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Evaluation of Caffeine Ingested Timing on Endurance Performance based on CYP1A2 rs762551 Profiling in Healthy Sedentary Young Adults

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Abstract

Background: Caffeine is generally suggested to increase VO_2max in endurance performance. Nevertheless, the response to caffeine ingestion does not seem to be uniform across individuals. Therefore, caffeine ingested timing on endurance performance based on the type of *CYP1A2* single nucleotide polymorphism rs762551, that were classified as fast and slow metabolizers, need to be evaluated.

Methods: Thirty participants participated in this study. DNA was obtained from saliva samples and genotyped using polymerase chain reaction-restriction fragment length polymorphism. Each respondent completed beep tests under three treatments blindly: placebo, 4 mg/kg body mass of caffeine one hour, and two hours before test.

Results: Caffeine increased estimated VO2max in fast metabolizers (caffeine=29.39±4.79, placebo=27.33±4.02, $p<0.05$) and slow metabolizers (caffeine=31.25±6.19, placebo=29.17±5.32, $p<0.05$) in one hour before test. Caffeine also increased estimated VO2max in fast metabolizers (caffeine=28.91±4.65, placebo=27.33±4.02, $p<0.05$) and slow metabolizers (caffeine=32.53±6.68, placebo=29.17±5.32, $p<0.05$) in two hour before test. However, for slow metabolizers, the increasing was greater when caffeine was administered two hours before test (slow=3.37±2.07, fast=1.57±1.62, $p<0.05$).

Conclusions: Genetic variance may affect the optimal caffeine ingestion timing, sedentary individuals who want to enhance their endurance performance may ingest caffeine 1 hour before exercise for fast metabolizers and 2 hours before exercise for slow metabolizers.

Keywords: Caffeine, *CYP1A2*, Performance Enhancer, Sedentary, VO_2max.

Introduction

Caffeine is known as a central nervous system stimulant and widely used as a performance enhancer in sports (1). Evidences showed that caffeine is generally proved to increase sport-specific endurance performance (2,3). Because of its effect, caffeine is commonly used as a supplement before heavy training or competition. The use of caffeine in endurance sports was even the highest than their counterparts in sports competitions (4,5).

The performance-enhancing effect is caused by inhibition of adenosine to its receptor. The presence of caffeine and its metabolites play a role in preventing adenosine, a compound that causes the sensation of fatigue and drowsiness, from binding to receptors in brain (1,6).

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Caffeine's metabolites were obtained from metabolism of caffeine in the liver by cytochrome P450 enzyme (7,8).

Some studies showed caffeine could improve endurance performance in one hour before exercise. Yet, the response to caffeine ingestion does not seem to be uniform across individuals (6,9,10). These inconsistencies might be due to inter-individual difference in caffeine metabolism related to caffeine ingested timing (11,12). The expression of cytochrome P450 enzyme, a key component of caffeine metabolism, is influenced by single nucleotide polymorphism (SNP) in *CYP1A2* gene (13,14). An A to C substitution at position 163 (-163 C>A) of SNP rs762551 in the *CYP1A2* gene impacts the speed of caffeine metabolism (8). Based on rate of CYP1A2 enzyme expression, individuals who possess AC or CC genotype are categorized as "slow metabolizers". Individuals who possess AA genotype are categorized as "fast metabolizers" (6,8,12).

Until now, "fast metabolizers" individuals are known can improve their endurance performance at the time of consumption one hour before exercise compared to "slow metabolizers" (12,15-17). On the other hand, the significance for "slow metabolizer" has not been explored yet. Therefore, this study aimed to evaluate the caffeine ingested timing on endurance performance based on *CYP1A2* rs762551 genotype profiling. Hopefully, this research can also provide recommendations for the best time to consume caffeine in order to enhance performance for both fast and slow caffeine metabolizers.

Materials and Methods

There were six main steps, consisted of participant recruitment, saliva sampling, DNA extraction, genotype profiling, endurance performance testing, and statistical analysis.

