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CAFFEINE SUPPLEMENTATION CAN MAKE RUNNERS RUN FURTHER AND IMPROVE PACE STRATEGY

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ABSTRACT

Caffeine can stimulate the central nervous system and thus increase exercise tolerance throughout rating perceived exertion (RPE) changes and it can change the pace strategy (PS) during a physical task. The aim of this study was to check if caffeine supplementation might promote changes in PS during a laboratory run test (LRT). In a double-blind, crossover, randomized and counterbalanced fashion, fifteen healthy-male (age: 24 ± 4.4 years; VO_2max. 53 ± 5 ml.Kg^{-1}.min^{-1}) ingested 6 mg•kg^{-1} of CAF or placebo supplementation, 60 minutes before the LRT. The LRT was three-minutes sets (at fixed speed, 1 km/h above Onset Blood Lactate Accumulation) until volitional exhaustion; Rest Time Interval (RTI) between sets were chosen by the participants in the first test (ranging from 30 to 60s). RPE, Heart Rate (HR) and blood plasma lactate concentration ([La]p) were collected at rest, immediately after each set and at the end of test. Time to exhaustion was higher for CAF (p= 0.014). RTI between sets was significantly lower in caffeine (p= 0.048) and this decreased significantly the time to perform a same distance (p= 0.034). Overall HR and [La]p was similar for both conditions (p= 0.252, p= 0.129, respectively). Despite similar overall RPE throughout test (p= 0.380), in caffeine, there was not a RPE abrupt increase similar to placebo. Caffeine supplementation can positively influence running PS (to decrease the RTI required for recovery between moments of high-intensity exercise), as well as, can make an individual run further (in the same event).

Key words: Time to Exhaustion. Pace Strategy. Ergogenic Aid. Rating Perceived Exertion.

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RESUMO

A suplementação de cafeína pode fazer corredores correrem mais e melhorar estratégia de ritmo

A cafeína pode estimular o sistema nervoso central e aumentar a tolerância ao exercício por meio de mudanças na percepção subjetiva de esforço (PSE), isso pode mudar a estratégia de ritmo (ER) durante uma corrida. Nosso objetivo foi verificar se a suplementação de cafeína promove mudanças na ER durante um teste de corrida de laboratório (TCL). De modo duplo-cego, cruzado, randomizado e contrabalançado, quinze homens saudáveis (idade: 24 ± 4,4 anos, VO_2max. 53 ± 5 ml.Kg^{-1}.min^{-1}) ingeriram 6 mg/kg^{-1} de cafeína ou placebo, 60 minutos antes do TCL. O TCL consistiu de séries de três minutos (velocidade fixada à 1 km/h acima do "*Onset Blood Lactate Accumulation*") até fadiga volicional; O Intervalo de Tempo de Descanso (ITD) entre as séries foi escolhido pelos participantes no primeiro teste (entre 30 a 60s). PSE, frequência cardíaca (FC) e lactato ([La]p) foram coletados em repouso, imediatamente após cada série e ao final do teste. O tempo de exaustão foi maior na situação cafeína (p= 0,014); o ITD foi significativamente menor na situação cafeína (p= 0,048), isso fez diminuir significativamente o tempo para realizar uma mesma distância (p= 0,034). A FC e [La]p foram semelhantes para ambas as condições (p= 0,252, p= 0,129, respectivamente). Apesar da semelhança da PSE ao longo do teste (p= 0,380), na situação cafeína não houve um aumento abrupto ao longo do teste como ocorreu na situação placebo. A suplementação de cafeína pode influenciar positivamente a ER durante uma corrida, além de aumentar a capacidade de correr mais (no mesmo evento).

Palavras-chave: Tempo de Exaustão. Estratégia de Ritmo. Auxílio Ergogênico. Percepção Subjetiva de Esforço.

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INTRODUCTION

Intermittent running appears to be an effective method to train athletes for high metabolic and psychological stress condition like a long-distance race, and to delay the exhaustion (Penteado and collaborators, 2014).

With this method, the athlete seems to be able to run a longer distance once the rest time interval (RTI) component (the moment between the high-intensity series) modulates the rate of perceived exertion (RPE).

In other words, RTI between high-intensity series prevents RPE from reaching critical values early, i.e., restores the ability of the central nervous system (CNS) to stimulate muscle work, thus keeping the athlete practicing physical exercise (Kay and collaborators, 2001; Marcora and collaborators, 2008; Minett e Duffield, 2014).

The intermittent running simulated by Penteado and collaborators (2014) indicates what really happens in real-life running condition (i.e., in endurance races of the time trial (TT) type with the use of Pacing Strategy) (Millet, 2011) and provides valuable information for situations of this kind: running a longer distance at a predetermined time (e.g., 1 h, 24 h or 6-days run).

In real life sports, athletes do not run until exhaustion. For example, in endurance events such as a 42 Km or 6 h-run, what happens is a TT condition (running with a predetermined distance or time, where the winner will be the person who does it in less time or greater distance, respectively).

In this type of event, to avoid exhaustion, before the finish line, the athlete adopts a pace strategy, alternating strong and weak intensity moments, and not running at the same intensity (De Koning and collaborators, 2011; Millet, 2011).

