Caffeine improved cycling trial performance in mentally fatigued cyclists, regardless of alterations in prefrontal cortex activation

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\textbf{ABSTRACT}

\textbf{Purpose:} To verify whether caffeine (CAF) could increase the prefrontal cortex (PFC) activation and improve 20 km cycling time trial (TT\textsubscript{20km}) performance in mentally fatigued cyclists.

\textbf{Methods:} After preliminary TT\textsubscript{20km}, twelve recreational cyclists (VO\textsubscript{2MAX} of 58.9 ± 6.2 ml.kg.m\textsuperscript{-1}) performed a familiarization with a cognitive test to induce mental fatigue (MF) and psychological scales. Thereafter, they performed: 2a) a baseline TT\textsubscript{20km} 3) a mentally fatigued TT\textsubscript{20km} (MF); 4 and 5) a mentally fatigued TT\textsubscript{20km} after CAF (MF + CAF) or placebo (MF + PLA) ingestion, in a double-blind, counterbalanced design. Performance and psychological responses were obtained throughout the TT\textsubscript{20km} while PFC electroencephalography (EEG) theta wave was obtained before and after the mental fatigue test.

\textbf{Results:} The mental fatigue-induced increase in EEG theta wave (↑ - 4.8%) was reverted with CAF (↑ 8.8%) and PLA ingestion (↑ 4.8%). CAF improved TT\textsubscript{20km} performance in mentally fatigued cyclists by reducing time (p = .00; ↓ - 1.7%) and increasing W\textsubscript{MEAN} (p = .00; ↑ - 3.6%), when compared to MF + PLA. The RPE power output ratio was lower (p = .01), but affect (p = .018), motivation (p = .033) and emotional arousal (p = .001) were greater throughout the TT\textsubscript{20km} in MF + CAF than in MF + PLA.

\textbf{Conclusions:} CAF ingestion improved TT\textsubscript{20km} performance and psychological responses in mentally fatigued cyclists, despite the unaltered PFC activation.

1. Introduction

A body of literature has shown that mental fatigue impairs endurance cycling performance\cite{1,2,3}. Recently, a study by Pires et al.,\cite{3} also found that mental fatigue reduced 20 km cycling time trial (TT\textsubscript{20km}) performance by -2.7% and -6.5% when results were expressed as time and mean power output (W\textsubscript{MEAN}), respectively. The authors related such an impaired cycling performance to a decreased prefrontal cortex (PFC) activation\cite{4,5}, as interpreted by the increased electroencephalography (EEG) theta wave at the 10th and 20th km of the trial. Importantly, this altered PFC activation during the TT\textsubscript{20km} may have resulted from the high-demanding cognitive task performed prior to the cycling trial, as an increased PFC EEG theta wave was readily observed during the cognitive test\cite{3}. Although the underlying mechanisms were not fully clarified, mental fatigue may have affected the higher-order cognitive control, thereby reducing the ability to deal with attentional control, encoding and storage of relevant information\cite{6}, leading to a greater perceived cost-future reward relationship and aversive sensations during exercise\cite{7}. In this regard, as the exercise disengagement/investment is related to the mental representation of the sense of effort\cite{8}, manipulations capable of unbalancing the incentive-performance and effort-performance relationship (such as mental fatigue) may affect performance during exercise\cite{9}. Therefore, recreational mentally fatigued cyclists may have less cognitive ability to use inhibitory control and deal with aversive sensations when regulating their pace during exercise.

Cycling trials in which a specific distance has to be covered as fast as possible are a realistic scenario that resembles conditions met in cycling training and competitions routines\cite{3,10}. In this scenario, cyclists may base their pace on psychological responses such as the ratings of perceived exertion (RPE), as it has been suggested that athletes avoid premature fatigue and maximize performance by using a RPE template.
formed from previous experiences, derived from the momentary RPE in relation to the endpoint [11,12]. Consequently, mental fatigue may be a threat for a successful cycling pacing and performance regulation, as mentally fatigued cyclists may perceive a higher than expected RPE for the same power output during the trial [13]. They may have insufficient motivation to overcome exercise-derived aversive sensations, thereby impairing performance [3,14]. Hence, interventions capable to counteract negative mental fatigue effects on endurance performance may be helpful to improve performance, particularly in recreational athletes, as they may regularly experience mental fatigue due to their high-load aerobic training routines combined with a strict-life style that encompasses dietary restrictions, longer workdays, reduced time for recovery and restricted social life [2,3].

