. Training status

The subgroup analysis for training status indicated no significant differences in maximal strength in trained ($p = 0.076$) and untrained individuals ($p = 0.144$). The meta-analysis of the three studies among untrained individuals was limited by small overall sample size ($n = 32$). It may be considered indicative that two of three individual studies reported significant differences in maximal strength with caffeine ingestion, but more individual studies on this topic are needed before drawing firm conclusions. Training status seems to play a significant role in response to caffeine intake in other forms of physical activity, such as swimming, with greater improvements observed in trained athletes (Collomp, Ahmaidi, Chatard, Audran, & Prefaut, 1992). However, it remains unclear whether the same applies to strength outcomes. More studies are needed before confidently drawing conclusions about the potential differences in effects of caffeine ingestion on muscle strength of trained and untrained individuals.

6.5.1.3. Sex

The subgroup analysis in males showed a significant improvement in strength with caffeine ingestion. The subgroup analysis for females was limited by small sample size, as only three studies (Arazi et al., 2016b; Goldstein et al., 2010b; Sabblah et al., 2015) were found meeting the inclusion criteria. The landmark study by Goldstein et al. (2010b) reported a significant increase in the 1RM bench press in a cohort of resistance trained females. However, the ES was very small (SMD = 0.07), thereby limiting the practical significance of the finding. Another study among female participants was performed by Sabblah et al. (2015). The researchers reported an SMD of 0.33 for increases in upper body strength with caffeine ingestion. However, the study employed a single-blind design and hence provided evidence of somewhat lower methodological quality compared to other studies. Additionally, the participants in the study

from Sabblah et al. (2015) exhibited lower levels of fitness than the participants in the study from Goldstein et al. (2010b), with marked disparities observed for 1RM strength (32 kg and 52 kg, respectively). None of the studies that included female participants controlled for the potential variability attributable to metabolic alterations across the menstrual cycle (Lane et al., 1992), which is a limitation of the current body of literature. Additional rigorously controlled studies are needed to provide clarity on the topic.

6.5.1.4. Caffeine form

The subgroup analysis indicated significant increases in strength after the ingestion of caffeine in the capsule form. The meta-analysis of the effects of the liquid form of caffeine included only three studies and did not report a significant effect. It is likely that the analysis was limited due to the small sample size (n = 50). Only one study (Martin, 2015) used caffeine in the form of a gel. Previous studies indicate that there are no practically meaningful pharmacokinetic differences between these routes of caffeine ingestion (Liguori, Hughes, & Grass, 1997); as such, it is unlikely that marked differences exist when comparing ergogenic effects of various forms of caffeine administration. Further investigations are needed for liquid forms of caffeine and others that have rarely or never been studied in this context, such as gum and gel.

6.5.2. Power outcomes

The meta-analysis supports caffeine as an effective ergogenic aid for achieving acute increases in muscle power expressed as vertical jump height. These results may have considerable applicability to many sports, including basketball and volleyball, in which muscle power and jumping ability are highly related to performance outcomes. The magnitude of acute improvement in vertical jump height found in the current analysis for a single caffeine ingestion is roughly equivalent to the effects of ~4 weeks of plyometric training (Markovic, 2007). The current analysis included only studies that used vertical jump as the power outcome; as such, it is possible that caffeine ingestion could produce somewhat different effects on other types of muscle power tests. However, a recent meta-analysis also showed a significant performance-enhancing effect of caffeine on the Wingate test, which is a common test of power (Grgic, 2018). Furthermore, most of the included studies used countermovement jump for assessing vertical jump; it remains to be explored whether the caffeine ingestion would produce different effects on other forms of vertical jumping. In addition, all of the included studies evaluated

these effects in isolated conditions that may not accurately reflect in-game, sport-specific jumping tasks. More evidence may be needed to determine if the performance-enhancing effects of caffeine would transfer in the context of individual sports and/or team-sport matches (Bishop, 2010).

While previous research (Abian-Vicen et al., 2014) has shown an increase in countermovement jump height after ingestion of a caffeine-containing energy drink, it was unclear if the effect was attributable to the caffeine content or the presence of other substances, such as taurine. A recent meta-analysis on caffeinated energy drinks found a significant association between their taurine content and performance, but not between their caffeine content and performance (Souza, Del Coso, Casonatto, & Polito, 2017). As postulated by Bloms et al. (2016), motor schema might play a role when assessing the association between caffeine and muscle power. Bloms et al. (2016) tested the effect of caffeine on muscle power among a cohort of athletes and reported significant increases in jumping height. By contrast, Gauvin (2016) reported no effects of caffeine ingestion on muscle power in a group of untrained men, with no previous experience in the exercise. The subgroup analysis for training status indicated a significant effect for athletes, but not for non-athletes. It may be suggested that future studies should control for this confounding factor by including only participants with or without previous experience in the task, or by performing initial familiarisation sessions.

None of the remaining subgroup analysis showed a significant effect of caffeine. These results might be due to the small sample sizes in different subgroup analysis. More studies are needed before reaching conclusions about context-specific effects of caffeine. Furthermore, while the body of evidence evaluating effects of caffeine on muscle power is still limited; the current meta-analysis shows promising findings, but more studies are needed on this topic. Specifically, studies including different forms of vertical jumping and sport-specific jumping tasks, different population groups, larger sample sizes, and different doses and forms of caffeine are required.

6.5.3. Methodological quality

The PEDro scale showed good to excellent quality among the included studies, suggesting that the results of the current meta-analysis were not confounded by the inclusion of studies with

poor research methodology. Only two studies (Gant et al., 2010; Williams et al., 2008) reported receiving funding from parties that may have had commercial interest for conducting the research, so it is improbable that the overall results of the current study were significantly affected by financial bias. To further improve the quality of evidence, future studies should use a double-blind rather than a single-blind design and assess the effectiveness of the blinding. Only three studies (Andrade-Souza et al., 2015; Astorino et al., 2008; Foskett et al., 2009) reported assessing the effectiveness of the blinding. This information is of importance as participants' recognition of the caffeine trial may influence outcomes (Saunders et al., 2017), because psychological effects of 'expectancy' and 'belief' might have an impact on performance (Tallis et al., 2016) In some studies, performance-enhancing responses were found with perceived 'caffeine' ingestion, when in fact, a placebo was consumed (Pollo et al., 2008). Future studies examining this topic should include a questionnaire of perception of the trials to prevent possible issues associated with such confounding.

While the inclusion of doctoral and master's theses may be considered as a limitation of this review, their inclusion is supported by their high methodological quality scores. Therefore, the inclusion of such studies may be regarded as a strength rather than a limitation, as it would be inappropriate to omit high-quality contributions to the literature from a comprehensive systematic review. A limitation of the current review is the low number of studies included in the subgroup analysis. Secondly, a limitation is that no studies were found for age groups other than adolescents and young adults. The findings, therefore, pertain mainly to young individuals and cannot be generalised to other age groups. Furthermore, due to the high degree of inter-individual variability of effects (Pickering & Kiely, 2018), these results should be interpreted with caution when it comes to prescribing caffeine supplementation to individuals. Individuals should also assess their susceptibility to possible side effects as reported in the literature, such as tremor, insomnia, elevated heart rate, headache, abdominal/gut discomfort, muscle soreness, and inability to verbally communicate and stay focused. These side effects may be enhanced in naive caffeine users (Astorino et al., 2008; Goldstein et al., 2010b), so extra precaution may be warranted in such individuals.

6.6. Conclusion

Caffeine appears to provide significant ergogenic effects on muscle strength and power. The expression of strength in the form of 1RM is most specific to the sport of powerlifting but may translate to performance improvements in a variety of other strength-power sports. The effects of caffeine on muscle power may apply to athletes in a variety of sports in which jumping is a predominant activity that affects the sport-specific performance. Subgroup-analyses suggested that the effects of caffeine on strength may be more pronounced in upper body muscles, but further research on this topic is warranted. The results of the present meta-analysis are based on limited evidence, and thus need to be interpreted with caution. Future studies should explore the optimal dosage and form of caffeine for maximizing effects on strength and power. Finally, responses to caffeine ingestion have a high degree of inter-individual variability, and as such, the applicability of the current findings must be assessed on a case-by-case basis, based on the specific characteristics of the individual and the sports activity or other physical tasks.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files

Competing interests

The authors declare that they have no competing interest

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7. Caffeine ingestion enhances Wingate performance: A meta-analysis

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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

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2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

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3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

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2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

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3. There are no other authors of the publication according to these criteria;
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Caffeine ingestion enhances Wingate performance: A meta-analysis

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7.1. Abstract

The positive effects of caffeine ingestion on aerobic performance are well-established; however, recent findings are suggesting that caffeine ingestion might also enhance anaerobic performance. A commonly used test of anaerobic performance and power output is the 30-second Wingate test. Several studies explored the effects of caffeine ingestion on Wingate performance, with equivocal findings. To elucidate this topic, this paper aims to determine the effects of caffeine ingestion on Wingate performance using meta-analytic statistical techniques. Following a search through PubMed/MEDLINE, Scopus, and SportDiscus®, 16 studies were found meeting the inclusion criteria (pooled number of participants = 246). Random-effects meta-analysis of standardised mean differences (SMD) for peak power output and mean power output was performed. Study quality was assessed using the modified version of the PEDro checklist. Results of the meta-analysis indicated a significant difference (p = 0.005) between the placebo and caffeine trials on mean power output with SMD values of small magnitude (0.18; 95% confidence interval: 0.05, 0.31; +3%). The meta-analysis performed for peak power output indicated a significant difference (p = 0.006) between the placebo and caffeine trials (SMD = 0.27; 95% confidence interval: 0.08, 0.47 [moderate magnitude]; +4%). The results from the PEDro checklist indicated that, in general, studies are of good and excellent methodological quality. This meta-analysis adds on to the current body of evidence showing that caffeine ingestion can also enhance components of anaerobic performance. The results presented herein may be helpful for developing more efficient evidence-based recommendations regarding caffeine supplementation.

7.2. Introduction

Caffeine is a 1,3,7 trimethylxanthine and is commonly found in foods and beverages. In a detailed review of literature, Glade (2010) concluded that consumption of caffeine (1) increases energy availability, (2) enhances cognitive performance, (3) decreases mental fatigue, (4) increases concentration and focus attention, (5) improves memory, and (6) increases problem-solving that requires reasoning, among others. Besides its impact on the aspects mentioned above, caffeine has received attention from researchers due to its ergogenic effects on sport and exercise performance.

The effects of caffeine ingestion on improving aerobic performance are well-established (Berglund & Hemmingsson, 1982; Bruce et al., 2000); however, there is considerable evidence suggesting that caffeine intake might also enhance anaerobic components of performance (Astorino & Roberson, 2010; Davis & Green, 2009; Grgic & Mikulic, 2017). One common test of anaerobic capacity and power output is the Wingate test. Briefly, the Wingate test consists of a short warm-up and of pedalling or arm cranking at a maximal speed for 30 seconds. This test is widely accepted and commonly used as it is inexpensive, non-invasive, and feasible for administration across populations (Bar-Or, 1987). Several studies explored the effects of caffeine intake on Wingate performance, with equivocal findings. For instance, Greer, McLean, and Graham (1998) reported an ergolytic effect of caffeine ingestion compared to placebo on power output, specifically, on the fourth Wingate bout. No significant effect was noted with caffeine ingestion in the follow-up work by the same author (Greer, Morales, & Coles, 2006). Interestingly, while not reaching significance, it is important to highlight that 12 out of the 18 participants in that study did experience an increase in peak power output when caffeine was ingested compared with placebo. In contrast to Greer et al. (1998), Salinero et al. (2017) reported that caffeine ingestion increased both peak power and mean power output during the Wingate test in a group of young men and women.

Most of the studies that explored this topic have small sample sizes, which can be underpowered to detect statistical significance (at an a priori alpha level of 0.05), when in fact, an actual effect might exist (type II error). A way to surmount these issues is to perform a meta-analysis. Such statistical techniques allow integration of findings from studies that are addressing the same issue while providing greater statistical power than individual studies. However, such an

analysis has yet to be done. Therefore, this paper aims to conduct a meta-analysis of studies that are investigating the effects of caffeine ingestion on Wingate performance.

7.3. Methodology

7.3.1. Inclusion criteria

To be included in the review, studies were required to meet the following criteria: (i) the original research was published in an English-language refereed journal; (ii) the study assessed the effects of caffeine ingestion in the form of capsule, liquid, gum or gel on performance in the 30-second Wingate test; (iii) the study employed a crossover design, and (iv) included apparently healthy human participants.

Coffee ingestion was not considered because coffee has other compounds that might moderate the impact of caffeine (Trexler et al., 2016). Furthermore, studies were not included if caffeine was co-ingested with other substances or potentially ergogenic compounds, such as taurine.

7.3.2. Search strategy

Searches were performed through PubMed/MEDLINE, Scopus, and SportDiscus®. The following word syntax was used for the search through titles, abstracts, and keywords: caffeine AND (Wingate OR anaerobic OR "peak power" OR "mean power"). No year restriction was applied to the search strategy. Secondary searches were performed by screening the reference lists of all selected studies and relevant review papers. The search concluded on August 8th, 2017.

7.3.3. Study coding and data extraction

The following information from the studies found meeting the inclusion criteria was extracted on an Excel spreadsheet: (i) sample characteristics including sample size, participant's sex and age; (ii) caffeine form, dosage, and time of ingestion before the testing sessions; (iii) main findings related to the placebo and caffeine trials; (iv) and reported side effects.

7.3.4. Methodological quality

To assess the methodological quality of the studies the previously validated 11-item PEDro scale was used (Maher et al., 2003). Details from the checklist can be found elsewhere (Maher et al., 2003). Due to the specificity of the topic, the scale was modified, and the following question (item 12) was added: "Did the study assess the effectiveness of the blinding to caffeine conditions?" With the addition of this question, the maximal score on the scale is 11, as the first item is not included in the total score. Each question is answered with a yes if the criteria are satisfied or with a no if the criteria are not satisfied. Based on the score, the studies were classified as being of excellent (10–11 points), good (7–9 points), fair (5–6 points) or poor (<5 points) methodological quality (McCrary et al., 2015).

7.3.5. Statistical analyses

A random-effects meta-analysis of standardised mean differences (SMD) expressed as Hedge's g was performed using the Comprehensive Meta-analysis software (Biostat Inc., Englewood, NJ, USA). SMDs and 95% confidence intervals (CI) were calculated using the sample size (n), the correlation between the conditions, and mean ± standard deviation values of the placebo and caffeine trials. None of the included studies reported correlation values; therefore, a conservative 0.5 correlation was assumed for all studies (Follmann et al., 1992). If a study measured Wingate performance under multiple conditions, such as multiple caffeine doses, the average values were used for the analysis. As presented by Cohen (1988), the SMDs were classified as: [i] small (≤0.2); [ii] moderate (0.2-0.5); [iii] large (0.5-0.8); and [iv] very large (>0.8). Sensitivity analysis was performed by excluding two studies performed in children and examining the outcomes (Turley et al., 2012; Turley, Eusse, Thomas, Townsend, & Morton, 2015). Statistical significance was set at $p < 0.05$. In addition to SMDs, percent changes were calculated. Heterogeneity was assessed using the I^2 statistic. I^2 values that were ≤50% indicated low heterogeneity, I^2 values from 50-75% indicated moderate heterogeneity and I^2 values >75% indicated a high level of heterogeneity. Standard error was plotted against Hedge's g for the funnel plots. The Trim-and-Fill method was used for assessing the asymmetry of the funnel plots.

7.4. Results

7.4.1. Search results

The search syntax resulted with a total of 540 results (PubMed/MEDLINE = 159; Scopus = 259; SportDiscus® = 122). Of the total results, 34 full-text articles were read. Eighteen studies were excluded as they did not meet the inclusion criteria, which resulted in the inclusion of 16 studies (Bell, Jacobs, & Ellerington, 2001; Bellar, Judge, Kamimori, & Glickman, 2012; Cakir-Atabek, 2017; Collomp et al., 1991; Duncan, 2009; Greer et al., 1998; Greer et al., 2006; Lorino, Lloyd, Crixell, & Walker, 2006; Mahdavi, Daneghian, Jafari & Homayouni, 2015; Pereira et al., 2010; Salinero et al., 2017; Turley et al., 2012; Turley et al., 2015; Warnock, Jeffries, Patterson, & Waldron, 2017; Williams et al., 2008; Woolf et al., 2008). Publication dates of the included studies ranged from 1991 to 2017. The pooled number of participants across the studies was 246 (median = 15; range = 6-26). All of the participants were classified as being young or children. Thirteen of the studies employed a double-blind design (Bell et al., 2001; Bellar et al., 2012; Cakir-Atabek, 2017; Greer et al., 1998; Greer et al., 2006; Lorino et al., 2006; Mahdavi et al., 2015; Pereira et al., 2010; Salinero et al., 2017; Turley et al., 2012; Turley et al., 2015; Williams et al., 2008; Woolf et al., 2008), two a single-blind design (Collomp et al., 1991; Warnock et al., 2017), while in one study there was no blinding (Duncan, 2009). Caffeine doses ranged from 1 mg/kg to 5 mg/kg, with two studies using a fixed dose of caffeine. Only one study used caffeine in the form of gum (Bellar et al. 2012), while in the rest either liquid of capsule was used. Time of caffeine ingestion before testing sessions was most commonly 60 minutes. All of the studies used the lower body Wingate test. Summary of individual studies can be found in Table 11.

Table 11. Summary of the studies included in the meta-analysis

Study	Sample	Caffeine form	Caffeine dosage	Timing of caffeine intake
Bell et al. (2001)	Young men (*n* = 16)	Capsule	5 mg/kg	90 minutes
Bellar et al. (2012)	Young men (*n* = 10)	Gum	Fixed dose of 100 mg	Exercise immediately after caffeine intake
Cakir-Atabek (2017)	Young men (*n* = 14)	Liquid	5 mg/kg	60 minutes
Collomp et al. (1991)	Young men (*n* = 3) and women (*n* = 3)	Capsule	5 mg/kg	60 minutes
Duncan (2009)	Young men (*n* = 8) and women (*n* = 6)	Liquid	5 mg/kg	60 minutes
Greer et al. (1998)	Young men (*n* = 9)	Capsule	6 mg/kg [1]	60 minutes
Greer et al. (2006)	Young men (*n* = 18)	Capsule	5 mg/kg	60 minutes
Lorino et al. (2006)	Young men (*n* = 16)	Capsule	6 mg/kg	60 minutes
Mahdavi et al. (2015)	Young women (*n* = 24)	Capsule	5 mg/kg	70 minutes
Pereira et al. (2010)	Young men (*n* = 7) and women (*n* = 7)	Capsule	6 mg/kg	60 minutes
Salinero et al. (2017)	Young men (*n* = 14) and women (*n* = 7)	Capsule	3 mg/kg [1]	60 minutes

Turley et al. (2012)	Boys (*n* = 24)	Liquid	5 mg/kg	60 minutes
Turley et al. (2015)	Boys (*n* = 26)	Liquid	1, 3, and 5 mg/kg	60 minutes
Warnock et al. (2017)	Young men (*n* = 7)	Capsule	5 mg/kg	60 minutes
Williams et al. (2008)	Young men (*n* = 9)	Capsule	Fixed dose of 300 mg	45 minutes
Woolf et al. (2008)	Young men (*n* = 18)	Liquid	5 mg/kg	60 minutes

7.4.2. Meta-analysis results

Meta-analysis for mean power output indicated a significant difference ($p = 0.005$) between the placebo and caffeine trials, with SMD values of 0.18 (95% CI: 0.05, 0.31; +3; $I^2 = 0.0\%$ [Figure 10]). The meta-analysis performed for peak power output indicated a significant difference (SMD = 0.27; 95% CI: 0.08, 0.47; +4%; $p = 0.006$; $I^2 = 52.1\%$ [Figure 11]) between the placebo and caffeine trials. The sensitivity analysis did not change the outcomes by a meaningful degree. Funnel plots did not indicate any substantial asymmetry in both analyses. The Trim-and-Fill method did not have an impact in either analysis.

Figure 10. Forest plot of studies comparing the effects of placebo and caffeine trials on mean power output. The size of the plotted squares reflects the statistical weight of the study. Horizontal lines denote the 95% confidence intervals. SMD = standardised mean difference

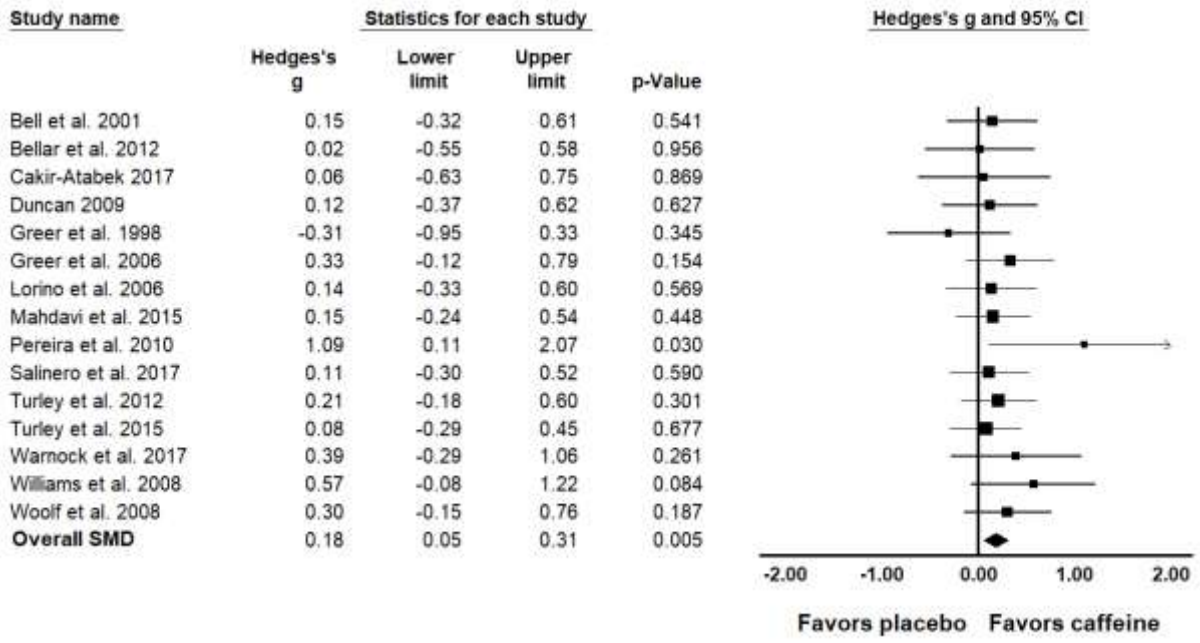
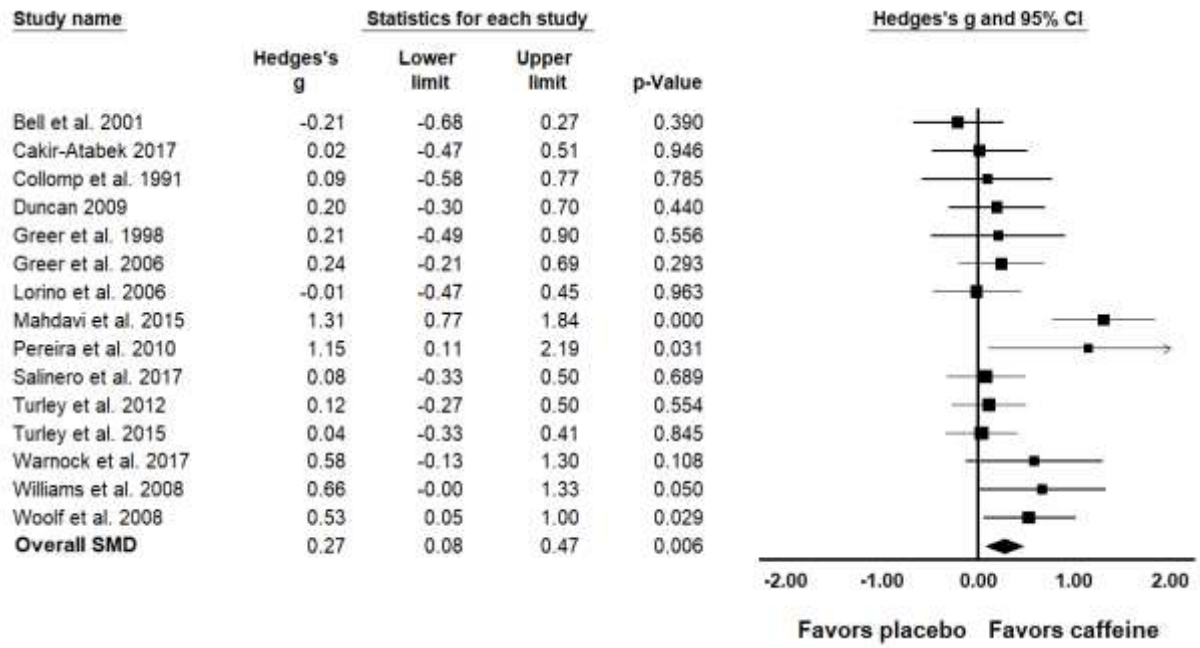


Figure 11. Forest plot of studies comparing the effects of placebo and caffeine trials on peak power output. The size of the plotted squares reflects the statistical weight of the study. Horizontal lines denote the 95% confidence intervals. SMD = standardised mean difference



7.4.3. Methodological quality

The average score on the PEDro scale was 9 ± 1. Nine of the studies were classified as being of excellent quality, six as being of good quality, and one as being of fair methodological quality. None of the studies satisfied the added item regarding the assessment of the effectiveness of the blinding. Only three studies specified who was eligible to participate in the study (checklist item 1). The scores from individual studies can be found in Table 12.

Table 12. Results from the PEDro checklist

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Total score
Bell et al. (2001)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Bellar et al. (2012)	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	9
Cakir-Atabek (2017)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Collomp et al. (1991)	No	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	7
Duncan (2009)	No	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	No	6
Greer et al. (1998)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Greer et al. (2006)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Lorino et al. (2006)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	9
Mahdavi et al. (2015)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	9
Pereira et al. (2010)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Salinero et al. (2017)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Turley et al. (2012)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Turley et al. (2015)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10

Warnock et al. (2017)	No	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	7
Williams et al. (2008)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	10
Woolf et al. (2008)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	9
Studies are classified as: excellent methodological quality (10-11 points); good methodological quality (7–9 points); fair methodological quality (5–6 points); poor methodological quality (<5 points)													

7.5. Discussion

The present study is the first to assess the effectiveness of caffeine ingestion on Wingate performance using meta-analytic statistical techniques. The results presented herein indicate that caffeine ingestion augments mean and peak power output on the Wingate test by +3% and +4%, respectively. This meta-analysis adds on to the current body of evidence supporting the notion that caffeine ingestion can also be ergogenic for anaerobic performance.

It is important to highlight that while caffeine ingestion can enhance performance on the Wingate test, the SMDs for mean and peak power output are classified as being of small and moderate magnitude, respectively. While athletes would likely benefit the most for such small improvements in performance, only four studies included that population (Duncan, 2009; Mahdavi et al., 2015; Warnock et al., 2017; Woolf et al., 2008). Therefore, the practical usability of these findings remains somewhat questionable.

In a review by Bar-Or (1987), the author concluded that the correlation between performance on the Wingate test and several other anaerobic tasks (e.g., short sprinting and swimming) is quite high ($r = 0.75$). However, it is relevant to emphasise that performance in the Wingate test does not necessarily reflect the performance in sports-specific activities. Therefore, the generalisability of these findings to other anaerobic tasks is limited. While a transfer of effects can be hypothesised, the current body of evidence prevents concrete conclusions regarding possible benefits of these findings to other sport and exercise activities.

Mechanisms by which caffeine ingestion might enhance anaerobic performance include an increase in calcium release from the sarcoplasmic reticulum, which may lead to an increase in tetanic tension, and the alterations that caffeine might have on the neuromuscular transmission (Davis & Green, 2009). However, discussion on the potential mechanisms is beyond the scope of this article (for a review the reader is directed to the work by Davis & Green, 2009).

Besides the study by Williams et al. (2008), which that reported a coefficient of variation (CV) of 1% to 5% on the Wingate test, none of the other included studies reported their CV for

repeated measures. It might be that some of the differences between the placebo and caffeine conditions are the effect of an error of the measurement and not truly related to the effects of the condition. Therefore, possible issues with measurement error between placebo and caffeine trials in the analysed studies should not be excluded. Most of the studies did include at least one practice trial to prevent any learning effects; however, two studies did not report any familiarisation sessions (Collomp et al., 1991; Greer et al., 2006), which presents a confounding factor to their results, and should be avoided in future research. Besides the differences in the protocols used, it is also important to note that some studies used a mechanically-braked ergometer (Bell et al., 2001), while others used an electrically-braked ergometer (Warnock et al., 2017), which might also be a reason for differences in estimates across studies (Astorino & Cottrell, 2012).

A confounding factor to the present findings is that none of the studies assessed the effectiveness of the blinding. Salinero et al. (2017) reported that they did ask the participants to indicate which trial they perceived to be the caffeine trial. However, the results of this assessment were not reported. Assessing the effectiveness of the blinding can be of significant impact due to the possible placebo effects of "caffeine" ingestion on performance (Beedie, Stuart, Coleman, & Foad, 2006). Therefore, future studies should assess the effectiveness of the blinding following the trials, to increase the robustness of their findings.

The current body of evidence suggests that caffeine ingestion might result in several side effects such as insomnia, headaches, nervousness, gastrointestinal problems, and muscle soreness, among others (Astorino et al., 2008; Goldstein et al., 2010b). Only three of the included studies assessed the side effects of caffeine ingestion in their experimental trials. Williams et al. (2008) reported that no side effects occurred. Lorino et al. (2006) reported that one of the participants vomited following caffeine ingestion, while Salinero et al. (2017) noted a slight increase in self-reported insomnia and nervousness following the caffeine trials. It seems that some of the side effects mentioned above may be augmented in individuals with low habitual caffeine intake so extra precaution might be necessary for these individuals (Astorino et al., 2008; Goldstein et al., 2010b). Future studies should consider tracking and reporting side effects to highlight the possible disadvantages of supplementing with caffeine.

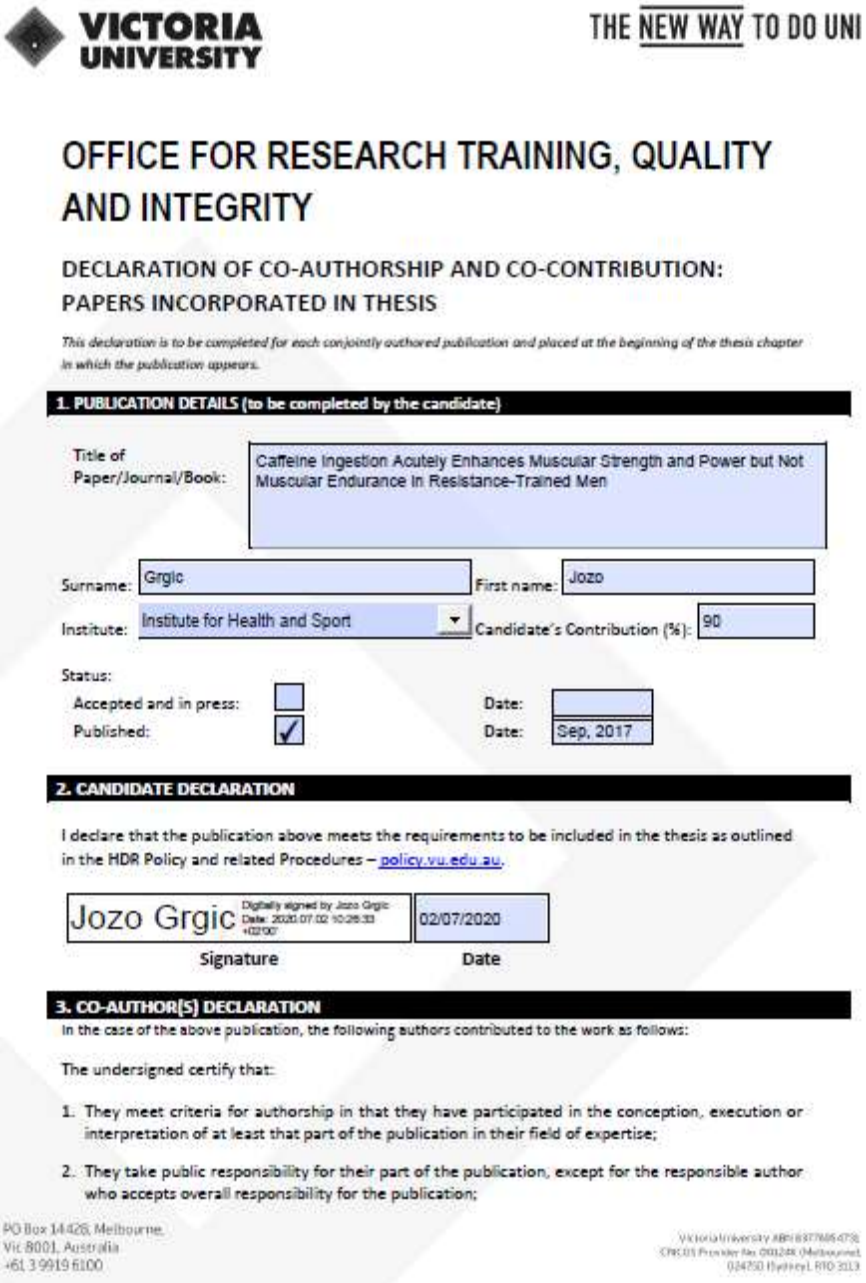
7.5.1. Future directions

None of the included studies used the upper-body Wingate test in their trials. Therefore, the results presented in this meta-analysis cannot be generalisable to upper body power, as it has been shown that the effects of caffeine ingestion might differ between upper and lower body (Grgic & Mikulic, 2017). This gap in the literature opens an avenue for future research to test the effects of caffeine ingestion on upper body Wingate performance. Furthermore, studies might consider exploring the effects of caffeine ingestion and Wingate performance in older adults, as to date, there are no such studies. More evidence is needed on females, as most of the included studies were performed in men. Some studies included a mixed-gender sample, but nonetheless, the number of female participants was small (pooled n = 23). Besides females, more studies are needed on athletes, in particular on those competing in anaerobic sports. It would be desirable for future studies to plot the individual values from the placebo and caffeine trials, to examine the variation in responses to caffeine ingestion.