The pace strategy is used by athletes to regulate consciously (Millet, 2011), or even unconsciously (Marino, 2014), the fatigue state avoiding exhaustion which would lead to the abandon of the activity.

The recovery of voluntary muscle activation trough CNS pathway on RTI, preceding high intensity physical activity (PA), is considered a key variable for the performance maintenance (i.e., to control fatigue), even in an unfavorable metabolic environment with high demand of the cardiovascular system and production of lactic acid (Kay and collaborators, 2001; Minett e Duffield, 2014).

One of the most successful nutritional strategies to prevent fatigue and increase performance is caffeine supplementation. Caffeine can stimulate the CNS (Burdan, 2015; Fernández-Dueñas and collaborators, 2014; Huang and collaborators, 2005; Ledent and collaborators, 1997; Meeusen, 2014; Spriet, 2014; Yang and collaborators, 2009) and improve athletic performance (Spriet, 2014).

It has been demonstrated in laboratory that individuals supplemented with caffeine show high voluntary activation of skeletal muscles (Warren and collaborators, 2010), decreased RPE for the same effort intensity, (De Morree and collaborators, 2014) increased Time to Exhaustion (TTE) (Doherty e Smith, 2005), improved TT (Ganio and collaborators, 2009), and higher pleasure throughout the PA (Astorino and collaborators, 2012).

Moreover, caffeine is beneficial in intermittent high intensity PA, being these effects credited to the stimulation of the CNS, and thereby, increasing performance (Spriet, 2014).

The effects of both caffeine (Spriet, 2014) and RTI between PA bouts (Marino, 2014; Millet, 2011) are related to the improvement and recovery, respectively, of CNS work capacity. However, in real-life, running and RTI between high-intensity PA bouts are not leak-proof, but are in accordance with the capability of regulating the running pace of each athlete, that is, according to the CNS's recovery ability to stimulate muscle work (Marino, 2014).

Therefore, it is appropriate to speculate that caffeine, being a CNS stimulant, could influence the RTI in an intermittent running, i.e., changing the athlete's pacing strategy.

Thus, the aim of this study is to verify if caffeine supplementation affects pace strategy (i.e., RTI) during a laboratory test run.

Additionally, the changes in metabolic ([La]p) and central (RPE and HR) values will also be analyzed.

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MATERIALS AND METHODS

Participants

We tested fifteen young adults Physical Education students, with large experience in running on treadmill, aged 25 ± SD 7 years, weight 75.3 ± 3.6 kg, height 176.1 ± 4.0 cm, VO2max 53 ± 5 ml.Kg-1.min-1, body fat 9 ± 2 % (assessed by a Lange skinfold calipers, according to Jackson and Pollock) (Jackson e Pollock, 1978), which did not use any ergogenic supplements in the preceding 6-months of the test.

All participants were recreationally active, practicing street racing and soccer. Participant recruitment and data collection was performed between July 2014 and October 2015 at the Laboratório do Estudo do Movimento Humano, Barueri, São Paulo, Brazil (at Mackenzie Presbyterian University) (Fig 1).

Participants were instructed to be at least 48-hours without practicing intense PA before the test; they were also instructed to not use any kind of food or substances that might interfere with performance (throughout CNS and peripheral physiological or biochemical reactions, such as alcohol or caffeine) for at least 24-hours before the test.

Additionally, 12-hours before the test, participants followed a standardized high-carbohydrate diet (~ 70% kcal), low fiber, and fat (~ 15% kcal) which was mimicked in the crossover trial. All participants were coffee drinkers, on average, three daily cups (i.e., ~ 180 mg of caffeine daily).

Ethics: All procedures were approved by São Judas Tadeu University Ethical Committee under the protocol number: CEP/USJT nº 309.368. Participants signed an informed consent form and were alerted that even that, they were free to abandon the protocol if desired. This study was registered at ClinicalTrials.gov (Identifier: RBR-5x8ttb); the study was not registered prior to enrolment of participants as the institution's ethical committee did not require this as it was not deemed a clinical trial under its descriptors.

Experimental approach

In two moments (separated by 7 days) participants underwent a caffeine or placebo supplementation experiment (followed by a physical exertion test). The experiment was a cross-over trial (where participants took control of themselves), counterbalanced (where half of participants would begin with the placebo test and the other half would start with the caffeine supplement), randomized and double-blind design (Fig 1).

