Journal of Physical Education and Sport ® (JPES), Vol. 22 (issue 9),Art 277, pp. 2173 - 2181, September, 2022
online ISSN: 2247 - 806X; p-ISSN: 2247 – 8051; ISSN - L = 2247 - 8051 © JPES

Original Article

The effects of different dosing strategies of caffeine ingestion during an endurance performance event in male half marathon runners

NATTHAPONG SEEPIKA[1], SONGDHASN CHINAPONG[2], NUNCHANOK PIAKAEW[3], WANIDA LAIWATTANAPAISAL[4], KASINEE KATELAKHA[5], NUTCHA LARPANT[6], CHANAWAT SANPASITT[7], PATTARAWUT KHAOSANIT[8], WANCHAI BOONROD[9], NATTIPORN NOKKAEW[10]
[1,7,8,9,10] Faculty of Sports Science, Chulalongkorn University, THAILAND
[2] Division of Sports Science, Faculty of Science, Chandrakasem Rajabhat University, THAILAND
[3] Faculty of Sports Science, Kasem Bundit University, THAILAND
[4,6] Biosensors and Bioanalytical Technology for Cells and Innovative Testing Device Research Unit, Department of Clinical Chemistry, Faculty of Allied Health Science, Chulalongkorn University, THAILAND
[5] The Halal Science Center, Chulalongkorn University, THAILAND

Published online: September 30, 2022
(Accepted for publication September 15, 2022)
DOI:10.7752/jpes.2022.09277

Abstract:
This study examined the acute effects of two different dosing strategies of caffeine ingestion and compared the effects of high dose (ingested before and during exercise) and moderate dose (only ingested before exercise) with placebo on free fatty acid responses and endurance performance in male half-marathon runners. Eight male half marathon runners were randomly assigned in a double-blind crossover design according to dosing strategy conditions: high dose (HIGH), ingested 6 mg·kg body mass^{-1} of caffeine before exercise and 3 mg·kg body mass^{-1} of caffeine during exercise (total caffeine ingested after both doses = 9 mg·kg body mass^{-1}); moderate dose (MOD), ingested 6 mg·kg body mass^{-1} of caffeine only before exercise (total caffeine ingested after both doses = 6 mg·kg body mass^{-1}), and placebo (PLA), performed to exhaustion test 60 min before each time to determine the endurance performance as well as serum caffeine, free fatty acids, glucose and lactate concentration, gastrointestinal symptoms, and rate of perceived exertion (RPE). There was a significant difference in the interaction effect for serum free fatty acids concentration at the time point of 120 min during exercise between the HIGH (0.086 ± 0.026 mmol/L) and PLA (0.051 ± 0.018 mmol/L) groups (p = 0.00). The ingestion of caffeine in moderate dose resulted in significantly longer time to exhaustion compared with placebo (127.46 ± 3.72 min vs. 124.61 ± 4.58 min, p = 0.02). In conclusion, consumption of a high dose of caffeine may help to improve endurance performance in male half-marathon runners via higher serum FFA concentration and increase fat oxidation at the proper rate to produce energy for prolonged aerobic exercise.
Keywords: **Caffeine, Half-marathon, Endurance performance, Free Fatty Acids**

Introduction

A half-marathon is a 21-km event with a significant energy cost. Academically, a half-marathon can be described as a long-duration, primarily aerobic activity that comprises rhythmic movements sustained by vast muscle groups and relies on oxidative metabolism for energy (Chen et al., 2017; Gómez-Molina et al., 2017; Doyenart et al., 2020). Carbohydrates (CHO) stored in the liver and contracting muscle and the fat stored in the muscle and adipose tissue are the primary fuels used during endurance running. CHO oxidation, mainly from muscle glycogen, dominates at higher exercise intensities, while fat oxidation is more important at lower intensities. Oxidation of muscle glycogen and fatty acids derived from muscle is highest during the early stages of endurance exercise and decreases as exercise duration is prolonged (Hargreaves et al., 2020; Ravindra et al., 2022). With prolonged exercise, muscle glycogen output may fall below muscle glycogen uptake, thus resulting in muscle glycogen depletion.

Because muscle glycogen depletion is considered to be the primary determinant of exhaustion during prolonged exercise, increasing fatty acid oxidation will delay the onset of glycogen depletion, enhancing endurance performance (Hunter et al., 2002). The intensity of exercise is likely to impact the relative amounts of CHO and fat oxidized while running because fat oxidation at moderate or high intensities is insufficient to meet the demands of the muscles for ATP (Spriet, 2007). Although endogenous fatty acid reserves are large, they do not supply ATP at rates that support increased exercise intensity with the high relative (70–90%) $\dot{V}O_2$max, and absolute work rates maintained by competitive athletes during endurance events lasting less than 2 h are not supported by rates of muscle fat oxidation (Noakes, 2022). One of the nutritional strategies to enhance endurance performance that has received widespread attention is caffeine (CAF) intake. Many studies have shown that acute caffeine intake can improve aerobic endurance, anaerobic performance, maximal strength and muscular endurance, movement velocity of resistance exercise, and sprint and agility performance (de Souza et al., 2022).

Corresponding Author: . NATTIPORN NOKKAEW, E-mail: Nattiporn.N@chula.ac.th

The potential mechanism for the ergogenic effects of CAF on endurance performance is the adrenaline (epinephrine)-induced enhanced FFA oxidation after CAF ingestion and consequent glycogen sparing, resulting in improved endurance performance (Guest et al., 2021).