7.6. Conclusions

In contrast to previous reviews which suggested that caffeine does not have an impact on Wingate performance, this meta-analysis provides findings that caffeine ingestion may increase both peak power output and mean power output during the Wingate test. Therefore, the results presented in this paper may be helpful for developing more efficient evidence-based recommendations regarding caffeine supplementation. While this would suggest that athletes who compete in anaerobic dominant sports might consider supplementing with caffeine, this remains tentative as it is unclear to which extent these effects could transfer in the sports context. Furthermore, the effects are not of a large magnitude which somewhat questions the practical usability of the findings. Because of the inter-individual response to caffeine ingestion, potential supplementation with caffeine needs to be adjusted on a case-by-case basis.

8. Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance trained men



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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book: Caffeine Ingestion Acutely Enhances Muscular Strength and Power but Not Muscular Endurance in Resistance-Trained Men

Surname: Grgic | First name: Jozo

Institute: Institute for Health and Sport | Candidate's Contribution (%): 90

Status:

Accepted and in press: ☐ Date:

Published: ☑ Date: Sep, 2017

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

Jozo Grgic Digitally signed by Jozo Grgic Date: 2020.07.02 10:26:33 +02'00' | 02/07/2020

Signature | Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

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3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following **location(s)**:

The original data has been electronically stored on VU R drive and the student's laptop for at least five years since September 2017.

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Pavle Mikulic	10	Conceptualization, data interpretation, and manuscript writing	[redacted]	02/07/2020

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Jozo Grgic & Pavle Mikulic (2017) Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance-trained men, European Journal of Sport Science, 17:8, 1029-1036, DOI: 10.1080/17461391.2017.1330362

Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance trained men

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Running head: Caffeine and athletic performance

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8.1. Abstract

The goal of this randomised, double-blind, crossover study was to assess the acute effects of caffeine ingestion on muscular strength and power, muscular endurance, rate of perceived exertion (RPE), and pain perception (PP) in resistance-trained men. Seventeen volunteers (mean ± SD: age = 26 ± 6 years, stature = 182 ± 9 cm, body mass = 84 ± 9 kg, resistance training experience = 7 ± 3 years) consumed placebo or 6 mg/kg of anhydrous caffeine one hour before testing. Muscular power was assessed with seated medicine ball throw and vertical jump exercises, muscular strength with one-repetition maximum (1RM) barbell back squat and bench press exercises, and muscular endurance with repetitions of back squat and bench press exercises (load corresponding to 60% of 1RM) to momentary muscular failure. RPE and PP were assessed immediately after the completion of the back squat and bench press exercises. Compared to placebo, caffeine intake enhanced 1RM back squat performance (+2.8%; ES = 0.19; $p = 0.016$), which was accompanied by a reduced RPE (+7%; ES = 0.53; $p = 0.037$), and seated medicine ball throw performance (+4.3%, ES = 0.32; $p = 0.009$). Improvements in 1RM bench press were not noted although there were significant ($p = 0.029$) decreases in PP related to this exercise when participants ingested caffeine. The results point to an acute benefit of caffeine intake in enhancing lower-body strength, likely due to a decrease in RPE; upper-, but not lower-body power; and no effects on muscular endurance, in resistance-trained men. Individuals competing in events in which strength and power are important performance-related factors may consider taking 6 mg/kg of caffeine pre-training/competition for performance enhancement.

8.2. Introduction

It is assumed that coffee is mainly consumed for the caffeine benefits, as these include increased wakefulness, focus, and alertness (Glade, 2010). Caffeine has received attention from researchers for its benefits related to the enhancement of athletic performance. The research examining the effects of caffeine on athletic performance initially mainly focused on endurance-type sports (i.e., cycling, rowing, distance running and cross-country skiing; Berglund & Hemmingsson, 1982; Bruce et al., 2000; Pasman et al., 1995; Wiles et al., 1992). In recent years, however, the general focus has shifted toward investigating the effects of caffeine intake on performance in resistance exercise protocols.

Caffeine is often consumed before resistance training sessions, most commonly in the form of a pre-workout supplement. Athletes report that the primary motives for the consumption of pre-workout drinks are to "increase athletic endurance" and "increase strength/power" (Sassone, 2016). However, discrepant evidence has been presented in the literature regarding the effects of caffeine on resistance-exercise performance or, more precisely, on muscular strength and power, and muscular endurance. For example, while some studies suggest that caffeine intake may acutely enhance muscular strength (Beck et al., 2006; Goldstein et al., 2010b), other studies indicate no improvement in strength-exercise performance (Astorino et al., 2008; Beck, Housh, Malek, Mielke, & Hendrix, 2008). A recent meta-analysis conducted by Warren et al. (2010) suggested that caffeine intake may improve maximal voluntary contraction in the knee extensors by approximately 7%. However, isometric exercise has low utility value to the everyday resistance training practice as most exercises intended to enhance muscular strength include traditional dynamic exercises involving coupled concentric and eccentric muscle actions.

A common caveat in studies investigating the effects of caffeine on resistance-exercise performance is that "further research is needed to draw stronger conclusions". We feel that, in particular, studies involving resistance-trained participants are lacking, as findings of studies involving untrained or recreationally trained individuals restrict the generalisability of conclusions to more advanced individuals and, as such, reduce the practical usability of recommendations for many trained individuals and athletes. Studies examining the effects of caffeine intake on muscular strength, power, and muscular endurance, are of significant value

to various competitive athletes since, as of 2004, caffeine is no longer listed on the World Anti-Doping Agency's (WADA) List of Prohibited Substances and Methods. With that in mind, the primary aim of the present study is to examine the effects of anhydrous caffeine ingestion (6 mg/kg) on muscular strength and power, muscular endurance, rating of perceived exertion (RPE), and pain perception (PP) in resistance-trained men.

We aimed to assess the impact of caffeine on strength using the barbell back squat exercise performance as a measure of lower-body strength. We selected the back squat exercise as it represents an integral part of most resistance training programs of athletes and trained individuals. Despite this and perhaps surprisingly enough, free-weight back squat has not been previously used in empirical studies aiming to assess the effects of caffeine on lower-body maximal strength performance.

We hypothesised that caffeine intake would enhance muscular strength and power as well as muscular endurance, and reduce RPE and PP. The findings of our study may benefit coaches and athletes regarding the optimisation of pre-training and pre-competition protocols aimed at athletic performance improvement.

8.3. Methods

8.3.1. Participants

Following the approval by the Committee for Scientific Research and Ethics of the Faculty of Kinesiology at the University of Zagreb, the research commenced. Twenty resistance-trained men satisfied the inclusion criteria and volunteered to participate in the study. The inclusion criteria were as follows: (a) free from neuromuscular and musculoskeletal disorders, aged 18-45 years; (b) the participants were able to perform back squat and bench press exercises with load corresponding to 125% and 100% of their current body mass, respectively; (c) the participants had a minimum of 12 months of experience in resistance training and were actively involved in resistance training at least 3 times per week over the last 6 months. The experimental procedures, including possible risks and discomforts, were verbally explained to the participants after which they signed informed consent. Of the 20 participants that started the study, 3 failed to complete all study protocols. Two participants reported discomforts during

the testing protocol (elbow and shoulder issues during the bench press exercise) and one participant dropped out due to private reasons, so the final number of participants included in the analysis was seventeen (mean ± SD: age = 26 ± 6 years, stature = 182 ± 9 cm, body mass = 84 ± 9 kg, resistance training experience = 7 ± 3 years). The participants also filled out the Physical Activity Readiness Questionnaire (PAR-Q) in order to confirm that there were no contraindicated health conditions. All participants answered "No" to all the questions on the PAR-Q.

8.3.2. Experimental protocol

This study used a randomised, double-blind, crossover design. A total of three sessions were completed. The first session was a familiarisation session during which the participants' performance of the back squat and the bench press exercises was checked by a certified personal trainer. To estimate their one-repetition maximum (1RM) for the back squat and bench press exercises during this first session, the participants performed a set of repetitions of both exercises to momentary muscular failure with a load at which they could perform a maximum of 12 successful repetitions. The estimation of 1RM was then calculated using the equation proposed by Brzycki (1993), where W stands for weight and R for repetitions: 1 RM = W x (36 / (37 – R)). The equation has been found to have a high correlation coefficient ($r > 0.95$) between the predicted and achieved 1RM both for the squat and the bench press exercises (LeSuer, McCormick, Mayhew, Wasserstein, & Arnold, 1997).

During the first session, the participants were also introduced to the Borg scale (Borg, 1970) for estimation of the RPE, and to the PP scale (described in Cook et al., 1998) which ranged from 0 to 10, with 0 marking "no pain at all" and 10 marking "extremely intense pain". They were also re-introduced to the scales before both subsequent assessment sessions. Before the second and the third sessions that contained identical assessment protocols, spaced 7 days apart, the participants ingested either caffeine or placebo in a randomised order.

The participants were instructed to follow their general nutrition and exercise practices. They were instructed to keep track of their calorie and caffeine intake using the "Myfitness pal" software (http://www.myfitnesspal.com). Calorie intake was tracked and replicated before the

third session. In addition, the participants had to refrain from caffeine intake after 6 pm the day prior to testing, as done in previous research (Duncan et al., 2013), to reduce withdrawal symptoms in caffeine users such as headaches and lethargy. In the 24 hours preceding the testing, as well as on the testing days, the participants refrained from vigorous exercise. Adherence to these regulations was checked with a brief questionnaire. Caffeine intake from 24-hour diet recall was calculated using a SELF Nutrition Data software (http://nutritiondata.self.com). Caffeine intake was equal to 58 ± 92 (range 0-320) mg/day.

8.3.3. Supplementation protocol

The amount of 6 mg/kg of caffeine was chosen because it has been shown to maximise plasma levels of caffeine (Graham and Spriet, 1995). The prescribed amount of anhydrous caffeine (Proteka, Split, Croatia) was diluted in 250 ml of water and 20 grams of granulated orange-tasting beverage (Cedevita, Zagreb, Croatia) containing 65 calories (0 grams of protein, 16 grams of carbohydrates, and 0 grams of fat). Placebo was administrated in the same fashion without the anhydrous caffeine. The beverage was served in opaque shaker bottles. The assignment to either condition was blinded both to the participants and the investigators.

8.3.4. Testing procedures

All assessments were performed at the same time of the day for each participant to avoid circadian variation. Sixty minutes after the consumption, when the plasma concentration of caffeine is considered to be at its highest (Graham, 2001), the testing procedure began. First, the participants warmed up for 5 minutes by cycling on a stationary bicycle. Then, they performed several repetitions of push-ups or "walkouts" to additionally activate the upper-body musculature. The sequence of measures is explained in the following sections. A 5-minutes rest interval was employed between performance tests.

Muscle power was assessed first. For the assessment of lower-body power, the vertical jump test was used (for a detailed description of the testing procedure, see Martinez et al., 2016). The assessment of upper-body power was conducted using the seated medicine ball throw test, as described by Clemons et al. (2010).

The barbell back squat was used for the assessment of lower-body strength. During the first visit, one-repetition maximum was estimated as described above. During the subsequent two visits, 50% of the estimated 1RM was used for the first set, during which a participant performed 12-15 repetitions. For the second set, 60% of the estimated 1RM was used for 5 repetitions, 75% of the estimated 1RM was used for the third set (3 repetitions), and 90% of the estimated 1RM for the fourth set (1 repetition). In the fifth set, a participant tried to perform a successful attempt with a load corresponding to the estimated 1RM. If unsuccessful, the load was decreased by 2.5 kg for further attempts until a successful attempt was recorded. If successful, the load was increased by 2.5 kg until the participant was no longer able to record a successful attempt. The participants rested for 3 minutes between sets. After the final 1RM attempt, the participants rested for 5 minutes, and then completed the repetitions to a momentary muscular failure of the back squat exercise with a load corresponding to 60% of 1RM. This exercise was used to assess lower-body muscular endurance. The barbell bench press was used for the assessment of the upper-body muscular strength and muscular endurance. The same procedures, as described for the barbell back squat exercise, were also used for the barbell bench press exercise. Within 5 seconds of the successful 1RM attempts for all back squat and bench press exercises, the participants were asked to indicate their levels of perceived exertion and pain on the relevant scales.

8.3.5. Statistical analyses

We tested the normality of data for all variables both numerically using a Shapiro-Wilk test of normality, and graphically by visually inspecting the normal Q-Q plots. A series of one-way repeated measures analysis of variance (ANOVA), provided in a computer software SPSS version 20 (Chicago, IL, USA), was used to compare the differences between conditions (caffeine, placebo) for all measures. Statistical significance was set at $p < 0.05$. Ninety-five percent confidence intervals (95% CI) were calculated using Microsoft Excel software (Microsoft Corporation, WA, USA). An ES (Cohen (1988)) was calculated for all differences. All results are presented as mean ± SD.

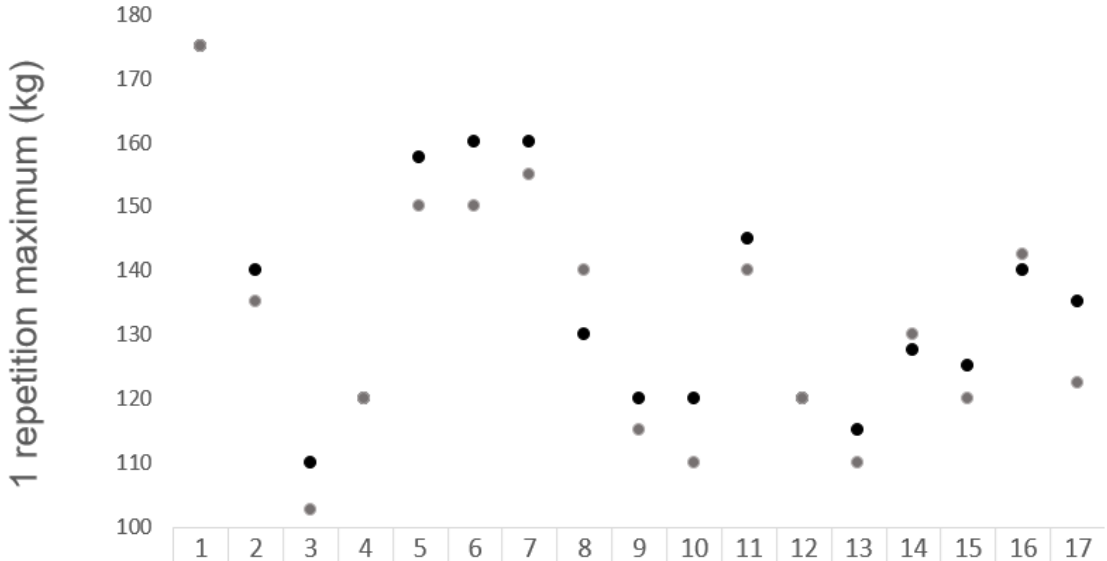
The following scale, proposed by Hopkins (2002), was observed to determine the magnitude of an effect: 0-0.2 was considered as trivial, 0.2-0.6 was considered as small, 0.6-1.2 was

considered as moderate, 1.2-2.0 was considered as large, and >2.0 was considered as very large magnitude of an effect. Relative differences (i.e., in percentages) between conditions were also calculated.

8.4. Results

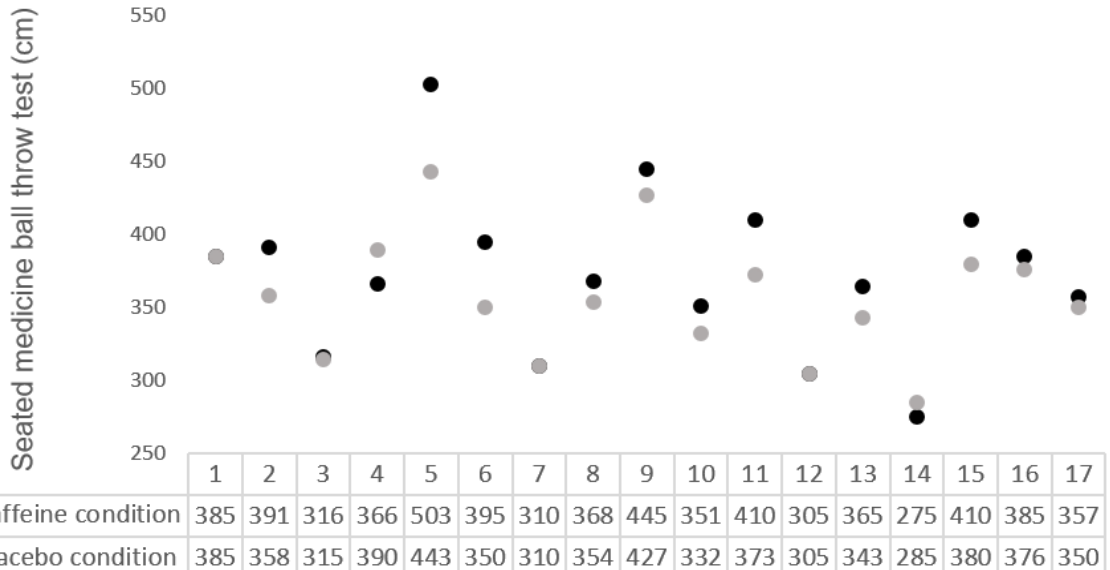
One-way ANOVA revealed a significant within-participants effect for the back squat exercise (p = 0.016; ES = 0.19), RPE for the back squat exercise (p = 0.037; ES = 0.53), the seated medicine ball throw (p = 0.009; ES = 0.32), and pain perception for the 1RM bench press exercise (p = 0.029; ES = 0.49). Individual responses for the 1 RM back squat and the seated medicine ball throw test are presented in Figure 12 and Figure 13, respectively. None of the other differences between conditions reached significance. The results for both the placebo and caffeine conditions for measures of performance responses and measures of subjective responses are presented in Table 13 and Table 14, respectively, along with the 95% CI. A total of 9 ESs were small, four ESs were trivial, and one ES was negative (i.e., an increase in pain perception in 1RM back squat exercise in caffeine condition). All participants tolerated caffeine well, with two participants reporting a feeling of slight nausea after ingestion.

Figure 12. Individual responses of the resistance-trained participants (n = 17) to the 1RM back squat test



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Caffeine condition	175	140	110	120	158	160	160	130	120	120	145	120	115	128	125	140	135
Placebo condition	175	135	103	120	150	150	155	140	115	110	140	120	110	130	120	143	123

Figure 13. Individual responses of the resistance-trained participants (n = 17) to the seated medicine ball throw test



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Caffeine condition	385	391	316	366	503	395	310	368	445	351	410	305	365	275	410	385	357
Placebo condition	385	358	315	390	443	350	310	354	427	332	373	305	343	285	380	376	350

Table 13. Differences in placebo vs. caffeine conditions in measures of performance responses

Measure	Placebo condition (mean ± SD)	Caffeine condition (mean ± SD)	Relative effects (%)	Effect size – magnitude	*p*-value
Vertical jump (cm)	66.1 ± 7.7	68.0 ± 7.1	2.8	0.25 – small	0.067
Seated medicine ball throw (cm)	357.4 ± 41.9	372.8 ± 54.9	4.3	0.32 – small	0.009*
1RM back squat (kg)	131.6 ± 19.2	135.3 ± 18.7	2.8	0.19 – trivial	0.016*
Back squat - repetitions to failure with 60% of 1RM	22.5 ± 8.4	23.4 ± 8.1	3.9	0.11 – trivial	0.484
1RM bench press (kg)	106.9 ± 11.9	107.9 ± 11.9	1.0	0.09 – trivial	0.275
Bench press - repetitions to failure with 60% of 1RM	20.8 ± 3.0	21.5 ± 3.0	3.1	0.21 – small	0.315
CI = confidence interval; * = statistically significant difference between conditions; 1RM = one-repetition maximum					

Table 14. Differences in placebo vs. caffeine conditions in measures of subjective responses

Measure	Placebo condition (mean ± SD)	Caffeine condition (mean ± SD)	Relative effects (%)	Effect size – magnitude	*p*-value
RPE for 1RM back squat	16.7 ± 1.9	15.5 ± 2.5	7.0	0.53 – small	0.037*
PP for 1RM back squat	2.7 ± 1.4	2.8 ± 1.5	-4.3	-0.08 – negative effect	0.778
RPE for back squat repetitions to failure	16.8 ± 2.6	16.0 ± 2.4	4.9	0.33 - small	0.115
PP for back squat repetitions to failure	5.4 ± 2.1	4.9 ± 2.4	9.2	0.22 – small	0.408
RPE for 1RM bench press	16.4 ± 2.4	15.5 ± 2.7	5.4	0.35 – small	0.140
PP for 1RM bench press	2.6 ± 1.4	2.0 ± 1.3	24.7	0.49 – small	0.029*
RPE for bench press repetitions to failure	15.6 ± 2.3	15.5 ± 2.2	0.4	0.03 – trivial	0.921
PP for bench press repetitions to failure	3.8 ± 1.2	3.2 ± 1.5	14.8	0.41 – small	0.106

CI = confidence interval; * = statistically significant difference between conditions; 1RM = one-repetition maximum; RPE = rating of perceived exertion (expressed on a 6-20 scale); PP = pain perception (expressed on a 0-10 scale)

8.5. Discussion

The current study evaluated the acute effects of caffeine ingestion on physical performance requiring muscular strength and power, and muscular endurance, in resistance-trained individuals. In addition, the effects on perception of pain and perceived exertion were also evaluated. The major finding of this study is that caffeine ingestion acutely enhances lower-body strength performance, and this enhancement in performance is accompanied by a reduced perception of exertion. Positive effects of caffeine ingestion were also observed for the upper- but not for the lower-body power. No effects were observed for the upper-body strength nor for the muscular endurance and corresponding RPE and pain perception values. Taken together, these results only partially confirm our initial hypothesis.

Our findings indicate that 6 mg/kg of caffeine acutely enhances lower- but not upper-body strength in resistance-trained men. Although only a trivial ES and a small percent increase were observed (0.19 and 2.8%, respectively), improvements in performance by as little as 3% in some events may mean the difference between winning and not even being at the podium (Le Meur et al., 2012; Pyne et al., 2009).

Our findings indicating enhanced strength performance following caffeine ingestion are in contrast with the current data. Brooks et al. (2015) found no increases in 1RM machine-based back squat exercise in a group of 7 trained males. Likewise, Trexler et al. (2016) and Astorino et al. (2008) found no improvements in lower-body strength using leg press exercise as an assessment tool. The discrepancies between the studies may be due to the following: Brooks et al. (2015) used back squat exercise performed on the Smith machine, a lower dose (5 mg/kg) and a different form (capsule) of caffeine. Trexler et al. (2016) used a fixed dose of anhydrous caffeine (i.e., 300 mg) which yielded a smaller mean amount (3.9 mg/kg; range 3-5 mg/kg) of caffeine per participant. Furthermore, Trexler et al. (2016) performed the testing sessions 30 minutes after caffeine ingestion, while in the present study the testing sessions were performed 60 minutes after caffeine ingestion. Ingestion of caffeine 60 minutes before exercise may be optimal as plasma concentrations approximate a maximum level in 1 hour (Graham, 2001). However, it seems that peak saliva levels vary depending on the source of caffeine. As shown by Liguori et al. (1997), saliva caffeine levels may peak sooner when caffeine is ingested via coffee (42 ± 5 min) and cola (39 ± 5 min) but later if ingested via the capsule (67 ± 7 min).

Astorino et al. (2008) reported a habitual intake of 110 ± 152 mg of caffeine per day, while our participants reported a smaller caffeine intake of 58 ± 92 mg per day, with 10 participants reporting no regular caffeine intake. While it may be hypothesised that a reduction in effects is caused by caffeine habits, the differences caused by caffeine habits do not appear to be major (Graham, 2001). However, it is important to emphasise that individual factors determine responsiveness, as there probably are "responders" and "non-responders" (Butler, Iwasaki, Guengerich, & Kadlubar, 1989). These variations in response to caffeine intake have been observed in the present study as well, as in some participants the back squat performance decreased with caffeine intake by 7%, while in one participant it increased by as much as 10%. These acute increases in strength performance may probably be attributed to better motor unit recruitment; however, discussing the physiological effects of caffeine is beyond the scope of this article (for a review, see Graham, 2001; Tarnopolsky, 2008).

Improvements in lower-body strength performance were accompanied by a reduction in RPE. By contrast, the perception of pain did not change significantly among conditions in the 1RM back squat exercise, while it was significantly lower for the caffeine condition in the 1RM bench press exercise. No differences in RPE were noted for the bench press exercise, possibly because the bench press exercise is a less complex and less demanding exercise than the squat; however, this remains unclear. It has been suggested by Warren et al. (2010) that smaller muscles, such as muscles of the upper arm, have a limited ability for increased motor unit recruitment with caffeine ingestion. Differences in the effects of caffeine on upper and lower body were also noted in a recent study by Black et al. (2015). These authors (Black et al., 2015) reported increases (+6.3%) in maximal voluntary strength in the lower (i.e., knee extensors), but not the upper body (i.e., elbow flexors) when assessed 60 minutes following the ingestion of a 5 mg/kg dose of caffeine. Further studies are warranted to assess for possible differences in upper- vs. lower-body strength after caffeine ingestion. Based on these findings, we may surmise that acute increases in strength may mainly be attributed to a reduction in perceived exertion that allows an individual to perform more work (Tarnopolsky, 2008). We would like to stress that the bench press exercise was the very last test performed in the assessment procedure, and the performance of the participants, therefore, may have been affected by the accumulated fatigue.

A novel finding of this study is that caffeine ingestion may enhance performance in exercises that require upper-body power. This is in contrast to the findings of Martinez et al. (2016) who showed that consuming a pre-workout supplement containing caffeine does not enhance upper-body power performance; however, the participants in that study refrained from caffeine ingestion only 3 hours prior to testing, while our participants ceased consumption the day prior to testing. The comparison of conditions for the lower-body power, as assessed using the vertical jump test, indicated no significant differences ($p = 0.067$), although an ES of 0.25 was observed. The prevailing body of literature indicates acute improvements in lower-body power (Bloms et al., 2016; Del Coso et al., 2012), with a dose of caffeine in the range of 3-6 mg/kg being the most desirable to reduce the possible side-effects such as jitters, increased heart rate and performance impairment (Graham and Spriet, 1995).

The effects of caffeine intake on muscular endurance in resistance-trained population were previously assessed in few studies (Astorino et al., 2008; Beck, et al., 2006; Hudson et al., 2008) with equivocal results. Tarnopolsky (2008) suggested that caffeine intake should have a considerable positive effect on muscular endurance; however, our results do not support this suggestion. We did not observe improvements in our participants' upper- nor lower-body muscular endurance with caffeine ingestion. Also, we did not observe a difference in RPE nor PP among conditions. Similar results were obtained by Richardson and Clarke (2016), who reported no improvement in muscular endurance performance assessed 60 minutes after ingestion of 5 mg/kg of anhydrous caffeine in a cohort of resistance-trained men. However, our findings are in contrast with the recent meta-analysis performed by Polito et al. (2016) who concluded that caffeine intake could have a significant performance improvement effect on muscular endurance when consumed 60 minutes before testing. We emphasise that, in our study, muscular endurance was assessed in the latter part of the testing sequence, so the accumulated fatigue may have played a role, and different outcomes might have been observed if muscular endurance had been assessed at the beginning of the testing session.

A limitation of the present study pertains to the fact that assessment procedures consisted of 6 exercise tests performed in succession. In a typical session lasting 70 to 90 minutes, this may have dampened performance in tests positioned later in the sequence. Also, only two testing sessions (placebo condition + caffeine condition) were employed. Future studies striving to

examine the acute effects of caffeine on a range of physical abilities may benefit from splitting the assessment procedures into multiple sessions, thus minimising the effects of accumulated fatigue and enabling the participants to give their maximal effort in each assessment procedure. On a final note, a limitation of the study also pertains to the lack of assessment of the effectiveness of blinding on the participants. Consequently, it is not entirely clear if the results could be ascribed to the effects of caffeine consumption, or if they are merely placebo-induced. From previous work on the topic (Astorino et al., 2008; Astorino et al., 2011; Duncan et al., 2013) we may only assume that the correct differentiation between the caffeine and placebo trials would have been in the 29-60% range. Researchers examining this issue in the future should circumvent these issues by asking the participants to indicate which trial they perceive to be the caffeine trial, and which trial they perceive to be the placebo trial.

8.6. Conclusion

Based on our findings, it may be suggested that trained individuals competing in events in which maximal strength and power are important performance-related factors (e.g., powerlifting, strongman, weightlifting etc.) might consider taking 6 mg/kg of caffeine pre-training/competition for performance enhancement. The mentioned dose may be consumed with minimal health risks; however, due to individual responsiveness, this should be tested for each athlete individually before important competitions.

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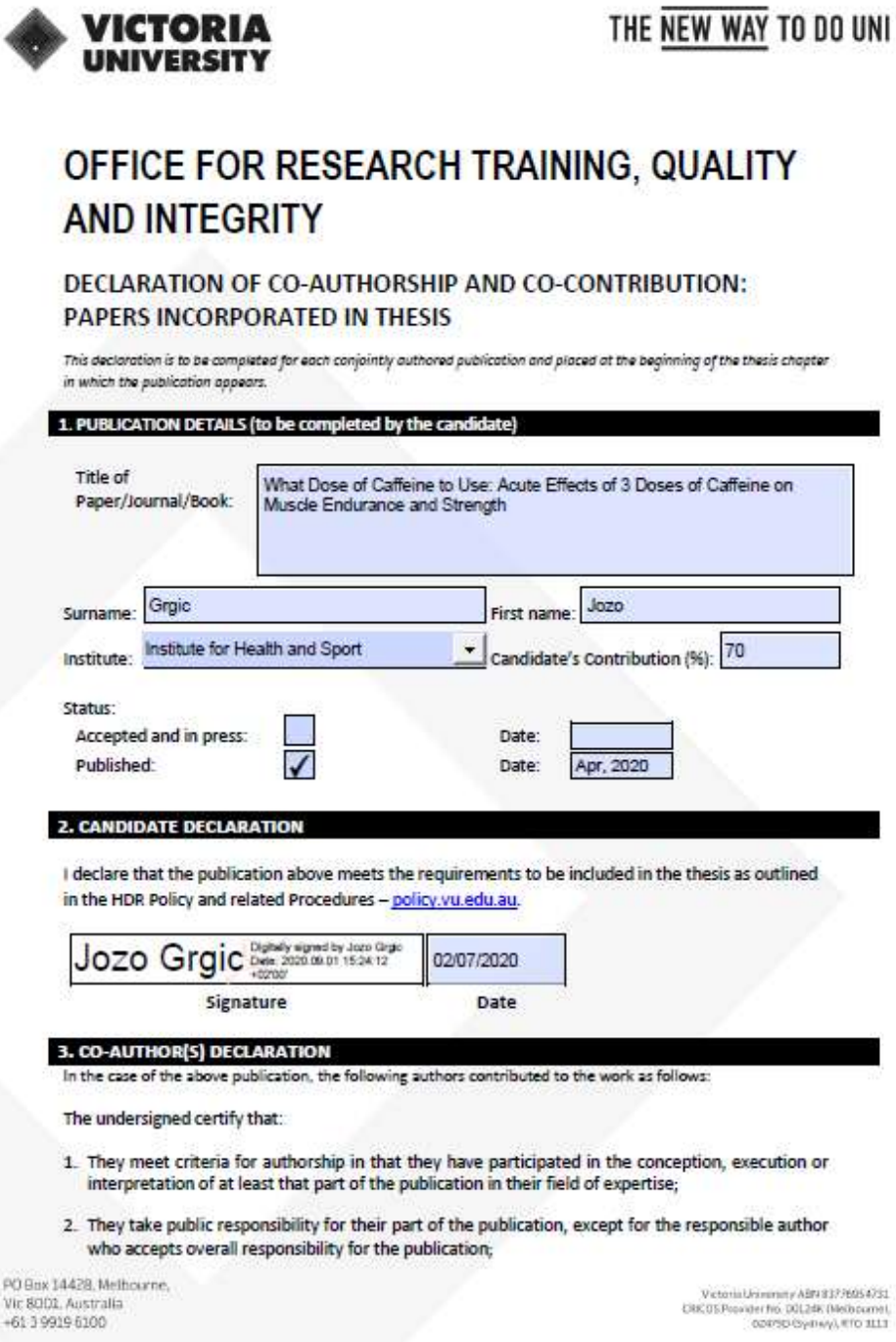
Conflict of interest statement: The authors declare that there are no conflicts of interest relevant to this study.

9. What dose of caffeine to use: acute effects of three doses of caffeine on muscle endurance and strength



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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book: What Dose of Caffeine to Use: Acute Effects of 3 Doses of Caffeine on Muscle Endurance and Strength

Surname: Grgic First name: Jozo

Institute: Institute for Health and Sport Candidate's Contribution (%): 70

Status:

Accepted and in press: ☐ Date:

Published: ☑ Date: Apr, 2020

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

Jozo Grgic Digitally signed by Jozo Grgic Date: 2020.09.01 15:24:12 +02'00' 02/07/2020

Signature Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

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3. There are no other authors of the publication according to these criteria;
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5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

The original data has been electronically stored on VU R drive and the student's laptop for at least five years since April 2020.

