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CYP1A2 genotype and acute effects of caffeine intake on growth hormone and testosterone response to resistance exercise

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Abstract

Background: Caffeine is widely recognized as a potent ergogenic aid commonly used to enhance exercise performance and recovery. However, individual responses to caffeine can vary significantly, a variability that might be explained by genetic differences.

Aim: This study aimed to investigate the influence of the CYP1A2 rs762551 SNP on the effects of caffeine (CAF) consumption on growth hormone (GH) and testosterone (TS) levels response to resistance exercise (RE) in male athletes.

Materials and Methods: Thirty resistance-trained men (mean age 21.72±4.06 years, weight 77.31±14.07 kg, height 179.31±5.08 cm) participated in a randomized, double-blind, placebo-controlled, crossover study. They consumed either CAF (6 mg/kg) or placebo (PL; 6 mg/kg maltodextrin) one hour before performing a RE protocol including three sets with 85% of 1RM and two-minute rest. CYP1A2 genotyping categorized participants as AA homozygous ("fast" metabolizers) or AC heterozygous ("slow" metabolizers). GH and TS levels were measured by ELISA methods.

Results: Repeated measures ANOVA showed significant differences in GH levels across time (F=10.94, *P*=0.000), with significant time-group (F=4.3, *P*=0.019) and time-genotype-group interactions (F=3.83, *P*=0.024). One-way ANOVA indicated significant differences in GH levels between CAF and PL conditions in AA individuals, but not in AC/CC genotypes. For testosterone, significant effects of time (F=14.88, *P*=0.000) and time-group interaction (F=3.197, *P*=0.045) were observed. Post-RE CAF supplementation significantly increased serum GH and TS levels in AA individuals compared to PL. Both CAF and PL groups showed increased serum hormone concentrations post-exercise.

Conclusion: In conclusion, the study demonstrated that caffeine consumption significantly increased serum levels of growth hormone and testosterone in individuals with the AA genotype of the CYP1A2 rs762551 SNP. These findings suggest that genetic variations play a role in the hormonal response to caffeine, which may have implications for exercise performance and recovery strategies.

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1. Introduction

Athlete performance is influenced by three crucial factors: training, nutrition, and genetics. Over the past two decades, sports scientists have conducted valuable research that has significantly enhanced advanced sports training techniques and effective nutritional strategies. As a result, there have been remarkable improvements in sports records. Among the various supplements used by athletes, caffeine (C8H10N4O2, CAF) is a popular choice due to its physiological role as an adenosine receptor antagonist [1] It is considered an ergogenic substance and is utilized in different exercise modalities [2, 3, 4, 5, 6, 7, 8, 9, 10, 11].

The actions of caffeine (CAF) are mediated through molecular mechanisms that involve the inhibition of cyclic AMP phosphodiesterase and the release of catecholamines from both the adrenal medulla and the central nervous system [12, 13, 14]. Caffeine's effects on performance are significantly mediated through its impact on hormonal responses, particularly GH and T. By inhibiting cyclic AMP phosphodiesterase and stimulating catecholamine release [12], caffeine increases cAMP levels, leading to stimulates the secretion of GH from the anterior pituitary gland, may also directly or indirectly affect the hypothalamic-pituitary-gonadal axis, leading to changes in TS secretion [15]. The resulting hormonal changes can improve muscle protein synthesis, recovery, and overall athletic performance.

The pharmacokinetics of caffeine in humans have been extensively studied and understood. Upon ingestion, caffeine is rapidly absorbed into the bloodstream, with 20% being absorbed by the stomach and 80% by the small intestine [14]. In healthy adult volunteers, the peak plasma concentration of caffeine is typically reached within 15 to 60 min after ingestion [14, 16].

For athletes, the suggested dosage of caffeine is approximately 6 mg/kg of body weight, to be taken around 60 min prior to exercise training [17]. However, studies examining the performance-enhancing effects of caffeine on strength, muscular endurance, power output, ballistic movements (such as throws and jumps), and sport-specific performance (e.g., basketball, soccer, volleyball) have yielded inconsistent findings [2, 10, 18, 19, 20, 21]. The conflicting results highlight the importance of further research into individual differences in response to caffeine consumption [20]. In recent years, there has been a surge in studies exploring genetic variations and their impact on caffeine metabolism and response, indicating the need for additional investigations in this area [21].