CAF has been for years a legal and widely used ergogenic aid or exercise performance enhancer among athletes at various levels of sports competition. CAF is currently available in many products, e.g., coffee, gels, energy bars, gum, and energy drinks. CAF administration in research is performed by instructing athletes to ingest tablets or capsules with water or by drinking coffee, which is the largest source of CAF used by athletes before a competition (Ganio et al., 2009; Wickham & Spriet, 2018). It has been reported that 74% of elite athletes use CAF before or during a competition (Southward et al., 2018). The highest prevalence rate for CAF use as an exercise performance enhancer is seen in endurance athletes, e.g., triathletes, cyclists, and marathoners; the second highest prevalence rate for CAF use is in game athletes (e.g., tennis players, volleyball players, and handball players) and strength athletes, e.g., weightlifters (Kyle et al., 2018; Del Coso et al., 2011). CAF has been found to improve sport and exercise performance through three different mechanisms: increasing intracellular calcium mobilization, acting as an adenosine receptor antagonist in the CNS to induce effects on both the central and peripheral nervous systems to reduce pain and exertion perception, improving motor recruitment and excitation–contraction coupling, and increasing free fatty acid (FFA) oxidation during exercise and subsequent glycogen sparing (Del Coso et al., 2011; Ganio et al., 2009; Martins et al., 2020).

The benefits of CAF supplementation for endurance performance have been demonstrated in time to exhaustion (TTE) tests. Bell and McLellan (2002), using a cycling time-to-exhaustion test, found an ergogenic effect of ingesting 5 $mg \cdot kg^{-1}$ BM of caffeine that was greater and lasted longer in caffeine nonusers than users, and Smirmaul et al. (2017) found a 12% improvement in TTE following ingestion of 4 $mg \cdot kg^{-1}$ BM of caffeine. In many studies, the dosage of CAF to enhance performance is between 3 and 6 $mg \cdot kg^{-1}$ BM, 60 min before exercise (Maughan et al., 2018; Goldstein et al., 2010; Hodgson et al., 2013). This 60 min period is the CAF absorption period or the time necessary for CAF to reach the maximum peak of the substance in plasma (Martins et al., 2020). However, recent studies have revealed that both low (≤3 $mg \cdot kg^{-1}$ BM) and moderate to high (i.e., 6–9 $mg \cdot kg^{-1}$ BM) intakes of CAF doses 60 min before exercise can also improve exercise performance (Spriet, 2014; Pickering and Kiely, 2018, 2019). Zhang et al. (2020) suggested that the lower doses of CAF (≤3 $mg \cdot kg^{-1}$ BM) had greater effects on cognitive function and brain activation compared to moderate and higher doses (6 or 9 $mg \cdot kg^{-1}$ BM); thus, a lower amount of CAF (≤3 $mg \cdot kg^{-1}$ BM) is sufficient to induce saturation of the effect of CAF on CNS (Zhang et al., 2020), and the use of moderate or higher doses (6 or 9 $mg \cdot kg^{-1}$ BM) is suitable for altering the energetic use of the lipids with the muscle glycogen preservation, resulting in enhanced aerobic exercise performance (Martins et al., 2020; Spriet, 2014; Taylor et al., 2011; Cruz et al., 2015).

Of note, acute higher CAF doses (9 $mg \cdot kg^{-1}$ BM) do not seem to increase the performance benefit, and such dosages may increase the risk of side effects such as nausea, anxiety, insomnia, and restlessness, which would counteract any performance-enhancing effects (Bruce et al., 2000; Burke, 2008).

CAF is typically excreted through sweat during endurance exercise (Hunter et al., 2002) and has a half-life of 3–5 h; consequently, endurance athletes with a faster metabolism may not experience the aerobic exercise performance effects of CAF in long-duration events (e.g., marathons, triathlons, and ultra-endurance events) if CAF is metabolized prior to the end of the event (Southward et al., 2018; Martins et al., 2020). Thus, athletes should consume CAF prior to, during, or after physical exercise to maintain peak plasma CAF levels (Hunter et al., 2002; Martins et al., 2020). Hunter et al. (2002) suggested that athletes should ingest a maintenance dose of 0.33 $mg \cdot kg^{-1}$ BM of CAF every 15 min during the exercise period. This dose was calculated to ensure the overall
CAF dosage not exceeding 9 $mg \cdot kg^{-1}$ BM, and this dose maintains mean urinary CAF levels below the IOC limit (12 $\mu g \cdot ml^{-1}$) (Hunter et al., 2002). However, the CAF dose should be administered based on the athlete's tolerance to CAF and the type of exercise.

Accordingly, this study examined the acute effects of two different dosing strategies of CAF ingestion, i.e., high dose (ingestion of 6 $mg \cdot kg^{-1}$ BM of CAF 60 min before exercise and 3 $mg \cdot kg^{-1}$ BM of CAF at 45 min during exercise, total CAF ingested after both doses was 9 $mg \cdot kg^{-1}$ BM) and moderate dose (ingestion of 6 $mg \cdot kg^{-1}$ BM of CAF only 60 min before exercise), in comparison with placebo on FFA responses and endurance performance in male half-marathon runners. We hypothesized that the high dose caffeine intake would result in an increase in serum FFAs during prolonged exercise compared to placebo, eliciting maximal fat oxidation, and resulting in improved endurance performance as measured by the time to exhaustion test.