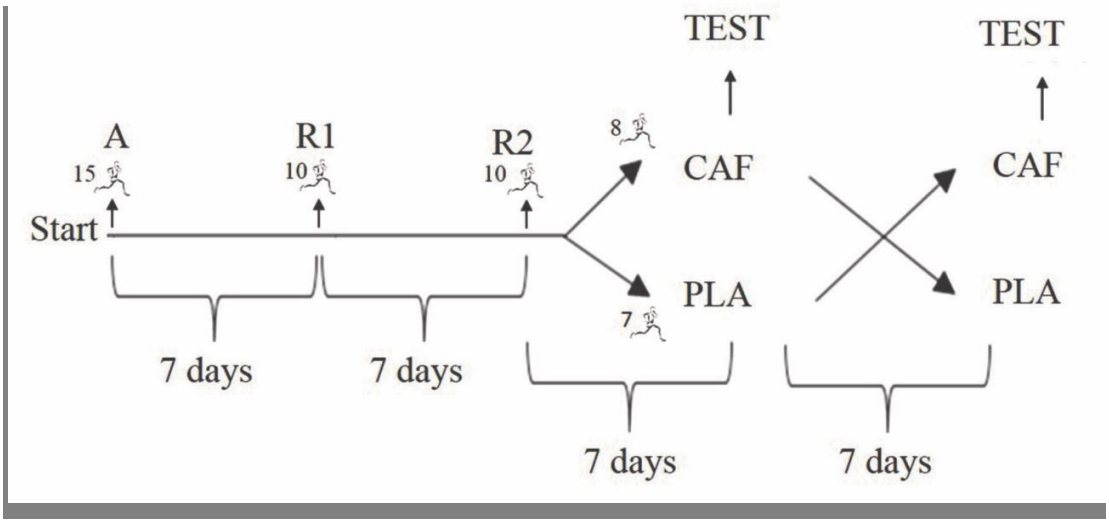

Figure 1 - Study design. A, assessment (anthropometric and Onset Blood Lactate Accumulation assessment); R1, reproducibility test (day 1); R2, reproducibility test (day 2); CAF, caffeine condition; PLA; placebo condition.

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Supplementation: Caffeine and resistant starch (placebo) supplements for this study were provided from Orion Labs Pharmacy (São Paulo, Brazil). The supplements were allocated in three gelatin capsules containing 6 mg·kg−1 of caffeine or resistant starch, both with the same color, taste and smell.

As all participants were chronic coffee drinkers and caffeine users (estimated in ~ 180 mg of caffeine daily), we choose a high, but safe dose of 6 mg·kg−1) (Goldstein and collaborators, 2010). Participants ingested the supplementation 60 minutes before the Laboratory Test Run (considered sufficient time to achieve a peak plasma level of caffeine) (Teekachunhatean and collaborators, 2013).

The tests were performed in double-blind fashion (the supplement was blinded with labels of A or B, by one of the researchers not involved in the data collection procedures), randomized and counterbalanced (at site www.randomization.com).

Participants came to laboratory on five different occasions, always in the afternoon, at the same time of the day and at the same day of week. Temperature was maintained in 23 ± 3 °C and humidity in 70 ± 5 %.

Briefly, the first visit was to present the study design, to sign the informed consent form, carry out anthropometric assessment followed by VO_2 max estimation and Onset Blood Lactate Accumulation (OBLA) (Denadai and collaborators, 2005) for the individualized prescription of the physical test intensity, in a motorized treadmill (detailed description of the VO2max estimation OBLA determination are below).

In the second and the third visit we carried out a reproducibility test for the physical effort test (a Laboratory Test Run) with ten participants, who were also experiment volunteers with caffeine supplementation (the overall result of the reproducibility test is available elsewhere) (De França and collaborators, 2016).

The last two visits were to carry out the laboratory test run condition with caffeine (CAF) or placebo (PLA) supplementation. From the first to the fifth visit the follow-up lasted ~ 35 days (Fig 1).

Determination of OBLA and VO2max estimation: This protocol is an adaptation of Denadai and collaborators (2005).

Participants completed a standard incremental treadmill test (Aegean 6200, Porto Alegre, RS) until volitional exhaustion.

The initial running speed was set in 8 km·h-1, with the treadmill grade set at 1%. Participants completed submaximal stages of 3-minute duration, with running speed increased by 1 km·h-1 between stages. At the end of each stage, participants supported their weight with their hands and moved their feet to the sides of treadmill belt.

A right-hand finger capillary blood sample (100 µl) was taken within 20–30 seconds, after which participants recommenced running. Blood lactate concentration was analyzed by an automated analyzer (YSI 1500 Sport Lactate Analyzer ™).

Heart rate (HR) was recorded during the whole test (Polar S810i). OBLA was determined according to Cheng and collaborators (1992). VO2max was determined according to ACSM (Heyward e Gibson, 2014) metabolic equation (for estimating gross VO2 at running).

Laboratory test run: The test was performed in the same motorized treadmill (with 1% inclination) where we analyzed the OBLA.

The protocol details were described elsewhere (De França and collaborators, 2016). Briefly, after a warm up, participants performed an intermittent run until volitional exhaustion, with series of 3-minute exercises bouts, interspersed with passive RTI, determined by the subject in the first test (detailed description of the RTI determination is below). To induce a higher rate of metabolic and mental stress during the test (Grassi and collaborators, 2015) the intensity of the run has been determined (by interpolation) in 1 km/h (fixed speed) above the speed at which the OBLA has been identified. During all tests, participants were encouraged by strong verbal stimulus (stimulation) to realize their maximum performance and they were informed of the exercise bout remaining. The water was consumed ad libitum throughout protocol (during RTI).

The rest time interval (RTI): The RTI protocol details and its reproducibility data were provided elsewhere (De França and collaborators, 2016). This variable has been created to test the hypothesis that caffeine could influence recovery length between

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exercise bouts and then influence the pace strategy.