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Filip Sabol	10	Data collection and interpretation, and manuscript writing	[redacted]	02/07/2020
Sandro Venier	2	Data collection and manuscript writing		02/07/2020
Ivan Mikulic	2	Data collection and manuscript writing		02/07/2020
Nenad Bratkovic	2	Data collection and manuscript writing		02/07/2020
Brad J. Schoenfeld	2	Data interpretation and manuscript writing		02/07/2020
Craig Pickering	2	Data collection and manuscript writing		02/07/2020

Updated: September 2019

3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
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Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
David J. Bishop	2	Data interpretation and manuscript writing	[redacted]	02/07/2020
Zeljko Pedisic	3	Data interpretation and manuscript writing		02/07/2020
Pavle Mikulic	5	Conceptualization, data interpretation, and manuscript writing		02/07/2020

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What dose of caffeine to use: acute effects of three doses of caffeine on muscle endurance and strength

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9.1. Abstract

Purpose: To explore the effects of three doses of caffeine on muscle strength and muscle endurance.

Methods: Twenty-eight resistance-trained men completed the testing sessions under five conditions: no-placebo control, placebo-control, and with caffeine doses of 2, 4, and 6 mg/kg. Muscle strength was assessed using the one-repetition maximum (1RM) test; muscle endurance was assessed by having the participants perform a maximal number of repetitions with 60% 1RM.

Results: In comparisons with both control conditions, only a caffeine dose of 2 mg/kg enhanced lower-body strength (d = 0.13–0.15). In comparisons with the no-placebo control condition, caffeine doses of 4 mg/kg and 6 mg/kg enhanced upper-body strength (d = 0.07–0.09) with a significant linear trend for the effectiveness of different doses of caffeine (p = 0.020). Compared to both control conditions, all three caffeine doses enhanced lower-body muscle endurance (d = 0.46–0.68). For upper-body muscle endurance, we did not find significant effects of caffeine.

Conclusions: We found a linear trend between the dose of caffeine and its effects on upper-body strength. This study found no clear association between the dose of caffeine and the magnitude of its ergogenic effects on lower-body strength and muscle endurance. From a practical standpoint, the magnitude of caffeine's effects on strength is of questionable relevance. A low dose of caffeine (2 mg/kg)—for an 80kg individual, this dose of caffeine contained in one to two cups of coffee—may produce substantial improvements in lower-body muscle endurance with the magnitude of the effect being similar to that attained using higher doses of caffeine.

9.2. Introduction

The use of caffeine is highly prevalent among both the general population and athletes (Graham, 2001; Van Thuyne & Delbeke, 2006). The International Olympic Committee has also identified caffeine as having strong scientific support for its ergogenic effects on exercise performance (Maughan et al., 2018). There is good evidence that caffeine ingestion can acutely enhance aerobic and muscle endurance, muscle strength, power, jumping height, and exercise speed (Grgic et al., 2020a; Pickering & Grgic, 2019).

In research studies, caffeine is often administered in moderate to high doses (3 to 6 mg/kg), with 6 mg/kg being the most common (Grgic et al., 2020a). There is, however, emerging interest in exploring the effects of lower doses of caffeine (≤3 mg/kg) on exercise performance as such doses generally provide an ergogenic benefit with minimal side-effects (Spriet, 2014). While lower doses are ergogenic for exercise performance, there is a lack of studies exploring whether they provide similar performance-enhancing effects as more conventionally recommended intakes (i.e., 3 to 6 mg/kg). Additionally, the evidence for the ergogenic effects of low doses of caffeine is largely based on studies using tests of aerobic endurance (Spriet, 2014). There is a paucity of studies exploring the effects of such doses of caffeine on high-intensity, short-duration exercise performance (such as resistance exercise) (Spriet, 2014).

Caffeine ingestion has been demonstrated to be ergogenic for muscle strength and muscle endurance (Grgic et al., 2019b). One meta-analysis (Grgic et al., 2018) reported a significant effect of caffeine ingestion on one-repetition maximum (1RM) strength. Of the ten studies included in that meta-analysis, nine used a single dose of caffeine (most commonly 6 mg/kg). One study used two different doses of caffeine (2 mg/kg versus 5 mg/kg); however, their results were inconclusive given that neither dose was associated with increased muscle strength (Arazi et al., 2016b). Another meta-analysis pooled the evidence for the effects of caffeine ingestion on muscle endurance (Polito et al., 2016). As with strength, the authors observed an ergogenic effect of caffeine. Of the sixteen studies that met the inclusion criteria for that review, all of them used a single caffeine dose (relative doses of ≥4 mg/kg). Therefore, minimal effective doses of caffeine for muscle strength and endurance remain unclear due to the lack of studies using multiple doses of caffeine.

To glean new insights into this topic, in the present study we aimed to explore the acute effects of three doses of caffeine (2, 4, and 6 mg/kg) on muscle strength and muscle endurance in resistance-trained men. We hypothesised that all doses of caffeine would enhance upper- and lower-body muscle strength and muscle endurance.

9.3. Methods

9.3.1. Participants

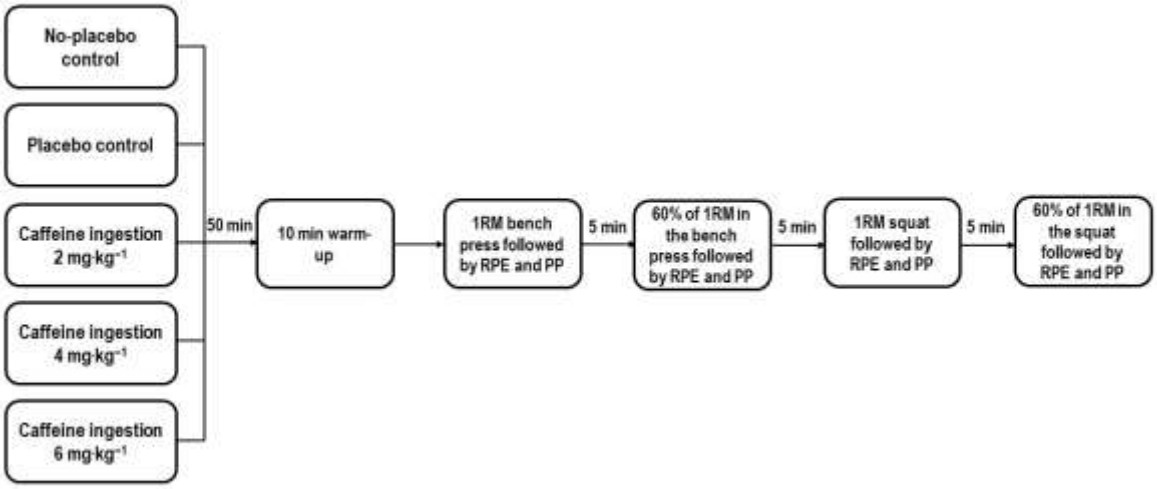
To be included in the present study, participants had to satisfy the following criteria: (a) be apparently healthy men, aged between 18 and 45 years; (b) be resistance-trained, defined as having a minimum of one year of resistance training experience with a minimum weekly training frequency of two times per week (on most weeks); and (c) have the ability to perform the bench press and back squat exercises with a load corresponding to at least 100% of their body mass. Based on a power analysis using the G*Power software (Germany, Düsseldorf, version 3), with an ES *f* of 0.10 for lower-body muscle endurance, alpha error of 0.05, statistical power of 80%, and *r* of 0.90 (Grgic & Mikulic, 2017), the minimum required sample size for this study was estimated to be 26 participants. To factor in possible dropouts, we initially recruited a sample of 32 men. During the study, four participants dropped out due to personal reasons. A sample of 28 participants (mean ± standard deviation of age: 25 ± 6 years, height: 185 ± 6 cm, body mass: 89 ± 11 kg), completed the trials. Habitual caffeine intake was assessed via a validated food frequency questionnaire (FFQ) (Bühler et al., 2014). A qualified nutritionist estimated the daily caffeine intake based on the responses to the FFQ. The mean ± standard deviation habitual caffeine intake of the whole sample was 112 ± 165 mg/day. Ethical approval was requested and granted from the Committee for Scientific Research and Ethics of the Faculty of Kinesiology at the University of Zagreb, where the study was conducted. All participants were informed about the study requirements, benefits, and risks and provided their written informed consent before the involvement in the study.

9.3.2. Experimental design

Following the familiarisation session, the participants were randomly assigned to five experimental conditions in a counterbalanced fashion. The conditions were: no-placebo control condition, placebo-control condition, and three caffeine conditions with caffeine doses of 2, 4,

and 6 mg/kg. The placebo and caffeine powders were weighted using a high precision electronic digital scale and were administered in capsules of identical appearance to maintain a double-blind design. The testing sessions consisted of upper- and lower-body muscle strength and muscle endurance tests (Figure 14).

Figure 14. An overview of the experimental protocol. 1RM: one repetition maximum, RPE: completing the rating of perceived exertion scale; PP: completing the pain perception scale; minutes above the arrows denote rest interval time. The order of the conditions was randomised



To ensure that the exercise performance was not affected by circadian variation, all testing sessions were conducted at the same time of the day for each participant (23 participants were tested in the evening hours and five were tested in the morning hours). The participants came to each session after a three-hour fasting period. Testing was then carried out sixty minutes after supplement ingestion. Sessions were separated by no less than five and no more than seven days. Between the conditions, the participants were advised to maintain their usual training routines. The participants were instructed not to perform any vigorous exercise, to maintain their usual hydration, dietary habits, and sleep patterns in the 24 hours prior to each session. Also, the participants were requested to refrain from any caffeine ingestion 12 hours before the five sessions. Caffeine has a half-life of four to six hours; therefore, stopping its ingestion around 12 hours before the testing session is deemed sufficient to avoid potential confounding by prior caffeine ingestion (Graham, 2001). To facilitate this process, the participants were

provided with a comprehensive list of food and drink products containing caffeine that they should avoid consuming in that period.

9.3.3. Testing protocol

Upper-body muscle strength and muscle endurance were assessed first, using the barbell bench press exercise. After the bench press exercise, lower-body muscle strength and muscle endurance were evaluated using the barbell back squat exercise. In the eccentric phase of the squat exercise, the participants were required to squat to a depth where the hips were at the same level as the knees for the attempt to be considered valid. None of the participants used knee wraps during the tests; five participants used a weight lifting belt, but its use was standardised across all conditions. Participants initially performed a self-selected warm-up lasting 10 minutes. For the 1RM, the first warm-up set included eight to ten repetitions with 50% of the participants' estimated 1RM. The second warm-up set included three to five repetitions with ~75% of the estimated 1RM. Participants then completed one repetition with ~95% of their estimated 1RM. Based on whether the participant successfully lifted the load or not, the weight was increased or decreased on subsequent attempts. Three to five minutes were given between the 1RM attempts, and all 1RM values were obtained within five attempts. After a five-minute rest period, muscle endurance was assessed with one 'all-out' set with a load corresponding to 60% of 1RM performed to momentary concentric failure. The test was terminated when the participants could not maintain the prescribed cadence (1-2 seconds for both concentric and eccentric muscle actions) and/or could not maintain the whole range of motion of for the exercise. Following a five minute rest, the same procedure was repeated for lower-body muscle strength and muscle endurance.

9.3.4. Rating of perceived exertion (RPE) and pain perception (PP)

Within five seconds of a successful 1RM attempt, as well as following the final repetition in the muscle endurance tests (after re-racking the weight), the participants indicated their perceived levels of exertion on the RPE scale (Borg, 1970). Furthermore, the participants indicated their levels of PP on a previously validated scale (Cook et al., 1998). For the RPE scale, the responses ranged from 6 to 20, while on the PP scale, the responses ranged from 0 to 10. Before the familiarisation session, the participants were instructed on the proper use of the scales. Before the subsequent assessments, the participants were re-introduced with the scales.

9.3.5. Assessment of blinding

We tested the effectiveness of blinding by asking the participants to identify the supplement they had ingested. The question for this assessment was based on the study by Saunders et al. (2017) and was phrased: "Which supplement do you think you have ingested?" Its response scale included five possible answers: (a) caffeine 2 mg/kg; (b) caffeine 4 mg/kg; (c) caffeine 6 mg/kg; (d) placebo; (e) do not know. This assessment was conducted pre- and post-exercise given that the opinion of participants might change pre- to post-exercise (Saunders et al., 2017).

9.3.6. Statistical analyses

A series of repeated-measures analysis of variance (ANOVA) was used to analyse the differences in performance and subjective responses between the conditions. In cases of a significant main effect, post hoc comparisons were conducted using Dunnett's test so that each caffeine condition was compared to the placebo-control condition (i.e., 2 mg/kg vs. placebo-control, 4 mg/kg vs. placebo-control, and 6 mg/kg vs. placebo-control) and to the no-placebo control condition (i.e., 2 mg/kg vs. no-placebo control, 4 mg/kg vs. no-placebo control, and 6 mg/kg vs. no-placebo control). We have also calculated *p*-values for the linear and quadratic trends between the doses of caffeine. The statistical significance threshold was set at $p < 0.05$. Relative ESs were calculated using Cohen's *d* with 95% confidence intervals (95% CI) for repeated measures. ESs of <0.20, 0.20 to 0.49, 0.50 to 0.79, and ≥0.80 were considered to represent trivial, small, moderate, and large effects, respectively. In addition to relative effect sizes, we also calculated the raw mean differences between the trials and their 95% CIs. The blinding data were examined using the Bang's Blinding Index with all three possible responses for caffeine (i.e., 2, 4, and 6 mg/kg) collapsed into a single caffeine response. The values in this index range from –1.0 which indicates opposite guessing to 1.0 which indicates complete unblinding; here, we reported these data as a percentage of individuals who identified the correct condition beyond chance. All analyses were performed using the STATISTICA software (version 13.0; StatSoft, Tulsa, OK, USA).

9.4. Results

9.4.1. Lower-body muscle strength

For 1RM strength in the back squat exercise a significant main effect of condition was observed (p = 0.008; Table 15). In comparisons with the no-placebo control condition, post hoc test revealed that a dose of 2 mg/kg of caffeine acutely enhanced lower-body strength (d = 0.15; +3.5 kg; p = 0.003). In comparisons with the no-placebo control condition, no significant differences were observed for 4 mg/kg (d = 0.09; +2.1 kg; p = 0.069) and 6 mg/kg of caffeine (d = 0.08; +2.0 kg; p = 0.083). In comparisons with placebo-control condition, post hoc tests revealed that a dose of 2 mg/kg of caffeine also acutely enhanced lower-body strength (d = 0.13; +3.0 kg; p = 0.009). In comparisons with placebo-control condition, no significant differences were observed for 4 mg/kg (d = 0.07; +1.6 kg; p = 0.159) and 6 mg/kg of caffeine (d = 0.06; +1.5 kg; p = 0.185). The linear trend for the effectiveness of different doses of caffeine was not significant (p = 0.162). The quadratic trend for the effectiveness of different doses of caffeine was not significant (p = 0.541).

9.4.2. Upper-body muscle strength

For 1RM strength in the bench press exercise a significant main effect of condition was observed (p = 0.025). In comparisons with the no-placebo control condition, post hoc test revealed that doses of 4 mg/kg (d = 0.07; +1.6 kg; p = 0.044) and 6 mg/kg (d = 0.09; +2.1 kg; p = 0.007) of caffeine acutely enhanced upper-body strength. In comparisons with no-placebo control condition, no significant differences were observed for 2 mg/kg (d = 0.01; +0.2 kg; p = 0.656). In comparisons with placebo-control condition, post hoc tests revealed no significant differences for 2 mg/kg (d = –0.03; –0.5 kg; p = 0.923), 4 mg/kg (d = 0.04; +0.9 kg; p = 0.287) and for 6 mg/kg (d = 0.06; +1.4 kg; p = 0.100) doses of caffeine. We found a significant linear trend for the effectiveness of different doses of caffeine (p = 0.020). The quadratic trend for the effectiveness of different doses of caffeine was not significant (p = 0.508).

9.4.3. Lower-body muscle endurance

For the number of repetitions in the back squat exercise a significant main effect of caffeine was observed (p = 0.004). As compared to no-placebo control condition, post hoc tests revealed that doses of 2 mg/kg (d = 0.55; +4.2 repetitions; p = 0.011), 4 mg/kg (d = 0.52; +3.3 repetitions;

p = 0.046), and 6 mg/kg (d = 0.46; +3.9 repetitions; p = 0.018) acutely enhanced lower-body muscle endurance. As compared to placebo-control condition, post hoc tests revealed that 2 mg/kg of caffeine (d = 0.67; +4.8 repetitions; p = 0.008), 4 mg/kg (d = 0.68; +3.9 repetitions; p = 0.032) and 6 mg/kg (d = 0.56; +4.5 repetitions; p = 0.014) acutely enhanced lower-body muscle endurance. The linear trend for the effectiveness of different doses of caffeine was not significant (p = 0.802). The quadratic trend for the effectiveness of different doses of caffeine was not significant (p = 0.633).

9.4.4. Upper-body muscle endurance

The repeated measures ANOVA conducted for the number of repetitions in the bench press exercise did not show a significant main effect (p = 0.470), and therefore no post hoc analysis was performed.

9.4.5. RPE and PP

None of the comparisons for the RPE or the PP were significant (p > 0.05 for all). All data are presented in Table 16.

9.4.6. Effectiveness of blinding

Just before exercise, in the placebo-control, and the 2, 4, and 6 mg/kg conditions, 1%, 11%, 29%, and 21% of the participants correctly guessed the treatment identity beyond chance, respectively. After exercise, in the placebo-control, and the 2, 4, and 6 mg/kg conditions, 14%, 32%, 29%, and 25% of the participants correctly guessed the treatment identity beyond chance, respectively.

Table 15. Summary of the study comparision between the conditions

Outcome	Comparision	Cohen's *d* (95% CI)	Raw mean difference (95% CI)	*r*
Weight lifted in the 1RM barbell back squat test	No-placebo control vs. 2 mg/kg	0.15 (0.08, 0.22)*	+3.5 kg (1.9, 5.1 kg)*	0.99
	No-placebo control vs. 4 mg/kg [1]	0.09 (–0.01, 0.19)	+2.1 kg (–0.2, 4.4 kg)	0.97
	No-placebo control vs. 6 mg/kg	0.08 (0.00, 0.17)	+2.0 kg (0.0, 4.0 kg)	0.98
	Placebo-control vs. 2 mg/kg	0.13 (0.06, 0.20)*	+3.0 kg (1.4, 4.6 kg)*	0.98
	Placebo-control vs. 4 mg/kg	0.07 (–0.03, 0.17)	+1.6 kg (–0.6, 3.8 kg)	0.97
	Placebo-control vs. 6 mg/kg	0.06 (–0.03, 0.16)	+1.5 kg (–0.5, 3.5 kg)	0.98
Weight lifted in the 1RM barbell bench press test	No-placebo control vs. 2 mg/kg	0.01 (–0.05, 0.06)	+0.2 kg (–1.0, 1.4 kg)	0.99
	No-placebo control vs. 4 mg/kg	0.07 (0.00, 0.15)*	+1.6 kg (0.0, 3.3 kg)*	0.98
	No-placebo control vs. 6 mg/kg	0.09 (0.03, 0.16)*	+2.1 kg (0.9, 3.4 kg)*	0.99
	Placebo-control vs. 2 mg/kg	–0.03 (–0.10, 0.05)	–0.5 kg (–2.0, 0.9 kg)	0.98
	Placebo-control vs. 4 mg/kg	0.04 (–0.05, 0.14)	+0.9 kg (–1.1, 2.9 kg)	0.97
	Placebo-control vs. 6 mg/kg	0.06 (0.00, 0.14)	+1.4 kg (0.0, 2.9 kg)	0.99
	No-placebo control vs. 2 mg/kg	0.55 (0.21, 0.92)*	+4.2 repetitions (1.9, 6.5 repetitions)*	0.76

Number of repetition in the lower-body muscle endurance test	No-placebo control vs. 4 mg/kg	0.52 (0.07, 0.97)*	+3.3 repetitions (0.6, 5.9 repetitions)*	0.46
	No-placebo control vs. 6 mg/kg	0.46 (0.01, 0.92)*	+3.9 repetitions (0.4, 7.3 repetitions)*	0.51
	Placebo-control vs. 2 mg/kg	0.67 (0.17, 1.21)*	+4.8 repetitions (1.4, 8.1 repetitions)*	0.36
	Placebo-control vs. 4 mg/kg	0.68 (0.22, 1.17)*	+3.9 repetitions (1.5, 6.3 repetitions)*	0.48
	Placebo-control vs. 6 mg/kg	0.56 (0.01, 1.16)*	+4.5 repetitions (0.1, 8.8 repetitions)*	0.08
1RM: one repetition maximum; CI: confidence interval; *: significant difference between the conditions				

Table 16. Summary of the exercise performance data and the responses to the rating of perceived exertion and pain perception scales under the five employed conditions

Variable	Control condition		Caffeine intake condition (dose)		
	No-placebo	Placebo	2 mg/kg	4 mg/kg	6 mg/kg
1RM barbell back squat (kg)	128.7 ± 23.8	129.2 ± 21.7	132.2 ± 22.7[a, b]	130.8 ± 22.8	130.7 ± 24.6
RPE for 1RM barbell back squat (6-20 scale)	16.4 ± 2.6	17.0 ± 2.0	17.0 ± 1.8	16.9 ± 1.8	16.4 ± 2.2
PP for 1RM barbell back squat (0-10 scale)	2.3 ± 2.2	2.3 ± 2.8	2.8 ± 2.8	2.2 ± 2.5	2.1 ± 2.5
1RM barbell bench press (kg)	106.2 ± 21.6	106.9 ± 21.9	106.3 ± 21.1	107.8 ± 20.7[a]	108.3 ± 22.5[a]
RPE for 1RM barbell bench press (6-20 scale)	16.3 ± 2.3	15.9 ± 2.8	15.3 ± 2.8	15.7 ± 2.6	15.9 ± 2.7
PP for 1RM barbell bench press (0-10 scale)	1.8 ± 2.4	1.4 ± 2.2	1.9 ± 2.5	1.8 ± 2.2	1.6 ± 1.9
Barbell back squat – repetitions to failure with 60% of 1RM (repetitions)	21.7 ± 6.2	21.1 ± 4.9	25.9 ± 8.4[a, b]	25.0 ± 6.1[a, b]	25.5 ± 9.5[a, b]
RPE for barbell back squat repetitions to failure (6-20 scale)	16.7 ± 2.6	16.9 ± 2.4	17.0 ± 2.3	17.1 ± 2.6	17.2 ± 2.4
PP for barbell back squat repetitions to failure (0-10 scale)	2.9 ± 2.7	3.2 ± 2.7	3.5 ± 3.1	3.5 ± 3.1	3.0 ± 3.0
Barbell bench press – repetitions to failure with 60% of 1RM (repetitions)	20.5 ± 3.9	20.5 ± 4.2	21.1 ± 3.8	21.2 ± 3.6	20.9 ± 4.0

RPE for barbell bench press repetitions to failure (6-20 scale)	16.8 ± 2.3	17.0 ± 2.4	16.6 ± 2.4	16.9 ± 2.4	17.0 ± 2.5
PP for barbell bench press repetitions to failure (0-10 scale)	2.4 ± 2.6	2.3 ± 2.7	2.5 ± 3.2	2.3 ± 2.2	2.3 ± 2.7
All data are presented as mean ± standard deviation, RPE: rating of perceived exertion, PP: pain perception, 1RM: one repetition maximum; [a]: significant difference as compared to no-placebo control [b]: significant difference as compared to placebo-control					

9.5. Discussion

This study found mixed effects of different doses of caffeine on muscle strength and endurance. Except for upper-body muscle strength, no clear dose-response trends were observed. The results suggested that only 2 mg/kg of caffeine was ergogenic for lower-body strength, as compared to both control conditions. When considering the comparison with the no-placebo control condition, caffeine doses of 4 mg/kg and 6 mg/kg enhanced upper-body strength. Compared to both control conditions, all three caffeine doses were effective for acute improvements in lower-body muscle endurance, whereas no significant effects were found for any of the three caffeine doses on upper-body muscle endurance.

9.5.1. Effects of caffeine on muscle strength

Our results indicate that a caffeine dose of 2 mg/kg acutely enhanced lower-body muscle strength. We did not find significant ergogenic effects for higher doses, even though the ESs favoured the caffeine conditions. For upper-body strength, only 4 mg/kg and 6 mg/kg doses of caffeine were ergogenic. However, it is important to consider that the results for the upper-body were statistically significant only when compared to no-placebo control, but not with the placebo-control condition.

Our results support the findings of a previous meta-analysis that caffeine ingestion may acutely enhance 1RM strength (Grgic et al., 2018). This meta-analysis found a pooled ES of caffeine on strength of 0.20 (Grgic et al., 2018). Even though caffeine was ergogenic in our study, the ES for strength ranged from 0.07 to 0.15 which can be considered as 'trivial'. Mean changes in weight lifted which ranged from +1.6 to +3.5 kg, can be considered relatively small from a practical perspective. Such increases in strength would likely only be worthwhile in strength-based sports such as powerlifting, in which, narrow margins determine the competition outcomes. While we did not include competitive powerlifters in the study, several of the participants did indeed exhibit very high levels of strength. One participant had a 1RM in the squat of 185 kg, and another successfully performed the 1RM in the bench press exercise with 147.5 kg. Such levels of strength are similar to those previously observed in national-level powerlifters (Bjørnsen et al., 2019). This coupled with the fact that all of the participants were resistance-trained individuals increases the generalisability of these findings to athletes competing in strength-based sports; however, future work examining these effects among

athletes from strength-based sports is warranted. For upper-body strength, we observed a significant linear trend between the dose of caffeine and strength performance. Indeed, average 1RM bench press values with caffeine doses of 2, 4, and 6 mg/kg amounted to 106.3 kg, 107.8 kg, and 108.3 kg, respectively. Again, it needs to be highlighted that these differences in weight lifted are relatively small, which may call into question the practical relevance of these findings for most individuals.

To date, only one study has explored the effects of multiple doses of caffeine on 1RM strength (Arazi et al., 2016b). In that study, the researchers did not find any significant effects of 2 and 5 mg/kg of caffeine on 1RM strength in the leg press exercise. There are several key differences in the study design between the present study and the work by Arazi et al. (2016b) that may explain inconsistent findings. The participants in our study were adult resistance-trained men, while the Arazi et al. (2016b) study was conducted in a sample of adolescent female karate athletes. This may be relevant given that the response to caffeine ingestion might not be uniform between men and women (Pickering & Grgic, 2019). Also, there were substantial differences in the total sample size (10 vs. 28 participants), which may have affected statistical inferences. The average ES for the effects of caffeine in the Arazi et al. (2016b) study was 0.35, which might suggest that the effects would be statistically significant if the study included a larger sample size.

9.5.2. Effects of caffeine on muscle endurance

For lower-body muscle endurance, all three doses of caffeine were found to be ergogenic, in comparison to both control conditions. The average relative ES spanned from 0.46 to 0.67, which is considered as an indication of a 'moderate' effect. The mean differences in the number of performed repetitions in the back squat exercise ranged from 3 to 5. Such acute improvements in muscle endurance following caffeine ingestion are similar to those observed after eight weeks of regimented resistance exercise, which highlights the magnitude of these effects (Mattocks et al., 2017; Schoenfeld et al., 2016). For upper-body muscle endurance, no significant differences were observed between the caffeine conditions versus the control conditions.

While caffeine is ergogenic for muscle endurance, these effects may be modulated by factors such as the size of the activated muscle (Warren et al., 2010). Previous research has suggested that the lower- and upper-body musculature exhibit divergent responses to caffeine ingestion with the effects being more pronounced in the lower-body musculature (Black et al., 2015; Grgic & Pickering, 2019). In support of this idea, Warren et al. (2010) reported that caffeine has a greater ergogenic effect on the knee extensor muscles as compared to the smaller muscle groups such as the elbow flexors. During maximal voluntary contractions, knee extensor activation level is generally 85% to 95% (Shield & Zhou, 2004). However, smaller muscle groups reach up to 99% of their maximum activation (Gandevia & McKenzie, 1988; Shield & Zhou, 2004). Given these baseline differences in muscle activation levels between muscle groups, Warren et al. (2010) suggested that larger muscles, such as the knee extensors, are more responsive to the ergogenic effects of caffeine. In one study, at baseline, the percentage of motor-unit recruitment of the knee extensors and elbow flexors during maximal contractions—as assessed using the interpolated-twitch electrical stimulation—was at 83% and 97%, respectively (Black et al., 2015). Due to the lower muscle activation level at baseline, after the ingestion of caffeine, performance was only improved for the lower- but not the upper-body (Black et al., 2015). These results might explain why we did not observe significant improvements in upper-body muscle endurance. Additionally, these results might explain why we did not find significant increases in upper-body strength following caffeine ingestion when compared to the placebo-control conditions.

Thus far, only Polito, Grandolfi, and De Souza (2019) conducted a study that had a similar design to ours. In this study, 14 resistance-trained men performed three upper-body resistance exercises (chest press, shoulder press, and biceps curl exercises) for three sets until exhaustion with 70% of 1RM after the ingestion of either 3 or 6 mg/kg of caffeine. The results indicated that both doses of caffeine acutely increased the number of repetitions performed in the three upper-body exercises. The reason for the discrepancies between the studies could be related to the protocol used. In the study by Polito et al. (2019) study, the participants performed a total of nine sets (three sets for each of the three exercises), whereas we used one ‘all-out’ set. Caffeine ingestion attenuates the fatigue-induced decline in muscle contractile properties (Pethick et al., 2017) which may explain why caffeine was effective for upper-body muscles over a multiple set protocol, as in the study by Polito et al. (2019), but not when using a single set. Given the overall lack of studies on this topic, future work is warranted to provide further

insights into the determinants of caffeine's effects on muscle endurance such as the exercise type (e.g., single vs. multiple sets).

9.5.3. RPE and PP

When analysing the responses of the participants in the RPE and PP scales, no significant effects between the conditions were observed. These results suggest that mechanisms other than a reduction in RPE or PP are responsible for the ergogenic effects of caffeine. The ergogenic effects of caffeine in the present study might be explained by caffeine's effects on increasing muscle fibre conduction velocity and motor unit recruitment (Bazzucchi et al., 2011; Warren et al., 2010). Nonetheless, it is also important to consider that the use of multiple tests of performance might have influenced the estimated effects of caffeine on RPE. For example, testing of strength in the bench press first in the testing session might have impacted the RPE responses in the upper-body muscle endurance test.

9.5.4. Limitations of the study

One of the limitations of the present study is that we did not measure blood caffeine concentrations, and therefore, the amount of caffeine absorption in the blood with different doses of caffeine remains unclear. Additionally, even though the majority of the participants were considered as 'low' habitual users (caffeine intake of <100 mg per day), several of the participants were moderate-to-high caffeine users with habitual intakes of >100 mg per day. Caffeine's ergogenic effect might be more pronounced in individuals with low habitual caffeine consumption (Evans et al., 2018). Even though the findings from the studies on this matter are equivocal (Evans et al., 2018; Gonçalves et al., 2017; Lara, Ruiz-Moreno, Salinero, & Del Coso, 2019), this still needs to be acknowledged as a potential limitation of the current study. The wide inter-individual variation in responses to caffeine has been associated with variation in the *CYP1A2* gene. The *CYP1A2* gene affects caffeine metabolism; individuals with the AA genotype seems to experience greater improvements in exercise performance than those with the AC/CC genotype (Rahimi, 2019). In this study, we did not collect data on genotype variations which is something that future studies may consider. Finally, the blinding of the participants was generally effective, even though the percentage of those that correctly guessed the treatment identity beyond chance increased pre to post-exercise. In this context, it is possible

that the pre-exercise responses are of greater importance, given that the post-exercise responses might be influenced by the improved performance (or lack thereof) during the testing session.

9.5.5. Practical implications

As little as 2 mg/kg of caffeine may enhance lower-body muscle endurance. While caffeine ingestion was ergogenic for lower and upper-body strength, the magnitude of these effects can be categorised as trivial.

9.6. Conclusions

In this study, we found a linear trend between the dose of caffeine and its effects on upper-body strength. However, this study found no clear association between the dose of caffeine and the magnitude of its ergogenic effects for lower-body strength and muscle endurance. While our findings indicate that caffeine ingestion may enhance upper- and lower-body strength, from a practical standpoint, the magnitude of this effect is of questionable relevance. A low dose of caffeine (i.e., 2 mg/kg)—for an 80 kg individual this dose of caffeine is contained in one to two cups of coffee—may produce substantial improvements in lower-body resistance exercise performance with the magnitude of the effect being similar to that attained using higher doses of caffeine.

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10. ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation

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I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

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3. CO-AUTHOR(S) DECLARATION

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ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation

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10.1. Abstract

Caffeine's ergogenic effects on exercise performance are generally explained by its ability to bind to adenosine receptors. *ADORA2A* is the gene that encodes A_{2A} subtypes of adenosine receptors. It has been suggested that *ADORA2A* gene polymorphisms may be responsible for the inter-individual variations in the effects of caffeine on exercise performance. In the only study that explored the influence of variation in *ADORA2A*—in this case, a common polymorphism (rs5751876)—on the ergogenic effects of caffeine on exercise performance, C allele carriers were identified as "non-responders" to caffeine. To explore if C allele carriers are true "non-responders" to the ergogenic effects of caffeine, in this randomised, double-blind study, we examined the acute effects of caffeine ingestion among a sample consisting exclusively of *ADORA2A* C allele carriers. Twenty resistance-trained men identified as *ADORA2A* C allele carriers (CC/CT genotype) were tested on two occasions, following the ingestion of caffeine (3 mg/kg) and a placebo. Exercise performance was evaluated with movement velocity, power output, and muscle endurance during the bench press exercise, countermovement jump height, and power output during a Wingate test. Out of the 25 analysed variables, caffeine was ergogenic in 21 (ES range: 0.14 to 0.96). In conclusion, *ADORA2A* (rs5751876) C allele carriers exhibited ergogenic responses to caffeine ingestion, with the magnitude of improvements similar to what was previously reported in the literature among samples that were not genotype-specific. Therefore, individuals with the CT/CC genotype may still consider supplementing with caffeine for acute improvements in performance.

