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Effects of caffeine on neuromuscular function in a non-fatigued state and during fatiguing exercise

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Abstract

Caffeine enhances exercise performance, but its mechanisms of action remain unclear. In this study, we investigated its effects on neuromuscular function in a non-fatigued state and during fatiguing exercise. Eighteen men participated in this randomized, double-blind, placebo-controlled crossover trial. Baseline measures included plantarflexion force, drop jump, squat jump, voluntary activation of triceps surae muscle, soleus muscle contractile properties, M-wave, α-motoneuron excitability (H-reflex), corticospinal excitability, short-interval intracortical inhibition, intracortical facilitation, silent period evoked by transcranial magnetic stimulation (SP) and plasma potassium and caffeine concentrations. Immediately after baseline testing, participants ingested caffeine (6 mg·kg^{-1}) or placebo. After a 1-h rest, baseline measures were repeated, followed by a fatiguing stretch–shortening cycle exercise (sets of 40 bilateral rebound jumps on a sledge apparatus) until task failure. Neuromuscular testing was carried out throughout the fatigue protocol and afterwards. Caffeine enhanced drop jump height (by 4.2%) and decreased the SP (by 12.6%) in a non-fatigued state. A caffeine-related decrease in SP and short-interval intracortical inhibition before the fatiguing activity was associated with an increased time to task failure. The participants who benefitted from an improved performance on the caffeine day reported a significantly lower sense of effort during

exercise and had an accelerated postexercise recovery of M-wave amplitude. Caffeine modulates inhibitory mechanisms of the CNS, recovery of M-wave amplitude and perception of effort. This study lays the groundwork for future examinations of differences in caffeine-induced neuromuscular changes between those who are deemed to benefit from caffeine ingestion and those who are not.

Keywords

central fatigue, peripheral fatigue, rate of perceived exertion, soleus, transcranial magnetic stimulation

Introduction

Caffeine is the most widely consumed drug worldwide. Several studies and a number of reviews have documented the ergogenic effects of caffeine for aerobic endurance, muscle strength, muscle endurance, anaerobic power, jumping and short-term high-intensity exercise performance (Burke, 2008; Davis & Green, 2009; Ganio, Klau, Casa, Amstrong, & Maresh, 2009; Graham, 2001; Grgic et al., 2019; Southward, Rutherfurd-Markwick, & Ali, 2018). The current leading hypothesis for its mechanism of action is the enhancement of neural drive attributable to the reversal of inhibitory effects of adenosine, by blocking its receptors in the central nervous system (CNS). This blockage enhances dopaminergic transmission, which is associated with increased arousal and motivation and with an increase of serotonin release presynaptically and serotonergic input postsynaptically (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). Furthermore, caffeine also seems to reduce sense of effort during exercise (Doherty & Smith, 2005), probably through a modulation of afferent feedback from the working muscles, corollary discharges associated with the central motor command, or both (Pageaux, 2016).

Effects of caffeine on the muscle itself are unlikely, because a number of studies were unable to demonstrate a significant effect of the drug on electrically evoked contractile properties (Black, Waddell, & Gonglach, 2015; Del Coso, Estevez, & Mora-Rodriguez, 2008; Eaton et al., 2016; Hespel, Op ’t Eijnde, & Van Leemputte, 2002; Kalmar & Cafarelli, 1999, 2004, 2006; Kalmar, Del Balso, & Cafarelli, 2006; Neyroud et al., 2019; Plaskett & Cafarelli, 2001; Smirmaul, de Moraes, Angius, & Marcora, 2016). However, to date five studies have shown a caffeine-induced effect on contractile properties (Bazzucchi, Felici, Montini, Figura, & Sacchetti, 2011; Bowtell et al., 2018; Cureton et al., 2007; Meyers & Cafarelli, 2005; Tarnopolsky & Cupido, 2000); therefore, this hypothesis should not be dismissed. The idea of caffeine acting on the CNS through adenosine receptor antagonism is very attractive, but further research is needed to clarify the exact sites of action.

Some research has shown a caffeine-induced attenuation of plasma K^+ concentrations (Lindinger, Graham, & Spriet, 1993) and attenuated muscle interstitial K^+ concentrations (Mohr, Nielsen, & Bangsbo, 2011), which could be translated into a beneficial effect at the neuromuscular junction. In fact, a caffeine-induced attenuation of neuromuscular transmission failure after fatigue, assessed by the maximal M-wave (M_{max}), has been demonstrated (Bowtell et al., 2018).

Effects of caffeine on the excitability of spinal motoneurons are likely but far from unequivocal. Some authors have shown a positive effect in a non-fatigued state (Kalmar & Cafarelli, 1999; Walton,

Kalmar, & Cafarelli, 2002, 2003) and during fatigue (Kalmar et al., 2006), whereas others did not observe a significant effect in a non-fatigued state (Behrens et al., 2015a, b; Kalmar et al., 2006). Some studies have attributed the ergogenic effects of caffeine to a decreased inhibition at the cortical or spinal level (Cerqueira, de Mendonça, Minez, Dias, & de Carvalho, 2006; de Carvalho, Marcelino, & de Mendonça, 2010) and an increased corticospinal excitability (Kalmar & Cafarelli, 2004, 2006), both of which may contribute to enhancement of descending neural drive. However, some other studies have also been unable to observe an effect of caffeine on inhibitory or excitatory mechanisms at the cortical or spinal level (de Carvalho et al., 2010; Orth, Amann, Ratnaraj, Patsalos, & Rothwell, 2005).

To date, only a handful of studies have investigated the effects of caffeine on neuromuscular function concurrently in a non-fatigued state, during fatigue and during recovery. It is also important to take into consideration that some of the aforementioned discrepant results could be related, in part, to differences in the research designs that could modulate the influence of caffeine on neuromuscular function, such as the nature of the exercise task, muscle of interest, dose of caffeine (Sabol, Grgic, & Mikulic, 2019) and time of day (Mora-Rodríguez et al., 2015). Another possible contributor is the known large inter-individual variability regarding the ergogenic effects of caffeine on exercise performance (Doherty, Smith, Davison, & Hughes, 2002; Graham & Spriet, 1991; Green, Olenick, Eastep, & Winchester, 2017; Guest, Corey, Vescovi, & El-Sohemy, 2018; Roelands et al., 2011; Skinner, Jenkins, Coombes, Taaffe, & Leveritt, 2010; Wiles, Coleman, Tegerdine, & Swaine, 2006). This interindividual variability is potentially driven by a variety of factors, such as sex and menstrual cycle stage (Lane, Steege, Rupp, & Kuhn, 1992), training status (Collomp, Ahmaidi, Chatard, Audran, & Prefaut, 1992), habitual caffeine use (Lara, Ruiz-Moreno, Salinero, & Del Coso, 2019) and genetic variations that modify caffeine metabolism (Guest et al., 2018; Koonrungsesomboon, Khatsri, Wongchompoo, & Teekachunhatean, 2018). Presumably, all these factors contribute to an increased likelihood of encountering a masking effect in caffeine 'responders', which might not be revealed in the averaged data. Thus, given that the degree of responsiveness of some individuals often contrasts to the average findings reported, it is necessary to take this inter-individual variability into consideration in order to gain a greater understanding of the magnitude of the caffeine-induced effects on different levels of the neuromuscular system. It is not yet known whether a differential modulation of caffeine on neuromuscular function is observed between those individuals who benefit from caffeine ingestion and those who do not.

The aim of the present study was to investigate the underlying neuromuscular mechanisms that might be responsible for any caffeine-induced ergogenic effects in a non-fatigued state and during a fatiguing stretch-shortening cycle (SSC) exercise and recovery. Our hypotheses were as follows: (i) caffeine would result in an improvement in exercise performance in a non-fatigued state and in an increased resistance to fatigue during a fatiguing SSC exercise; and (ii) caffeine would not modulate contractile properties of the muscle, but would upregulate excitatory mechanisms and downregulate inhibitory mechanisms in the CNS.

2 Methods

2.1 Ethical approval

Participants were fully informed of any risks and discomforts associated with the procedures before giving their informed written consent to participate. The procedures were approved by the Ethics Committee of the University of Jyväskylä and performed according to the 1964 Declaration of Helsinki and its later amendments, except registration in a database.

2.2 Participants

Eighteen healthy men (age, 26.6 ± 3.1 years; body mass, 75.3 ± 7.4 kg; and height, 179.3 ± 5.2 cm), who engaged in recreational physical exercise, volunteered to participate in this study. The participants were non-smokers and were not overweight or obese, because cigarette smoking and adiposity may enhance the rate of caffeine degradation (Kamimori, Somani, Knowlton, & Perkins, 1987; Parsons & Neims, 1978). Participants had an average daily consumption of caffeine of 184 ± 157 mg (six light consumers, 21 ± 26 mg; and 12 regular consumers, 266±125 mg), assessed by a 7 day questionnaire on intake of food and beverages containing caffeine.

2.3 Procedures

The participants visited the laboratory on three different occasions. The first visit was a familiarization session, in which they were introduced to the electrophysiological techniques and the sledge apparatus, and the stimulation sites were marked for the upcoming sessions. In this session, the maximal jumping height was also determined in the sledge apparatus, by dropping the participants from progressively greater heights (Horita, Komi, Nicol, & Kyröläinen, 1996). The sledge was inclined at 23 deg for all testing, with the exception of one participant, for whom it was necessary to incline the sledge apparatus to 27 deg to allow maximal rebounding. The drop jumps

were preceded by a 5 min warm-up on a cycle ergometer at an intensity of 60–70% of individual predicted maximal heart rate, and 10 submaximal jumps in the sledge with increasing effort.

