



## Effects of caffeine ingestion on the diurnal variation of cognitive and repeated high-intensity performances

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### ABSTRACT

The purpose of this study was to evaluate the effects of caffeine ingestion on the daily variation of cognitive (*i.e.*, reaction time (RT), attention) and repeated high-intensity exercise performances.

Fifteen active males (age:  $20 \pm 1$  years, height:  $174.3 \pm 4.3$  cm, body-mass:  $70.8 \pm 3.5$  kg) performed cognitive and physical tasks under two different circumstances [after a placebo or caffeine ingestion (6 mg/kg of body-mass)] at six different time-of-day (07 h00, 09 h00, 11 h00, 13 h00, 15 h00 and 17 h00) in a randomized double-blind balanced crossover design. During each session, RT, attention and 5-m multiple shuttles run test performances were recorded.

During both the placebo and the caffeine conditions, a significant diurnal variation was found with improvement of cognitive performances recorded at 11 h00 (*e.g.*, RT:  $0.37 \pm 0.02$ -s and  $0.36 \pm 0.02$ -s for placebo and caffeine respectively) and 17 h00 (*e.g.*, RT:  $0.37 \pm 0.02$ -s and  $0.35 \pm 0.03$ -s for placebo and caffeine respectively) compared to (*i.e.*, worst performances) 07 h00 (*e.g.*, RT:  $0.41 \pm 0.02$ -s and  $0.38 \pm 0.02$ -s for placebo and caffeine respectively) and 13 h00 (*e.g.*, RT:  $0.41 \pm 0.02$ -s and  $0.38 \pm 0.02$ -s for placebo and caffeine respectively) ( $p < 0.05$ ). For physical performance, improved values were recorded at 17 h00 (*e.g.*, total distance:  $730.00 \pm 43.92$ -m and  $733.93 \pm 43.08$ -m for placebo and caffeine respectively) compared to 07 h00 (*e.g.*, total distance:  $698.14 \pm 45.39$ -m and  $709.21 \pm 43.78$ -m for placebo and caffeine respectively) ( $p < 0.05$ ). Compared to placebo, cognitive (*e.g.*, RT: by 6.4% at 07 h00, 4.1% at 09 h00, 3.4% at 11 h00, 6.0% at 13 h00, 3.8% at 15 h00 and 3.8% at 17 h00) and physical (*e.g.*, total distance: 1.6% at 07 h00, 0.9% at 09 h00, 0.1% at 11 h00 ( $p > 0.05$ ), 0.5% at 13 h00, 1.0% at 15 h00 and 0.5% at 17 h00) performances increased at all time-of-day ( $p < 0.05$ ). In conclusion, cognitive and physical performances are time-of-day dependent and caffeine is an effective ergogenic aid to improves both cognitive and physical performances especially at the moment of their lowest values.

### 1. Introduction

Diurnal fluctuations of short-term maximal performance has been widely confirmed in various tasks such as the 30-s Wingate test (Chtourou et al., 2011, 2013a) and the repeated sprint ability (RSA) exercise (Racinais et al., 2010; Chtourou et al., 2012b). During the Wingate test, peak and mean power fluctuate during the day reaching an acrophase (*i.e.*, time of maximal level) around 18 h00 (Souissi et al., 2004). Few studies have investigated the impact of time-of-day on RSA performance (Chtourou et al., 2018) and most of them consistently reported higher muscle power in the end of the afternoon or early evening compared to the morning (Racinais et al., 2010; Hammouda

et al., 2011; Aloui et al., 2013).

Also, previous studies have shown that cognitive (Valdez et al., 2014) and mental performances (Touitou and Bégué, 2010) were influenced by the time-of-day and more accurately by time since awake (Edwards et al., 2008) with higher values registered in the afternoon. This can be explained by the fact that early in the morning, performance might be poorer due to the combined effects of lower body temperature (*i.e.*, not having risen far enough above its nocturnal minimum) and sleep inertia; while, immediately after lunch, performance is reduced due to a post lunch dip effect (Valdez et al., 2008).

As physical and cognitive performances were at low levels in the morning, some strategies have been proposed in order to improve

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performances at this period of the day (*i.e.*, especially for athletes who compete in the morning hours) such as active warm-up (Souissi et al., 2010), listening to music (Chtourou et al., 2012a), regular training in the morning (Chtourou et al., 2012c, 2012d, 2014, 2015) and caffeine ingestion (Souissi et al., 2013a).

