The Association of Gene Polymorphisms Linked to Caffeine Use with Athletic Performance

Kafein Kullanimina Etki Eden Gen Polimorfizmlerinin Atletik Performans ile İlişkisi

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ABSTRACT

Caffeine's use as a performance-enhancing supplement among professional athletes progressively increased after it was taken off the World Anti-Doping Agency's (WADA) list of banned substances. The present review looks at the interactions caused by different levels of caffeine ergogenicity in athletes and gene polymorphisms that affect caffeine ergogenic effect in the current literature. Therefore, a comprehensive analysis of the studies available in the literature and the respective study results have evaluated caffeine ergogenicity in athletes and gene polymorphisms. The search for studies was performed through five databases. Individual responses to caffeine consumption may differ from person to person due to multigene and multifactorial interactions. Adenosine A2A receptor (*ADORA2A*) and Cytochrome P450 1A2 (*CYP1A2*) are two genes thought to have the most effects on caffeine's ergogenicity. The function of these two genes accounts for most inter-individual variations in studies after caffeine ingestion. Based on the findings, it is understood that the contribution of caffeine use to individual performance development varies over time due to changes in both environmental and epigenetic interactions, as well as parameters such as circadian rhythm, training loads, nutritional routine, habitual caffeine use, drug intake, expectation, time of consumption, and heredity. As a result, individual caffeine responses are highly complex and involve multiple modifiers.

Key Words: Caffeine, athlete, exercise, athletic performance, *CYP1A2*, *ADORA2A*

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ÖZET

Kafein kullanımı, Dünya Anti Doping Ajansı (WADA) tarafından yasaklı maddeler listesinden çıkarılmasının ardından elit sporcular arasında performans artırıcı bir destek ürünü olarak kullanım oranı da giderek artış göstermeye başlamıştır. Bu doğrultuda, bu araştırmanın amacı sporcularda kafein alımına verilen farklı seviyelerdeki yanıtların neden olduğu etkileşimlerin ve kafein ergojenitesine etki eden gen polimorfizmlerinin güncel literatür kapsamında incelenmesidir. Kafein alımı sonucunda ortaya çıkan bireysel tepkiler çok gen ve çok faktörlü etkileşimler neticesinde bireyden bireye farklılık gösterebilmektedir. Cytochrome P450 1A2 (*CYP1A2)* ve Adenozine A_{2A} reseptorü (*ADORA2A)*, kafeinin ergojenitesi üzerinde en büyük etkiye sahip olduğu düşünülen genlerden ikisidir. Bu iki genin rolü, kafein alımını takiben yapılan çalışmalarda bireyler arası farklılıkların büyük bir bölümünü açıklamaktadır. Bugüne kadar elde edilen bulgular ışığında kafein kullanımının bireysel performans gelişimine sağladığı katkıların sirkadiyen ritim, antrenman yüklenmeleri, beslenme rutini, alışılmış kafein kullanımı, ilaç alımı, beklenti, kullanım zamanı ve soyaçekim gibi parametrelerle birlikte hem çevresel hem de epigenetik etkileşimlerin zaman içinde değişkenlik göstermesi sebebiyle farklılık gösterdiği anlaşılmaktadır. Sonuç olarak, kafein kullanımındaki bireysel tepkiler oldukça karmaşıktır ve birden fazla değiştirici parametre söz konusudur. Bu derlemeden elde edilen bulgular, atletik performans gelişimine yönelik hazırlanan kafein kullanım programlarının bireysel etkileşimdeki farklılıkların göz önünde bulundurularak düzenlenmesine katkı sağlayacağı düşünülmektedir.

Anahtar Sözcükler: Kafein, sporcu, egzersiz, atletik performans, *CYP1A2*, *ADORA2A*