Participants visited the laboratory on two subsequent experimental days, for a placebo and a caffeine condition session, in a randomized order. Participants were asked to abstain from consumption of caffeine-containing foods and beverages, alcohol consumption and physical exercise within 72 h of the experimental sessions. Each experimental session began with a pre-capsule test, which was followed by the oral administration (double-blind) of a capsule containing either caffeine (6 mg·kg^{-1}) or placebo (maltodextrin). An identical post-capsule protocol took place 60 min after the ingestion of the capsule. The post-capsule test was followed by the fatigue protocol and a 15 min recovery period. The overall design of the experimental sessions is presented schematically in Figure 1. The sessions took place 14 days apart. The participants were asked to wear the same shoes during the three visits.

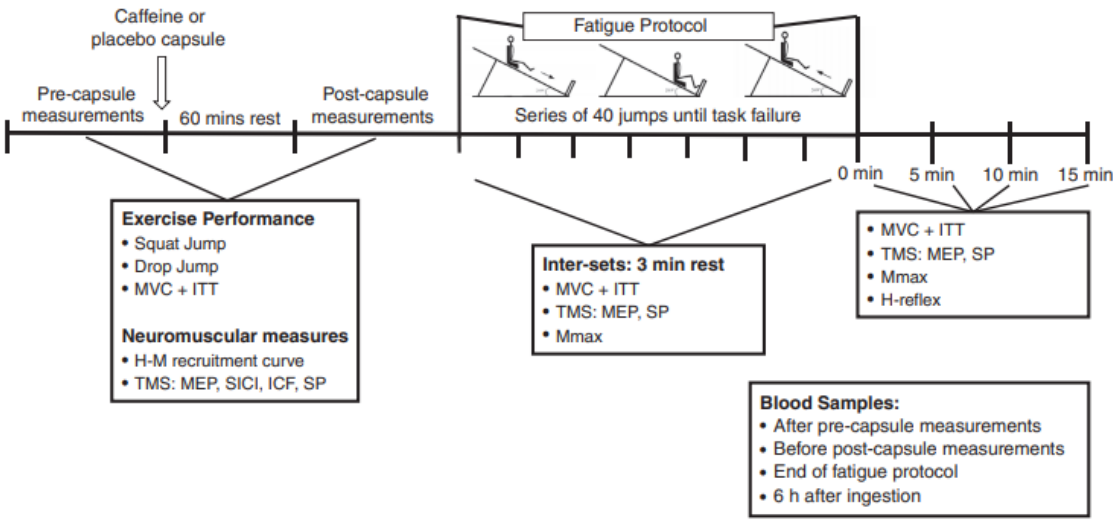

Figure 1 Experimental protocol. Pre- and post-capsule measurements were identical and included H- and M-wave recruitment curves followed by transcranial magnetic stimulation (TMS). During TMS, unconditioned motor evoked potentials (MEPs), short-interval intracortical inhibition (SICI), intracortical facilitation (ICF) and the duration of the TMS-evoked silent period (SP) were assessed. Baseline measures also included evaluation of squat and drop jump height and maximal voluntary contractions (MVCs). Electrical stimulation was applied immediately before the MVCs to assess the maximal compound muscle action potential (M_{max}), and during and after the MVCs (interpolated twitch technique, ITT) to assess voluntary activation and contractile properties. A capsule was ingested at the end of the pre-capsule measurement, and the post-capsule test was conducted 1 h later. The fatigue protocol took place after the post-capsule test. Neuromuscular testing was carried out during the inter-series periods, at task failure and during the recovery period. Blood samples were collected immediately after the pre-capsule measurements, before the post-capsule test, after the fatigue protocol and exactly 6 h after ingestion (~3 h after the end of the fatigue protocol).

2.4 Pre- and post-capsule measurements

The pre-capsule test began with a series of stimuli applied to the tibial nerve to evoke H-reflex and M-wave recruitment curves in the soleus (SOL) muscle. Transcranial magnetic stimulation (TMS) was then used to elicit evoked responses in SOL. The motor threshold (MT) was assessed, and, in a randomized order that was constant for each participant throughout the study, corticospinal excitability through unconditioned motor evoked potentials (MEPs), short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were assessed.

Participants then performed a warm-up identical to the one performed in the familiarization session, followed by the last part of the neuromuscular testing, which included maximal isometric voluntary contractions (MVCs) of the plantarflexors, single-pulse TMS during three voluntary contractions at 50% of MVC to assess the TMS-evoked silent period (SP), and assessment of maximal squat jump and drop jump heights. A rest period of 2 min and 30 s was given between the MVC trials, and at least three attempts were completed. Subsequent trials were performed if the participant reached a force value >10% higher than his previous best attempt. Visual feedback of the force trace was continuously provided on a screen in front of the participant, and the participants were instructed to contract as hard and as fast as possible. Standardized verbal encouragement was provided by the same investigator in all trials. During the MVC trials, a supramaximal single-pulse stimulation was applied to the tibial nerve before MVC to assess M_{max}, and a supramaximal double-pulse stimulation was applied during and 2 s after each attempted MVC to quantify voluntary activation (VA) of the triceps surae muscle through the interpolated twitch technique (Merton, 1954). The amplitude of the twitch after MVC was also used as an estimate of contractile properties.

A minimum of three trials were performed for squat and drop jumps, with 1 min rest between trials. Immediately after each squat jump, the force curve was inspected to identify any countermovement jump that would invalidate the jump. To perform the drop jumps, participants were dropped from their optimal height, assessed during the familiarization session. They were instructed to keep a knee angle of ∼90 deg on landing, to keep their heels off the force plate and to be as explosive as possible. These criteria were inspected visually by a research assistant.

MVC, squat jump and drop jump trials were performed in a randomized order that was constant for each participant throughout the study, with SP being assessed immediately after MVCs.

2.5 Fatigue protocol

Participants performed a fatiguing SSC exercise on a sledge apparatus by repeating series of 40 submaximal bilateral jumps, with inter-series periods of 3 min, during which neuromuscular function was assessed. During the fatiguing sets, participants were asked to reach a rebound height of 70% of the best drop jump height that was reached either in the familiarization session or during the pre-capsule measurements of the first session. One research assistant gave feedback to the participant after every jump regarding the rebound height, with the oral commands 'Good', 'Higher' and 'Not so high'. Another research assistant gave feedback to ensure that the participant satisfied the jump criteria stated above. The exercise was stopped when the participant could not reach the submaximal rebound height 10 times in a row.

During the inter-series periods, the following measures were performed: rating of perceived exertion (RPE), a train of 10 TMS single pulses, one plantarflexion MVC attempt performed with peripheral electrical stimuli, and TMS during three voluntary contractions at 50% of MVC to assess SP. Recovery measures were performed at 0, 5, 10 and 15 min from task failure. Recovery measures included the protocol applied during the inter-series periods of the fatigue protocol, followed by evoked H-reflexes with concomitant M-waves with a size equivalent to a predetermined percentage of M_{max}.

2.6 Rating of perceived exertion

Rating of perceived exertion was reported using a 6–20 Borg scale, and the participants received an exercise-specific description of how to rate perception of effort: 'How hard is it for you to drive your legs and how heavy is your breathing?'. To provide a point of reference, exercise anchoring was performed, and the participants were told that maximal exertion corresponds to the effort they experienced at exhaustion during an incremental test (Pageaux, 2016).

2.7 Position in the sledge for electrical stimulation, TMS and for MVCs

Participants assumed a seated position in the sledge apparatus, with the forefoot on a built-in force plate and the knees in full extension. This ensured a small activation of the plantarflexors with the ankles in a neutral position. The hips were at 50 deg of flexion (0 deg = extended neutral position). The sledge was locked in an unmovable position.

2.8 Electrical stimulation

Electrical stimuli were given using a constant-current stimulator (model DS7AH; Digitimer Ltd, Welwyn Garden City, UK); a circular cathode with a surface area of 0.77 cm^2 (Unilect 4535M, Ag/AgCl; Unomedical Ltd, Redditch, UK) was placed over the tibial nerve on the popliteal fossa, and an oval-shaped anode (5.08 cm × 10.16 cm) positioned superior to the patella (V-trodes; Mettler Electronics Corporation, Anaheim, CA, USA). Square pulses of 1-ms pulse duration were applied to evoke M-waves and H-reflexes in SOL. The H- and Mwave recruitment curves were generated with a series of single pulses with interstimulus intervals that ranged randomly between 8 and 10 s, in order to avoid postactivation H-reflex depression (Crone & Nielsen, 1989). The stimulus intensity was increased gradually by steps of 2 mA until the descending part of the H-reflex recruitment curve was reached and by steps of 10 mA thereafter until the M-wave amplitude reached a plateau. The stimulation intensity was reassessed during the post-capsule test. When M_{max} was reached, the intensity of the electrical stimulation was decreased in order to evoke five control H-reflexes on the ascending portion of the recruitment curve preceded by a stable M-wave amplitude. For the measurement of VA and contractile properties, the intensity was set at 150% of M_{max}, and two square-wave pulses with a duration of 1 ms and an interstimulus interval of 10 ms were applied over the isometric force plateau and ∼2 s after each MVC.

2.9 Transcranial magnetic stimulation

Magnetic stimuli were given with a concave double-cone coil (110 mm diameter) attached to a BiStim 200[2] magnetic stimulator (Magstim Co., Whitland, UK) to elicit MEPs in SOL. The coil was oriented to induce current in the posterior-to-anterior direction. Participants were seated in the sledge, as previously described, performing a low-intensity sustained contraction. The hotspot of the SOL area of the motor cortex was identified in the familiarization session by placing the centre of the coil 1 cm to the left of the vertex and 1 cm posterior. The coil was then moved in 1 cm steps in the lateral–medial and anterior– posterior directions to identify the optimal location. The hotspot was defined as the location eliciting the greatest SOL MEP amplitude. Upon identification, the hotspot was marked on the head with a permanent marker, and the lateral–medial and anterior–posterior distances were measured for the upcoming sessions.