Participant Recruitment

A total of 16 males and 14 females who fit the criteria participated in the present study. The criteria in this experiment were aged 18-25 years, not an active smoker, sedentary physical activity (exercised less than 150 minutes per week), low caffeine consumption (caffeine consumed less than 70 mg per day), and physically healthy. Participants were asked to complete International Physical Activity Questionnaire (IPAQ), Caffeine Consumption Questionnaire (CCQ), and Physical Activity Readiness Questionnaire (PARQ), honestly (18- 21).

Each participant was measured their body composition using Body Composition Monitor HBF 375 (Omron, Kyoto, Japan). Participants with normal BMI (18.5-22.9) (based on Asia-Pacific BMI classification), normal fat percentage (8.0-19.9 for males and 20.0-30.0 for females), and normal muscle percentage (33.3-39.3 for males and 24.0-30.0 for females) would be determined as qualified participants (22,23).

Saliva Sampling

Sampling was taken from the participant's saliva independently. Prior to collection, participants were told to avoid eating or drinking (other than mineral water) for 30 minutes before salivating. Participants were also told to rinse their mouth with clean water before salivating. To stimulate saliva with high DNA yield, participants were told to swab their inner cheeks with their tongue. Participants were instructed to drooled the produced saliva into the collection tube containing sample buffer. After 2.5 mL saliva was collected, the collection tube was inverted several times.

DNA Extraction and Genotype Analysis

After saliva was well mixed with the sample buffer, DNA was extracted using phenol chloroform protocol. Genotype profiling was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. DNA was amplified using GoTaq Green Master Mix (Promega, Madison, United States) with the forward primer (5'-CAACCCTGCCAATCTCAAGCAC-3') and reverse primer (5'-AGAAGCTCTGTGGCC GAGAAGG-3'). The PCR amplification reaction conditions were as follows: initial denaturation at 95 °C for 2 minutes, followed

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by 30 cycles at 95 °C for 30 seconds, 62 °C for 30 seconds, 72 °C for 1 minute, with final elongation at 72 °C for 5 mintes.

The PCR product was digested with ApaI restriction enzyme (New England Biolabs, Ipswich, United Kingdom). Digested and undigested PCR products were evaluated using agarose visualization (1.5% (w/v) in TAE buffer). The gel was then stained in dilute ethidium bromide and visualized by UV light. The presence of a 920 bp was identified as AA genotype, while the presence of 920 bp, 709 bp, and 211 bp was identified as AC genotype, and the presence of 709 bp and 211 bp was identified as CC genotype.

Endurance Performance Testing

Endurance testing was performed by beep test method, also known as multi-stage fitness test. This study was blinded experiment. The test conducted three times with different treatments. First, as placebo, participants were given 2 mg of decaffeinated coffee (Nestle, Vevey, Switzerland) dissolved in 150 mL water one hour before the test. Second, as treatment 1, participants were given 2 mg of decaffeinated coffee and 4 mg/kg body mass of caffeine anhydrous (Soho Global Health, Jakarta, Indonesia) dissolved in 150 mL water one hour before the test. Third, as treatment 2, participants were given 2 mg of decaffeinated coffee and 4 mg/kg body mass of caffeine anhydrous dissolved in 150 mL water two hours before the test.

Each test was held at 1-week intervals to allow complete recovery and caffeine wash off. During three weeks of endurance performance testing, the participants were committed to not doing any sports. Every time before the test, each participant was also given breakfast to eat before drinking the coffee. Calorie's menu was designed to its calculation, basal metabolic rate multiplied by 1.2 then multiplied by 20% (24,25). The breakfast menu consisted of white breads (50 kcal per slice) (Sari Roti, Jakarta, Indonesia) and strawberry jam (tera up to calorie calculation) (Morin, Jakarta, Indonesia). Before the beep test, participants were warmed up using dynamic stretching led by an investigator for 5 minutes.