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INTRODUCTION

Following the removal of caffeine from the list of banned substances by the World Anti-Doping Agency (WADA), its use as a performance-enhancing supplement among elite athletes has been on the rise. Previous studies report that caffeine intake provides a positive ergogenic effect on the development of athletic performance in numerous sports disciplines and that the levels of increased effort observed (during training and competition) create significant differences between individuals (1). Caffeine is a supplement widely used by many athletes due to its physiological effects on adenosine receptors in the central nervous system (2). Caffeine acts as a competitive adenosine receptor antagonist in the central nervous system (CNS), reducing the excitability and down-regulation of nerve activity induced by adenosine. Furthermore, the binding of caffeine to adenosine receptors increases neurotransmitter release and muscle activation, stimulates adrenaline secretion, alters substrate utilization (3), and raises the focus and resistance threshold while also decreasing the effort and pain threshold, leading to a series of physiological reactions that contribute to the acceleration of exercise performance (4). The effects of adenosine are inhibited through competition with caffeine at adenosine receptor sites. In addition to caffeine, caffeine metabolites theophylline and paraxanthine have a structure similar to adenosine, which allows them to bind to adenosine receptors in the body. The antagonism of adenosine by caffeine and theophylline molecules has arguably a substantial effect on caffeine's ergogenicity. This improves alertness, stimulation, and motivation to exert effort by reducing the effects of adenosine and the consequent feelings of fatigue, particularly during endurance exercise. Previous studies indicate that caffeine's effect is most likely due to the fact that it acts as an antagonist of the adenosine receptor, which blocks movements in the brain (5).

The use of caffeine as an ergonomic aid significantly improves workout performance. In literature, caffeine has positive effects on both aerobic (endurance) and anaerobic (strength, explosive power) metabolism (2). While the energy provided by caffeine triggers muscle strength development, it also increases aerobic endurance and delays fatigue. Athletes who perform training loads where aerobic capacity and prolonged use of energy are important are more inclined to use caffeine. Caffeine primarily provides a ready energy source by allowing the body's fat stores to be used as fuel. In several studies, it has been observed that the ergogenic effect of caffeine has a direct effect on fat stores and increases lipolysis by stimulating the release of cortisol and norepinephrine. In contrast, it has been suggested that increased lipid mobilization is also related to caffeine use (6). It has been hypothesized that the increased rate of this lipid breakdown delays the potential accumulation of lactate during aerobic endurance training by allowing the preservation of glycogen resources (7).

In previous studies on caffeine use in athletes, it has been reported that most elite athletes use caffeine supplementation during training loads and competitions, which positively affects performance enhancement, the focus threshold and reaction time [8]. Energy drinks containing caffeine are also used as ergogenic supplements to improve physical performance in various sports disciplines (8). Several studies conducted within the framework of the assessment of caffeine intake by the International Society of Sports Nutrition (ISSN) have reported that caffeine supplementation acutely improves various aspects of exercise performance and positively affects aerobic and anaerobic capacity (9). On the other hand, it was also found that the effects of caffeine use differed between individuals, physical activities requiring prolonged effort associated with aerobic endurance provided more improvement compared to caffeine use, and caffeine gradually improved exercise performance when consumed at doses of approximately 3-6 mg/kg. Additionally, certain studies have stated that very high doses of caffeine (9 mg/kg) do not produce any ergogenic effect due to side effects (10). In contrast, other studies have found that caffeine does not significantly accelerate lipid metabolism during exercise (1, 5).

Despite the positive developments observed in exercise performance following caffeine intake (5), caffeine intake responses vary significantly between individuals and across studies (11). Previous studies have demonstrated that some individuals do not respond positively to caffeine supplementation, that their athletic performance status did not change between placebo and caffeine trials, and that performance decreased following caffeine supplementation. Similar studies show that approximately 33% of individuals failed to improve their aerobic endurance performance following caffeine intake. The worldwide popularity of caffeine can be attributed to its ability to promote alertness, enhance mood and cognition, and produce stimulant effects (12).

Use of caffeine may result in bronchodilation, diuresis, and a rise in some people's systolic blood pressure. (13). Caffeine has modest euphoric effects, increases alertness, and enhances cognitive function when used in small doses. The effects of caffeine in individuals with addiction are quite different from those in individuals who do not use caffeine. There are distinct individual differences in responses to caffeine. For example, some individuals are sensitive to the anxiogenic effects of caffeine (14) and others to caffeine-induced sleep disturbances and insomnia (15). Caffeine can exacerbate anxiety and precipitate panic attacks in patients with anxiety and panic disorders, often leading to reduced consumption (16). Several factors are likely to contribute to individual differences in responses to caffeine, including demographic and environmental factors such as age, use of banned substances, circadian factors, and sleep hygiene. The variance in caffeine consumption is significantly influenced by genetic inclination. Genetic differences between individuals may positively or negatively alter the metabolic impact of performance enhancers used to increase the effects of training. In addition to ergogenic effect like creatine, caffeine, arginine, and carnitine, banned performance-enhancing substances, such as Erythropoietin (EPO), growth hormone (GH), and insulin-like growth factor-1 (IGF-1) may contribute positively to some. In contrast, for others, they do not provide any positive development, and side effects may occur (17). Individual genetic variations, which are important to take into account in the development of athletic performance, as well as training load types and dietary practices, might positively or negatively change the impact of these performance enhancers (17).