The MT was defined as the minimal intensity needed to evoke an MEP of >200 μV in three out of five trials in the tonically active SOL (Škarabot et al., 2016), and MT showed a very high level of day-to-day reliability (day-to-day coefficient of variation of 4.40%) in our study.

During the protocol, trains of 10 TMS pulses were used. Corticospinal excitability was examined by eliciting MEPs at a stimulus intensity of 130% of MT. The intracortical circuits measured were SICI and ICF with paired-pulse TMS, whereby a conditioning stimulus was delivered before the test stimulus. The conditioning stimulus intensity was 80% of MT, and the test stimulus intensity was 130% of MT. The interstimulus intervals were 2 ms during the blocks that assessed SICI and 12 ms during the blocks that assessed ICF (Sanger, Garg, & Chen, 2001). During the TMS trains, the interstimulus intervals ranged randomly between 5 and 10 s in order to avoid anticipation. To assess SP, single-pulse TMS was delivered while participants performed a voluntary contraction at 50% of MVC. Three trials were performed, with an interval of 4 s between trials, at a test stimulus intensity of 130% of MT.

2.10 Force

Data were sampled at 1000 Hz via an A/D converter (CED 1401; Cambridge Electronics Design, Cambridge, UK) and low-pass filtered (cut-off frequency, 50 Hz).

2.11 Electromyography

Electromyographic (EMG) signals from SOL were recorded using an EISA 16-2 EMG system (University of Freiburg, Freiburg, Germany) and bandpass filtered (10–500 Hz). Oval-shaped, bipolar, pre-gelled silver chloride self-adhesive surface electrodes (Ambu Blue Sensor N; Ambu A/S, Ballerup, Denmark) were placed over the mid-dorsal line of the posterior shank, below the gastrocnemius muscle. The interelectrode distance was 2 cm. To keep the inter-electrode resistance low (<2 kΩ), the skin under the electrodes was shaved, abraded with sandpaper and swabbed with a 60% ethanol solution. Data were sampled at 1000 Hz (gain 500) via an A/D converter (CED 1401; Cambridge Electronics Design). A permanent marker was used to mark the location of the EMG electrodes in the familiarization session to ensure consistent electrode placement between sessions.

2.12 Muscle soreness

Participants self-reported soreness of the triceps surae muscle on the experimental days (immediately after the fatigue protocol and in the evening) and on the following 3 days (in the morning and in the evening). Participants were asked to draw a straight line on a visual analog scale from 0 to 10 cm, where zero represents no soreness and 10 maximal soreness.

2.13 Blood samples

Venous blood samples were collected immediately after the pre-capsule testing, immediately after the fatigue protocol and 6 h after the pre-capsule testing. Samples were collected into serum tubes using needles (Venosafe; Terumo Medical Co., Leuven, Belgium) for the basic blood count (XP300; Sysmex Corporation, Kobe, Japan) before the maximal test on the sledge apparatus. Whole blood was centrifuged at 2500 g (Megafuge 1.0R; Heraeus, Hanau, Germany) for 10 min, after which serum was removed and stored at −80◦C until analyses for serum caffeine and K^+.

Serum caffeine concentrations were assessed using high-performance liquid chromatography (Agilent 1100 Series HPLC equipment; Agilent Technologies, Santa Clara, CA, USA). A 5 μm, 100 mm ×4.6 mm reversed-phase C18 column and a 4.6 mm C18 guard cartridge insert (Phenomenex SecurityGuard ULTRA; Phenomenex Kinetex, Torrance, CA, USA) were used. The mobile phase consisted of 1% acetic acid (A) and methanol (B). The mobile phase was degassed and delivered as a gradient (methanol, from 15 to 40%), with a flow rate of 1 mL·min^{-1} and a pressure of 12–15 MPa. Absorbance was monitored at 280 nm and at a sensitivity of 0.01 A, recording peak integrals. A standard curve was prepared for each set of samples using caffeine concentrations of 1.25, 2.5, 5.0 and 10.0 mg l^{-1}. Standards were prepared by diluting 100 mg l^{-1} caffeine in 1% acetic acid with serum from individuals who had abstained from methylxanthines for 3 days. Caffeine was extracted from standards and serum samples using solid-phase extraction columns (Phenomenex Strata C18-E; Phenomenex Kinetex) according to the manufacturer's instructions. Columns were conditioned by passing 1 ml of methanol and 2 × 1 ml of distilled water through them under vacuum. Two hundred microlitres of serum were applied and, after 1 min, drawn through the column under vacuum. After an equilibration period of 2 min, the columns were washed with 2 × 1 ml of distilled water. Caffeine was eluted with 400 μl of methanol. The solvent was evaporated under nitrogen and the residue reconstituted in 400 μl of mobile phase. Potassium was assessed by the spectrophotometric method with Konelab 20 XTi (Thermo Fisher Scientific, Vantaa, Finland).

2.14 Data analysis

R.N.O.M was blinded to the experimental conditions and performed all analyses of the raw data. Peak-to-peak amplitude of H-reflexes, M-waves, MEPs and the twitch following the MVCs [potentiated twitch (PTw)], in addition to VA and the greatest force value during MVCs, were obtained through automated analysis in Spike2 software (v.6.17; Cambridge Electronic Design). The duration of SP was analysed manually in the same software. After decoding, the data obtained in each experimental session were grouped for statistical analysis.

2.14.1 H-reflexes and M-waves

The highest peak-to-peak amplitude of the H-reflex during the recruitment curve was determined and normalized to the peak-to-peak amplitude of M_{max}. Immediately before and after the fatigue protocol and during the recovery period, the H-reflex was taken as the average of the five control H-reflexes that were evoked with a concomitant stable M-wave, and normalized to the M_{max} evoked at that time point. The concomitant evoked M-wave was 7.6 ± 4.3% of M_{max} on the placebo day and 7.4 ± 3.8% on the caffeine day. The M_{max} value used for analysis from the pre- and post-capsule measurements was the highest value evoked either during the recruitment curve protocol or before MVCs.

2.14.2 Motor evoked potentials

Peak-to-peak unconditioned MEP amplitudes (single-pulse stimulation) were averaged from each train of 10 stimuli and normalized to M_{max}. Regarding the paired-pulse protocol (SICI and ICF) in the pre- and post-capsule measurements, the average of the amplitudes of each conditioned test MEP was expressed as a percentage of the average MEP size (control MEP) of the train of single-pulse stimuli from the same time point.

2.14.3 Transcranial magnetic stimulation-evoked silent period

The duration of the SP was measured from the end of the MEP evoked by the test stimulus to the resumption of any level of sustained EMG activity. The SP onset and offset were identified by visual inspection. The presented SP is the average value of the three trials.

2.14.4 Voluntary activation and contractile properties

Voluntary activation was calculated with the linear equation: VA (%) = [1 − (SIT/PTw)] × 100, according to the interpolated twitch technique, where SIT is the amplitude of the superimposed twitch and PTw is the amplitude of the potentiated twitch evoked at rest after MVC. The VAs presented from the pre- and post-capsule measurements are the averaged values of the three trials. To examine contractile properties, PTw was analysed.

2.14.5 Force and rate of force development

The MVC peak was defined as the greatest force achieved before the stimulation. In the pre- and post-capsule measurements, the highest MVC peak was considered. The rate of force development (RFD) was assessed by analysing the average slope values of the force–time curve over time intervals of 0–100 ($RFD_{0–100}$) and 0–200 ms ($RFD_{0–200}$), starting from the onset of contraction (identified through automated analysis in Spike2 software), in addition to the maximal instantaneous ascending rate of force development (RFD_{max}). For the analysis of RFD in the pre- and post-capsule measurements, the trial with the highest RFD_{max} was considered.

2.14.6 Squat jump and drop jump performance

Jump height was determined by subtracting the relative standing height of the participant on the sledge from the highest position of the sledge odometer, which was determined by visual inspection in each jump. The best trial was considered.

2.15 Statistical analysis

Data are presented as means ± SD. Statistical analysis was performed using SPSS (v.20; SPSS Inc., Chicago, IL, USA). Statistical significance was set at an α level of 0.05. Normality of the data was examined using Shapiro–Wilk test. For VA, the combinations of the independent variables were normally distributed after an arc-sin transformation of the square root of the raw values [arc-sin √(raw value) − 0.2854]. Student's two-tailed paired t test was used to analyse the effects of caffeine on the number of sets completed in the fatigue protocol. Pre- to post-capsule data, in addition to fatigue and recovery data, were analysed using repeated-measures ANOVA, with time and drug as independent factors. Additionally, exploratory analysis was conducted through repeated-measures

mixed ANOVA, which was used to investigate differences between two subgroups of the present sample. These subgroups were identified through a k-means cluster analysis, based on the placebo-to-caffeine change in the number of sets during the fatigue protocol. To determine the optimal number of clusters, k-means clustering was initially computed for different values of k. For each value of k, the squared average distance of each point within a cluster to the cluster centroid was calculated and then averaged across all clusters. The lowest mean value across clusters was then used to determine the optimal value of k. The least significant difference (LSD) method was used for post hoc comparisons. For all ANOVAs, sphericity was assessed using Mauchly's test of sphericity. When sphericity was violated, the Greenhouse–Geisser correction was used.

Effect sizes were reported as Cohen's d_z for the t test, and as partial eta squared (η^2_p) for ANOVAs. Magnitudes of d_z were interpreted using the following thresholds: trivial, < 0.2; small, 0.2–0.6; moderate, 0.6–1.2; large, 1.2–2.0; or very large, > 2.0. Regarding correlations, Pearson product–moment correlation was used when data were normally distributed and Spearman's rank order correlation for non-normally distributed data.

2.16 Data availability

Non-identifiable datasets generated during and/or analysed during the present study are available from the corresponding author on reasonable request.