It has been reported that caffeine has an ergogenic effect on long duration exercise performance (*i.e.*, muscular endurance; Warren et al., 2010), RSA (Jordan et al., 2012), reaction time (RT) and the 30-s Wingate test (Souissi et al., 2012, 2014).

It is, now, believed that the mechanism by which caffeine enhances performance stem from its effects on the central nervous system (CNS) through adenosine receptor antagonism. Indeed, adenosine and caffeine have opposite effects to each other in regulating cellular activities. Indeed, it has been reported that the ingestion of caffeine inhibits the effects of adenosine on neurotransmission, perceived exertion and arousal (Chia et al., 2017). The caffeine's ergogenic effect could be explained, also, by a reduction in the sensation of fatigue induced by the physical exercise (Motl et al., 2003) and a stimulation of the CNS (Lopes et al., 1983). In this context, it has been showed that the effect of caffeine was related to the stimulation of the CNS by improving vigilance, mood, and attention as well as reducing the RT (Ruijter et al., 2000).

However, the literature is unclear concerning the effect of caffeine ingestion on short-duration high-intensity maximal exercise performance. One of the major reasons for these inconclusive results is the “uncontrolled time-of-day” of testing. Indeed, to the best of the authors' knowledge, few existing studies took into account the effect of time-of-day on their experimental design. Souissi et al. (2013a) demonstrated that caffeine ingestion in the morning is recommended to minimize the diurnal fluctuations (*i.e.*, morning-afternoon difference) of short-term maximal performance during the Wingate test. In the same way, Souissi et al. (2015) revealed that caffeine ingestion in the afternoon has no-significant effect on muscle power and fatigue during the Wingate test and, thus, there was no-ergogenic effect of caffeine ingestion at this time of day.

Likewise, a number of studies testing the effect of 6 mg/kg of caffeine ingestion on RSA reported significant enhancement of performance (Foskett et al., 2009; Jordan et al., 2012). In view of the above consideration, the present study aimed to investigate the effect of time-of-day and caffeine ingestion on cognitive and repeated short-duration high-intensity efforts performances.

The hypothesis of the present study was that caffeine ingestion may improve physical as well as cognitive performance during all moments of the day with greater enhancement in the early morning.

## 2. Methods

### 2.1. Participants

The software G\*Power was utilized to calculate the required sample size using alpha and power level of 0.05 and 0.95. Based on the results of Mora-Rodríguez et al. (2015) and after authors' discussion, effect sizes was fixed on 0.95 (large effect). At minimum fourteen participants are required for the present study to reach the desired power.

Fifteen healthy male physical education students ( $20 \pm 1$  years,  $174.3 \pm 4.3$  cm,  $70.8 \pm 3.5$  kg; mean  $\pm$  SD) volunteered to participate in the study. They were fully informed about the protocol of the study and signed a consent form before participating. They had no tobacco dependence assessed through the Fagerström test (1978). They did not consume caffeine, alcoholic beverages, anti-oxidants or anti-inflammatory drugs during the experiment. To ensure that participants were all of “intermediate type”, they were further selected on the basis of their scores in the Horne and Östberg's (1976) questionnaire. This last criterion resulted in a sample of participants who shared the same timing in terms of rising time ( $\sim 06\text{h}00 \pm 1\text{h}$ ) and bedtime ( $\sim 22\text{h}00 \pm 1\text{h}$ ).