10.2. Introduction

The effects of caffeine on exercise have received substantial attention in the scientific literature (Graham, 2001; Grgic & Pickering, 2019; Grgic et al., 2018; Grgic et al., 2019b; Grgic, 2018; Grgic et al., 2020a; McLellan et al., 2016; Salinero, Lara, & Del Coso, 2019). Currently, it is well established that acute ingestion of caffeine doses in the range from 2 to 6 mg per kilogram of body mass enhances exercise performance (Graham, 2001; Grgic & Pickering, 2019; Grgic et al., 2018; Grgic et al., 2019b; Grgic, 2018; Grgic et al., 2020a; McLellan et al., 2016; Salinero, Lara, & Del Coso, 2019). Caffeine's ergogenic effects are apparent in different components of exercise. For example, a recent umbrella review reported that caffeine ingestion enhances muscle strength and endurance, aerobic endurance, power output, and jumping performance (Grgic et al., 2020a). Even though research indicates that caffeine ingestion may be acutely ergogenic for a wide range of exercise tasks, between-person variability in responses to this dietary supplement seems substantial (Pickering & Kiely, 2018). The ergogenic effects of caffeine are generally explained by its interaction with adenosine A_1, A_{2A}, and A_{2B} receptors (Davis et al., 2003; Fredholm, Yang, & Wang, 2017). Adenosine concentrations in the brain progressively increase during waking hours, resulting ultimately in sensations of fatigue; the concentrations of adenosine also decrease during sleep. Caffeine's molecular structure is similar to that of adenosine. Therefore, after ingestion, caffeine binds to adenosine receptors, subsequently resulting in reduced fatigue, increased vigilance, and ergogenic effects on exercise performance (Davis et al., 2003; Fredholm, Yang, & Wang, 2017).

Researchers have suggested that the inter-individual variation in caffeine response may be due to polymorphisms within two genes, namely *CYP1A2* and *ADORA2A* (Pickering & Kiely, 2018). Cytochrome P450 1A2 (an enzyme responsible for up to 95% of caffeine metabolism) is encoded by the *CYP1A2* gene (Pickering & Kiely, 2018). A single nucleotide polymorphism rs762551 within CYP1A2 affects the speed of caffeine metabolism. Specifically, individuals with the AA genotype are commonly classified as "fast caffeine metabolisers", whereas C allele carriers (AC/CC genotypes) are considered to be "slow caffeine metabolisers", respectively (Djordjevic, Ghotbi, Jankovic, & Aklillu, 2010). The influence of *CYP1A2* (rs762551) on the acute effects of caffeine supplementation on exercise performance has been explored in several studies (Algrain et al., 2016; Giersch et al., 2018; Guest et al., 2018; Klein et al., 2012; Pataky et al., 2016; Puente et al., 2018; Rahimi, 2019; Salinero et al., 2017; Womack et al., 2012). However, the evidence in these studies remains inconsistent, with some reporting no effect of

the polymorphism on the ergogenic effects of caffeine supplementation and others showing a modifying effect, but in different directions (Algrain et al., 2016; Giersch et al., 2018; Guest et al., 2018; Klein et al., 2012; Pataky et al., 2016; Puente et al., 2018; Rahimi, 2019; Salinero et al., 2017; Womack et al., 2012).

ADORA2A is the gene that encodes A_{2A} subtypes of adenosine receptors (Cornelis et al., 2007). Previous research has suggested that this receptor represents the primary target of caffeine action in the central nervous system, and thus, polymorphic variations in the *ADORA2A* gene may impact the responses to caffeine ingestion (Cornelis et al., 2007). The rs5751876 polymorphisms in the *ADORA2A* gene are comprised of a C-to-T substitution at nucleotide position 1083 (rs5751876) (also known as 1976C>T) (Cornelis et al., 2007). Interestingly, as compared to TT homozygotes, *ADORA2A* C allele carriers have higher habitual caffeine consumption, which may suggest that these individuals need higher doses of caffeine to obtain a pharmacological effect (Cornelis et al., 2007).

Only one study has explored the influence of variation in this gene—in this case, a common polymorphism (rs5751876)—on the ergogenic effects of caffeine on exercise performance (Loy et al., 2015). The study included 12 participants (6 TT homozygotes and 6 C allele carriers [i.e., CC/CT genotype]). These participants were untrained women who completed 20 min of cycling at a work rate eliciting 60% of VO_{2peak} followed by two 10-min cycling time trials. The exercise task was performed on two occasions, following the ingestion of 5 mg/kg of caffeine or a placebo. Results indicated that caffeine ingestion was ergogenic for TT homozygotes but not for C allele carriers. Based on this study, C allele carriers were identified as “non-responders” to caffeine (Loy et al., 2015).

Given the limited data on this topic, the aim of this study was to explore the influence of *ADORA2A* (rs5751876) on the acute effects of caffeine supplementation on exercise performance, by using exercise tests for which caffeine had previously been shown to be ergogenic (Grgic et al., 2020a).

10.3. Materials and methods

10.3.1. Experimental design

In this double-blind, randomised, crossover trial, all participants attended four laboratory sessions (in the morning hours between 07:00 to 12:00 h) that were from 4 to 7 days apart. The first two sessions consisted of familiarisation with the exercise protocol. The third and fourth sessions were the main sessions. Twenty-four hours before the main trials, participants were asked the following: (a) to avoid any intense exercise; (b) to track their energy and macronutrient intake; and (c) to refrain from caffeine intake after 6 pm on the day before testing. The participants performed the two main sessions in a fasted state (overnight fast). Caffeine and placebo supplementation was provided on different days. Caffeine (Pure Lean Nutrition, Melbourne, Australia) was administered in a gelatin capsule with a dose of 3 mg/kg of body mass, while the placebo gelatin capsule contained 3 mg/kg of body mass of dextrose. All capsules were of identical appearance. Placebo and caffeine powders were weighed using a high precision electronic digital scale (Precisa, XT 120A, Dietikon, Switzerland) and then packaged into capsules. Capsules were prepared in the laboratory by an experienced researcher while other researchers performed the blinding. Capsules were ingested 60 min before the start of the exercise session under the supervision of the research staff, as in previous research (Graham, 2001; Grgic & Mikulic, 2017; Grgic et al., 2020d). The participants' genotype was determined using a buccal swab. Ethical approval was requested and granted from the Victoria University Human Research Ethics Committee (number: HRE19-019), and every participant signed an informed consent form.

10.3.2. Participants

The study included a sample of 22 resistance-trained men, defined herein as having a minimum of six months of resistance training experience with a minimum weekly training frequency of two times on most weeks. Exclusion criteria were the existence of any health limitations and prior use of anabolic steroids (self-reported). All participants completed all sessions with no injuries or adverse events. Participants' characteristics are presented in Table 17.

Table 17. Characteristics of the participants.

Variable	Mean ± standard deviation
Age (years)	29.3 ± 4.8
Body mass (kg)	80.3 ± 11.2
Height (cm)	183.1 ± 5.9
1RM in the bench press (normalised per body mass)	1.1 ± 0.2
Habitual caffeine intake (mg/day)	143 ± 113
1RM: one repetition maximum	

10.3.3. Exercise protocol

Exercises involving the upper body were performed prior to those that predominately activated the lower body, to avoid any transfer of muscle fatigue from one exercise task to another. At the beginning of the exercise protocol, the participants performed the bench press exercise with different loads (i.e., 25%, 50%, 75%, and 90% of one-repetition maximum (1RM)—performed in that order) (Pallarés et al., 2013). 1RM was established during the first familiarisation session. At each respective load, the participants performed two sets of one repetition, separated by a 3-min rest interval. The better repetition at each load was used for the analysis. The eccentric phase lasted 2 s, there was no pause at the bottom phase, and the concentric action was performed with maximal velocity. Mean power (W), mean concentric velocity (m/s), peak power (W), and peak concentric velocity (m/s) were measured for each repetition using the GymAware linear position transducer device (GymAware Power Tool, Kinetic Performance Technologies, Canberra, Australia) that was attached to the barbell.

After the second set that was performed with 90% of 1RM, the participants were provided with five minutes of rest. Then, we tested upper-body muscular endurance with a task that involved performing repetitions to momentary muscular failure in the bench press exercise with a load of 85% of 1RM. In this test, we collected data on the total number of repetitions, as well as power and velocity output of each repetition using the linear position transducer attached to the barbell. The tempo was the same as in the previous task. For the statistical analysis, we

compared the total number of repetitions between the placebo and caffeine conditions. In addition, to explore the "quality" of performed repetitions, we matched the number of repetitions between the placebo and caffeine conditions and examined their average power and velocity. For example, one participant performed 7 and 8 repetitions following the ingestion of the placebo and caffeine, respectively. In this case, we only examined the velocity and power of the first 7 repetitions in both conditions.

After the muscular endurance test, the participants rested for three minutes. Then the participants performed a short warm-up consisting of one minute of light running, followed by ten bodyweight squats. After the warm-up, participants performed a countermovement jump (CMJ) without an arm swing on a force platform (400S Isotronic Fitness Technology, Skye, Australia). The participants positioned themselves in an upright starting position and received commands from the computer software associated with the force platform that was positioned in front of the platform. This software visually counted down, "3, 2, 1" and provided "Set" and "Go" commands. After the "Go" command, the participants had five seconds to complete the jump. The participants performed a fast knee flexion (where their lowest position was a semi-squat position) (Venier, Grgic, & Mikulic, 2019a; Venier, Grgic, & Mikulic, 2019b). Immediately after reaching this point (i.e., no pause at the bottom phase), the participants rapidly extended the hip, knee, and ankle joints with prior instructions to jump as quickly and "explosively" as possible to achieve maximal vertical jump height (Venier et al., 2019a, 2019b). A total of three attempts was provided with one minute of rest between them. The best jump was used for the analysis. The outcome in the CMJ test was vertical jump height.

After the CMJ, the participants rested for three minutes. Then, the participants performed the Wingate test on an Excalibur Sport Cycle Ergometer (Lode, Groningen, The Netherlands). The Wingate test started with a 5-min warm-up consisting of pedaling at 100 W at 60–80 rpm (Frikha, Chaâri, Mezghanni, & Souissi, 2016). Following the warm-up, participants performed a 30-s "all-out" sprint on the bike. The flywheel resistance was set at 0.075 Nm/kg. The participants were instructed to remain seated during the 30-s sprint.

10.3.4. Assessment of blinding

In both main trials (i.e., caffeine and placebo), before and after the testing session, participants responded to the following question: "Which supplement do you think you have ingested?" (Saunders et al., 2017). This question was used to explore the effectiveness of the blinding and had three possible responses: (a) "caffeine", (b) "placebo", and (c) "I do not know" (Saunders et al., 2017). If the participants responded with "a" or "b", they were also asked to state the reason for choosing their respective response.

10.3.5. Genetic testing

Genetic testing was performed using a commercially available testing kit from DNAfit Life Sciences. The procedure used for genetic testing is explained in detail elsewhere (Pickering, Kiely, Suraci, & Collins, 2018). Briefly, the buccal swab sample was collected using OCR-100 kits by DNAGenotek. For the analysis, these samples were sent to IDna Genetics Laboratory (Norwich, UK). DNA was: (a) extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Kent, UK); and (b) amplified using polymerase chain reaction (PCR) on an ABI 7900 real-time thermocycler (Applied Biosystem, Waltham, MA, USA). The collected samples were analysed for the *ADORA2A* (rs5751876) single-nucleotide polymorphism. Genotype analyses were performed after the exercise performance data collection was finalised. Therefore, researchers and participants were blinded to genotype variations of the sample during the exercise performance data collection.

10.3.6. Statistical analysis

Two participants who were *ADORA2A* TT homozygotes were excluded, leaving a total of 20 C allele carriers (CC and CT) in the analysis. One-way repeated-measures analysis of variance (ANOVA) was used to analyse the exercise performance data. Relative ESs (and their 95% confidence intervals; 95% CI) were expressed using Hedges' g for repeated measures. The ESs were classified as follows: trivial (<0.20); small (0.20–0.49); moderate (0.50–0.79); and large (≥0.80). The effectiveness of blinding was examined using the Bang's Blinding Index, as explained elsewhere (Venier et al., 2019a). All analyses were performed using the Statistica software (version 13.0; StatSoft; Tulsa, OK, USA). The significance level was set at $p < 0.05$.

10.4. Results

10.4.1. Exercise performance

For movement velocity and power, we found significant effects of caffeine ingestion for all outcomes except for mean velocity at 25% of 1RM, and mean velocity, peak power, and peak velocity at 50% of 1RM (Figure 15). The significant ESs ranged from 0.16 to 0.53. For muscular endurance, we found significant effects of caffeine ingestion on the total number of performed repetitions and the quality of repetitions when matched for repetitions between the conditions. Here, the ESs ranged from 0.27 to 0.96 (Table 18). We also found a significant effect of caffeine ingestion on vertical jump height with an ES of 0.13. For power output in the Wingate test, we found significant effects of caffeine ingestion on peak, mean, and minimum power. The ESs ranged from 0.34 to 0.41.

Figure 15. The effects of caffeine vs. placebo on peak power (upper left section), peak velocity (lower left section), mean power (upper right section), and mean velocity (lower right section) in the bench press with 25%, 50%, 75%, and 90% of one repetition maximum (1RM). Data are presented as mean ± standard deviation. * denotes significant differences between the conditions

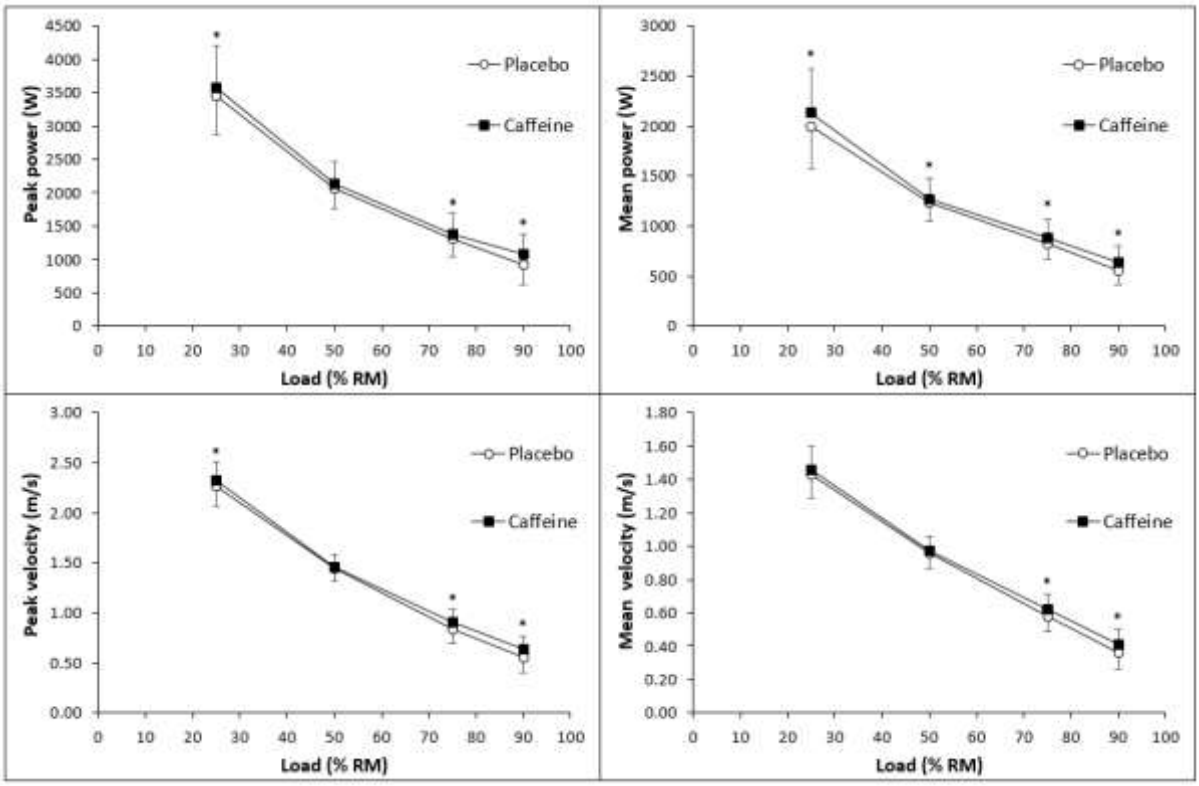

Table 18. Effects of caffeine ingestion on performance in the muscular endurance test, countermovement jump, and Wingate: results from a series of one-way repeated measures analyses of variance.

Variable	Placebo	Caffeine	Hedge's *g* and 95% CI	*p*-value
Muscular endurance test				
Maximum repetitions at 85% 1RM	6.9 ± 2.2	8.2 ± 2.1	0.58 (0.29, 0.91)	< 0.001
Mean power matched for repetitions (W)	418 ± 116	492 ± 138	0.56 (0.32, 0.83)	< 0.001
Mean velocity matched for repetitions (m/s)	0.27 ± 0.05	0.32 ±0.05	0.96 (0.58, 1.41)	< 0.001
Peak power matched for repetitions (W)	669 ± 250	740 ± 258	0.27 (0.14, 0.42)	< 0.001
Peak velocity matched for repetitions (m/s)	0.41 ± 0.08	0.46 ± 0.07	0.64 (0.38, 0.94)	< 0.001
CMJ				
Vertical jump height (cm)	35.0 ± 6.1	35.8 ± 5.9	0.13 (0.02, 0.25)	0.034
Wingate test				
Peak power in the Wingate test (W)	859 ± 237	948 ± 229	0.37 (0.21, 0.55)	< 0.001
Mean power in the Wingate test (W)	598 ± 101	634 ± 100	0.34 (0.17, 0.54)	< 0.001
Minimum power in the Wingate test (W)	349 ± 103	392 ± 96	0.41 (0.07, 0.78)	0.020
1RM: one repetition maximum: CMJ: countermovement jump; CI: confidence interval				

10.4.2. Assessment of blinding

Before the start of the exercise session, 50% and 65% of the participants correctly guessed (beyond chance) the placebo and caffeine conditions, respectively. After finishing the exercise session, 65% and 75% of the participants correctly guessed the placebo and caffeine conditions beyond chance, respectively. Participants who correctly identified caffeine reported "feeling more energised" and/or "more alert", or they associated the improvements in exercise performance with caffeine ingestion.

10.5. Discussion

The main finding of this study is that caffeine ingestion may be ergogenic for *ADORA2A* (rs5751876) C allele carriers in a range of exercise performance outcomes. Therefore, these results do not support the theoretical supposition that *ADORA2A* C allele carriers do not experience improvements in exercise performance following caffeine ingestion.

Our findings are not in accord with the Loy et al. (2015) study, which proposed that *ADORA2A* C allele carriers do not experience an ergogenic response to caffeine supplementation. The main differences between our study and Loy et al. (2015) are the sex of the participants and the exercise tests employed. Specifically, we included male participants, whereas Loy and colleagues included females. Therefore, it might be that female *ADORA2A* C allele carriers experience a different response to caffeine ingestion as compared to their male counterparts. However, this explanation is perhaps less plausible because recent evidence suggests that female and male participants experience similar ergogenic responses to caffeine ingestion in aerobic-, anaerobic- and strength-based exercise tasks (Mielgo-Ayuso et al., 2019; Sabblah et al., 2015; Skinner et al., 2019). Importantly, the present study and the work by Loy et al. (2015) also differed in the selection of performance tests; while we assessed changes in power, muscular endurance, and sprinting performance, Loy and colleagues focused on aerobic endurance. It may be that caffeine affects performance in these components of exercise performance through different mechanisms. The possible impact of genetic variations might be more expressed in some tests and less in others. Given the scarce evidence on the influence of polymorphisms in *ADORA2A* on the individual variation in responses to caffeine, this topic certainly requires further research. Finally, given that we report here that *ADORA2A* C allele carriers improve performance following caffeine ingestion, this might suggest that other

genotypes that were not tested herein (e.g., *CYP1A2* AA and AC/CC genotypes) are more important for the individual responses to caffeine ingestion.

Interestingly, the effects of caffeine on exercise performance in this study were very similar in size to the effects previously reported in the literature. For example, the increases in muscular endurance in our study are similar to the performance benefits of caffeine recorded in a previous study that included individuals with *CYP1A2* (rs762551) AA genotype—which are suggested to experience the most profound ergogenic benefits of caffeine (Rahimi, 2019). Furthermore, the increases in movement velocity, vertical jump height, and power output in the Wingate test are comparable to the improvements reported in meta-analyses of these outcomes among samples that were not genotype-specific (Grgic et al., 2018; Grgic, 2018; Raya-González et al., 2020). For example, one meta-analysis (Grgic, 2018) reported that caffeine ingestion acutely enhances Wingate peak power by an ES of 0.27 (95% CI: 0.08, 0.47), which is very similar to the ES of 0.37 (95% CI: 0.21, 0.55) observed in this study.

10.5.1. Strengths and limitations

The main strength of the present study is the use of a randomised, double-blind study design, which is identified as the gold standard in sports nutrition (Burke, 2008). Additionally, the strength of the present study is in the use of exercise tests for which caffeine has been shown to be ergogenic.

The main limitation of this study is that 50% to 75% of the participants were able to identify caffeine and placebo conditions beyond chance. However, these results are not a likely explanation of the differences in findings between our study and the Loy et al. (2015) study given that the majority of participants (>75%) in the Loy et al. study were able to guess the content of the capsules correctly. Additionally, given the small number of *ADORA2A* TT homozygotes in our sample, we could not assess whether they experience different responses to caffeine ingestion compared with C allele carriers, an area that should be explored in future research. The low number of participants classified as TT homozygotes could be explained by the estimate that around 85% of the population possess the CC/CT genotype at rs5751876 (Erblang et al., 2019).

Finally, to avoid any potential confounding by prior food and caffeine ingestion (Roberts et al., 2010; Yeo, Jentjens, Wallis, & Jeukendrup, 2005) we opted to test the participants in a fasted state. This needs to be acknowledged as a limitation given that caffeine supplementation and exercise in a fasted state is likely not a "real-life" practice of many individuals, and is not in line with the current sports nutrition recommendations (Aird, Davies, & Carson, 2018). Future studies may consider further exploring this topic, by using caffeine supplementation protocols that mirror those more commonly observed in practice.

10.6. Conclusions

Our findings suggest that *ADORA2A* (rs5751876) C allele carriers respond positively to caffeine supplementation. Therefore, individuals with the CT/CC genotype may still consider supplementing with caffeine for acute improvements in performance. Future research is needed to explore if *ADORA2A* TT homozygotes experience different responses to caffeine supplementation than C allele carriers.

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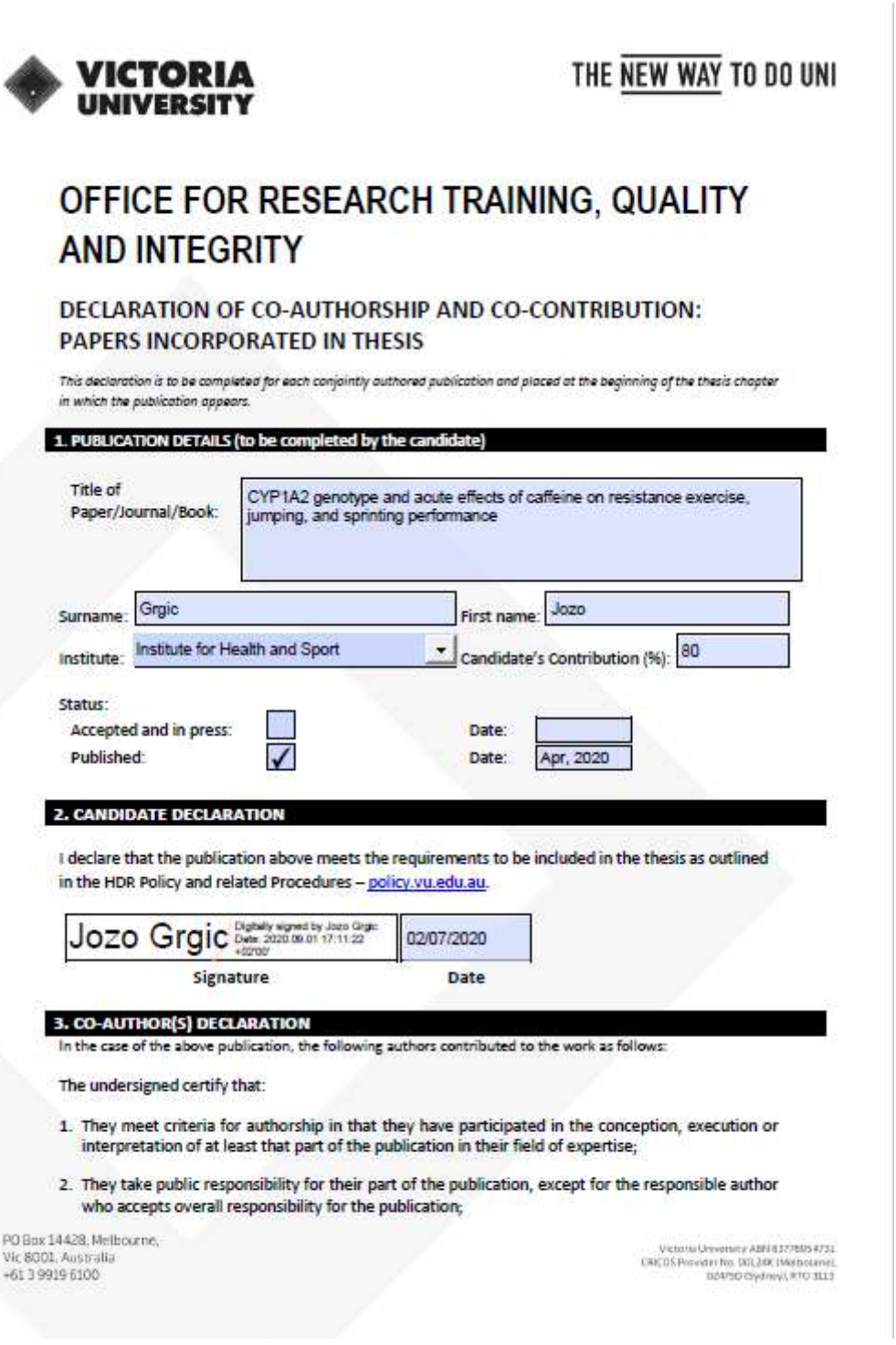
11. CYP1A2 genotype and acute effects of caffeine on resistance exercise, jumping, and sprinting performance



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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

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Surname: Grgic First name: Jozo

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2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

Jozo Grgic Digitally signed by Jozo Grgic Date: 2020.09.01 17:11:22 +02'00' 02/07/2020

Signature Date

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
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3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following location(s):

The original data has been electronically stored on VU R drive and the student's laptop for at least five years since April 2020.

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Craig Pickering	5	Data collection and interpretation, and manuscript writing	[redacted]	02/07/2020
David J. Bishop	5	Conceptualization, data interpretation, and manuscript writing		02/07/2020
Brad J. Schoenfeld	2.5	Data interpretation and manuscript writing		02/07/2020
Pavle Mikulic	2.5	Data interpretation and manuscript writing		02/07/2020
Željko Pedisic	5	Conceptualization, data interpretation, and manuscript writing		02/07/2020

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CYP1A2 genotype and acute effects of caffeine on resistance exercise, jumping, and sprinting performance

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11.1. Abstract

Background: It has been suggested that polymorphisms within *CYP1A2* impact inter-individual variation in the response to caffeine. The purpose of this study was to explore the acute effects of caffeine on resistance exercise, jumping, and sprinting performance in a sample of resistance-trained men, and to examine the influence of genetic variation of *CYP1A2* (rs762551) on the individual variation in responses to caffeine ingestion.

Methods: Twenty-two men were included as participants (AA homozygotes $n = 13$; C allele carriers $n = 9$) and were tested after the ingestion of caffeine (3 mg/kg of body mass) and a placebo. Exercise performance was assessed with the following outcomes: (a) movement velocity and power output in the bench press exercise with loads of 25%, 50%, 75%, and 90% of one-repetition maximum (1RM); (b) quality and quantity of performed repetitions in the bench press exercise performed to muscular failure with 85% 1RM; (c) vertical jump height in a countermovement jump test; and (d) power output in a Wingate test.

Results: Compared to placebo, caffeine ingestion enhanced: (a) movement velocity and power output across all loads (ES: 0.20–0.61; $p < 0.05$ for all); (b) the quality and quantity of performed repetitions with 85% of 1RM (ES: 0.27–0.85; $p < 0.001$ for all); (c) vertical jump height (ES: 0.15; $p = 0.017$); and (d) power output in the Wingate test (ES: 0.33–0.44; $p < 0.05$ for all). We did not find a significant genotype × caffeine interaction effect (p-values ranged from 0.094 to 0.994) in any of the analysed performance outcomes.

Conclusions: Resistance-trained men may experience acute improvements in resistance exercise, jumping, and sprinting performance following the ingestion of caffeine. The comparisons of the effects of caffeine on exercise performance between individuals with the AA genotype and AC/CC genotypes found no significant differences.

11.2. Background

Caffeine is one of the most consumed psychoactive stimulants in the world (Graham, 2001). The effects of caffeine supplementation on exercise performance have received considerable attention in the literature, and the evidence on its ergogenic effects is well-established (Graham, 2001; Grgic et al., 2020a; McLellan et al., 2016). For example, a recent umbrella review of 21 published meta-analyses reported that caffeine ingestion is acutely ergogenic for aerobic endurance, muscle strength, muscle endurance, power, jumping performance, and exercise speed (Grgic et al., 2020a). Despite these established performance-enhancing effects of caffeine, it is also commonly acknowledged that there is a large degree of variation in response to caffeine supplementation between individuals (Pickering & Kiely, 2018). Studies that have reported individual participant data suggest that some individuals experience an increase in performance following caffeine ingestion, whereas others do not (Grgic & Mikulic, 2017; Pickering & Grgic, 2019; Womack et al., 2012). In order to develop more effective guidelines for caffeine supplementation in sport and exercise settings, the scientific focus has recently been placed on examining and understanding the reasons for the between-individual variation in responses (Pickering & Grgic, 2019; Pickering & Kiely, 2018).

One potential driver of this individual response is inter-individual genetic variation (Pickering & Kiely, 2018). The gene *CYP1A2* encodes cytochrome P450 1A2, an enzyme responsible for up to 95% of caffeine metabolism (Gu et al., 1992). The speed of caffeine metabolism is affected by a single nucleotide polymorphism, rs762551, within this gene (Gu et al., 1992). Individuals with the AA genotype at rs762551 are commonly classified as 'fast caffeine metabolisers', while C allele carriers (AC/CC genotypes) tend to have a slower clearance of caffeine and are, therefore, commonly classified as 'slow caffeine metabolisers' (Sachse et al., 1999). Significantly greater ergogenic effects of caffeine on aerobic endurance have been reported for individuals with the AA genotype, compared with C allele carriers (Guest et al., 2018; Womack et al., 2012). However, for high-intensity exercise tasks of a shorter duration, the evidence is less clear.

In a recent study of 19 basketball players, acute ingestion of 3 mg/kg of caffeine produced similar effects on vertical jump performance in individuals with the AA genotype and AC/CC genotypes (Puente et al., 2018). These results are in accord with a study that utilised a 30-second

Wingate sprint test, while improvement in peak and mean power output was noted following caffeine ingestion, the researchers did not find differences in responses between genotypes (Salinero et al., 2017). Based on the results of these two studies, it seems variations in the *CYP1A2* genotype may not affect the ergogenic effects of caffeine ingestion on high-intensity exercise performance. However, a recent study reported that caffeine ingestion enhances the number of performed repetitions in a resistance exercise session in individuals with the AA genotype but not AC/CC genotypes (Rahimi, 2019).

Given the conflicting evidence on this topic, the aim of this randomised, double-blind crossover study was to explore the acute effects of caffeine on resistance exercise, jumping, and cycle ergometer sprint performance in a sample of resistance-trained men and the influence of genetic variation of *CYP1A2* (rs762551) on the individual variation in responses. We hypothesised that caffeine ingestion would be ergogenic across all exercise tasks and that individuals with the AA genotype would experience greater improvements in exercise performance following caffeine ingestion than those with AC/CC genotypes.

11.3. Methods

11.3.1. Experimental design

This study employed a double-blind, randomised, crossover design. All participants attended four laboratory sessions. All trials were performed in the morning hours (between 7 am and noon), and at the same time of the day across the sessions for each participant, to ensure that the results were not affected by circadian variation (Grgic et al., 2019a). The trials took place four to seven days apart. The first and second session included familiarisation with the exercise protocol (explained in detail in the "Exercise protocol" section). The two main sessions (i.e., caffeine and placebo sessions) were conducted in a randomised and counterbalanced order. The participants were randomly assigned to the two conditions; half of the participants ingested caffeine in the first session and a placebo in the second session, while the other half ingested a placebo in the first session and caffeine in the second session. Participants were asked not to perform any strenuous exercise for at least twenty-four hours before the main trials. The participants were also asked to keep a food diary for 24 hours using "MyFitnessPal" software, and to match their dietary intakes on the days before the two main sessions as much as possible.

The participants were required to refrain from caffeine intake after six pm on the day prior to the testing (Graham, 2001). In order to assist with caffeine restriction, we provided the participants with a list of the most common foods and drinks that contain caffeine. The participants arrived at the laboratory following overnight fasting. Caffeine was administered in capsule form, with a dose of 3 mg/kg of body mass (equivalent to the caffeine dose contained in approximately two cups of coffee). The placebo capsule was identical in appearance to the caffeine capsule, but, instead of caffeine, it contained 3 mg/kg of dextrose. The capsules were ingested 60 minutes before the start of the exercise session (Graham, 2001). Genotype was determined using a buccal swab. A validated Food Frequency Questionnaire was used to estimate habitual caffeine intake (Bühler et al., 2014). Prior to the study, the trial was registered in the Australian New Zealand Clinical Trials Registry ID: ACTRN12619000885190.