It is well established that the liver enzyme CYP1A2 plays a significant role in metabolizing caffeine (CAF) through demethylation [22]. This process leads to the formation of paraxanthine (84%), theobromine (12%), and theophylline (4%) as metabolites [23]. Research indicates that the activity of the CYP1A2 enzyme can be influenced by the CYP1A2 genotype (–163 C > A, rs762551), resulting in individuals with an AC genotype being categorized as slow caffeine metabolizers, while those with an AA genotype are considered fast caffeine metabolizers [24].

Previous studies comparing the effects of CAF on performance based on CYP1A2 genotypes have shown significantly greater improvements in performance among individuals with AA genotypes [25, 26, 27].

Studies have shown that fast

metabolizers (AA genotype) tend to experience greater improvements in exercise performance following caffeine ingestion. For example, caffeine has been found to enhance endurance performance by 7.1% in fast metabolizers compared to a placebo [28]. Additionally, fast metabolizers may benefit more from caffeine during resistance exercise, jumping, and sprinting tasks [29]. However, the impact of caffeine on exercise performance in slow metabolizers is less consistent. Some studies suggest that slow metabolizers may not experience the same level of ergogenic benefits from caffeine as fast metabolizers [30, 31].

In addition to its ergogenic effects, caffeine possesses various physiological properties, including metabolic effects, anti-inflammatory and antioxidant properties, and hormonal modulation [3, 4, 7, 8, 14, 22].

TS and GH play crucial roles in response to RE. Both hormones contribute to the anabolic environment necessary for muscle hypertrophy and strength gains, making them essential for effective resistance training outcomes [32]. Their acute elevations post-exercise is vital for long-term adaptations in muscle tissue.

While the ergogenic effects of caffeine (CAF) have been extensively studied, there is a lack of research on the hormonal response to resistance exercise (RE) and the influence of CYP1A2 genotype on this response. *In vitro* studies have shown that both CAF and its metabolite theophylline can stimulate the secretion of growth hormone (GH) in cultured rat and bovine anterior pituitary cells [33]. However, existing studies on the impact of CAF on GH and testosterone (TS) response to RE have produced conflicting results [6, 34, 35]. Thus, further investigation is needed to better understand the effects of CAF on hormonal response during resistance exercise.

Previous research has yielded conflicting findings regarding the effects of caffeine (CAF) on testosterone (TS) and growth hormone (GH) secretion. Some studies have reported an increase in TS secretion with CAF supplementation [34], while others have shown a decrease in GH [35, 36] and TS levels [35, 37]. Additionally, no previous study has investigated the influence of CYP1A2 genotype (-163 C>A, rs762551) on the endogenous TS and GH responses to CAF ingestion during resistance exercise (RE) in athletes. Therefore, a randomized, double-blind, placebo-controlled, crossover study was conducted to examine the GH and TS response to acute CAF ingestion in individuals with AA, AC, and CC genotypes of the CYP1A2 gene (-163 C>A, rs762551) before, during, and after RE.

2. Materials and Methods

2.1. Participation

A total of 30 men who were experienced in resistance training (age: 21.72±4.06 years, weight: 77.31±14.07 kg, height: 179.31±5.08 cm) volunteered to participate in the study. These individuals had been engaging in resistance training for at least one year, three days a week. Based on G*Power v3.1, the total sample size required based on effect size 0.56, α=0.05, number of measurement=3 and 4 conditions was 16 [7]. During the familiarization session, the researchers provided an explanation of the research objectives, methods, and potential risks to the participants. Following this, the participants completed the consent form, health questionnaire, and caffeine questionnaire. The participants' genotypes for the

CYP1A2 gene (AA n=14, AC/CC n=16; rs762551) were determined using amplification of refractory mutation system-polymerase chain reaction (ARMS-PCR) as previously described in genetics laboratory of University of Guilan [38]. The genotype distribution indicated a Hardy-Weinberg equilibrium with a Chi-squared value of 0.39. Based on the caffeine consumption questionnaire [39], the participants were classified as light caffeine consumers. To be eligible for the study, participants had to meet certain criteria, including abstaining from caffeine-containing drugs for the previous two weeks, avoiding anti-inflammatory and performance-enhancing supplements for the past three months, having no history of cardiovascular, metabolic, or neuromuscular disorders, and being non-smokers.