For this, before the first test, participants were informed that in the first test they could choose the RTI between exercises bouts on the treadmill: the minimum RTI would be 30 seconds and the maximum would be no more than 60 s.

It has also been settled that the RTI values of the first test would be the maximum RTI of the next test, for example, if in the first test the RTI was 40 s between the first and second exercise bouts, and 50 s between the second and third exercise bouts, these same RTI values would be the maximum amount (of RTI) to the next test in their respective moments of bouts on the treadmill.

An important point is that they could rest less time if they feel the will to resume the test and perform another series of 3 minutes of exercise (this point was created to test the effectiveness of caffeine on RTI). When the subject felt that he could not perform, or was not even willing to try to perform another series of 3 min, the test was given as completed.

Materials and data collection procedures

Data collection was performed before the start of the test (i.e., at rest) immediately after each exercise bout and at the end. At all times, before the volunteers begin the test, and immediately after each exercise bouts, we assessed RPE (by Borg scale) (Borg e Noble, 1974). HR values (with a Polar S810i series heart rate monitor, with HR measurement capacity every 5 s) and [La]p (through the YSI 1500 Sport Lactate Analyzer ™; device with an error up to 0.10 ± 5 mmol/L).

The collection of blood samples for [La]p analysis was obtained by collecting around 100 µl/l of blood from the ring finger of the participants' right hand (for that we used an automatic Softclix II AccuCheck lancing device from Roche and the blood was collected to a heparin capillary tube), then, it was injected into the lactate analyzer device using a 50 µl/l pipette.

The collection at rest was made before the start of the laboratory test run, 55 minutes after CAF or PLA supplementation. Between the time of supplementation and the beginning of the laboratory test run, participants were sitting and talking in a relaxed environment with colleagues.

The same three researchers collected all data from exercise bouts, one for blood collection, one for collection of HR and the third for RPE report according to the Borg scale.

Statistical analysis

The results are presented as mean (for parametric data) or as median (for non-parametric data) ± Standard Deviation. After the assessment of the normality of the data (with Shapiro-Wilk test) we used the paired Student t test (on RTI, HR, [La]p and RPE) and Wilcoxon signed-rank test (on TTE) to verify the effect of caffeine supplementation.

In addition, we evaluated the magnitude difference (Effect Size-ES) between the two treatments in these variables by Cohen's d (for parametric data) (Fritz and collaborators, 2012) and by Cliff's Delta (for non-parametric data) (Macbeth and collaborators, 2011).

Furthermore, we evaluated Confidence Interval (CI) at 95%. ANOVA two-way (supplement x time) with Tukey's post hoc test was used to determine the influence of caffeine supplementation in HR, RPE and [La]p. Partial correlation, controlled by the exercise bouts and supplementation/treatment, was performed to verify the relationship of [La]p, RPE and HR with TTE.

To verify if the RTI from both treatment (CAF or PLA) influenced [La]p, RPE and HR variables, a Pearson (r) correlation was made to verify the relationship between them (ie, correlation between [La]p, RPE and HR with RTI before exercise bouts).

Also, a Pearson (r) correlation was made in order to verify if the [La]p, RPE and HR from both treatment (CAF or PLA) influenced RTI length (i.e., correlation between [La]p, RPE and HR with RTI after exercise bouts). All statistical tests were conducted using the Statistical Package for the Social Sciences 20. Significance was set at $p < 0.05$.

RESULTS

Performance and Pace Strategy

Individuals supplemented with caffeine were able to run longer (12.79 ± 20.67 %) than the PLA condition (PLA, 9.00 ± 3.09 min *vs.* CAF, 11.00 ± 4.82 min, 95% IC= 1.29 to 4.00