Materials and Methods

Participants

Eight male, well-trained half-marathon runners, from local running clubs volunteered to participate in this study (mean ± S.D.: age of 33.25 ± 3.19 years; body mass (BM) of 71.80 ± 7.35 kg; height of 174.37 ± 4.86 cm; maximal oxygen consumption ($\dot{V}O_2max$) of 52.75 ± 2.76 $mL \cdot kg^{-1} \cdot min^{-1}$). Eligibility criteria included: males 30–39 years old; experience in half-marathon races for 1–5 years; engaged in training for at least 3–5

occasions each week, >180 min/week of exercise training; no allergies or intolerance to CAF; not naïve to CAF usage and consuming CAF <500 mg/day; free from heart conditions and/or did not recently experience shortness of breath, chest pain, bone, or joint problems and has no symptom of gastrointestinal (GI) discomfort, and has the approval of attending physician. All participants must not participate in any experimentation and/or endurance road race and have no supplementation at least two weeks before the study begins and during the data collection period in this study.

Design

This study used a double-blind, placebo-controlled, and repeated measures crossover design to determine the effect of different doses of CAF on FFAs and endurance capacity in half-marathon runners. The experiments took place at the Exercise and Sports Performance Laboratory (ESPL), the Faculty of Sports Science, Chulalongkorn University, Thailand. The participants were fully informed of any associated risks and discomforts and provided written informed consent prior to the study. The Research Ethics Review Committee for Research involving Human Research Participants, Group I, Chulalongkorn University, Thailand, has approved the study, and the study was performed in accordance with Belmont Report 1979, Declaration of Helsinki 2013, Council for International Organizations of Medical Science (CIOM) 2016, Standards of Research Ethics Committee (SREC) 2017, and National Policy and guidelines for Human Research 2015 (COA No. 062/2022).

Preliminary Testing

Anthropometric characteristics were measured during the first visit to the laboratory. Body composition measurements without shoes and minimal clothing were taken with a bioelectrical impedance body composition analyzer (ACCUNIQ BC510, Korea). Participants completed an exhaustive ramp incremental test on a motorized treadmill (h/p/cosmos®, Germany) to determine the maximal oxygen uptake ($\dot{V}O_2max$), first ventilatory threshold (VT_1), and second ventilatory threshold (VT_2). After a 3-min warm-up at a speed of 4.5 $km \cdot h^{-1}$, the test started at an initial velocity of 4.95 $km \cdot h^{-1}$ for 1 min, thereafter an increase in speed of 0.65 $km \cdot h^{-1}$ every 1 min along with a an increase in the treadmill inclination from the initial 0° by 0.4% every 1 min until volitional exhaustion. Oxygen uptake was measured with an open-circuit breath-by-breath gas and volume analyzer (Cortex Metamax 3B, Germany). Heart rate (HR) was recorded telemetrically with a chest strap sensor (Polar, Finland) and was synchronized to the gas analysis system software, allowing the continuous recording of heart rate expressed in beats per minute (bpm). The statistical analysis was conducted using the average gas, volume, and heart rate values for each 30-s period, with the highest values being defined as the maximum values. The determination criteria for exhaustion were as follows: 1) heart rate (HR) ≤10 beats/min or ≤5% of the age-predicted (220-age) maximum, 2) respiratory exchange ratio (RER) greater than 1.10–1.15, and 3) rating of perceived exertion (RPE) based on the 6-20 Borg scale as 18 (extremely hard) (Midgley et al., 2007; Poole et al., 2008; Poole and Jones, 2017). The initial velocities corresponding to VT_1 were obtained to determine the exercise intensity of the experiment trial (Chuychai et al., 2022).

Experimental Procedure

Each participant completed three trials, randomly, under double-blind conditions. All trials were conducted at the same time of day (mornings) to eliminate the influence of circadian variation, with a 7-day wash-out period between each session. The trials were performed in the same laboratory, on the same ergometer, and under stable environmental conditions (~20–21 °C, ~60% relative humidity). Participants reported to the laboratory for each visit early in the morning following an overnight fast (only water was permitted). Participants recorded food and beverage intake as they were consumed and physical activity throughout three days prior to the first laboratory visit, replicated this prior to the next visit, and abstained from all dietary sources of caffeine, alcohol, vigorous physical activity, and any pre-workout supplement for 24 h preceding each experimental trial. The participants were randomly assigned in a double-blind crossover design to dosing strategy conditions: 1) high dose (HIGH); the participants ingested 150 mL of fluid containing 6 $mg \cdot kg^{-1}$ BM of CAF 60 min before exercise and 150 mL of fluid containing 3 $mg \cdot kg^{-1}$ BM of CAF at 45 min during exercise; total CAF ingested after both doses was 9 $mg \cdot kg^{-1}$ BM; 2) moderate dose (MOD); the participants ingested 150 mL of fluid containing 6 $mg \cdot kg^{-1}$ BM of CAF 60 min before exercise and 150 mL of fluid with placebo at 45 min during exercise; total CAF ingested after both doses was 6 $mg \cdot kg^{-1}$ BM; 3) placebo (PLA); the participants ingested 150 mL of fluid placebo 60 min before exercise and 150 mL of fluid placebo at 45 min during exercise, with the instruction to consume the treatment fluid in 5 min.

After the treatment fluid ingestion for 30 min, the participants performed the time to exhaustion (TTE) test on a motorized treadmill (h/p/cosmos®, Germany) to determine the endurance capacity. After a warm-up at a self-selected running speed for 5 min, the running speed increased to the initial velocity corresponding to VT_1 until voluntary exhaustion. BM with minimal clothing was measured before and after the trial to determine the volume of fluid loss using a bioelectrical impedance body composition analyzer (Accuniq, Korea). RPE was assessed every 30 min during TTE using the RPE 6–20 scale, and gastrointestinal symptoms were assessed before ingestion, immediately prior to and post-exercise, and every 30 min during TTE using the visual analogue scale (VAS) score for gastrointestinal symptoms.