11.3.2. Participants

The study involved resistance-trained men as participants. Being resistance-trained was defined in this study as having a minimum of six months of resistance training experience with a minimum weekly training frequency of two times on most weeks. Based on an *a priori* power analysis done using G*Power software (version 3.1; Germany, Dusseldorf) for repeated-measures Analysis of Variance (ANOVA) (within-between interaction, i.e., in the context of this study genotype × caffeine interaction), with an assumed true ES *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 80% for this study was 18 participants. To factor in possible dropouts, we recruited 22 participants. The exclusion criteria were: (i) prior use of anabolic steroids; and (ii) the existence of any health limitations. Ethical approval for this study was granted by the Victoria University Human Research Ethics Committee (HRE19-019). The remaining data of the project are published elsewhere (Grgic et al., 2020a). Before enrolling in the study, every participant signed an informed consent and filled out a Physical Activity Readiness Questionnaire (PAR-Q). Only participants who responded with 'No' to all PAR-Q items were included in the study. In line with previous research (Puente et al., 2018; Rahimi, 2019; Salinero et al., 2017; Womack et al., 2012) we combined participants with the AC and CC genotypes into one group (AC/CC group) for the analysis.

11.3.3. Exercise protocol

11.3.3.1. One repetition maximum testing

The first two sessions included familiarisation with the exercise protocol. These sessions were the same as the main sessions (i.e., placebo and caffeine sessions), with the exception that the first one included one-repetition maximum (1RM) testing in the bench press exercise. For the 1RM test, the participants performed sets of one repetition with progressive increases in load until they reached their estimated 1RM. The load was initially set to 20 kg and subsequently increased by 10 kg increments if the mean concentric velocity of the repetition was 0.4 m/s or higher (as determined by a linear position transducer attached to the barbell). If the mean velocity was lower than 0.4 m/s, the load for the next attempt was adjusted using smaller increases (e.g., 5 kg or 2.5 kg, determined based on consultation with the participants). The participants performed 1RM attempts with progressively increasing loads until the mean velocity was ≤0.2 m/s (González-Badillo & Sánchez-Medina, 2010). When the mean velocity of a successful 1RM attempt reached these values, the load was considered as a valid estimate of the 1RM (González-Badillo & Sánchez-Medina, 2010). Three minutes were allowed between 1RM attempts.

11.3.3.2. Movement velocity and power in the bench press exercise

In the first session, upon determining the 1RM, the participants performed the bench press exercise with loads of 25%, 50%, 75%, and 90% of 1RM (Orange et al., 2020). The second, third, and fourth sessions started with the assessment of movement velocity in the bench press exercise with different loads, as the 1RM test was only performed in the first session. The external load was first set at 25% of 1RM and was progressively increased to 90% of 1RM. With each load, the participants performed two sets of one repetition and were instructed to lift the load as fast as possible. The better repetition (in the context of higher movement velocity and power output) was used for the analysis. Each repetition was followed by a 3-min rest interval. During each repetition, a GymAware linear position transducer (GymAware Power Tool, Kinetic Performance Technologies, Canberra, Australia) was attached to the barbell and used to measure mean concentric velocity (m/s), mean power (W), peak concentric velocity (m/s), and peak power (W). Previous research has established that this device has good test-retest reliability for power and velocity outcomes in the bench press (Orange et al., 2020).

11.3.3.3. Muscle endurance

After the final repetition with 90% of 1RM, participants were provided with 5 minutes of passive rest. After the rest interval, muscle endurance was assessed with a test that involved performing repetitions to momentary muscle failure with a load corresponding to 85% of 1RM in the bench press exercise, as in the study by Rahimi (2019). Besides the total number of repetitions, we also measured velocity and power output for each repetition using the linear position transducer attached to the barbell. For the purpose of statistical analyses, we compared the total number of repetitions in the placebo and caffeine conditions. We also explored movement velocity and power output of all repetitions by matching the number of repetitions between the placebo and caffeine conditions. For example, if a participant performed eight repetitions following the ingestion of placebo and nine following the ingestion of caffeine, for this part of the analysis, we only considered movement velocity and power output in the first eight repetitions. This approach allowed us to objectively quantify the average quality of the repetitions during the test and examine if caffeine ingestion had an effect on movement velocity and power output when the total number of repetitions was matched.

11.3.3.4. Countermovement jump

After the muscle endurance test, participants rested passively for three minutes and then performed one minute of light running, followed by ten bodyweight squats, in order to warm-up for the countermovement jump (CMJ). The participants performed a CMJ on a force platform (400S Isotronic Fitness Technology, Skye, South Australia, Australia). The CMJ was performed without an arm swing. The participants started CMJ testing from an upright standing position on the force platform. The participants positioned themselves in the starting position and then received commands from the software displayed on a computer screen that was in front of the platform. The software counted down, “3, 2, 1” and provided “Set” and “Go” commands. After the “Go” command, the participants had five seconds to complete the jump. From the starting position, the participants performed a downward countermovement (i.e., a fast knee flexion) where their lowest position was a semi-squat position (knee ~90° and trunk/hips in a flexed position) (Venier et al., 2019a). Immediately after reaching this point, the participants performed an ‘explosive’ extension of the legs (Venier et al., 2019a). The participants were given instructions to jump as quickly and ‘explosively’ as possible to achieve maximal vertical jump height (Venier et al., 2019a). The participants had one warm-up jump and three official attempts. Each attempt was followed by one minute of rest. For the analysis,

the best jump from three official attempts was used. The outcome in the CMJ test was vertical jump height, determined by an algorithm based on the flight time.

11.3.3.5. Wingate test

After the CMJ test, the participants were provided another three minutes of passive rest before starting the Wingate test. The Wingate test was performed using a Lode Excalibur Sport Cycle Ergometer (The Netherlands, Groningen). Individual setup of the cycle ergometer; namely, saddle and handlebar height and length, was determined in the first session and was maintained throughout all subsequent trials. The Wingate test started with a 5-minute warm-up (100 W at 60-80 rpm) (Frikha et al., 2016). After the warm-up, participants performed a 30-second 'all-out' sprint while the resistance placed on the flywheel remained constant at 0.075 Nm/kg. The participants remained seated during the 30-second sprint. During the test, peak power, mean power, and minimum power were recorded using the Lode Ergometry Manager 10 software. Peak power was defined as the greatest power value recorded during the 30-seconds; mean power was the arithmetic mean of power during the test, and minimum power was the lowest power recorded during the sprint.

11.3.4. Side effects

Side effects of caffeine and placebo supplementation were evaluated at two time points: (1) immediately after the completion of the testing sessions; and (2) in the following mornings, upon waking. The participants responded to an 8-item survey regarding the incidence of side effects ("yes/no" response scale). This survey was also used to examine side effects in previous research that explored effects of caffeine on exercise performance (Diaz-Lara et al., 2016; Venier et al., 2019a, 2019b).

11.3.5. Assessment of blinding

Both in the caffeine and the placebo trials, before and after the exercise session, participants responded to the following question: "Which supplement do you think you have ingested?" (Saunders et al., 2017). The question had three possible responses: (a) "caffeine", (b) "placebo" and (c) "I do not know" (Saunders et al., 2017). In case participants respond with "a" or "b", they were required to state the reason for choosing their response.

11.3.6. Genetic testing

The participants underwent genetic testing using a commercially available testing kit from DNAfit Life Sciences (London, UK), as in other studies (Pickering et al., 2018). Samples were collected using buccal swab devices, with OCR-100 kits by DNAGenotek (Ottawa, Canada). The participants were required to avoiding eating or drinking for at least 60 minutes prior to the sample collection. All samples were collected according to the manufacturer guidelines. The samples were sent to IDna Genetics Laboratory (Norwich, UK), where the analysis was performed. DNA was extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Cell Projects Ltd, Kent, UK), and amplified through polymerase chain reaction (PCR) on an ABI 7900 real-time thermocycler (Applied Biosystem, Waltham, USA). The samples were analysed for the *CYP1A2* rs762551 single-nucleotide polymorphism. This analysis was performed after the exercise performance data collection; thus, the researchers and participants were blinded to genotype variations of the cohort until the data collection process was finalised.

11.3.7. Statistical analysis

One-way ANOVA was used to test the differences between genotype groups in age, body mass, height, 1RM, and habitual caffeine intake. We used a two-way, repeated-measures ANOVA to test genotype (AA genotype vs. AC/CC genotypes) × caffeine (placebo vs. caffeine) interaction effect on performance data, separately for each performance variable. In the absence of significant genotype × caffeine interaction effects, we conducted no stratified analyses of the effects of caffeine by genotype groups. Relative ESs were calculated as Hedge's *g* for repeated measures and presented together with their respective 95% confidence intervals (95% CIs). ESs of <0.20, 0.20 to 0.49, 0.50 to 0.79, and ≥0.80 were considered to represent trivial, small, moderate, and large effects, respectively. McNemar's test was used in the comparison of the incidence of side effects between the placebo and caffeine conditions. The blinding data were summarised using the Bang's Blinding Index [26]. The values in this index range from –1.0 (denoting opposite guessing) to 1.0 (denoting complete unblinding) (Bang, Ni, & Davis, 2004). For this study, we reported the data from this index as a percentage of individuals who identified the correct treatment condition beyond chance (Bang et al., 2004; Venier et al., 2019a). All analyses were performed using the Statistica software (version 13.4.0.14; TIBCO Software Inc., Palo Alto, CA, USA). The significance level was set at $p < 0.05$.

11.4. Results

11.4.1. Study participants

All participants completed all testing procedures and were included in the final analysis. Of the whole sample, 13, 7, and 2 participants were categorised as having the AA, AC, or CC genotype, respectively. The participants' characteristics are presented in Table 19. There were no significant differences between the genotype groups for age, body mass, height, 1RM, or habitual caffeine intake.

Table 19. Characteristics of the participants

Variable	AA group (n = 13)	AC/CC group (n = 9)	p-values from one-way ANOVA
Age (years)	27.0 ± 5.6	29.8 ± 3.6	0.205
Body mass (kg)	78.2 ± 6.5	80.9 ± 14.8	0.559
Height (cm)	182.2 ± 5.5	183.2 ± 5.7	0.658
1RM in the bench press (normalised per body mass)	1.1 ± 0.1	1.2 ± 0.2	0.240
Habitual caffeine intake (mg/day)	133 ± 123	117 ± 68	0.286
Data reported as mean ± standard deviation; 1RM: one repetition maximum; habitual caffeine intake was estimated using a Food Frequency Questionnaire			

11.4.2. Movement velocity and power output in the bench press exercise

We did not find a significant main effect for group ($p > 0.05$ for all) or a genotype × caffeine interaction effect for any of the 16 analysed variables for movement velocity and power output in the bench press exercise (mean power, mean velocity, peak power, and peak velocity at 25%, 50%, 75%, and 90% 1RM; Table 20). For all variables, except peak power output at 50% 1RM, there was a significant main effect for caffeine ($p < 0.05$). The ESs, favouring caffeine conditions in all outcomes, ranged from 0.20 to 0.29 for all outcomes recorded at 25% 1RM,

from 0.21 to 0.23 for all outcomes at 50% 1RM, from 0.31 to 0.50 for all outcomes at 75% 1RM, and from 0.57 to 0.61 for outcomes at 90% 1RM.

11.4.3. Muscle endurance

For the maximum number of repetitions in the bench press exercise with 85% 1RM, we did not find a significant main effect for genotype ($p = 0.397$) or a genotype × caffeine interaction effect ($p = 0.454$), while there was a significant main effect favouring caffeine ($p < 0.001$; ES = 0.53). For peak velocity, mean power output, and peak power output (matched for repetitions between placebo and caffeine conditions), we did not find a significant main effect for genotype ($p > 0.05$ for all) or a genotype × caffeine interaction effect ($p > 0.05$ for all), while there was a significant main effect for caffeine in all three variables ($p < 0.001$ for all). The ESs ranged from 0.27 to 0.53. For mean velocity, there was a significant main effect for genotype ($p = 0.034$), with the AC/CC genotypes producing greater movement velocity than the AA genotype, and a significant main effect favouring caffeine ($p < 0.001$; ES = 0.85), while we found no significant genotype × caffeine interaction effect ($p = 0.094$).

11.4.4. Countermovement jump

For vertical jump height in the CMJ test, we did not find a significant main effect for genotype ($p = 0.447$) or a genotype × caffeine interaction effect ($p = 0.752$), while there was a significant main effect favouring caffeine ($p = 0.017$; ES = 0.15).

11.4.5. Wingate test

For peak power in the Wingate test, we did not find a significant main effect for genotype ($p = 0.998$) or a genotype × caffeine interaction effect ($p = 0.542$), while there was a significant main effect favouring caffeine ($p < 0.001$; ES = 0.33). For mean power in the Wingate test, we did not find a significant main effect for genotype ($p = 0.517$) or a genotype × caffeine interaction effect ($p = 0.583$), while there was a significant main effect favouring caffeine ($p < 0.001$; ES = 0.35). For minimum power in the Wingate test, we did not find a significant main effect for genotype ($p = 0.505$) or a genotype × caffeine interaction effect ($p = 0.396$), while there was a significant effect favouring caffeine ($p = 0.011$; ES = 0.44).

Table 20. Effects of caffeine on resistance exercise, jumping, and sprinting performance: results from the two-way, repeated-measures ANOVA

Variable	**AA genotype (placebo)**	**AA genotype (caffeine)**	**AC/CC genotypes (placebo)**	**AC/CC genotypes (caffeine)**	**Main effect for genotype** *p*-**value**	**Main effect for caffeine** *p*-**value**	**Genotype × caffeine interaction effect** *p*-**value**	**Effect size for condition and its 95% CI**
Movement velocity and power in the bench press with different loads								
MP at 25% 1RM (W)	1892 ± 299	2012 ± 325	2152 ± 501	2279 ± 517	0.139	0.001	0.918	0.29 (0.12, 0.46)
MV at 25% 1RM (m/s)	1.41 ± 0.12	1.44 ± 0.14	1.46 ± 0.16	1.49 ± 0.15	0.411	0.035	0.566	0.20 (0.02, 0.39)
PP at 25% 1RM (W)	3287 ± 374	3409 ± 384	3598 ± 688	3703 ± 804	0.215	0.033	0.868	0.20 (0.03, 0.37)
PV at 25% 1RM (m/s)	2.21 ± 0.18	2.27 ± 0.18	2.31 ± 0.20	2.35 ± 0.17	0.244	0.008	0.806	0.26 (0.07, 0.46)
MP at 50% 1RM (W)	1182 ± 145	1217 ± 154	1279 ± 214	1333 ± 249	0.196	0.008	0.545	0.22 (0.06, 0.39)
MV at 50% 1RM (m/s)	0.94 ± 0.08	0.97 ± 0.08	0.96 ± 0.11	0.98 ± 0.10	0.711	0.019	0.955	0.21 (0.02, 0.42)
PP at 50% 1RM (W)	1979 ± 201	2036 ± 220	2122 ± 394	2203 ± 406	0.228	0.090	0.753	0.21 (-0.03, 0.46)
PV at 50% 1RM (m/s)	1.41 ± 0.09	1.43 ± 0.09	1.44 ± 0.18	1.48 ± 0.16	0.468	0.031	0.489	0.23 (0.03, 0.45)
MP at 75% 1RM (W)	789 ± 144	838 ± 151	849 ± 148	928 ± 198	0.281	< 0.001	0.229	0.36 (0.19, 0.56)
MV at 75% 1RM (m/s)	0.56 ± 0.07	0.60 ± 0.07	0.58 ± 0.10	0.63 ± 0.10	0.618	< 0.001	0.514	0.48 (0.27, 0.72)

PP at 75% 1RM (W)	1210 ± 238	1289 ± 233	1369 ± 207	1453 ± 293	0.128	0.007	0.940	0.31 (0.10, 0.54)
PV at 75% 1RM (m/s)	0.80 ± 0.12	0.88 ± 0.09	0.86 ± 0.17	0.91 ± 0.17	0.433	< 0.001	0.243	0.50 (0.26, 0.77)
MP at 90% 1RM (W)	501 ± 128	582 ± 132	588 ± 109	675 ± 143	0.103	< 0.001	0.850	0.61 (0.31, 0.93)
MV at 90% 1RM (m/s)	0.33 ± 0.06	0.38 ± 0.07	0.38 ± 0.12	0.43 ± 0.09	0.182	< 0.001	0.909	0.57 (0.28, 0.89)
PP at 90% 1RM (W)	821 ± 225	970 ± 231	994 ± 301	1165 ± 308	0.099	< 0.001	0.789	0.57 (0.25, 0.91)
PV at 90% 1RM (m/s)	0.50 ± 0,09	0.59 ± 0.11	0.59 ± 0.18	0.67 ± 0.13	0.117	< 0.001	0.966	0.59 (0.27, 0.95)
Muscle endurance test								
Maximum repetitions at 85% 1RM	6.8 ± 2.3	8.2 ± 2.2	7.8 ± 2.4	8.8 ± 2.2	0.397	< 0.001	0.454	0.53 (0.27, 0.81)
MP matched for repetitions (W)	376 ± 86	449 ± 96	476 ± 122	531 ± 159	0.074	< 0.001	0.406	0.53 (0.31, 0.79)
MV matched for repetitions (m/s)	0.25 ± 0.04	0.30 ± 0.04	0.30 ± 0.05	0.33 ± 0.04	0.034*	< 0.001	0.094	0.85 (0.50, 1.25)
PP matched for repetitions (W)	607 ± 178	674 ± 187	741 ± 297	808 ± 300	0.201	< 0.001	0.994	0.27 (0.14, 0.41)
PV matched for repetitions (m/s)	0.38 ± 0.06	0.43 ± 0.05	0.44 ± 0.09	0.48 ± 0.08	0.108	< 0.001	0.198	0.51 (0.28, 0.77)
CMJ								
CMJ vertical jump height (cm)	34.8 ± 6.2	35.6 ± 5.9	36.6 ± 5.2	37.6 ± 5.4	0.447	0.017*	0.752	0.15 (0.03, 0.28)
Wingate								

PP in the Wingate test (W)	874 ± 208	943 ± 197	864 ± 273	954 ± 260	0.998	< 0.001	0.542	0.33 (0.16, 0.52)
MP in the Wingate test (W)	583 ± 77	614 ± 67	606 ± 120	646 ± 132	0.517	< 0.001	0.583	0.35 (0.20, 0.52)
MinP in the Wingate test (Watts)	338 ± 108	372 ± 79	350 ± 109	414 ± 114	0.505	0.011	0.396	0.44 (0.09, 0.81)
MP: mean power; MV: mean velocity; PP: peak power; PV: peak velocity; 1RM: one repetition maximum: MinP: minimum power; CMJ: countermovement jump; CI: confidence interval								

Table 21. Perceived side effects based on questionnaires completed immediately after the testing session and the following morning

Variable	**AA group – placebo**	**AA group – caffeine**	**AC/CC group – placebo**	**AC/CC group – caffeine**
	Immediately after testing session			
Muscle soreness	46%	23%	0%	0%
Increased urine production	0%	23%	0%	11%
Tachycardia and heart palpitations	8%	8%	0%	0%
Increased anxiety	0%	23%	0%	0%
Headache	8%	8%	11%	11%
Abdominal/gut discomfort	0%	0%	0%	0%
Increased vigour/activeness	23%	62%	0%*	67%*
Perception of improved performance	15%	62%	11%*	100%*
	The following morning			
Muscle soreness	23%	8%	0%	22%
Increased urine production	8%	0%	0%	11%
Tachycardia and heart palpitations	0%	0%	0%	0%

Increased anxiety	0%	0%	0%	0%
Headache	8%	8%	22%	0%
Abdominal/gut discomfort	0%	0%	0%	0%
Insomnia	8%	0%	0%	11%
Increased vigor/activeness	0%	0%	0%	33%
* Significant difference between the placebo and caffeine conditions within a group				

11.4.6. Side effects

In the responses recorded immediately post-exercise, we found a significant difference between the placebo and caffeine conditions only in items "Increased vigour/activeness" and "Perception of improved performance" in the AC/CC genotypes (Table 21). In the responses 24-hours after capsule ingestion, we did not find any significant differences in the incidence of side effects between the placebo and caffeine conditions.

11.4.7. Assessment of blinding – AA genotype

Before starting the exercise session, in the placebo and caffeine conditions, respectively, 62% and 54% of the participants with the AA genotype correctly guessed the treatment identity beyond chance. After exercise, in the placebo and caffeine conditions, respectively, 85% and 69% of the participants with the AA genotype correctly guessed the treatment identity beyond chance.

11.4.8. Assessment of blinding – AC/CC genotypes

Before starting the exercise session, in both the placebo and caffeine conditions, 55% of the participants with the AC/CC genotypes correctly guessed the treatment identity beyond chance. After exercise, in the placebo and caffeine conditions, respectively, 44% and 78% of the participants with the AC/CC genotypes correctly guessed the treatment identity beyond chance, respectively.

11.5. Discussion

The results of the present study demonstrate that the acute ingestion of a moderate dose of caffeine (3 mg/kg) may produce significant improvements in: (a) movement velocity and power output in the bench press using loads ranging from 25% to 90% of 1RM; (b) maximum number of repetitions performed to momentary muscle failure in the bench press exercise, as well as the average quality (i.e., higher movement velocity and power output) of the performed repetitions; (c) vertical jump height; and (d) peak, mean, and minimum power in the 30-second Wingate test. No significant differences in the effects of caffeine were found between the individuals with the AA genotype and the individuals with the AC/CC genotypes in any of the performance tests used in the present study.

11.5.1. Effects of caffeine on exercise performance

In the bench press exercise, caffeine ingestion enhanced peak and mean velocity and consequently, mean and peak power, when exercising with low, moderate, and high loads. These results are generally in line with previous findings (Pallarés et al., 2013; Venier et al., 2019a, 2019b). One of the early studies (Pallarés et al., 2013) conducted on this topic reported that high doses of caffeine (9 mg/kg) are required for acute increases in movement velocity when exercising with very high loads (90% 1RM). However, our results suggest that a dose of 3 mg/kg is effective for enhancing velocity across a wide range of external loads, suggesting that very high doses might not be needed. This is especially relevant to highlight as the ESs in our study are very similar to those reported for the bench press exercise by Pallarés et al. (2013).

A recent meta-analysis found that caffeine ingestion enhances mean and peak movement velocity in resistance exercise (Raya-González et al., 2020). The researchers also noted that the effects of caffeine on mean velocity (ES = 0.80) were higher than those for peak velocity (ES = 0.41) (Raya-González et al., 2020). However, the studies included in that meta-analysis assessed either mean or peak velocity: that is no studies included in the meta-analysis measured both outcomes in the same group of participants (Raya-González et al., 2020). In the present study, we found that the ESs were very similar for both mean and peak velocity, and this was a constant finding across all the employed loads (i.e., 25% to 90% of 1RM).

The muscle endurance test used in this study further confirmed that caffeine ingestion is ergogenic for this fitness component in resistance-trained men. This study adds to the body of evidence showing improvements in muscle endurance following caffeine ingestion (Cook et al., 2012; Grgic et al., 2020d; Polito et al., 2016; Warren et al., 2010). However, a more novel finding is that caffeine is ergogenic for power and velocity outputs when the number of repetitions between the caffeine and placebo conditions is matched. Specifically, when matching the number of repetitions between conditions, we found that the effects of caffeine, as compared to placebo, amounted to 0.27 for peak power, 0.51 for peak velocity, 0.53 for mean power, and 0.85 for mean velocity. Several studies that explored the effects of caffeine on muscle endurance did not find a difference in the number of performed repetitions between the caffeine and placebo conditions (Goldstein et al., 2010b; Rahimi, 2019; Woolf et al., 2009).

However, as we demonstrated in the present study, even with an equal number of repetitions between conditions, caffeine might have still produced considerable improvements in the quality of the performed repetitions, that is, greater movement velocity and consequently, greater power output (which was not tested in the aforementioned studies). As compared to placebo, caffeine ingestion most commonly produced moderate improvements in the number of performed repetitions (generally one to three additional repetitions). We propose that in some contexts, improvements in the overall quality of the performed repetitions may be more important for training adaptations than simply performing a greater number of repetitions. This hypothesis is in line with recent findings that training at a velocity loss of 20% produced greater improvement in CMJ performance than training at a 40% velocity loss (Pareja-Blanco et al., 2017). Improvements in squat strength were similar for both training conditions, even though the group that trained with a velocity loss of 20% performed 40% fewer repetitions.

Caffeine ingestion resulted in increased vertical jump height in the CMJ. The ES magnitude of 0.15 observed in this study is very similar to the pooled ES of 0.17 reported in a recent meta-analysis of 10 studies (Grgic et al., 2018). This result, therefore, confirms that caffeine ingestion may have a relatively small performance-enhancing effect on vertical jump height (Bloms et al., 2016; Grgic et al., 2018; Sabol et al., 2019). The acute improvement in vertical jump height following caffeine ingestion is comparable to the improvement in jump height found as a result of four weeks of plyometric training (Chimera, Swanik, Swanik, & Straub et al., 2004; Markovic, 2007). Even though the improvement in performance was relatively small (approximately 1 cm), it might still be practically meaningful in sports where jump height directly impacts athletic outcomes.

In the Wingate test, we found a significant ergogenic effect of caffeine on peak, mean, and minimum power. These results are in line with the findings of a recent meta-analysis that reported ergogenic effects of caffeine on mean and peak power in the ES magnitude of 0.18 and 0.27, respectively (Grgic, 2018). Of the 16 studies included in the meta-analysis (Grgic, 2018), 12 studies used caffeine doses of 5 or 6 mg/kg. Therefore, it could be argued that the findings of the meta-analysis should primarily be generalised to these doses of caffeine. In the present study, we found that even a lower dose of caffeine (namely, 3 mg/kg), increases

performance in this test and that the ES is very similar to that reported by studies using higher caffeine doses (Grgic, 2018).

11.5.2. The influence of the *CYP1A2* genotype

We did not find significant genotype × caffeine interaction effects in any of the analysed performance variables. It might be that the effects of caffeine ingestion are similar between different *CYP1A2* genotypes, at least for the performance tests used in the present study. The results reported herein are generally in line with the current body of evidence. Two studies (Puente et al., 2018; Salinero et al., 2017) that explored the effects of caffeine on jumping and Wingate test performance reported similar improvements in these outcomes following the ingestion of 3 mg/kg of caffeine in groups of participants with the AA and AC/CC genotypes. However, a recent study (Rahimi, 2019) that used a resistance exercise protocol, found that caffeine is ergogenic only for individuals with the AA genotype. On average, individuals with the AA genotype were able to complete one more repetition with the consumption of caffeine, as compared to placebo, whereas the number of repetitions was the same in the placebo and caffeine conditions among those with the AC/CC genotypes. The main methodological difference between the current studies exploring this topic was the dose of caffeine administered to the participants. Specifically, we and two other studies that reported similar results utilised 3 mg/kg of caffeine. We opted to utilise a lower dose of caffeine as higher doses of caffeine do not seem to produce greater increases in performance (Grgic et al., 2020d). In the study by Rahimi (2019), the dose was considerably higher (i.e., 6 mg/kg). It might be that the differences in responses between genotypes become apparent only at higher doses of caffeine. Future dose-response studies might consider exploring this hypothesis further. The effectiveness of the blinding was not explored by Rahimi (2019) thus limiting the comparison of the results in this aspect of the study design.

Even though Rahimi (2019) reported that caffeine ingestion is ergogenic for AA but not AC/CC genotypes in resistance exercise, the main outcome of their study was the number of performed repetitions in the bench press exercise with 85% 1RM, which can be considered as a somewhat crude test of performance. As mentioned previously, we demonstrated that even when matched for the number of repetitions, caffeine, as compared to placebo, increases the average movement velocity and power output of the performed repetitions (ES range = 0.27 to 0.85). Therefore,

even though Rahimi (2019) reported that in the AC/CC genotypes the total number of repetitions was the same following the ingestion of caffeine and placebo, caffeine might have still enhanced the average velocity and power of these repetitions. Therefore, we would suggest that future research in this area explores both the quality and quantity of the performed repetitions, to provide a more comprehensive assessment of possible effects of caffeine.

11.5.3. Strengths and limitations

Some of the key strengths of this study are: (a) the standardisation of testing conditions, including nutritional intake, physical activity, and the time of day at which the testing is conducted; (b) the inclusion of trained individuals as study participants; (c) a broad range of exercise performance variables that were assessed as outcomes; (d) assessment of performance across a wide-range of loads in the bench press exercise and both quantity and quality of repetitions, when examining muscle endurance as the outcome variable.

There are several potential limitations of this study that need to be acknowledged. First, due to the low number of individuals with the CC genotype, we combined the AC and CC genotypes into one group. This is fairly common in this line of research, as the number of individuals with the CC genotype in the population is suggested to be ~10% (Sachse et al., 1999). To get around 10 to 12 participants with the CC genotype a study would need to screen from 100 to 120 potential study participants. However, despite the fact this is a common practice, it could have confounded findings, as the effects of caffeine might not be uniform between individuals with the AC vs. CC genotype (Guest et al., 2018; Koonrungsesomboon, Khatsri, Wongchompoo, & Teekachunhatean, 2018). In the current study, we could not test this further, because the number of individuals with the CC genotype was $n = 2$. Of note, the exclusion of these two participants from the analysis did not alter the study results.

The second limitation is related to the efficacy of blinding (Saunders et al., 2017). Previous research has established that correct supplement identification may impact the outcomes of a given exercise test and, therefore, bias the results. In the present study, around 50% – 60% of the participants were able to correctly identify the placebo and caffeine condition beyond random chance in the pre-exercise assessment. In the post-exercise assessment, this percentage

generally stayed the same or slightly increased. We believe that the pre-exercise responses are of greater importance, given that the improvements during the testing session (or lack thereof) may influence the post-exercise responses. Tallis et al. (2013) tested their participants in four conditions: (1) “told caffeine, given caffeine”; (2) “told caffeine, given placebo”; (3) “told placebo, given placebo”; and (4) “told placebo, given caffeine”. Equal improvements were found on both occasions when the participants indeed ingested caffeine (i.e., “told caffeine, given caffeine” and “told placebo, given caffeine” conditions), thus suggesting that this limitation of our study might not have greatly affected our findings.

11.6. Conclusions

This study found that caffeine is acutely ergogenic for movement velocity, power output, and muscle endurance in resistance exercise, vertical jump height, and peak, mean, and minimum power in a Wingate test. These performance-enhancing effects were observed following the ingestion of using a moderate dose of caffeine (3 mg/kg), which resulted in minimal side effects. The comparisons of the effects of caffeine on exercise performance between individuals with the AA genotype and AC/CC genotypes found no significant differences.

Abbreviations

1RM: one repetition maximum

ANOVA: Analysis of variance

CI: confidence interval

CMJ: countermovement jump

ES: effect size

PAR-Q: Physical Activity Readiness Questionnaire

PCR: polymerase chain reaction

Contributions

JG, DJB, and ZP conceived and designed the study. JG performed the experiments, analysed the data, and wrote the first draft. ZP, DJB, CP, BJS, and PM critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Victoria University Human Research Ethics Committee (HRE19-019). The research was performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. Craig Pickering is a former employee of DNAFit Ltd, a genetic testing company. He received no financial incentives for the preparation of this manuscript.

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12. Discussion

This thesis thoroughly explored the effects of caffeine—one of the most popular ergogenic aids—on muscle strength, power, and endurance. Overall, the main findings are that: (a) caffeine ingestion acutely enhances exercise performance in various exercise tasks; (b) lower doses of caffeine may produce ergogenic effects comparable to those of higher doses of caffeine; (c) *ADORA2A* C allele carriers exhibit acute ergogenic effects to caffeine ingestion, with the magnitude of improvements similar to what was previously reported in the literature among samples that were not genotype-specific; and (d) there was no significant difference in the effects of caffeine on exercise performance between individuals with the *CYP1A2* AA genotype and AC/CC genotype.

Studies 3, 5, and 6 of this thesis showed that caffeine ingestion is acutely ergogenic for both upper and lower-body strength. Specifically, Study 5 established that caffeine ingestion in the dose of 6 mg/kg acutely enhanced 1RM squat strength by 3% (Grgic & Mikulic, 2017). The finding was reinforced in Study 3, which presented meta-analytical evidence regarding caffeine's ergogenic effects on strength (Grgic et al., 2018). Most commonly, caffeine supplementation is provided at a relatively high dose of 6 mg/kg (Spriet, 2014). However, such doses of caffeine may produce side-effects, such as nausea and insomnia. Therefore, Study 6 explored the effects of ingesting 2, 4, and 6 mg/kg of caffeine on strength and found that all three doses of caffeine were comparably ergogenic (Grgic et al., 2020d). Muscle strength is one of the most important muscular qualities in resistance exercise (Kell et al., 2001; Suchomel et al., 2016), and the prevalence of caffeine supplementation is very high among athletes in strength-based sports (Van Thuyne et al., 2006). Studies 3, 4, and 5 provided sound evidence in support of the use of caffeine to acutely improve muscle strength, which may be of practical importance for a large number of athletes, particularly when making decisions whether to use caffeine or not and what magnitude of the ergogenic effects can be expected. Given that in Study 6 smaller and larger doses of caffeine were found to be comparably ergogenic (Grgic et al., 2020d), athletes should consider using smaller doses of caffeine. It may be that such a strategy would reduce the incidence and severity of side-effects, while providing substantial improvements in muscle strength, potentially very similar to the ones provided by higher doses of caffeine. This finding may be particularly important for athletes who are more susceptible to side-effects of caffeine. Another important finding from these three studies was that caffeine's overall effect on strength was generally small (ES: 0.07 to 0.20). Taken this into account, it

may be that the ergogenic effects of caffeine are of practical importance primarily for professional athletes in strength-based sports, for whom even a small difference in performance at competition or small cumulative effects of improved performance in training sessions may play a significant role.