Mental fatigue is likely associated with an elevated ATP hydrolysis and increased adenosine concentrations in the central nervous system (CNS) [15,16]. This condition has been associated with an inhibited release of excitatory neurotransmitters (such as dopamine) which reduces arousal, spontaneous behavior and affect (i.e. pleasure) during exercise [16–18]. In this regard, with the potential to counteract mental fatigue effects is caffeine (CAF), as it has been suggested that CAF increases neuronal activity and excitability of the CNS by blocking neuronal A1 adenosine receptors [17,19]. For example, a neuroimaging study observed that CAF improved the tissue oxygen extraction and reduced the cerebral metabolic rate of oxygen consumption [20]. Consequently, one may hypothesize that CAF counteracts the mental fatigue-altered PFC activation, thereby improving cycling performance in mentally fatigued cyclists. In fact, a recent study [1] provided insightful results as mentally fatigued individuals increased their cycling time-to-exhaustion after CAF ingestion (when compared to a mental fatigue trial without CAF). However, neither PFC EEG measures during the high-demanding cognitive task nor psychological measures such as motivation, affect and emotional arousal during exercise were included, therefore inferences to a more realistic distance-based time trial scenario are still required [3].

The present study verified whether CAF improved PFC activation and TT20km performance in mentally fatigued cyclists. We also verified if CAF ingestion altered psychological responses to a TT20km in mentally fatigued cyclists. We hypothesized that CAF ingestion would attenuate the mental fatigue-reduced PFC activation [3], improving TT20km performance of mentally fatigued cyclists. Moreover, we expected that CAF would reduce RPE, and increase affect, motivation and emotional arousal during exercise.

2. Materials and methods

2.1. Participants

The sample size was calculated through an equation suggested elsewhere \( n = \frac{8e^2}{d^2}; n, e, \text{ and } d \text{ denote the required sample size, coefficient of variation and magnitude of the treatment, respectively} \) [21], assuming \( e = 0.11\% \) for TT20km performed by recreational cyclists [22] and a conservative \( d = 1.0\% \), thus resulting in \( n = 10 \) participants. However, considering a possible sample loss of \( -20\% \), 12 non-professional trained cyclists (means and SDs of 34.3 ± 6.2 years; 179.3 ± 5.1 cm; 77.6 ± 6.8 kg) classified as performance level 3 (means and SDs of \( \text{VO}_{2,\text{MAX}} = 58.9 \pm 6.2 \text{mL.kg.min}^{-1} \); \( W_{\text{peak}} = 367.0 \pm 32.5 \text{W} \)) according to criteria suggested elsewhere [23] volunteered to take part in this study. They had a training frequency of \( 4.7 \pm 2.3 \) sessions/week (283.7 ± 138.6 km/week) and a training experience of \( -6.5 \) years (competing at regional and national tournaments) when the study was conducted. They were non-smokers and free from cardiovascular, visual, auditory and cognitive disorders. Three of them were non-consumers (\( \approx 40 \text{mg of CAF per day} \)), five were occasional consumers (\( \approx 250 \text{mg of CAF per day} \)) and four were daily consumers (\( \approx 250 \text{mg of CAF per day} \)), according to a proposed classification [24]. Importantly, CAF has been suggested as an ergogenic aid capable of improving endurance performance, regardless of habitual caffeine consumption [25,26]. They were oriented to avoid consumption of stimulant (coffee, energy drink etc.) and alcoholic beverages, as well as intense exercise for the 48 h preceding the sessions. Experimental procedures, risks, and benefits were explained before collecting their written consent form signature. The procedures were previously approved by a local Ethics Committee (Process: 63787816.1.0000.5390) and performed according to the Declaration of Helsinki.