The total of beep test levels achieved by participants was noted and then converted as an estimated VO_2max value based on Coulson & Archer. Its equation was calculated using formula by Leger & Lambert before (26,27).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 24 (IBM, New York, United States). Participants' descriptive data were compared between groups using Mann Whitney U test. Estimated VO_2max of placebo treatment between fast and slow metabolizer groups was also using Mann Whitney U test. Then, the effect of caffeine intake for 1 hour and 2 hours before the test compared to its placebo were compared using Wilcoxon signed-rank test. Increase of estimated VO_2max value between fast and slow metabolizer groups were also compared using Mann Whitney U test. $P<0.05$ was considered as significant in all cases.

Results

Out of the 30 participants, 50% (8 males and 7 females) were homozygous for the A variant (AA genotype) classified as fast metabolizers. As much as 50% (8 males and 7 females) were heterozygous (AC genotype) classified as slow metabolizers. Participants' descriptive data are shown in Table 1. There were no significant differences ($p>0.05$) between two groups for age, body mass, BMI, and muscle percentage. As for fat percentage in males, although it was significantly different, they were in normal range (8.0–19.9) (25). In addition to the data description, VO_2max in the placebo treatment between fast and slow metabolizer groups was also not significantly different (Table 2).

The sub-analyses were also conducted to see the difference between caffeine ingestion response in males and females. The effect of caffeine intake in male participants was similar to the all-gender results, including the effect of caffeine intake for 1 hour and 2 hours before the test compared to placebo, also the increase of estimated VO2max between fast and slow

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metabolizer groups (Tables 2 and 3). Yet, the results for female participants were slightly different. As in Table 3, the effect of caffeine intake for 2 hours before test compared to placebo was just significantly different for slow metabolizer groups. Then, there is no difference for increase of estimated VO_2max between fast and slow metabolizer groups (Table 3).

Table 1. Participants' descriptive data.

	Groups	Age	Body Mass (kg)	BMI	Fat (%)	Muscle (%)
Males	Fast (n=8)	22.00±1.20	67.95±6.17	22.71±1.30	19.73±4.89	34.55±2.11
	Slow (n=8)	21.00±0.76	61.70±7.94	21.49±2.41	14.30±3.83	36.63±1.73
	p value	0.062	0.141	0.292	0.036[a]	0.059
Females	Fast (n=7)	21.00±0.00	54.66±8.70	21.83±2.27	26.86±2.96	26.93±1.22
	Slow (n=7)	21.00±0.58	56.50±8.18	21.53±2.16	27.51±2.07	26.87±0.87
	p value	> 0.999	0.482	0.798	0.749	0.370
All	Fast (n=15)	21.53±0.99	61.77±9.96	22.30±1.81	23.05±5.41	31.02±4.29
	Slow (n=15)	21.00±0.65	59.07±7.83	21.51±2.22	20.56±7.30	32.04±5.17
	p value	0.174	0.436	0.345	0.389	0.653

[a]Mann Whitney U test, significant difference at α = 5%.

Table 2. Effect of caffeine intake for treatments 1 and 2.

Treatment	Gender	Metabolizers group	n	Placebo (mL/kg/min)	Test (mL/kg/min)	*p* value
1	Male	Fast	8	29.55±4.13	31.89±4.76	0.012[a]
		Slow	8	32.27±4.79	35.01±5.58	0.018[a]
	Female	Fast	7	24.80±1.93	26.53±3.05	0.017[a]
		Slow	7	25.63±3.43	26.96±3.57	0.016[a]
2	Male	Fast	8	29.55±4.13	31.20±4.45	0.012[a]
		Slow	8	32.27±4.79	36.99±5.24	0.012[a]
	Female	Fast	7	24.80±1.93	26.29±3.51	0.058
		Slow	7	25.63±3.43	27.44±3.92	0.027[a]

[a]Wilcoxon signed rank test, significant difference at α=5%.

Table 3. Increase of estimated VO_2max in fast and slow metabolizer groups.