Another important muscular quality in resistance exercise is muscle endurance. The work presented in this thesis supports previous findings regarding caffeine's ergogenic effects on muscle endurance (Polito et al., 2016). For example, in Study 6, we found that caffeine ingestion allowed the participants to complete four more repetitions in a set of barbell squats performed to muscle failure with 60% of 1RM (Grgic et al., 2020d). Additionally, we demonstrated that lower doses of caffeine produced comparable effects on muscle endurance as higher doses of caffeine. Given that caffeine ingestion may acutely enhance muscle endurance and increase total volume load, future research should explore the long-term effects of caffeine on resistance training adaptations such as muscle strength and muscle hypertrophy. Previous research has established that muscle strength and muscle hypertrophy are enhanced with greater training volumes (Ralston, Kilgore, Wyatt, & Baker, 2017; Schoenfeld et al., 2019). Given that caffeine may increase training volume, it may also have a positive effect on these adaptations. From a practical standpoint, this is one of the most important areas for future research given that individuals interested in caffeine supplementation are likely to consume caffeine over the long-term, not only acutely.

Generally, the primary goal of power-oriented resistance training programs is to move the force-velocity curve to the right, denoting an athlete's ability to lift greater loads at higher velocities (Haff & Nimphius, 2012). Therefore, studies in recent years started to focus on caffeine's effects on velocity and power in resistance exercise (Pallares et al., 2013). Study 7 found that ingesting 3 mg/kg of caffeine 60 minutes before exercise enhances velocity and power in resistance exercise (Grgic et al., 2020b). Interestingly, the ergogenic effects on caffeine velocity and power were observed when exercising with low (<50% of 1RM), moderate (75% of 1RM), and high loads (90% of 1RM). Furthermore, it seems that the effects of caffeine tend to be greater at higher loads. Specifically, the relative ESs favouring caffeine intake (compared with placebo) ranged from 0.20 to 0.29 for loads up to 50% of 1RM, from 0.36 to 0.50 at 75% 1RM, and from 0.57 to 0.61 at 90% 1RM (Grgic et al., 2020c). While there is a potential relationship

between ergogenic effects of caffeine and the external load, we should also consider that: (a) the 95% CIs in this study overlapped between the analyses for different loads; and (b) the loads used in each testing session were increased from lowest to highest (i.e., the order of loads was not randomised). Therefore, future research is needed to examine further the association between the ergogenic effects of caffeine and the load used in resistance exercise.

Studies 3, 4, 7 and 8 found that caffeine ingestion may increase jump height during vertical jumps as well as peak and mean power during the Wingate test (Grgic et al., 2018; Grgic et al., 2020b; Grgic et al., 2020c; Grgic, 2018). Early studies that examined the effects of caffeine supplementation on jump height and sprint performance reported equivocal findings (Andrade-Souza et al., 2015; Collomp et al., 1991). Some of the discrepancies in findings between the studies might have been due to the small sample sizes. For example, the study by Collomp et al. (1991) did not find ergogenic effects of caffeine on Wingate test performance, but it also included only 6 participants. Pooled estimates from Studies 3 and 4 helped solve the ambiguity. However, while the pooled estimates from Study 3 and 4 show an ergogenic effect of caffeine on these components of exercise performance when they are evaluated in the laboratory, future work is needed to explore if caffeine ingestion may enhance jump height and sprint performance in sport-specific situations (Grgic et al., 2018; Grgic, 2018). Jumping and sprinting performance is important in many sports (Vescovi & McGuigan, 2008). Therefore, finding the best nutritional strategies that may enhance these components of exercise in sport-specific situations may be highly practically relevant.

By collating the results and scrutinising the methods employed in 21 published meta-analyses, Study 1 showed that caffeine ingestion is ergogenic for different components of exercise performance, such as aerobic endurance, muscle strength, muscle endurance, power, jumping performance, and exercise speed (Grgic et al., 2020a). However, caffeine's ergogenic effects on muscle endurance, muscle strength, anaerobic power, and aerobic endurance were substantiated by moderate-quality evidence from moderate-to-high quality systematic reviews. The evidence for other outcomes was based on moderate-quality reviews that presented evidence of very low or low quality. Meta-analyses are useful as they allow the pooling of outputs from different studies to obtain a summary estimate. In sports nutrition, they are commonly used to support establishing evidence-based guidelines and decision making for the

effective prescription of nutritional supplements and ergogenic aids. However, the methods used and the quality of the included studies will ultimately determine the robustness of the findings presented in any given meta-analysis. When it comes to caffeine supplementation, several individual meta-analyses were published in recent years, but Study 1 was the first umbrella review to critically evaluate their methods and summarise their results. The findings of Study 1 may be useful to inform future evidence-based guidelines on caffeine supplementation in sport and exercise.

While caffeine tends to improve exercise performance when looking at mean differences, studies that plot individual participant data commonly observe that caffeine's effects range from ergogenic to ergolytic (Jenkins et al., 2008). Variations in *ADORA2A* and *CYP1A2* genotypes are suggested to play a role in determining the effects of caffeine on exercise performance (Pickering & Kiely, 2018). Study 7 found small to moderate ergogenic effects of caffeine ingestion on movement velocity, muscular endurance, jumping, and sprinting performance in a sample of 20 *ADORA2A* (rs5751876) C allele carriers (CC/CT genotype). This was only the second study in the current literature that explored the influence of *ADORA2A* genotype on the acute effects of caffeine on exercise performance. A limitation of Study 4 was that we could not compare the effects observed among C allele carriers to those with the TT genotype. Specifically, out of the 22 participants, only two possessed the TT genotype. As suggested by Erblang et al. (2019), only around 15% of the population has the *ADORA2A* TT genotype. Theoretically, researchers should recruit 100 participants to get around 15 participants with the TT genotype. Therefore, future studies with larger sample sizes are needed to explore potential differences in the ergogenic effects of caffeine between individuals with different variations of the *ADORA2A* genotype. Furthermore, Study 8 found that caffeine ingestion is ergogenic for resistance exercise performance, vertical jump height, and power output in the Wingate test, but no significant differences were found between the *CYP1A2* AA and AC/CC genotypes were found. While the lack of significant between genotype differences might be due to the small sample size, the results of this study were similar to the recent finding by Spineli et al. (2020). In the Spineli et al. (2020) study, caffeine ingestion enhanced aerobic and muscle endurance, but there was no genotype × caffeine interaction effect, even though a sample of 100 male adolescents was recruited—a much larger sample than in Study 8. Rahimi (2019) found that the *CYP1A2* genotype moderated the ergogenic effects of caffeine, given that participants (young resistance-trained men) with the AA genotype improved performance following caffeine

ingestion, while exercise performance did not improve following caffeine ingestion in those with the AC/CC genotype. However, the difference in exercise performance found by Rahimi (2019) was small, as caffeine improved performance in the AA genotype group by an average of one repetition in a set with 85% of 1RM. Overall, it seems that *CYP1A2* and *ADORA2A* genotype variation might not determine the individual response to caffeine ingestion, or that the between-genotype differences are small, but this needs to be confirmed in larger samples and using different measures of exercise performance.

13. Conclusions

The main findings of this thesis are that: (a) caffeine ingestion acutely enhances performance in various exercise tasks; (b) lower doses of caffeine may produce ergogenic effects comparable to those of higher doses of caffeine; and (c) the individual responses to caffeine ingestion may not be moderated by *ADORA2A* and *CYP1A2* genotype variation. The findings on ergogenic effects of different doses of caffeine and the influence of genotype on individual responses to caffeine need to be confirmed in future studies with larger sample sizes. These findings may be useful to athletes, coaches, and sports nutritionists in making evidence-based decisions about caffeine supplementation.

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Appendix 1. Published papers

Grgic J, Grgic I, Pickering C, et al Wake up and smell the coffee: caffeine supplementation and exercise performance—an umbrella review of 21 published meta-analyses
British Journal of Sports Medicine 2020;54:681-688.

http://dx.doi.org/10.1136/bjsports-2018-100278

Grgic, J., Mikulic, P., Schoenfeld, B.J. et al. The Influence of Caffeine Supplementation on Resistance Exercise: A Review. Sports Med 49, 17–30 (2019).

https://doi.org/10.1007/s40279-018-0997-y

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Journal of the International Society of Sports Nutrition

REVIEW Open Access

Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis

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Abstract

Background: Caffeine is commonly used as an ergogenic aid. Literature about the effects of caffeine ingestion on muscle strength and power is equivocal. The aim of this systematic review and meta-analysis was to summarize results from individual studies on the effects of caffeine intake on muscle strength and power.

Methods: A search through eight databases was performed to find studies on the effects of caffeine on: (i) maximal muscle strength measured using 1 repetition maximum tests; and (ii) muscle power assessed by tests of vertical jump. Meta-analyses of standardized mean differences (SMD) between placebo and caffeine trials from individual studies were conducted using the random effects model.

Results: Ten studies on the strength outcome and ten studies on the power outcome met the inclusion criteria for the meta-analyses. Caffeine ingestion improved both strength (SMD = 0.20; 95% confidence interval [CI]: 0.03, 0.36; $p = 0.023$) and power (SMD = 0.17; 95% CI: 0.00, 0.34; $p = 0.047$). A subgroup analysis indicated that caffeine significantly improves upper (SMD = 0.21; 95% CI: 0.02, 0.39; $p = 0.026$) but not lower body strength (SMD = 0.15; 95% CI: -0.05, 0.34; $p = 0.147$).

Conclusion: The meta-analyses showed significant ergogenic effects of caffeine ingestion on maximal muscle strength of upper body and muscle power. Future studies should more rigorously control the effectiveness of blinding. Due to the paucity of evidence, additional findings are needed in the female population and using different forms of caffeine, such as gum and gel.

Keywords: Ergogenic aid, Performance, Power, Data synthesis

Background

Caffeine's ergogenic potential has been extensively studied in the sports science literature, with research dating back to 1907 [1]. From investigating caffeine's effects on aerobic exercise, in recent years the research focus has shifted to anaerobic exercise performance outcomes, such as muscular endurance, muscle strength, and jumping tasks that require muscle power. While caffeine has been found to significantly enhance muscular endurance [2], the effects of caffeine ingestion on maximal muscle strength (commonly operationalized as one repetition maximum [1RM]) and muscle power (commonly operationalized as vertical jump) remain unclear, and the practical utility of caffeine ingestion for enhancing performance in such physical tasks has not been fully elucidated.

The pioneering work on caffeine's effects on strength by Astorino et al. [3] reported no significant strength-enhancing effects with caffeine ingestion in a group of resistance trained men. Recent work by Grgic and Mikulic [4], however, found a significant 3% increase in lower body strength with caffeine ingestion using the barbell back squat 1RM as a measure of maximal strength. Goldstein et al. [5] reported a significant increase in upper body strength with caffeine ingestion, while Williams et al. [6] reported no ergogenic effect. The inconsistent results of individual studies prevent drawing sound conclusions regarding the ergogenic potential of caffeine for maximal strength outcomes.

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Equivocal findings have also been presented for the effects of caffeine intake on muscle power. A recent study by Ali et al. [7] reported no effect on countermovement jump height with caffeine ingestion. However, the findings of Bloms et al. [8] support conclusions about caffeine as an effective ergogenic aid for achieving acute improvements in countermovement jump height and peak force. Given the importance of jumping abilities for many common sports, it would be of both scientific and practical significance to determine a reasonably precise estimate regarding the potential performance-enhancing impact of caffeine ingestion on muscle power.

Several aspects that vary between studies, including the exercise used, participants' characteristics (e.g., age, sex, and training experience), and caffeine form, might be responsible for the inconsistency of findings. Most importantly, small sample sizes often limited the statistical power to detect significant effects [9]. A meta-analysis of individual studies is needed to circumvent these issues and provide in-depth, evidence-based scrutiny of the current body of evidence. The first meta-analytic investigation on the topic of caffeine and strength was performed by Warren et al. [10], who found a mean increase of approximately 7% in lower body maximal voluntary contraction with caffeine ingestion. A limitation of the meta-analysis is that only two of the included studies tested the effects of caffeine ingestion on 1RM, which significantly restricted the findings to isometric and isokinetic strength outcomes.

The latest meta-analysis on the topic, done by Polito et al. [2], found no significant effect of caffeine intake on performance in 1RM strength tests. However, only three studies met the inclusion criteria for the meta-analysis. The total number of pooled participants was relatively low ($n = 46$), potentially indicating issues with the statistical power of the analysis. Furthermore, the small number of included studies prevented subgroup analyses for possible moderators that may potentially impact the ergogenic potential of caffeine. Since the review by Polito et al. [2], a number of experimental trials have been published [4, 11–16], presenting novel findings for females [14], trained [4, 16] and untrained men [11, 13], athletes [15], and adolescents [12]; as such, an updated review appears to be warranted.

No previous meta-analyses have pooled the results of individual studies on the effects of caffeine on muscle power. The aim of this systematic review was, therefore, twofold: (a) to perform an updated meta-analysis of the acute effects of caffeine ingestion on maximal muscle strength; and (b) to conduct the first meta-analysis of acute effects of caffeine ingestion on muscle power assessed by vertical jump tests. The results may benefit athletes and practitioners in a variety of sports in which muscle strength and/or power are important determinants of performance.

Methods

Search strategy

The systematic literature search was performed following the PRISMA guidelines [17]. A search of the following databases was performed: PubMed/MEDLINE, Scopus, Cochrane Library, Web of Science (including Science Citation Index Expanded, Social Sciences Citation Index, and Arts & Humanities Citation Index), Google Scholar, Networked Digital Library of Theses and Dissertations, ProQuest Dissertation & Theses and Open Access Theses and Dissertations. The search for the studies on the effects of caffeine on strength was restricted to the documents published from 2015 onwards as the review by Polito et al. [2], with a search performed in March 2015 was used as a reference point. The review by Polito and colleagues [2] was assessed for rigor and deemed as of high-quality. Thus, the studies [3, 5, 6] included in the work by Polito et al. [2] were also included in the present review. The following syntax was used for the primary search: caffeine AND ("muscle strength" OR "ergogenic aid" OR performance OR "resistance exercise" OR "resistance training" OR recovery OR "strength training").

A separate search was done for the studies on the effects of caffeine on power outcomes. The following syntax with no time restriction was used: caffeine AND ("vertical jump" OR "countermovement jump" OR "squat jump" OR plyometrics OR height OR "drop jump" OR "depth jump" OR "jump training").

The search results were downloaded and filtered in EndNote software (X8; Clarivate Analytics, New York, USA). A secondary search was performed by screening the reference lists of all selected studies, and by conducting forward citation tracking (using Google Scholar and Scopus) of studies found meeting the inclusion criteria. The search concluded on April 19th, 2017.

Inclusion criteria

To warrant inclusion in the current analysis potential studies were required to meet the following criteria:

(a) an experimental trial published in English in a peer-reviewed journal, or a doctoral or a master's thesis;
(b) assessed the effects of caffeine ingestion in the form of capsule, liquid, gum or gel on dynamic maximal muscle strength (i.e. the greatest amount of weight lifted in a single repetition – 1RM) using constant external resistance, and/or on muscle power assessed using a vertical jump test (both peak force and vertical jump height were considered);
(c) caffeine was not co-ingested with other drugs/substances or potentially ergogenic compounds;
(d) employed a single or double-blind, randomized crossover design;

(e) used human participants without known chronic disease or injury.

Studies were excluded from the analysis if any of the above criteria were violated. Caffeine ingestion via coffee was not considered as coffee has several other biologically active compounds that might moderate the impact of caffeine.

Study coding and data extraction

For all studies meeting the inclusion criteria, the following information was tabulated on a predefined coding sheet using Microsoft Excel software (Microsoft Corporation, WA, USA):

(a) author(s), title and year of publication;
(b) sample size, participants' sex, participants' age (categorized as: adolescents [10–18 years]; young adults [18–39 years]; middle-aged adults [40–64 years];and seniors [≥65 years], and participants' experience in resistance training (categorized as: untrained [less than 1 year of experience]; and trained [more than 1 year of experience]) for studies assessing strength outcomes, and experience in sport training using the same categories as above for studies assessing muscle power.
(c) caffeine form, dosage, and time of ingestion before the experimental session(s);
(d) the exercises used for assessing muscle strength and power with the accompanying mean ± standard deviation (SD) data for the placebo and caffeine trials;
(e) habitual caffeine intake by the participants;
(f) the number of participants indicating which trial they perceived to be the caffeine trial;
(g) reported side effects;
(h) reported funding for conducting the studies.

Methodological quality

The 11-point PEDro scale was used for the assessment of the methodological quality of studies [18]. The first item concerns external validity and is not included in the total score; hence, the maximal score on the scale is 10. Studies were classified as in McCrary et al. [19]. Two authors of the article (JG and BL) performed the search, coding, and appraisal of methodological quality independently, with discussion and consensus over any observed differences. Before correcting for observed differences, the overall agreement between the two independent data extractions was very high (Cohen's kappa = 0.94).

Statistical analysis

The meta-analysis was performed using the Comprehensive Meta-analysis software, version 2 (Biostat Inc., Englewood, NJ, USA). Standardized mean differences (Hedge's g [SMD]) and 95% confidence intervals (CI) were calculated between the placebo and caffeine trials based on their means and standard deviations in 1RM (kg) and vertical jump (cm) tests, the correlations between the trials, and the number of participants. An analysis of peak force in the vertical jump test was not performed as only two studies reported such outcomes [8, 16]. Since none of the studies reported correlation, a 0.5 correlation was assumed for all trials, as recommended by Follmann et al. [20]. When a study measured muscle strength and/or power under multiple conditions (e.g. used more than one caffeine dose, tested more than one muscle group), SMDs and variances were averaged across the different conditions. SMDs of ≤0.2, 0.2–0.5, 0.5–0.8, and > 0.8 were considered to represent small, medium, large and very large effects, respectively [9]. The random effects model was used for analysis of both muscle strength and muscle power outcomes. The statistical significance threshold was set a priori at $p < 0.05$.

Subgroup analyses for the effects of caffeine on muscle strength were performed for the following study characteristics: (a) upper body strength; (b) lower body strength; (c) the capsule form of caffeine; (d) the liquid form of caffeine; (e) females; (f) males; (g) untrained; and (h) trained. Subgroup analyses for the effects of caffeine on muscle power were performed for the following characteristics: (a) the capsule form of caffeine; (b) the liquid form of caffeine; (c) females; (d) males; (e) athletes; (h) non- athletes; (f) countermovement and squat jump tests; and (g) Sargent jump tests.

The I^2 statistic was used to assess the degree of heterogeneity, with values from ≤50% indicating low heterogeneity, 50–75% moderate heterogeneity and > 75% high level of heterogeneity. Funnel plots were constructed for both muscle strength and muscle power outcomes, plotting standard error against Hedge's g. Funnel plot asymmetry arising from potential publication bias was assessed using the Trim-and-Fill method [21].

Results

The literature search yielded a total of 2533 documents. After a preliminary screening of titles and abstracts, 71 full-text studies were scrutinized. In total, ten studies were found meeting the inclusion criteria for strength outcomes [3–6, 11–16] (Table 1) with a total of 149 participants (males $n = 116$, females $n = 33$). Ten studies were found assessing muscle power outcomes [4, 7, 8, 15, 22–26] with a total of 145 participants (males $n = 116$, females $n = 29$). According to their age, all participants were classified as adolescents or young adults. Three studies [4, 12, 15] assessed both muscle strength and muscle power. The results of the search and study selection process are depicted in Fig. 1.

Fifteen studies were published in peer-reviewed journals, while two studies were master's theses [14, 26]. The

Table 1 Studies included in the analysis: summary of study designs

Study	Study design	Participants age (years)	Sample size and sex	Resistance/sport training experience	Habitual caffeine intake ($mg.d^{-1}$)[a]	Caffeine form	Caffeine dosage ($mg.kg^{-1}$)	Timing of caffeine ingestion before the experimental session(s) [minutes])	Exercise(s) used for the muscle strength/ power assessment	PEDro score
Ali et al. [7] 2016	RDB	24 ± 4	10 females	Athletes	0–300	Capsule	6	60	CMJ	10
Andrade-Souza et al. [22] 2014	RDB	25 ± 3	11 males	Athletes	N/A	Capsule	6	60	CMJ	8
Arazi et al. [12] 2016*a*	RDB	17 ± 1	10 females	Untrained/ Athletes	< 60	Capsule	2 and 5	60	LP and ST	10
Arazi et al. [11] 2016*b*	RDB	21 ± 4	15 males	Untrained	N/A	Capsule	6	60	BP and LP	10
Astorino et al. [3] 2008	RDB	23 ± 4	22 males	Trained	110 ± 152	Capsule	6	60	BP and LP	10
Bloms et al. [8] 2016	RSB	20 ± 1	9 females	Athletes	N/A	Capsule	5	60	CMJ and SJ	8
		21 ± 2	16 males							
Brooks et al. [13] 2015	RDB	21 ± 3	14 males	Untrained	N/A	Capsule	5	60	MBS	10
Clarke et al. [23] 2016	RDB	21 ± 2	8 males	Athletes	N/A	Capsule	3	60 and during the testing sessions	CMJ	10
Diaz-Lara et al. [15] 2016	RDB	29 ± 3	14 males	Trained/ Athletes	< 60	Capsule	3	60	BP and CMJ	10
Foskett et al. [24] 2009	RDB	24 ± 5	12 males	Athletes	0–350	Liquid	6	60	CMJ	10
Gant et al. [25] 2010	RDB	21 ± 3	15 males	Athletes	N/A	Liquid	260 (fixed) 3.7 on average	60 and during the testing sessions	CMJ	10
Gauvin [26] 2016	RDB	22 ± 2	23 males	Untrained/ Non-athletes	< 200 per week	Capsule	7	60	CMJ	9
Goldstein et al. [5] 2010	RDB	25 ± 7	15 females	Trained	< 250 ($n = 8$) > 250 ($n = 7$)	Liquid	6	60	BP	10
Grgic et al. [4] 2017	RDB	26 ± 6	17 males	Trained/ Non-athletes	58 ± 92	Liquid	6	60	BP, BBS and ST	9
Martin [16] 2015	RDB	20 ± 1	12 males	Trained	N/A	Gel	75 (fixed) - 0.9 on average	60	BP and BBS	10
Sabblah et al. [14] 2015	RSB	24 ± 3	7 females	Trained	N/A	Liquid	5	60	BP and MBS	8
		28 ± 6	10 males							
Williams et al. [6] 2008	RDB	26 ± 4	9 males	Trained	'Low' (no exact values)	Capsule	300 (fixed) - 3.6 on average	45	BP and LPD	10

[a]intake per day unless stated otherwise; *RDB* randomized double-blind study, *RSB* randomized single-blind study, *CMJ* countermovement jump, *SJ* squat jump, *LP* leg press, *ST* Sargent test, *BP* bench press, *MBS* machine-based squat, *LPD* lat pulldown, *BBS* barbell back squat

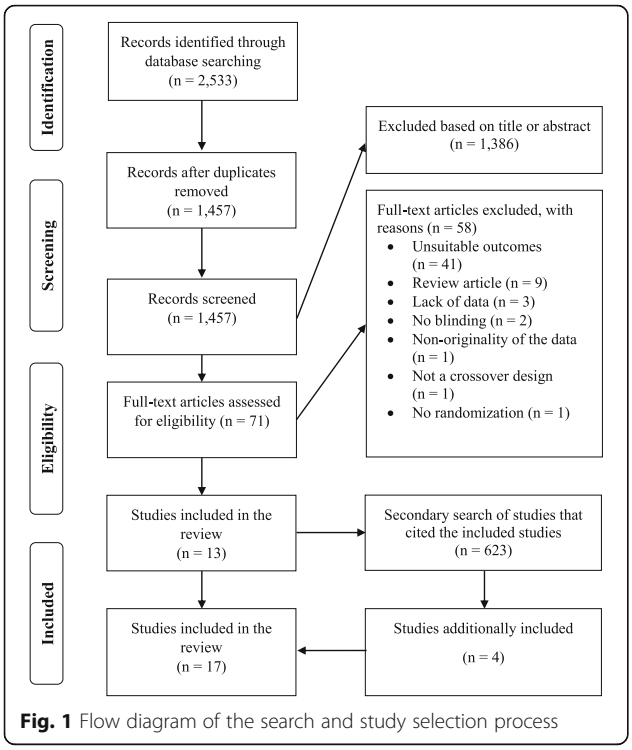

Fig. 1 Flow diagram of the search and study selection process

median number of participants per study was 14. Most of the studies used a double-blind design (i.e., 15 studies), with two studies [8, 14] using a single-blind design. Caffeine dosage varied from 0.9 $mg.kg^{-1}$ to 7 $mg.kg^{-1}$. Only one study administered caffeine in the form of gel [16], while the rest used capsule or liquid forms. Only nine studies reported habitual caffeine intake, with Astorino et al. [3] and Goldstein et al. [5] reporting a large range of habitual caffeine intakes among the participants (0–600 $mg.kg^{-1}$ per day). Only three studies [3, 22, 24] reported assessing the effectiveness of the blinding, with 60%, 50% and 33% of the participants correctly differentiating between the placebo and the caffeine trials, respectively. Individual characteristics of the included studies are reported in Table 1.

Results of the meta-analysis indicated a significant difference ($p = 0.023$) between the placebo and caffeine trials on measures of maximal strength (Fig. 2). The pooled SMD for the effects of caffeine ingestion on muscle strength was 0.20 (95% CI: 0.03, 0.36). A subgroup analysis indicated that caffeine significantly improves upper (SMD = 0.21; 95% CI: 0.02, 0.39; $p = 0.026$; Fig. 3) but not lower body strength (SMD = 0.15; 95% CI: -0.05, 0.34; $p = 0.147$; Fig. 4). Results from all of the remaining subgroup analysis may be found in Table 2.

The meta-analysis performed for muscle power indicated a significant difference (SMD = 0.17; 95% CI: 0.00, 0.34; $p = 0.047$) between the placebo and caffeine trials (Fig. 5). Results from all of the subgroup analysis can be found in Table 2.

The I^2 statistic showed low heterogeneity for the studies assessing muscle strength and muscle power ($I^2 = 0.0$; $p = 0.981$, and $I^2 = 0.0$; $p = 0.933$, respectively). The analysis of funnel plots did not reveal substantial asymmetry for muscle strength or muscle power outcomes. The Trim-and-Fill method changed the pooled SMD for muscle power from 0.17 (95% CI: 0.00, 0.34) to 0.12 (95% CI: -0.01, 0.26). The Trim-and-Fill method did not have an impact on the pooled effect size for muscle strength outcomes.

The mean PEDro methodological quality score was 9.6, with the values for individual studies ranging from 8 to 10. Three studies [8, 14, 22] were categorized as being of "good methodological quality" (PEDro score = 8), while all other studies were classified as being of "excellent quality".

Discussion

The results of the meta-analysis show that caffeine may be an effective ergogenic aid for muscle strength and power. The pooled effects of caffeine on performance were small to medium. It is important to note that even small improvements in performance in some sports may translate to meaningful differences in competitive outcomes [27, 28]. A previous meta-analysis did not show a significant effect of caffeine supplementation on muscle strength [2], and the results of individual studies investigating caffeine's effects on muscle power have not been previously pooled in a meta-analysis. Our novel results showing that caffeine may induce practically meaningful improvements in muscle strength and power can, therefore, be used to inform athletes, coaches, and sports nutritionists, as well as future research endeavors in this area, about the ergogenic potential of caffeine.

Strength outcomes

Upper and lower body strength

The subgroup analysis indicated a significant increase in upper body, but not lower body strength, with caffeine ingestion. These results are somewhat unexpected, as Warren et al. [10] suggested that larger muscles, such as those of the lower body, have a greater motor unit recruitment capability with caffeine intake than smaller muscles, such as those of the arm. Motor unit recruitment, in addition to the reduced rate of perceived exertion and the central effects of adenosine on neurotransmission, arousal, and pain perception, are considered to be underlying mechanisms by which caffeine can enhance performance, although the exact mechanisms remain to be fully elucidated [29, 30]. Based on the current results, it may be surmised that caffeine is a useful ergogenic aid for achieving acute increases in maximal upper body strength. In the included studies, lower body maximal strength was evaluated using only leg press and squat (machine-based and

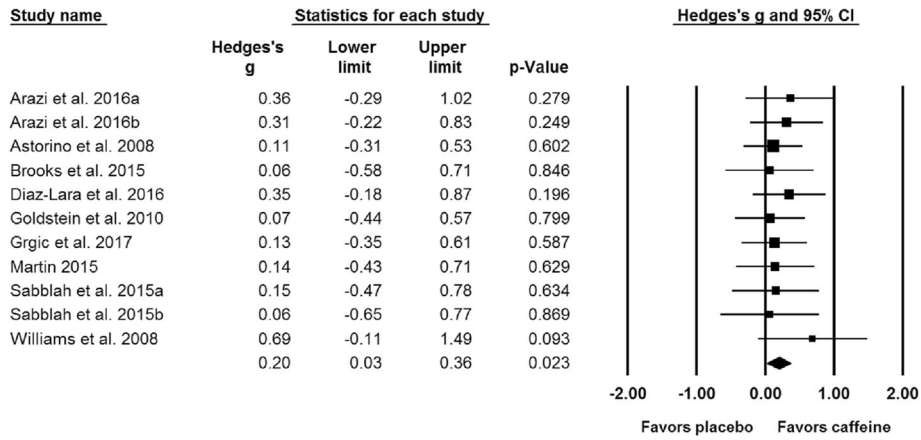

Fig. 2 Forest plot showing differences between the effects of placebo and caffeine trials on measures of maximal muscular strength. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the *x*-axis denote the standardized mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

free weight) tests. Two studies [4, 16] used a free weight exercise (barbell back squat), and both reported a significant increase in lower body strength. Warren et al. [10] concluded that caffeine ingestion might increase lower body isometric strength. Our findings do not indicate a strength increasing effect with caffeine ingestion for lower body dynamic strength. It is worth noting that in general, the included studies did not report on the reliability of their strength assessment, indicating potential reasons for the surprising findings for lower body strength. Further research is needed to examine the effects of caffeine on dynamic strength. Such studies may benefit from using a larger variety of dynamic lower body strength tests, as the current findings are mostly limited to a small selection of primarily machine-based tests.

Training status

The subgroup analysis for training status indicated no significant differences in maximal strength in trained ($p = 0.076$) and untrained individuals ($p = 0.144$). The

Table 2 Results from the subgroup meta-analyses

Subgroup analysis	SMD [95% CI]	*p*-value	Mean caffeine dose (mg.kg^{-1}[range])
Strength outcomes			
Upper body strength	0.21 [0.02, 0.39]	0.026	4.7 [0.9–6]
Lower body strength	0.15 [−0.05, 0.34]	0.147	4.8 [0.9–6]
Capsule form of caffeine	0.27 [0.04, 0.50]	0.023	4.7 [2–6]
Liquid form of caffeine	0.11 [−0.17, 0.39]	0.462	6 [6]
Males	0.21 [0.02, 0.41]	0.034	4.7 [0.9–6]
Females	0.15 [−0.13, 0.43]	0.294	5 [2–6]
Trained participants	0.18 [−0.02, 0.37]	0.076	4.8 [0.9–6]
Untrained participants	0.27 [−0.09, 0.63]	0.144	4.8 [2–5]
Power outcomes			
Capsule form of caffeine	0.14 [−0.06, 0.34]	0.174	4.6 [2–7]
Liquid form of caffeine	0.24 [−0.06, 0.54]	0.124	5.2 [3.7–6]
Males	0.16 [−0.02, 0,34]	0.081	5.3 [3–7]
Females	0.23 [−0.23, 0.69]	0.323	4.8 [2–6]
Athletes	0.23 [0.03, 0.42]	0.025	4.4 [2–6]
Non athletes	0.03 [−0.33, 0.40]	0.854	6.5 [6–7]
Countermovement jump	0.14 [−0.04, 0.32]	0.138	5.0 [3.7–7]
Sargent test	0.31 [−0.09, 0.70]	0.129	4.3 [2–6]

SMD standardized mean difference, *CI* confidence interval

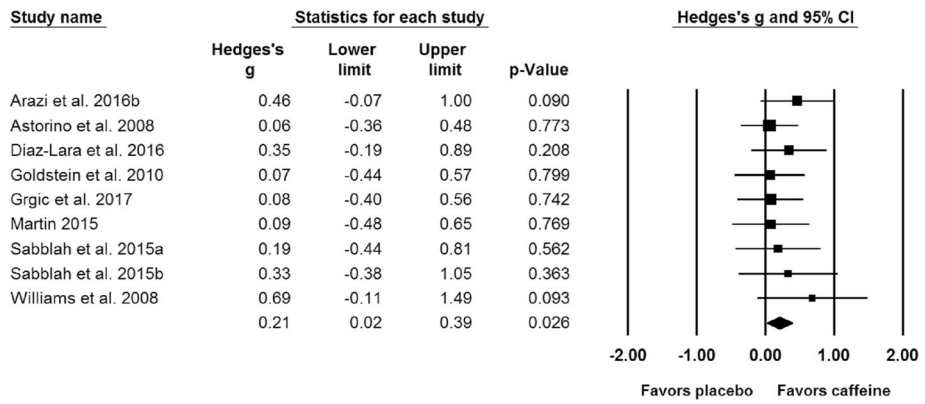

Fig. 3 Forest plot showing differences between the effects of placebo and caffeine trials on measures of upper-body maximal muscle strength. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the *x*-axis denote the standardized mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

meta-analysis of the three studies among untrained individuals was limited by small overall sample size ($n = 32$). It may be considered indicative that two of three individual studies reported significant differences in maximal strength with caffeine ingestion, but more individual studies on this topic are needed before drawing firm conclusions. Training status seems to play a significant role in response to caffeine intake in other forms of physical activity, such as swimming, with greater improvements observed in trained athletes [31]. However, it remains unclear whether the same applies to strength outcomes. More studies are needed before confidently drawing conclusions about the potential differences in effects of caffeine ingestion on muscle strength of trained and untrained individuals.