### 2.2. Experimental design

During the two weeks preceding the experiment, participants visited the research unit during various times (i) to undergo anthropometric measurements, (ii) to be familiarized with the test procedure and (iii) to determine the caffeine dose for each participant. They performed the cognitive and the 5-m shuttle run tests twice before the beginning of the study. The amount of 6 mg/kg of caffeine was selected because it was demonstrated that this dose could increase plasma levels of caffeine (Graham and Spriet, 1995) and has been reported (i) to be a moderate dose in comparison with 9 mg/kg considered as high dose (Goldstein et al., 2010; Pickering and Kiely, 2018) and (ii) to improve jump and sprint performances (Chia et al., 2017). Although it is still on the watched list (Maughan, 2018), this dose of caffeine allows an ergogenic effect without approaching the legal limit fixed by the International Olympic Committee IOC (Graham, 2001). Instructions about sleep and diet were given to each participant prior to the experiment. They had the same standard isocaloric meal during the day of each session. After that, only water was allowed *ad libitum*. They must maintain their routine of physical activities during the experimental period and avoid any intense activity during the 24 h preceding each test session. All test sessions took place in similar conditions of temperature and relative humidity ( $28.2\text{--}29.3$  °C and  $45.1\text{--}46.7\%$  respectively). Participants performed 12 test sessions separated by at least three recovery days: at 07 h00, 09 h00, 11 h00, 13 h00, 15 h00 and 17 h00 in a randomized, balanced crossover design after placebo or caffeine ingestion. Placebo and caffeine were ingested using a double-blind protocol. During each testing session, participants were requested to attend the laboratory two hours before the beginning of the test session. One hour after the caffeine or the placebo ingestion, participants performed the following tests in the same order: RT, attention (*i.e.*, number cancellation test), ten minutes of warm-up and stretching exercises and, then, the 5-m multiple shuttles run test.

#### 2.2.1. Attention (Hatta et al., 2012) (numbers cancellation test)

The numbers cancellation test is composed of four forms. Each form contains 600 numbers containing from one (*e.g.*, 5) to five (*e.g.*, 46,972) digits arranged in 36 lines. The target number (*i.e.*, that contain three digits) appeared two to eight times per line in a series of other random numbers. Numbers was separated by a dot and preceded and followed by a space. One mark was allocated for each of the targets correctly detected. Each participant had one minute to cross out as quickly as possible the maximum 3-digit numbers. The score was calculated in terms of the number of 3-digit numbers correctly identified.

#### 2.2.2. Reaction time (ms)

RT was used as an index of cognitive performance. It was used as a measure of general alertness and motor performance and was assessed using the software “React” as previously described by Jarraya et al. (2012). Participants were instructed to press a key of the micro-computer as quickly as a visual stimulus appeared.

#### 2.2.3. 5-m multiple shuttles run test (Boddington et al., 2001)

The 5-m multiple shuttles test was performed in a gymnasium. Six beacons were placed 5 m apart in a straight line to cover a total distance of 25 m. The participants were instructed to perform six repetitions of maximal sprints during 30-s with a recovery period of 35-s in-between. They began the test in line with the first beacon, they sprinted 5 m to a second beacon, and returned back to the first beacon, they sprinted 10 m to a third beacon and back to the first beacon, *etc.*, until the end of the 30-s. The distance covered by each participant was recorded to the nearest of 1-m during each 30-s shuttle.

The data recorded during the tests were further used to calculate:

- Peak distance (PD) = the longest distance covered during a 30-s shuttle;

- Total distance (TD) = the total distance covered during the 6 × 30-s shuttles;
- Fatigue index (FI) = calculated according to the following equation:

$$[(\text{Shuttle 1} + \text{Shuttle 2}) - (\text{Shuttle 5} + \text{Shuttle 6}) / (\text{Shuttle 1} + \text{Shuttle 2})] \times 100$$

### 3. Statistical analysis

Data were analyzed using the STATISTICA Software (StatSoft, France). The Shapiro-Wilk test revealed that the data were normally distributed. All variables were analyzed using a two-way ANOVA (2 Caffeine × 6 Time-of-day) with repeated measures. When appropriate, significant differences between means were tested using the Bonferroni post-hoc test. A probability level of 0.05 was selected as the criterion for statistical significance ( $p < 0.05$ ). Furthermore, effect sizes using partial eta squared ( $\eta_p^2$ ) were calculated, and were defined as small ( $\geq 0.01$ ), moderate ( $\geq 0.06$ ) or large ( $\geq 0.14$ ) (Cohen, 1992). Relative differences in percentages between conditions were also calculated as follows:

$$\Delta (\%) = [(\text{Peak value} - \text{Minimum value}) / \text{peak value}] \times 100$$