3 Results

3.1 Plasma values of caffeine

Blood analysis revealed a non-existence of caffeine in the bloodstream before capsule administration. Caffeine increased over time during caffeine trials [$F(1.858, 31.582) = 368.785$, $P < 0.001$, $\eta^2_p = 0.956$], reaching an average peak level ($P < 0.001$) of 8.51 $mg \cdot l^{-1}$ (range, 7.22–10.94 $mg \cdot l^{-1}$) 60 min after capsule ingestion. Immediately after the fatigue protocol [7.04 (5.38–9.02) $mg \cdot l^{-1}$] and 6 h after ingestion [4.77 (1.78–7.02) $mg \cdot l^{-1}$] it was significantly decreased ($P < 0.001$ for both pairwise comparisons). Large inter-individual variability was observed in plasma caffeine concentrations throughout the protocol. Nevertheless, the degree of responsiveness to caffeine in the fatigue protocol (number of sets performed, as the percentage change from placebo) was not

significantly correlated with plasma caffeine values either 60 min after ingestion (r = 0.10, P = 0.693) or immediately after exhaustion (r = 0.24, P = 0.345). Participants finished the fatigue protocol ∼3 h (196 ± 29 min) after ingestion of the caffeine capsule.

3.2 Pre- to post-capsule measurements

3.2.1 Exercise performance variables

Analysis of variance revealed no significant main effect of time (P = 0.403), drug (P = 0.529) or interaction between these two factors (P = 0.476) for MVC [pre-capsule on the placebo day (PREPLA), 1918.6 ± 345.1 N; post-capsule on the placebo day (POSTPLA), 1962.4 ± 374.4 N; pre-capsule on the caffeine day (PRECAFF), 1893.0 ± 347.9 N; and post-capsule on the placebo day (POSTCAFF), 1898.3 ± 401.6 N]. Likewise, there was no significant effect of time, drug or time*drug interaction for $RFD_{0–200}$ (PREPLA, 5086.3 ± 1451.9 N s^{-1}; POSTPLA, 5208.4 ± 1403.2 N s^{-1}; PRECAFF, 4676.3 ± 2077.5 N s^{-1}; and POSTCAFF, 4875.9 ± 2059.8 N s^{-1}). The $RFD_{0–100}$ and RFD_{max} behaved in the same way.

Squat jump height was increased after capsule ingestion [F(1, 17) = 4.825, P = 0.042, η^2_p = 0.221], but no significant main effect of drug (P = 0.176) or time*drug (P = 0.460) was found (PREPLA, 78 ± 8 cm; POSTPLA, 79 ± 7 cm; PRECAFF, 79 ± 9 cm; and POSTCAFF, 81 ± 9 cm).

For drop jump height, a significant main effect of drug was not observed (P = 0.309). However, there was a significant effect of time [F(1, 17) = 11.064, P = 0.004, η^2_p = 0.394] and a significant time*drug effect [F(1, 17) = 59.587, P = 0.001, η^2_p = 0.485]. Post hoc analysis (Figure 2a) revealed a significant 4.2 ± 3.7% improvement after caffeine administration (93 ± 9 versus 97 ± 10 cm, P < 0.001) which was not observed in the placebo trial (93 ± 10 versus 94 ± 11 cm, P = 0.704).

3.2.2 H-reflex

Analysis of variance revealed no significant main effect of time (P = 0.202), drug (P = 0.493) or time*drug interaction (P = 0.485) on the maximal H-reflex (PREPLA, 53.8±15.3%; POSTPLA, 54.7±14.4%; PRECAFF, 55.4 ± 16.6%; and POSTCAFF, 58.4 ± 15.9%).

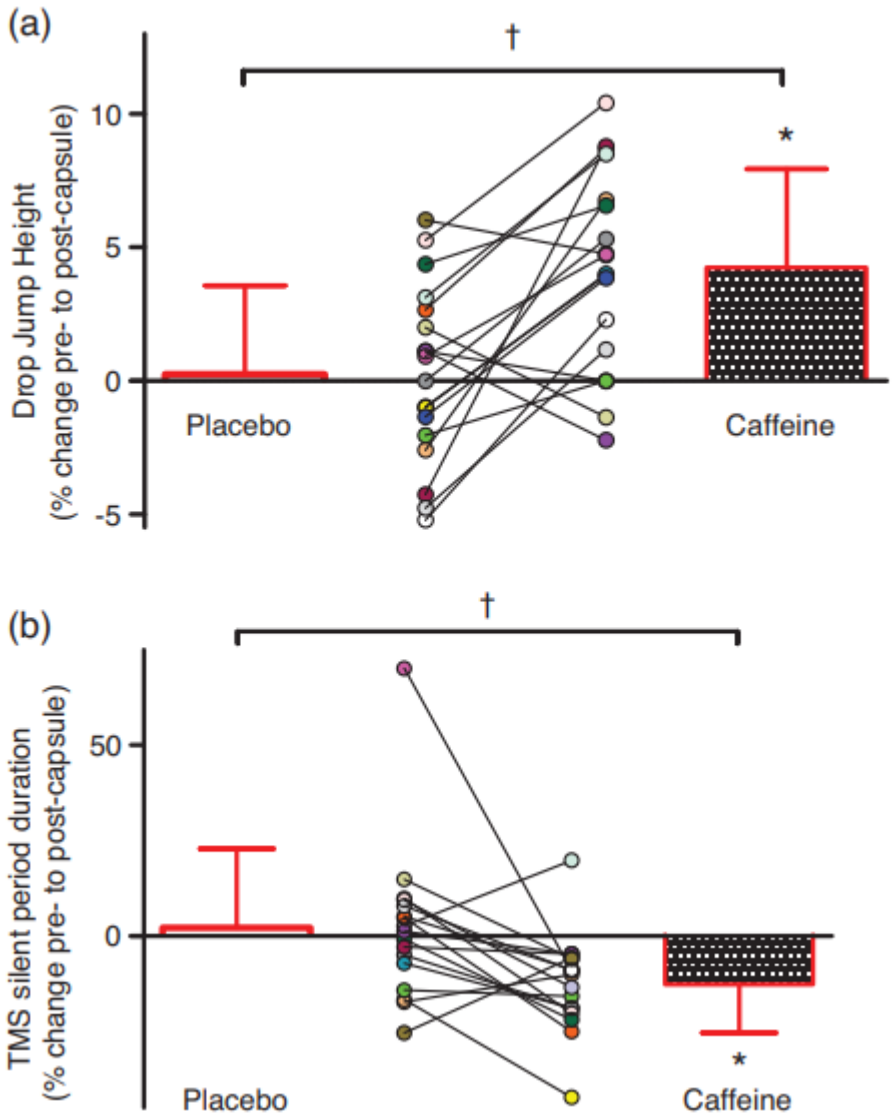

*Figure 2 Pre- to post-capsule changes. (a) Drop jump height. (b) Transcranial magnetic stimulation (TMS)-evoked silent period duration. Values are means (SD). Significant difference between time points: *P < 0.05. Significant difference between the post-capsule measurement of the caffeine trial and the placebo trial: †P < 0.05.*

3.2.3 Transcranial magnetic stimulation measures

Analysis of variance revealed no significant main effect of time (P = 0.780), drug (P = 0.897) or time*drug interaction (P = 0.151) on MT (PREPLA, 36 ± 6%; POSTPLA, 35 ± 5%; PRECAFF, 35 ± 7%; and POSTCAFF, 36 ± 7%). No significant changes were found for the unconditioned MEP amplitude either (PREPLA, 12.3 ± 4.3%; POSTPLA, 11.7±5.5%; PRECAFF, 12.3±5.6%; and POSTCAFF, 11.3±5.0%), regarding time (P = 0.950), drug (P = 0.110) or time*drug interaction (P = 0.756).

Regarding SICI, there was no significant main effect of drug (P = 0.431) or time*drug interaction (P = 0.418). However, the amplitude of the conditioned MEP was significantly higher in the postcapsule measurement for both conditions [F(1, 17)=12.869, P=0.002, η^2_p = 0.431; PREPLA, 32.0 ± 14.3%; POSTPLA, 38.2 ± 16.8%; PRECAFF, 31.3 ± 12.9%; and POSTCAFF, 34.2 ± 13.2%].

On average, the protocol to induce ICF did not elicit a facilitated conditioned MEP (PREPLA, 60.3 ± 25.1%; POSTPLA, 64.7 ± 32.8%; PRECAFF, 47.1 ± 20.3%; and POSTCAFF, 53.7 ± 21.3%). ANOVA for ICF did not reveal a significant effect of time (P = 0.215), drug (P = 0.052) or time*drug interaction (P = 0.818).

A significant effect of drug (P = 0.682) was not observed for SP. However, there was a significant effect of time [F(1 16) = 5.142, P = 0.038, η^2_p = 0.243] and a significant effect of time*drug interaction [F(1, 16) = 10.764, P = 0.005, η^2_p = 0.402]. Post hoc analysis revealed a significant 12.6 ± 12.7% decrease of SP after caffeine ingestion (78 ± 17 versus 67 ± 12 ms, P = 0.002), which was not observed in the placebo conditions (71 ± 15 versus 72 ± 16 ms, P = 0.854; Figure 2b). Further analysis revealed that the exclusion of an outlier who showed a ∼70% increment of the SP on the placebo day did not affect the pattern of the aforementioned results.

3.2.4 Voluntary activation and potentiated twitch

Analysis of variance revealed no significant main effect of time (P = 0.613), drug (P = 0.179) or time*drug interaction (P = 0.496) on VA, which ranged from 95 to 100%. Merged placebo and caffeine data revealed that PTw was significantly potentiated following three MVCs (P = 0.006, η^2_p = 0.368), but caffeine had no effect on the degree of potentiation.