2.2. Study design

The design of the present study encompassed 5 sessions, as depicted in Fig. 1. Firstly, cyclists performed a TT20km during a preliminary session (visit 1) and those who completed the trial within 33 min, were eligible to participate in the study. This criterion was based on previous TT20km studies and adopted to homogenize the sample and reduce the data variability [3,27–29]. Afterwards, eligible cyclists were familiarized with a short version (\(-5\) min) of the rapid visual information processing; EEG is electroencephalography.
Caffeine improved cycling trial performance in mentally fatigued cyclists, regardless of alterations in prefrontal cortex activation [3]. Hence, rather than assuming it, we confirmed that mental fatigue impaired TT20km performance (a proof-of-concept) by comparing session 2 vs session 3 (baseline TT20km vs MF TT20km). Consequently, sessions 4 and 5 were designed to investigate if CAF may improve TT20km performance in mentally fatigued cyclists, so that MF + CAF and MF + PLA trials were performed in a double-blinded, counterbalanced order. The study was finished within 30 days, the sessions were interspersed by a 3–7 days washout period, performed at the same time of the day, under controlled temperature (−24°C) and humidity (50–60%). Psychological responses such as RPE, motivation, emotional arousal and affect were measured every 2 km through the TT20km, while EEG, motivation, emotional arousal, and mental fatigue sensation were also obtained before and after the RVIP test. After the study conclusion, cyclists performed a maximal graded test (25 W min⁻¹ increments until voluntary exhaustion) in order to obtain their VO₂MAX and peak power output (WPEAK).

2.3. Mental fatigue protocol

The RVIP test was performed in a silent and illuminated room [30]. Cyclists sat comfortably on a chair, frontally to a 17 in. colored monitor, and wore an earphone damper to avoid noise distractions. The RVIP test consisted of a 40 min high-demanding cognitive task, which randomly displayed single numbers (numbers from 1 to 9 being displayed isolated) in a white box in the center of the monitor, in a rate of 100 numbers per minute (one number per 600 milliseconds). They were asked to press the space bar of a standard keyboard every time they identified a sequence of three even (e.g., 2, 4, 6, 4, 6, 8 etc.) or odd numbers (e.g., 3, 5, 7, 3, 9, 7 etc.), shown eight times a minute. Cognitive performance was measured as false alarms (expressed as arbitrary units; a.u.), reaction time (s) and percentage of accuracy answers (i.e. correct numerical sequences %).

2.4. Caffeine and placebo ingestion

We followed the recommendations of the International Society of Sports Nutrition (ISSN) position for CAF ingestion [24]. Briefly, it has been suggested that 3 to 6 mg kg⁻¹ of body mass of CAF significantly improve endurance performance in trained athletes approximately 1 h post-ingestion [24]. In order to accomplish this recommendation, participants ingested 5 mg kg⁻¹ of body mass of CAF or PLA immediately before the RVIP test (−50 min before the cycling TT20km commence-ment). The CAF and PLA capsules were formulated to have the same appearance (i.e. form, size and color) and contain the same taste and smell, thereby ensuring that cyclists could not identify differences between them. CAF was manipulated as previously reported in a mental fatigue-caffeine study [1]. In contrast, PLA was manipulated in cellulose capsules containing inert substances such as a lubricant, magnesium stearate, and magnesium silicate. Importantly, neither participants nor researchers appointed to the data collection were aware of the intervention. Likewise, researchers appointed to data analysis were blinded to manipulations, thus characterizing the present study as a truly double-blind study.

2.5. Cycling time trial (TT20km)

Cyclists performed the baseline, MF, MF + CAF and MF + PLA TT20km, having only distance as available feedback, that is they were blinded to feedback such as time, cadence, speed, power output, and heart rate. Cyclists used a road bicycle (Giant*, New York, USA) attached to a cycle simulator (Computrainer, Racer Mate* 8000, Seattle, USA) that provided power output (W), cadence (rpm) and speed (km h⁻¹) data throughout the trials. The device was calibrated before each test according to the manufacturer's instructions. The bike was individually adjusted according to cyclists' preferences and they were allowed to drink water ad libitum during the trials. The time to complete the TT20km and the W̄_MEAN recorded throughout the trial were used as performance measures. Furthermore, power output data were averaged every 2 km to analyze pacing strategy.

3. Measures and instruments

3.1. Electroencephalography (EEG)

Previous EEG studies have suggested that EEG theta wave is a slow frequency EEG band sensitive to distinguish a mental fatigue state [3-5]. Additionally, theta rather than alpha wave may be a reliable distinguisher of changes in cognitive processing as mental fatigue progresses, as frontal cortex EEG theta wave is correlated with the percentage of accuracy answers (i.e. error rate) during high-demanding cognitive tasks [5]. Hence, PFC activation was continuously obtained through an EEG unit (Emsa*, EEG BNT 36, TiEEG, Brazil) at the Fp1 position, according to the international EEG 10–20 system [31]. This position was ensured according to frontal and sagittal planes, referred to mastoid. The EEG was recorded at a 600 Hz sampling frequency, through active electrodes (Ag-AgCl) with resistance ~5 KΩ. After exfoliation and cleaning, electrodes were fixed with a conductive gel, adhesive tape, and medical strips. The EEG signal was recorded during 3 min rest, immediately before and after the RVIP test, when participants were completely calm, maintaining their eyes closed and avoiding head and trunk movements.