Based on the principle of individuality of branch selection, nutrition, and training in the light of genetic information, it may be an advantage for coaches, athletes, and sedentary individuals to be able to evaluate the use of performance-enhancing substances and the effects of loading on the metabolism within the framework of both athletic performance and wellness. These advantages may contribute to continuity in training adaptation resulting from accurate branch selection, as well as to the reduction of the risk level of mortality due to metabolic and chronic diseases and the development of public health as part of wellness and exercise programs that take individual differences into account. Considering that nutrition can also affect post-exercise gene signaling, it can be said that the changes caused by the combination of lifestyle and epigenetic factors together with genes and the familiar environment directly affect athletic performance (17, 18). This study will address the interactions caused by varied quantities of caffeine ergogenicity in athletes, as well as gene polymorphisms that alter caffeine ergogenic effects.

The Effect Mechanism of Caffeine, Individual Limits and Times of Use in Different Exercises

Literature searches were conducted in five electronic databases including Open Access Theses, PubMed/MEDLINE, Scopus, SPORTDiscus, and Web of Science. In all of these databases, we used the following syntax: (*CYP1A2*; *ADORA2A* OR genotype OR genetics OR polymorphism) AND (caffeine) AND (exercise OR training OR ergogenic OR performance). The search for studies concluded on December 30th, 2022.

Caffeine is a performance-enhancement product widely used at all levels of sport. The use of caffeine, which has an ergogenic effect, can affect the level of performance in an individual training session, thereby differentiating the overall adaptation process (5, 11, 13). The caffeine usage limits specified by the International Olympic Committee (IOC) are approximately 600 to 800 mg (approximately 4 to 7 cups of coffee to be consumed over a 30-minute period). At the 1972 Summer Olympics, a judo athlete was banned from competitions and stripped of his prize after an overdose of caffeine was detected. In reality, it is well-known that the other athletes on the podium also consumed large amounts of caffeine but avoided punishment (8). Despite the ban imposed by WADA in 2004 on high doses of caffeine (12µg.ml1) by athletes for performance-enhancing purposes in Olympic competitions, studies report that 4/3 of the samples tested as part of the anti-doping process contained measurable levels of caffeine (19).

Seven thousand four hundred eighty-eight urine samples from athletes competing in official competitions in Spain were analyzed for urinary caffeine concentration, and it was found that the percentage of samples with detectable caffeine (i.e., >0.1 µg/mL) increased from 70.1% in 2004-2008 to 75.7% in 2015. The mean urinary caffeine concentration recorded in 2015 (0.85 µg/mL) was higher than in other years.

It was determined that urinary caffeine concentration increased significantly from 2004 to 2015 in water sports, athletics, boxing, judo, football, weightlifting, and rowing while cycling, athletics and rowing were among the disciplines with the highest urinary caffeine concentration in 2015. The findings of previous studies show that although caffeine use gained further momentum following the removal of caffeine from the list of banned substances, caffeine concentrations in urine samples indicate that caffeine use is at a moderate level (8). Since the lifting of the ban on caffeine use, caffeine use has remained consistent and similar rates of caffeine use have been observed in urine samples (20). However, significant differences have been found in individuals' responses following caffeine intake, both for performance enhancement and for issues such as sleep disturbances and anxiety.

High concentrations of caffeine appear in the blood 15 minutes after intake and peak approximately 60 minutes later with a half-life of 3 to 4 hours. Caffeine is metabolized in the liver. It is transported by the bloodstream through the hepatic portal vein. In hepatocytes, microsomal cytochrome P450, especially in the hepatic isozyme *CYP1A2*, is responsible for about 95% of the primary metabolism, demethylating caffeine and transforming it into theobromine, paraxanthine, and theophylline. Caffeine and its metabolites can promote the stimulation of the intermediary metabolism and present many effects in different tissues such as the CNS, skeletal muscle, cardiovascular, renal, and pulmonary tissue through the effect related to critical enzymes to metabolism (21). These substances (theobromine, paraxanthine, and theophylline) also contribute to the performance-enhancing effects of caffeine. Furthermore, Cytochrome P450 expression and activity within the nervous system raises the possibility that caffeine metabolism is partially triggered by these enzymes (22). The biological effects of caffeine are closely related to 3 main modulatory points; antagonistic effect on adenosine receptors (A1 and A2A receptors), antagonistic action of caffeine and its secondary metabolites to adenosine at its receptors in the Central Nervous System (CNS), increasing alertness and decreasing perceived exertion in exercise. Inhibition of phosphodiesterases; effect of preserving muscle glycogen from a greater distribution of fatty acids in the bloodstream and energy use under some conditions, and calcium mobilization; increased release of calcium ions by a neuromuscular stimulus, enhancing contraction power in muscle fibers (21).