Treatment	Gender	Metabolizers group	n	(mL/kg/min)	*p* value
1	Male	Fast	8	2.34±1.26	0.430
		Slow	8	2.75±1.47	
	Female	Fast	7	1.73±1.82	0.547
		Slow	7	1.33±0.51	
2	Male	Fast	8	1.65±0.88	0.003[a]
		Slow	8	4.73±1.78	
	Female	Fast	7	1.49±2.28	0.122
		Slow	7	1.81±1.00	

[a]Mann Whitney U test, significant difference at α=5%.

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Comparing the estimated VO_2max between 1 hour before test and placebo treatment, the results were significantly different in the fast and slow metabolizer groups (Fig. 1). The same results were also obtained, both in the fast and slow metabolizer groups, which resulted in significantly different estimated VO_2max values between 2 hours before test and control. However, when observed from the increase of estimated VO_2max, in 2 hours before the test, the slow metabolizer group resulted in a significantly higher increase than the fast caffeine metabolism. While in 1 hour before the test, the increase in estimated VO_2max between the two groups was not significantly different (Fig. 2).

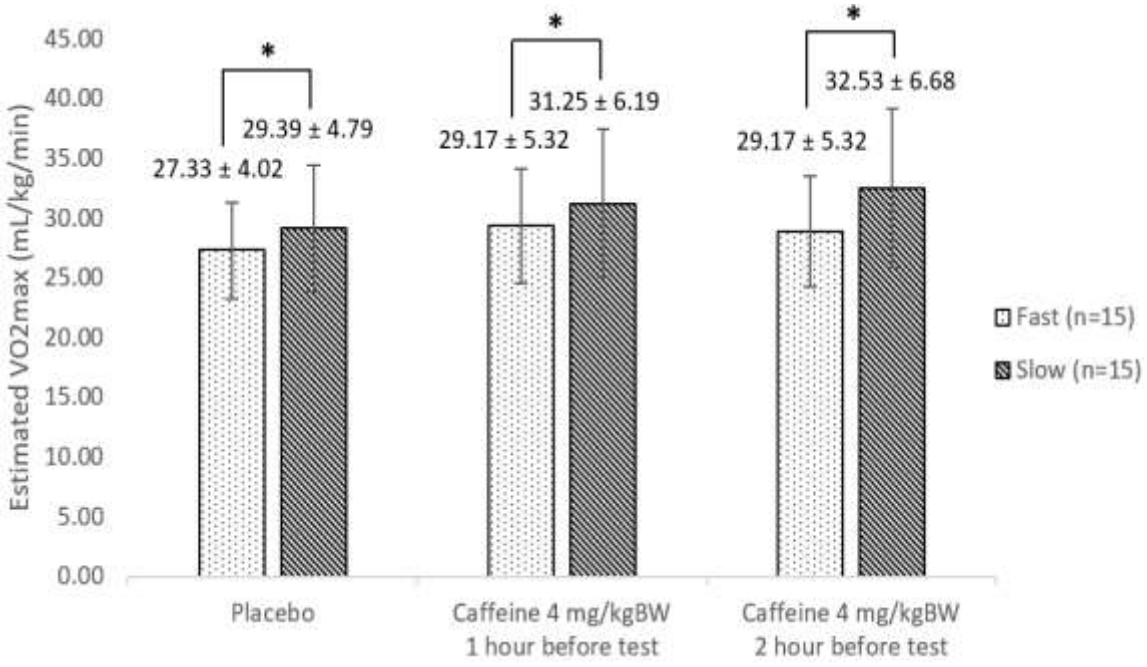

Fig. 1. The effect of caffeine intake for 1 and 2 hours before test compared to placebo.
*Wilcoxon signed rank test, significant difference at α=5%.