The EEG signal was amplified (gain of 1 × 10⁵) and filtered with a digital notch (60 Hz), thereafter a 1–30 Hz bandpass filter was applied. EEG signal showing spectral leakage, defined as a signal ≥100 μV, were considered as artifacts (n = 2–2, depending on the moment of the experimental setup) and were excluded from the analysis [31]. Furthermore, data recorded during the first and last 30s of a 180 s time window were removed to avoid eventual noise associated with the participants' movements when expecting the EEG record start and stop. Thereafter, EEG data were analyzed in frequency domains through a fast-Fourier transformation so that the total power spectral density (PSD) of the theta wave (3–7 Hz) was calculated over the most steady (i.e. lowest SD) 30 s window (determined through an algorithm implemented in Matlab® environment).

3.2. Psychological responses

Responses of mental fatigue sensation, emotional arousal and motivation were obtained before and immediately after the RVIP test. Briefly, the mental fatigue sensation was rated through a 100 mm visual analogue scale (VAS), then cyclists were required to answer "How mentally fatigued you feel now?" having 0 (zero) as "none at all" and 100 as "maximal" mental fatigue, as reported elsewhere [32]. The emotional arousal was assessed through a 6-points felt arousal scale (FAS) that ranks the emotional arousal within categories ranging from "low activation" to "high activation" [33]. The perception of high emotional arousal may be interpreted as a state of "worked-up" whilst perception of low emotional arousal, as a state of "relaxation". Moreover, motivation was assessed through a 10 points Likert scale having two opposite motivational descriptors, that is 0 (zero) as "not all
motivated” and 10 as “extremely motivated” [34,35]. These responses, expressed as arbitrary units (a.u.), were compared between pre and post RVIP test.

Furthermore, emotional arousal, motivation, affect and RPE responses were obtained every 2 km of the TT\textsubscript{20km}. Affect responses (pleasure/displeasure) were obtained by using the 11-points feeling scale (FS), as suggested elsewhere [36]. This single-item bipolar scale (-5 to +5) uses descriptors as “neutral” (zero), “very good” (+5) and “very bad” (-5) to rate the affective valence. Furthermore, RPE was obtained through a 15-points Borg scale, as suggested elsewhere [37]. In order to verify possible mental fatigue-induced psychological alterations during exercise, motivation, FAS, FS and RPE (expressed as a.u.) were analyzed every 2 km. Given the comparable absolute RPE responses in control and mental fatigue, although the reduced power output values under mental fatigue [3], we also calculated the RPE-power output ratio (RPE\textsubscript{PO}) for every 2 km of the TT\textsubscript{20km}.

### 3.3. Statistical analysis

Gaussian distribution and homoscedasticity were previously checked through Shapiro-Wilk and Levene tests, respectively, and results were reported as mean and standard deviation (±SD).

Firstly, we checked the reliability on performance measures by comparing preliminary TT\textsubscript{20km} (session 1) and baseline TT\textsubscript{20km} (session 2), and reporting the typical error of measurement (expressed as a variation of the grand mean) and the correlation coefficient between them [21].

Secondly, as a proof-of-concept of mental fatigue effects we verified if performance in MF TT\textsubscript{20km} was impaired when compared to baseline TT\textsubscript{20km}. Therefore, time and W\textsubscript{MEAN} responses in baseline and FM TT\textsubscript{20km} were compared through a paired T-student test (session 2 vs session 3). Particularly in MF session (session 3), we also compared pre to post RVIP test alterations in EEG theta power, VAS, FAS and motivation through a paired T-student test.

Effects of CAF ingestion on mentally fatigued cyclists were assessed in different ways. Firstly, to mitigate the impact of inter-individual variability (between sessions) we expressed EEG and psychological responses (i.e. sensation of fatigue, emotional arousal, and motivation) as Δ values from pre-treatment (pre to post RVIP measures) and compared MF + CAF and MF + PLA responses through a paired T-student test. Secondly, we compared cognitive performance (i.e. false alarms, reaction time and accuracy of answers averaged during the RVIP test) between MF + CAF and MF + PLA sessions through a paired T-student test.