3.3 Fatigue protocol and recovery

On average, the number of sets completed in the fatigue protocol was not significantly different between conditions, with a moderate effect size favouring caffeine [t(17) = −1.474, P = 0.159, d_z = 0.347] between placebo (7 ± 5) and caffeine (8 ± 6)]. However, a k-means clustering approach was used, revealing that the lowest within-cluster sum of squares was observed for k = 3, with a cluster of participants who did not benefit from caffeine ingestion or with a trivial magnitude of response (n = 8; range, −29.4 to 2.4% of change from placebo to caffeine), a cluster of participants with a considerable improvement on the caffeine day (n = 9; range, 11.20–74.29% change from placebo to

caffeine) and a cluster with only one participant with a considerably higher level of responsiveness (141.5% change from placebo to caffeine), who was considered an outlier. The remaining two subgroups were used as an independent variable for exploratory analysis. A significant effect of this between-subjects factor was observed only for M_{max} and RPE, and is described below. Preliminary analysis revealed that the exclusion of the outlier in this exploratory analysis did not affect the pattern of results. Individual data regarding the relative change of the number of sets between conditions can be seen in Figure 3.

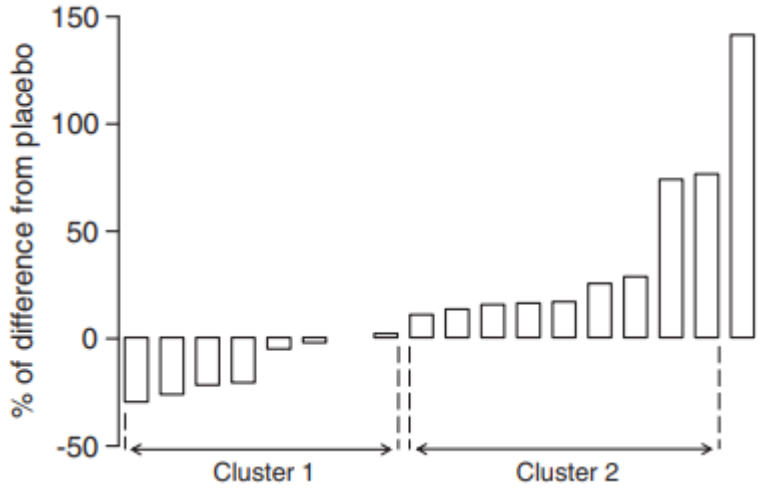

Figure 3 Percentage of change from placebo, regarding the number of sets completed for each participant. Each bar represents a different participant, and positive values indicate a longer endurance time with caffeine. A k-means clustering approach was used, revealing that the lowest within-cluster sum of squares was observed for k = 3, with a cluster of participants who did not benefit from caffeine ingestion (n = 8; range, −29.4 to 2.4% of change from placebo to caffeine), a cluster of participants with a considerable improvement on the caffeine day (n = 9; range, 11.20–74.29% change from placebo to caffeine) and a cluster with only one participant with a considerably higher level of responsiveness (141.5% change from placebo to caffeine), who was considered an outlier.

The number of sets completed in the caffeine trial revealed a weak to moderate correlation with the pre- to post-capsule caffeine-induced decrease in SICI (Figure 4a) and a significant moderate correlation with the pre- to post-capsule caffeine-induced decrease in SP (Figure 4b). The degree of responsiveness to caffeine (percentage change from placebo, regarding the number of sets performed) was not associated with the daily habitual consumption of caffeine ($r = 0.100$, $P = 0.693$) or the order of trials.

Means and SDs of the neuromuscular measures presented below can be found in Table 1.

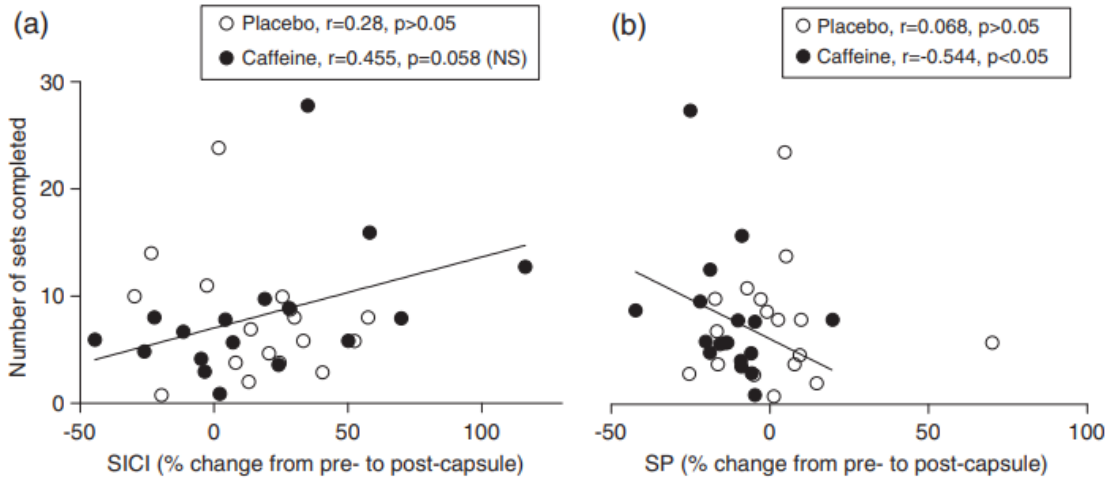

Figure 4 (a) Correlation between pre- to post-capsule changes in short-interval intracortical inhibition (SICI) and number of sets completed during the fatigue protocol in both trials. (b) Correlation between pre- to post-capsule changes in the duration of the corticospinal silent period (SP) and number of sets completed during the fatigue protocol in both trials. The percentage change in SICI is related to the amplitude of the unconditioned motor evoked potential (MEP). Thus high positive percentages reveal a decrease in SICI. The lines in the graphs are fitted to the caffeine data points only, because there was no significant correlation between the variables in the placebo condition. Abbreviation: NS, non-significant.

The MVC remained depressed (Figure 5) throughout the fatiguing activity and recovery [$F(2.360, 35.394) = 22.469$, $P < 0.001$, $\eta^2_p = 0.6$], with no significant main effect of drug ($P = 0.129$) or time*drug interaction ($P = 0.814$). Likewise, there was a significant main effect of time [$F(6, 90) = 8.213$, $P < 0.001$, $\eta^2_p = 0.354$], but not drug ($P = 0.082$) or time*drug interaction ($P=0.249$) for $RFD_{0–200}$. Analysis of variance for $RFD_{0–100}$ and RFD_{max} revealed the same, with a decline of RFD during fatigue and recovery.

Regarding VA, ANOVA revealed a significant main effect of time [$F(2.924, 40.937) = 6.683$, $P = 0.001$, $\eta^2_p = 0.323$] but not drug ($P = 0.847$) or time*drug interaction ($P = 0.411$). A significant main effect of time was also found for PTw [$F(6, 90) = 9.089$, $P < 0.001$, $\eta^2_p = 0.377$], with no significant main effect of drug ($P = 0.861$) or time*drug interaction ($P = 0.861$). The VA and PTw were significantly depressed at exhaustion and throughout recovery (Figure 5).

The M_{max} was significantly depressed during fatigue and recovery [$F(2.142, 29.991) = 10.614$, $P < 0.001$, $\eta^2_p = 0.431$], with no significant effect of drug ($P = 0.241$) or time*drug interaction ($P = 0.591$). Notably, a significant interaction effect between time, drug and the two subgroups of participants was found for this variable in the repeated-measures mixed ANOVA [$F(2.938, 38.196) = 2.761$, $P = 0.005$, $\eta^2_p = 0.277$; Figure 6].

Table 1 Changes in neuromuscular variables during fatigue and recovery.

		Fatigue protocol			Recovery (min)		
Variable	**Prefatigue**	**40%**	**60%**	**Task failure**	**5**	**10**	**15**
MVC (N)							
Placebo	1962.4 ± 374.4	1744.1 ± 460.6	1714.3 ± 397.4	1581.2 ± 339.8	1509.0 ± 483.2	1515.8 ± 417.2	1522.5 ± 437.6
Caffeine	1898.3 ± 401.6	1633.8 ± 388.2	1578.0 ± 355.9	1456.3 ± 424.7	1395.5 ± 394.7	1405.1 ± 421.6	1471.2 ± 368.4
RFD_{0-100} ($N\ s^{-1}$)							
Placebo	5613.5 ± 1446.3	4939.6 ± 2182.6	4296.4 ± 1652.3	3749.7 ± 2109.4	3444.2 ± 1797.9	3500.9 ± 1516.1	3855.2 ± 1886.5
Caffeine	5129.7 ± 2185.9	4185.4 ± 1997.9	3971.4 ± 1942.9	3156.3 ± 2063.3	3113.1 ± 1586.4	3660.8 ± 2169.2	3503.6 ± 1953.0
RFD_{0-200} ($N\ s^{-1}$)							
Placebo	5208.4 ± 1403.2	4727.9 ± 1484.0	4134.0 ± 1276.1	3932.0 ± 1652.2	3463.5 ± 1475.1	3863.4 ± 1179.0	3906.8 ± 1757.2
Caffeine	4875.9 ± 2059.8	4005.5 ± 1717.9	3923.8 ± 1161.0	3218.7 ± 1616.7	3383.7 ± 1311.1	3443.5 ± 1614.6	3466.9 ± 1620.8
RFD_{MAX} ($N\ s^{-1}$)							
Placebo	11,190.4 ± 2986.0	9109.1 ± 3085.1	8217.1 ± 2817.9	8239.7 ± 2496.0	6672.3 ± 2612.1	6674.3 ± 2109.1	7223.6 ± 2640.2
Caffeine	9911.2 ± 4490.4	7783.3 ± 2942.5	8124.7 ± 3024.4	7436.2 ± 2978.3	6481.3 ± 2837.1	6503.8 ± 2219.4	6886.5 ± 1859.2
VA (%)							
Placebo	97.9 ± 2.3	96.0 ± 6.8	92.9 ± 12.3	90.5 ± 11.3	86.4 ± 13.0	91.5 ± 11.5	93.9 ± 7.0
Caffeine	99.1 ± 0.9	95.0 ± 6.4	95.7 ± 4.9	86.7 ± 15.3	83.4 ± 20.9	94.3 ± 16.0	91.7 ± 16.2
PTw (N)							
Placebo	195.5 ± 55.1	188.2 ± 46.2	188.0 ± 47.4	169.3 ± 45.8	172.0 ± 48.0	164.1 ± 37.3	159.5 ± 36.7
Caffeine	199.2 ± 44.8	196.1 ± 50.1	183.2 ± 40.2	180.3 ± 38.1	168.3 ± 48.6	173.2 ± 55.1	169.6 ± 47.6
M_{MAX} (mA)							
Overall							
Placebo	40.5 ± 1.51	3.34 ± 1.33	3.32 ± 1.47	3.50 ± 1.38	3.50 ± 1.37	3.50 ± 1.26	3.57 ± 1.49
Caffeine	4.46 ± 1.67	3.37 ± 1.40	3.25 ± 1.33	3.59 ± 1.42	3.74 ± 1.73	3.60 ± 1.80	3.86 ± 1.68
M_{MAX} (mA)							