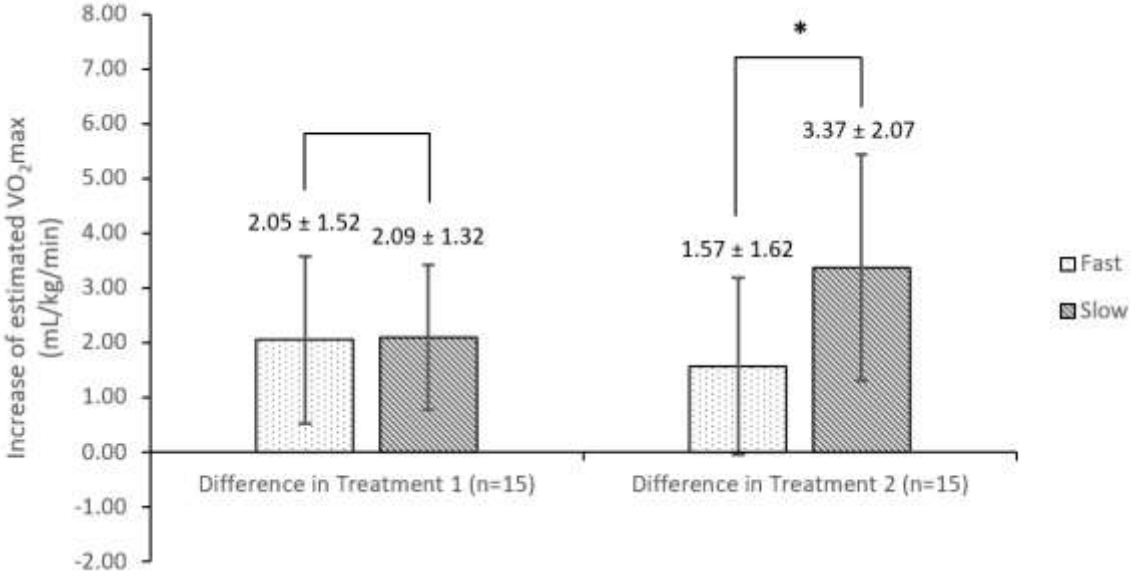

Fig. 2. Increase of estimated VO_2max between fast and slow metabolizer groups.
*Mann Whitney U test, significant difference at α=5%.

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Discussion

The main finding of this study is caffeine could improve estimated VO_2max value at one and two hours before beep test trial for fast and slow metabolizers respectively (Fig. 1). Yet, the improvement was better for slow metabolizers at two hours before exercise (Fig. 2).

Our result showed that both fast and slow metabolizers could improve performance endurance with 4 mg/kg body mass of caffeine intake in one hour before exercise (Fig. 1, Table 2). Its result, caffeine dosage, and one of timing treatments were in accordance with Guest et al. (11). It showed that 4 mg/kg body mass of caffeine intake one hour before exercise could improve 10-km cycling time trial for fast and slow metabolizers. Even though the exercise trial methods were different with Guest et al. (11), beep test method in our study was also used by Sepriani et al. (28) and Usman et al. (29). Both of these studies found caffeine supplementation could improve beep test performance.

The result in Fig. 1 and Table 2 is the new finding from this study. It investigated the effect of 4 mg/kg body mass of caffeine intake 2 hours before exercise for fast and slow metabolizers. The idea of this experimental design was in line with Pickering (30) and Pickering & Kiely (31) suggestion. Our study was designed because previous studies found the significance of caffeine supplementation on endurance performance for fast metabolizers in one hour before exercise (6,11,12). Meanwhile, caffeine supplementation was known to improve in performance between caffeine users and nonusers between one to six hours before exercise (32). Moreover, it seems plausible to discover AC or CC genotypes, classified as slow metabolizers, to experience in longer time than fast metabolizers. Based on this research, two hours treatment showed better trend for slow metabolizer group (Fig. 2, Tables 2 and 3).

The results in Fig. 2 are also advanced results for the new finding of this study. From Fig. 1, it seems equivocal to determine which genotype profiling was better for each timing ingestion of caffeine intake. So, we looked further to compare the increase in estimated VO_2max of treatment of placebo between fast and slow metabolizer groups. It showed, in two hours before exercise, slow metabolizers resulted in a significantly higher increase than fast metabolizers. While in one hour before exercise, the increase in estimated VO_2max between the two groups was not significantly different.