Furthermore, we confirmed if CAF improved TT\textsubscript{20km} performance (W\textsubscript{MEAN} and time) in mentally fatigued cyclists. Accordingly, to mitigate the impact of inter-individual variability we expressed TT\textsubscript{20km} performance as Δ values from MF TT\textsubscript{20km} and compared MF + CAF and MF + PLA through a paired T-student test. In addition, we analyzed pacing (i.e. power output) and psychological responses (i.e., RPE, RPE\textsubscript{PO}, motivation, FAS and FS) during the MF + CAF and MF + PLA through a 10 × 2 mixed model having distance (2nd, 4th up to 20th km) and condition (MF + CAF vs MF + PLA) as fixed factors, and cyclists as the random one. The AIC index (Akaike’s information criterion) determined the covariance matrix that best fitted to the dataset (Compound Symmetric, First-order Autoregressive homogeneous and heterogeneous, First-order Autoregressive Moving Average, and Toeplitz homogeneous and heterogeneous), and the Bonferroni test corrected p values in multiple comparisons.

We reported the post-hoc ES analysis (expressed as d-Cohen) to make eventual comparisons with previous studies possible [3,28], so that ES was interpreted as small (r < 0.2), moderate (0.2 to 0.6), large (0.6 to 1.2), very large (1.2 to 2.0), and extremely large (≥2.0), as suggested elsewhere [38]. Results were significant when p ≤ 0.05.

### 4. Results

As part of the study control, we checked the reliability of performance measures. There was no difference in time (p = .81; d = 0.074, small ES) and W\textsubscript{MEAN} (p = .27; d = 0.066, small ES) between preliminary (32.8 ± 1.3 min and 262.3 ± 37.5 W) and baseline (32.7 ± 1.4 min and 260.0 ± 32.0 W) sessions. The typical error of measurement and correlation between preliminary (trial 1) and baseline (trial 2) sessions were 0.8% and r = 0.94, and 3.1% and r = 0.96 for time to complete the trial and W\textsubscript{MEAN}, respectively.

#### 4.1. Proof-of-concept of mental fatigue effects

As a proof-of-concept, we verified if TT\textsubscript{20km} performance was impaired by mental fatigue, given the 0.9 ± 0.7% increase in time to complete the trial (32.7 ± 1.4 min vs 33.0 ± 1.4 min; p = .00; d = 2.41, extremely large ES) and the 2.2 ± 1.6% reduction in W\textsubscript{MEAN} (260 ± 32 W vs 254.3 ± 29.7 W; p = .00; d = 2.87, extremely large ES) in mental fatigue trial when compared to baseline. Fig. 2 depicts the percentage of alteration in time (panel A) and power output (panel B) from baseline TT\textsubscript{20km}.

Furthermore, we observed that the RVIP test changed PFC activation in MF session, as we observed a 4.8 ± 7.1% increase in EEG theta band from pre to post RVIP test (p = .03; d = 1.53, very large ES). Accordingly, cyclists rated increased mental fatigue sensation (35.0 ± 16.9 vs 73.3 ± 12.1 a.u.; p = .000; d = 3.40, extremely large ES), reduced motivation (7.6 ± 1.9 vs 6.0 ± 2.9 a.u.; p = .009; d = 3.17, extremely large ES) and lower emotional arousal (4.7 ± 1.4 vs 3.8 ± 1.5 a.u.; p = .002 d = 2.73, extremely large ES) when comparing pre to post RVIP test responses. Mentally fatigued cyclists showed a reaction time of 37.0 ± 12.4 s, false alarms of 22.4 ± 17.4 and accuracy of 41.8 ± 16.1% during the RVIP test.
4.2. Caffeine effects on EEG, psychological and cognitive performance responses in mentally fatigued cyclists

In contrast to the $-4.8 \pm 7.1\%$ increase in EEG theta wave found in MF condition, we observed a $-8.8 \pm 13.9\%$ and $-4.8 \pm 17.9\%$ reduction in EEG theta wave from pre to post RVIP test in MF + CAF and MF + PLA sessions, respectively. Importantly, the $\Delta$ alteration in PFC activation was comparable between MF + CAF and MF + PLA sessions ($p = .25; d = 0.50$, moderate ES).