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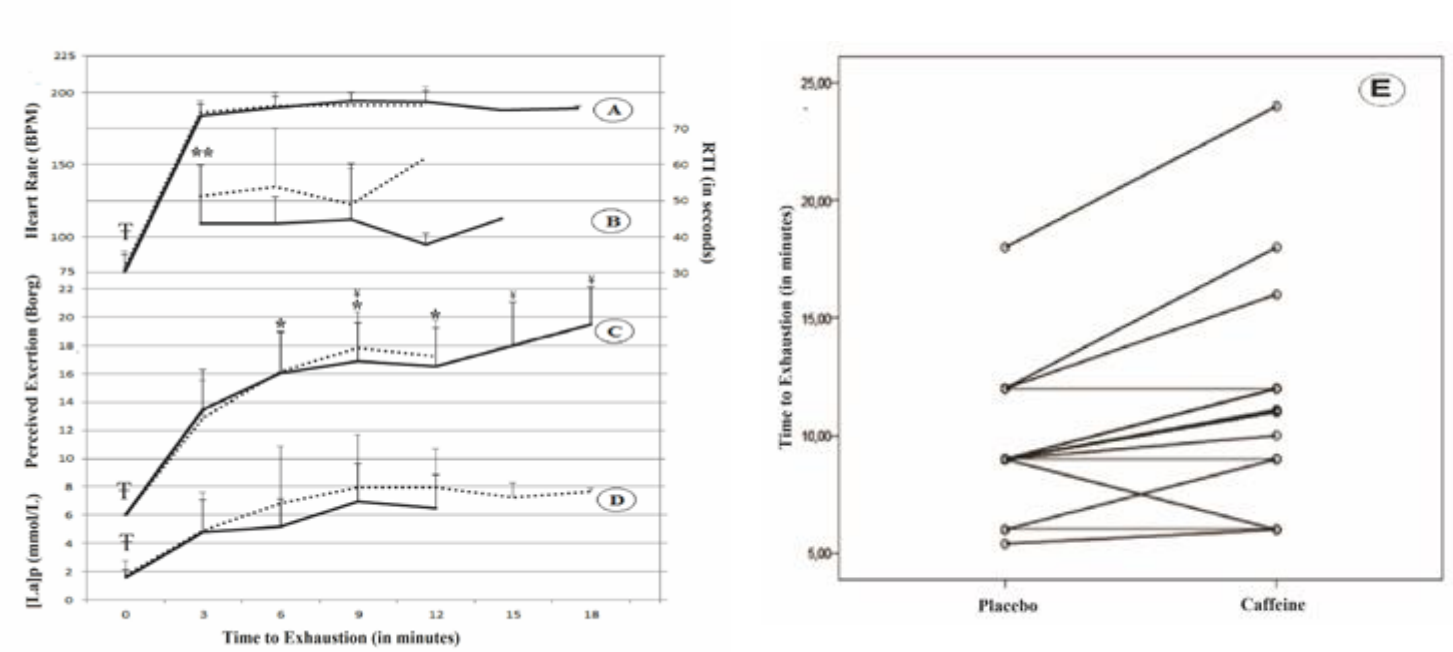
min, *p*= 0.014, ES= - 0.288), as shown in Figure 2e. The RTI were shorter for CAF condition in comparison to PLA condition (PLA 52.19 ± 8.60 s *vs.* CAF 47.95 ± 10.36 s, CI= -8.44 to -0.02 s, *p*= 0.048, ES= - 0.445), as presented in Figure 2b.

Before entering volitional exhaustion, participants in the CAF treatment ran 17% (CI: 9.17 to 25.96) more than in the PLA treatment (PLA 2,708 ± 1.141 meters *vs.* CAF 3,274 ± 1.167 m, CI= 143 to 816 m, *p*= 0.008, ES= -0.415).

Due to a shorter RTI between exercise bouts (p= 0.048), participants in the CAF treatment had a tendency to perform at higher average speed than when they were in PLA treatment (1.49 % ± 3.00%, CI: - 0.64 to 3.64, *p*= 0.127, ES= - 0.457), this increase in average speed reduced the running time for the CAF condition, to perform the same distance (ie, 2,708 m) when compared to PLA condition (-3.50 ± 18.40 seconds, CI: - 35.49 to – 0.50, p= 0.034, ES= 0.130- non-parametric tests performed).



Legend: Heart Rate (A); RTI (B), Rate of Perceived Exertion (C), [La]p- Plasma Lactate Concentration (D), Time to exhaustion of all participants (E). Dashed lines, placebo condition; solid lines, caffeine condition. Values are Mean and Standard Deviation (SD); * p < 0.05 (Fig. C) RPE of PLA condition compared to their respective 3 minutes test value; ¥ p < 0.05 (Fig. C), RPE of CAF condition compared to their respective 3 minutes test value; Ŧ p < 0.05 (Fig. A, C and D) compare to the values during the laboratory run test. ** p < 0.05 (Fig. B) rest time interval higher in PLA than CAF condition.

Figure 2 - The effect of caffeine supplementation during a high-intensity intermittent run with three-minute bouts of exercise and with Rest Time Interval (RTI) in a range of 30 to 60s (RTI were self-chosen by participants in the first test, but not in the second one).

Rate of Perceived Exertion

Figure 2c shows the RPE values during the test. The results between CAF and PLA condition showed that caffeine supplementation provides a RPE slightly lower, but not significant, during the test compared to placebo (Δ= - 0.18 ± 2.32 %, CI= - 0.88 to 0.50, p= 0.380, ES= 0.057).

In addition, in Figure 2c, no significant differences were observed between the conditions when we compare them in a paired form in 3, 6, 9 and 12 minutes.

However, the ES and CI values demonstrated a suppressive effect of small magnitude in the RPE with CAF supplementation in 9 and 12 minutes of the test (3 min: CI= -0.66 to 1.79 Borg values, p=

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0.389, ES= - 0.205; 6 min: CI= -1.19 to 0.92, p= 0.579, ES= 0.046; 9 min: CI= - 3.05 to 1.05, p= 0.270, ES= 0.370; and 12 min: CI= -5.13 to 3.65, p= 0.581, ES= 0.253).

The RPE during the laboratory test run (from 3 min) increased significantly for both conditions (p< 0.05), when compared to rest. Interestingly, RPE for PLA treatment from 6 min was significantly higher than 3 min. However, the CAF treatment RPE was statistically higher for 9, 15 and 18 min when compared to 3 min. In addition, for 12 minutes of the CAF treatment RPE was not statistically different from its respective 3 min value. Together these data show that caffeine supplementation prevented an abrupt increase of the RPE (see Figure 2c).