Recommendations for caffeine use include a caffeine intake of 3-9 mg/kg approximately 60 minutes before exercise, with no additional benefit associated with higher doses (23). However, recent studies have shown that the ergogenic effects of caffeine can emerge with a wide range of caffeine doses and timings. For example, two recent studies focused on the effects of low doses of caffeine (<3 mg/kg) on performance enhancement and found that lower caffeine intake tended to exert ergogenic effects (24). Grgic (2021) stated that lower doses of caffeine (2-3 mg/kg) produced greater ergogenic effects compared to high doses (6 mg/kg), that minimally effective doses of caffeine were around 1.5 mg/kg, and that alternative caffeine sources such as caffeinated chewing gum, gels and coffee also contributed to the improvement of resistance exercise performance (25). The most common time of caffeine intake is 60 minutes before exercise. The ideal timing of caffeine intake is likely to depend on the source of the caffeine. For example, caffeinated chewing gum may require a shorter waiting period after consumption until the start of the exercise session.

Caffeine intake improves physical performance in both trained and untrained individuals. In addition to inter-individual differences in athletic and exercise performance, adverse effects on sleep or feelings of anxiety following caffeine intake have been attributed to genetic variation associated with caffeine metabolism and physical and psychological response. Other factors, such as habitual caffeine intake, also affect inter-individual response variation. Caffeine use is reported to be ergogenic effect for cognitive functions, including attention and alertness, in most individuals and improves cognitive and physical performance in some individuals under conditions of sleep deprivation. Caffeine dosages of 3 to 6 mg/kg during prolonged exertion at high temperatures and approximately 4-6 mg/kg in hypoxic environments positively support performance.

Alternative caffeine sources such as caffeinated chewing gum, mouthwash, energy gels and chewing have been found to improve both anaerobic and aerobic performance (10).

A previous study examining the ergogenic effects of caffeine dependence on strength endurance and jumping performance as well as perceptual responses, 36 strength-trained individuals were classified according to their habitual caffeine consumption as low (20±11 mg/day), moderate (88±33 mg/day) and high doses (281±167 mg/day) and tested for vertical jump and endurance performance following supplementation with 6 mg/kg caffeine and placebo or no supplementation (control). It was found that acute caffeine supplementation improved vertical jump performance and total repetitions regardless of caffeine habituation, with caffeine promoting a significantly greater improvement in both vertical jump performance and total repetitions compared to placebo. The side effects of caffeine were observed similarly in consumers of low, moderate, and high (26).

In another study investigating the impact of habitual caffeine intake on the acute effects of strength training, jumping, and Wingate performance, male individuals who had learned the application technique of strength training were tested following ingestion of 3 mg/kg caffeine and 3 mg/kg dextrose as a placebo. The participants were classified as low caffeine users and moderate to high caffeine users, and their exercise performance was measured in terms of movement speed, strength and muscular endurance in the bench press, jumping and Wingate test. As a result of the study, the average increases in speed and power in resistance exercise were observed to range from 0.02 to 0.08 m/s and 42 to 156W, respectively. The number of repetitions performed increased by 1-2, and jump height increased by 0.9 cm while the power in the Wingate test was between 31 and 75 W (27). The optimal intake time of caffeine tablets is usually 30-60 minutes before exercise. Caffeinated gums and gels can improve performance in resistance exercises even when consumed 10 minutes before exercise. Caffeine use is presumed to improve performance in resistance exercise. However, a small part of the ergogenic effect of caffeine is also thought to be placebo-based (25).