2.2. Experimental design

To investigate the acute effects of caffeine ingestion on growth hormone (GH) and testosterone (TS) response to resistance exercise (RE) in resistance-trained men, a randomized, double-blind, placebo-controlled, crossover design was employed. The study procedures were approved by Research Ethics Committees (IR.UOK.REC.1397.030, RCT20170405033231N2) and conducted accordingly. Prior to the experimental treatments, participants attended two consecutive laboratory sessions. During the first session, participants provided informed consent, completed the caffeine consumption questionnaire [39], and filled out a health and exercise history questionnaire.

During the participants' second visit, various measurements were taken, including height, body mass, body composition, and one-repetition maximum (1RM) for exercises such as bench press (BP), leg press (LP), seated cable row (SR), and shoulder press (SP) [7]. To minimize circadian variance, the experimental trials were conducted in the exercise physiology laboratory during two separate sessions, with a 7-day interval between them, at the same time of day [38].

Participants were instructed to abstain from consuming caffeine and engaging in any exercise for a minimum of 48 hours prior to the two visits. Subsequently, participants consumed caffeine (C0750 Sigma-Aldrich, Germany) and placebo (PL) in a counterbalanced order, one hour before the resistance exercise (RE) protocol. Following one hour of caffeine ingestion, participants completed the RE protocol, and blood samples were collected at three time points: before (Pre), immediately after (Post), and 15 min after (15 min Post) the RE session.

2.3. Supplementation

During the experimental treatment sessions, participants consumed either caffeine (6 mg kg^{-1} body mass) or a placebo (6 mg kg^{-1} body mass of maltodextrin). The ingestion was done in a randomized, double-blind, placebo-controlled, and crossover manner. The capsules used for both caffeine and placebo were identical in terms of shape, color, and size. Participants took the capsules along with 250 mL of water, 60 min before the resistance exercise (RE) protocol.

2.4. Resistance exercise protocol

Prior to the resistance exercise (RE) protocol, all participants completed a standardized warm-up consisting of exercises. Following a 60-min period after ingesting caffeine (CAF) and placebo (PL), participants performed three sets of bench press (BP), leg press (LP), seated cable row (SR), and shoulder press (SP) until failure,

which means they were unable to complete the repetitions with proper technique. The intensity for these sets was set at 85% of their one-repetition maximum (1RM), with 2-min rest intervals between sets and exercises [40]. Previous studies demonstrated that this intensity is enough to increase GH and TS responses [7, 27, 41].

2.5. Genotyping

The participants' genotype in the CYP1A2 gene (rs 762551) was determined using a previously reported method. Briefly, blood samples were collected 60 min after the ingestion of caffeine (CAF) and placebo (PL) for the extraction of human genomic DNA. The TIANamp Genomic DNA Kit (Cat.No. DP304) was used for this purpose. The amplification of refractory mutation system-polymerase chain reaction (ARMS-PCR) method was employed to identify the single-nucleotide polymorphism (SNP) in the intron 1 of the human CYP1A2 gene (rs 762551). The SNP -163A>C was amplified using the allele A primer (forward): 5'-CAAAGGGTGAGCTCTGTGGACA-3', allele C primer (forward): 5'-CAAAGGGTGAGCTCTGTGGTCC-3', and reverse primer: 5'-GAGGCGATGGAGAAGGTGTTGA-3' (Macrogen Korea). The PCR thermal cycler (Thermal Cycler, Analytik Jena, Germany) was used for the amplification process.