Sex

The subgroup analysis in males showed a significant improvement in strength with caffeine ingestion. The subgroup analysis for females was limited by small sample size, as only three studies [5, 12, 14] were found meeting the inclusion criteria. The landmark study by Goldstein et al. [5] reported a significant increase in the 1RM bench press in a cohort of resistance trained females. However, the effect size was very small (SMD = 0.07), thereby limiting the practical significance of the finding. Another study among female participants was performed by Sabblah et al. [14]. The researchers reported an SMD of 0.33 for increases in upper body strength with caffeine ingestion. However, the study employed a single-blind design and hence provided evidence of somewhat lower methodological quality compared to other studies. Additionally, the participants in the study from Sabblah et al. [14] exhibited lower levels of fitness than the participants in the study from Goldstein et al. [5], with marked disparities observed for 1RM strength (32 kg and 52 kg, respectively). None of the studies that included female participants controlled for the potential variability attributable to

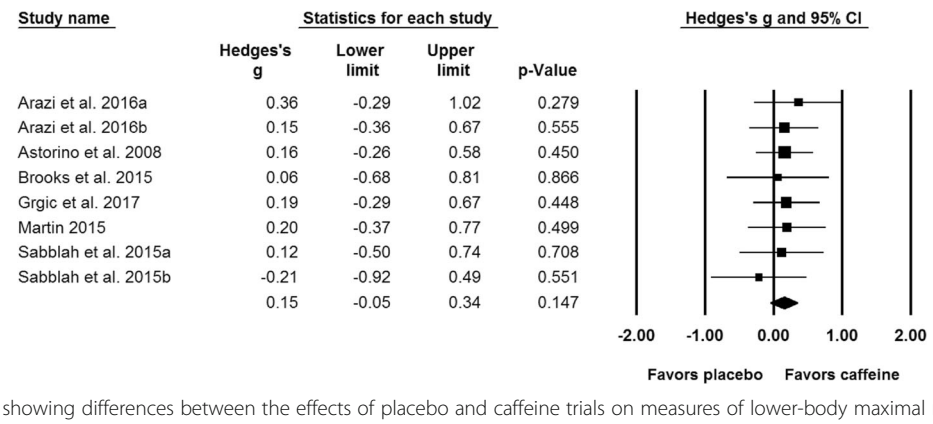

Fig. 4 Forest plot showing differences between the effects of placebo and caffeine trials on measures of lower-body maximal muscle strength. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the *x*-axis denote the standardized mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

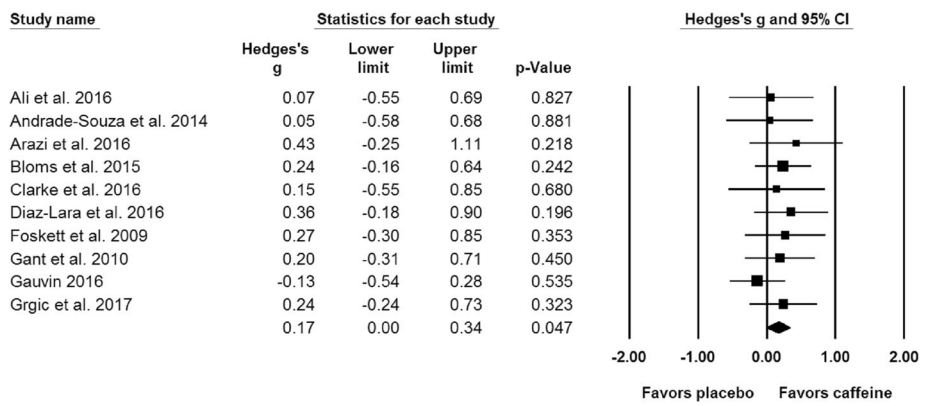

Fig. 5 Forest plot showing differences between the effects of placebo and caffeine trials on measures of muscle power expressed as vertical jump height. The size of the plotted squares reflects the relative statistical weight of each study. The numbers on the *x*-axis denote the standardized mean differences expressed as Hedge's g. The horizontal lines denote the respective 95% confidence intervals (CI)

metabolic alterations across the menstrual cycle [32], which is a limitation of the current body of literature. Additional rigorously controlled studies are needed to provide clarity on the topic.

Caffeine form

The subgroup analysis indicated significant increases in strength after the ingestion of caffeine in the capsule form. The meta-analysis of the effects of the liquid form of caffeine included only three studies and did not report a significant effect. It is likely that the analysis was limited due to the small sample size ($n = 50$). Only one study [16] used caffeine in the form of a gel. Previous studies indicate that there are no practically meaningful pharmacokinetic differences between these routes of caffeine ingestion [33]; as such, it is unlikely that marked differences exist when comparing ergogenic effects of various forms of caffeine administration. Further investigations are needed for liquid forms of caffeine and others that have rarely or never been studied in this context, such as gum and gel.

Power outcomes

The meta-analysis supports caffeine as an effective ergogenic aid for achieving acute increases in muscle power expressed as vertical jump height. These results may have considerable applicability to many sports, including basketball and volleyball, in which muscle power and jumping ability are highly related to performance outcomes. The magnitude of acute improvement in vertical jump height found in the current analysis for a single caffeine ingestion is roughly equivalent to the effects of ~ 4 weeks of plyometric training [34]. The current analysis included only studies that used vertical jump as the power outcome; as such, it is possible that caffeine ingestion could produce somewhat different effects on other types of muscle power tests. However, a recent meta-analysis also showed a significant performance-enhancing effect of caffeine on the Wingate test, which is a common test of power [35]. Furthermore, most of the included studies used countermovement jump for assessing vertical jump; it remains to be explored whether the caffeine ingestion would produce different effects on other forms of vertical jumping. In addition, all of the included studies evaluated these effects in isolated conditions that may not accurately reflect in-game, sport-specific jumping tasks. More evidence may be needed to determine if the performance-enhancing effects of caffeine would transfer in the context of individual sports and/or team-sport matches [36].

While previous research [37] has shown an increase in countermovement jump height after ingestion of a caffeine-containing energy drink, it was unclear if the effect was attributable to the caffeine content or the presence of other substances, such as taurine. A recent meta-analysis on caffeinated energy drinks found a significant association between their taurine content and performance, but not between their caffeine content and performance [38]. As postulated by Bloms et al. [8], motor schema might play a role when assessing the association between caffeine and muscle power. Bloms et al. [8] tested the effect of caffeine on muscle power among a cohort of athletes and reported significant increases in jumping height. By contrast, Gauvin [26] reported no effects of caffeine ingestion on muscle power in a group of untrained men, with no previous experience in the exercise. The subgroup analysis for training status indicated a significant effect for athletes, but not for non-athletes. It may be suggested that future studies should control for this confounding factor by including only participants with or without previous experience in the task, or by performing initial familiarization sessions.

None of the remaining subgroup analysis showed a significant effect of caffeine. These results might be due to the small sample sizes in different subgroup analysis.

More studies are needed before reaching conclusions about context-specific effects of caffeine. Furthermore, while the body of evidence evaluating effects of caffeine on muscle power is still limited; the current meta-analysis shows promising findings, but more studies are needed on this topic. Specifically, studies including different forms of vertical jumping and sport-specific jumping tasks, different population groups, larger sample sizes, and different doses and forms of caffeine are required.

Methodological quality

The PEDro scale showed good to excellent quality among the included studies, suggesting that the results of the current meta-analysis were not confounded by the inclusion of studies with poor research methodology. Only two studies [6, 25] reported receiving funding from parties that may have had commercial interest for conducting the research, so it is improbable that the overall results of the current study were significantly affected by financial bias. To further improve the quality of evidence, future studies should use a double-blind rather than a single-blind design and assess the effectiveness of the blinding. Only three studies [3, 22, 24] reported assessing the effectiveness of the blinding. This information is of importance as participants' recognition of the caffeine trial may influence outcomes [39], because psychological effects of 'expectancy' and 'belief' might have an impact on performance [40]. In some studies, performance-enhancing responses were found with perceived 'caffeine' ingestion, when in fact, a placebo was consumed [41]. Future studies examining this topic should include a questionnaire of perception of the trials to prevent possible issues associated with such confounding.

While the inclusion of doctoral and master's theses may be considered as a limitation of this review, their inclusion is supported by their high methodological quality scores. Therefore, the inclusion of such studies may be regarded as a strength rather than a limitation, as it would be inappropriate to omit high-quality contributions to the literature from a comprehensive systematic review. A limitation of the current review is the low number of studies included in the subgroup analysis. Secondly, a limitation is that no studies were found for age groups other than adolescents and young adults. The findings, therefore, pertain mainly to young individuals and cannot be generalized to other age groups. Furthermore, due to the high degree of inter-individual variability of effects [42], these results should be interpreted with caution when it comes to prescribing caffeine supplementation to individuals. Individuals should also assess their susceptibility to possible side effects as reported in the literature, such as tremor, insomnia, elevated heart rate, headache, abdominal/gut discomfort, muscle soreness, and inability to verbally communicate and stay focused. These side effects may be enhanced in naive caffeine users [3, 5], so extra precaution may be warranted in such individuals.

Conclusion

Caffeine appears to provide significant ergogenic effects on muscle strength and power. The expression of strength in the form of 1RM is most specific to the sport of powerlifting but may translate to performance improvements in a variety of other strength-power sports. The effects of caffeine on muscle power may apply to athletes in a variety of sports in which jumping is a predominant activity that affects the sport-specific performance. Subgroup-analyses suggested that the effects of caffeine on strength may be more pronounced in upper body muscles, but further research on this topic is warranted. The results of the present meta-analysis are based on limited evidence, and thus need to be interpreted with caution. Future studies should explore the optimal dosage and form of caffeine for maximizing effects on strength and power. Finally, responses to caffeine ingestion have a high degree of inter-individual variability, and as such, the applicability of the current findings must be assessed on a case-by-case basis, based on the specific characteristics of the individual and the sports activity or other physical tasks.

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Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions
JG and ZP conceived the idea and conceptualized the review. JG and BL conducted the study selection, data extraction, and methodological quality assessment. JG conducted the meta-analysis. JG drafted the initial manuscript. JG, ET, BL, and ZP contributed to writing the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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ADORA2A C Allele Carriers Exhibit Ergogenic Responses to Caffeine Supplementation

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Abstract: Caffeine's ergogenic effects on exercise performance are generally explained by its ability to bind to adenosine receptors. *ADORA2A* is the gene that encodes A_{2A} subtypes of adenosine receptors. It has been suggested that *ADORA2A* gene polymorphisms may be responsible for the inter-individual variations in the effects of caffeine on exercise performance. In the only study that explored the influence of variation in *ADORA2A*—in this case, a common polymorphism (rs5751876)—on the ergogenic effects of caffeine on exercise performance, C allele carriers were identified as "non-responders" to caffeine. To explore if C allele carriers are true "non-responders" to the ergogenic effects of caffeine, in this randomized, double-blind study, we examined the acute effects of caffeine ingestion among a sample consisting exclusively of *ADORA2A* C allele carriers. Twenty resistance-trained men identified as *ADORA2A* C allele carriers (CC/CT genotype) were tested on two occasions, following the ingestion of caffeine (3 mg/kg) and a placebo. Exercise performance was evaluated with movement velocity, power output, and muscle endurance during the bench press exercise, countermovement jump height, and power output during a Wingate test. Out of the 25 analyzed variables, caffeine was ergogenic in 21 (effect size range: 0.14 to 0.96). In conclusion, *ADORA2A* (rs5751876) C allele carriers exhibited ergogenic responses to caffeine ingestion, with the magnitude of improvements similar to what was previously reported in the literature among samples that were not genotype-specific. Therefore, individuals with the CT/CC genotype may still consider supplementing with caffeine for acute improvements in performance.

Keywords: caffeine; ergogenic aid; genetics; mean velocity

1. Introduction

The effects of caffeine on exercise have received substantial attention in the scientific literature [1–8]. Currently, it is well established that acute ingestion of caffeine doses in the range from 2 to 6 mg per kilogram of body mass enhances exercise performance [1–8]. Caffeine's ergogenic effects are apparent in different components of exercise. For example, a recent umbrella review reported that caffeine ingestion enhances muscle strength and endurance, aerobic endurance, power output, and jumping performance [3]. Even though research indicates that caffeine ingestion may be acutely ergogenic for

a wide range of exercise tasks, between-person variability in responses to this dietary supplement seems substantial [9,10]. The ergogenic effects of caffeine are generally explained by its interaction with adenosine A_1, A_{2A}, and A_{2B} receptors [11,12]. Adenosine concentrations in the brain progressively increase during waking hours, resulting ultimately in sensations of fatigue; the concentrations of adenosine also decrease during sleep. Caffeine's molecular structure is similar to that of adenosine. Therefore, after ingestion, caffeine binds to adenosine receptors, subsequently resulting in reduced fatigue, increased vigilance, and ergogenic effects on exercise performance [11,12].

Researchers have suggested that the inter-individual variation in caffeine response may be due to polymorphisms within two genes, namely *CYP1A2* and *ADORA2A* [10]. Cytochrome P450 1A2 (an enzyme responsible for up to 95% of caffeine metabolism) is encoded by the *CYP1A2* gene [10]. A single nucleotide polymorphism rs762551 within *CYP1A2* affects the speed of caffeine metabolism. Specifically, individuals with the AA genotype are commonly classified as "fast caffeine metabolizers", whereas C allele carriers (AC/CC genotypes) are considered to be "slow caffeine metabolizers", respectively [13]. The influence of *CYP1A2* (rs762551) on the acute effects of caffeine supplementation on exercise performance has been explored in several studies [14–23]. However, the evidence in these studies remains inconsistent, with some reporting no effect of the polymorphism on the ergogenic effects of caffeine supplementation and others showing a modifying effect, but in different directions [14–23].

ADORA2A is the gene that encodes A_{2A} subtypes of adenosine receptors [24]. Previous research has suggested that this receptor represents the primary target of caffeine action in the central nervous system, and thus, polymorphic variations in the *ADORA2A* gene may impact the responses to acute caffeine ingestion [24]. The rs5751876 polymorphisms in the *ADORA2A* gene are comprised of a C-to-T substitution at nucleotide position 1083 (rs5751876) (also known as 1976C>T) [24]. Interestingly, as compared to TT homozygotes, *ADORA2A* C allele carriers have higher habitual caffeine consumption, which may suggest that these individuals need higher doses of caffeine to obtain a pharmacological effect [24].

Only one study has explored the influence of variation in this gene—in this case, a common polymorphism (rs5751876)—on the ergogenic effects of caffeine on exercise performance [25]. The study included 12 participants (6 TT homozygotes and 6 C allele carriers [i.e., CC/CT genotype]). These participants were untrained women who completed 20 min of cycling at a work rate eliciting 60% of VO_{2peak} followed by two 10-min cycling time trials. The exercise task was performed on two occasions, following the ingestion of 5 mg/kg of caffeine or a placebo. Results indicated that caffeine ingestion was ergogenic for TT homozygotes but not for C allele carriers. Based on this study, C allele carriers were identified as "non-responders" to caffeine [25].

Given the limited data on this topic, the aim of this study was to explore the influence of *ADORA2A* (rs5751876) on the acute effects of caffeine supplementation on exercise performance, by using exercise tests for which caffeine had previously been shown to be ergogenic [3].

2. Materials and Methods

2.1. Experimental Design

In this double-blind, randomized, crossover trial, all participants attended four laboratory sessions (in the morning hours between 07:00 to 12:00 h) that were from 4 to 7 days apart. The first two sessions consisted of familiarization with the exercise protocol. The third and fourth sessions were the main sessions. Twenty-four hours before the main trials, participants were asked the following: (a) to avoid any intense exercise; (b) to track their energy and macronutrient intake; and (c) to refrain from caffeine intake after 6 pm on the day before testing. The participants performed the two main sessions in a fasted state (overnight fast). Caffeine and placebo supplementation was provided on different days. Caffeine (Pure Lean Nutrition, Melbourne, Australia) was administered in a gelatin capsule with a dose of 3 mg/kg of body mass, while the placebo gelatin capsule contained 3 mg/kg of body mass of dextrose. All capsules were of identical appearance. Placebo and caffeine powders were weighed using

a high precision electronic digital scale (Precisa, XT 120A, Dietikon, Switzerland) and then packaged into capsules. Capsules were prepared in the laboratory by an experienced researcher while other researchers performed the blinding. Capsules were ingested 60 min before the start of the exercise session under the supervision of the research staff, as in previous research [1,26,27]. The participants' genotype was determined using a buccal swab. Ethical approval was requested and granted from the Victoria University Human Research Ethics Committee (number: HRE19-019), and every participant signed an informed consent form.

2.2. Participants

The study included a sample of 22 resistance-trained men, defined herein as having a minimum of six months of resistance training experience with a minimum weekly training frequency of two times on most weeks. Exclusion criteria were the existence of any health limitations and prior use of anabolic steroids (self-reported). All participants completed all sessions with no injuries or adverse events. Participants' characteristics are presented in Table 1.

Table 1. Characteristics of the participants.

Variable	Mean ± Standard Deviation
Age (years)	29.3 ± 4.8
Body mass (kg)	80.3 ± 11.2
Height (cm)	183.1 ± 5.9
1RM in the bench press (normalized per body mass)	1.1 ± 0.2
Habitual caffeine intake (mg/day)	143 ± 113

1RM: one repetition maximum.

2.3. Exercise Protocol

Exercises involving the upper body were performed prior to those that predominately activated the lower body, to avoid any transfer of muscle fatigue from one exercise task to another. At the beginning of the exercise protocol, the participants performed the bench press exercise with different loads (i.e., 25%, 50%, 75%, and 90% of one-repetition maximum (1RM)—performed in that order) [28]. 1RM was established during the first familiarization session. At each respective load, the participants performed two sets of one repetition, separated by a 3-min rest interval. The better repetition at each load was used for the analysis. The eccentric phase lasted 2 s, there was no pause at the bottom phase, and the concentric action was performed with maximal velocity. Mean power (W), mean concentric velocity (m/s), peak power (W), and peak concentric velocity (m/s) were measured for each repetition using the GymAware linear position transducer device (GymAware Power Tool, Kinetic Performance Technologies, Canberra, Australia) that was attached to the barbell.

After the second set that was performed with 90% of 1RM, the participants were provided with five minutes of rest. Then, we tested upper-body muscular endurance with a task that involved performing repetitions to momentary muscular failure in the bench press exercise with a load of 85% of 1RM. In this test, we collected data on the total number of repetitions, as well as power and velocity output of each repetition using the linear position transducer attached to the barbell. The tempo was the same as in the previous task. For the statistical analysis, we compared the total number of repetitions between the placebo and caffeine conditions. In addition, to explore the "quality" of performed repetitions, we matched the number of repetitions between the placebo and caffeine conditions and examined their average power and velocity. For example, one participant performed 7 and 8 repetitions following the ingestion of the placebo and caffeine, respectively. In this case, we only examined the velocity and power of the first 7 repetitions in both conditions.

After the muscular endurance test, the participants rested for three minutes. Then the participants performed a short warm-up consisting of one minute of light running, followed by ten bodyweight

squats. After the warm-up, participants performed a countermovement jump (CMJ) without an arm swing on a force platform (400S Isotronic Fitness Technology, Skye, Australia). The participants positioned themselves in an upright starting position and received commands from the computer software associated with the force platform that was positioned in front of the platform. This software visually counted down, "3, 2, 1" and provided "Set" and "Go" commands. After the "Go" command, the participants had five seconds to complete the jump. The participants performed a fast knee flexion (where their lowest position was a semi-squat position) [29,30]. Immediately after reaching this point (i.e., no pause at the bottom phase), the participants rapidly extended the hip, knee, and ankle joints with prior instructions to jump as quickly and "explosively" as possible to achieve maximal vertical jump height [29,30]. A total of three attempts was provided with one minute of rest between them. The best jump was used for the analysis. The outcome in the CMJ test was vertical jump height.

After the CMJ, the participants rested for three minutes. Then, the participants performed the Wingate test on an Excalibur Sport Cycle Ergometer (Lode, Groningen, The Netherlands). The Wingate test started with a 5-min warm-up consisting of pedaling at 100 W at 60–80 rpm [31]. Following the warm-up, participants performed a 30-s "all-out" sprint on the bike. The flywheel resistance was set at 0.075 Nm/kg. The participants were instructed to remain seated during the 30-s sprint.

2.4. Assessment of Blinding

In both main trials (i.e., caffeine and placebo), before and after the testing session, participants responded to the following question: "Which supplement do you think you have ingested?" [32]. This question was used to explore the effectiveness of the blinding and had three possible responses: (a) "caffeine", (b) "placebo", and (c) "I do not know" [32]. If the participants responded with "a" or "b", they were also asked to state the reason for choosing their respective response.

2.5. Genetic Testing

Genetic testing was performed using a commercially available testing kit from DNAfit Life Sciences. The procedure used for genetic testing is explained in detail elsewhere [33]. Briefly, the buccal swab sample was collected using OCR-100 kits by DNAGenotek. For the analysis, these samples were sent to IDna Genetics Laboratory (Norwich, UK). DNA was: (a) extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Kent, UK); and (b) amplified using polymerase chain reaction (PCR) on an ABI 7900 real-time thermocycler (Applied Biosystem, Waltham, MA, USA). The collected samples were analyzed for the *ADORA2A* (rs5751876) single-nucleotide polymorphism. Genotype analyses were performed after the exercise performance data collection was finalized. Therefore, researchers and participants were blinded to genotype variations of the sample during the exercise performance data collection.

2.6. Statistical Analysis

Two participants who were *ADORA2A* TT homozygotes were excluded, leaving a total of 20 C allele carriers (CC and CT) in the analysis. One-way repeated-measures analysis of variance (ANOVA) was used to analyze the exercise performance data. Relative effect sizes (and their 95% confidence intervals; 95% CI) were expressed using Hedges' g for repeated measures. The effect sizes were classified as follows: trivial (<0.20); small (0.20–0.49); moderate (0.50–0.79); and large (≥0.80). The effectiveness of blinding was examined using the Bang's Blinding Index, as explained elsewhere [29]. All analyses were performed using the Statistica software (version 13.0; StatSoft; Tulsa, OK, USA). The significance level was set at $p < 0.05$.

3. Results

3.1. Exercise Performance

For movement velocity and power, we found significant effects of caffeine ingestion for all outcomes except for mean velocity at 25% of 1RM, and mean velocity, peak power, and peak velocity at 50% of 1RM (Figure 1). The significant effect sizes ranged from 0.16 to 0.53. For muscular endurance,

we found significant effects of caffeine ingestion on the total number of performed repetitions and the quality of repetitions when matched for repetitions between the conditions. Here, the effect sizes ranged from 0.27 to 0.96 (Table 2). We also found a significant effect of caffeine ingestion on vertical jump height with an effect size of 0.13. For power output in the Wingate test, we found significant effects of caffeine ingestion on peak, mean, and minimum power. The effect sizes ranged from 0.34 to 0.41.

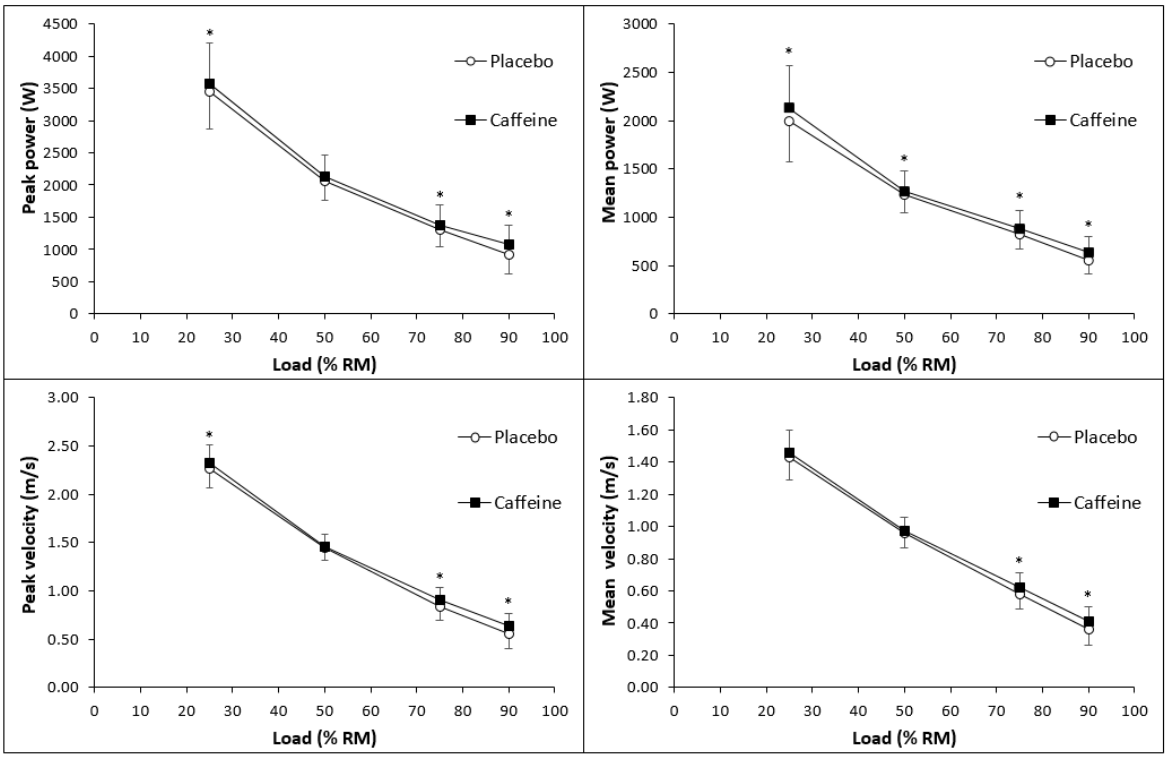

Figure 1. The effects of caffeine vs. placebo on peak power (upper left section), peak velocity (lower left section), mean power (upper right section), and mean velocity (lower right section) in the bench press with 25%, 50%, 75%, and 90% of one repetition maximum (1RM). Data are presented as mean ± standard deviation. * denotes significant differences between the conditions.

Table 2. Effects of caffeine ingestion on performance in the muscular endurance test, countermovement jump, and Wingate: results from a series of one-way repeated measures analyses of variance.

Variable	Placebo	Caffeine	Hedges' g and 95% CI	*p*-Value
Muscular endurance test				
Maximum repetitions at 85% 1RM	6.9 ± 2.2	8.2 ± 2.1	0.58 (0.29, 0.91)	<0.001
Mean power matched for repetitions (W)	418 ± 116	492 ± 138	0.56 (0.32, 0.83)	<0.001
Mean velocity matched for repetitions (m/s)	0.27 ± 0.05	0.32 ± 0.05	0.96 (0.58, 1.41)	<0.001
Peak power matched for repetitions (W)	669 ± 250	740 ± 258	0.27 (0.14, 0.42)	<0.001
Peak velocity matched for repetitions (m/s)	0.41 ± 0.08	0.46 ± 0.07	0.64 (0.38, 0.94)	<0.001
CMJ				
Vertical jump height (cm)	35.0 ± 6.1	35.8 ± 5.9	0.13 (0.02, 0.25)	0.034
Wingate test				
Peak power in the Wingate test (W)	859 ± 237	948 ± 229	0.37 (0.21, 0.55)	<0.001
Mean power in the Wingate test (W)	598 ± 101	634 ± 100	0.34 (0.17, 0.54)	<0.001
Minimum power in the Wingate test (W)	349 ± 103	392 ± 96	0.41 (0.07, 0.78)	0.020

1RM: one repetition maximum: CMJ: countermovement jump; CI: confidence interval.

3.2. Assessment of Blinding

Before the start of the exercise session, 50% and 65% of the participants correctly guessed (beyond chance) the placebo and caffeine conditions, respectively. After finishing the exercise session, 65% and 75% of the participants correctly guessed the placebo and caffeine conditions beyond chance, respectively. Participants who correctly identified caffeine reported "feeling more energized" and/or "more alert", or they associated the improvements in exercise performance with caffeine ingestion.

4. Discussion

The main finding of this study is that caffeine ingestion may be ergogenic for *ADORA2A* (rs5751876) C allele carriers in a range of exercise performance outcomes. Therefore, these results do not support the theoretical supposition that *ADORA2A* C allele carriers do not experience improvements in exercise performance following caffeine ingestion.

Our findings are not in accord with the Loy et al. [25] study, which proposed that *ADORA2A* C allele carriers do not experience an ergogenic response to caffeine supplementation. The main differences between our study and Loy et al. [25] are the sex of the participants and the exercise tests employed. Specifically, we included male participants, whereas Loy and colleagues included females. Therefore, it may be that female *ADORA2A* C allele carriers experience a different response to caffeine ingestion as compared to their male counterparts. However, this explanation is perhaps less plausible because recent evidence suggests that female and male participants experience similar ergogenic responses to caffeine ingestion in aerobic-, anaerobic- and strength-based exercise tasks [34–36]. Importantly, the present study and the work by Loy et al. [25] also differed in the selection of performance tests; while we assessed changes in power, muscular endurance, and sprinting performance, Loy and colleagues focused on aerobic endurance. It may be that caffeine affects performance in these components of exercise performance through different mechanisms. The possible impact of genetic variations may be more expressed in some tests and less in others. Given the scarce evidence on the influence of polymorphisms in *ADORA2A* on the individual variation in responses to caffeine, this topic certainly requires further research. Finally, given that we report here that *ADORA2A* C allele carriers improve performance following caffeine ingestion, this may suggest that other genotypes that were not tested herein (e.g., CYP1A2 AA and AC/CC genotypes) are more important for the individual responses to caffeine ingestion.

Interestingly, the effects of caffeine on exercise performance in this study were very similar in size to the effects previously reported in the literature. For example, the increases in muscular endurance in our study are similar to the performance benefits of caffeine recorded in a previous study that included individuals with *CYP1A2* (rs762551) AA genotype—which are suggested to experience the most profound ergogenic benefits of caffeine [22]. Furthermore, the increases in movement velocity, vertical jump height, and power output in the Wingate test are comparable to the improvements reported in meta-analyses of these outcomes among samples that were not genotype-specific [5,7,37]. For example, one meta-analysis [7] reported that caffeine ingestion acutely enhanced Wingate peak power by an effect size of 0.27 (95% CI: 0.08, 0.47), which is very similar to the effect size of 0.37 (95% CI: 0.21, 0.55) observed in this study.

Strengths and Limitations

The main strength of the present study was the use of a randomized, double-blind study design, which is identified as the gold standard in sports nutrition [38]. Additionally, the strength of the present study was in the use of exercise tests for which caffeine had been shown to be ergogenic.

The main limitation of this study was that 50% to 75% of the participants were able to identify caffeine and placebo conditions beyond chance. However, these results were not a likely explanation of the differences in findings between our study and the Loy et al. [25] study, given that the majority of participants (>75%) in the Loy et al. study were able to guess the content of the capsules correctly.

Additionally, given the small number of *ADORA2A* TT homozygotes in our sample, we could not assess whether TT homozygotes experience different responses to caffeine ingestion compared with C allele carriers, an area that should be explored in future research. The low number of participants classified as TT homozygotes could be explained by the estimate that around 85% of the population possess the CC/CT genotype at rs5751876 [39].

Finally, to avoid any potential confounding by prior food and caffeine ingestion [40,41], we opted to test the participants in a fasted state. This needs to be acknowledged as a limitation given that caffeine supplementation and exercise in a fasted state is likely not a "real-life" practice of many individuals, and is not in line with the current sports nutrition recommendations [42]. Future studies may consider further exploring this topic by using caffeine supplementation protocols that mirror those more commonly observed in practice.

5. Conclusions

Our findings suggest that *ADORA2A* (rs5751876) C allele carriers respond positively to caffeine supplementation. Therefore, individuals with the CT/CC genotype may still consider supplementing with caffeine for acute improvements in performance. Future research is needed to explore if *ADORA2A* TT homozygotes experience different responses to caffeine supplementation than C allele carriers.

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CYP1A2 genotype and acute effects of caffeine on resistance exercise, jumping, and sprinting performance

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Abstract

Background: It has been suggested that polymorphisms within CYP1A2 impact inter-individual variation in the response to caffeine. The purpose of this study was to explore the acute effects of caffeine on resistance exercise, jumping, and sprinting performance in a sample of resistance-trained men, and to examine the influence of genetic variation of CYP1A2 (rs762551) on the individual variation in responses to caffeine ingestion.

Methods: Twenty-two men were included as participants (AA homozygotes $n = 13$; C-allele carriers $n = 9$) and were tested after the ingestion of caffeine (3 mg/kg of body mass) and a placebo. Exercise performance was assessed with the following outcomes: (a) movement velocity and power output in the bench press exercise with loads of 25, 50, 75, and 90% of one-repetition maximum (1RM); (b) quality and quantity of performed repetitions in the bench press exercise performed to muscular failure with 85% 1RM; (c) vertical jump height in a countermovement jump test; and (d) power output in a Wingate test.

Results: Compared to placebo, caffeine ingestion enhanced: (a) movement velocity and power output across all loads (effect size [ES]: 0.20–0.61; $p < 0.05$ for all); (b) the quality and quantity of performed repetitions with 85% of 1RM (ES: 0.27–0.85; $p < 0.001$ for all); (c) vertical jump height (ES: 0.15; $p = 0.017$); and (d) power output in the Wingate test (ES: 0.33–0.44; $p < 0.05$ for all). We did not find a significant genotype × caffeine interaction effect (p-values ranged from 0.094 to 0.994) in any of the analyzed performance outcomes.

Conclusions: Resistance-trained men may experience acute improvements in resistance exercise, jumping, and sprinting performance following the ingestion of caffeine. The comparisons of the effects of caffeine on exercise performance between individuals with the AA genotype and AC/CC genotypes found no significant differences.

Trial registration: Australian New Zealand Clinical Trials Registry. ID: ACTRN12619000885190.

Keywords: Supplements, Ergogenic effects, Genetic, Variation

Background

Caffeine is one of the most consumed psychoactive stimulants in the world [1]. The effects of caffeine supplementation on exercise performance have received considerable attention in the literature, and the evidence on its ergogenic effects is well-established [1–3]. For example, a recent umbrella review of 21 published meta-analyses reported that caffeine ingestion is acutely ergogenic for aerobic endurance, muscle strength, muscle endurance, power, jumping performance, and exercise speed [3]. Despite these established performance-enhancing effects of caffeine, it is also commonly acknowledged that there is a large degree of variation in response to caffeine supplementation between individuals [4]. Studies that have reported individual participant

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data suggest that some individuals experience an increase in performance following caffeine ingestion, whereas others do not [4–6]. In order to develop more effective guidelines for caffeine supplementation in sport and exercise settings, the scientific focus has recently been placed on examining and understanding the reasons for the between-individual variation in responses [4, 7].