Regarding the ideal timing of intake, in contrast to the studies mentioned above, it was observed that 6 mg/kg of caffeine consumed 60 minutes prior to exercise was no more effective than six doses of 1 mg/kg of caffeine spread over the exercise period. Caffeine intake is generally recommended for performance enhancement during prolonged and moderately intense effort (marathon, half marathon, triathlon, etc.). The widespread use of caffeine observed in the sporting world makes it clear that there is a standard, predictable response to caffeine among individuals. As with other complex phenotypes, individual responses following caffeine intake may differ from one individual to another due to multigene and multifactorial interactions (28).

Genes Linked to Caffeine Use

Caffeine use has become widespread among athletes following its removal from the World Anti-Doping Agency's list of prohibited substances, with approximately 75% of competitive athletes using caffeine. While the literature supports that caffeine has a small positive ergogenic effect for most sports branches and exercise types, significant inter-individual differences have been reported in responses to caffeine intake and its subsequent effect on exercise performance. Cytochrome P450 1A2 (*CYP1A2)* (hepatic enzymes related to caffeine degradation) and Adenosine A_{2A} receptor (*ADORA2A)* (genetic factors related to the structures of adenosine receptors in the central nervous system) are two of the new genes that are thought to have the greatest influence on the ergogenic effect of caffeine. *CYP1A2* is responsible for most caffeine metabolism, whereas *ADORA2A* is linked to caffeine-induced anxiety (1). The role of these two genes explains a large proportion of the inter-individual variance reported in studies following caffeine intake. Clarifying the extent to which these genes moderate responses to caffeine during exercise will ensure that caffeine supplementation programs can be tailored for individual athletes to maximize the potential ergogenic effect.

The single nucleotide polymorphism in the *CYP1A2* gene responsible for caffeine metabolism has been described as a critical factor influencing acute responses to caffeine intake. However, the existing literature regarding the effect of this polymorphism on the ergogenic effects of caffeine is controversial. Although most studies have shown no differences in reactions to caffeine use between *CYP1A2* genotypes, some studies have identified a possible advantage or disadvantage for C-allele carriers (4).

In the findings of studies examining the effect of the *CYP1A2*-163C>A polymorphism on the ergogenic effects of caffeine, it was stated that individuals with AA or AC/CC genotypes exhibited varying levels of performance improvement following caffeine intake, with AC and CC genotypes responding positively to performance improvement in comparison to AA. It was also stated that *CYP1A2* genotype variations might modulate the ergogenic effects of caffeine [55]. Barreto et al. (2021) report that further studies are needed on how *CYP1A2* polymorphisms may modulate the ergogenic effect of caffeine and its magnitude, and whether *CYP1A2* polymorphisms should be taken into account prior to caffeine supplementation (4).

The *CYP1A2* gene encodes Cytochrome P450 1A2, an enzyme responsible for 95% of all caffeine metabolism. An SNP in this gene, rs762551, determines the duration of the effects of caffeine on the metabolism. AA homozygotes (fast metabolizers) tend to produce more of this enzyme. C allele carriers (slow metabolizers) tend to have slower caffeine clearance (29, 30). Among the observed health effects of *CYP1A2* polymorphisms, it was found that slow metabolizers consuming moderate amounts of coffee (3-4 cups) had an increased risk of myocardial infarction and hypertension, while fast metabolizers experienced a protective effect from moderate coffee consumption (31). Womack et al. (2012) found that the *CYP1A2* genotype was an important factor in the performance-enhancing effects of caffeine in two 40 km cycle time trials following the consumption of 6 mg/kg of caffeine or placebo before exercise in elite-level male cyclists and observed an improvement of 4.9% in fast metabolizers and 1.8% in slow metabolizers. In fast metabolizers, caffeine performance improved by at least one minute in 15 out of 16 participants, while in the others, only 10 out of 19 participants improved by more than one minute (32). These findings suggest that exercise increases *CYP1A2* expression and that trained and untrained individuals metabolize caffeine differently. Another study in this context examined the acute effects of caffeine on resistance exercise, jumping and sprinting performance in a sample of resistance-trained males and the effects of the genetic variation of *CYP1A2* (rs762551) on individual variation in the responses to caffeine. In the study, twenty-two male participants were tested after receiving caffeine (3 mg/kg body mass) and a placebo. No significant genotype-caffeine interaction effect was found for any of the performance outcomes analyzed. It was reported that resistance-trained males may experience acute improvements in resistance exercise as well as jumping and sprinting performance following caffeine intake, whereas no significant differences were found in the comparison of the effects of caffeine on exercise performance between individuals with the AA genotype and the AC/CC genotype (33).