The sub-analysis for male participants was in line with all-gender results. The slow metabolizers could result of estimated VO_2max increase in a significantly higher than fast metabolizers (Table 3). Yet the increase of estimated VO_2max in female participants was not significantly different (Table 3). Since Table 2 showed that the slow metabolizers have estimated VO_2max significantly higher, we can still conclude that, in female participants, slow metabolizers also have better endurance performance than fast metabolizers in two hours before exercise. It was also supported by Table 2, although the increase of estimated VO_2max was not significantly higher, the slow metabolizers have better estimated VO_2max value than fast metabolizers. Difference in caffeine response between females and males was hypothesized by menstrual cycle and steroid hormone changes. The steroid hormone would contribute to physiological response of caffeine (33,34).

In general, our findings were aligned to systematic reviews about caffeine effects to increase endurance performance (2,3,6). Many theories and hypotheses were published about its increasing effect. Nevertheless, the primary and common theory of its effect was due to the inhibition of adenosine to its receptors by caffeine and its metabolites. Adenosine itself was produced from the breakdown of energy, the more energy brokedown the more adenosine produced. Because the adenosine receptors were connected to central nervous system, adenosine produced would become a signal for the body to feel fatigue and drowsiness (1,2,6).

Specifically, in our study, the participants were also divided into two groups of genotype profiling, fast and slow metabolizers. The result was in line with Guest et al. (11). It also resulted in significant improvement of endurance performance with 4 mg/kg body mass of caffeine

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intake at one hour before exercise for fast and slow metabolizers, respectively. The primary reason that suggested its effect is the higher binding affinities of caffeine metabolites to adenosine receptors. By cytochrome P450 enzyme in liver, caffeine would be metabolized and produce three main metabolites, as much as 84% to paraxanthine, 12% to theobromine, and 4% to theophylline (7,8,35). Paraxanthine, the most abundant metabolite, and also theophylline were known to have higher binding affinities to adenosine receptors than caffeine. It was considered as the performance-enhancing effect experienced in one hour treatment because 99% of caffeine was absorbed within 45 minutes (8,36).

Furthermore, as the new finding, the significance of two hours treatment was suggested caused by half-life of caffeine about 3-6 hours. Half-life of caffeine is the time it takes for the amount of caffeine in the body to be reduced by half (2,3). Moreover, slow metabolizers were better at two hours before exercise is also a new finding. We could suggest it was due to the rapid accumulation of caffeine metabolites that were estimated performed after one hour (3). It was considered the performance-enhancing effect of caffeine performed up to 6 hours after its intake (30). It was also considered the half-life of caffeine up to six hours, too (2).
This study also has potential limitations. The endurance performance test was based on beep test method and VO_2-max value. Common studies about endurance performance were reported in the time trial and long duration (10-12,37). Our study was in the same as Sepriani et al. (28) and Usman et al. (29) that used beep test as a parameter. In this test, estimated VO_2max was calculated, represent endurance performance. Vary studies use it as gold standard of overall fitness that represents body's ability to deliver oxygen (38,39). Also the time span being tested is still quite far (1 hour), so that if the time span is narrowed, it may be possible to see even more differences in the effect of caffeine on exercise in fast and slow metabolizer groups.

This study implied that genetic variance of *CYP1A2* rs762551 may affect the optimal caffeine ingestion timing. This study is also the first to investigate the significant performance of slow metabolizers on two hours treatment. Our results suggested that caffeine could improve endurance performance at 1 and 2 hours before exercise. Yet, the improvement was better for slow caffeine metabolizers to consume 2 hours before exercise because the increase of estimated VO_2max was found higher. For the conclusion, individuals who want to enhance their endurance performance, this study recommends consuming caffeine 1 hour before exercise for fast caffeine metabolizers and 2 hours before exercise for slow caffeine metabolizers.

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Conflicts of Interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Ethical Approval
Ethical approval was obtained from The Institution of Research and Community Services Atma Jaya Catholic University of Indonesia (approval No. 0003A/III/LPPM-PM.10.05/01/2021). All participants provided written informed consent, and were informed that they could terminate their participation any time.

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