## 4. Results

### 4.1. Cognitive function

#### 4.1.1. Attention

There was a significant main effect of Time-of-day for attention ( $F = 56.62$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.80$ ). During the placebo and the caffeine sessions, attention improved at 07 h00 compared to 09 h00, 11 h00, 15 h00 and 17 h00, decreased at 09 h00 compared to 11 h00 and 17 h00 and increased at 09 h00 compared to 13 h00 ( $p < 0.05$ ). Attention increased at 11 h00 compared to 13 h00 and 15 h00 and decreased at 13 h00 compared to 15 h00 and 17 h00 and at 15 h00 compared to 17 h00 ( $p < 0.05$ ). The lowest values of attention were observed at 07 h00 ( $66.13 \pm 2.89$  and  $68.07 \pm 2.74$  during the placebo and caffeine test sessions respectively) and 13 h00 ( $65.88 \pm 2.94$  and  $68.10 \pm 2.66$  during the placebo and caffeine test sessions respectively) and the highest values were observed at 11 h00 ( $71.48 \pm 3.52$  and  $71.82 \pm 3.31$  during the placebo and caffeine test sessions respectively) and 17 h00 ( $71.45 \pm 3.56$  and  $71.50 \pm 3.40$  during the placebo and caffeine test sessions respectively). The difference between the highest and lowest value (*i.e.*, amplitude of the diurnal variation) was 7.8% and 5.2% during the placebo and caffeine test sessions respectively.

Also, a significant main effect of Caffeine ( $F = 289.15$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.95$ ) and a significant interaction Time-of-day × Caffeine ( $F = 85.89$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.86$ ) were reported. Compared to placebo, attention increased by 2.9% at 07 h00 ( $p < 0.05$ ), 1.5% at 09 h00 ( $p < 0.05$ ), 0.5% at 11 h00 ( $p < 0.05$ ), 3.4% at 13 h00 ( $p < 0.05$ ), 1.31% at 15 h00 ( $p < 0.05$ ) and 0.1% at 17 h00 ( $p > 0.05$ ) after caffeine ingestion (Table 1).

#### 4.1.2. The reaction time

The statistical analysis showed a significant Time-of-day main effect for RT ( $F = 48.51$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.78$ ). During the placebo and the caffeine sessions, RT increased at 07 h00 compared to 09 h00, 11 h00, 15 h00 and 17 h00 and at 09 h00 compared to 11 h00 and 17 h00 and decreased at 09 h00 compared to 13 h00 ( $p < 0.05$ ). RT decreased at 11 h00 compared to 13 h00 and 15 h00 and improved at 13 h00 compared to 15 h00 and 17 h00 and at 15 h00 compared to 17 h00 ( $p < 0.05$ ). The highest values of RT were observed at 07 h00 ( $0.41 \pm 0.02$  s and  $0.38 \pm 0.02$  s during the placebo and caffeine test sessions respectively) and 13 h00 ( $0.41 \pm 0.02$  s and  $0.38 \pm 0.02$  s during the placebo and caffeine test sessions respectively) and the lowest values were observed at 11 h00 ( $0.37 \pm 0.02$  s and

$0.36 \pm 0.02$  s during the placebo and caffeine test sessions respectively) and 17 h00 ( $0.37 \pm 0.02$  s and  $0.35 \pm 0.03$  s during the placebo and caffeine test sessions respectively). The difference between the highest and lowest value was 10.2% and 7.8% during the placebo and caffeine test sessions respectively.

Likewise, a significant main effect of Caffeine ( $F = 185.0$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.93$ ) and a significant interaction Time-of-day × Caffeine ( $F = 5.77$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.29$ ) were reported. After caffeine ingestion, RT was reduced by 6.4% at 07 h00 ( $p < 0.05$ ), 4.1% at 09 h00 ( $p < 0.05$ ), 3.4% at 11 h00 ( $p < 0.05$ ), 6.0% at 13 h00 ( $p < 0.05$ ), 3.8% at 15 h00 ( $p < 0.05$ ) and 3.8% at 17 h00 ( $p < 0.05$ ) compared to placebo (Table 1).