When controlled for the exercises bouts and treatment (CAF/PLA), RPE showed a negative and significant moderate correlation with TTE (r= - 0.492, p= 0.001).

Remarkably, there was a significant trend for a weak and negative correlation between the RPE and before RTI to the exercise bouts for the CAF condition, but not for the PLA condition (CAF: r= - 0.354, p= 0.076; PLA: r= 0.140, p= 0.584). However, there was no correlation between RPE and RTI length after the exercise bouts for both groups (CAF: r= - 0.187, p= 0.430; PLA: r= 0.119, p= 0.547).

These results show that, in the laboratory run test, caffeine supplementation seems to promote a fine tune between the RPE and exercise intensity. Nevertheless, RPE (at the end of the exercise bouts) did not seem influence the RTI length for any conditions (in a range from 30 to 60 seconds).

Blood Plasma Lactate Concentration

In Figure 2d, when CAF was compared to PLA condition (paired form), despite having, on average, a higher [La]p, the difference between the treatments was not statistically significant, regarding p level (CAF vs PLA: 0.64 ± 3.01%, CI= - 0.17 to 1.31 p= 0.129, ES= - 0.185).

However, the ES values, when analyzed in each series of exercise bouts, show an increase of moderate magnitude for [La]p in caffeine treatment (rest: CI= - 0.39 to 0.71 mmol/L [La]p, p= 0.550, ES= - 0.206; 3 min: CI= - 1.20 to 1.49, p= 0.820, ES= - 0.053; 6 min: CI= - 0.83 to 4.11, p= 0.177, ES= - 0.531; 9 min: CI= - 1.75 to 2.74, p= 0.625, ES= - 0.302; 12 min: CI= -4.61 to 11.70 p= 0.283, ES= - 0.830).

As the RPE and HR (see below), the [La]p presented a negative and significant moderate correlation with the TTE (r= -0.448, p= 0.001), when controlled for exercise bouts and treatment (CAF/PLA).

In the same way as RPE, the [La]p was significantly correlated with the before RTI of the exercise bouts for CAF condition (r= - 0.462, p= 0.026), but not for PLA condition (r= - 0.018, p= 0.943), possibly indicating that the lactate production also was related to muscle work intensity only for CAF condition.

Also similar to the results of RPE and HR, there was no correlation between [La]p and subsequent RTI for both conditions (CAF: r= - 0.106, p= 0.657; PLA: r= - 0.104, p= 0.599), demonstrating that the [La]p is not related to the RTI length after exercise bouts for both treatments.

Heart Rate

Throughout the treatment HR was similar for both the conditions (CAF vs PLA: Δ= - 1.42 ± 9.46, CI= - 3.88 to 1.04, p=0.252, ES= 0.028), without a significant difference comparing from rest to 12 min within the test. In addition, the ES values demonstrate that caffeine supplementation apparently did not increase HR in this test (rest: CI= - 10 to 4.72, p= 0.425, ES= 0.245; 3 min: CI: - 5.61 to 1.87, p= 0.302, ES= -0.235; 6 min: CI= - 5.17 to 2.51, p= 0.469, ES= 0.166; 9 min: CI= -3.07 to 6.27, p=0.732, ES= - 0.363; and 12 min: CI: - 18.70 to 23.70, p= 0.732, ES= - 0.210) (see Figure 2a).

As the RPE and [La]p, when controlled by the exercise bouts and treatment (CAF/PLA), the HR presented a negative and significant weak correlation with the TTE (r= - 0.257, p=0.003).

However, there was no correlation between RTI and HR before (CAF: r= - 0.161, p= 0.421; PLA: r= 0.107, p= 0.653) or after (CAF: r= 0.221, p= 0.350; PLA: r= 0.000, p= 1.000) the exercises bouts. This shows that, in both treatments (CAF/PLA), HR did not influence the RTI, conversely, HR was not influenced by the RTI length, between exercise bouts.

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DISCUSSION

The main result of this study was that supplementation of 6 mg·kg−1 caffeine influences positively the pace strategy (compared to the placebo condition), to decrease the RTI required for recovery between moments of high-intensity exercise. In addition, caffeine supplementation has the ability to make an individual run further in the same event, even against a high metabolic and mental stress.

In our experiment we managed to reduce bias from variables that would affect the pace strategy, such as mood and hormonal variation (tests were made on the same day and time- but they were not assessed), nutrition and hydration (a standard nutrition and hydration plan was set 24 hours before the test), motivational (during the collections we encouraged individuals to put their maximum effort in the test) and test environment (we ensured that the tests occurred at similar temperature and humidity).

Thus, other variables that would also influence pace strategy during the test (i.e., RPE and cognitive variables such as knowledge of the length of each exercise range, RTI and previous experiences of RPE for exercises at various intensities and durations (Marcora, 2010) would represent less bias to the caffeine supplementation study.

Our blinding supplementation was effective, as only one subject responded correctly to the both PLA and CAF condition. Two participants responded correctly only to caffeine. Three participants responded correctly only to placebo.

The increase in average speed (p= 0.138), but non-significant, in CAF condition was possible due to the significant improvement in RTI (p = 0.048) for the subsequent bout of exercise. Moreover, even at a higher intensity, the individuals when supplemented with caffeine ran further (p= 0.014).