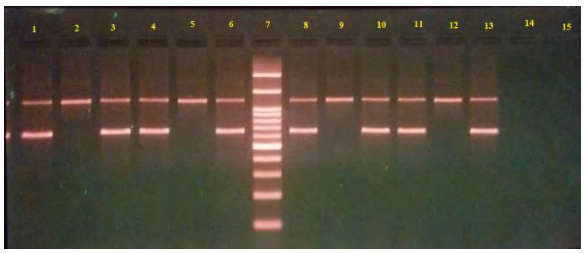


Figure 1. The CYP1A2 gene ARMS–PCR amplification products. 1 and 2: CC genotype, 3 and 4: AC genotype, 5 and 6: AA genotype, 7: DNA lader

To validate the PCR reactions, the mitochondrial genome primers were used as an internal control. The primers used for this purpose were the L strand: 5'-CTCCACCATTAGCACCCAAAGC-3' and H strand: 5'-CCTATTTGTTTATGGGGTGATG-3', which generated a 250-bp fragment. Each PCR sample was run in duplicate, and two negative controls were included. Participants were categorized based on the rs762551 single nucleotide polymorphism in the CYP1A2 gene. Those with the AA genotype were classified as "fast" metabolizers, while those with the AC genotype were classified as "slow" metabolizers (Figure 1).

2.6. Blood sampling and analysis

Peripheral blood samples were collected from the antecubital forearm vein at three time points: before the resistance exercise (Pre), immediately after the exercise (Post), and 15 min after the exercise (15 min Post). The blood samples were kept at room temperature for 10 min and then centrifuged at 3000 rpm for 10 min. The resulting serum samples were stored at -70°C until analysis. The concentration of serum hormones was assessed using ELISA kits from Monobind Inc. (USA), and testosterone levels were measured using an ELISA kit from IBL. All ELISA samples were analyzed in duplicate.

2.7. Statistical analyses

The collected data was analyzed using SPSS software. The normal distribution of the data was assessed using the Shapiro-Wilk test. For normally distributed data, statistical analysis was conducted using the repeated measures analysis of variance test. In cases where a significant difference was found between groups, the one-way ANOVA test was employed. To examine intra-group differences, the correlated t-test

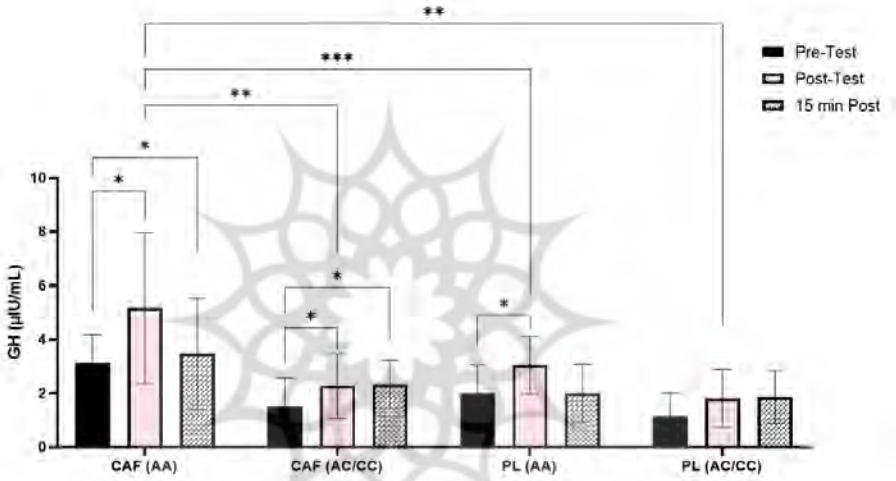
with Bonferroni correction was utilized. The significance level was set at α=0.05.

3. Results

3.1. Findings on growth hormone

The findings of the present research on growth hormone, using the analysis of variance test with repeated measurements, indicated significant differences over time (F=10.94, *P*=0.000), a significant time-group interaction (F=4.30, *P*=0.019), and a significant time-genotype-group interaction (F=3.83, *P*=0.024). One-way analysis of variance revealed a significant difference in the two conditions of caffeine consumption and placebo in the AA genotype between the post-tests.

Specifically, the concentration of growth hormone in the post-test was significantly higher in individuals with the AA genotype compared to those with the CA/CC allele, both in the condition of consuming caffeine and in the condition of taking a placebo. No significant difference was observed in individuals with the AC/CC allele between the two conditions of caffeine and placebo (Figure 2).