### 4.2. 5-m multiple shuttles test

#### 4.2.1. Total distance (m)

The statistical analysis showed a significant Time-of-day main effect for total distance ( $F = 87.82$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.86$ ). During the placebo and the caffeine sessions, TD decreased at 07 h00 compared to 09 h00, 11 h00, 13 h00, 15 h00 and 17 h00, at 09 h00 compared to 11 h00, 13 h00, 15 h00 and 17 h00 and at 11 h00 compared to 13 h00, 15 h00 and 17 h00 ( $p < 0.05$ ). TD, also, decreased at 13 h00 compared to 15 h00 and 17 h00 and at 15 h00 compared to 17 h00 ( $p < 0.05$ ). The lowest values of TD were observed at 07 h00 ( $698.14 \pm 45.39$  m and  $709.21 \pm 43.78$  m during the placebo and caffeine test sessions respectively) and the highest values were observed at 17 h00 ( $730.00 \pm 43.92$  m and  $733.93 \pm 43.08$  m during the placebo and caffeine test sessions respectively). The difference between the highest and lowest value was 4.4% and 3.4% during the placebo and caffeine test sessions respectively.

Also, a significant main effect of caffeine ( $F = 148.92$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.91$ ) and a significant interaction Time-of-day × Caffeine ( $F = 26.28$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.65$ ) were reported. Compared to placebo, total distance increased by 1.6% at 07 h00 ( $p < 0.05$ ), 0.9% at 09 h00 ( $p < 0.05$ ), 0.1% at 11 h00 ( $p > 0.05$ ), 0.5% at 13 h00 ( $p < 0.05$ ), 1.0% at 15 h00 ( $p < 0.05$ ) and 0.5% at 17 h00 ( $p < 0.05$ ) after caffeine ingestion (Table 1).

#### 4.2.2. Peak distance (m)

The statistical analysis showed a significant Time-of-day main effect ( $F = 153.38$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.92$ ). During the placebo and the caffeine sessions, PD decreased at 07 h00 compared to 09 h00, 11 h00, 13 h00, 15 h00 and 17 h00, at 09 h00 compared to 11 h00, 13 h00, 15 h00 and 17 h00 and at 11 h00 compared to 13 h00 (*i.e.*, only during the caffeine session) 15 h00 and 17 h00 ( $p < 0.05$ ). PD, also, decreased at 13 h00 compared to 15 h00 and 17 h00 and at 15 h00 compared to 17 h00 (*i.e.*, only during the placebo session) ( $p < 0.05$ ). The lowest values of PD were observed at 07 h00 ( $129.36 \pm 7.85$  m and  $134.64 \pm 8.01$  m during the placebo and caffeine test sessions respectively) and the highest values were observed at 17 h00 ( $146.36 \pm 9.39$  m and  $146.93 \pm 9.72$  m during the placebo and caffeine test sessions respectively). The difference between the highest and lowest value was 11.6% and 8.4% during the placebo and caffeine test sessions respectively.

Likewise, a significant main effect of Caffeine ( $F = 170.61$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.92$ ) and a significant interaction Time-of-day × Caffeine ( $F = 33.0$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.70$ ) were reported. After caffeine ingestion, peak distance increased by 4.1% at 07 h00 ( $p < 0.05$ ), 2.3% at 09 h00 ( $p < 0.05$ ), 0.4% at 11 h00 ( $p > 0.05$ ), 2.5% at 13 h00 ( $p < 0.05$ ), 2.6% at 15 h00 ( $p < 0.05$ ) and 0.4% at 17 h00 ( $p > 0.05$ ) in comparison to placebo (Table 1).

#### 4.2.3. Fatigue index (%)

The statistical analysis showed a significant Time-of-day main effect for fatigue index ( $F = 33.09$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.72$ ). During the placebo and the caffeine sessions, FI decreased at 07 h00 compared to

**Table 1**

Evolution of attention, reaction time and total and peak distance and the fatigue index during the 5-m shuttle run test recorded at 07 h00, 09 h00, 11 h00, 13 h00, 15 h00 and 17 h00.