Cluster (caffeine: +)							
Placebo	4.18 ± 1.51	3.87 ± 1.03*	3.47 ± 1.15	3.51 ± 1.12	3.37 ± 0.97	3.37 ± 0.95	3.38 ± 0.99
Caffeine	4.60 ± 1.53	3.23 ± 0.66*	3.29 ± 0.82*	3.61 ± 1.16*	4.25 ± 1.24†	4.20 ± 1.29†	4.40 ± 1.34†
M_{MAX} (mA)							
Cluster (caffeine: =/-)							
Placebo	3.95 ± 1.61	3.14 ± 1.59*	3.18 ± 1.78*	3.50 ± 1.65	3.62 ± 1.71	3.61 ± 1.53	3.74 ± 1.87
Caffeine	4.34 ± 1.88	3.51 ± 1.87*	3.22 ± 1.72*	3.56 ± 1.70*	3.30 ± 2.04*	3.07 ± 2.09*	3.39 ± 1.89*
MEP (%)							
Placebo	11.5 ± 5.2	14.2 ± 6.6	16.0 ± 7.0	13.9 ± 5.3	14.4 ± 6.2	13.0 ± 5.2	14.6 ± 5.8
Caffeine	11.7 ± 5.4	13.5 ± 6.5	14.6 ± 7.8	13.3 ± 6.7	14.1 ± 6.1	15.8 ± 9.6	15.6 ± 8.8
SP (ms)							
Placebo	73 ± 16	61 ± 18	59 ± 13	62 ± 13	63 ± 13	62 ± 15	64 ± 13
Caffeine	68 ± 12	63 ± 11	61 ± 11	62 ± 13	62 ± 15	62 ± 15	64 ± 16
H-reflex (%)							
Placebo	44.2 ± 13.3	-	-	40.3 ± 18.9	40.3 ± 16.6	43.5 ± 14.6	43.2 ± 15.8
Caffeine	41.9 ± 17.6	-	-	40.3 ± 17.7	42.7 ± 15.1	46.1 ± 14.3	43.4 ± 22.7

Values are means ± SD. Significant difference from the post-capsule value: * $P < 0.05$. When an asterisk is placed above the number, the difference was observed for the merged placebo and caffeine data. Significant difference between conditions: †$P < 0.05$. Abbreviations: MEP, motor evoked potential; M_{max}, maximal compound muscle action potential; MVC, maximal voluntary contraction; PTw, potentiated twitch; RFD, rate of force development; SP, transcranial magnetic evoked silent period; VA, voluntary activation.

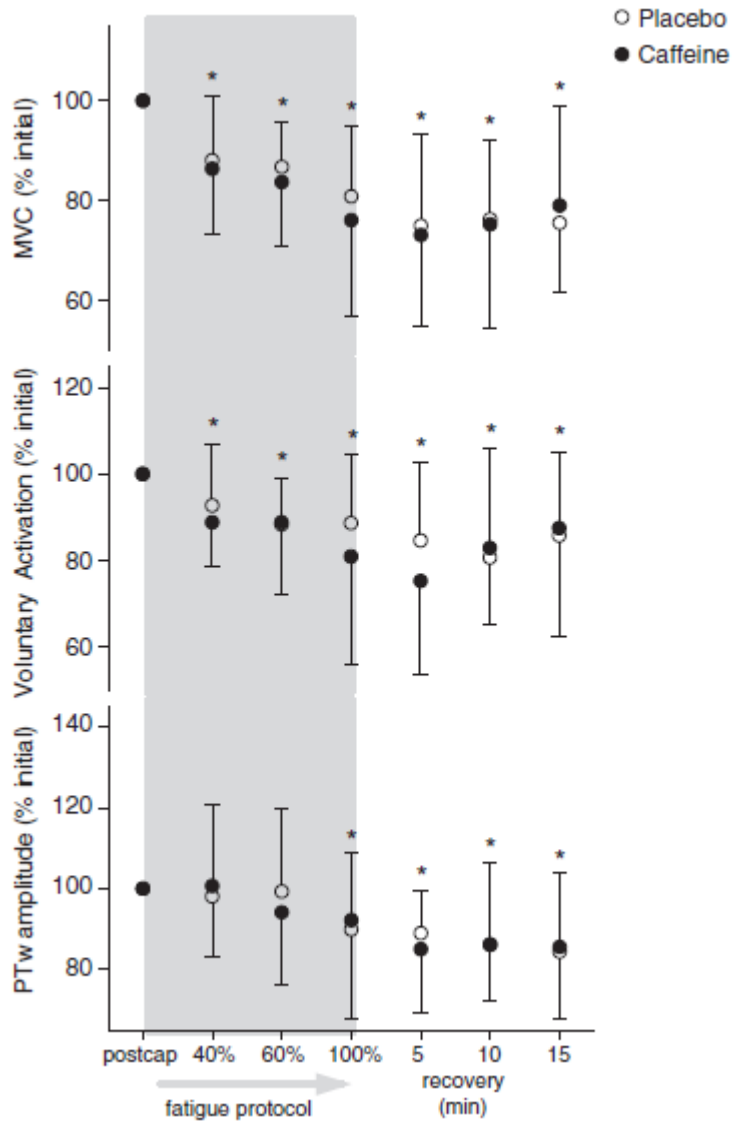

*Figure 5 Changes in maximal voluntary contraction (MVC), voluntary activation and potentiated twitch (PTw) during fatigue and recovery. The MVC, voluntary activation and PTw are expressed as a percentage of the post-capsule value in both trials. Values are means (SD). Significant difference from the post-capsule value for the merged placebo and caffeine data: *P < 0.05.*

Analysis of variance revealed no significant main effect of time (P = 0.07), drug (P = 0.895) or time*drug interaction (P = 0.378) for the MEP amplitude. The SP was 11% shorter at task failure and remained depressed during the early stage of recovery [F(3.011, 39.138)=3.793, P = 0.018, η^2_p = 0.226], with no effect of drug (P = 0.869) or interaction between these two factors (P=0.577). Analysis of variance revealed no significant main effect of time (P=0.514), drug (P=0.761) or time*drug interaction (P = 0.832) for the H-reflex measured in the ascending part of the recruitment curve.

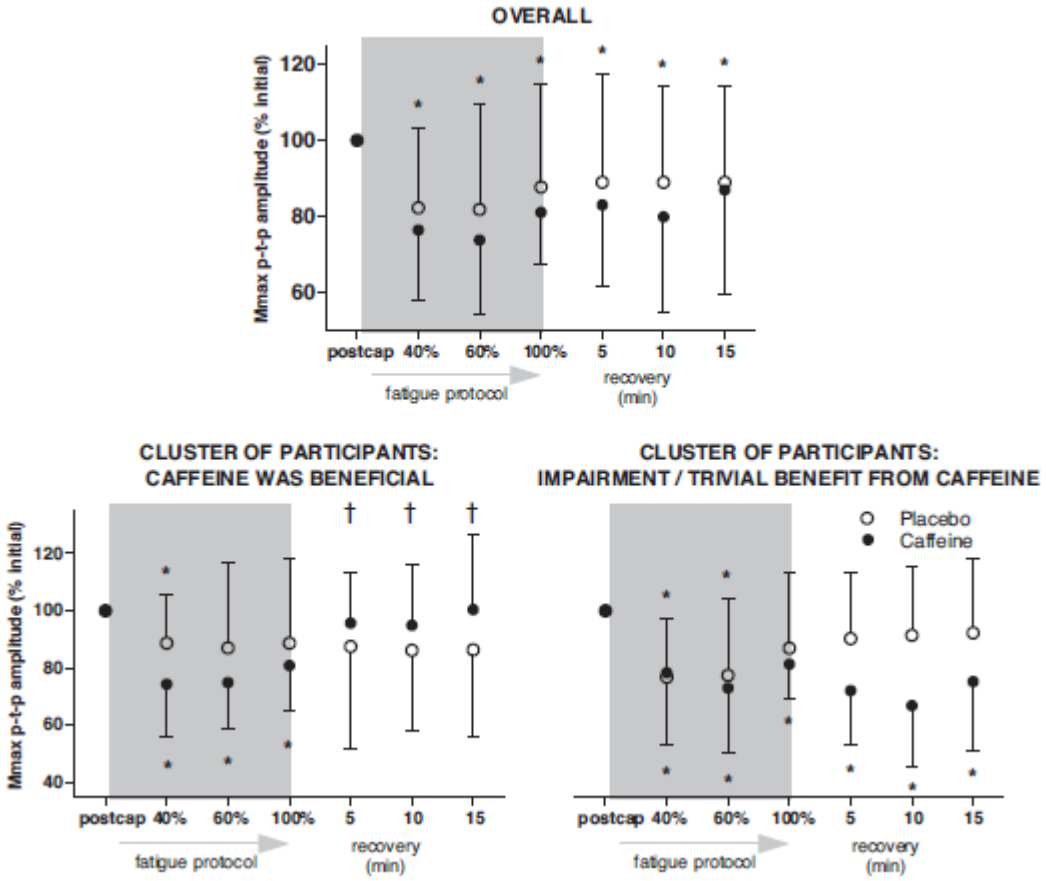

*Figure 6 Changes in maximal compound muscle action potential (M_{max}) during fatigue and recovery. The M_{max} is expressed as a percentage of the post-capsule value in both trials. Abbreviation: p-to-p, pre- to post- capsule. Values are means (SD). Significant difference from the post-capsule value: *P < 0.05. Significant difference between conditions: †P < 0.05*

Regarding RPE, ANOVA showed a significant main effect of time [F(1.172, 15.236) = 28.515, P < 0.001, η^2_p = 0.687] and drug [F(1;13) = 5.698, P = 0.033, η2 p = 0.305], but no significant interaction (P = 0.297). The RPE increased throughout the protocol, and merged data from all time points revealed significantly lower values in the caffeine conditions in the first three sets (Figure 7). Furthermore, mixed ANOVA showed that this significant caffeine-induced lower RPE was observed in the subgroup of participants who responded to caffeine (P = 0.006), but not in the other participants with a trivial benefit or with a performance impairment on the caffeine day (P = 0.534).