Regarding the RVIP test-induced psychological alterations, mental fatigue sensation increased from pre to post RVIP test in both MF + CAF ($\Delta 65.7 \pm 105.4\%$) and MF + PLA sessions ($\Delta 114.8 \pm 113.0\%$), but the $\Delta$ alteration was significantly higher in MF + PLA than in MF + CAF ($p = .02; d = 0.70$, large ES). In contrast, there was an increase in emotional arousal in MF + CAF ($\Delta 11.4 \pm 15.8\%$) but a decrease in MF + PLA ($\Delta 18.1 \pm 24.2\%$), thus $\Delta$ alteration from pre to post RVIP test was significantly different between conditions ($p = .01; d = 1.51$, very large ES). Furthermore, motivation changed slightly from pre to post RVIP test in MF + CAF ($\Delta 4.6 \pm 15.5\%$) and MF + PLA ($\Delta 3.3 \pm 31.7\%$), therefore no significant $\Delta$ alterations were observed between conditions ($p = .67; d = 0.15$, small ES).

Comparable cognitive performance was observed between MF + CAF and MF + PLA, as $\Delta$ alterations of reaction time ($38.0 \pm 14.5s$ vs $39.8 \pm 13.8s$, respectively; $p = .39; d = 0.13$, small ES), false alarms ($19.7 \pm 18.1s$ vs $13.4 \pm 11.2s$, respectively; $p = .25; d = 0.42$, moderate ES) and accuracy ($46.4 \pm 16.1\%$ vs $46.8 \pm 17.3\%$, respectively; $p = .83; d = 0.024$, small ES) were not significantly different between conditions.

4.3. Caffeine effects on TT20km performance and pacing in mentally fatigued cyclists

Mentally fatigued cyclists significantly improved TT20km performance in CAF when compared to PLA ingestion. The $1.8 \pm 1.4\%$ improvement in time to complete the trial with CAF ingestion ($32.2 \pm 1.2\min$) was significantly greater ($p = .002; d = 2.36$, extremely large) than the $0.99 \pm 1.5\%$ improvement with PLA ingestion ($33.0 \pm 1.2\min$). Accordingly, the $4.8 \pm 4.1\%$ improvement in $W_{\text{mean}}$ in MF + CAF ($265.8 \pm 28.2\W$) was significantly greater ($p = .001; d = 2.72$, extremely large ES) than the $0.7 \pm 3.9\%$ improvement in MF + PLA session ($256.0 \pm 25.3\W$).

Cyclists adopted a similar “J-shaped” pacing profile throughout the MF + CAF and MF + PLA trials. Multiple comparisons revealed a condition ($F = 11.62, p = .005, d = 1.45$, very large ES) and a distance main effect ($F = 17.49, p = .000, d = 1.78$, very large ES) in power output, despite no condition by distance interaction effect was observed ($F = 0.28, p = .97, d = 0.23$, small ES). Fig. 3 (panels A and B) showed performance $\Delta$ values from MF TT20km while Fig. 4 depicted pacing responses.

4.4. Caffeine effects on TT20km psychological responses in mentally fatigued cyclists

Comparable results were observed in absolute RPE values, as neither a condition main effect ($F = 2.24; p = .16; d = 0.63$, very large ES) nor a condition by distance interaction effect ($F = 1.18; p = .33; d = 0.46$, large ES) was detected, despite the distance main effect in absolute RPE values ($F = 12.27, p = .000, d = 1.43$ extremely large ES). However, there was a significant condition main effect ($F = 10.32; p = .005, d = 1.37$, extremely large ES) as well as a distance main effect ($F = 4.28, p = .001, d = 0.82$, large ES) in RPEW data, as the increase in RPEW during the TT20km was lower in CAF than in PLA. However, no condition by distance interaction effect was found in RPEW ($F = 1.29, p = .278, d = 0.48$, large ES). Overall RPE responses were shown in Fig. 5 (panel A and B).

Fig. 3. Performance changes in mentally fatigued cyclists after caffeine (MF + CAF) and placebo (MF + PLA) ingestion. Data of time to complete the TT20km (panel A) and W_{\text{mean}} (panel B) were reported as mean ± SD. * indicates that time to complete TT20km ($p = .002$) and W_{\text{mean}} ($p = .001$) were significantly different.