It should be noted that, to cover the same distance of the placebo treatment (2,708 m), CAF condition spent less time. We believe that if we had a longer distance to compare (Placebo ran only ~2,708m instead ~3,274m in CAF treatment), we would have seen a higher statistical difference (in average speed).

Another important element was that, we set a fixed speed instead to allow a self-choose speed (which also enhances pace strategy) (Black and collaborators, 2015), thus in our study the intensity could only be increased with the reduction of RTI (which in fact happened to the CAF condition).

Studies with endurance condition, such as TT, with greater duration than our study, in which the intensities were self-selected by the participants, also showed decreasing time values to cover the same distance (Ganio and collaborators, 2009; Spriet, 2014).

In addition, a running study with metabolic demand similar to ours (with a running distance of 1,550m) that monitored the first 1,110m demonstrated that when participants are allowed to choose the speed in the last 400 m (to complete the test), individuals in caffeine condition were able to run at an average speed significantly higher than the placebo condition (Targum and collaborators, 2014).

Therefore, studies like these have great relevance to elite sport, where the difference between the first and second place in TT tests with similar distance in this study have a variation of less than 1% (Currell e Jeukendrup, 2008).

In the paired comparison performed in this study, there was no significant difference (at p level) for [La]p production, HR or RPE for CAF, compared to PLA condition. These results agree with those reported by the literature, in which doses of ≤ 6 mg·kg−1 do not affect parameters that might be considered side effects of caffeine (namely excessive calcium release, increased values of the following: HR, [La]p, free fatty acids, catecholamine's hormones release and glycerol concentration during exercise) (Spriet, 2014). These metabolic variables are changed in studies with higher doses (> 6 mg·kg−1) (Spriet, 2014).

Previously, some authors (Donghia and collaborators, 2016) presented similar data to this study (on TTE, when an exercise was performed above the anaerobic threshold, whit fixed RTI at 1 minute) caffeine supplementation did not promoted significant change compared to placebo treatment in HR, [La]p or RPE.

Although we found no significant difference between treatments (CAF vs. PLA) we identified that CAF condition showed a

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delay in increasing RPE throughout the test. This delay in increasing RPE was expected for CAF condition (Doherty e Smith, 2005) due to, probably, the suppressor effect of caffeine on corollary discharges from premotor and motor areas of the cortex (De Morree and collaborators, 2014).

It is known that the intensity of the exercise performed is directly proportional to the increase in the processes of corollary discharges (De Morree and collaborators, 2014; De Morree e Marcora, 2015). Thus, the delay in RPE increase assessed in our study appears to be linked to TTE increased, probably mediated by this phenomenon.

The fact that we found no significant difference between groups or correlation of HR with the RTI between exercise sets in this study can be explained by our severe intensity protocol, in which the final average of HR in both tests was 193.8 ± 10.15 BPM, showing that participants came very close to their maximum HR.

A trend was observed for higher [La]p production on CAF condition (p= 0.129), but we are unable to distinguish if this was probably due to a more intense exercise carried out by the participants in this condition or this might be a side effect related to caffeine dose used in this study (6 mg·kg−1) (Glaister and collaborators, 2014; Morita and collaborators, 2014; Spriet, 2014). However, no significant difference was found, just as in other studies that used this dose (≤ 6 mg·kg−1) (Spriet, 2014).

Interestingly, both [La]p and RPE in CAF condition were correlated negatively with the RTI preceding the series of exercises. It is likely that correlation is not related to this length of RTI (statistically smaller in the CAF condition) (Glaister and collaborators, 2014; De Morree e Marcora, 2015), because the correlation should repeat itself for the PLA condition, which did not occur (neither in the reproducibility tests performed in this study, provided elsewhere) (De França and collaborators, 2016).

Therefore, an explanation for the negative correlation trend between the RPE and RTI before the exercise bouts for CAF condition may result from the effect of it on the individual's cognition (De Morree and collaborators, 2014; Franke e Bagusat, 2015), since the RPE has a cognitive component (De Morree e Marcora, 2015).

In agreement with De Morree and Marcora (2015), a change in cognition state implies RPE modulation relatively to exercise intensity, for instance, cognitive fatigue (or cognitive demand during exercise) tends to overestimate the RPE regarding physical effort (Smith and collaborators, 2014). Therefore, supplementation with caffeine, probably due to its positive effects on cognition state, provided a more accurate perception of effort with respect to intensity changes during testing at each exercise bout, avoiding the overestimation, allowing more effort and consequently more performance. These findings might be useful for individuals with chronic fatigue, (Targum and collaborators, 2014) as well as athletes.

The significant correlation between [La]p and the RTI before exercise bouts (for CAF condition) might be influenced by the increase in the anaerobic glycolysis metabolism promoted by caffeine (Morita and collaborators, 2014), because lactacidemia is classically higher during high-intensity physical exercise in caffeine condition (Glaister and collaborators, 2014).

Unsurprisingly caffeine supplementation increases the TTE (Doherty e Smith, 2005) and improves the TT (Ganio and collaborators, 2009) in physical exercise tests. To our knowledge, this is the first study to demonstrate that caffeine supplementation might change RTI (decrease) and TTE (increased) in the same event.