Analysis of variance on [K^+] revealed no significant effect of time (P = 0.07) and no significant interaction effect (P = 0.207). A significant effect of drug was, however, observed [F(1, 17) = 13.679, P = 0.002, η^2_p = 0.446]. In the caffeine trial, [K^+] was significantly lower (4.091 ± 0.245 versus 3.922 ± 0.271 mmol·l^{-1}, placebo and caffeine conditions for the merged pre- and postfatigue data).

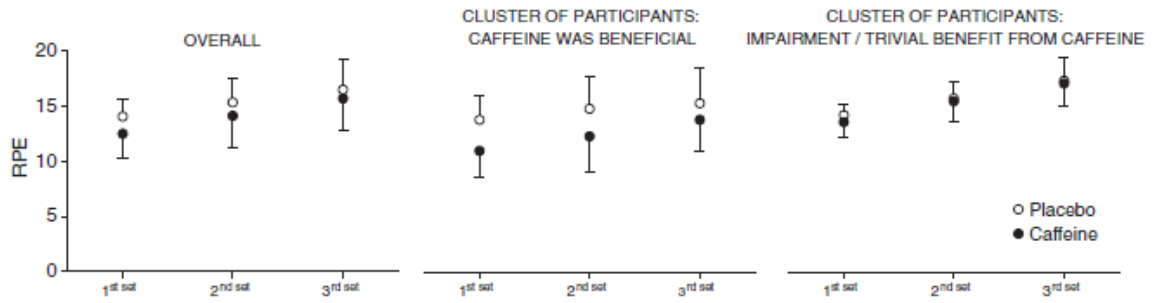

Figure 7 Changes in rate of perceived exertion (RPE) during the first sets of fatigue. ANOVA revealed a significant main effect of time and a significant main effect of drug. Post hoc analysis showed a reduced RPE in the caffeine condition. Values are means (SD). Mixed ANOVA showed that this significant main effect of drug was present in those with observed improvements in the number of sets on the caffeine day (P = 0.006), but not in those who did not benefit from caffeine (P = 0.534).

For muscle soreness, there was a significant main effect of time [F(2.729, 43.665) = 21.546, P < 0.001, η^2_p = 0.574] and session [F(1, 16) = 13.997, P = 0.002, η^2_p = 0.467] and a significant interaction between them [F(2.064, 33.019) = 5.078, P = 0.011, η^2_p = 0.241]. A delayed onset of muscle soreness was observed after both sessions and attenuated after the second session, in a time window between the following morning and 2 days later in the evening (+1 day morning, 4.9 ± 2.8 versus 3.3 ± 2.3; +1 day evening, 5.0 ± 2.8 versus 3.5 ± 2.5; +2 days morning, 4.9 ± 2.4 versus 2.6 ± 2.1; and +2 days evening: 3.5 ± 2.1 versus 2.0 ± 1.8).

4 Discussion

The aim of the present study was to investigate the underlying neuromuscular mechanisms that might be responsible for any caffeine-induced ergogenic effects in a non-fatigued state and during fatiguing SSC exercise. In this randomized, double-blind, placebo-controlled crossover trial, it was found that in a non-fatigued state, caffeine enhanced drop jump height and decreased inhibition at the cortical and/or spinal level, as revealed by the SP duration. Although caffeine administration did not significantly increase the number of sets completed during the fatiguing SSC task when looking at the averaged data, a high level of inter-individual variability was observed. Caffeine did not affect the decline of MVC and RFD at exhaustion and did not potentiate recovery in the fatigue protocol. Central and peripheral fatigue, demonstrated throughout the fatiguing protocol and recovery, were also not attenuated by this psychoactive drug. Nonetheless, the present study shows an association between pre- to post-capsule caffeine-induced changes in the CNS and a subsequent improvement of exercise performance. Furthermore, the subgroup of participants who responded to caffeine

benefitted from a decreased sense of effort during the activity and an accelerated recovery of M_{max} after fatiguing exercise.

4.1 In a non-fatigued state, caffeine improved drop jump height, but not squat jump height, MVC or RFD

Caffeine administration enhanced SSC performance (drop jump) by 4.2 ± 3.7%, without a significant ergogenic effect on isometric performance (MVC and RFD) or concentric performance (squat jump). It could be hypothesized that the known effects of this psychoactive drug on vigilance (Koelega, 1993), sustained attention (Lorist & Tops, 2003) and arousal (Barry et al., 2009), and on inhibitory and excitatory central mechanisms that were shown in the present study, might explain its selective ergogenicity in intensive and complex tasks (e.g. rebounding as high as possible after being dropped from a certain height), without an ergogenic association in tasks with less complexity (e.g. performing a squat jump). Previous studies (Abian et al., 2015; Bloms, Fitzgerald, Short, & Whitehead, 2016; Del Coso et al., 2014; Pérez-López et al., 2015) have shown an increased squat jump height after caffeine ingestion. However, these studies did not include a precapsule measurement and, therefore, these data should be interpreted with caution, because day-to-day variability was not controlled (for example, in our study squat jump height had a day-to-day coefficient of variation of 5.2%).

4.2 Effects of caffeine on the CNS in a non-fatigued state and during the fatigue protocol

The antagonism of adenosine receptors in the CNS is consistently identified as the leading hypothesis to explain the ergogenic effects of caffeine. In the present study, pre- to post-capsule changes in the CNS were associated with a subsequent improvement of exercise performance. In a non-fatigued state, caffeine administration resulted in a significant 12.6 ± 12.7% decrease of SP, which was not observed in the placebo condition. The SP has usually been used as a measure of intracortical inhibition that is activated by long-lasting GABAB receptors (McDonnell, Orekhov, & Ziemann, 2006). More recently (Škarabot, Mesquita, Brownstein, & Ansdell, 2019), it has been suggested that both cortical and spinal inhibitory mechanisms might contribute to the SP duration. Cerqueira et al. (2006) found a decrease in this measure in the biceps brachii and abductor digiti minimi, which was similar to the present study, whereas Orth et al. (2005) found an unaltered SP in the first dorsal interosseus muscle. This inconsistency might be attributed to the use of different

doses of caffeine, different muscles under investigation and different methodological procedures used to examine SP.

All these confounding factors might also explain the divergent results regarding fatigue-induced changes of SP. In the present study, SP was decreased in both conditions throughout fatigue, reaching a value that was 11% lower at task failure, and remained depressed during the early stage of recovery. Thus, it is very likely that the participants experienced a fatigue-induced decrease in cortical and/or spinal inhibition. However, there are some limitations and confounding factors associated with this measure that should be acknowledged. It is possible that fatigue-induced changes in neural drive might have modulated SP duration. However, this is unlikely given that participants were asked to perform a submaximal contraction, which reached a force level that was relative to the new MVC. Furthermore, most studies have been unable to demonstrate a relationship between neural drive and the duration of SP (e.g. Säisänen et al., 2008). In the present experiment, SP was analysed from the end of MEP, as suggested by Säisänen et al. (2008), in order to reflect the period solely marked by inhibitory influence. Another option would have been to use a relative SP measured from the onset of MEP or from the TMS delivery (Škarabot et al., 2019). Although a significant change in the MEP amplitude was not found in the present study after fatigue, it should be remembered that any changes in the characteristics of MEP associated with fatigue might influence the duration of SP to some extent. However, it is important to note that the exact time point of the start of the inhibitory mechanisms that explain SP and the beginning of the excitatory mechanisms reflected in the onset of MEP might not coincide. Nevertheless, a similar behaviour has been observed in previous studies (Girard, Bishop, & Racinais, 2013; Kirk, Trajano, Pulverenti, Rowe, & Blazevich, 2019; Latella, Hendy, Vanderwesthuizen, & Teo, 2018; Mira et al., 2017). We hypothesize that this suppression of inhibitory mechanisms might be the expression of a compensatory phenomenon to try to overcome a deficit of VA, which was observed throughout the fatiguing protocol and recovery.

De Carvalho et al. (2010) showed a decrease of SP (of ∼17%) after a 2-min MVC in caffeine conditions, which was not observed in the placebo trial. Although caffeine did not have an influence on the changes of SP during fatigue and recovery in the present study, the caffeine-related decline of SP before the fatiguing exercise was associated with a better performance in the caffeine trial. It is possible that the observed shortening of SP in a non-fatigued state after caffeine ingestion and after the fatigue protocol might be attributable to a decrease of the long-lasting activity of cortical

GABAergic neurons (Krnjević, Randić, & Straughan, 1966). However, other mechanisms of spinal origin cannot be ruled out (Škarabot et al., 2019).