Fig. 4. Power output responses of mentally fatigued cyclists throughout the TT20km after caffeine (MF + CAF, filled circles) and placebo (MF + PLA, open circles) ingestion. # is condition main effect ($p = .005$); * is distance main effect ($p = .000$). Data were reported as mean ± SD.

Regarding the remaining psychological responses, a condition main effect ($F = 5.72, p = .018, d = 1.02$ large ES) and a distance by condition interaction effect ($F = 2.29, p = .019, d = 0.65$ large ES) was found in affect, as cyclists reported higher affect in MF + CAF than in MF + PLA when they were spurring at the end of the trial ($p = .000$). However, no distance main effect was detected in affective valence ($F = 1.47, p = .169, d = 0.52$ moderate ES). In contrast, neither a distance main effect ($F = 0.45, p = .90, d = 0.29$ moderate ES) nor a distance by condition interaction effect ($F = 0.87, p = .55, d = 0.40$ moderate ES) was observed in motivation. Nevertheless, a condition main effect ($F = 4.61, p = .033, d = 0.92$, large ES) was observed so that motivation was greater in mentally fatigued cyclists after in MF + CAF trial. Accordingly, although neither a distance main effect ($F = 0.78, p = .64, d = 0.38$ moderate ES) nor a distance by condition interaction effect ($F = 0.88, p = .54, d = 0.40$ moderate ES) was found in emotional arousal. However, mentally fatigued cyclists rated higher...
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arousal throughout the TT_{20km} in CAF than PLA (F = 11.03, p = .001, d = 1.42 very large ES) (Fig. 6).

5. Discussion

The present study was designed to investigate if CAF ingestion may revert mental fatigue effects on PFC activation, thus improving cycling time trial performance in mentally fatigued recreational cyclists. Results showed that CAF reverted the mental fatigue-reduced TT_{20km} performance, despite the comparable CAF and PLA effects on PFC activation. Additionally, CAF reduced RPE and changed other psychological responses throughout the trial. Then, results suggest that CAF is capable to revert the mental fatigue-reduced cycling time trial performance, but challenged its role in cortical activation.

5.1. Proof-of-concept of mental fatigue effects on cycling performance

Most studies have shown that mental fatigue impairs endurance performance, but only showed that mental fatigue impaired TT_{20km} performance [3]. This study suggested that the reduced TT_{20km} performance was possibly related to a mental fatigue-reduced PFC activation. Hence, as a proof-of-concept, firstly we confirmed that mental fatigue affected PFC activation and TT_{20km} performance. We found a change in PFC activation after the RVIP test, indicated by the increased slow-frequency EEG band suggested to distinguish mental fatigue states [3–5]. Moreover, cyclists rated a higher fatigue sensation and lower motivation and emotional arousal after this high-demanding cognitive task. Accordingly, when comparing baseline and MF trials we observed that mental fatigue reduced cycling performance outcomes after the reliability measures have evidenced that performance was steady (i.e. no learning or training effects from preliminary to baseline trial). Thus, together with others [3], this part of the present study reinforced the notion of a likely connection between PFC activation and impaired TT_{20km} performance. Briefly, it has been proposed that TT_{20km} is a self-paced exercise that requires superior inhibitory control and ability to deal with aversive sensations [2,9], and that PFC is involved in proactive, goal-directed behavior [3,26,39,40]. Therefore, although we have not measured PFC activation during TT_{20km} we found an altered PFC activation readily after the RVIP test, showing that PFC activation may have played a role on TT_{20km} performance in mentally fatigued cyclists [3].
5.2. Caffeine effects on high-demanding cognitive task responses

Although the mechanism underlying mental fatigue effects is not fully understood, the reduced PFC activation could be a result of an enhanced cerebral ATP hydrolysis-derived adenosine concentration during cognitive overload [15,16]. Then, we had also hypothesized that CAF ingestion may counteract mental fatigue effects as CAF blocks neuronal A1 adenosine receptors and improves the neuronal activity and excitability of the CNS [17,19]. However, we observed that both CAF and PLA similarly increased the PFC activation when expressed as pre-to-post RVIP changes. Accordingly, a recent study also reported similar cortical changes to CAF and CAF-perceived PLA ingestion, thereby challenging the effects of CAF ingestion on cortical activation [29]. It has been proposed that the expectation of receiving a given substance (such as CAF) during a PLA ingestion may induce cortical changes in the direction of the active substance [29,41]. Thus, perhaps the cyclists may have experienced some PLA effects as reported elsewhere [29], although we have used a true double-blind design in the present study. Therefore, although knowing that they had 50% chance of ingesting CAF or PLA in each experimental session, the uncertainty about the substance ingested may have led them to expect some CAF effects. However, PLA effects in laboratory settings have been poorly understood and require future studies.