One potential driver of this individual response is inter-individual genetic variation [4]. The gene CYP1A2 encodes cytochrome P450 1A2, an enzyme responsible for up to 95% of caffeine metabolism [8]. The speed of caffeine metabolism is affected by a single nucleotide polymorphism, rs762551, within this gene [8]. Individuals with the AA genotype at rs762551 are commonly classified as "fast caffeine metabolizers", while C allele carriers (AC/CC genotypes) tend to have a slower clearance of caffeine and are, therefore, commonly classified as "slow caffeine metabolizers" [9]. Significantly greater ergogenic effects of caffeine on aerobic endurance have been reported for individuals with the AA genotype, compared with C allele carriers [6, 10]. However, for high-intensity exercise tasks of a shorter duration, the evidence is less clear.

In a recent study of 19 basketball players, acute ingestion of 3 mg/kg of caffeine produced similar effects on vertical jump performance in individuals with the AA genotype and AC/CC genotypes [11]. These results are in accord with a study that utilized a 30-s Wingate sprint test, while improvement in peak and mean power output was noted following caffeine ingestion, the researchers did not find differences in responses between genotypes [12]. Based on the results of these two studies, it seems variations in the CYP1A2 genotype may not affect the ergogenic effects of caffeine ingestion on high-intensity exercise performance. However, a recent study reported that caffeine ingestion enhances the number of performed repetitions in a resistance exercise session in individuals with the AA genotype but not AC/CC genotypes [13].

Given the conflicting evidence on this topic, the aim of this randomized, double-blind crossover study was to explore the acute effects of caffeine on resistance exercise, jumping, and cycle ergometer sprint performance in a sample of resistance-trained men and the influence of genetic variation of CYP1A2 (rs762551) on the individual variation in responses. We hypothesized that caffeine ingestion would be ergogenic across all exercise tasks and that individuals with the AA genotype would experience greater improvements in exercise performance following caffeine ingestion than those with AC/CC genotypes.

Methods

Experimental design

This study employed a double-blind, randomized, crossover design. All participants attended four laboratory sessions. All trials were performed in the morning hours (between 7 am and noon), and at the same time of the day across the sessions for each participant, to ensure that the results were not affected by circadian variation [14]. The trials took place 4 to 7 days apart. The first and second session included familiarization with the exercise protocol (explained in detail in the "Exercise protocol" section). The two main sessions (i.e., caffeine and placebo sessions) were conducted in a randomized and counterbalanced order. The participants were randomly assigned to the two conditions; half of the participants ingested caffeine in the first session and a placebo in the second session, while the other half ingested a placebo in the first session and caffeine in the second session. Participants were asked not to perform any strenuous exercise for at least 24 hours before the main trials. The participants were also asked to keep a food diary for 24 h using "MyFitnessPal" software, and to match their dietary intakes on the days before the two main sessions as much as possible. The participants were required to refrain from caffeine intake after 6 pm on the day prior to the testing [1]. In order to assist with caffeine restriction, we provided the participants with a list of the most common foods and drinks that contain caffeine. The participants arrived at the laboratory following overnight fasting. Caffeine was administered in capsule form, with a dose of 3 mg/kg of body mass (equivalent to the caffeine dose contained in approximately two cups of coffee). The placebo capsule was identical in appearance to the caffeine capsule, but, instead of caffeine, it contained 3 mg/kg of dextrose. The capsules were ingested 60 min before the start of the exercise session [1]. Genotype was determined using a buccal swab. A validated Food Frequency Questionnaire was used to estimate habitual caffeine intake [15]. Prior to the study, the trial was registered in the Australian New Zealand Clinical Trials Registry ID: ACTRN12619000885190.

Participants

The study involved resistance-trained men as participants. Being resistance-trained was defined in this study as having a minimum of 6 months of resistance training experience with a minimum weekly training frequency of two times on most weeks. All participants were non-smokers. Based on an a priori power analysis done using G*Power software (version 3.1; Germany, Dusseldorf) for repeated-measures Analysis of Variance (ANOVA) (within-between interaction, i.e., in the context of this study genotype × caffeine 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 80% for this study was 18 participants. To factor in possible dropouts, we recruited 22 participants. The exclusion criteria were: (i) prior use of anabolic steroids; and (ii) the existence of any health limitations. Ethical approval for this study was granted by the Victoria University Human Research Ethics Committee (HRE19–019). The remaining data of the project are published elsewhere [16]. Before enrolling in the study, every participant signed an informed consent and filled out a Physical Activity Readiness Questionnaire (PAR-Q). Only participants who responded with 'No' to all PAR-Q items were included in the study. In line with previous research [6, 11–13], we combined participants with the AC and CC genotypes into one group (AC/CC group) for the analysis.

Exercise protocol

One repetition maximum testing The first two sessions included familiarization with the exercise protocol. These sessions were the same as the main sessions (i.e., placebo and caffeine sessions), with the exception that the first one included one-repetition maximum (1RM) testing in the bench press exercise. For the 1RM test, the participants performed sets of one repetition with progressive increases in load until they reached their estimated 1RM. The load was initially set to 20 kg and subsequently increased by 10 kg increments if the mean concentric velocity of the repetition was 0.4 m/s or higher (as determined by a linear position transducer attached to the barbell). If the mean velocity was lower than 0.4 m/s, the load for the next attempt was adjusted using smaller increases (e.g., 5 kg or 2.5 kg, determined based on consultation with the participants). The participants performed 1RM attempts with progressively increasing loads until the mean velocity was ≤0.2 m/s [17]. When the mean velocity of a successful 1RM attempt reached these values, the load was considered as a valid estimate of the 1RM [17]. Three minutes were allowed between 1RM attempts.

Movement velocity and power in the bench press exercise In the first session, upon determining the 1RM, the participants performed the bench press exercise with loads of 25, 50, 75, and 90% of 1RM [18]. The second, third, and fourth sessions started with the assessment of movement velocity in the bench press exercise with different loads, as the 1RM test was only performed in the first session. The external load was first set at 25% of 1RM and was progressively increased to 90% of 1RM. With each load, the participants performed two sets of one repetition and were instructed to lift the load as fast as possible. The better repetition (in the context of higher movement velocity and power output) was used for the analysis. Each repetition was followed by a 3-min rest interval. During each repetition, a GymAware linear position transducer (GymAware Power Tool, Kinetic Performance Technologies, Canberra, Australia) was attached to the barbell and used to measure mean concentric velocity (m/s), mean power (W), peak concentric velocity (m/s), and peak power (W). Previous research has established that this device has good test-retest reliability for power and velocity outcomes in the bench press [19].

Muscle endurance After the final repetition with 90% of 1RM, participants were provided with 5 min of passive rest. After the rest interval, muscle endurance was assessed with a test that involved performing repetitions to momentary muscle failure with a load corresponding to 85% of 1RM in the bench press exercise, as in the study by Rahimi [13]. Besides the total number of repetitions, we also measured velocity and power output for each repetition using the linear position transducer attached to the barbell. For the purpose of statistical analyses, we compared the total number of repetitions in the placebo and caffeine conditions. We also explored movement velocity and power output of all repetitions by matching the number of repetitions between the placebo and caffeine conditions. For example, if a participant performed eight repetitions following the ingestion of placebo and nine following the ingestion of caffeine, for this part of the analysis, we only considered movement velocity and power output in the first eight repetitions. This approach allowed us to objectively quantify the average quality of the repetitions during the test and examine if caffeine ingestion had an effect on movement velocity and power output when the total number of repetitions was matched.

Countermovement jump After the muscle endurance test, participants rested passively for 3 minutes and then performed 1 minute of light running, followed by 10 bodyweight squats, in order to warm-up for the countermovement jump (CMJ). The participants performed a CMJ on a force platform (400S Isotronic Fitness Technology, Skye, South Australia, Australia). The CMJ was performed without an arm swing. The participants started CMJ testing from an upright standing position on the force platform. The participants positioned themselves in the starting position and then received commands from the software displayed on a computer screen that was in front of the platform. The software counted down, "3, 2, 1" and provided "Set" and "Go" commands. After the "Go" command, the participants had 5 seconds to complete the jump. From the starting

position, the participants performed a downward countermovement (i.e., a fast knee flexion) where their lowest position was a semi-squat position (knee ~ 90° and trunk/hips in a flexed position) [20]. Immediately after reaching this point, the participants performed an "explosive" extension of the legs [20]. The participants were given instructions to jump as quickly and "explosively" as possible to achieve maximal vertical jump height [20]. The participants had one warm-up jump and three official attempts. Each attempt was followed by 1 minute of rest. For the analysis, the best jump from three official attempts was used. The outcome in the CMJ test was vertical jump height, determined by an algorithm based on the flight time.

Wingate test After the CMJ test, the participants were provided another 3 minutes of passive rest before starting the Wingate test. The Wingate test was performed using a Lode Excalibur Sport Cycle Ergometer (The Netherlands, Groningen). Individual setup of the cycle ergometer; namely, saddle and handlebar height and length, was determined in the first session and was maintained throughout all subsequent trials. The Wingate test started with a 5-min warm-up (100 W at 60–80 rpm) [21]. After the warm-up, participants performed a 30-s "all-out" sprint while the resistance placed on the flywheel remained constant at 0.75 Nm/kg. The participants remained seated during the 30-s sprint. During the test, peak power, mean power, and minimum power were recorded using the Lode Ergometry Manager 10 software. Peak power was defined as the greatest power value recorded during the 30-s; mean power was the arithmetic mean of power during the test, and minimum power was the lowest power recorded during the sprint.

Side effects

Side effects of caffeine and placebo supplementation were evaluated at two time points: (1) immediately after the completion of the testing sessions; and (2) in the following mornings, upon waking. The participants responded to an 8-item survey regarding the incidence of side effects ("yes/no" response scale). This survey was also used to examine side effects in previous research that explored effects of caffeine on exercise performance [20, 22, 23].

Assessment of blinding

Both in the caffeine and the placebo trials, before and after the exercise session, participants responded to the following question: "Which supplement do you think you have ingested?" [24]. The question had three possible responses: (a) "caffeine", (b) "placebo" and (c) "I do not know" [24]. In case participants respond with "a" or "b", they were required to state the reason for choosing their response.

Genetic testing

The participants underwent genetic testing using a commercially available testing kit from DNAfit Life Sciences (London, UK), as in other studies [25]. Samples were collected using buccal swab devices, with OCR-100 kits by DNAGenotek (Ottawa, Canada). The participants were required to avoiding eating or drinking for at least 60 min prior to the sample collection. All samples were collected according to the manufacturer guidelines. The samples were sent to IDna Genetics Laboratory (Norwich, UK), where the analysis was performed. DNA was extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Cell Projects Ltd., Kent, UK), and amplified through polymerase chain reaction (PCR) on an ABI 7900 real-time thermocycler (Applied Biosystem, Waltham, USA). The samples were analyzed for the CYP1A2 rs762551 single-nucleotide polymorphism. This analysis was performed after the exercise performance data collection; thus, the researchers and participants were blinded to genotype variations of the cohort until the data collection process was finalized.

Statistical analysis

One-way ANOVA was used to test the differences between genotype groups in age, body mass, height, 1RM, and habitual caffeine intake. We used a two-way, repeated-measures ANOVA to test genotype (AA genotype vs. AC/CC genotypes) × caffeine (placebo vs. caffeine) interaction effect on performance data, separately for each performance variable. In the absence of significant genotype × caffeine interaction effects, we conducted no stratified analyses of the effects of caffeine by genotype groups. Relative effect sizes (ES) were calculated as Hedge's *g* for repeated measures and presented together with their respective 95% confidence intervals (95% CIs). ESs of < 0.20, 0.20 to 0.49, 0.50 to 0.79, and ≥ 0.80 were considered to represent trivial, small, moderate, and large effects, respectively. McNemar's test was used in the comparison of the incidence of side effects between the placebo and caffeine conditions. The blinding data were summarized using the Bang's Blinding Index [26]. The values in this index range from − 1.0 (denoting opposite guessing) to 1.0 (denoting complete unblinding) [26]. For this study, we reported the data from this index as a percentage of individuals who identified the correct treatment condition beyond chance [19, 26]. All analyses were performed using the Statistica software (version 13.4.0.14; TIBCO Software Inc., Palo Alto, CA, USA). The significance level was set at $p < 0.05$.

Results

Study participants

All participants completed all testing procedures and were included in the final analysis. Of the whole sample, 13, 7, and 2 participants were categorized as having the AA, AC, or CC genotype, respectively. The participants' characteristics are presented in Table 1. There were no significant differences between the genotype groups for age, body mass, height, 1RM, or habitual caffeine intake.

Movement velocity and power output in the bench press exercise

We did not find a significant main effect for genotype ($p > 0.05$ for all) or a genotype × caffeine interaction effect for any of the 16 analyzed variables for movement velocity and power output in the bench press exercise (mean power, mean velocity, peak power, and peak velocity at 25, 50, 75, and 90% 1RM; Table 2). For all variables, except peak power output at 50% 1RM, there was a significant main effect favoring caffeine ($p < 0.05$). The ESs, favoring caffeine conditions in all outcomes, ranged from 0.20 to 0.29 for all outcomes recorded at 25% 1RM, from 0.21 to 0.23 for all outcomes at 50% 1RM, from 0.31 to 0.50 for all outcomes at 75% 1RM, and from 0.57 to 0.61 for outcomes at 90% 1RM.

Muscle endurance

For the maximum number of repetitions in the bench press exercise with 85% 1RM, we did not find a significant main effect for genotype ($p = 0.397$) or a genotype × caffeine interaction effect ($p = 0.454$), while there was a significant main effect favoring caffeine ($p < 0.001$; ES = 0.53). For peak velocity, mean power output, and peak power output (matched for repetitions between placebo and caffeine conditions), we did not find a significant main effect for genotype ($p > 0.05$ for all) or a genotype × caffeine interaction effect ($p > 0.05$ for all), while there was a significant main effect favoring caffeine in all three variables ($p < 0.001$ for all). The ESs ranged from 0.27 to 0.53. For mean velocity, there was a significant main effect for genotype ($p = 0.034$), with the AC/CC genotypes producing greater movement velocity than the AA genotype, and a significant main effect favoring caffeine ($p < 0.001$; ES = 0.85), while we found no significant genotype × caffeine interaction effect ($p = 0.094$).

Countermovement jump

For vertical jump height in the CMJ test, we did not find a significant main effect for genotype ($p = 0.447$) or a genotype × caffeine interaction effect ($p = 0.752$), while there was a significant main effect favoring caffeine ($p = 0.017$; ES = 0.15).

Wingate test

For peak power in the Wingate test, we did not find a significant main effect for genotype ($p = 0.998$) or a genotype × caffeine interaction effect ($p = 0.542$), while there was a significant main effect favoring caffeine ($p < 0.001$; ES = 0.33). For mean power in the Wingate test, we did not find a significant main effect for genotype ($p = 0.517$) or a genotype × caffeine interaction effect ($p = 0.583$), while there was a significant main effect favoring caffeine ($p < 0.001$; ES = 0.35). For minimum power in the Wingate test, we did not find a significant main effect for genotype ($p = 0.505$) or a genotype × caffeine interaction effect ($p = 0.396$), while there was a significant effect favoring caffeine ($p = 0.011$; ES = 0.44).

Side effects

In the responses recorded immediately post-exercise, we found a significant difference between the placebo and caffeine conditions only in items "Increased vigor/activeness" and "Perception of improved performance" in the AC/CC genotypes (Table 3). In the responses 24-h after capsule ingestion, we did not find any significant differences in the incidence of side effects between the placebo and caffeine conditions.

Assessment of blinding – AA genotype

Before starting the exercise session, in the placebo and caffeine conditions, respectively, 62% and 54% of the participants with the AA genotype correctly guessed the treatment identity beyond chance. After exercise, in the placebo and caffeine conditions, respectively, 85% and 69% of the participants with the AA genotype correctly guessed the treatment identity beyond chance.

Table 1 Characteristics of the participants

Variable	AA group ($n = 13$)	AC/CC group ($n = 9$)	p-values from one-way ANOVA
Age (years)	27.0 ± 5.6	29.8 ± 3.6	0.205
Body mass (kg)	78.2 ± 6.5	80.9 ± 14.8	0.559
Height (cm)	182.2 ± 5.5	183.2 ± 5.7	0.658
1RM in the bench press (normalized per body mass)	1.1 ± 0.1	1.2 ± 0.2	0.240
Habitual caffeine intake (mg/day)	133 ± 123	117 ± 68	0.286

Data reported as mean ± standard deviation; *1RM* one repetition maximum; habitual caffeine intake was estimated using a Food Frequency Questionnaire

Table 2 Effects of caffeine on resistance exercise, jumping, and sprinting performance: results from the two-way, repeated-measures ANOVA

Variable	AA genotype (placebo)	AA genotype (caffeine)	AC/CC genotypes (placebo)	AC/CC genotypes (caffeine)	Main effect for genotype ***p***-value	Main effect for caffeine ***p***-value	Genotype × caffeine interaction effect ***p***-value	Effect size for condition and its 95% CI
Movement velocity and power in the bench press with different loads								
MP at 25% 1RM (W)	1892 ± 299	2012 ± 325	2152 ± 501	2279 ± 517	0.139	0.001	0.918	0.29 (0.12, 0.46)
MV at 25% 1RM (m/s)	1.41 ± 0.12	1.44 ± 0.14	1.46 ± 0.16	1.49 ± 0.15	0.411	0.035	0.566	0.20 (0.02, 0.39)
PP at 25% 1RM (W)	3287 ± 374	3409 ± 384	3598 ± 688	3703 ± 804	0.215	0.033	0.868	0.20 (0.03, 0.37)
PV at 25% 1RM (m/s)	2.21 ± 0.18	2.27 ± 0.18	2.31 ± 0.20	2.35 ± 0.17	0.244	0.008	0.806	0.26 (0.07, 0.46)
MP at 50% 1RM (W)	1182 ± 145	1217 ± 154	1279 ± 214	1333 ± 249	0.196	0.008	0.545	0.22 (0.06, 0.39)
MV at 50% 1RM (m/s)	0.94 ± 0.08	0.97 ± 0.08	0.96 ± 0.11	0.98 ± 0.10	0.711	0.019	0.955	0.21 (0.02, 0.42)
PP at 50% 1RM (W)	1979 ± 201	2036 ± 220	2122 ± 394	2203 ± 406	0.228	0.090	0.753	0.21 (− 0.03, 0.46)
PV at 50% 1RM (m/s)	1.41 ± 0.09	1.43 ± 0.09	1.44 ± 0.18	1.48 ± 0.16	0.468	0.031	0.489	0.23 (0.03, 0.45)
MP at 75% 1RM (W)	789 ± 144	838 ± 151	849 ± 148	928 ± 198	0.281	< 0.001	0.229	0.36 (0.19, 0.56)
MV at 75% 1RM (m/s)	0.56 ± 0.07	0.60 ± 0.07	0.58 ± 0.10	0.63 ± 0.10	0.618	< 0.001	0.514	0.48 (0.27, 0.72)
PP at 75% 1RM (W)	1210 ± 238	1289 ± 233	1369 ± 207	1453 ± 293	0.128	0.007	0.940	0.31 (0.10, 0.54)
PV at 75% 1RM (m/s)	0.80 ± 0.12	0.88 ± 0.09	0.86 ± 0.17	0.91 ± 0.17	0.433	< 0.001	0.243	0.50 (0.26, 0.77)
MP at 90% 1RM (W)	501 ± 128	582 ± 132	588 ± 109	675 ± 143	0.103	< 0.001	0.850	0.61 (0.31, 0.93)
MV at 90% 1RM (m/s)	0.33 ± 0.06	0.38 ± 0.07	0.38 ± 0.12	0.43 ± 0.09	0.182	< 0.001	0.909	0.57 (0.28, 0.89)
PP at 90% 1RM (W)	821 ± 225	970 ± 231	994 ± 301	1165 ± 308	0.099	< 0.001	0.789	0.57 (0.25, 0.91)
PV at 90% 1RM (m/s)	0.50 ± 0,09	0.59 ± 0.11	0.59 ± 0.18	0.67 ± 0.13	0.117	< 0.001	0.966	0.59 (0.27, 0.95)
Muscle endurance test								
Maximum repetitions at 85% 1RM	6.8 ± 2.3	8.2 ± 2.2	7.8 ± 2.4	8.8 ± 2.2	0.397	< 0.001	0.454	0.53 (0.27, 0.81)
MP matched for repetitions (W)	376 ± 86	449 ± 96	476 ± 122	531 ± 159	0.074	< 0.001	0.406	0.53 (0.31, 0.79)
MV matched for repetitions (m/s)	0.25 ± 0.04	0.30 ± 0.04	0.30 ± 0.05	0.33 ± 0.04	0.034	< 0.001	0.094	0.85 (0.50, 1.25)
PP matched for repetitions (W)	607 ± 178	674 ± 187	741 ± 297	808 ± 300	0.201	< 0.001	0.994	0.27 (0.14, 0.41)
PV matched for repetitions (m/s)	0.38 ± 0.06	0.43 ± 0.05	0.44 ± 0.09	0.48 ± 0.08	0.108	< 0.001	0.198	0.51 (0.28, 0.77)
CMJ								
CMJ vertical jump height (cm)	34.8 ± 6.2	35.6 ± 5.9	36.6 ± 5.2	37.6 ± 5.4	0.447	0.017	0.752	0.15 (0.03, 0.28)

Table 2 Effects of caffeine on resistance exercise, jumping, and sprinting performance: results from the two-way, repeated-measures ANOVA *(Continued)*

Variable	AA genotype (placebo)	AA genotype (caffeine)	AC/CC genotypes (placebo)	AC/CC genotypes (caffeine)	Main effect for genotype ***p***-value	Main effect for caffeine ***p***-value	Genotype × caffeine interaction effect ***p***-value	Effect size for condition and its 95% CI
Wingate								
PP in the Wingate test (W)	874 ± 208	943 ± 197	864 ± 273	954 ± 260	0.998	< 0.001	0.542	0.33 (0.16, 0.52)
MP in the Wingate test (W)	583 ± 77	614 ± 67	606 ± 120	646 ± 132	0.517	< 0.001	0.583	0.35 (0.20, 0.52)
MinP in the Wingate test (Watts)	338 ± 108	372 ± 79	350 ± 109	414 ± 114	0.505	0.011	0.396	0.44 (0.09, 0.81)

MP mean power, *MV* mean velocity, *PP* peak power, *PV* peak velocity, *1RM* one repetition maximum, *MinP* minimum power, *CMJ* countermovement jump, *CI* confidence interval

Assessment of blinding – AC/CC genotypes

Before starting the exercise session, in both the placebo and caffeine conditions, 55% of the participants with the AC/CC genotypes correctly guessed the treatment identity beyond chance. After exercise, in the placebo and caffeine conditions, respectively, 44% and 78% of the participants with the AC/CC genotypes correctly guessed the treatment identity beyond chance, respectively.

Discussion

The results of the present study demonstrate that the acute ingestion of a moderate dose of caffeine (3 mg/kg) may produce significant improvements in: (a) movement velocity and power output in the bench press using loads ranging from 25 to 90% of 1RM; (b) maximum number of repetitions performed to momentary muscle failure in the bench press exercise, as well as the average quality (i.e., higher movement velocity and power output) of the performed repetitions; (c) vertical jump height; and (d) peak, mean, and minimum power in the 30-s Wingate test. No significant differences in the effects of caffeine were found between the individuals with the AA genotype and the individuals with the AC/CC genotypes in any of the performance tests used in the present study.

Table 3 Perceived side effects based on questionnaires completed immediately after the testing session and the following morning

Variable	AA group – placebo	AA group – caffeine	AC/CC group – placebo	AC/CC group – caffeine
Immediately after testing session				
Muscle soreness	46%	23%	0%	0%
Increased urine production	0%	23%	0%	11%
Tachycardia and heart palpitations	8%	8%	0%	0%
Increased anxiety	0%	23%	0%	0%
Headache	8%	8%	11%	11%
Abdominal/gut discomfort	0%	0%	0%	0%
Increased vigor/activeness	23%	62%	0%[a]	67%[a]
Perception of improved performance	15%	62%	11%[a]	100%[a]
The following morning				
Muscle soreness	23%	8%	0%	22%
Increased urine production	8%	0%	0%	11%
Tachycardia and heart palpitations	0%	0%	0%	0%
Increased anxiety	0%	0%	0%	0%
Headache	8%	8%	22%	0%
Abdominal/gut discomfort	0%	0%	0%	0%
Insomnia	8%	0%	0%	11%
Increased vigor/activeness	0%	0%	0%	33%

[a]Significant difference between the placebo and caffeine conditions within a group

Effects of caffeine on exercise performance

In the bench press exercise, caffeine ingestion enhanced peak and mean velocity and consequently, mean and peak power, when exercising with low, moderate, and high loads. These results are generally in line with previous findings [18, 20, 22]. One of the early studies [18] conducted on this topic reported that high doses of caffeine (9 mg/kg) are required for acute increases in movement velocity when exercising with very high loads (90% 1RM). However, our results suggest that a dose of 3 mg/kg is effective for enhancing velocity across a wide range of external loads, suggesting that very high doses might not be needed. This is especially relevant to highlight as the ESs in our study are very similar to those reported for the bench press exercise by Pallarés et al. [18].

A recent meta-analysis found that caffeine ingestion enhances mean and peak movement velocity in resistance exercise [27]. The researchers also noted that the effects of caffeine on mean velocity (ES = 0.80) were higher than those for peak velocity (ES = 0.41) [27]. However, the studies included in that meta-analysis assessed either mean or peak velocity; that is, no studies included in the meta-analysis measured both outcomes in the same group of participants [27]. In the present study, we found that the ESs were very similar for both mean and peak velocity, and this was a constant finding across all the employed loads (i.e., 25 to 90% of 1RM).

The muscle endurance test used in this study further confirmed that caffeine ingestion is ergogenic for this fitness component in resistance-trained men. This study adds to the body of evidence showing improvements in muscle endurance following caffeine ingestion [28–32]. However, a more novel finding is that caffeine is ergogenic for power and velocity outputs when the number of repetitions between the caffeine and placebo conditions is matched. Specifically, when matching the number of repetitions between conditions, we found that the effects of caffeine, as compared to placebo, amounted to 0.27 for peak power, 0.51 for peak velocity, 0.53 for mean power, and 0.85 for mean velocity. Several studies that explored the effects of caffeine on muscle endurance did not find a difference in the number of performed repetitions between the caffeine and placebo conditions [13, 33, 34]. However, as we demonstrated in the present study, even with an equal number of repetitions between conditions, caffeine might have still produced considerable improvements in the quality of the performed repetitions, that is, greater movement velocity and consequently, greater power output (which was not tested in the aforementioned studies). As compared to placebo, caffeine ingestion most commonly produced moderate improvements in the number of performed repetitions (generally one to three additional repetitions) [28, 31]. We propose that in some contexts, improvements in the overall quality of the performed repetitions may be more important for training adaptations than simply performing a greater number of repetitions. This hypothesis is in line with recent findings that training at a velocity loss of 20% produced greater improvement in CMJ performance than training at a 40% velocity loss [35]. Improvements in squat strength were similar for both training conditions, even though the group that trained with a velocity loss of 20% performed 40% fewer repetitions.

Caffeine ingestion resulted in increased vertical jump height in the CMJ. The ES magnitude of 0.15 observed in this study is very similar to the pooled ES of 0.17 reported in a recent meta-analysis of 10 studies [36]. This result, therefore, confirms that caffeine ingestion may have a relatively small performance-enhancing effect on vertical jump height [36–38]. The acute improvement in vertical jump height following caffeine ingestion is comparable to the improvement in jump height found as a result of 4 weeks of plyometric training [39, 40]. Even though the improvement in performance was relatively small (approximately 1 cm), it might still be practically meaningful in sports where jump height directly impacts athletic outcomes.

In the Wingate test, we found a significant ergogenic effect of caffeine on peak, mean, and minimum power. These results are in line with the findings of a recent meta-analysis that reported ergogenic effects of caffeine on mean and peak power in the ES magnitude of 0.18 and 0.27, respectively [41]. Of the 16 studies included in the meta-analysis [41], 12 studies used caffeine doses of 5 or 6 mg/kg. Therefore, it could be argued that the findings of the meta-analysis should primarily be generalized to these doses of caffeine. In the present study, we found that even a lower dose of caffeine (namely, 3 mg/kg), increases performance in this test and that the ES is very similar to that reported by studies using higher caffeine doses [41].

The influence of the CYP1A2 genotype

We did not find significant genotype × caffeine interaction effects in any of the analyzed performance variables. It might be that the effects of caffeine ingestion are similar between different CYP1A2 genotypes, at least for the performance tests used in the present study. The results reported herein are generally in line with the current body of evidence. Two studies [11, 12] that explored the effects of caffeine on jumping and Wingate test performance reported similar improvements in these outcomes following the ingestion of 3 mg/kg of caffeine in groups of participants with the AA and AC/CC genotypes. However, a recent study [13] that used a resistance exercise protocol, found that caffeine is ergogenic only for individuals with the AA genotype. On average,

individuals with the AA genotype were able to complete one more repetition with the consumption of caffeine, as compared to placebo, whereas the number of repetitions was the same in the placebo and caffeine conditions among those with the AC/CC genotypes. The main methodological difference between the current studies exploring this topic was the dose of caffeine administered to the participants. Specifically, we and two other studies that reported similar results utilized 3 mg/kg of caffeine. We opted to utilize a lower dose of caffeine as higher doses of caffeine do not seem to produce greater increases in performance [28]. In the study by Rahimi [13], the dose was considerably higher (i.e., 6 mg/kg). It might be that the differences in responses between genotypes become apparent only at higher doses of caffeine. Future dose-response studies might consider exploring this hypothesis further. The effectiveness of the blinding was not explored by Rahimi [13] thus limiting the comparison of the results in this aspect of the study design.

Even though Rahimi [13] reported that caffeine ingestion is ergogenic for AA but not AC/CC genotypes in resistance exercise, the main outcome of that study was the number of performed repetitions in 4 different resistance exercises with 85% 1RM, which can be considered as a somewhat crude test of performance. As mentioned previously, we demonstrated that even when matched for the number of repetitions, caffeine, as compared to placebo, increases the average movement velocity and power output of the performed repetitions (ES range = 0.27 to 0.85). Therefore, even though Rahimi [13] reported that in the AC/CC genotypes the total number of repetitions was the same following the ingestion of caffeine and placebo, caffeine might have still enhanced the average velocity and power of these repetitions. We would suggest that future research in this area explores both the quality and quantity of the performed repetitions, to provide a more comprehensive assessment of possible effects of caffeine.

Strengths and limitations

Some of the key strengths of this study are: (a) the standardization of testing conditions, including nutritional intake, physical activity, and the time of day at which the testing is conducted; (b) the inclusion of trained individuals as study participants; (c) a broad range of exercise performance variables that were assessed as outcomes; (d) assessment of performance across a wide-range of loads in the bench press exercise and both quantity and quality of repetitions, when examining muscle endurance as the outcome variable.

There are several potential limitations of this study that need to be acknowledged. First, due to the low number of individuals with the CC genotype, we combined the AC and CC genotypes into one group. This is fairly common in this line of research, as the number of individuals with the CC genotype in the population is suggested to be ~ 10% [9]. To get around 10 to 12 participants with the CC genotype a study would need to screen from 100 to 120 potential study participants. However, despite the fact this is a common practice, it could have confounded findings, as the effects of caffeine might not be uniform between individuals with the AC vs. CC genotype [10, 42]. In the current study, we could not test this further, because the number of individuals with the CC genotype was $n = 2$. Of note, the exclusion of these two participants from the analysis did not alter the study results.

The second limitation is related to the efficacy of blinding [24]. Previous research has established that correct supplement identification may impact the outcomes of a given exercise test and, therefore, bias the results. In the present study, around 50–60% of the participants were able to correctly identify the placebo and caffeine condition beyond random chance in the pre-exercise assessment. In the post-exercise assessment, this percentage generally stayed the same or slightly increased. We believe that the pre-exercise responses are of greater importance, given that the improvements during the testing session (or lack thereof) may influence the post-exercise responses. Tallis and colleagues [43] tested their participants in four conditions: (1) "told caffeine, given caffeine"; (2) "told caffeine, given placebo"; (3) "told placebo, given placebo"; and (4) "told placebo, given caffeine". Equal improvements were found on both occasions when the participants indeed ingested caffeine (i.e., "told caffeine, given caffeine" and "told placebo, given caffeine" conditions), thus suggesting that this limitation of our study might not have greatly affected our findings.

Conclusions

This study found that caffeine is acutely ergogenic for movement velocity, power output, and muscle endurance in resistance exercise, vertical jump height, and peak, mean, and minimum power in a Wingate test. These performance-enhancing effects were observed following the ingestion of using a moderate dose of caffeine (3 mg/kg), which resulted in minimal side effects. The comparisons of the effects of caffeine on exercise performance between individuals with the AA genotype and AC/CC genotypes found no significant differences.

Abbreviations

1RM: One repetition maximum; ANOVA: Analysis of variance; CI: Confidence interval; CMJ: Countermovement jump; ES: Effect size; PAR-Q: Physical Activity Readiness Questionnaire; PCR: Polymerase chain reaction

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Authors' contributions
JG, DJB, and ZP conceived and designed the study. JG performed the experiments, analyzed the data, and wrote the first draft. ZP, DJB, CP, BJS, and PM critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study protocol was approved by the Victoria University Human Research Ethics Committee (HRE19–019). The research was performed in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests. Craig Pickering is a former employee of DNAFit Ltd., a genetic testing company. He received no financial incentives for the preparation of this manuscript.

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