* Significant difference with pre-exercise

** Significant difference with AA genotype

*** Significant difference with AA genotype in PL condition

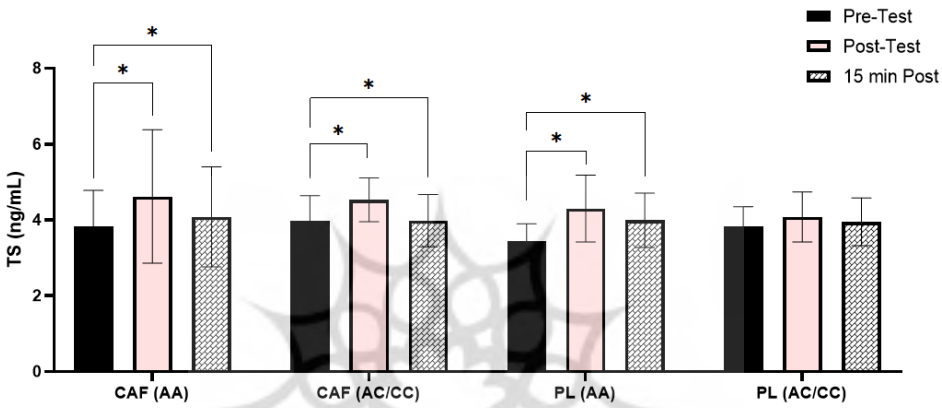
Figure 2. Growth hormone response to caffeine supplementation in athletes with AA and AC/CC genotypes of CYP1A2 rs762551 gene after resistance exercise

To assess intra-group differences, the t-test with Bonferroni correction was used. The results showed a significant increase in growth hormone concentration from pre-test to post-test (*P*=0.019, t=2.66) and 15 min after resistance activity (*P*=0.018, t=2.7) in individuals with the AA genotype under caffeine consumption conditions. Similarly, individuals with the AC/CC allele experienced a significant increase in growth hormone concentration from pre-test to post-test (*P*=0.001, t=4.63) and 15 min after resistance activity (*P*=0.008, t=3.06) under caffeine consumption conditions.

In placebo conditions, no significant intra-group difference was observed in the CA/CC genotype. However, there was a significant increase in growth hormone from pre-test to post-test (*P*=0.001, t=4.47) and up to 15 min after resistance activity (*P*=0.001, t=5.12) in placebo conditions. The significance level was corrected from α=0.05 to α=0.016 (Figure 2).

3.2. Findings on testosterone hormone

The results of the variance analysis with repeated measurements for testosterone hormone indicated that the effect of time (*P*=0.000, F=14.884) and the interaction of time × group (caffeine and placebo; *P*=0.045, F=3.197) were significant. To examine intra-group changes, the t-test with Bonferroni correction was utilized. The results showed a significant increase in testosterone levels in individuals with the AA genotype and the AC/CC allele in the condition of caffeine consumption from pre-test to post-test and up to 15 min after resistance activity (Figure 3). In placebo conditions, only individuals with the AA genotype showed a significant increase in testosterone from pre-test to post-test and 15 min after resistance activity (Figure 3). The significance level was corrected from α=0.05 to α=0.016.



⁎ Significant difference with pre exercise

Figure 3. Growth hormone response to caffeine supplementation in athletes with AA and AC/CC genotypes of CYP1A2 rs762551 gene after resistance exercise

4. Discussion

4.1. Hormonal responses to resistance exercise

Measuring GH and testosterone levels after resistance exercise is critical to understanding the physiological adaptations associated with strength training. GH stimulates the production of insulin-like growth factor 1 (IGF-1), which causes muscle protein synthesis and hypertrophy. Known as the primary anabolic hormone, testosterone increases muscle mass by increasing protein synthesis and decreasing protein breakdown [42].

Studies have shown that resistance exercise acutely increases GH and testosterone levels, although the magnitude of this response can vary based on factors such as age, sex, and training status [43]. Understanding the hormonal responses to resistance exercise has significant implications for improving the performance of athletes and the health of populations at risk for sarcopenia, obesity, and metabolic disorders. By investigating how GH and testosterone levels respond to different resistance training protocols and supplements or ergogenic elements, exercise professionals can develop appropriate exercise and nutrition programs that target hormonal responses for specific populations, such as athletes, the elderly, and the elderly, or people with chronic diseases to maximize [42, 43].