Blood Analysis

Arterialized fingertip blood samples were collected before ingestion, immediately prior to and post-exercise, and every 30 min during TTE. Blood lactate concentrations were analyzed using a lactate analyzer (Lactate-scout, EKF diagnostics, UK) immediately after blood collection. Antecubital venous blood was sampled before ingestion, immediately prior to and post-exercise, and every 30 min during TTE. The sample was homogenized in 200 µL of 1% (w/v) Triton X-100 in chloroform solution. The samples were centrifuged at 13,000 × g for 10 min to remove insoluble material. The organic phases (lower phase) were collected, and chloroform was removed by air drying at 50°C. The dried lipids were dissolved in a 200-µL fatty acid assay buffer by extensive vortexing for 5 min. Then, the concentration of FFAs was determined by a Free Fatty Acid Quantitation Kit (Sigma-Aldrich, USA). To evaluate concentrations after CAF ingestion, serum CAF concentrations were measured using high-performance liquid chromatography (HPLC; Spectra SERIES P100/UV 100, UK). Serum glucose concentration was determined by the Hexokinase/G-6-PDH method using Architect Analyzer (Abbott Laboratories, Abbott Park, IL).

Statistical Analysis

All data are presented as the mean ± standard deviation (S.D.). Statistical analysis was performed using IBM SPSS Statistics software, version 22.0 (SPSS, IBM Statistics, New York, US). All analyzed variables of interest were screened for normality using the Shapiro–Wilks test. Baseline differences (i.e., serum CAF concentrations, serum FFA concentrations, serum glucose concentrations, blood lactate concentration, gastrointestinal symptoms VAS score, and RPE) and differences between conditions (HIGH, MOD, and PLA) were tested using a two-way analysis of variance (ANOVA). If a significant difference ($p < 0.05$) was observed, Bonferroni post hoc analyses were conducted. The differences in time to exhaustion between conditions (HIGH, MOD, and PLA) were tested using a one-way analysis of variances (ANOVA), followed by Bonferroni correction for post hoc tests. Statistical significance was accepted at $p < 0.05$ for all tests.

Results

Serum Caffeine Concentrations

Serum CAF concentrations immediately before exercise testing (0 min) were not significantly different ($p > 0.05$) between the HIGH (11.17 ± 10.53 µg/L), MOD (5.34 ± 8.89 µg/L), and PLA (0.00 ± 0.00 µg/L) groups. During exercise testing, a significant effect was observed in mean serum CAF concentrations at the time point of 60 min during exercise testing between the HIGH (30.64 ± 36.36 µg/L) and PLA (0.00 ± 0.00 µg/L) groups ($p = 0.00$) and between the MOD (12.35 ± 19.63 µg/L) and PLA (0.00 ± 0.00 µg/L) groups ($p = 0.03$). At the time point of 90 min, serum CAF concentrations were significantly different between the HIGH (16.17 ± 9.79 µg/L) and MOD (4.62 ± 8.53 µg/L) groups ($p = 0.04$) and between the HIGH (16.17 ± 9.79 µg/L) and PLA (0.00 ± 0.00 µg/L) groups ($p = 0.00$). At the time point of 120 min, serum CAF concentrations were significantly different between the HIGH (13.24 ± 7.11 µg/L) and PLA (0.00 ± 0.00 µg/L) groups ($p = 0.00$), respectively (Fig. 1).

Serum Free Fatty Acid Concentrations

During exercise testing, a significant main effect group was observed in the mean serum FFA concentrations at the time point of 120 min during exercise testing between the HIGH (0.086 ± 0.026 mmol/L) and PLA (0.051 ± 0.018 µg/L) groups ($p = 0.00$) (Fig. 2).

Serum Glucose Concentrations

There was no significant difference in mean serum glucose concentrations between the HIGH, MOD, and PLA groups in the period of exercise testing (Fig. 3).

Blood Lactate Concentrations

Baseline blood lactate concentrations were not significantly different between the HIGH (1.32 ± 0.16 mmol/L), MOD (0.87 ± 0.43 mmol/L), and PLA (1.30 ± 0.26 mmol/L) groups ($p < 0.05$). During exercise testing, a significant effect was observed in mean blood lactate concentrations at the time point of 30 min during exercise between the HIGH (1.60 ± 0.66 mmol/L) and MOD (0.97 ± 0.43 mmol/L) groups ($p = 0.02$) and between the HIGH (1.60 ± 0.66 mmol/L) and PLA (0.87 ± 0.49 mmol/L) groups ($p = 0.00$). At the time point of 120 min, blood lactate concentrations were significantly different between the HIGH (1.57 ± 0.55 mmol/L) and MOD (1.02 ± 0.67 mmol/L) groups ($p = 0.01$) and between the HIGH (1.57 ± 0.55 mmol/L) and PLA (0.82 ± 0.43 mmol/L) groups ($p = 0.00$), respectively (Fig. 4).

Time to Exhaustion

The two-way repeated-measures ANOVA indicated a significant effect of CAF ingestion on the time to exhaustion ($F = 3.75$; $p < 0.04$). Post hoc analyses of the effect of CAF ingestion indicated a significant increase in time to exhaustion after the intake of CAF in the MOD (127.46 ± 3.72 min) compared to the PLA (124.61 ± 4.58 min) groups ($p = 0.02$) (Table 1).