This finding has direct application in endurance events of the TT type in which the pacing strategy is what will determine the average speed and consequently the performance produced in the competition (Millet, 2011).

In addition, the TTE increase in this study shows that, in fact, exercise tolerance was increased, and, hypothetically, the participants still would have a margin of resistance to run more intensely, if the speed was self-selected.

It is well known that acute doses of caffeine can increase the mood, motor skills, cognition, alertness and reduce fatigue and sleepiness (Burdan, 2015).

It is worth to highlight that poor performance of these capabilities are fatigue-related symptoms seen in patients with CNS disorder (Targum and collaborators, 2014) and metal fatigue (i.e., because of a high cognitive

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work), that are also detrimental to physical performance, resulting in increase in RPE (Smith and collaborators, 2014; Wright, 2008).

Thus, the change in pace strategy and a concomitant increase in the TTE without any substantial change in HR seems to have occurred by CNS stimulation suggested by the delay in the increases of RPE, registered in the CAF condition.

Recently, a 'flushmodel' (Millet, 2011) was proposed to explain that in the endurance races of the TT type, the adoption of pace strategy is a conscious and unconscious control resulting from fatigue condition to avoid exhaustion (Marino, 2014; Millet, 2011).

In this model, RPE would be as a float inside a water container that goes up or down depending on the degree of filling or emptying. The race intensity (or, running pace) is a major factor for the filling (i.e., increasing the negative feedback) or emptying (i.e., power output recovery) of the container. If the intensity of the race increases, enough causing the float (i.e., RPE) to reach critical values, the individual ends up disengaging the activity.

Thus, to avoid exhaustion, they decrease the intensity, which would be like a water escape to empty the container. This theory is consistent with the physical exercise proposed for patients with chronic fatigue (individuals who have high RPE values for any exercise intensity when compared to other populations of healthy individuals or individuals with other types of disease).

In other words, use intermittent exercise to prevent the RPE to reach critical values is considered the best method to delay fatigue since it provides less negative feedback to the CNS (Marino, 2014).

In addition, the use of CNS stimulants that promotes catecholamine release and pleasure during exercise (Astorino and collaborators, 2012; Burdan, 2015; Meeusen, 2014) (such as caffeine or painkillers) can also change the sensitivity of the float increase to the critical limit that it can reach.

Therefore, as noted in this study the [La]p, HR and RPE were negatively correlated with the volitional exhaustion, i.e., they are indicators of the end of the test (working as float).

Despite the fixed speed, participants supplemented with caffeine ran more time and tended to run faster (the latter due to the fact of shorter RTI during the race). Therefore, we believe that the increased TTE and a tendency for higher mean velocity were due to the altered sensitivity of the float (i.e. RPE) and thus inhibited the sensation of effort during activity (in the caffeine condition), thus allowing increase in work capacity (see Figure 2c).

Marino (2014) postulated that fatigue is determined by the ability to regulate body processes that bring the body back to the homeostasis state. This fact was verified in our study when we demonstrate (by the negative correlations between TTE vs. HR, [La]p and RPE) that individuals who have the highest HR and [La]p and RPE values were the ones with the lowest values of TTE.

Thus, individuals who present a greater emptying capacity (recovery), or a greater filling space (i.e. greater tolerance to exercise stress) are those that can run more effectively (longer and faster), or will be less likely to get into exhaustion (Millet, 2011).

This fact seems to be independent of caffeine (i.e. increase HR, [La]p and RPE during physical exercise), however, due to the crossover design of our study it became clear that caffeine supplementation delayed fatigue onset.

As it can be seen in our Figures 2a, 2c and 2d (similar to data reported by Marino (2014) and Kay and collaborators (2001), the values that indicate a high peripheral ([La]p) and central (HR and RPE) metabolic stress do not reflect the behavior of the muscular performance during physical exertion.

In other words, although we note a shorter RTI for the caffeine treatment, a longer TTE compared to placebo treatment was showed.

Moreover, as evidenced by the weak and non-significant correlations between mental and metabolic stress indicators with subsequent RTI from participants (i.e. HR, [La]p and RPE vs. RTI), for both treatments, we observed that, indeed, there is another kind of intensity control mechanism (which cannot be measured by the variables used in this study).

For instance, a control mechanism used by the CNS, such as, corollary discharges. Together, these data suggest that caffeine supplementation can suppress the anticipation of fatigue mechanism improving the pace strategy and effort tolerance.

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CONCLUSION

In conclusion, caffeine supplementation can positively influence the pace strategy to decrease the RTI required for recovery between moments of high intensity exercise bouts. This decrease in RTI may be related to a less abrupt increase of the RPE on CAF condition.

Moreover, even with the decrease of the RTI (which increases the intensity of the activity) caffeine supplementation increases exercise tolerance, and thus, increases the TTE. Indeed, the anaerobic glycolysis seems to be enhanced with caffeine supplementation.

However, more research is needed to determine the full mechanisms of influence of caffeine in these phenomena and how it is relevant to increasing performance.

Together, these findings demonstrate that in a running of high mental and metabolic stress caffeine supplementation can make an individual run further besides reducing RTI.

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