Interestingly, both CAF and PLA also improved cognitive performance responses to the RVIP test. However, CAF attenuated the mental fatigue-induced negative sensations rather than PLA, as indicated by the lower fatigue sensation and higher emotional arousal in CAF than in PLA after the RVIP test. Somehow, the cycling performance in MF + CAF trial may have benefited from an alleviated mental fatigue-induced negative sensations before starting the TT20km.

5.3. Caffeine effects on TT20km performance and psychological responses in mentally fatigued cyclists

Actually, regardless of a “J-shaped” pacing strategy adopted in all experimental sessions, CAF ingestion improved TT20km performance expressed as time and W\text{\textsubscript{MAX}} when compared to PLA. Interestingly, mentally fatigued cyclists showed an “improved psychological state” after CAF ingestion, given the reduced RPE\textsubscript{max} ratio, and increased affect, motivation and emotional arousal during TT20km.

It has been proposed that a successful distance-based cycling trial performance such as a TT20km, is related to the cognitive ability to preserve inhibitory control while dealing with aversive sensations [2,9], because cyclists would be required to adequately evaluate the perceived cost-future reward relationship during exercise in order to maximize their pace and complete the trial as fast as possible. Consequently, mental fatigue is considered as a threat to a successful TT20km performance. When compared to PLA, CAF reduced the mental fatigue-negative sensations after the RVIP test. Likewise, mentally fatigued cyclists completed the TT20km reporting lower RPE\textsubscript{max}, higher affective valence, motivation and emotional arousal after CAF ingestion. Perhaps, CAF prevented cyclists from the RVIP test-induced cognitive depletion before the trial, thereby allowing them to complete the TT20km under an “improved psychological state” when compared to PLA [2]. Somehow, these improved psychological responses are likely associated with an improved cycling performance as reported elsewhere [1].

5.4. Methodological aspects and practical implications

CAF has been suggested as a powerful aid in improving endurance performance regardless of habitual caffeine consumption [25], mainly through its action on the CNS [1,19,42]. It should be pointed out that peripheral effects such as an increased glycolytic flux, mitochondrial oxidation rate and lipid oxidation-induced muscle glycogen sparing [42] have been reported in millimolar doses (supra-physiological) of CAF [19,43]. Therefore, considering that we used oral doses ~100 times lower than millimolar dosage [43], it is unlikely that mentally fatigued cyclists have improved cycling performance due to a peripheral CAF action [44].

Recently, a study showed that CAF reverted negative mental fatigue effects on cycling time-to-exhaustion performance [1] while another verified that mental fatigue impaired TT20km performance [3]. Thus, we combined both hypotheses, as cyclists may experience mental fatigue and use supplements as CAF in training and competitions. In fact, a study by Stewart et al., [45] verified that cyclists committed to the sport may perceive pressure to use supplements to improve performance. In contrast, cyclists may experience mental fatigue due to the high-load aerobic training routines combined with a strict-life style in daily activities [2,3]. Thus, our results have practical implications as we verified that CAF counteracted the mental fatigue-reduced performance during a cycling trial that resembles the conditions met in cycling competitions and training sessions [10,27].

In order to potentiate our manipulation we administered CAF readily before the RVIP test. Because the oral CAF ingestion has a ~45-60 min time course [24] cyclists had to ingest CAF immediately before the 40 min RVIP test, as the ingestion after the RVIP test could have missed some mental fatigue effects. Despite most effects likely occurring from 45 to 60 min after CAF ingestion, we cannot ensure that cyclists did not experience some CAF effects during the RVIP. Actually, cyclists reported attenuated psychological changes readily after the RVIP test when they ingested CAF. Future studies may verify if other soluble central-action compounds or fasting CAF (instead of ingesting) may also counteract mental fatigue effects on performance.

6. Conclusions

The present study showed that CAF improved TT20km performance in mentally fatigued cyclists, regardless of alterations in PFC activation. Furthermore, CAF ingestion attenuated the mental fatigue-induced negative sensations, thus reducing RPE and increasing affect and emotional arousal during the cycling trial.

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