Table 1. Average time to exhaustion (min ± S.D.) in the HIGH, MOD, and PLA groups (n = 8)

Condition (n = 8)	Time to Exhaustion (min) Error! ± S.D.	F	P-value
HIGH	126.52 ± 4.54	3.758	.049*
MOD	127.46 ± 3.72#		
PLA	124.61 ± 4.58#		

* Significant difference ($p < 0.05$) between groups. # Significant difference ($p < 0.05$) between MOD and PLA

Gastrointestinal Symptoms

During exercise testing, a significant effect was observed in the mean abdominal pain VAS score at the time point of 90 min during exercise testing between the HIGH (87 ± 1.45 mm) and MOD (0.00 ± 0.00 mm) groups ($p = 0.01$) and between the HIGH (87 ± 1.45 mm) and PLA (0.00 ± 0.00 mm) groups ($p = 0.01$). There was no significant difference in the mean abdominal bloating and nausea VAS scores between the HIGH, MOD, and PLA groups during the exercise test.

Rating of Perceived Exertion

RPE revealed a predictable pattern of physiological responses that are typical for exercising, and RPE gradually increased throughout the exercise test. There were no significant differences between the groups.

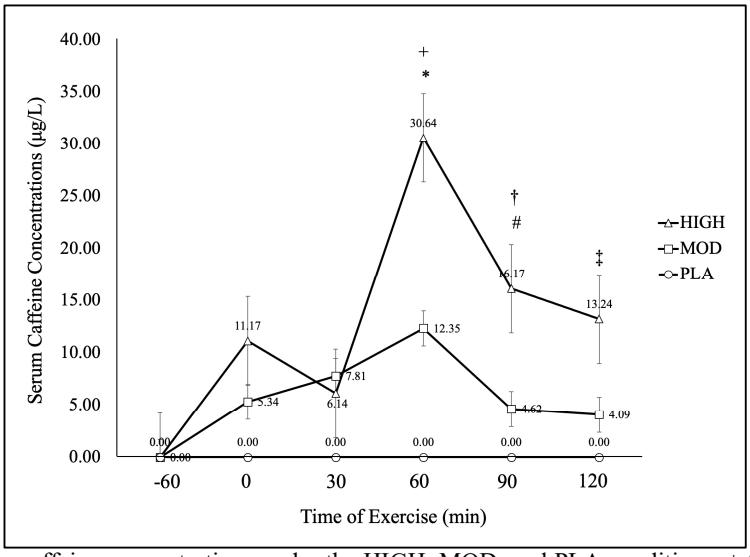

Fig 1. Serum caffeine concentrations under the HIGH, MOD, and PLA conditions. + Significant difference ($p < 0.05$) between HIGH and PLA at 60 min. * Significant difference ($p < 0.05$) between MOD and PLA at 60 min. † Significant difference ($p < 0.05$) between HIGH and MOD at 90 min. # Significant difference ($p < 0.05$) between HIGH and PLA at 90 min. ‡ Significant difference ($p < 0.05$) between HIGH and PLA at 120 min

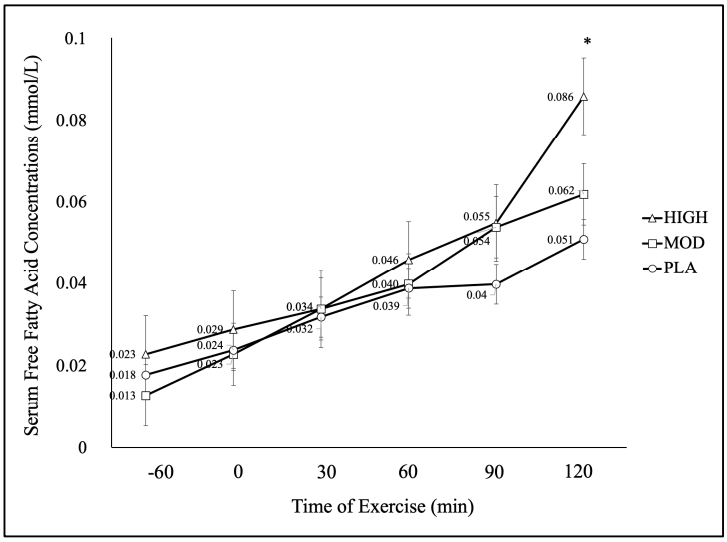

Fig 2. Serum free fatty acid concentrations under the HIGH, MOD, and PLA conditions.
* Significant difference ($p < 0.05$) between HIGH and PLA at 120 min