In another study, it was investigated whether variations in 163 C>A *CYP1A2* (rs762551) genotypes (AA, AC and CC) alter the ergogenic effects of caffeine on strength, power, muscular endurance, and agility in adolescent athletes. The participants were administered caffeine (6 $mg.kg^{-1}$) or placebo (300 mg cellulose) 1 hour before performing a series of physical tests. In the study's findings, it was concluded that caffeine did not have a significant ergogenic effect depending on genotype in the comparison between placebo and caffeine intake [34]. This framework states that caffeine use improves muscular endurance and aerobic performance in adolescent athletes regardless of 163 C>A *CYP1A2* genotype. Another study examined the effect of the genetic variations of the *CYP1A2* (rs762551) gene on the performance-enhancing effects of moderate caffeine intake (n= 21 healthy active participants; 29.3 ± 7.7 years). The participants were administered 3 mg of caffeine or a placebo per kilogram of body weight in test sessions separated by one week, and visual attention and side effects were evaluated with the 30 s Wingate performance test. The study identified two different polymorphisms: AA homozygotes (n=5) and C-allele carriers (n=16). Caffeine ingestion increased peak power (682±140 vs. 667±137 W) and mean power (527±111 vs. 518±111 W) during the Wingate test, and no difference was observed between AA homozygotes and C alleles. Similarly, reaction times did not differ between the two polymorphisms (276±31 vs. 269±71 milliseconds). However, 31.3% of the C-allele carriers exhibited increased irritability following caffeine intake, suggesting that the genetic variations of the *CYP1A2* polymorphism do not affect the ergogenic effects and disadvantages resulting from moderate caffeine intake (35).

The restorative effects of caffeine on exercise performance are directly related to its ability to bind to adenosine receptors. The *ADORA2A* gene does not encode A2A subtypes of adenosine receptors. It has been argued that *ADORA2A* gene polymorphisms may be responsible for inter-individual variation in the effects of caffeine on exercise performance (36).

An SNP in the adenosine receptor gene *ADORA2A*, rs5751876, affects habitual caffeine use and sleep disturbances following caffeine use (37). In a previous study, 12 female participants underwent a randomized, double-blind crossover experiment consisting of two 10-minute trials following caffeine intake (5 mg/kg) or a placebo. Caffeine use led to performance enhancement in TT homozygotes, but in C allele carriers, the performance improvement was observed in only one of the six individuals who exhibited an ergogenic effect [38]. Furthermore, although *CYP1A2* polymorphism positively affects physical performance, it is unknown whether these improvements enhance team sport performance. Another study investigated the effect of *ADORA2A* and *CYP1A2* genotypes on caffeine's physiological and ergogenic effects. As a result of the study (n= 66 cyclists), it was found that caffeine affects physiological responses to exercise and improves time trial performance regardless of the *ADORA2A* or *CYP1A2* genotypes (38).

Genetic factors make a difference in individual responses to caffeine intake. However, the mechanisms by which genetic variation alters caffeine ergogenicity are also unclear. AA alleles of the *CYP1A2* gene are thought to metabolize caffeine more rapidly (33). Guest et al. (2018) reported that C allele carriers metabolize caffeine at a slower rate, possibly limiting performance in endurance activities where the transfer of oxygen and nutrients to active muscles is crucial due to prolonged vasoconstriction [39]. Therefore, the timing of caffeine use is of great importance. The effects of caffeine are longer lasting in C allele carriers. Given caffeine's many different mechanisms of action, it is likely that each mechanism has polymorphisms that alter ergogenic effects. For example, since caffeine reduces the severity of exercise-induced pain (39), it is predicted that gene polymorphisms related to pain tolerance may alter this effect.

In a previous study investigating the effect of variation in *ADORA2A* (rs5751876) on the ergogenic effects of caffeine on exercise performance, C allele carriers were identified as "non-responders" to caffeine. In order to investigate whether C allele carriers are true "non-responders" to the ergogenic effects of caffeine, this randomized, double-blind study examined the acute effects of caffeine intake among a sample of *ADORA2A* C allele carriers only. Twenty males who had undergone strength training and were identified as *ADORA2A* C allele carriers (CC/CT genotype) were tested on two occasions following an intake of caffeine (3 mg/kg) and a placebo. During the Wingate test, exercise performance was assessed by movement speed, power output and muscular endurance during bench press repetition, vertical jump height, and power output. Caffeine intake produced ergogenic effects (effect size range: 0.14 to 0.96) in 21 out of 25 variables analyzed (33). In conclusion, caffeine supplementation is predicted to improve performance development in individuals with the *ADORA2A* (rs5751876) CT/CC genotype.