The relationship between CYP1A2 genotype, caffeine intake and hormonal responses to resistance exercise have been a

topic of interest in sports nutrition and exercise physiology. Research suggests that genetic variations in the CYP1A2 gene may affect individual responses to caffeine, particularly in terms of performance and hormonal changes. The CYP1A2 gene is responsible for encoding the enzyme that metabolizes caffeine. 90% of caffeine clearance is mediated by CYP1A2. Variants of this gene, especially the single nucleotide polymorphism (SNP) rs762551, categorize people into different types of metabolizers: AA genotype, fast metabolizers, AC genotype, moderate metabolizers, and CC genotype, considered slow metabolizers of caffeine. Studies have shown that these genetic differences can lead to changes in the way people react to caffeine supplements, especially during exercise, although the type of exercise can also affect this response. In a study, acute consumption of caffeine at the rate of 6 mg/kg of body weight significantly increased resistance exercise performance in 30 trained men with the A allele of the rs762551 polymorphism of the CYP1A2 gene, but no significant change was reported for C allele carriers [38].

Wang et al. (2023) recently showed in an overall analysis of a review and meta-analysis that cycling times were significantly improved after caffeine consumption, that this improvement was only for individuals with the A allele. The results of changes regarding the C allele were not significant. However, caffeine supplementation and CYP1A2 genotype did not affect Wingate [44].

4.2. Effect of caffeine on hormonal responses

Research shows that caffeine consumption can significantly affect GH and testosterone levels following resistance training, but this relationship seems complex. Conversely, other research suggests that caffeine may reduce the GH or testosterone response. It seems in addition to gender, time and dose of caffeine consumption [45], genotype is also one of the effective factors in this contradiction of results. For example, some studies have shown that caffeine supplementation can lead to an increase in GH and testosterone levels after exercise. A study on resistance trained men showed that one hour after consuming caffeine and performing intense acute resistance exercise, TT genotype carriers had significantly higher testosterone and GH levels than ADORA2A gene C allele carriers [7].

4.3. Role of CYP1A2 genotype in GH and testosterone responses

According to our knowledge, there has not been a study that investigated the possible effect of rs762551 polymorphism of CYP1A2 gene on GH and testosterone response after caffeine consumption in men. The results of our study showed that GH and testosterone response to resistance exercise. It was found that a significant increase in the GH levels of the placebo group occurred only in AA allele carriers, and in the caffeine group, despite the increase in its levels in both AA and AC/CC allele carriers. There was a greater increase in AA allele carriers. A significant increase in testosterone to resistance exercise was observed only in the AA allele in the placebo group, and in the caffeine group, both AA and AC/CC alleles had a similar increase in testosterone levels together with AA allele carriers in the placebo group.

Although studies show that acute resistance training generally causes significant increases in GH levels, the amount of this increase can vary widely depending on several factors including exercise characteristics (intensity and volume of exercise), individual

characteristics (age and gender), hormonal and genetics [46]. Our results showed that a bout of acute resistance exercise with an intensity of 85% of maximum repetitions, in the placebo group, was associated with a significant increase in GH only in those subjects who carried the AA allele. In other words, a number of subjects had a greater increase than other subjects in GH levels, despite their similarities (gender, age, training records, etc.) being controlled. Individuals with the AA allele may benefit more from high-intensity resistance training in terms of GH secretion, which can enhance muscle growth and recovery. Conversely, those without this allele may require different exercise strategies or intensities to achieve similar hormonal responses.

The most important result of our study was that the increase in GH immediately and 15 min after resistance exercise was significant in the group that consumed caffeine, that this increase was observed in both groups of people with AA and AC/CC alleles, and that the increase in GH levels. In people carrying AA allele, it was significantly higher than AC/CC allele immediately after exercise. The reason for the lack of GH increase in AC/CC allele carriers is not known. The interaction between GH and CYP1A2 may occur through different hormonal pathways. For example, GH affects insulin-like growth factor 1 (IGF-1) levels, which is associated with changes in CYP1A2 activity. Furthermore, the pattern of GH secretion (pulsatile vs. continuous) appears to affect CYP1A2 differently, with pulsatile administration of GH leading to decreased CYP1A2 activity, whereas continuous administration may increase it [47].