		07 h00	09 h00	11 h00	13 h00	15 h00	17 h00
Attention (number of correct response)	Placebo	66.13 ± 2.89	68.43 ± 2.98 <sup>a</sup>	71.48 ± 3.52 <sup>a,b</sup>	65.88 ± 2.94 <sup>b,c</sup>	68.55 ± 2.99 <sup>a,c,d</sup>	71.45 ± 3.56 <sup>a,b,d,e</sup>
	Caffeine	68.07 ± 2.74	69.43 ± 2.93 <sup>a</sup>	71.82 ± 3.31 <sup>a,b</sup>	68.10 ± 2.66 <sup>b,c</sup>	69.45 ± 2.96 <sup>a,c,d</sup>	71.50 ± 3.40 <sup>a,b,d,e</sup>
	Δ (%)	2.9 <sup>f</sup>	1.5 <sup>f</sup>	0.5 <sup>f</sup>	3.4 <sup>f</sup>	1.31 <sup>f</sup>	0.1
Reaction time (s)	Placebo	0.41 ± 0.02	0.39 ± 0.02 <sup>a</sup>	0.37 ± 0.02 <sup>a,b</sup>	0.41 ± 0.02 <sup>b,c</sup>	0.39 ± 0.02 <sup>a,c,d</sup>	0.37 ± 0.03 <sup>a,b,d,e</sup>
	Caffeine	0.38 ± 0.02	0.37 ± 0.02 <sup>a</sup>	0.36 ± 0.02 <sup>a,b</sup>	0.38 ± 0.02 <sup>b,c</sup>	0.37 ± 0.02 <sup>a,c,d</sup>	0.35 ± 0.03 <sup>a,b,d,e</sup>
	Δ (%)	6.4 <sup>f</sup>	4.1 <sup>f</sup>	3.4 <sup>f</sup>	6.0 <sup>f</sup>	3.8 <sup>f</sup>	3.8 <sup>f</sup>
Total distance (m)	Placebo	698.14 ± 45.39	706.29 ± 43.99 <sup>a</sup>	716.50 ± 41.98 <sup>a,b</sup>	719.79 ± 42.22 <sup>a,b,c</sup>	723.21 ± 43.72 <sup>a,b,c,d</sup>	730.00 ± 43.92 <sup>a,b,c,d,e</sup>
	Caffeine	709.21 ± 43.78	712.86 ± 44.54 <sup>a</sup>	717.00 ± 42.08 <sup>a,b</sup>	723.71 ± 41.32 <sup>a,b,c</sup>	730.71 ± 41.13 <sup>a,b,c,d</sup>	733.93 ± 43.08 <sup>a,b,c,d,e</sup>
	Δ (%)	1.6 <sup>f</sup>	0.9 <sup>f</sup>	0.1	0.5 <sup>f</sup>	1.0 <sup>f</sup>	0.5 <sup>f</sup>
Peak distance (m)	Placebo	129.36 ± 7.85	135.00 ± 8.95 <sup>a</sup>	139.07 ± 9.64 <sup>a,b</sup>	138.29 ± 9.51 <sup>a,b</sup>	142.00 ± 9.96 <sup>a,b,c,d</sup>	146.36 ± 9.39 <sup>a,b,c,d,e</sup>
	Caffeine	134.64 ± 8.01	138.14 ± 8.83 <sup>a</sup>	139.64 ± 9.56 <sup>a,b</sup>	141.79 ± 9.52 <sup>a,b,c</sup>	145.71 ± 9.57 <sup>a,b,c,d</sup>	146.93 ± 9.72 <sup>a,b,c,d</sup>
	Δ (%)	4.1 <sup>f</sup>	2.3 <sup>f</sup>	0.4	2.5 <sup>f</sup>	2.6 <sup>f</sup>	0.4
Fatigue index (%)	Placebo	6.05 ± 3.10	8.74 ± 2.77 <sup>a</sup>	10.00 ± 3.05 <sup>a</sup>	11.07 ± 3.71 <sup>a,b</sup>	11.68 ± 3.45 <sup>a,b,c</sup>	16.00 ± 2.84 <sup>a,b,c,d,e</sup>
	Caffeine	5.52 ± 2.90	8.45 ± 2.42 <sup>a</sup>	8.54 ± 2.50 <sup>a</sup>	11.06 ± 3.61 <sup>a,c</sup>	11.97 ± 3.69 <sup>a,b,c</sup>	13.72 ± 2.87 <sup>a,b,c,d</sup>
	Δ (%)	8.7	3.2	14.6 <sup>f</sup>	0.01	2.5	14.3 <sup>f</sup>

<sup>a</sup> Significant difference compared to 07 h00.

<sup>b</sup> Significant difference compared to 09 h00.

<sup>c</sup> Significant difference compared to 11 h00.

<sup>d</sup> Significant difference compared to 13 h00.

<sup>e</sup> Significant difference compared to 15 h00.