Genetic variation is also likely to influence exercise performance indirectly. *ADORA2A* polymorphisms may predispose individuals to increased anxiety following caffeine intake (40). This is notable for individuals with high levels of pre-competition anxiety who may benefit from high stimulation. *ADORA2A* polymorphisms are also associated with increased sleep disturbances following caffeine intake. This may affect athletes participating in evening competitions or those participating in daily competitions organized at frequent intervals due to caffeine-induced sleep problems. Given that genetic variation can modify sleep disturbances after caffeine intake, individuals more likely to suffer from these disorders may consume less caffeine in an evening competition than in a morning competition (40). This leads to the possibility that certain genotypes may consume less caffeine in competitions where anxiety is higher, such as the Olympic Games or the World Cup final, and more caffeine in competitions where anxiety is lower, such as a league match.

Caffeine's ergogenic activity may be modified by a variety of non-genetic factors. Caffeine doses must be increased for habitual users, who are thought to potentially diminish the ergogenic benefit of caffeine. Similar non-genetic factors affect the rate of caffeine metabolization by increasing Cytochrome P450 activity. These include smoking, dietary vegetable intake, oral contraceptive use, pregnancy, menstrual cycle stage, training status and hormone replacement therapy. Other non-genetic but controllable factors influencing caffeine ergogenicity include caffeine dose, source, timing, time of day and education status (41).

Furthermore, the positive benefits expected from caffeine use may also influence performance improvement. Certain genotypes appear to be more sensitive to placebo effects. Participants appeared to have improved performance even when they received a placebo instead of caffeine (42). Caffeine use induces epigenetic modifications, which may affect caffeine clearance by altering *CYP1A2* activity (43).

However, it is not entirely clear how epigenetic factors may alter the ergogenic effects of caffeine. Long-term caffeine use is predicted to potentially lead to habituation, both mediated by epigenetic modifications in Cytochrome P450 genes through increased caffeine clearance and a reduction in the threshold for caffeine-induced stimulation, possibly through the inhibition of genes affecting dopaminergic.

Previous studies on the effects of caffeine use (twin studies) generally assess the level of use, toxicity, tolerance, inadequate use and direct effects such as caffeine-induced sleep disturbances. In these studies, it was found that the heritability of coffee consumption habits from different populations ranged from 0.30 to 0.60. Twin studies explain the important role of genetics in determining caffeine intake. In several studies, overall caffeine consumption was found to have a heritability of 0.43, and the heritability of >625 mg of caffeine per day was reported as 0.77, while a twin (male) study in which one cup of coffee was consumed per day, the heritability of coffee consumption was found to range from 0.36-0.38 [87, 88]. These values, with results ranging from 0.38 to 0.58, are similar to the results of studies conducted on other twin populations using similar modeling techniques (44). The heritability effects of caffeine consumption may vary at different stages of life. In a retrospective study evaluating caffeine use in males (from early adolescence to middle adulthood), the variance of genetic contribution was found to range from 0.30-0.45 (45). Therefore, it was suggested that genetic contribution becomes more pronounced during adolescence and is then influenced by various genetic factors that remain constant throughout adulthood.

Another process through which genetics may influence caffeine use is the adaptation of individuals to certain positive or negative effects, such as sensitivity to caffeine withdrawal symptoms or effects on sleep. Kendler and Prescott (1999) observed that the heritability of genetics in twins (female) for individual susceptibility to caffeine toxicity, tolerance and withdrawal was 0.45, 0.40 and 0.35, respectively (45). In another study, the heritability of sleep disorders attributed to caffeine and its relationship with other sleep disorders was determined. It was stated that females generally exhibited slightly higher levels of caffeine-induced insomnia and more sleep disorders than males. The overall heritability of coffee attributed to insomnia was found to be 0.40, with three-quarters of the genetic variance reported to be unrelated to the overall sleep factor. It was also observed that the possible polychoric-phenotypic correlations between insomnia and other types of insomnia attributed to coffee ranged from 0.23 to 0.39, which is lower than the reciprocal correlations between non-coffee sleep disorders ranging from 0.40 to 0.79 (46). The results of the studies suggest that the genetic mechanisms affecting caffeine-induced sleep disturbance differ from other types of sleep disorders.