Individuals with the AA allele may benefit more from high-intensity resistance training in terms of GH secretion, which can enhance muscle growth and recovery. Conversely, those without this allele may require different exercise strategies or intensities to achieve similar hormonal responses. The most important result of our study was that the increase in GH immediately and 15 min after resistance exercise was significant in the group that consumed caffeine. This increase was observed in both groups of people with AA and AC/CC alleles. The increase in GH levels in people carrying AA allele, was significantly higher than AC/CC allele immediately after exercise.

A few studies in the past have reported that caffeine consumption has been associated with no significant increase [35] or increase [7, 9] in GH after exercise using different exercise protocols and different doses of caffeine. The mechanism or mechanisms of increasing GH with caffeine consumption were not investigated in this study, however, caffeine, like other xanthine phosphodiesterase inhibitors, has been proposed to stimulate GH secretion by direct action on pituitary cells [33]. In fact, methylxanthines are phosphodiesterase inhibitors that lead to an increase in pituitary cyclic AMP, which is involved in GH release.

In addition, caffeine may also affect neurotransmitters. Caffeine has been shown to increase the circulation of norepinephrine and serotonin in the brain. Both norepinephrine and serotonin stimulate GH secretion in adult rats and humans [48].

In this study, we observed that individuals carrying the AA allele had a greater increase in GH levels in the CYP1A2 rs762551 gene than the AC/CC allele. This appears to be mainly due to differences in caffeine metabolism and related enzyme activity. CYP1A2 is a key

enzyme responsible for caffeine metabolism, and genetic variations in the CYP1A2 gene affect its activity [49]. The AA genotype is associated with higher enzyme activity levels, leading to more efficient caffeine metabolism. This increase in metabolism can increase the physiological effects of caffeine, such as the release of GH. Research shows that people with the AA genotype metabolize caffeine faster than people with the AC or CC genotype. Consequently, after caffeine consumption, AA carriers may experience more pronounced physiological responses, including greater increases in GH levels. In contrast, AC and CC genotypes, which are associated with lower enzyme activity, may not experience the same degree of hormonal response due to slower metabolism of caffeine [50].

Testosterone is a vital hormone for building and maintaining muscle mass, especially in resistance training. Higher testosterone levels are associated with greater muscle protein synthesis and hypertrophy. In addition to anabolic effects, testosterone also prevents the destruction of muscle protein, and by reducing muscle damage and inflammation after intense exercise, testosterone causes faster recovery and prevents muscle breakdown [51]. Acute resistance exercise of sufficient volume and high intensity increases testosterone, although the increase in testosterone varies depending on many variables such as the use of large muscle groups, the use of free weights or functional training, body fat percentage, and average age [43].

The results of the present study showed that testosterone levels increased without caffeine consumption in AA allele carriers of the placebo group, but this increase was not significant in AC/CC allele carriers of this group. In the context of acute resistance exercise without caffeine consumption, individuals with the AA genotype may have a different hormonal response than individuals with the AC or CC genotype. This could be due to differences in how their bodies manage stress and recover, potentially affecting testosterone levels. The exact mechanisms are still being studied, but it is believed that the AA genotype may have a more favorable response to exercise-induced stress, leading to increased testosterone levels [52]. The relationship between caffeine and testosterone is complex. While some studies suggest that caffeine may lead to increased testosterone levels, especially in non-teenage and elderly populations [43], others suggest that excessive caffeine consumption can impair testosterone production [35]. A study on mice has shown that the injection of a high dose of caffeine (30 mg and 60 mg per body weight) has increased plasma testosterone concentration [53].

In addition, the use of doses of 200, 400 and 800 mg of caffeine one hour before resistance exercise in a study showed that the concentration of testosterone increased to a very small amount (15%) during the exercise, which caffeine increased this concentration in doses higher has increased a greater amount (21%) [34].

We observed that caffeine consumption was associated with an increase in testosterone levels in the AC/CC allele of the respective group compared to the placebo group, although it did not cause a significant increase in testosterone secretion in AA allele carriers compared to those carrying the same allele in the placebo group. The exact mechanism by which caffeine increases testosterone is not fully understood, but it is believed that caffeine acts as a non-selective phosphodiesterase

(PDE) inhibitor, specifically targeting PDE-4. This inhibition prevents the breakdown of cyclic adenosine monophosphate (cAMP), which is crucial for testosterone production.