<sup>f</sup> Significant difference between placebo and caffeine.

09 h00, 11 h00, 13 h00, 15 h00 and 17 h00, at 09 h00 compared to 13 h00, 15 h00 and 17 h00, at 11 h00 compared to 13 h00 (*i.e.*, only for the placebo session) 15 h00 and 17 h00, at 13 h00 compared to 17 h00 and at 15 h00 compared to 17 h00 ( $p < 0.05$ ). The lowest values of FI were observed at 07 h00 ( $6.05 \pm 3.10\%$  and  $5.52 \pm 2.90\%$  during the placebo and caffeine test sessions respectively) and the highest values were observed at 17 h00 ( $16.00 \pm 2.84\%$  and  $13.72 \pm 2.87\%$  during the placebo and caffeine test sessions respectively). The difference between the highest and lowest value was 62.2% and 59.7% during the placebo and caffeine test sessions respectively.

A significant main effect of Caffeine ( $F = 7.32$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.36$ ) and a significant interaction Time-of-day  $\times$  Caffeine ( $F = 6.43$ ;  $p < 0.05$ ;  $\eta_p^2 = 0.33$ ) were reported. After caffeine ingestion, FI was reduced by 8.7% at 07 h00 ( $p > 0.05$ ), 3.2% at 09 h00 ( $p > 0.05$ ), 14.6% at 11 h00 ( $p < 0.05$ ), 0.01% at 13 h00 ( $p > 0.05$ ), and 14.3% at 17 h00 ( $p < 0.05$ ) and increased by 2.5% at 15 h00 ( $p < 0.05$ ) (Table 1).

## 5. Discussion

The present study was designed to explore the effects of caffeine ingestion on the diurnal fluctuations of cognitive and physical (*i.e.*, during the 5-m multiple shuttles run test) performances. The main findings were that cognitive and physical performances are time-of-day dependent with better performances observed during the late morning (*i.e.*, 11 h00) and afternoon (*i.e.*, 17 h00) for attention and reaction time and during the end of the afternoon (*i.e.*, 17 h00 h) for the 5-m multiple shuttles run test performance (*i.e.*, total distance, peak distance). The fatigue index was higher during the end of the afternoon (*i.e.*, 17 h00 h). Also, the data demonstrated that caffeine improved both cognitive and physical performances during the day; but this improvement was better when cognitive or physical performances were at their lowest values during the day (*i.e.*, the early morning and the early afternoon for RT and attention and the early morning for the 5-m multiple shuttles run test).

Concerning cognitive function, the findings of the present study are in agreement with those of Valdez et al. (2014) who reported that the higher values of attention and reaction time were recorded from 10 h00 to 14 h00 and from 16 h00 to 22 h00 and these values decreased from 14 h00 to 16 h00 and after 22 h00. Cognitive performances are at a low level early in the morning and immediately after lunch (Valdez et al.,

2014). In addition, as previously reported, sleep inertia and post lunch dip occurs at these times, which also contributes to the low level in cognitive performances (Wright et al., 2012).

The present results involving the 5-m multiple shuttle run test at different time-of-day showed that total distance, peak distance and fatigue index were higher at 17 h00 compared to the other time-of-day points. In this context, previous reports showed significant diurnal fluctuations in short-term maximal performances with higher values typically achieved at the end of the afternoon (Reilly and Waterhouse, 2009; Chtourou and Souissi, 2012; Gueldich et al., 2017). Also, a number of studies reported significant daily variations of RSA' performance with higher muscle power or speed in the end of the afternoon or early evening compared to the morning (Racinais et al., 2010; Hammouda et al., 2011; Aloui et al., 2013). Time-of-day effect on peak distance (*i.e.*, recorded in 30-s) shown in the present study was in agreement with previous studies on the 30-s Wingate test (Chtourou et al., 2012a, 2012b, 2013a, 2015). These authors reported that muscle power recorded during the 30-s Wingate test was lower in the morning compared to the end of the afternoon.