One of the main questions from genetic studies is whether the hereditary factor predisposes an individual to caffeine in particular or whether substance use predominates. Epidemiological studies show that smokers drink more coffee than non-smokers (46), but it is unclear whether these associations are related to genetic factors, drug interactions, social conditioning, or other variables. Kendler and Prescott (1999) reported that the heritability of caffeine use was not associated with the heritability of alcohol, nicotine, and illicit drug use (45). However, some studies observed that the heritability of coffee use overlapped with that of nicotine and alcohol, although 0.72 of the total heritability was specific to caffeine. Another study (twin pairs) showed that the genetic contribution of caffeine uses addiction towards heritability for lifetime addiction and abuse of cannabis, cocaine, alcohol, nicotine and caffeine was not associated with heritability for addiction or abuse of illicit substances such as heroin and cocaine (47).

Studies on dietary sources of caffeine consider the measure used to assess daily caffeine intake. The preference for caffeine use may have social or cultural underpinnings that may confound genetic influences. In a study on twins, heritability was estimated to be 0.51 for coffee and 0.26 for tea consumption (46). It has been suggested that environmental factors play a greater role in tea consumption than coffee consumption and that the social environment influences tea and coffee drinking habits differently, with tea consumption having a higher common environment contribution and a lower heritability. A similar study (twins) found a heritability of 0.41 for coffee and 0.36 for tea preferences (49). Another twin study on the preference for coffee over tea showed a heritability of 0.62 (44). One of the reasons for an individual's preference for coffee may be due to taste preference. Caffeine itself may taste bitter to some individuals. A taste preference test conducted among Australian adolescent and young adult twins showed that the perceived bitterness of caffeine has a wide heritability of 0.30 after adjusting for age, gender, and other covariates (50).

The study by Laitala et al. (2008) examined the influence of genetic factors on caffeine consumption in twins (3409 identical and 7307 non-identical twins). It concluded that caffeine consumption is influenced by additive genetic and unique environmental factors, each playing an almost equally important role (48). Long-term caffeine use has been found to be mainly influenced by several fixed genetic factors. In the study conducted by Luciano et al. (2007) to determine the heritability of caffeine-induced sleep disturbance and the chromosomal regions that influence this trait (1799 identical and 2009 non-identical twins). It was reported that the heritability of coffee-induced sleep disturbance (measured by self-report) was approximately 0.40 and that three-quarters of this genetic variance was accounted for by genes unrelated to the general sleep disturbance factor (46).

A previous study on 4495 twins was conducted by Vink et al. (2009) in which a genetic analysis of caffeine consumption was performed on Dutch twins. In conclusion, genetic influences (39%), shared environmental factors (21%) and unique environmental factors (40%) affecting coffee consumption were found. The variance in coffee preference was explained by genetic factors (62%) and unique environmental factors (38%) (48). In the study conducted by Kendler and Prescott (1999) on caffeine intake and tolerance in female twins (486 identical and 335 non-identical), caffeine was found to be an addictive substance and, similar to previous findings on other legal and illegal psychoactive drugs, it was observed that caffeine use was largely influenced by genetic factors (45).

Our analysis does have some limitations. Firstly, we have not included data on genetic markers linked to dietary supplements or habitual caffeine consumption, withdrawal effects, coffee dosage, ingestion time, time of day training, degree of training, or gender. These markers have previously been well described (51). Second, due to word limitations, not all research (ethnicity, particular sporting disciplines, sample size) has been fully detailed.

CONCLUSIONS

Based on the current findings, it is evident that the impact of caffeine consumption on individual performance enhancement is subject to variability influenced by factors such as environmental conditions, epigenetic interactions, circadian rhythm, training intensity, dietary habits, habitual caffeine intake, concomitant drug use, expectations, timing of consumption, and genetic factors. Furthermore, it is well established that the ergogenic effects of caffeine differ significantly among individuals, with some experiencing positive improvements while others observe no discernible benefits. Consequently, understanding the complexities of individual responses to caffeine involves considering multiple influencing factors. Continued research in this area can contribute to the elucidation of underlying physiological mechanisms.

Conflict of interest

No conflict of interest was declared by the authors.

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