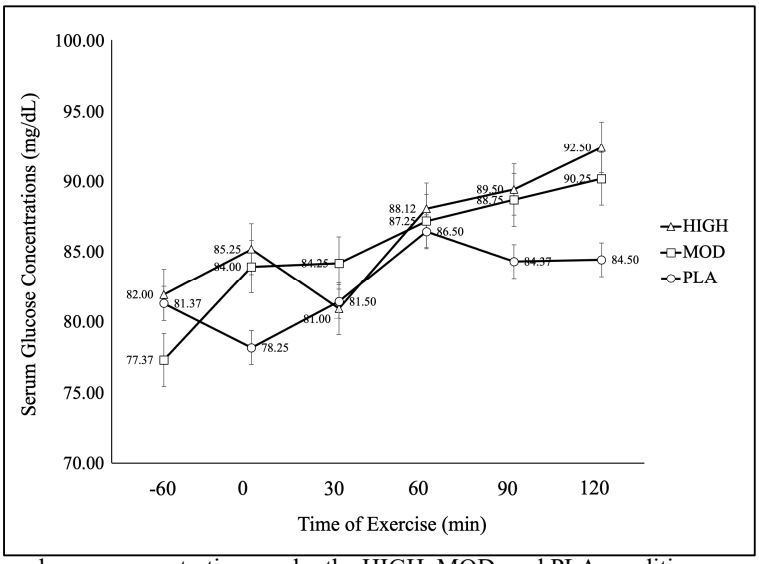

Fig 3. Serum glucose concentrations under the HIGH, MOD, and PLA conditions

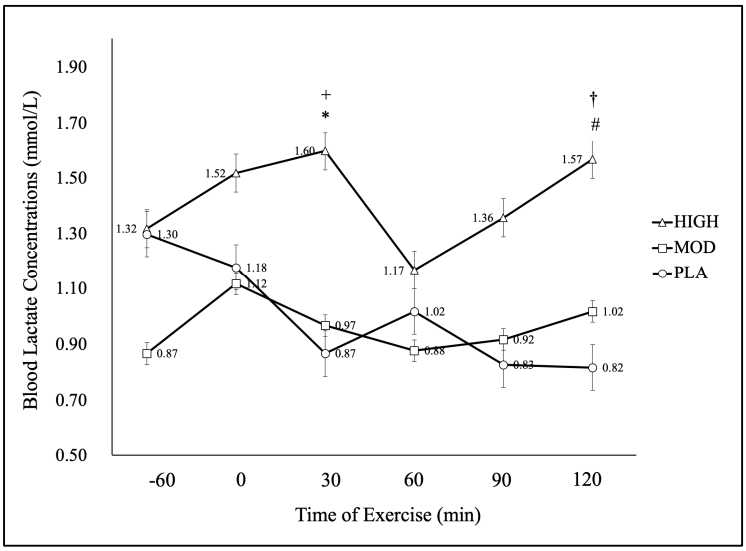

Fig 4. Blood lactate concentrations under the HIGH, MOD, and PLA conditions. + Significant difference ($p < 0.05$) between HIGH and MOD at 30 min. * Significant difference ($p < 0.05$) between HIGH and PLA at 30 min. † Significant difference ($p < 0.05$) between HIGH and MOD at 120 min. # Significant difference ($p < 0.05$) between HIGH and PLA at 120 min

Discussion

The HIGH group, which consumed two doses of CAF (for a total of 9 mg·kg^{-1} BM), had the highest levels of serum FFAs, followed by the MOD group, which consumed one dose (for a total of 6 mg·kg^{-1} BM), and the PLA group, which had the lowest levels. The HIGH group had the highest serum FFAs levels at 120 min during the exercise test compared to the MOD and PLA groups. This occurred possibly because the HIGH group took their second CAF dose after 45 min during the exercise test. The high serum FFAs levels may indicate that the body can increase FFA oxidation at the proper rate to produce energy for prolonged aerobic exercise. As a result, the running performance of the HIGH group was better than that of the PLA group.

CAF contains three metabolites that improve energy efficiency in endurance athletes, i.e., paraxanthin, theophylline, and theobromine. These metabolites can stimulate the body's breakdown of fat into FFAs and glycerol. CAF dosages of 3 to 6 mg·kg^{-1} BM, 60 min before exercising, are well-accepted to improve performance; whereas the high doses of CAF (e.g., 9 mg·kg^{-1} BM) are associated with a high incidence of side effects and do not appear to be required to elicit an ergogenic effect (Maughan et al., 2018; Guest et al., 2021). A previously published critical review recently highlighted that both low (3 mg·kg^{-1} BM) and moderate to high (i.e., 6–9 mg·kg^{-1} BM) CAF dosages can improve exercise performance; the abovementioned study also

demonstrated that, while low doses of CAF (3 $mg \cdot kg^{-1}$ BM) have the potential to be ergogenic, it is unclear whether such doses are as ergogenic as higher doses (Pickering & Kiely, 2019). According to some studies, prolonged aerobic exercises that are associated with moderate and high doses of CAF (i.e., 6–9 $mg \cdot kg^{-1}$ BM) may change the oxidative flow of substrates. In those situations, CAF changed how lipids were used for energy while maintaining muscle glycogen stores (Taylor et al., 2011; Cruz et al., 2015). The consumption of a high dose of CAF prior to exercise sessions improved results in aerobic performance tests (Cruz et al., 2015). A previous study by Olcina et al. (2011) suggested that the lower respiratory exchange ratio (RER) values, which may indicate a lesser reliance on glycogen, were associated with an increase in relative fat oxidation due to CAF's ergogenic effect and, consequently, lower lactate production (glycolytic metabolites). These results may eventually result in a longer time to exhaustion.

The obtained results showed that different caffeine doses had no effect on blood glucose levels during prolonged exercise. This finding is consistent with the findings of Siahpoosh and Nesaei (2016), who investigated the effect of caffeine consumption at different doses on blood lactate and glucose levels after middle-distance running and concluded that none of the caffeine doses had a significant effect on blood lactate and glucose levels both before and after the middle-distance exercise. Several other studies have observed that caffeine ingestion had no effect on the levels of blood glucose during exercise (Van Soeren & Graham, 1998; Battram et al., 2005). Hulston and Jeukendrup (2008) demonstrated that CAF may only have an effect on muscle glucose uptake during resting and possibly during the recovery period following exercise but not during exercise. However, their findings indicated that relative to placebo, the ingestion of a high dose of caffeine resulted in significantly increased blood lactate concentration during prolonged exercise. The effects of caffeine ingestion on blood lactate concentration during exercise have received little attention, and in the few studies that focused on this aspect, the findings regarding the glycogen-sparing property of caffeine are contradictory. Some studies attribute higher lactate concentrations with caffeine ingestion to a catecholamine-induced increase in the rate of muscle glycogenolysis. Indeed, epinephrine infusion can increase muscle glycogenolysis and lactate formation during steady-state exercise (Watt et al., 2001; Hulston and Jeukendrup, 2008). The increased blood lactate concentration can indicate lactate production (glycolytic metabolites) by active muscles or decreased blood clearance. Of note, blood lactate concentration should not increase if glycogen sparing occurs because FFAs are the primary source of fuel during exercise (Siahpoosh and Nesaei, 2016). Another controversial finding of Hulston and Jeukendrup (2008) suggests that higher lactate concentrations after caffeine consumption may result from decreased clearance by other nonexercising tissues (possibly the liver or resting muscles) instead of having a direct impact on the exercising muscles. Although in this study blood lactate concentrations are significantly different between the HIGH, MOD, and PLA groups, the blood lactate concentration values lower than 4 $mmol \cdot L^{-1}$ are also known as the onset of blood lactate accumulation (OBLA). The intensity of exercise testing in this study corresponded to VT_1, which is moderate intensity. As a result, the body circulates lactic acid into the liver to restore glucose balance, and lactic acid accumulation in the blood is low.