The result of the present study showed that cognitive and physical performances reached a peak at 17 h00. Previous studies had reported that a higher core temperature was recorded at the end of the afternoon (Nicolas et al., 2005; Souissi et al., 2012; Chtourou et al., 2013b) that may enhance metabolic reactions, increase the extensibility of connective tissue, reduce muscle viscosity and increase the conduction velocity of action potentials (Shephard, 1984). Thus, previous studies have suggested a possible link between the diurnal fluctuation of short-term maximal performance and the daily variation of core temperature (Chtourou and Souissi, 2012; Chtourou et al., 2013a; Souissi et al., 2013b). Also, it has been suggested that the diurnal variation of short-term maximal performance is linked to the daily fluctuation of attention and motivation (Reilly and Edwards, 2007).

Fatigue index during the 5-m multiple shuttles run test was higher in the end of the afternoon than the morning confirming previous results obtained during the 30-s Wingate test (Chtourou et al., 2013a), repeated muscle contractions (Nicolas et al., 2005) and RSA (Racinais et al., 2010; Hammouda et al., 2011; Zarrouk et al., 2012). The highest level of muscular fatigue recorded in the afternoon was explained by a high initial power achieved during the 30-s Wingate test in the afternoon compared to the morning (Chtourou et al., 2013b; Souissi et al., 2013b).

Compared to the placebo test session, the results of the present study indicated that cognitive and physical performances increased after the ingestion of 6 mg/kg of caffeine. Nevertheless, a significant difference between the percentages of improvement was observed. The ergogenic effect of caffeine ingestion was greater during the moments of the lowest performance and was lower during its peak value despite the significant decrease of the fatigue index for the physical task.

The increase in attention and the decrease in the reaction time after the caffeine ingestion are consistent with previous reports (Ruijter et al., 2000; Van Dongen et al., 2001). These studies have shown that caffeine eliminates psychomotor attention deficits from sleep inertia and affects the CNS by increasing vigilance, mood and attention as well as reducing the reaction time. Although, Hogervorst et al. (2008) showed no-significant effect of caffeine ingestion on the reaction time and memory. Foskett et al. (2009) confirm the findings of the present study by proving that caffeine has potential benefits on cognitive parameters and intermittent sprint activities using the same dose (*i.e.*, 6 mg/kg).

With regard to short-term maximal performance, the present study showed that the ingestion of 6 mg/kg of caffeine enhanced the peak and the total distance during the 5 m shuttle run test at all time-of-day, especially during the moments of the lower values of performance. These results are in line with previous studies that have demonstrated that caffeine ingestion induces significant increases in short-term maximal performance (Souissi et al., 2012, 2013a), RSA (Jordan et al., 2012), muscle speed and strength (Grgic and Mikulic, 2017) and muscle metabolism and time until exhaustion (Bell et al., 2001). To date, few studies have examined the effect of time-of-day of caffeine ingestion on the short-term maximal performance and they showed that caffeine ingestion was able to reduce the amplitude of the diurnal fluctuation of physical and cognitive parameters (Rodríguez et al., 2012; Souissi et al., 2013a).

The current result demonstrated a significant decrease in fatigue index after caffeine ingestion, especially during its peak values. These results agree with those of Terzi et al. (2009) and Giles et al. (2012) who reported that caffeine ingestion reduces the sensation of fatigue. But, the absence of caffeine effect on cognitive and physical performances when reaching the higher values of the day could be related to the increase of core temperature at these periods. In this context, Suvit et al. (2016) have demonstrated that caffeine ingestion has no-effect on rectal and cutaneous temperatures, mood and short-term memory when temperature values were high (*i.e.*, 42 °C).

In conclusion, the results of the present study revealed that cognitive and physical (*i.e.*, 5-m multiple shuttle run test) performance are time-of-day dependent. Indeed, RT and attention were better at 11 h00 and 17 h00 and performance during the 5-m multiple shuttle run test was better at 17 h00. Also, the data showed that 6 mg/kg of caffeine ingestion was able to enhance cognitive and physical performances at the different time-of-day, especially when performances were at their lowest values during the day (*i.e.*, 07 h00 and 13 h00 for RT and attention and 07 h00 for the 5-m multiple shuttles run test). Thus, caffeine is an effective ergogenic aid to improve both cognitive and physical performances and counteract its decline during the day. Therefore, athletes could ingest caffeine (6 mg/kg) 60 min before intense and repeated efforts especially in the morning and early afternoon in order to counteract the effect of time-of-day on cognitive and physical performances.

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## Declaration of interest

The authors report no conflicts of interest with this study or its results.

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