The relationship between suppression of inhibitory mechanisms before exercise and subsequent exercise performance was also corroborated by a weak to moderate correlation between the preto post-capsule caffeine-induced decrease in SICI and the number of sets completed in the caffeine trial. SICI is a form of inhibition that is modulated by the activation of $GABA_A$ receptors within the primary motor cortex (Ziemann, Lönnecker, Steinhoff, & Paulus, 1996). To the best of our knowledge, this is the first time that a caffeine-induced decrease of inhibitory mechanisms in the CNS has been associated with subsequent exercise performance. At the group level, however, caffeine did not influence the pre- to post-capsule decrease in SICI, which is consistent with data obtained by Orth et al. (2005) and de Carvalho et al. (2010).

Surprisingly, when using the ICF protocol, the amplitude of the conditioned MEP was lower than the amplitude of the unconditioned MEP. This has also been observed earlier (e.g. Brownstein et al., 2018). Previous studies did not show a significant effect of caffeine on intracortical excitatory mechanisms (de Carvalho et al., 2010; Orth et al., 2005), but we cannot be sure that we were, in fact, testing this association owing to our inability to induce an average ratio of conditioned/unconditioned MEP amplitude >100%. The cortical mechanisms underlying ICF are not yet understood fully, but it has been suggested that it results concurrently from excitatory glutamatergic responses and from weak $GABA_A$ inhibitory responses (Ziemann et al., 1996). These results challenge the validity and applicability of investigating intracortical excitatory mechanisms in the SOL.

As expected, caffeine had no effect on MT. This is in agreement with previous studies (Cerqueira et al., 2006; de Carvalho et al., 2010; Orth et al., 2005). We also did not observe a caffeine-induced effect on the MEP amplitude during a non-fatigued state, which is consistent with earlier studies (Cerqueira et al., 2006; de Carvalho et al., 2010; Kalmar & Cafarelli, 2004; Orth et al., 2005).

Central fatigue experienced by our participants, reflected by a marked decrease of VA throughout fatigue and recovery, was not attenuated by caffeine ingestion. Such an attenuation has been reported only once (Del Coso et al., 2008), which is in contrast to a considerable number of studies (Cureton et al., 2007; Eaton et al., 2016; Kalmar & Cafarelli, 2006; Kalmar et al., 2006;Meyers & Cafarelli, 2005; Smirmaul et al., 2016).

In the present study, caffeine had no effect on the size of the Hreflex. This is consistent with data obtained by Kalmar et al. (2006) and by Behrens et al. (2015a, b), but contrary to other studies where an enhancement of the peak-to-peak maximal normalized H-reflex (Kalmar & Cafarelli, 1999) and of the normalized slope of the Hreflex recruitment curve (Walton et al., 2003) were observed. Walton et al. (2002) also showed an effect of caffeine on the strength of persistent inward current activity of spinal motoneurons. Further research should elucidate the effects of caffeine on spinal mechanisms. Kalmar et al. (2006) observed a protective effect of caffeine on the decline of α-motoneuron excitability after fatigue, but this measure was unaltered in the present study. Consistent with our findings, Boerio, Jubeau, Zory, and Maffiuletti (2005) and Laurin, Dousset, Carrivale, Grélot, and Decherchi (2012) also did not observe significant changes after fatigue. H-reflexes might not be attributed to a single and isolated spinal mechanism (Zehr, 2002), and other approaches might be more sensitive to examine the effects of caffeine and fatigue on spinal excitability (e.g. cervicomedullary motor-evoked potentials). Unlike the evoked H-reflex, cervicomedullary motor-evoked potentials are not affected by presynaptic inhibition (Nielsen & Petersen, 1994).

4.3 Caffeine did not affect contractile properties

As expected, caffeine did not have an effect on contractile properties. An extensive number of studies in vivo have corroborated the implausibility of peripheral ergogenic effects of caffeine (e.g. Neyroud et al., 2019), with only three studies showing an attenuated decrease of contractile measures during fatigue (Bazzucchi et al., 2011; Cureton et al., 2007; Meyers & Cafarelli, 2005) and another study showing an offset of the decline of low-frequency tetanic force (Tarnopolsky & Cupido, 2000).

4.4 Caffeine enhanced the recovery of neuromuscular transmission, but only in responders

To the best of our knowledge, this is the first study to show an effect of caffeine on the recovery of neuromuscular transmission after fatiguing SSC exercise, which was accelerated only in the subgroup of participants who had a considerable improvement on the caffeine day. The fatiguing task resulted in a significant reduction of M_{max}, which remained suppressed in those who did not considerably enhance their performance on the caffeine day but recovered to baseline values in those who did.

These findings support the idea of a caffeine-related enhancement of peripheral neuromuscular transmission, which has previously been observed through an attenuation of plasma K^+ concentrations (Lindinger et al., 1993), attenuated muscle interstitial K^+ concentrations (Mohr et al., 2011) and a better preservation of M_{max} at task failure (Bowtell et al., 2018). A caffeine-induced effect on peripheral membrane excitability would attenuate postsynaptic neuromuscular transmission failure and possibly prevent other K^+ mediated mechanisms, such as failure of axonal propagation of action potentials (Adelman, Palti, & Senft, 1973) and activation of small-diameter group III–IV afferents (Rybicki, Waldrop, & Kaufman, 1985), with a possible inhibitory effect on the CNS (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016) in some motoneuron pools. The current caffeine-related findings on recovery of neuromuscular transmission are corroborated, in part, by an observed significant main effect of drug on plasma concentrations of $[K^+]$, which were attenuated in the caffeine conditions. Although a significant interaction effect of time*drug with a caffeine-related attenuation of K^+ accumulation in the bloodstream would be expected, it could be argued that venous blood samples are an indirect measure, which cannot directly translate what is occurring within the muscle. This has been demonstrated by Mohr et al. (2011), where plasma $[K^+]$ was not a sensitive measure of muscle interstitial $[K^+]$ changes. In fact, it is likely that an efflux of K^+ into the bloodstream resulted in an uptake of K^+ into inactive tissues (e.g. resting muscles), as noted by Lindinger, Heigenhauser, McKelvie, and Jones (1990).

4.5 Effects of caffeine on sense of effort

Interestingly, those with a considerable improvement of performance during the fatigue protocol on the caffeine day demonstrated a caffeine-induced attenuated sense of effort during the first three trials of the fatigue protocol, whereas the other subgroup of participants did not benefit from this effect. Perception of effort might be modulated through the effects of caffeine on adenosine receptors (Doherty & Smith, 2005) at multiple sites of the CNS. Caffeine might have influenced the neurocognitive processing of corollary discharges from premotor and motor areas of the cortex, which would be consistent with data obtained by de Morree, Klein, and Marcora (2014), who showed an association between caffeine ingestion and a reduction in the activity of cortical premotor and motor areas. Alternatively, caffeine could also have modulated the afferent feedback from group III/IV muscle afferents (Amann et al., 2010). Although the participants of the present study received exercise-specific instructions about how to rate perception of effort, we cannot absolutely rule out the possibility of inclusion of other exercise-related sensations in their rating of effort, such as muscle pain or the fatigue-related burning sensation in the muscles. Given that

humans are able to dissociate between perceptual physical effort and perception of pain (O'Connor & Cook, 2001), further research should be carried out to examine the effects of caffeine on perception of effort and other sensations related to physical exercise with specific psychophysical scales.

4.6 Repeated bout effect was observed after 14 days

In this randomized, double-blind, placebo-controlled crossover trial, a lower amount of muscle soreness was observed after the second session. This protective effect against muscle damage from a single bout of SSC exercise is known as the repeated bout effect (McHugh, 2003). As an interesting aside, we found that a repeated bout effect can be verified after 14 days.

4.7 Categorization of sample participants according to the degree of responsiveness

Given that group mean changes might not tell the whole story, an exploratory analysis based on k-means clustering was conducted, dividing our study sample into those with a considerably large improvement on the caffeine day and those with a trivial benefit or with a performance impairment. Although such an approach has been used before to identify clusters of individuals with different degrees of responsiveness (Bamman, Petrella, Kim, Mayhew, & Cross, 2007), a note of caution is required here, because this classification could have led to false inferences about individuals who are deemed to respond/not respond to caffeine, owing to within-subject variation between the two sessions. Future research could explore differences of neuromuscular mechanisms between responders and non-responders to caffeine with larger sample sizes and more robust sample responder counting that would take into consideration, for example, a measurement error statistic of the performance measure (e.g. two times the 'typical error'; Ross, de Lannoy, & Stotz, 2015). In the present study, this approach could not be taken.

4.8 Conclusions

In this study, we elucidated the mechanisms of action of caffeine that might explain its ergogenic effects on exercise performance and contributed to a better understanding of neuromuscular changes during fatiguing activity. A caffeine dose of 6 $mg \cdot kg^{-1}$ improves drop jump height in a non-fatigued state and decreases SP. A caffeine-induced decrease of inhibitory mechanisms seems to be associated with increased performance in subsequent exercise. Those who benefit from caffeine

ingestion experience a lower sense of effort during the exercise and an accelerated recovery of M-wave amplitude. Ergogenic effects of caffeine are highly dependent on individual responsiveness, and thus investigation of the effects of this drug on an individual basis is highly recommended. Further research could explore the effect of caffeine on the modulation of intracortical mechanisms during fatiguing exercise and provide a better description of the relationship between genetic polymorphisms and inter-individual variation in the response to caffeine and its neuromuscular effects.

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Competing Interests

None declared.

Author Contributions

R.N.O.M., J.H. and J.A. contributed to the study conception and design. Acquisition and analysis of the data were performed by R.N.O.M., N.J.C. and J.H., and all authors contributed to the interpretation of data. The first draft of the manuscript was written by R.N.O.M., and all authors contributed critically to early versions of the manuscript for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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