According to the findings of this study, CAF consumption improves endurance performance as measured by the time to exhaustion test. This occurs possibly because the higher serum FFA levels in the HIGH group have the potential to increase fat oxidation during prolonged exercise, resulting in improved endurance performance. The ability of the body to synthesize sufficient ATP to allow to perform exercise for the entire distance is essential for long-distance running. According to the study's findings, the HIGH and MOD groups, which both consume CAF, had a more significant increase in the serum FFA concentrations after 90 min of exercising compared to the PLA group, which did not consume CAF. Typically, the conversion of FFAs to ATP produces more ATP than the conversion of other macronutrients, which gives the body adequate energy reserves. Additionally, this process can reduce the increase in blood lactic acid concentration; consequently, the endurance running performance was superior to that of the caffeine-free group (Burke et al., 2019).

Conclusions

In conclusion, consumption of a high dose of caffeine, i.e., ingestion of 6 $mg \cdot kg^{-1}$ BM of CAF 60 min before exercise and 3 $mg \cdot kg^{-1}$ BM of CAF after 45 min during exercise (for a total of 9 $mg \cdot kg^{-1}$ BM) may help to improve endurance running performance in male half-marathon runners via higher serum FFA concentration, increase fat oxidation at the proper rate to produce energy for prolonged aerobic exercise, and reduce the increase in blood lactic acid concentration. These findings expand the usefulness of CAF as an ergogenic aid in athletes. However, CAF ingestion can have several drawbacks and may produce undesirable side effects. Further studies should examine the occurrence of side effects associated with different dosages of CAF ingestion among the athletes which may be beneficial to establish CAF supplementation strategies that enhance athletic performance while maintaining athletes' safety and well-being.

Acknowledgments

We gratefully acknowledge all the participants involved in the study. We also thank The Faculty of Sports Science, Chulalongkorn University for financial support. Finally, the authors would like to thank Falcon Scientific Editing (https://falconediting.com) for proofreading the English language in this paper.

References:

Battram, D.S., Graham, T.E., Richter, E.A. & Dela, F. (2005), The effect of caffeine on glucose kinetics in humans – influence of adrenaline. *J Physiol, 569*, 347-355.

Bell, D.G., & McLellan, T.M. (2002). Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers. *J Appl Physiol, 93*(4), 1227–1234.

Burke, L.M. (2008). Caffeine and sports performance. *Appl Physiol Nutr Metab, 33*(6), 1319–1334.

Burke, L.M., Jeukendrup, A.E., Jones, A.M., & Mooses, M. (2019). Contemporary nutrition strategies to optimize performance in distance runners and race walkers. *Int J Sport Nutr Exerc Metab, 49*(2), 117–129.

Bruce, C.R., Anderson, M.E., Fraser, S.F., Stepto, N.K., Klein, R., Hopkins, W.G., & Hawley, J.A. (2000). Enhancement of 2000-m rowing performance after caffeine ingestion. *Med Sci Sports Exerc, 32*(11), 1958–1963.

Chen, C., Nakagawa, S., An, Y., Ito, K., Kitaichi, Y., & Kusumi, I. (2017). The exercise-glucocorticoid paradox: How exercise is beneficial to cognition, mood, and the brain while increasing glucocorticoid levels. *Front Neuroendocrinol, 44*, 83–102.

Chuychai, J., Srihirun, K., Chinapong, S., Sanpasitt, C., Khaosanit, P., & Nokkaew, N. (2022). Fluid containing highly branched cyclic dextrin: an alternative ergogenic aid to enhance endurance exercise performance in long-distance runners. *J Exerc Physiol Online, 25*(4), 1–13.

Cruz, R.S., de Aguiar, R.A., Turnes, T., Guglielmo, L.G., Beneke, R., & Caputo, F. (2015). Caffeine affects time to exhaustion and substrate oxidation during cycling at maximal lactate steady state. *Nutrients, 7*(7), 5254–5264.

de Souza, J.G., Del Coso, J., Fonseca, F.d.S., Silva, B.V.C., de Souza, D.B., Gianoni, R.L.d.S., et al. (2022). Risk or benefit? Side effects of caffeine supplementation in sport: a systematic review. *Eur J Nutr.*

Del Coso, J., Muñoz, G., & Muñoz-Guerra, J. (2011). Prevalence of caffeine use in elite athletes following its removal from the world anti-doping agency list of banned substances. *Appl Physiol Nutr Metab, 36*, 555–561.

Doyenart, R., Olímpio, M, Boeira, D., Sombrio, F., Milhomes, Yuri, P., et al. (2020). Psycho-physiological changes after a half marathon in amateur runners. *J Phys Educ Sport, 20*(4), 1768–1774.

Ganio, M.S., Klau, J.F., Casa, D.J., Armstrong, L.E., & Maresh, C.M. (2009). Effect of caffeine on sport-specific endurance performance: a systematic review. *J Strength Cond Res, 23*(1), 315–324.