By maintaining higher levels of cAMP, caffeine can increase testosterone synthesis [54]. Studies show that people with the AC/CC genotype may have different hormonal responses to caffeine compared to people with the AA genotype [7, 38]. This is due to the slower metabolism of caffeine, which may prolong its stimulating effects on the adrenal glands, potentially leading to increased testosterone production. It was previously mentioned that individuals with the AC/CC genotype may have different hormonal responses to caffeine compared to individuals with the AA genotype. This is due to the slower metabolism of caffeine, which may prolong its stimulating effects on the adrenal glands, potentially leading to increased testosterone production.

Due to the relatively small sample size of the present study, the analysis and generalization of our results is limited. In adults, CYP1A2 activity is higher in males than in females [47], and we only studied males. In addition, we did not use different doses of caffeine. Any interpretation of the results of our study should be made with these limitations in mind.

The present study is the first to investigate how CYP1A2 genotype may be associated with GH and testosterone levels after resistance exercise in men. We observed that caffeine consumption before resistance exercise is associated with an increase in GH secretion, and this effect is greater in the AA allele of the CYP1A2 rs762551 gene than in the AC/CC allele. But regarding testosterone, we observed that caffeine can increase testosterone secretion in AC/CC allele.

5. Conclusions

This study established that caffeine consumption prior to resistance exercise significantly enhances serum levels of growth hormone and testosterone in male athletes possessing the AA genotype of the CYP1A2 rs762551 SNP, identifying them as "fast" metabolizers. The results underscore the influence of genetic predispositions on the physiological responses to caffeine, specifically in the context of athletic performance and hormonal regulation. Notably, while caffeine increased hormone levels post-exercise in AA individuals, this effect was not mirrored in AC or CC genotypes, indicating a genotype-specific response. These findings suggest that the metabolic rate of caffeine can significantly impact its ergogenic effects on hormone fluctuations during and after resistance exercises.

6. Practical Implications for Athletes

The study highlights the importance of considering genetic factors when utilizing caffeine as an ergogenic aid. Athletes with the AA genotype of the CYP1A2 rs762551 SNP may experience more pronounced benefits from caffeine consumption, particularly in terms of enhanced GH and T levels, which can improve performance and recovery. Athletes with the AC/CC genotype may need to adopt a more personalized approach to caffeine supplementation. Overall, these findings underscore the need for athletes to consider their genetic profile when optimizing their nutrition and supplementation strategies.

7. Limitations and Future Directions

The study observed significant increases in serum GH levels but not T levels in the AA genotype group following caffeine consumption. This lack of significance for T levels in the AA group warrants further

investigation. The dose of caffeine (6 mg/kg) and the resistance exercise protocol (three sets with 85% of 1RM and two-minute rest) may not have been optimal to elicit a significant response in T levels. Higher doses of caffeine or different exercise protocols might yield different results. There could be substantial individual variability in how athletes with the AA genotype respond to caffeine, which might not have been fully captured by the study.

Our study included resistance-trained men with a specific age range (mean age 21.72±4.06 years). The findings may not be generalizable to other populations, such as women, older adults, or athletes with different training backgrounds. Future studies should consider including a more diverse population to understand how genetic variations interact with caffeine across different demographics.

The study focused on the CYP1A2 rs762551 SNP, which is just one of many genetic factors that could influence the response to caffeine. Other genetic variants may also play a role in how individuals respond to caffeine, particularly regarding T levels. Future research should adopt a more comprehensive approach, considering multiple genetic variants and their interactions to provide a more complete picture of the genetic influence on caffeine's effects.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design. M.R.R., H.F. and M.K. conceptualized and designed the study. H.F. and M.K. collected the data. M.R.R. and H.F. wrote the original draft of the manuscript. M.R.R. and H.F. reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Ethical considerations

The authors have completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. The study procedures were approved by Research Ethics Committees (IR.UOK.REC.1397.030, RCT20170405033231N2) and conducted accordingly.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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