Goldstein, E. R., Ziegenfuss, T., Kalman, D., Kreider, R., Campbell, B.,Wilborn, C., et al. (2010). International society of sports nutrition position stand: caffeine and performance. *J Int Soc Sports Nutr, 7*:5.

Gómez-Molina, J., Ogueta-Alday, A., Camara, J., Stickley, C., Rodríguez-Marroyo, J. A., & García-López, J. (2017). Predictive Variables of Half-Marathon Performance for Male Runners. *J Sports Sci Med, 16*(2), 187–194.

Guest, N.S., Van Dusseldorp, T.A., Nelson, M. T., Grgic, J., Schoenfeld, B.J., Jenkins, N., et al. (2021). International society of sports nutrition position stand: caffeine and exercise performance. *J Int Soc Sports Nutr, 18*(1), 1.

Hargreaves, M., & Spriet, L.L. (2022). Skeletal muscle energy metabolism during exercise. *Nat Metab, 2,* 817–828.

Hodgson, A.B., Randell, R.K., & Jeukendrup, A.E. (2013). The Metabolic and performance effects of caffeine compared to coffee during endurance exercise. *PLoS ONE, 8*(4): e59561.

Hunter, A.M., Gibson, A.S.C., Collins, M., Lambert, M. & Noakes, T.D. (2002). Caffeine ingestion does not alter performance during a 100-km cycling time-trial performance. *Int J Sport Nutr Exerc Metab*, *12*, 438–452.

Hulston, C.J., Jeukendrup, A.E. (2008). Substrate metabolism and exercise performance with caffeine and carbohydrate intake. *Med Sci Sports Exerc, 40*(12), 2096–2104.

Martins, G. L., Guilherme, J. P. L. F., Ferreira, L. H. B., de Souza-Junior, T. P. & Lancha Jr., A. H. (2020). Caffeine and exercise performance: possible directions for definitive findings. *Front Sports Act Living, 2*, 574854.

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., et al. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *Br J Sports Med, 52*(7), 439–455.

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., et al. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *Int J Sport Nutr Exerc Metab, 28*, 104–125.

Midgley, A.W., McNaughton, L.R., Polman, R., & Marchant, D. (2007). Criteria for determination of maximal oxygen uptake. *Sports Med, 37*(12), 1019–1028.

Noakes, T. D. (2022). What is the evidence that dietary macronutrient composition influences exercise performance? a narrative review. *Nutrients, 14*: 862.

Olcina, G., Maynar, M., Muñoz, D., Timon, R., Caballero, M.J., & Maynar, J. (2011). Plasma total fatty acid responses to exercise following caffeine ingestion. *Eur J Sport Sci, 11*(2), 111-118

Pickering, C., & Kiely, J. (2018). Are the current guidelines on caffeine use in sport optimal for everyone? Inter-individual variation in caffeine ergogenicity, and a move towards personalised sports nutrition. *Sports Med, 48,* 7–16.

Pickering, C., & Kiely, J. (2019). Are low doses of caffeine as ergogenic as higher doses? A critical review highlighting the need for comparison with current best practice in caffeine research. *Nutrition, 67-68*, 110535.

Poole, D.C., Wilkerson, D.P., & Jones, A.M. (2008). Validity of criteria for establishing maximal O_2 uptake during ramp exercise tests. *Eur J Appl Physiol, 102*, 403–410.

Poole, D.C. & Jones, A.M. (2017). Measurement of the maximum oxygen uptake $\dot{V}O_2$max: $\dot{V}O_2$peak is no longer acceptable. *J Appl Physiol, 122*, 997–1002.

Ravindra, P.V., Janhavi, P., Divyashree, S. & Muthukumar, S.P. (2022). Nutritional interventions for improving the endurance performance in athletes. *Arch Physiol Biochem, 128*(4), 851-858

Siahpoosh A.K, & Nesaei A. (2016). The effect of caffeine supplementation on blood lactate and glucose after 800 and 1500 meter run. *Biomed Pharmacol J, 9*(1).

Smirmaul, B.P.C., de Moraes, A.C., Angius, L., & Macora, S.M. (2017). Effects of caffeine on neuromuscular fatigue and performance during high-intensity cycling exercise in moderate hypoxia. *Eur J Appl Physiol, 117*(1), 27–38.

Spriet, L.L. (2007). Regulation of substrate use during the marathon. *Sports Med, 37*(4-5), 332–336.

Spriet, L.L. (2014). Exercise and sport performance with low doses of caffeine. *Sports Med, 44*, 175–184.

Southward, K., Rutherfurd-Markwick, K. J. & Ali1, A. (2018). The effect of acute caffeine ingestion on endurance performance: a systematic review and meta–analysis. *Sports Med, 48*, 1913–1928.

Taylor, C., Higham, D., Close, G. L., & Morton, J. P. (2011). The effect of adding caffeine to postexercise carbohydrate feeding on subsequent high-intensity interval-running capacity compared with carbohydrate alone. *Int J Sport Nutr Exerc Metab, 21,* 410–416.

Van Soeren, M. H., & Graham, T. E. (1998). Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal. *J Appl Physiol, 85*(4), 1493–1501.

Watt, M. J., Howlett, K. F., Febbraio, M. A., Spriet, L. L., & Hargreaves, M. (2001). Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. *J Physiol, 534*(Pt 1), 269–278.

Wickham, K.A., & Spriet, L.L. (2018). Administration of caffeine in alternate forms. *Sports Med, 48* (Suppl 1): S79–S91.

Zhang, B., Liu, Y., Wang, X., Deng, Y., & Zheng, X. (2020). Cognition and brain activation in response to various doses of caffeine: a nearinfrared spectroscopy study. *Front Psychol, 11*:1393.